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Interim Report: "Microbiology of juvenile *Porites astreoides* in the Florida Keys"

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SUMMARY

In this collaborative research proposal titled "Microbiology of juvenile *Porites astreoides* in the Florida Keys", we proposed a series of experiments to determine: 1) the composition of bacterial communities in *P. astreoides* juveniles in post-settlement developmental stages; 2) whether the bacterial communities associated with coral recruits reflect what is in the surrounding seawater; and 3) whether macroalgae and/or their compounds can alter the bacterial communities in *P. astreoides* recruits. The underlying aim of this series of studies is to determine how juvenile corals interact with seawater microbes in their earliest life stages – while establishing their bacterial communities.

As juvenile corals continue to develop, are juveniles colonized by whatever bacteria are in the surrounding seawater, or are they able to "select" particular bacterial species? Furthermore, does macroalgal overgrowth, which is common throughout Florida Keys reefs, alter bacterial community composition in the juvenile corals? Does an increased amount of macroalgae on reefs affect the bacterial component of juvenile corals, and more importantly, their survival? This proposal aims to identify factors that control the timing and specificity of bacterial colonization in juvenile coral stages. The processes that regulate colonization of coral mucus and tissues are currently unknown, and characterization of the timing and species-specificity of bacterial colonization of corals will provide important insight into determinants of coral health and disease. Though data from our previous research indicate that *P. astreoides* acquires a consistent community of bacterial species regardless of location (Florida or Belize) and time (2006-2009 collections), it is unclear whether changes in the seawater and on the reef can alter this acquisition process. This study has the potential to influence conservation and management strategies significantly by identifying mechanisms by which corals in the Florida Keys National Marine Sanctuary – and potentially other regions in Florida and the Caribbean – acquire and maintain populations of specific bacteria. Investigations of the specificity and diversity of coral-associated microbial communities will further the understanding of their potential roles in coral health, including their responses to shifting bacterial communities as a result of global change.

Furthermore, does macroalgal overgrowth, which is common throughout Florida Keys reefs, alter bacterial community composition in the juvenile corals? Does an increased amount of macroalgae on reefs affect the bacterial component of juvenile corals, and more importantly, their survival? The work we propose here aims to identify factors that control the timing and specificity of bacterial colonization in juvenile coral stages. Our results will provide critical

insight into communication among the multiple partners in the coral holobiont and identify significant determinants in host vulnerability to bacterial disease and other threats in today's reef habitats.

OBJECTIVES AND HYPOTHESES

Objectives

The overall research objective that we originally proposed was to evaluate whether bacterial attachment and infection of coral mucus and tissues is a controlled and specific process. The processes that regulate colonization of coral mucus and tissues are currently unknown, and characterization of the timing and species-specificity of bacterial colonization of corals will provide important insight into determinants of coral health and disease.

Hypotheses

We proposed to test the following three hypotheses:

1. *P. astreoides* juvenile recruits acquire specific amounts and types of bacteria from the seawater.
2. Alterations of bacteria in the seawater surrounding settled polyps will not be reflected in the bacterial communities on the coral mucus layer or inside the coral tissue.
3. Exposure of juvenile recruits to macroalgae and macroalgal compounds will alter the bacterial communities associated with *P. astreoides* recruits.

PROGRESS TO DATE

1. Determine the composition of bacterial communities in *P. astreoides* juveniles in post-settlement developmental stages.

In the field, we were successful at harvesting *P. astreoides* larvae and at getting them to settle on conditioned glass microscopy slides and then develop on those slides in mesh-lined buckets in the seagrass beds (Fig 1). We harvested recruits in stages as late as 16 days after release, and these samples now represent the most mature samples we have collected. We are planning to start the molecular analysis this month to identify what types of bacteria are present in the recruits.

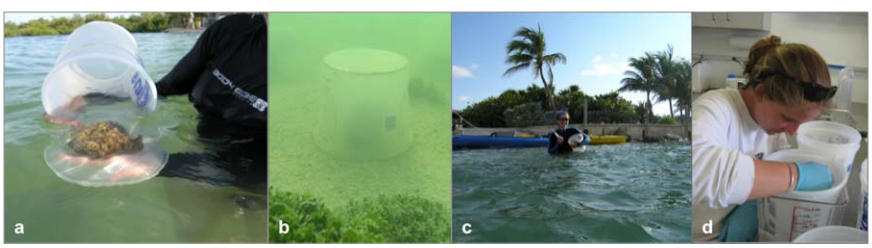

Figure 1. Collection buckets for larval harvesting in native reef water. Panels show: a) a coral colony being placed in the bucket for larval harvesting. b) a bucket containing a coral deployed on the reef flat; c) collection of larvae from the buckets; and d) "picking" larvae in the laboratory for manipulation.

2. Test whether the bacterial communities associated with coral recruits reflect what is in the surrounding seawater.

We had a large diversity of bacterial strains available to us this year, based on K. Ritchie's collection of bacterial strains that she isolated from *Symbiodinium* cultures. We used these bacteria to test a) if the bacteria would attach to the coral recruits, swimming larvae, or the biofilmed slide surface; and b) if the presence of the bacterial isolates affected the settlement rate of *P. astreoides* larvae. The settlement assays were run in the laboratory. Microscopy slides conditioned in reef water were placed in 25ml Petri dishes filled with sterile seawater, rinsed larvae, and either medium/seawater control or liquid inoculum of the test bacterial cultures. (Fig. 2).

We have performed fluorescence in situ hybridization (FISH) to compare the bacterial abundance on slides with and without treatments added. We performed these assays in May 2010 and then repeated them in June 2010. Microscopy analysis of the May 2010 settlement slides indicated that the bacterial cultures we added did not result in any marked difference in bacterial abundance on the settlement slides. It also suggested that the bacteria added did not attach to the swimming larvae or the recruits. We are currently interpreting these results to indicate that the bacteria may have remained in the seawater, which we did not collect after the settlement experiment.

Though the bacteria do not appear to have attached to the larvae or to the settlement surface, it appears that the addition of the strain *Roseivivax sp*. 46E8 significantly increased *P. astreoides* settlement (Fig 3). We were particularly encouraged by this result, especially since it was consistent with our findings last year. In addition, other bacterial strains that we added did not appear to affect larval settlement (Fig 3).

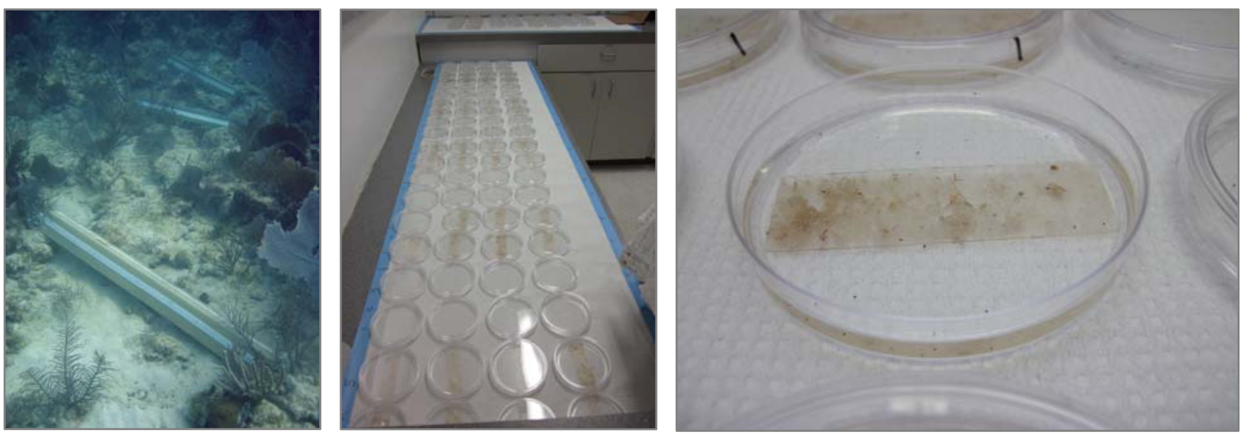

Figure 2. Settlement Assays. a) a strip containing double-sided adhesive with glass microscopy slides deployed on the reef. b) petri dish setup for settlement assays on the lab bench. c) dish with conditioned settlement slide, seawater, and larvae.

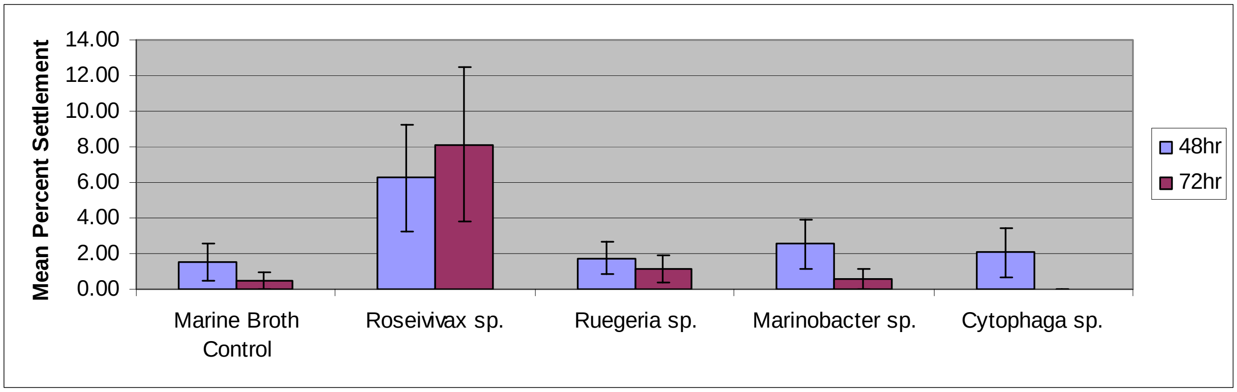

Figure 3. Settlement assays testing the impacts of various bacterial strains on *P. astreoides* larval settlement. *Roseivivax* strain 46E8 exhibited a significant increase in larval settlement compared to the control (addition of sterile growth medium), in contrast to no observed impact by addition of other tested strains, including three strains that were also isolated from *Symbiodinium sp.*

3. Test whether macroalgae and/or their compounds can alter the bacterial communities in *P. astreoides* recruits.

Instead of doing the macroalgal experiments, we expanded our experimental design to use a very diverse panel of specific bacterial isolates to screen for strains that influence *P. astreoides* larval settlement. Our results of the May 2010 and June 2010 fieldwork experiments (see above) were quite interesting. Because of limited numbers of larvae, we did not perform the algal experiments. However, were have been able to do the molecular and microscopy analysis in a smaller budget than expected. Because we still have funds available, we request a 6-month no-cost extension to continue our larval work next spring to continue testing the reproducibility of our settlement assay results, in addition to setting up experiments to determine how bacterial community composition shifts in response to the exposure of macroalgae common to Florida reefs, including *Dictyota* and *Halimeda* species.

EDUCATION AND OUTREACH

In its ongoing efforts to make marine research and education available to a wider audience, the outreach arm of SMS, the Smithsonian Marine Ecosystems Exhibit (SMEE), partnered with the Newspapers in Education (NIE) program at Scripps Treasure Coast Newspapers in 2008 to design, print and distribute an educational newspaper supplement entitled, “One World, One Ocean,” and a full-color, half-page educational advertisement, bringing Smithsonian research directly to a broad audience (see attachments A and B). The printing and release of both were timed to prepare students for “World Ocean Day” in June 2008, an international day of celebrating the importance of the ocean and all the life in it.

We proposed to partner again with NIE and to print a new educational newspaper supplement and a full-color, half-page educational advertisement. The supplement and ad would highlight the importance of coral reef ecosystems, current threats to reefs in Florida, and the importance of scientific research for informing reef management and conservation. The material will also stress that Mote Marine Laboratory “Protect Our Reefs” License Plates Grant Program plays a major role in funding high-quality research that benefits our local natural resources.

After careful review of the time table for the scheduled distribution of the educational supplement on coral reefs to 6th-, 7th-, and 8th-grade classrooms in the St. Lucie County School

District, it was determined that the piece would have the most impact if distributed earlier in the school year. Releasing the materials in May 2010, at the end of the school year, would provide only a narrow gap in which teachers would be able to utilize them effectively. Further delaying the release also provides the opportunity to highlight the updated POR-funded research. We are now planning to print and distribute the supplements in early March 2011. The statewide assessment of 8^{th}-grade science is scheduled later than usual this year (April 11-22), so teachers will be able to use the material to supplement exam preparation. The full-color, half-page educational advertisements will be printed shortly after the release of the supplements and will also serve to promote the annual Open House of the Smithsonian Marine Station. The SMS Open House is an event that allows the local community to visit the research station and learn about the scientific studies being conducted there by the researchers themselves. We are confident that delivering these educational supplements directly to the classroom is a way for the Smithsonian Marine Station and Mote Marine Laboratory to contribute to the science education process and reassert their presence as important educational resources to the community. We look forward to the Open House as an opportunity to feature our research, as well as acknowledge the POR program as a significant funding source for Smithsonian research in Florida.

2010 PROJECT TIMELINE

We are on target with most of the elements of our proposed timeline (see below): we had two successful field trips this summer, yielding recruits at stages later than we have previously collected, in addition to consistent, significant results in the larval settlement assays. The microscopy for evaluating the bacterial communities in the larvae and recruits challenged with different bacterial strains was performed, yielding unexpected but important results. Clone library construction and sequencing analysis are currently underway on the older recruit samples. The sequencing results from the DNA analysis will be used to guide design of specific probes to probe for particular bacterial species over the winter. We request a 6-month extension of the funding in order to repeat our larval settlement assays this spring, in addition to running experiments to determine how macroalgae influence bacterial community composition in early life stages of corals. K. Sharp has presented the results of these studies as oral presentations at international, national, and regional meetings, including the Boston Bacterial Meeting (Harvard University, June 2010), the International Symposium on Microbial Ecology (Seattle, August 2010), the Florida Keys Workshop “Linking Science to Management” (Duck Key, FL, October 2010), and the American Society of Microbiology Beneficial Microbes Meeting (Miami, October 2010).

Task	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Larval sampling and rearing	**X**	**x**										
Field “bacterial challenge experiment”	**x**	**x**										
Microscopy			**x**	**x**					**x**	**x**	**x**	**x**
DNA analysis					**x**	**X**	**X**	**x**	**x**			