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Publication Date
2012-12-13

Pink Pigmented Lesions on *Massive Porites* in Mo'orea: Distribution and Environmental Factors

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Abstract. Much of the recent decline in coral reefs can be attributed to coral disease; however, very little is known about coral immunity. Pink non-nomral pigmented immune response lesions have been seen on massive *Porites* coral in Mo'orea. Field surveys were conducted around the island measuring; sedimentation, water flow rate, location in the fringing or back reef and the number of *Dendropoma maximum* and *Spirobranchus giganteus* embedded in the coral to test for an association with the immune response. Multivariate linear regression reveals a nearly significant positive association between *Spirobranchus giganteus* and pink lesions. This study suggests *S. giganteus* may play a role in this immune response or be linked to some other confounding factor. Thus, *S. giganteus* could be a potential bio-indicator for coral disease to help aid reef conservation efforts.

Key words: Coral immune response, Spirobranchus giganteus, Dendropoma maximum, pink pigmentations, French Polynesia, Mo'orea.

INTRODUCTION

Coral reefs, one of the most biologically diverse ecosystems on Earth, have been dying off at an alarming rate. An estimated 33% of coral species face possible extinction (Carpenter et al., 2008, Mydlarz, L D. et al 2010). Indo-Pacific reefs are projected to deteriorate annually at a rate of 2% per year (Bruno and Selig, 2007). Much of the changing landscape in Caribbean and Indo- Pacific reefs in the past 30 years has been attributed to coral disease (Gladfelter 1982, Willis et al. 2004, Miller et al. 2006, 2009, Weil et al. 2009, Hoegh-Guldberg et al), yet surprisingly there are only a few known coral diseases (Gladfelter 1982, Willis et al. 2004, Miller et al. 2006, Aeby1998). Understanding the pathology and vectors of coral diseases could help aid reef conservation reverse the current trajectory.

Reef building scleractinia coral colonies are made up of genetically identical fleshy polyps embedded within a calcium carbonate exoskeletal structure. Within the epidermis of these polyps the mucociliary system expels mucus to trap or repel antigens (Mullen al et 2004). Phagocytosis is carried out by granular cells found within the gastrodermis of coral polyps (Palmer, C.V., Traylor-Knowles, N. 2012). Melanin-synthesis pathway products found in these cells and is believed to help in the immune response by creating a physical barrier and encapsulating invading pathogens (Mullen, Peters & Harvell 2004). Elevated levels of melanin production in infected pigmented tissues correlates with decreased zooxanthellae, indicating that tissue health is compromised in non-normally pigmented *Porites* coral. (Palmer, C. V., Mydlarz, L. D. Willis, B. L. 2008).

On *Porites compressa*, one cause of non-normal pink spotting is the disease trematodies caused by a parasitic flatworm, *Podocotyloides stenometra*. *P. stenometra* initially infects mollusks in the larval stage and is eventually eaten off the coral by butterfly fish (Aeby, GS 1998). A different pink pigmentation response has been found on other species of massive Porites parts of the Indo-Pacific region, including Mo'orea (Gacnik, A 2010, Weil, E 2010, Hoegh-Guldberg et al,). Causation has not been with this immune response on the dominate coral in this region (Trapon, M.L., Pratchett, M.S., Penin, L. 2011) as seen in trematodis, burrowing parasitic organisms maybe associated.

Pink lesions have been seen on corals embedded with *D. maximum* (lecture by Jeff Shima). *Dendropoma maximum* are vermated snails found embedded into the exoskeleton of coral. They have been found to cause up to an 81% reduction in coral growth and may impact coral health up to 52% (Shima, 2010). *Spirobranchus giganteus,* polychaete tube worms are another common organisms embedded in coral. *S. giganteus* use cilia and extended radioloes to create

feeding currents. They have been found to have a symbiosis with coral by protecting coral polyps from predation by *Acanthaster planci*, crown-of-thorns starfish (DeVantier L. M., Reichelt R. E., Bradbury R. H. 1986).

The goal of this project was to assess prevalence and distribution of non-normal pigmentation pink lesions on massive Porites on Mo'orea. This study also attempted to identify possible causal associations and factors that may affect the distribution of pink lesions on massive Porites. I hypothesized that: (1) there is a higher prevalence in the fringing reef which is exposed to more pollution and runoff than the back reef (Salvat B, Hutchings P, Aubanel A et al. 2000); (2) areas with higher water flow rates and sedimentation such as Temae would have a higher prevalence because increased stress from sedimentation and flow increases immune responses in coral (3) Massive *Porites* coral heads with more live *D. maximum* and *S. giganteus* embedded in the coral have a higher incidence of pigmented lesions.

METHODS

Study sites

The results from preliminary pilot surveys indicated that there was a high prevalence of Massive Porites with lesions in back reefs at every site sampled around the island. Six sites were selected on the island of Mo'orea to survey. The sites (Opunohu, Haapiti, Cooks Bay and Temae) were based on the different environmental factors of flow rate and sedimentation rates as well as accessibility to the sites and special distribution around the island. (figure 1)

Back reef versus fringing reef

To compare the differences in the abundance between the two types of reefs, a back reef was matched to a fringing reef at all sites but Temae Public Beach which does not have a fringing reef.

Sampling:

Sampling was done by snorkeling between October and November of 2012. At each of the seven sites, a spot was randomly chosen to run five 27m transects perpendicular to the shore and 10 meters apart from one another. Starting at the zero mark, one *Massive Porites* coral head was sampled every three meters. In total, ten coral heads were sampled for every transect run. If the transect tape did not fall on a coral head, the closest head was chosen. The coral was identified in the field to the genus level, and a head was considered any contiguous mass of coral of the same colony.

On each coral head, the number of active pink lesion spots was counted and recorded. (appendix)

D. maximum

The number of D. *maximum* embedded in the live coral head was counted as well as snails embedded nearby with mucus nets on any portion of the live coral.

S. giganteus

The number of holes and not number of *S. giganteus* individuals in the live portions of *Porites* were counted.

Water flow and sedimentation rates

Three sedimentation traps were placed at each site at the height of the coral heads to measure the amount of sediment that fell on the coral heads. To compare the average flow rate, three plaster of pasris clod cards were randomly placed at each site (Thompson L, Glen E 1994). weight of the clod cards was measured before and after they were placed at the sites. The difference in weight over the time was measured to compare that average flow rates among the different sites. The depth was measured from the top of each coral head

Coral health:

Coral bleaching percentage was used as proxy to estimate coral health. Coral heads fell into the following three health categories: good, okay and poor, which corresponded to 0-25% bleached, 26-50% bleached, and >50% bleached, respectively. These categories were made due to the recent damage of the reef.

Data analysis:

Coral size was estimated using three different sized PVC square quadrates: .3x.3 m, 1x1 m and 2x2 m, corresponding to the sizes small, medium and large, respectively. To determine which size quadrate the coral fell under, a PVC quadrate of a particular size was placed over the top and sides of the coral, making a cube around the coral to assess fit. If the coral did not fit within the quadrate cube, the larger quadrate was used. Coral smaller than .

1m across the smallest dimension or larger than 2m across the largest dimension was excluded from the study. Coral within each size category, small, medium and large was assigned to the median size 20 m2, 180 m2 or 1125m2 respectively. The number of pink lesions, *D. maximum*, and *S. giganteus* holes per coral of a median size category was counted. To standardize for size, the data was then converted to a 500m2 coral head. To adjust for the non-normal distribution, the data was transformed using y'=log (y+ .01). All statistical tests were analyzed in JMP (SAS Institute Inc).

RESULTS

Study sites

A one-way ANOVA of pink lesions and sites indicates that the null hypothesis is rejected and that there is a statistically significant difference in the abundance of pink lesions between the sites (P-value 0.0048 F Ratio 3.1733). A bivariate fit of pink lesions by sites indicates that there are significantly more lesions at Opunohu back reef (P-value <0.0001 t-ratio 4.21, estimate 107.347). The other sites did not show any statistical difference in the abundance of lesions on massive Porites. A Tukey-Kramer HSD test of the sites and pink lesions showed that the Opunohu back reef (A) was different in the abundance of pink lesions than the back and fringing reefs at Haapiti and Cooks Bay (B), but similar to the Opunohu Fringing reef and Temae (AB) (figure 2). Since Opunohu Back reef was similar to Opunohu Fringing reef and Temae the sites were pooled.

Back reef versus fringing reef

T-tests comparing the difference in the prevalence of lesions in the fringing reef vs. back reef reveal statistically more pink lesions in the back reef (p-value 0.0457, t-ratio -1.695, DF 2225.77) when the sites were pooled. T-tests of each of the paired back and fringing reefs showed a statically significant difference at Haapiti (P-value of 0.0066, t ratio -2.527 DF 93.83). Cooks Bay and Opunohu were not significant with p-values of 0.617 and 0.0627, respectively. (figure 3)

D. maximum

A bivariate linear regression of *D. maximum* and pink lesions for all the sites pooled indicates there is no significant association between *D. maximum* and pink lesions (Effect size on pink lesions 7.738, t-ratio 0.42, P-value 0.6783). A multivariate fit least square of *D. maximum* and *S. giganteus* at all the sites revealed an even less significant result for *D. maximum* (Effect size on pink lesions 0.421, t-ratio 0.02, P-value 0.9823).
A multivariate fit least square of *D. maximum* and *S. giganteus* by pink lesions for each site indicated a significant positive association of *D. maximum* and pink lesions only at Cooks Bay back reef (Estimate -27.917, t ratio -2.24, p value 0.0302).

S. giganteus

The bivariate linear regression of all the sites pooled of pink lesions by *S. giganteus* found a very close to significant positive association between pink lesions and *S. giganteus* (Effect size on pink lesions 49.461, t-ratio1.96, P-value 0.0512). A multivariate fit least square of *D. maximum* and S. giganteus by pink lesions when all of the sites were pooled revealed a similar, nearly significant positive association between *S. giganteus* and pink lesions (Effect size on pink lesions 49.345, t-ratio 1.91, P-value 0.0571). Individual multivariate fit least squares test at each site for *D. maximum* and *S. giganteus* by pink lesions indicated no significant positive association between *S. giganteus* and pink lesions at any site.

Coral health

Coral bleaching does not appear to be associated with pink lesions. The one-way ANOVA shows that coral bleaching and pink lesions are not associated (p-value 0.1686, F ratio 1.7910, degrees of freedom 2). The null hypothesis, that there is no difference in the mean number of pink lesions with differences in coral health, fails to be rejected.

Flow rate and sedimentation

A multivariate linear regression of mean sedimentation rates and mean flow rates by pink lesions indicates that there was no significant association between sedimentation rates and flow rate at my sites.

DISCUSSION

Back reef vs. fringing

Comparing the abundance of lesions in the back reef to the paired fringing reef, a statistical difference at Haapiti and a close to significant difference at Opunohu were demonstrated. At Cooks Bay, however, the back and fringing reefs

were not significantly different in the abundance of pink lesions. Comparing the pooled back reefs to pooled fringing reefs did show a statically larger amount of pink lesions in the back reefs than in the fringing reefs. Due to the difficulty in measuring the surface area of coral in the field, the adjustments to the data to account for size may have affected these results. The methods for adjusting size of coral were, however, consistent across the different sites.

There was greater variation in the abundance of lesions between the three sites than between matched back and fringing reefs, which may be explained by the variation in the reef's landscapes at the different sites. For example, one possible reason for these results is that there is a larger lagoon separating the fringing from back reef at Haapiti compared to Cooks Bay and Opunohu. This larger spatial separation may affect the dispersal of the pathogens or environmental factor affecting Massive *Porites*. Also, both Cooks Bay and Opunohu are affected by a northern swell during the austral summer while Haapiti is exposed to a constant strong south swell throughout the year. This difference in swell may affect the prevalence lesions on Massive *Porites* (Penin al et. 2007).

Although there was greater variation of pink lesions between the three sites, than within their matched back and fringing reefs, all of the back reefs had a higher prevalence of pink lesions than the fringing reefs. These results were consistent with the findings looking at non-normal pigmentation on the algal-coral margins (Gacnik 2010). This indicates that my hypothesis, that fringing reefs would have higher abundances of pink lesions, was wrong. Run-off and nitrification, which more often affect the fringing reef, have been found to exasperate coral immune responses. It was expected that the fringing reef would have poorer water quality due to its proximity to land and, thus, have more pink lesions. This, however, was not observed. Additionally, there was no association between coral health and pink lesions. While using percent coral bleaching as a proxy for coral health is not a comprehensive measure of coral health the findings were consistent with results on back reefs. The deleterious, anthropogenic effects on fringing reef coral, found in previous studies, may not be associated with these pink lesions on Massive *Porites* (Salbat B, Hutchings P, Aubanel A et al. 2000, Reopanichkul P, Schlacher TA, Carter RW, Worachananant S. 2009). Water quality was not tested to confirm this, due to resource constraints that made it difficult to obtain precise measurements of water quality.. While

Other abiotic or biotic factors might be affecting the abundance of pink lesions on back reefs but not fringing reefs (Mullen, Peters & Harvell 2004).

D. maximum

The data shows that *D. maximum* does not appear to have an association with pink lesions on massive Porites except at Cooks Bay back reef. Due to strong currents and the large populations of Dendropoma spp. on coral heads, there were some challenges with data collection. The data collection method, however, was consistent throughout the sites.

S. giganteus

The results indicate that there was a near significant positive association between *Spirobranchus giganteus* and lesions on massive *Porites* when all the sites are pooled together. The result of the pooled data may be due to two data points that appear to skew the distribution of the pooled data. To help alleviate this, the data was transformed to decrease the spread. More sites should be sampled to test if this association is seen at different sites around the island. When the sites are looked at individually, this same association is not evident. Rather than being a causal adjent, *S. giganteus* may be associated with pink lesions due to an additional confounding factor, such as, larval settling preference. *S. giganteus* which my prefer coral with features that also make coral more susceptible to an immune response.

Flow rate and sedimentation rate

Flow rate and sedimentation rates have been shown to negatively impact coral health and induce coral immune responses. Based on the results of this study, flow rate and sedimentation rates do not appear to be associated with pink lesions. Due to time constraints, measurements were only taken once; therefore, temporal suto-replication may be affecting the results of this study. Additionally, only two measurements were taken at some sites, which may not give an accurate representation of flow and sedimentation and their possible effects on pink lesions on massive *Porites.*

Conclusion

The results from this study reveal some of the environmental factors that may be affecting the distribution and abundance of non-normal lesions on massive *Porites* in the reefs of Mo'orea. One of those factors is *S. giganteus,* which has previously been thought to cause only structural damage to the calcium carbonate exoskeleton of corals and have no real negative consequences to its host. These news findings suggest otherwise; *S. giganteus* may play a role in the non-normal pigmented immune response on massive Porites. Further research is needed to study whether *S. giganteus* is a causal agent in this response or if it is linked to a confounding variable. Additionally, more research is needed to see if *S. giganteus* may be used as an indicator of reef health to help aid in conservation research in areas dominated by massive Porites.

As Massive *Porites* is one of the most abundant corals in Pacific reefs, the health of this coral is vital for the survival of coral reefs in this region. Further research should be conducted to examine other environmental factors that are associated with non-normal pigmented lesions on Massive *Porites* and the effects these lesions have on overall coral health. A natural history of this response should be done to address the prognosis of this response. Isolating and understanding the cause of this coral immune response is an important step in exploring the relatively new field of coral disease. Ultimately, this knowledge can aid coral reef protection and ideally help to conserve these important reef communities and the biodiversity that lies within them.

ACKNOWLEDGMENTS

I thank the Mo'orea class 2012 for their support and help in and out of the field. The hardworking GSI's Rosemary Romero, Darcy Kato-Ernst and Matthew Luskin. All the wonderful professors, Dr. Vincent Resh, Dr. Brent Mishler, Dr. Jonathon Stillman and Dr. George Roderick for their advice and assistance. Norma Kobzina for library assistance and the Gump station.

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