IDENTIFICATION OF LOCAL ADAPTATION IN *PORITES ASTREOIDES* INHABITING THE FLORIDA REEF TRACT: BIOTIC STRESS, A DISREGARDED FORCE OF CHANGE AFFECTING CORAL REEFS

By

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ABSTRACT

IDENTIFICATION OF LOCAL ADAPTATION IN *PORITES ASTREOIDES* INHABITING THE FLORIDA REEF TRACT: BIOTIC STRESS, A DISREGARDED FORCE OF CHANGE AFFECTING CORAL REEFS

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Up to 98 % of the total carbon acquired by scleractinian anthozoans (coral) is provided by carbon fixation from an algal symbiont (*Symbiodinium spp.*). In return, the host provides protection, a source of nitrogen, and CO_2 for carbon fixation to the symbiont. The obligate nature of this symbiosis places constraints on the environmental conditions capable of sustaining growth, reproduction, and reef expansion. Although many environmental factors can be detrimental to corals climate warming and disease have drastically altered coral communities over the past 40 years. Coral cover along the Florida Reef Tract (FRT) has decreased by 70 % and as a result the benthic reef community has transitioned from a coral dominated to a macro-algal dominated ecosystem. The primary contributors to the observed phase shift are disease and climate change. Increasing our understanding of coral resistance and sensitivity to disease and climate change related stressors is paramount when the future of this ecosystem is considered. Differential habitation of the inshore patch reef system and offshore bank reef system is identified with a multivariate statistical approach among three coral species, *Porites astreoides*, *Montastraea cavernosa*, and *Siderastrea siderea*. All three species were observed to have increased size and abundance at inshore reefs despite the characterization of inshore patch reefs as a habitat with increased levels of thermal stress and eutrophication compared to offshore bank reef habitats. These results point to disease as a contributing factor affecting the offshore bank reef system. Based on the extensive history of disease associated with the offshore bank reef system, I hypothesized that offshore coral communities increase immune expression to deter disease. Because corals utilize an innate immune system to detect and respond to harmful microorganisms, I applied quantitative reverse transcription real-time PCR of genes associated with immunity to estimate the degree of this response.

Reciprocal transplantation of *P. astreoides* collected from a representative inshore and offshore reef enabled the distinction between environmental and population dependent effects. Upregulation of TNF receptor associated factor 3, a protein critical to bacterial and viral responses, was identified in corals experiencing the offshore habitat during the summer indicating increased disease related stress during warmer periods. Corals originating offshore upregulated expression of adenylate cyclase associated protein 2 compared to inshore corals indicating an adaptive response to increased disease related stress. Population-dependent adaptive responses to temperature and pathogen related stress in *P. astreoides* were confirmed by challenging fragments originating from each site (n = 6) for 8 h with a control (28°C), increased temperature (32°C), or a treatment of increased seawater temperature and 5 $\mu g\ ml^{-1}$ lipopolysaccharide. Offshore coral fragments exposed to the synergistic treatment and inshore coral fragments exposed to increased SWT displayed responsive upregulation of genes. Responsive upregulation of genes was associated with increased variation in SWT for inshore corals and increased variation in pathogen associated molecular patterns response in offshore corals. The observed increase in expression of the immune system by the offshore population was also associated with decreased coral abundance (survival) and decreased colony size, which in *P. astreoides* contributes to decreased fecundity. Therefore, survival of offshore corals in the face of pathogenic organisms likely comes at costs to other measures of fitness whereas inshore corals do not experience these pathogen related costs. Therefore, two populations of *P. astreoides* inhabit the FRT. As SWTs continue to increase from carbon emissions, thermal stress will likely exacerbate the effects of pathogen related stress impacting the offshore population and SWT stress will further impact the inshore population. Both populations are in peril but we identify pathogen associated stress as a concealed stressor.

ACKNOWLEDGMENTS

A doctoral education has been a lesson in perseverance, adaptation, and imagination. This experience has extended across state and country boundaries and because of this I was afforded the opportunity to learn from a wonderfully diverse group of individuals that deserve recognition beyond what I can hope to provide here. I would like to first thank my family for their continued support during struggles and successes despite the distance between us. Secondly, I would like to thank Dr. Kevin Strychar for providing the opportunity to study a remarkable organism and ecosystem as well as the freedom to develop a project of my own design. I hope that this experience has been as rewarding for you as it has been for me. Third, I would like to thank both Nathaniel and Peggy Ostrom for accepting me into their lab and lives as my doctoral education migrated across state boundaries. The experience I have acquired at Michigan State University through both of you has been an integral part of my personal and professional development. Hasand Gandhi, Erich Bartels, and Jeff Landgraf also deserve more accolades than I can possibly provide. This research would have never come to fruition without their support and guidance in the laboratory. I also thank Dr. James Cervino for his unwavering support of this project. My friends, which now span the globe, also deserve recognition. I hope that our shared experiences have benefited you all as much as they have enriched my life. Briana Hauff, I thank you most of all, not only for accepting the challenge of this project with me but also for your support in overcoming the countless hurdles we experienced together.

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CHAPTER 1

DECOUPLED SEASONAL STRESS AS AN INDICATION OF CHRONIC STRESS IN *MONTASTRAEA CAVERNOSA* AND *PORITES ASTREOIDES* INHABITING THE FLORIDA REEF TRACT

1.1 Abstract

Scleractinian coral abundance and diversity increases along inshore to offshore transects across the Florida Reef Tract (FRT). I identify this trend among coral reefs throughout the middle and lower Florida Keys Region from Coral Reef Environmental Monitoring Program (CREMP) datasets as well as an inshore and offshore reef at similar depths. Although mass mortality from disease and climate anomalies are largely to blame for rapid losses in offshore reef coral cover, the failure of extant coral populations to recolonize this zone is puzzling given improvements to water quality and the mild seawater temperature regime compared to corals inhabiting the patch reef zone. Applying exploratory statistical methods I identified two abundant species, *Montastraea cavernosa* and *Porites astreoides*, inhabiting both zones to varying degrees. Following reciprocal transplantation of conspecifics between a representative bank reef and patch reef zone (6 m depth), I monitored monthly coral colony brightness (a measurement related to algal symbiont density) over a two-year period to examine symbiont loss, a common stress response in scleractinian corals. Although species-specific stress patterns were not identified, zone-specific variation was evident. Trigonometric regression of stress level by month revealed a significant relationship supporting an annual stress and recovery period at the inshore patch reef zone. Contrary to this result, conspecifics transplanted to the offshore zone followed a positive linear trend indicating the absence or diminishment of a recovery phase resulting in continued chronic bleaching over the two-year period. My results implicate the importance of turbidity in alleviating stress at inshore sites and the importance of extending greater protection to reefs within this zone.

1.2 Introduction

The Florida Keys Reef Tract has experienced dramatic decreases in coral cover (Aronson and Precht, 2001), shifts in reef communities (Pandolfi et al., 2005; Ruzicka et al., 2013), and decreases in reef architectural complexity (Alvarez-Filip et al., 2009), which have all resulted in decreased biodiversity. This trend is most apparent along the 15 offshore reef preservation areas of the Florida Keys National Marine Sanctuary. Located between 1 and 8 m depths and 5 to7 km offshore, these once highly productive reef crest and fore reef ecosystems no longer support accreting scleractinian coral communities and can be considered relict in comparison to historical baselines (Palandro et al., 2008). This imperiled status of the offshore reef zone is not a result of a single stressor. Diseases and macro-algal overgrowth arising from human interaction, decreased coral resilience following destructive hurricanes and anomalous hypothermic (Hudson et al., 1976; Shinn et al., 2003; Walker et al., 1982) and hyperthermic seawater temperature events have all greatly affected this region. Presently, conditions are such at offshore reefs that corals should display net accretion, however reef building coral populations remain at low abundances. Observing transects extending from inshore to offshore, coral abundance decreases to the offshore bank reef zone (Lirman and Fong, 2007; Roberts et al., 1982; Schutte et al., 2010) with approximately two-times lower abundance at offshore reefs. In contrast, sustained growth has been observed throughout the inshore patch reef system despite far fewer protected areas in contrast to offshore reefs, increased frequency of hyper and hypothermic seawater temperature events experienced (Soto et al., 2011), increased dissolved inorganic nitrogen, soluble reactive phosphorus, and chlorophyll a, increased turbidity, greater macro algal biomass (Lapointe et al., 2004), and closer proximity to anthropogenic sources of stress (Ginsburg et al., 2001). Several hypotheses have been proposed regarding the proliferation of inshore reefs and the deterioration of offshore reefs including (1) continued persistence of large fecund colonies in near-shore reef zones resulting in increased near-shore recruitment, (2) diet supplementation from increased turbidity and

nutrient concentration as a means to alleviate stressful near-shore conditions, and (3) differences in seawater temperature variance resulting in a wider temperature range allowing inshore reefs an opportunity to experience a broader range of temperatures acclimation (Soto et al., 2011).

Recruitment does not appear to be responsible for the differences in coral abundance between adjacent inshore and offshore sites. The current state of knowledge emphasizes the importance of local recruitment for the maintenance of coral population abundance (Mumby and Stenick, 2008; Steneck et al., 2009) and low frequency immigrant settlement promotes genetic diversity (Noreen et al., 2009; Sammarco and Andrews, 1989). Co-implementation of larval dispersal models, genetic population connectivity (Foster et al., 2012) and sensitive identification and quantification of recruits (Hsu et al., 2014; Schmidt-Roach et al., 2008) has increased the accuracy of coral recruitment estimation. In the Florida Keys annual recruitment at inshore and offshore reefs is highly variable (Moulding, 2005; Shearer and Coffroth, 2006) and not a locally defining characteristic (Miller et al., 2000). Post settlement processes and stress (i.e. hypotheses 2 and 3) are therefore more likely correlated to the difference in reef growth and community structure observed.

I hypothesize that corals inhabiting inshore reef zones are able to respond to a wider range of stress levels as a consequence of frequent occurrences of non-lethal stress characteristic of this zone. The history of abiotic and biotic stress experienced by a reef affects the inhabitants response, growth, selection for resistant individuals or in the case of corals establishment of resistant symbionts (Haslun et al., 2011; Oliver and Palumbi, 2011) following aberrant levels of stress. Gradients of increased levels of abiotic factors associated with stress extend from offshore reefs to inshore reefs in the Florida Keys (Lapointe et al., 2004) and supporting this hypothesis a negative correlation exists when between both coral cover and colony size, and distance from shore (Lirman and Fong, 2007). Although gradients may

exist along this transect the detriment of each potential stressor (e.g. nutrients, temperature, and light) to a species may not be biologically relevant despite significant location-dependent differences. Further, the stress level experienced by an organism is most likely not a reflection of any one stressor but rather the cumulative effect of stressors present. The level of one factor may provide a refuge from a factor the organism is more susceptible to decreasing the severity of a potentially lethal stressor. For example, increases in chlorophyll a occur concomitantly with increases in turbidity. Increased turbidity may in-turn, decrease photic stress on scleractinian corals during increased water temperatures resulting in an overall decreased level of stress.

Identification of abiotic factors contributing to the community level differences in abundance and size of corals inhabiting inshore and offshore reefs is critical to the management of this habitat (Lirman and Fong, 2007). This study integrated and applied coral cover data from the coral reef environmental monitoring project (CREMP), environmental data from the water quality monitoring project (WQMP), and coral community and environmental data from ongoing research projects to describe the asymmetry of coral cover currently observed between the inshore and offshore bank reefs. I identify four coral species with differential abundances within the nearshore and offshore zones, the study of which may increase our understanding of the differences in coral growth between these environments. To provide a more complete understanding of the community dynamics of a symbiotic organism I also observed the seasonal photosynthetic competence (symbiont photo-physiology) and colony color of the identified species at two sites, one representative of an inshore patch reef and the other representative of an offshore bank reef, over a two-year period. From this information the seasonal response strategy of each species was determined.

1.3 Materials and Methods

1.3.1 Coral communities of the patch reef and bank reef systems

Relative abundances of corals inhabiting offshore shallow and middle channel patch reefs, spanning a 16-year period, were obtained from the Coral Reef Evaluation and Monitoring Project (CREMP). This dataset has been applied to the detection of potential causal factor(s) for regional differences in benthic communities coupled with Florida Keys National Marine Sanctuary (FKNMS) water quality monitoring project (WQMP) (Maliao et al., 2008). Maliao et al. (2008) also determined that the CREMP monitoring strategy correlates well with the results of other sampling efforts including the Atlantic and Gulf Rapid Assessment protocol (AGRRA). I analyzed CREMP and WQMP datasets spanning a 15-year period from 1997-2012 to determine the variability in the communities of corals and environmental regimes associated reefs grouped as either offshore bank reef or as part of the inshore patch reef rather than at the regional scale. Of the available reefs within the two datasets I selected reefs within the lower and middle keys for three reasons. Firstly, throughout the lower and middle keys regions there are a number of offshore bank reefs as well as patch reef formations that spatially parallel one another (Figure 1). Secondly, for the past three years I monitored an inshore patch reef and an offshore bank reef centralized within this range, Birthday and Acer 24 reefs respectively (Figure 1). Thirdly, this region has been characterized by decreasing gradients of nutrients, turbidity and temperature from the inshore to offshore zones. Differences in these factors among sites and between the inshore and offshore zones (i.e. nutrients, turbidity, and temperature) may provide a foundation to understand the evident variation in coral cover.

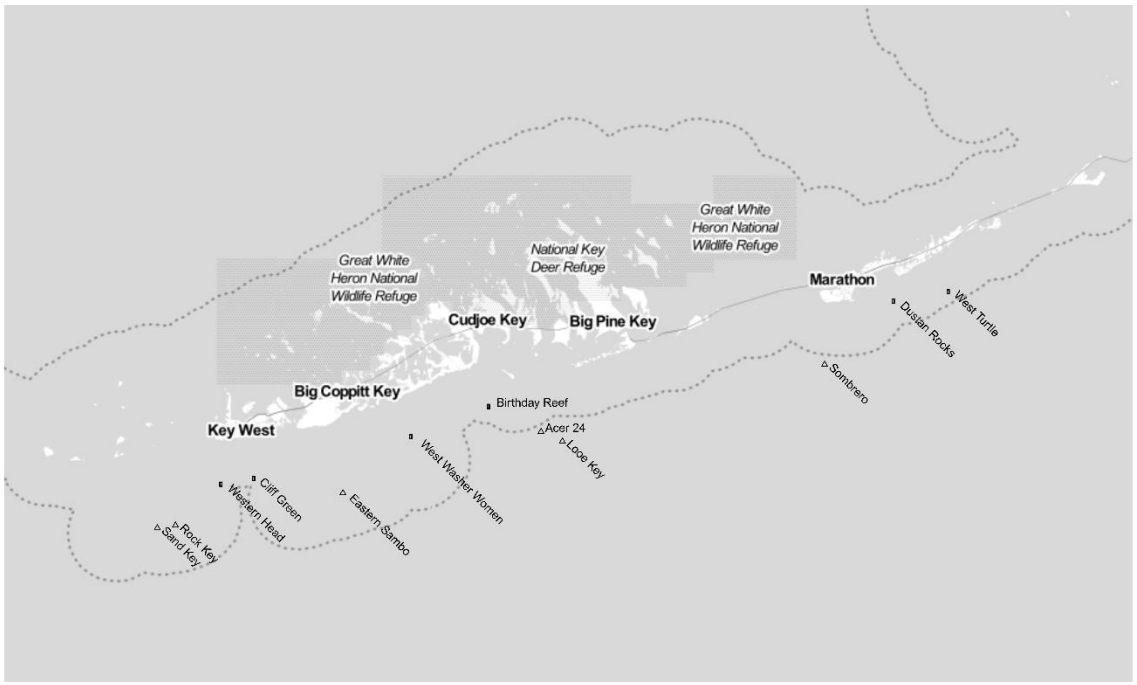


Figure 1: Map of Lower and Middle Florida Keys Study Sites. Sites were either selected from the Coral Reef Environmental Monitoring Project (Diagonal Labels) or from sites established for this study (Horizontal Labels). Sites identified with open triangles indicate offshore sites (n = 6) while those with open circles indicate inshore patch reef sites (n = 6).

Reefs with greater than 10% total coral cover as of 1996 were included. Reefs with cover less than 10% as of 1996 may have already become relict or may never have supported a large community of corals and were therefore excluded. From this criteria, five CREMP sites remained as representative of each of the two zones. Inshore sites included Western Head, Cliff Green, West Washerwoman, Dustan Rocks (also referred to as East Washerwoman), and Western Turtle Shoal. West Washerwoman is the largest patch reef of this set and is considered the largest in the lower keys. East Washerwoman and Western Turtle Shoal are also considerably large patch reef groups while Western Head and Cliff Green are singular patch reefs (see Lidz et al. (2007) for mapping and description of these patch reefs). Offshore shallow reefs included Sand Key, Rock Key, Eastern Sambo, Looe Key, and Sombrero Key. Each of these offshore bank reefs is characterized by spur and groove formations preceded inshore by patch reefs.

1.3.2 Coral community characteristics at inshore and offshore reefs

Colony size and abundance of corals were assessed biannually from 2011 to 2013 using photo-transects established at our inshore patch reef site (Birthday reef: 24.57917' N, -81.49692' W) and offshore bank reef site (Acer24 reef: 24.55268' N, -81.43741' W), both located at depths of 6.5 m (Figure 1). Assessments were conducted by divers using self-contained underwater breathing apparatus (SCUBA) during winter (February) and summer (August) seasons. Divers photographed the benthos at random non-overlapping points (n = 15) along 30 m transects (n = 3) with a Nikon D5100 camera affixed to a 0.5 x 0.5 m quadrapod (Coyer et al., 1999). Lighting was supplied by a Fantasea NanoFlash strobe. Transects were measured using surveyor tape and extended out from a central location in three randomly assigned directions for each season. The photo-quadrat method has been shown to be the most cost effective method to assess coral and sessile benthic communities without sacrificing accuracy (Leujak and Ormond, 2007). Images were analyzed using Coral Point Count with Excel (CPCe) extensions (Kohler and Gill, 2006). Coral species richness, abundance, area, percent cover, as well as benthos composition including coral, sponge, algae, sand, and rubble were determined. Further, all individual *Montastraea cavernosa* and *Porites astreoides* colonies within 1 m of transect were analyzed for photosynthetic capacity using a Diving Pulse Amplitude Flourometer (PAM: Walz). I was unable to assess field corals in a dark adapted state and therefore obtained the effective quantum yield of photochemical energy conversion ($\Delta F/F_m'$). The change in F (ΔF) represents the change in fluorescence from the maximal fluorescent yield of the coral in an illuminated environment (F_m') following a saturating pulse from the instrument. The use of these two measurements has been found to be highly correlate with quantum yield (Genty et al., 1989). The effective quantum yield was determined at the apex of colonies with the fiber quantum sensor held within the Surface Holder, to maintain a distance of 10 mm between it and the colony tissue (Cervino et al., 2012).

1.3.3 Location-dependent colony brightness, a bleaching characteristic

Following the identification of species characteristic of the inshore patch reef and offshore bank reef systems, a reciprocal transplant was carried out between Birthday Reef and Acer 24 with two species of coral, *M. cavernosa* and *P. astreoides*. Coral fragments (16 x 16 cm) of both species were collected from colonies at least 10 m apart at each reef using a steel mallet and cold chisel. Permission for field work at Birthday Reef and Acer 24 was granted by National Oceanic and Atmospheric Administration National Marine Sanctuaries (Permit # FKNMS-2011-107). Fragments were transferred in large coolers filled with site-derived water to the Mote Marine Laboratory Tropical Research Laboratory (MML), where they were immediately sectioned into two 8 x 8 cm fragments with a tile saw lubricated and cooled with sterile artificial sea water (Instant Ocean) sprayed on the blade with a wash bottle. Following sectioning, fragments were transferred to MML flow-through seawater raceways. Raceways were shaded from direct sunlight to decrease stress. Following 2 days of recovery, fragments were attached to pucks (1 part concrete: 3 parts aragonite sand) with a two-part epoxy (All Fix Epoxy; Philadelphia, PA USA). After another 3 days of recovery, corals were transferred in large coolers filled with seawater from the MML flow-through system, to each field site. Each field site consisted of 6 concrete blocks affixed to the calcium carbonate substrate using a 1 part plaster: 2 parts concrete mixture in a hexagonal shape. Each concrete block consisted of 6-7 randomly assigned coral fragments of a single species. Fragments were attached to each block with All Fix Epoxy. Neighboring blocks harbored different species.

Images of each cinder block and its associated coral fragments, were taken monthly over the course of two years with a Nikon D500 in a Fantasea underwater housing. Illumination was provided by the built-in camera flash and housing diffuser. Each image was analyzed with Image J to determine the mean fragment brightness. The brightness of gray-scale images is highly correlated with symbiont density (Siebeck et al., 2006) and thus, colony darkening (decreased brightness) reflects increased

concentrations of the coral endosymbiont (Siebeck et al., 2006). Analysis proceeded by first transforming an image to 8-bit format. The scale of each image was set using the average width of a cinder block (15.24 cm). Regions were drawn around each fragment including only live coral tissue using the polygon tool and minimum, maximum, and mean brightness values obtained. Differences in the background brightness between different blocks and/or site dependent water quality (turbidity) was corrected for by adding the corresponding value or subtracting the corresponding value to reach that of the first sampling period for each block.

1.3.4 Statistical analyses

Statistical analyses were performed with R version 3.0.2 (Team, 2013) and all figures were created with ggplot2 (Wickham, 2009).

We first analyzed the CREMP dataset for variation in coral cover among sites and zones across the 16-year sampling period *via* two-way repeated measures analysis of variance (ANOVA). The aov() function within the statistical package R was applied with reef zone and sites as predictors of total coral cover and an error term described by sample year to create a repeated measures design. Assumptions of ANOVA were visually evaluated with the plot() function, which produces figures including residuals *vs*. the fitted model, standardized residuals *vs*. theoretical quantiles (qq-plot), square root of the standardized residuals *vs*. the fitted values, and the standardized residuals at each factor level. Non-linearity in these figures is indicative of a deviation from a normal distribution. Homogeneity of variances was assessed using the Bartlett test (bartlett.test()), which tests the null hypothesis that variances are equal at all levels.

Site community richness and evenness was assessed across the 16-year CREMP dataset and the 2-year dataset from our field sites using Rényi diversity profiles (Jost, 2006). The renyi () function from the vegan package served as the platform for this analysis (Oksanen, 2013). Mean relative abundances of coral species across the 15-year period were applied to this function. The shape of each profile provides information with respect to the evenness of a community. Horizontal lines represent perfectly even communities where all species are present in equal abundances. Alpha values of 0, 1, 2, and infinity indicate species richness, Shannon diversity index, Simpson index (reciprocal), and the proportion of the most abundant species present, respectively.

Coral community differences were identified using a multivariate approach. Principal coordinate analysis (PCoA) was performed on the annual relative abundances of coral species at each site. To accomplish this, Bray-curtis dissimilarities were calculated with the vegdist () function from the vegan package (Oksanen, 2013). Eigen analysis was performed by the application of the cmdscale () function on this dissimilarity matrix in 2 dimensions to produce eigen values. Confidence intervals (95%) were calculated from these eigen values across years for each site. The correlations between the original relative abundances and eigen values were used as loadings to determine the association of species with particular sites.

Ad-hoc contrasts were used to identify differences between transplant groups, within a general linear model framework. Contrasts included (1) month dependent shifts in brightness between corals transplanted to and from the site of origin and (2) site and transplant dependent shifts in the mean brightness of a species. Seasonal patterns in mean brightness were identified with trigonometric linear modeling, applying a period of 12 to accommodate our monthly data interval.

1.4 Results

1.4.1 16-year comparison of zonal coral communities

Analysis of the CREMP dataset revealed that coral cover declined at 8 of 10 reefs between 1996 and 2011 (Figure 2). Decreases in coral cover were greatest between 1999 and 2000. Coral cover declined from 25-15 % at the three western-most middle channel sites (Western Head, Cliff Green, and West Washer Women) between 1999 and 2000 followed by marginal loss or stability in the remaining coral cover. The eastern most sites, Dustan Rocks and Western Turtle Shoal, maintained coral cover during this period while coral cover at all offshore bank reef sites decreased significantly between 1996 and 2000. Despite similar trends of coral cover loss, the total mean coral cover was significantly greater at the monitored inshore patch reefs compared to offshore bank reefs (RM-ANOVA: $p < 0.0001$).

In addition to the significant differences in coral cover, we identified community dissimilarities between offshore shallow and middle channel patch reefs. Species richness was significantly greater at middle channel sites (ANOVA: $p < 0.05$), with 9 additional species present when rare species were considered. Removing species accounting for less than 0.1 % of the mean benthos cover resulted in a single species difference between the zones. Rényi profiles of coral abundance indicated that all sites were dominated by a few species and therefore, are characterized by low species evenness (Figure 3). The most speciose sites, Looe Key from offshore reefs and Western Head from inshore reefs, displayed the least evenness. Offshore sites other than Looe Key reef, displayed similar profiles, indicated by the similar slopes of Rényi profiles. Although middle channel sites displayed decreased variation in species richness, diversity profiles indicate a range of characteristics. Dustan Rocks and Western Washer Woman were the most similar of the middle channel sites while Cliff Green with very similar richness to these two sites, was less even.

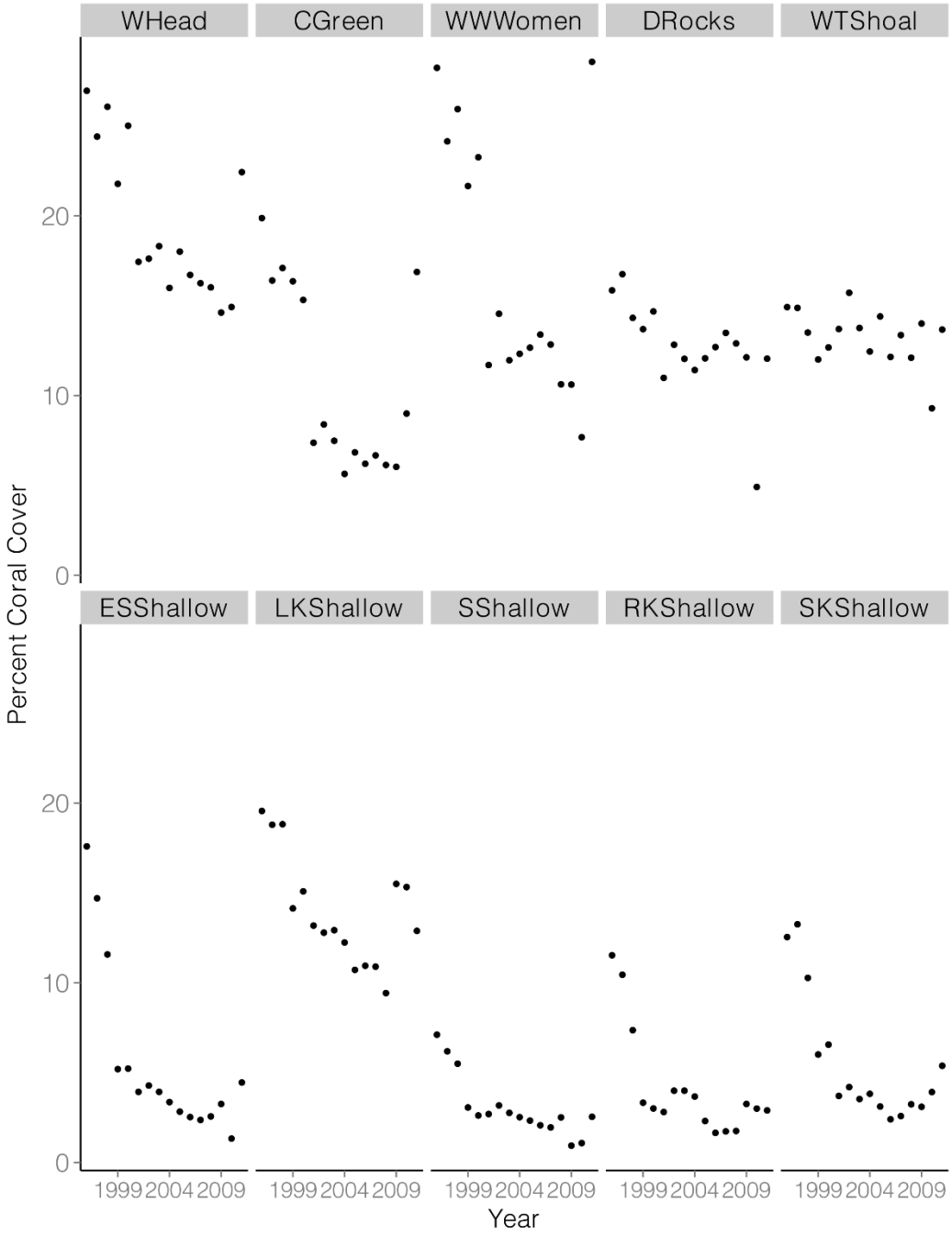


Figure 2: Annual Percentage (%) of Scleractinian Coral Cover from 1996-2011. Offshore shallow reef sites are grouped along the top row while inshore patch reef sites are grouped along the bottom row. WHead = Western Head, CGreen = Cliff Green, WWWomen = Western Washer Women, DRocks = Dustan Rocks, WTShoal = West Turtle Shoal, ESShallow = Eastern Sambo Shallow, LKShallow = Looe Key Shallow, SShallow = Sombrero Shallow, RKShallow = Rock Key Shallow, SKShallow = Sand Key Shallow. See figure 1 for site location.

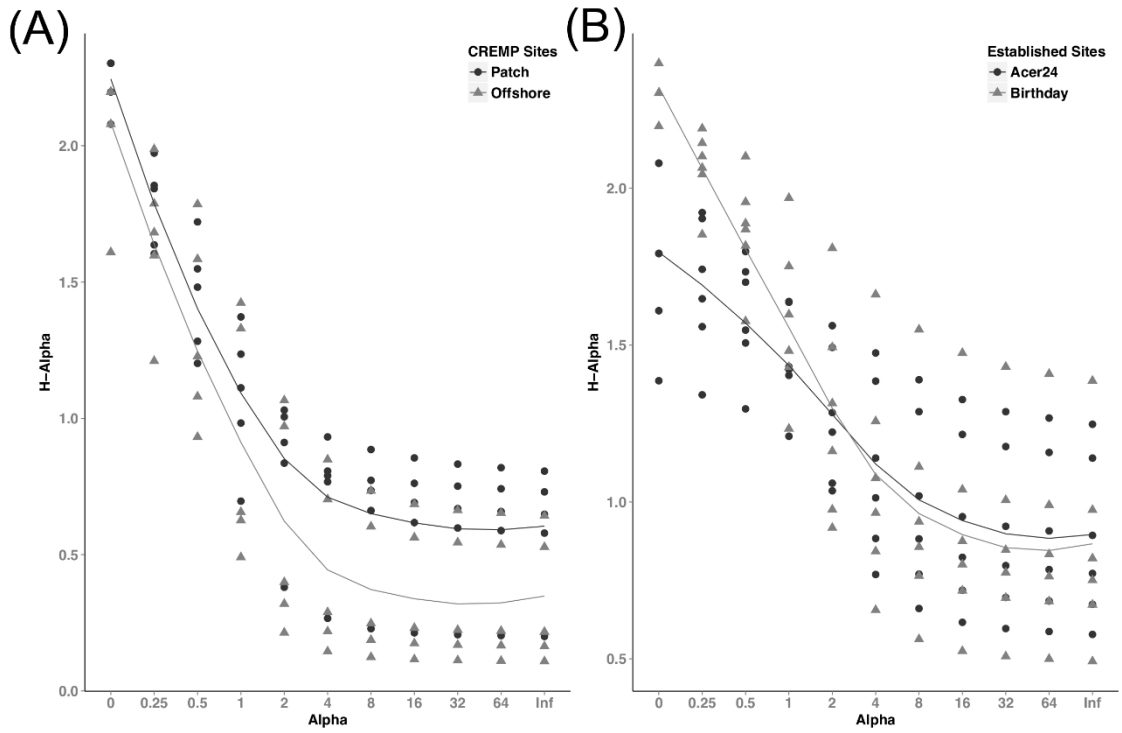


Figure 3: Mean Rényi Diversity Profiles of CREMP and Established Patch and Offshore Reef Sites. Patch reef points (grey triangles) and offshore points (black circles) are identified similarly for CREMP data (A) and established sites (B). CREMP data points reflect the mean value at each location across the sampling period from 1996-2011, while points for established sites reflect mean Rényi values of transects carried out in the summer and winter of 2012 (n = 6 per site). Loess regression was applied to visualize each trend.

Zonal ordination of coral communities was identified following PCoA (Figure 4). Middle channel sites ordinated closely together in quadrant 3 while offshore shallow reefs, although displaying more variability, ordinated in the periphery away from this cluster in quadrants 1, 2, and 4, centering around quadrant 2 (Figure 4). Evaluating the loadings, along the first two axes, the abundances of some coral species were dissimilar between locations. Inshore patch reefs were characterized by an increased prevalence of *Montastraea cavernosa* and *Sidereastrea siderea*, while *Porites astreoides* and *Acropora palamata* were more common at offshore bank reefs.

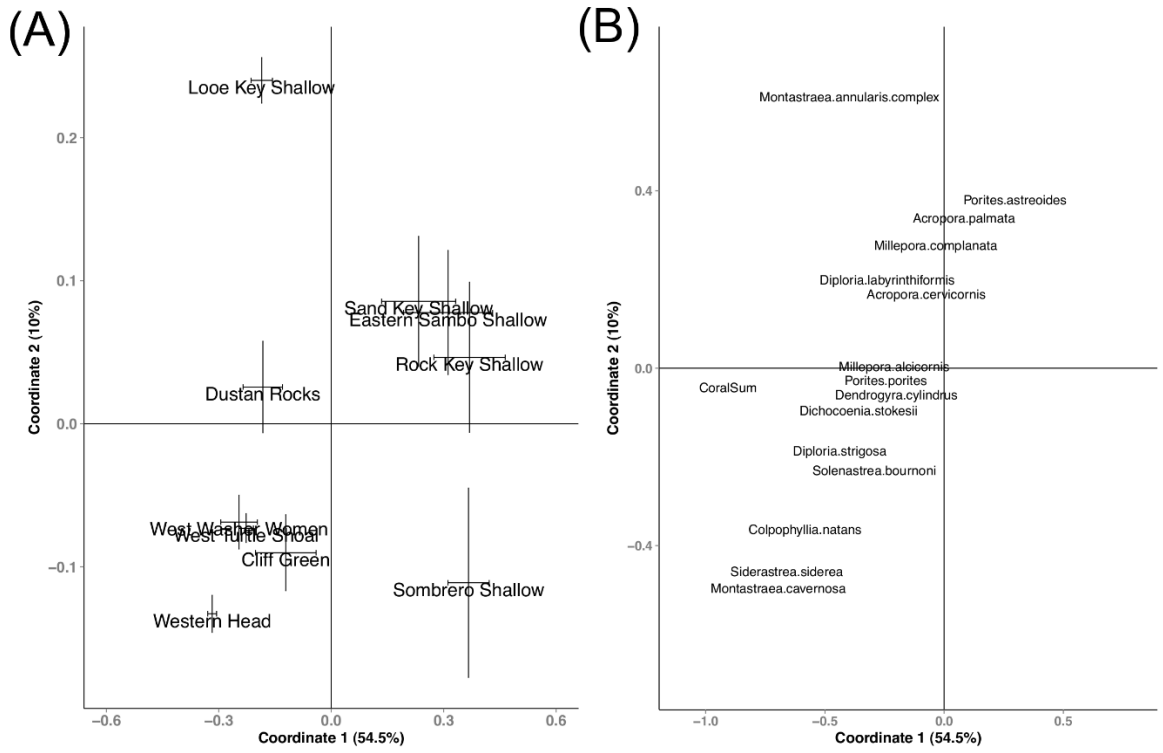


Figure 4: Principal Coordinate Analysis of Species Occurrence from 1996-2011. The scores for each site (A) and loadings for each species (B) following principal coordinate analysis are presented. The first two coordinates accounting for 64.5 % of variation in the data are plotted. Error bars (A) indicate 95 % confidence intervals across the 15-year period. Species present in a particular quadrant of the loadings plot are more likely to be associated with a site in the same quadrant of the score plot.

Analysis of CREMP data supports the ongoing observation of increased growth at inshore patch reefs, however this data set is limited to relative abundance. To circumvent this issue we selected two reference sites to analyze in greater detail, focusing on coral community characteristics, particularly colony abundance, colony size, and benthic community composition. The mean seasonal benthos composition at Acer 24 reef (an offshore reef) and Birthday reef (a patch reef) along 30 m transects is presented in Figure 5. Scleractinian coral cover was significantly greater at Birthday Reef (ANOVA: $p < 3.83e-07$), while the benthic community at Acer 24 contained significantly greater gorgonian coral cover (ANOVA: $p < 2.01e-08$), supporting our observations from the CREMP data analyses. Gorgonian cover

also displayed seasonality at Acer 24 reef, with a greater percent cover observed during winter sampling (ANOVA: $p < 0.003$). This pattern was not evident at Birthday reef. Macro algal and turf algal cover was not significantly different between the reference sites and accounted for greater than 30 % of all reef cover. Increased macro algal cover was observed during winter months, but this difference was not significant compared to summer.

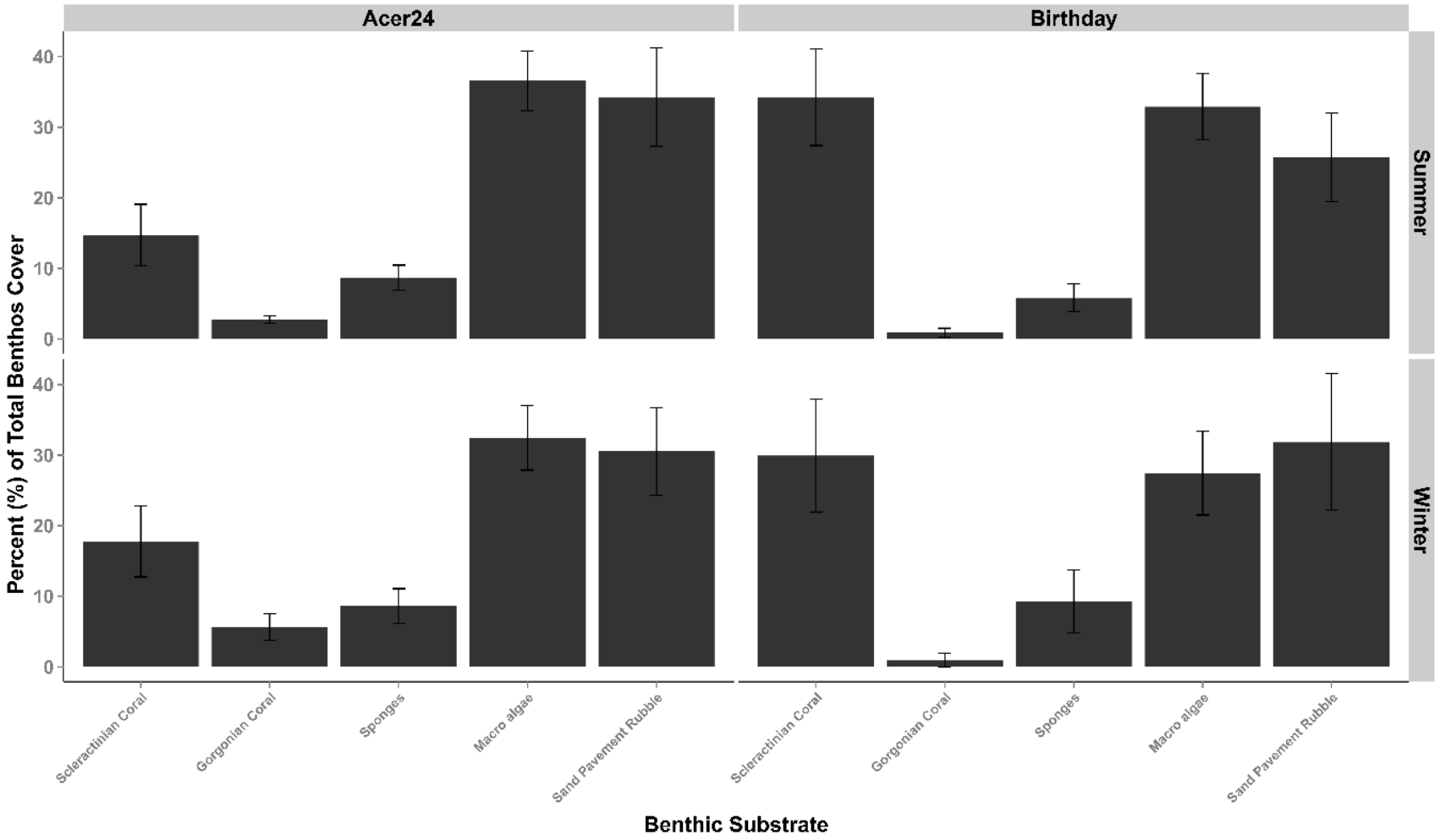


Figure 5: The Mean Percent Cover of Benthic Substrata at Birthday and Acer24 Reefs. Benthic substrate information was collected during the summer (August) and winter (February) from 2011-2012 along 30 m transects (n = 6). Error bars represent 95 % confidence intervals.

Coral community composition, species' abundances, and colony size were important components of the dissimilarity between the two sites. Rényi diversity profiles support the dissimilarity in scleractinian coral community composition (Figure 3). Species richness was greater at Birthday reef as indicated by an increased diversity value at alpha = 0. Fourteen scleractinian coral species were present at Birthday reef while only ten of these species were observed at Acer 24 reef. Additionally, both sites were characterized by low species evenness indicated by diversity values > 1 for alpha = Infinity. The most frequently observed colonies inhabiting Acer 24 reef were *Siderastrea siderea*, *Montastrea cavernosa*, *Porites astreoides*, and *Stephananocoenia michelinii* (Figure 6). In addition to these four species, *Orbicella annularis* was frequently observed at Birthday reef. Larger colonies were more frequently observed at Birthday Reef, as indicated by the greater positive skew of total coral frequency distributions (Figure 7). Amongst the most common species identified, *P. astreoides*, *S. siderea*, and *M. cavernosa* all occurred in similarly comparable zone dependent distributions.

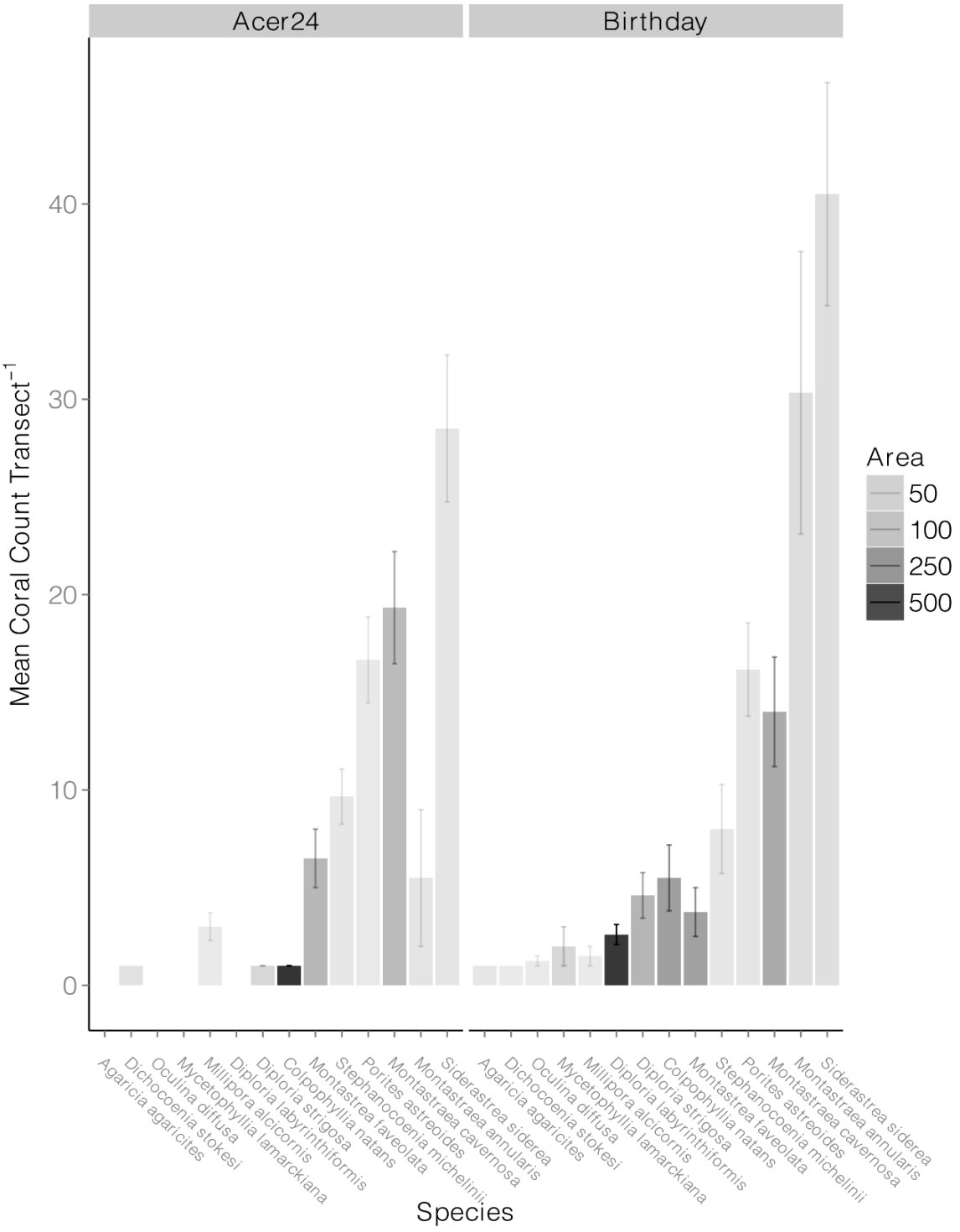


Figure 6: The Mean Abundance and Area of Corals in Transects of Established Sites. Bars indicate the mean count of a particular species of coral. Error bars represent the standard error for each bar. The color of each bar represents the mean area (cm^{-2}) along transects for each species at a given site. Acer 24 is representative of an offshore site while Birthday reef is representative of a patch reef.

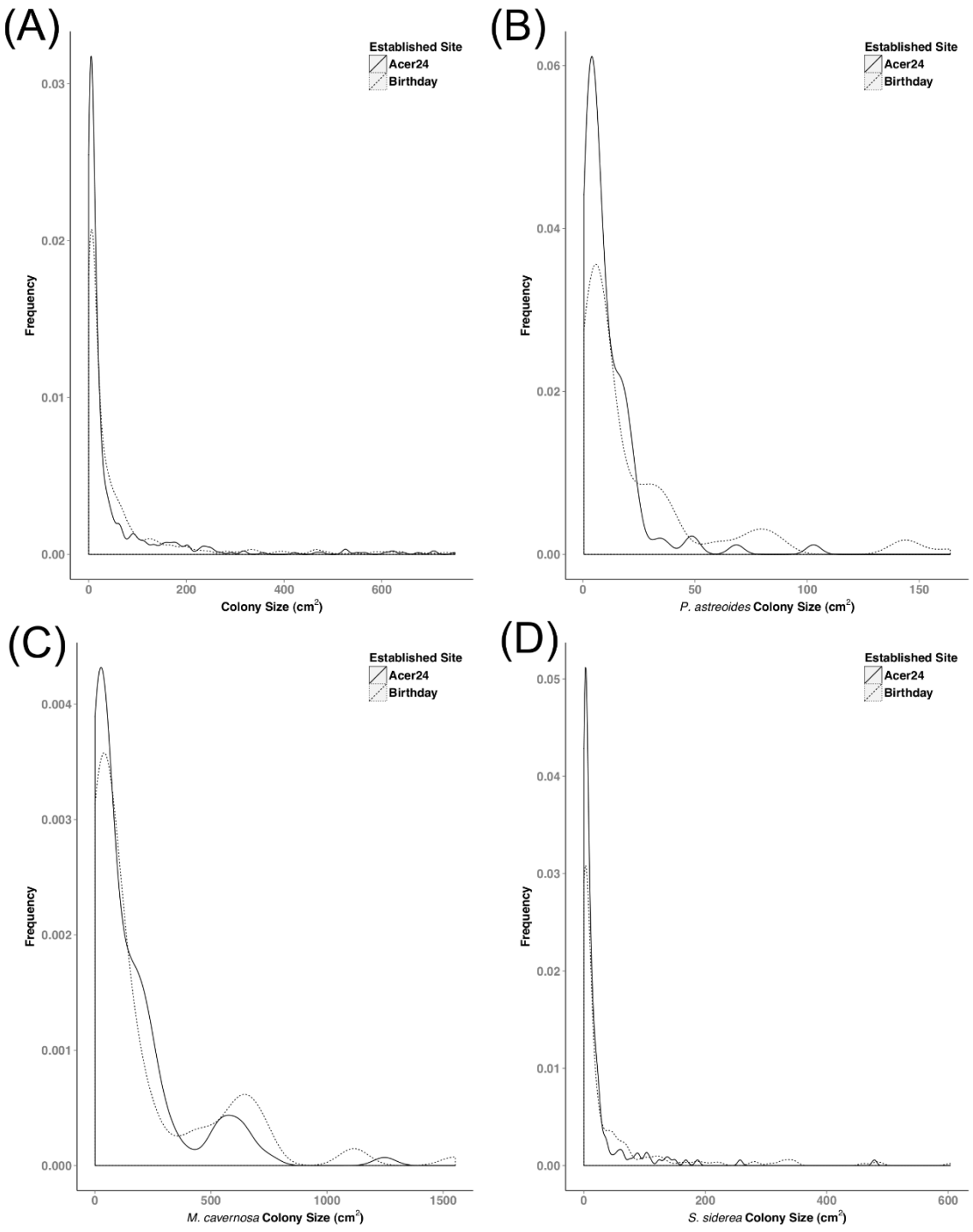


Figure 7: The Frequency Distributions of Sizes for all Corals and Three Selected Species at Established Sites. The four figures show the total coral colony size distribution (A), as well as the size distributions of *Porites astreoides* (B), *Montastraea cavernosa* (C), and *Siderastrea siderea* (D), the three most abundant species.

1.4.2 Zonal abiotic characteristics

Principal component analysis (PCA) was applied to winter and summer subsets of the WQMP dataset to identify factors that may be associated with stress during these periods and influence the coral communities of inshore and offshore reefs. During winter months (January and February) 55 % of the variation between sites was accounted for by three components. Variation along principal component 1 (22.9 %) was influenced by water chemistry attributes. Total organic nitrogen (TON) ordinated sites negatively while the inorganic nutrients ammonium and silicate ordinated sites positively along this axis. These three variables did not vary significantly by region or location, however the marginal assurance in variation associated with silicate (ANOVA; $p = 0.0652$) provides a potential difference between patch and offshore reefs during winter months. Along principal component 2 (19.2 %), variation between sites was dictated by nitrate, soluble reactive phosphorus (SRP), chlorophyll a (Chl a), and total organic carbon (TOC). Nitrate was significantly greater at eastern sites than western sites and independent of location (ANOVA; $p = 0.0062$), while SRP, Chl a, and TOC remained statistically similar across regions and locations. The variation along principal component 3 (13.1 %) was dictated by the variation in the physical characteristics turbidity and temperature. Turbidity was significantly greater at western sites regardless of location (ANOVA; $p = 0.0113$), while seawater temperature (SWT) did not was not significantly different for either region or location. Additionally, during winter months, greater variation in SWT was observed at inshore reefs (18-29°C) than on offshore reefs (20-25°C).

Plotting each of these components against one another resulted in regional ordination (Figure 8). With the exception of Rock Key, the western most sites West Washer Woman, Eastern Sambo, Cliff Green, Western Head and Sand Key, oriented away from eastern sites: Looe Key, Sombrero Reef, Dustan Rocks and Western Turtle Shoal. Variation in SWT, turbidity, and TOC appear responsible for the observed region dependent ordination during winter months, indicated by the direction and magnitude of arrows

for each environmental variable. Within this structure, the three most speciose inshore sites, Western Head, Cliff Green, and West Washer Woman ordinated near one another.

Principal component analysis of summer WQMP data indicated 48.8% of the environmental variability among all sites could be explained by the first three components. Similar to PCA of winter months, regional ordination of sites was evident during the summer (Figure 9). The three speciose western sites Western Washerwoman, Cliff Green, and Western Head ordinated away from the eastern sites Sand Key, Rock Key and Sombrero Reef, and Looe Key, while the remaining sites, Eastern Sambo, Western Turtle Shoal, and Dustan Rocks were found between these two groups. This separation was not a distinct as that identified during winter months and may provide an indication of the large potential for site or location dependent environmental variability during summer months for reefs of the middle and lower Florida Keys.

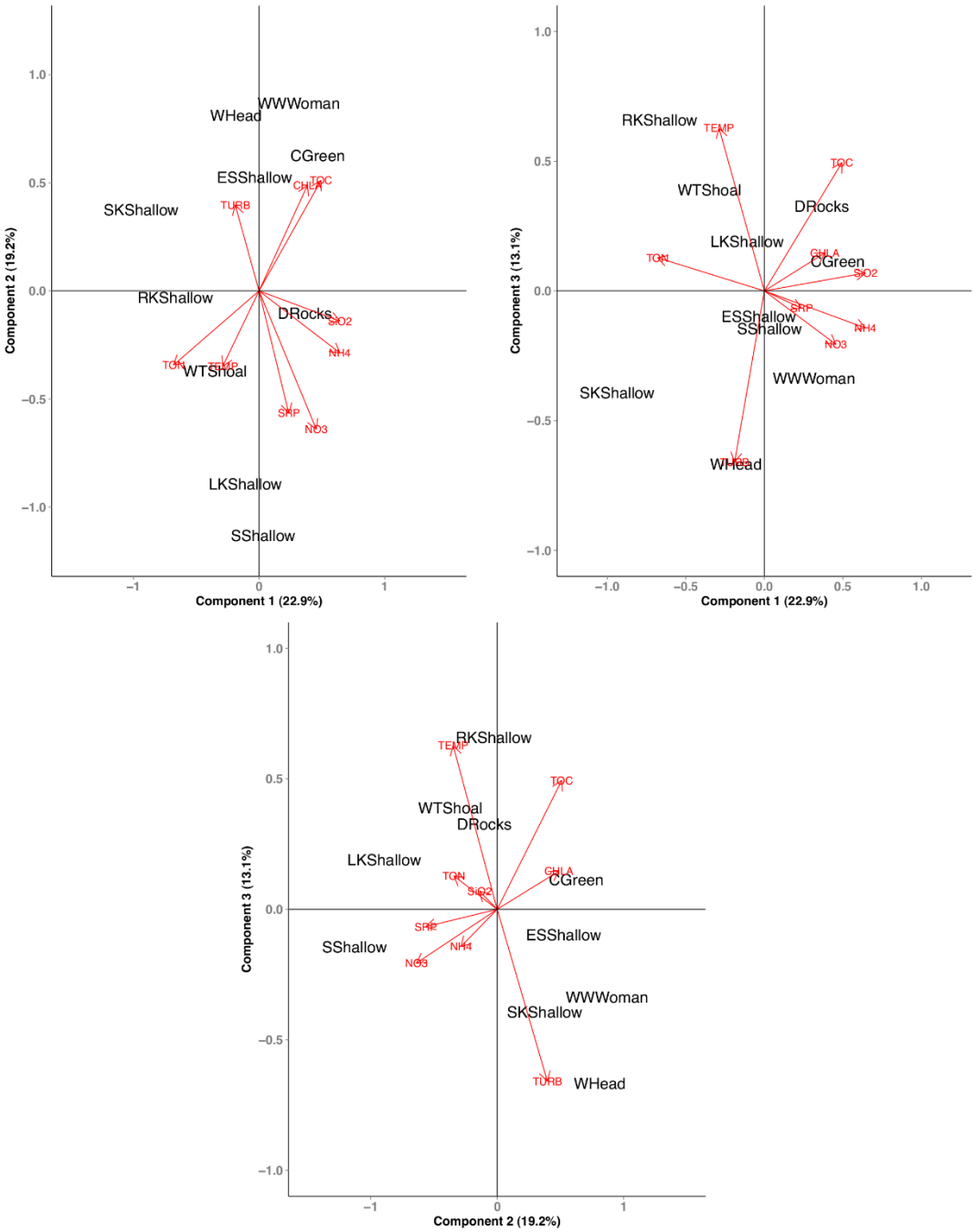


Figure 8: Principal Component Bi-plots of Winter Environmental Data from Patch Reef and Offshore Reef Sites from 1996-2011. Three bi-plots represent combinations of the first three components representing 55 % of the variation in the dataspace; component 1-component 2 (A), component 1-component 3 (B), and component 2-component 3 (C). Site names are positioned at the score associated with that site. Red arrows indicate the loading of a given environmental variable on the ordination of sites. The length and direction of the arrow is proportional to its effect.

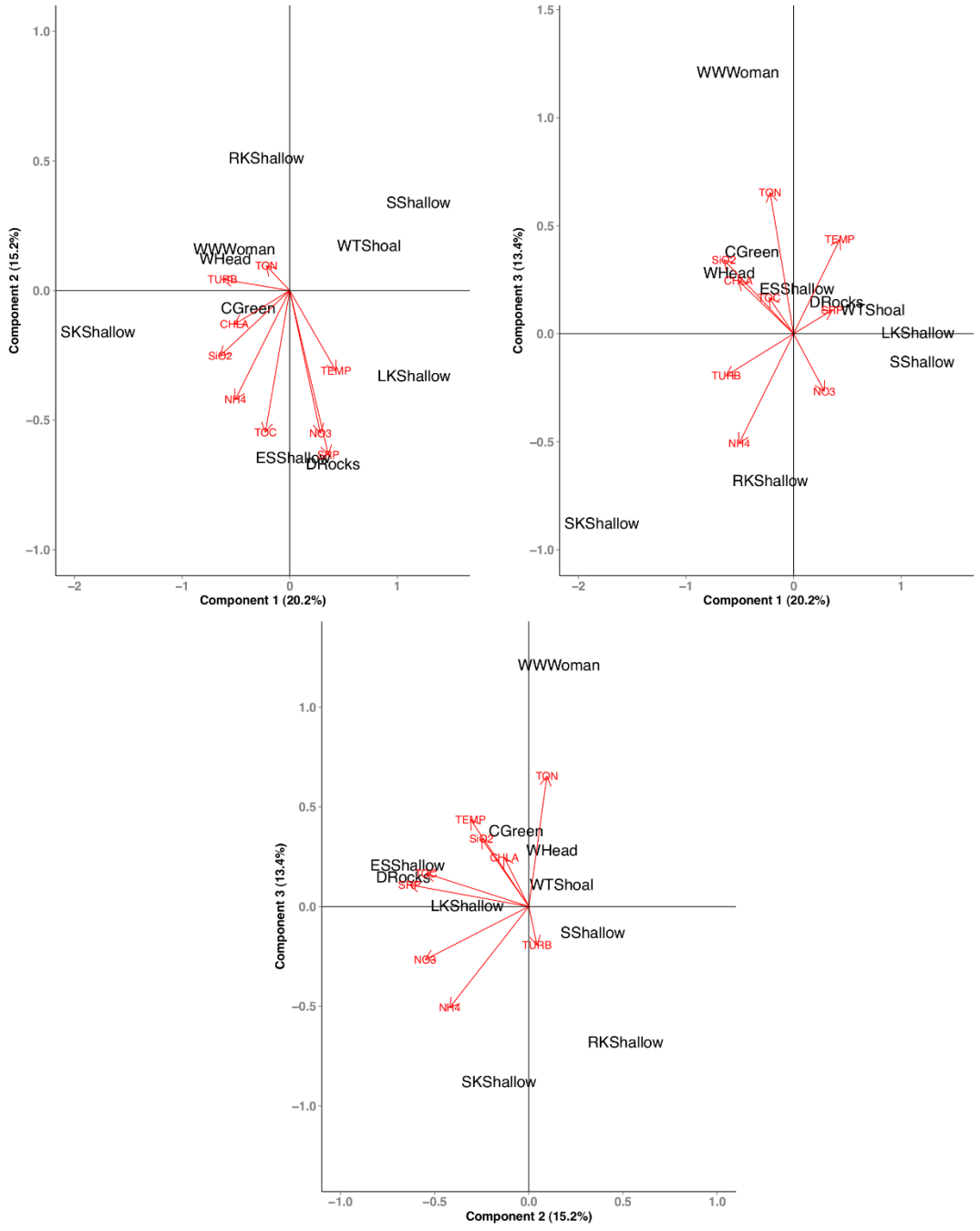


Figure 9: Principal Component Bi-plots of Summer Environmental Data from Patch Reef and Offshore Reef Sites from 1996-2011. Three bi-plots represent combinations of the first three components representing 48.8 % of the variation in the dataspace; component 1-component 2 (A), component 1-component 3 (B), and component 2-component 3 (C). Site names are positioned at the score associated with that site. Red arrows indicate the loading of a given environmental variable on the ordination of sites. The length and direction of the arrow is proportional to its effect.

Despite explaining only 13.3 % of the total variation, PC3 appeared largely responsible for the regional ordination of sites within the data-space. Clear ordination was evident only in bi-plots including this component as an axis. Therefore the environmental variables TON, ammonium, and SWT, which were most influential along PC3, may be important factors governing summer environmental differences between these regions and sites. Upon closer inspection, SWTs did not vary significantly between regions, however inshore sites were significantly warmer than offshore sites by 1°C (ANOVA; $p = 0.0183$). Further, SWT at inshore sites was consistently warmer during summer months (32-27°C), while offshore site SWTs were typically cooler but also ranged more widely than inshore sites during this time (31-20°C). Significant differences were not identified at the location or region level for TON (ANOVA; $p = 0.166$ and $p = 0.214$ respectively) or ammonium (ANOVA; $p = 0.42$ and $p = 0.06$ respectively).

Principal components 1 and 2, despite limited influence on the regional ordination of sites, imparted greater percentages of variation to the summer environment data-space than PC3. Influential variables along principal component 1 (20.2 %) included factors that contribute to turbidity including Chl a and silicates. Western sites were 0.7 NTU more turbid than eastern sites (ANOVA; $p = 0.022$) and also significantly greater in Chl a (ANOVA; $p = 0.013$). Silicates displayed significant variation at the zone level (ANOVA; $p = 0.0392$) but not regionally. Variation along PC2 was influenced by SRP, nitrate, and TOC, however significant variation was not identified for any of these variables at the regional or location level.

Increasing the resolution of temperature data to daily monitoring at a reference inshore and offshore site provided greater support to these findings. During three years of temperature monitoring (2011-2013) annual mean seawater temperatures (SWT) at the inshore site, Birthday Reef, and offshore site, Acer 24 reef, did not significantly differ (ANOVA $p = 0.828$); 26.74°C and 26.77°C respectively. Monthly

variance in SWT, however was 0.5°C less at Acer 24 (ANOVA $p < 0.05$). Additionally, the frequency of daily SWTs greater than 30°C and less than 23°C were 44 % and 50 % more frequent at Birthday reef than Acer 24.

1.4.3 Location-dependent shifts in brightness, a bleaching related characteristic

Fragments of *M. cavernosa* and *P. astreoides* were significantly darker in brightness at Birthday Reef (ANOVA; $p = 2e^{-16}$), the inshore site, relative to the offshore site Acer 24 (Figure 10). Although the occurrence of severely bleached corals (Brightness ≥ 150) was rare, the frequency of stressed corals (101 ≤ Brightness ≥ 149) was significantly greater at the offshore site, Acer 24. We did not observe a transplant dependent effect on the brightness of corals when analyzing the total frequency of occurrences at the three brightness ranges. Observing the quantum yield of photochemical energy conversion (Yield: $\Delta F/F_m'$) of corals inhabiting each location, we found significant differences at the species ($p = 2.23e^{-06}$) and site ($p = 0.0198$) levels during summer months (Figure 11). The probability of photons entering photosystem II (PSII) was greater for *P. astreoides* and colonies of these two species inhabiting Birthday reef. During the winter, yields did not vary significantly from that of summer months indicating similar levels of photic stress.

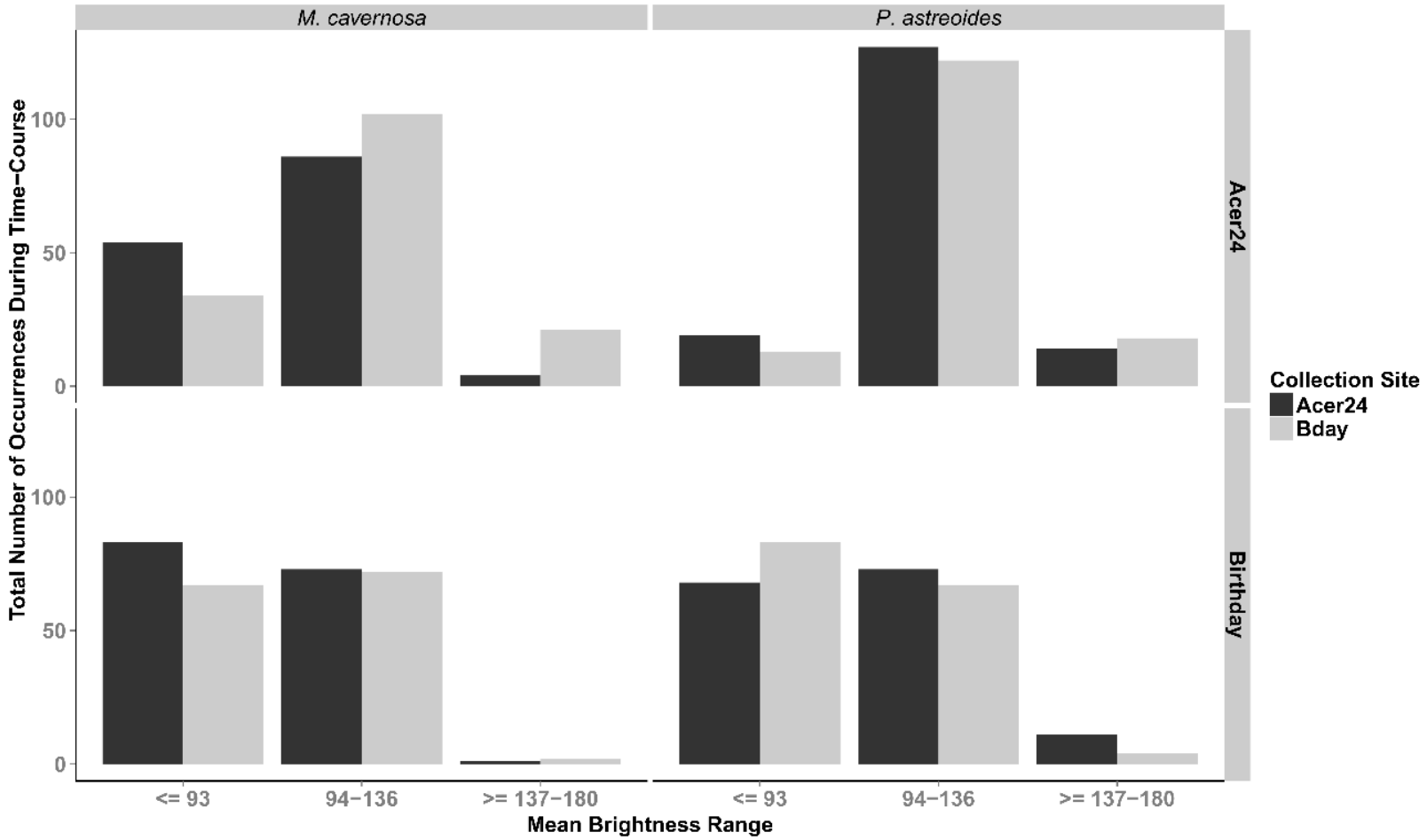


Figure 10: Frequency of Occurrence for Three Brightness States Following a Reciprocal Transplant of *Porites astreoides* and *Montastraea cavernosa*. Three bins were created for mean brightness values, each spanning approximately 40 units. The four graphs are separated along the y-axis by the site to which a coral was transplanted. Bar color indicates collection site (Black = Acer 24, Grey = Birthday). The graphs are further separated by species along the x-axis. Bar height is indicative of the cumulative number of occurrences within a particular bin from monthly images taken from September 2011-April 2013.

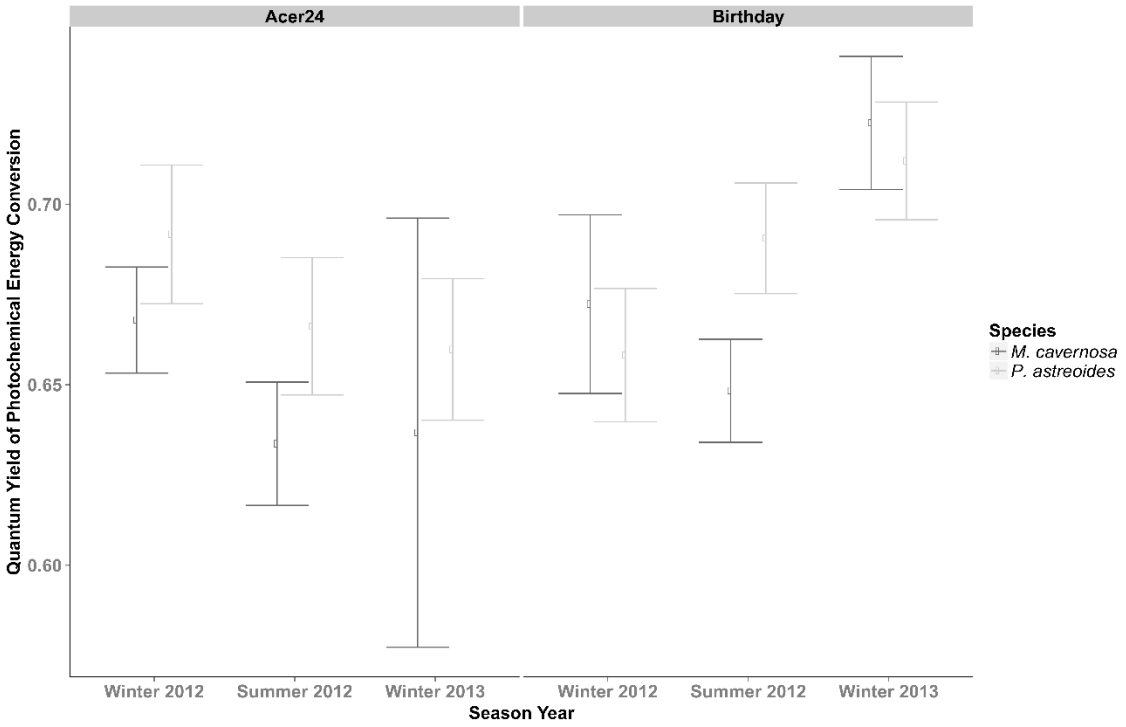


Figure 11: Quantum Yield of Photochemical Energy Conversion of *Montastraea cavernosa* and *Porites astreoides* Inhabiting an Offshore Reef (Acer 24) or an Inshore Reef (Birthday) within the Florida Reef Tract. The yields of all corals of the two species within 1 m of 90 m transects (n = 3) were obtained for a given sampling event. Points represent means and error bars indicate 95 % confidence intervals.

I observed a seasonal change in the monthly brightness of coral fragments transplanted to Birthday Reef, the inshore site, following the application of a Loess smoothing function (Cleveland and Devlin, 1988). The lightest shade (greater brightness value) was observed during September and October while the darkest (lower brightness value) were observed during February and March. Due to the large variance in monthly brightness values between coral fragments, trigonometric linear regression was applied to determine if a significant trend existed. Changes in brightness significantly fit a cosine function with 12-month period (ANOVA; $p = 0.04$), representative of a significant annual pattern in coral brightness at Birthday reef (Figure 12). A significant cosine pattern was not observed for corals at Acer 24. Instead, linear regression of coral fragment brightness at this site indicated that mean brightness

values of corals transplanted to this site increased from September 2011 to May 2013. Therefore these corals became progressively lighter (more bleached) during this period of time.

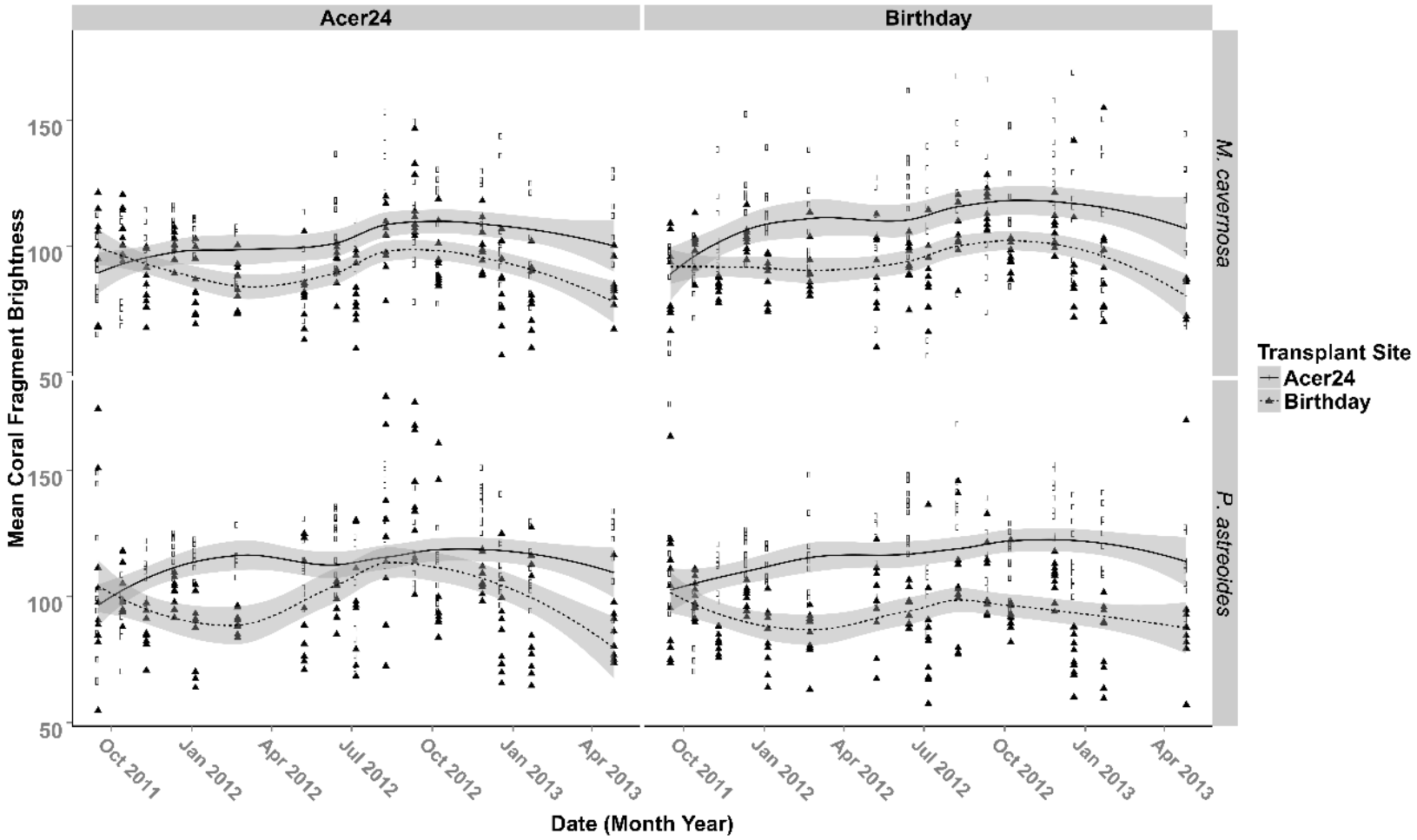


Figure 12: Brightness Time Series of Reciprocally Transplanted *Montastraea cavernosa* and *Porites astreoides* from September 2011-April 2013. The monthly brightness value of each individual coral within the transplant study is presented. Points identify the site a coral was transplanted to (Circle = Acer 24, Triangle = Birthday). Loess regression lines have been fitted to each subset of corals also based upon the site a coral was transplanted to (Line = Acer 24, Dash = Birthday). Confidence intervals (95 %) have been place around each line. The four graphs are separated along the y-axis by collection site and along the x-axis by species.

1.5 Discussion

The inshore patch reef system of the Florida Keys National Marine Sanctuary (FKNMS) has maintained stable and productive scleractinian coral communities since the last large scale decreases in coral cover around 1999. The neighboring bank reef communities experienced the greatest losses to coral cover during this period and have yet to rebound (Lirman and Fong, 2007; Schutte et al., 2010). Our analysis of the past 16-years of data from CREMP indicates that this trend has continued (Figure 2). We observed a greater percentage of live coral cover and greater coral diversity at five sites within the inshore reef system spanning the lower and middle keys compared to five bank reef sites. Community level analysis of an external patch reef and offshore reef site, further support that scleractinian corals inhabiting inshore sites are not only more abundant (Figure 2) and more diverse (Figure 3), but mean colony size is significantly greater (Figure 7). Analysis of the 10 reefs from the CREMP indicated that the inshore patch reef system now accounts for greater than 70 % of the coral cover remaining throughout the FKNMS regardless of the more consistent environment associated with offshore reefs. Bank reef habitats were characterized by lower SWTs along with a narrower range of thermal experience compared to inshore patch reefs (Figure 9). Additionally I identified significantly decreased levels of silicates at bank reef sites and a mean turbidity half that of inshore sites, providing support to previously reported trends of nutrients and turbidity in this area of the FKNMS (Lapointe et al., 2004). Although the rapid loss of dominant acroporiid corals from the bank reef system caused a dramatic shift in reef biota (Patterson et al., 2002) as a consequence of the epizootic pathogen *Serratia marsescens* (Patterson et al., 2002; Sutherland et al., 2011), this event alone does not explain the failure of the currently dominant coral species (Burman et al., 2012) to re-establish this zone over the past 20-years given the apparent absence of other potential environmental constraints.

My analysis indicates that patch reefs and bank reefs of similar depth (6 m) within the middle and lower FKNMS support distinct scleractinian coral communities. The abundances of four species greatly influenced the ordination of inshore sites away from offshore sites; *Acropora palmata*, *Siderastrea siderea*, *Montastraea cavernosa*, and *Porites astreoides* (Figure 4). The influence of *A. palmata* was least revelatory as this known specialist inhabits a narrow habitat range including high relief and inner line spur and groove habitats found only in the bank reef zone (Miller et al., 2008). The current endangered status of *A. palmata* also precluded it's inclusion in our transplant study. *Siderastrea siderea*, the most common scleractinian coral currently inhabiting the FKNMS (Burman et al., 2012), was highly prevalent regardless of the zone. Because the goal of this study was to identify sources of variation in the coral cover of inshore and offshore sites, the known generalist nature of *S. siderea* (Burman et al., 2012) precluded this species as well. The remaining two species, *P. astreoides* and *M. cavernosa*, although considered eurytopic, inhabited inshore and offshore zones with differing degrees of prevalence suggesting dissimilar tolerances to the stressors inherent to each zone. I selected these species as a proxy to identify site and species dependent factors that may contribute to the continued trend of decreased coral cover at offshore bank reefs compared to inshore patch reefs.

Reciprocal transplantation of *M. cavernosa* and *P. astreoides* between Birthday Reef (inshore site) and Acer 24 (offshore site), revealed that fragments from both species transplanted to the inshore site, displayed significantly lower brightness in images than conspecifics and ramets transplanted to the offshore site regardless of the collection site (Figure 11). I interpret this result to signify decreased levels of stress at inshore reefs resulting in greater symbiont concentrations within the host or increased pigment concentration within the algal symbionts (*Symbiodinium spp.* commonly referred to as zooxanthellae). Although increases in symbiont density can also occur when inorganic nitrogen concentrations increase (Marubini and Davies, 1996; Muscatine et al., 1989), my study of the abiotic

differences between the patch reef and bank reef zone did not yield a significant difference in inorganic nitrogen forms between zones. Instead, inshore sites displayed increased seawater temperatures and a wider range of turbidities. Further, the two CREMP sites nearest to our transplant sites, Western Washer Women (inshore) and Looe Key (offshore), displayed significantly different turbidities during the winter (μ = 3.1 NTU and μ = 0.26 NTU respectively) and summer (μ = 0.8218 NTU and μ = 0.480 NTU respectively). From this information differences in saturation and brightness of transplanted coral colonies may be a function of the effects of turbidity and temperature. Temperature and turbidity have been identified as mediators of growth for *Orbicella annularis* populations near Key Largo, FL (Hudson, 1981).

While transplantation site affected the brightness of coral fragments, the conditions previously experienced by a transplanted fragment (Collection Site) did not have a significant effect on this measure of symbiont response. Although this finding is by no means conclusive as to the nature of symbiont responses during this period, it provides evidence that the differences in stressors identified between these two sites were not significant enough to elicit a visible response. This finding is contradictory to studies that have reported significant interactions between the environmental history experienced by these two species and their response to future stress (Haslun et al., 2011; Kenkel et al., 2011). For instance, we have shown previously (Haslun et al., 2011) that *M. cavernosa* from the Flower Garden Banks National Marine Sanctuary (FGBNMS), a well-developed and thermally stable scleractinian reef ecosystem, are more susceptible to increased thermal stress than conspecifics from the FKNMS, where increased SWTs are experienced. Similarly, *P. astreoides* has been shown to display experience dependent acclamatory responses *via* the expression of genes associated with calcification and metabolism when exposed to different temperature regime (Kenkel et al., 2013b). Both of the aforementioned studies reported that pronounced differences in seawater temperature existed

between the collection locations. In this study, mean maximum and mean minimum temperatures for summer and winter respectively, deviated by approximately 1°C between inshore and offshore zones, which is less than that observed between the FGBNMS and the FKNMS (Haslun et al., 2011) or the more extreme temperature regimes at near shore sites (< 1km from shore) of the FKNMS (Kenkel et al., 2013b). It is possible that the dissimilarity in SWT observed between our sites was not acute enough to illicit an acclamatory effect in the observed brightness of *P. astreoides* and *M. cavernosa*.

Bleaching (loss of symbionts) of corals during summer seasons is a common response to increased stress from light and temperature levels (Fitt et al., 2000) and not necessarily detrimental to the organism. The primary purpose of this response is to limit the host's cellular stress caused by symbiont malfunction (Strychar et al., 2004). Seasonal and widespread bleaching are hypothesized to be critical to the adaptation of corals in the face of the increasing magnitude and frequency of stressors (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004; Guest et al., 2012). Coral fragments experiencing the conditions at the inshore site displayed seasonality in colony brightness, characterized by a summer stress period and a winter compensatory period (Figure 12). The seasonal pattern was also reflected in the significant relationship identified between temperature and brightness for these corals. Therefore, seasonal stress levels from temperature remain coupled to bleaching at Birthday reef and may be critical to the continued success of coral at inshore reefs. Seasonality in coral fragment brightness was not detected for fragments transplanted to Acer 24. On the contrary, we observed a significant positive linear trend between brightness and month, indicating that fragments of both species of coral became progressively lighter over this two-year period. Therefore, bleaching (the loss of symbionts) and temperature stress can be considered uncoupled for *P. astreoides* and *M. cavernosa* experiencing the conditions at Acer 24. Given the narrower temperature range characteristic of this site, this result is intriguing. From our results we hypothesize that stress resulting from increased

irradiance (i.e. decreased turbidity), characteristic of offshore sites, provides an additional source of stress increasing the cumulative level during summer and winter periods beyond that of corals at inshore sites. The increased cumulative stress level may decrease a colony's potential for recovery during the winter season resulting in a state of chronic bleaching.

Chronic bleaching (an archetype of chronic distress) can be difficult to observe given its non-lethal nature (Lasker and Coffroth, 1998). Horizontal linear extension was evident at both transplant sites throughout the course of our study (pers. obs.), however we were unable to determine whether or not the degree of growth differed between sites. In order to detect potential differences in fitness associated with chronic distress, we investigated site dependent community characteristics. Eventual mortality may occur when organisms must allocate resources to protection and maintenance (Lasker and Coffroth, 1998) rather than fecundity and growth. We observed a significant difference between the mean colony area of *M. cavernosa* and *P. astreoides* at Birthday reef and Acer 24, as well as another commonly observed species, *S. siderea* (a known generalist). Similar observations have been noted outside of the Florida Keys (Edmunds and Elahi, 2007). Following dramatic decreases in the abundance of *O. annularis* (formerly *Montastraea annularis*) at a reef near St. John Island in the United States Virgin Islands, the mean size of corals continued to decrease over a five-year period, from 1999-2003, despite the stabilization of percent coral cover (Edmunds and Elahi, 2007). The authors projected that this species of coral would become locally extinct if the level of stressors remained unchanged over the next 30-50 years. Corals at offshore reefs of the FKNMS appear to be experiencing this trend now.

1.6 Conclusion

Offshore bank reefs harbor relict communities of scleractinian corals and these communities do not show signs of rebounding. Although the stressors associated with this zone appear lower than those associated with more successful inshore patch reefs our results indicate that corals inhabiting offshore shallow reefs are experiencing more significant levels of stress compared to conspecifics from inshore patch reefs. This result may not be reflected in the acuteness of stress but rather the absence of a phase to recover from seasonal stressors. In support of this claim we provide several tracts of evidence. First, the brightness of corals (an indication of symbiont density) transplanted to an offshore site became progressively lighter over a two-year period regardless of the site of collection while corals transplanted to an inshore site displayed seasonality in brightness. Second, the linear relationship between seawater temperature and brightness was significant for corals transplanted to inshore sites but this relationship did not exist for corals transplanted to an offshore site. Third, the mean colony size of the two species used for transplantation as well as a common generalist, were smaller at offshore sites. Finally, we identified a factor, turbidity that provides an explanation supporting the potential for chronic stress to occur at offshore sites. We propose that decreased turbidity results in increased irradiance at offshore sites resulting in greater cumulative stress than is present at inshore reefs. The results presented in this study provide support for increased protection and awareness of reefs inshore to the bank reef habitat. It also provides important information regarding the important synergism between changing temperature and irradiance experienced by corals. Increased turbidity provides both a refuge from increased irradiance as well as a potential food source to counteract decreases in algal carbon molecule production for the cnidarian host during periods of eutrophication as long as sedimentation is not significant (Bongiorni et al., 2003).

CHAPTER 2

LOCAL ADAPTATION OF *PORITES ASTREOIDES* BETWEEN INSHORE AND OFFSHORE METAPOPULATIONS INHABITING THE FLORIDA REEF TRACT

2.1 Abstract

Dramatic changes to the coral communities of the Florida Reef Tract (FRT) have been observed over the past 30 years. Coral cover throughout this reef system is now disproportionately distributed with greater than 70% of the remaining coral cover accounted for within the inshore patch reef zone (< 2 km from shore) compared to 30% within the offshore bank reef zone (> 5 km from shore). Coral community changes along the FRT have been attributed to mortality stemming mainly from thermal stress and disease, although other stressors undoubtedly contribute (e.g. irradiance). Offshore patch reefs, however, experience a smaller seawater temperature (SWT) range than inshore patch reefs (i.e. less thermal stress) suggesting that disease contributes more to declines in coral cover than SWT at offshore reefs. I examined the degree to which the immune system was activated in *Porites astreoides* originating from the inshore and offshore reef environments to determine if increased activation was associated with decreased coral cover. Colonies from an inshore and offshore site were reciprocally transplanted and the expression of three genes determined biannually for two years (two summer, two winter periods). Variation in the expression of eukaryotic translation initiation factor 3, subunit H (eIF3H), an indicator of cellular stress in *P. astreoides*, did not follow patterns of SWT change indicating the contribution of other stressors to this response (e.g. irradiance, disease). Greater expression of TNF receptor associated factor 3 (TRAF3), a signaling protein of the inflammatory response activated following pathogen recognition, was observed among corals transplanted to or located within the offshore environment indicating that the offshore habitat stimulates the immune response to a greater degree than the inshore habitat ($p < 0.001$). Corals collected from the offshore site upregulated the expression of adenylyl cyclase associated protein 2 (ACAP2), which decreases innate immune system inflammatory responses, indicating a counteractive adaptive response to increased stimulation of the

immune system. Therefore, the offshore *P. astreoides* population, which is characterized by smaller mean coral colony size and decreased colony abundance was also associated with an increase in the immune response and a locally adaptive response indicating potential fitness tradeoffs. Activation of the immune response is metabolically costly. Therefore, increased immune system activation at offshore reefs is likely a contributing factor to coral community dynamics and declines along the FRT.

2.2 Introduction

Over the past 30 years *Porites astreoides* has become increasingly abundant along the Florida Reef Tract (FRT) surpassing the previously dominant *Acropora spp.* and *Orbicella annularis* (Lirman and Fong, 2007). Commonly described as a generalist scleractinian coral, *P. astreoides* is ubiquitous throughout the FRT (Crabbe, 2009). Lirman and Fong (2007) speculate that the change in species dominance is a response to climatic and anthropogenic stressors. An increase in the frequency and severity of warming events in conjunction with the spread of coral disease is hypothesized to have driven these community level changes (Harvell et al., 2002).

Several autecological factors have been identified in *P. astreoides* that contribute to its increased survivorship. First, among shallow (< 2 m depth) nearshore reefs a green color morph is prevalent due to increased levels of a particular mycosporine-like amino acid, asterina-330, that confers resistance to ultraviolet radiation (Gleason, 1993). Second, in contrast to other coral species, *P. astreoides*, focuses breeding efforts during the milder spring temperatures and some colonies remain gravid throughout the year. This reproductive strategy may provide the host with ample time to resupply energy stores after reproduction and increase survival during high temperatures in summer (Chornesky and Peters, 1987). Using gene expression methodologies Kenkel et al. (2011) showed that resistance to thermal stress in *P. astreoides* is primarily dependent upon the degree of prior exposure (Kenkel et al., 2013b). Additionally, we have shown that the thermal stress response of *Montastraea cavernosa* is also dependent upon the experienced non-lethal thermal history of a colony (Haslun et al., 2011). These two cases suggest that prior exposure to thermal stress may be an important factor affecting survivorship of coral species.

Along the FRT, corals inhabiting inshore patch reefs experience greater summer and winter variation in seawater temperature (SWT) than is experienced by corals at offshore bank reefs and increased coral

cover is linked to increased SWT variation along the FRT (Soto et al., 2011). Mean summer maximum and mean winter minimum SWTs at inshore reefs are at least 1°C more extreme than experienced offshore (Haslun et al., 2015 in review). *Porites astreoides* inhabiting inshore reefs can therefore be expected to have a smaller population relative to the offshore due to their exposure to increased levels of thermal stress as increased stress places constraints on fitness related traits. Contrary to this prediction, the abundance of *P. astreoides* colonies is considerably lower at offshore sites than in inshore sites. Moreover, the mean colony diameter of this species decreases from 16 cm at inshore reefs to 8 cm at offshore reefs (Haslun et al., 2015 in review), and colonies ≥ 40 cm in diameter have only been reported at inshore reefs (Lirman and Fong, 2007; Haslun et al., 2015 in review). A high abundance of small *P. astreoides* colonies (< 10 cm diameter) along this gradient indicates efficient recruitment at both sites whilst the scarcity of large colonies (> 30 cm diameter) offshore is indicative of an increased potential for mortality and stress offshore. While gene expression studies suggest that *P. astreoides* is resistant to thermal stress (Kenkel et al., 2011), and there is lower incidence of colony bleaching and increased colony growth during warmer SWTs (Kenkel et al., 2013a), these observations do not adequately explain differences in abundance and colony size between inshore and offshore reefs in this study. In addition to increased temperature stress, microbial stress and disease have severely impacted FRT offshore bank reefs. *Acropora cerviconis* and *A. palmata* have been functionally extirpated from the offshore bank reef habitats by a variety of "white" diseases; white plague disease types I, II, and III (Richardson et al., 1998), white band disease types I and II (Ritchie and Smith, 1998) and white pox (Patterson et al., 2002). Yellow band disease, and dark spots disease are also common throughout the Caribbean and impact many corals including *P. astreoides* (Cervino et al., 2001). The spread of these diseases has been linked to increased summer temperatures, while colder winter temperatures tend to diminish disease spread and reduce coral mortality by decreasing bacterial numbers and pathogenicity (Bruno et al., 2007; Cervino et al., 2004; Ward, Jessica, Kiho, Kim, Harvell,

2007). Therefore lower winter temperatures effectively decrease the activation of the host immune response to biotic stress. The milder SWTs of offshore bank reefs likely exacerbate disease related stress by failing to reduce the abundance of potential activators of disease during winter (e.g. microbe abundance and pathogenicity). Therefore I would expect to observe amplified immune activation in corals experiencing the offshore reef where warmer winter temperatures constrain pathogenic microbe activity to a lesser degree. Moreover, increased activation of the innate immune system has been shown to decrease invertebrate fitness (Armitage et al., 2003; Sheldon and Verhulst, 1996). Offshore reefs display both decreased coral colony size and decreased coral abundance (Haslun et al., 2015 in review; Lirman and Fong, 2007) resulting in decreased fecundity for *P. astreoides* (Chornesky and Peters, 1987).

In this study, I reciprocally transplanted *P. astreoides* colonies from an inshore patch reef and offshore bank reef. This form of transplantation allows for the detection of differences in an organisms' or populations' response to home vs. a foreign environment. We monitored the expression of three host-specific genes. Eukaryotic translation initiation factor 3, subunit H (eIF3H) provided an indication of a general stress response whereas TNF receptor associated factor 3 (TRAF3) and adenylyl cyclase associated protein 2 (ACAP2) provided indications of activation of the immune system biannually (summer and winter) over a duration of two years. In doing so, we determined (1) the effect of the site-dependent environment on gene expression, (2) the influence of SWT on gene expression in *P. astreoides* transplanted to each site and (3), we examined if inshore and offshore populations displayed adaptive responses to each environment.

2.3 Methods

The two sites selected for this study, Birthday Reef (24.57917' N, -81.49692' W) and Acer24 Reef (24.55268' N, -81.43741' W), are representative of an inshore patch reef and offshore bank reef environment, respectively (Figure 13). The reefs are located adjacent to one another along Hawk Channel within the lower region of the Florida Keys National Marine Sanctuary (FKNMS) and at similar depths (6 m). Birthday reef is characterized by greater abundance and diversity of scleratinian coral, increased variance in SWTs (including greater minimum and maximum temperatures during the winter and summer respectively), and greater turbidity than Acer 24 (Haslun et al., 2015 in review).

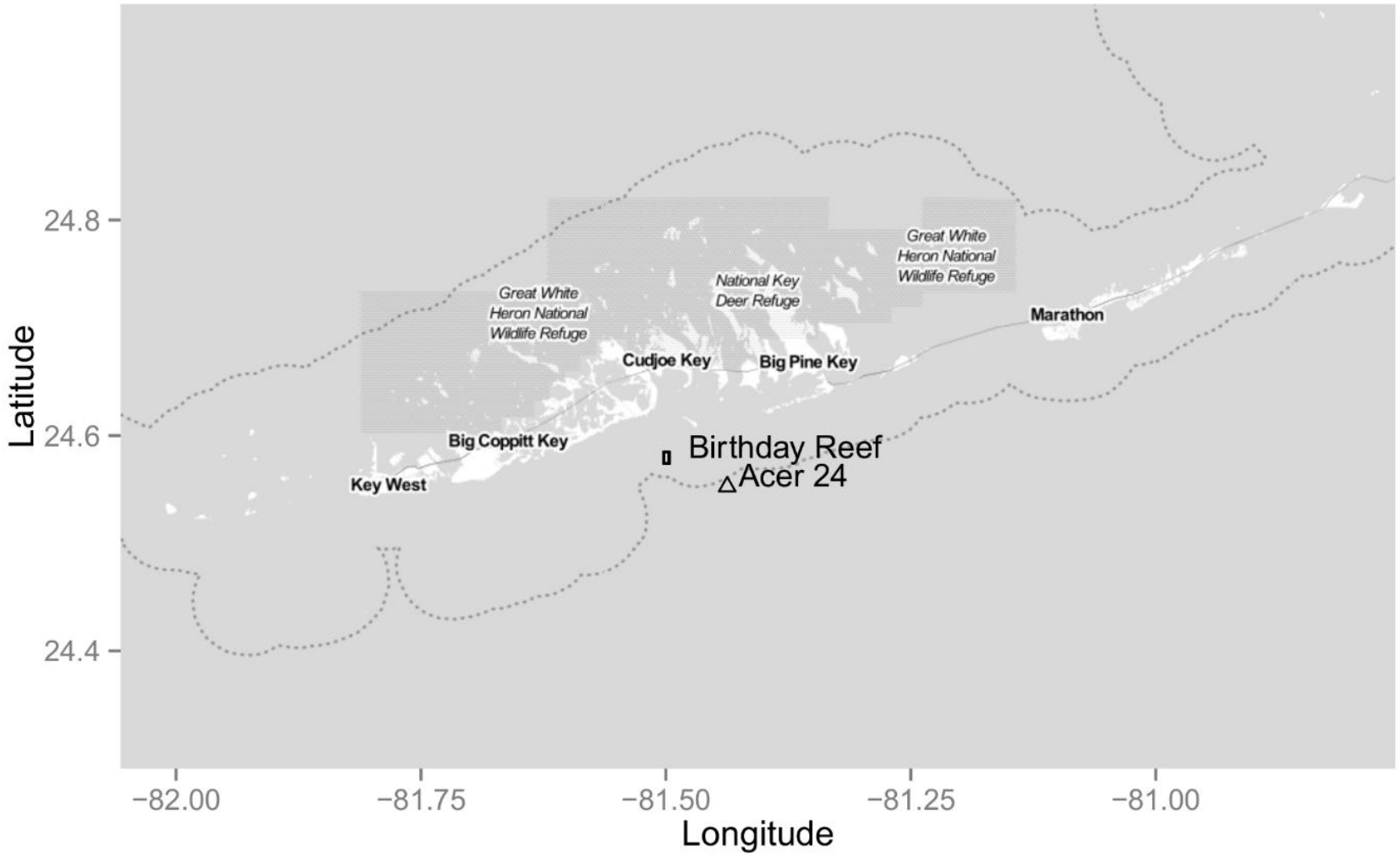


Figure 13: The Lower Region of the Florida Keys is Presented Along with Several Orientating Locations. The locations of each sample site are indicated by symbols. The inshore patch reef Birthday Reef is represented by a square and the offshore bank reef Acer 24 reef by a triangle.

2.3.1 Collection and transplantation

Collection and transplantation of coral in this study was conducted under NOAA National Marine Sanctuaries Permit # FKNMS-2011-10. *Porites astreoides* fragments from Birthday and Acer 24 reefs were reciprocally transplanted. Fragments (16 cm^2 x 16 cm^2) of *P. astreoides* colonies (n = 6) were collected (from each site at a depth of 6 m by divers using SCUBA. Fragments were removed from a colony using a cold chisel and mallet. Following collection, fragments were transported by boat to the Mote Marine Laboratory Tropical Research Laboratory (MML TRL) in a cooler containing site-derived water. Each fragment was halved with an electric brick-saw that was cooled and lubricated with sterile seawater. The resulting coral samples were placed in a shaded flow-through seawater system and equilibrated for 24 h. Following the recovery period, fragments were affixed to 6 cm x 6 cm concrete and aragonite (3:1) bricks with All-Fix epoxy (Cir-Cut: Lafayette Hill, PA). Coral fragments were then allowed an additional 48 h to equilibrate prior to being returned to the site of origin or transplanted to alternate site (Haslun et al., 2015). Each fragment was sub-sampled bi-annually for two years during the summer (August - September) and winter (January – February) by divers equipped with SCUBA. Sub-samples (2 x 2 cm) were removed with a hack saw equipped with a tungsten carbide blade (Milwaukee CPO: Pasadena, CA) and a cold chisel and mallet. This method prevented dislodging of fragments from the study site concrete blocks. On-board the ship, fragments were rinsed with sterile sea water, wrapped in combusted tin-foil, and then flash frozen in liquid nitrogen (LN_2). The entire process from collection to processing lasted less than 5 min for any single sample. Fixed samples were shipped to Michigan State University (East Lansing, MI) or the Annis Water Resources Institute (Muskegon, MI) on dry ice and stored at -80°C prior to RNA extraction.

2.3.2 Sample processing and RNA isolation

Coral fragments were first pulverized using a stainless steel dounce-style homogenizer chilled with LN_2 to retain sample integrity. Excess skeletal fragments were removed with forceps and the remaining sample further pulverized with a ceramic mortar and pestle in a shallow pool of LN_2 within the mortar. Mortar and pestles were cleaned with Alconox (Alconox Inc.: White Plains, NY), rinsed three times with de-ionized water, treated with RNase (RNase zap: Sigma-Aldrich, St. Louis, MO), and rinsed with ultra-pure deionized water (E-Pure; Thermo Fisher Scientific Inc.) between samples. Sample powder was transferred to a microcentrifuge tube and stored at -80°C prior to RNA isolation.

RNA was isolated from 110 mg of processed sample powder using a mixture of guanidine thiocyanate and phenol in a monophase solution (TRI Reagent: Sigma-Aldrich, St. Louis, MO). Materials used for weighing were chilled in LN_2 and the spatula treated for RNase as previously described. TRI Reagent (1 mL) was added to the sample and cell disruption facilitated *via* pipet aspiration. The sample was incubated for 10 min at room temperature and centrifuged (10,000 rcf, 10 min, 4°C) to achieve phase separation. The aqueous phase was then transferred to a new tube with 250 µL of a 0.8 M sodium citrate and 1.2 M sodium chloride solution and shaken vigorously for 5 sec to remove polysaccharide contaminants. Isopropanol (neat, 250 µL) was added and the microcentrifuge tube shaken vigorously for 15 sec. Samples in isopropanol were incubated (room temperature 10 min) and centrifuged (10,000 rcf, 10 min, 4°C) to pellet the RNA. The RNA pellet was washed twice with 75% ethanol and centrifuged (7,500 rcf, 5 min, 4°C). The resulting RNA extract was dissolved in RNase and DNase-free water (100 µL) and evaluated for RNA integrity with a Caliper Lab Chip GX (Perkin Elmer: Waltham, MA). RNA quality scores (RQS) greater than 6 were deemed of sufficient quality for two-step reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) (Fleige and Pfaffl, 2006). RNA extraction yielded approximately 2 µg of RNA.

2.3.3 Two-step qRT-PCR

Isolated RNA (300 ng) was treated with 1 unit of DNase 1 (Life Technologies: Waltham, MA) in accordance with the manufacturer's recommendations. DNase treated RNA (260 ng) was reverse transcribed with Superscript III first strand synthesis supermix (Life Technologies: Waltham, MA). Reverse transcription reactions were carried out in a 96-well plate using an Eppendorf Mastercycler and the following thermal profile: 10 min 25°C, 30 min 50°C, 5 min 85°C. After denaturation of the enzyme at 85°C, RNase H (1 µL) was added to each reaction and incubated (37°C, 5 min) to degrade the remaining template RNA.

Reverse transcribed cDNA was purified from the reaction via precipitation. To remove potential inhibitors to qRT-PCR, 7.5 M ammonium acetate (Sigma Aldrich: St. Louis, MO) (6 µL) was added followed by -20°C isopropanol (neat, 50 µL). The sample was inverted 10 times to mix and chilled (-80°C, 1 h) to precipitate the cDNA. Precipitated samples were centrifuged (18,000 rcf, 15 min) to pellet the cDNA. The precipitation reagents were decanted and pellet washed twice with 75% ethanol (1 mL). Washed cDNA was centrifuged (18,000 rcf, 15 min). The pellet was allowed to air dry (room temperature, 10-15 min) and re-suspended in ultra-pure water (104 µL) to yield a final cDNA concentration of 2.5 ng μL^{-1}. cDNA was stored at -20°C for less than 1 week prior to qRT-PCR.

2.3.4 qRT-PCR validation

Sequences of transcripts of interest were obtained from the *P. astreoides* SymBioSys database (http://sequoia.ucmerced.edu/SymBioSys/). Primers were created with Primer3 (Rozen and Skaletsky, 1998) software applying the selection criteria outlined in Table 1. The top scoring primer pair was selected for qRT-PCR validation. A standard curve relating the threshold cycle (Cq) to the log concentration of cDNA along a 2-fold serial dilution was used to validate primers (5 ng μL^{-1}, 2.5 ng μL^{-1},

1.25 ng μL^{-1}, 0.625 ng μL^{-1}, and 0.3715 ng μL^{-1}). Primer pairs that amplified a single product according to dissociation curves, displaying a highly linear relationship between Cq and concentration ($R^2 > 0.99$) and adequate amplification efficiency ($3.0 < E < 3.6$), were considered valid for analysis (Table 2).

Real-time PCR reactions were 10 µL in volume (1 µL template cDNA, 5 µL Power SYBR green master mix (Life Technologies: Waltham, MA), 1.5 µL primer pair (final concentration 250 nM), 2.5 µL DNase and RNase-free water). Amplification and detection of transcripts was performed on an Applied Biosystems 7900HT real-time PCR system in 384-well plate format following the manufacturer's recommendations. To prevent potential run-to-run variation and the need for inter-run calibrator samples, all samples were run in duplicate on a single 384-well plate for each gene of interest (Derveaux et al., 2010).

Table 1: Selection Criteria for Primers Used to Amplify Transcripts for Quantitative Real-Time Polymerase Chain Reaction.

Primer Selection Parameter	Criteria
Product size	50-150
Number of results returned	5
Max repeat mispriming	12
Max template mispriming	12
Max 3' stability	9
Pair max repeat mispriming	24
Pair max template mispriming	24
Primer size	18 < 20 > 22
Primer Tm	57 < 59 > 61
Max Tm difference	1
Primer GC %	20 < 50 > 80
Max self-complementarity	2
Max # N's	0
First base index	1
Max 3' self-complementarity	3
Max poly-X	4

Table 2: The Amplification Efficiency, Primer Sequences of Each Gene of Interest, and Control Genes are Presented in the Table.

Genes of Interest	Abbreviation	Primer Sequence (5'-3')	Efficiency
TNF receptor-associated factor 3	TRAF3	F: GTCTGGCTCCTCCCATCTTT R: GCCTCCAGCATTCTAACCTG	2.03
Adenylate cyclase associated protein 2	ACAP2	F: TCGTCTGGAGTCTGCTGCT R: TCTGCCACTTTGCCGTTTA	2.04
Eukaryotic initiation factor 3, subunit H	EIF3H	F: TTGATTGATACCAGCCCACA R: ACAAACTGCTTTGCTTTCCC	1.97
Control Genes			
60S ribosomal protein L11	RPL11	F: TTTCAAGCCCTTCTCCAAGA R: GACCCGTGCTGCTAAAGTTC	1.94
Cathepsin L	CATL	F: GGAAGGATTACTGGCTGGTC R: GGATAGATGGCGTTTGTGG	2

2.3.5 qRT-PCR analysis

A Bayesian model-based approach (Matz et al., 2013) was applied to analyze qRT-PCR Cq data. Raw Cq values were transformed to molecular counts creating values with a linear rather than exponential relationship. A Markovian Chain Monte Carlo generalized linear mixed model (MCMCglmm) with Poisson log-normal distribution was then applied. Applying an MCMC-based approach had several advantages over the traditional delta delta Cq methodology for this study (Livak and Schmittgen, 2001). First, it is rare that control genes behave perfectly stable in a natural setting. The MCMC-based approach allows for modeling of the variance associated with control genes and builds this error into the predictions. Secondly, this approach allows for the addition of random error terms that allow and correct for unequal template loading, resulting in normalization of the data. Thirdly, the hierarchical model produced allows for simultaneous determination of the treatment effects across all genes of

interest relative to control genes improving upon the gene by gene analysis applied with the delta delta Cq method (Matz et al., 2013).

I separated the analysis into two models *a priori* to identify transplantation site-dependent effects (Equation 1) and collection site-dependent effects (Equation 2). Molecular counts were the single response variable. The variable "*gene*" represented the different levels of gene expression, "*TransplantationSite"* represented coral fragments grouped by site of transplant, "*CollectionSite*" represented coral fragments grouped by site of origin, and "*SeasonYear*" represented the season by year combination (*i.e.* Summer 2012, Winter 2012). Conditions in brackets indicate random error terms and include the sample specific error, the gene specific sample error, and the gene specific error in the order presented in each model.

Equation 1:

$$\begin{aligned}\text{count} = {} & gene + gene{:}\,TransplantationSite + gene{:}\,SeasonYear \\ & + gene{:}\,TranplantationSite{:}\,SeasonYear + [sample] + [gene{:}\,sample] \\ & + [gene{:}\,residual]\end{aligned}$$

Equation 2:

$$\begin{aligned}\text{count} = {} & gene + gene{:}\,CollectionSite + gene{:}\,SeasonYear + gene{:}\,CollectionSite{:}\,SeasonYear \\ & + [sample] + [gene{:}\,sample] + [gene{:}\,residual]\end{aligned}$$

Model parameters included 15,000 iterations, a thinning interval of 10, and sample size of 1,000. A less-informative inverse Wishart prior with assumed variance of 1 and the degree of belief parameter at 0 was used for calculating variance components of all non-control genes. Control genes were allowed to vary on average 1.2 fold across the explanatory variables. Credible intervals (95% posterior probabilities) for fixed factors were calculated based on MCMC sampling.

2.3.6 Genes of interest

2.3.6.1 Tumor necrosis factor receptor associated factor 3 (TRAF3)

The protein TNF receptor associated factor 3 (TRAF3) perpetuates the activation of immune responses following host detection of bacterial and viral pathogen associated molecular patterns (PAMPs) in the surrounding environment (Bagchi et al., 2007; Häcker et al., 2011; Medzhitov and Janeway Jr., 2002; Rowley and Powell, 2007). Therefore, expression of TRAF3 provides an indication of PAMP detection and the resulting response.

2.3.6.2 Adenylate cyclase associated protein 2 (ACAP2)

Adenylate cyclase associated proteins (ACAPs) are activated to prevent over-activation of the inflammatory response (Montminy, 1997; Serezani et al., 2008; Shima et al., 2000, 1997). Therefore ACAP2 reduces responses of the immune system that may be detrimental to the host.

2.3.6.3 Eukaryotic translation Initiation Factor 3, subunit H (eIF3H)

Eukaryotic translation initiation factors (eIFs) regulate the translation of cytoplasmic mRNAs and degradation of proteins (Zhang et al., 2008). Eukaryotic translation initiation factor 3, subunit H (eIF3H) is a component of the translation initiation complex that is upregulated in *P. astreoides* during periods of increased SWT (Matz, 2013). Therefore this gene is included as an indicator of thermal stress.

2.4 Results

2.4.1 Site temperature regime

The two sites displayed similar trends in SWT change as a function of season reflecting their close proximity (Figure 14). Seawater temperatures at Acer 24 remained lower during the summer and greater during the winter compared to those observed at Birthday reef (Table 3). During winter lows and summer highs, SWT differed by 0.5 to 1°C between the sites. Additionally, the frequency of mean daily temperatures above 30°C was greater at Birthday than Acer 24 reef. The preceding 8 days to sampling during the winter of 2012, seawater temperature increased significantly at Birthday reef (3°C increase) and Acer 24 (1°C increase) ($p = 0.0024$ and $p = 0.037$ respectively; Table 3). Conversely, during the winter of 2013 a significant decrease in temperature was observed the 8 days preceding sampling at Birthday reef (3°C decrease) and Acer 24 (2°C decrease) ($p = 0.001$ and $p = 0.000$ respectively; Table 3). The magnitude of the temperature increase during the winter of 2013 was greater than that which occurred during the winter of 2012. There were no significant changes in temperature during the summer months over the week preceding sampling.

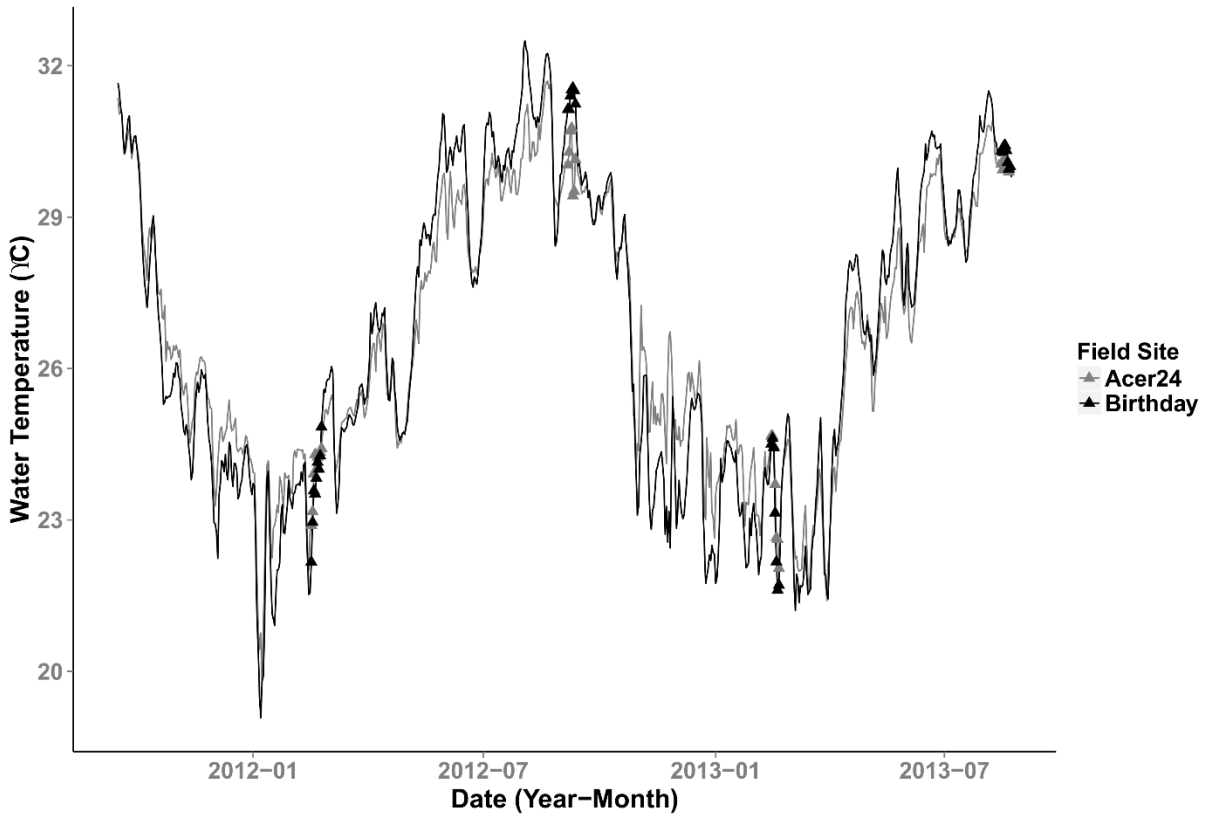


Figure 14: The Figure Displays Hourly Water Temperature for Birthday Reef (Inshore Patch Reef) and Acer 24 (Offshore Bank Reef) Over the Course of the Two-Year Reciprocal Transplantation Experiment. Daily water temperature means are presented for the day of sampling for each of the four sample periods as well as the mean daily water temperatures for the previous 6 days prior to sampling corals at the Acer 24 and Birthday Reef field site.

Table 3: Seawater Temperatures Reported at the Date of Collection at the Offshore Site (Acer 24 Reef) and the Inshore Site (Birthday Reef). The slope of temperature change during the week preceding sampling and p-value associated with the linear regression are also displayed.

Field Site	Date (Year-Month)	SWT at Collection (°C)	One-week SWT Slope	Regression p-value
Acer24 Reef	Winter 2012	24.5	0.06592	0.0377 *
	Summer 2012	30.6	-0.08914	0.418
	Winter 2013	22.9	-0.49137	0.000467 ***
	Summer 2013	30.0	-0.01571	0.513

Table 3 Cont'd

Birthday Reef	Winter 2012	25.2	0.19554	0.0024 **
	Summer 2012	31.5	0.04378	0.244
	Winter 2013	22.0	-0.59509	0.00115 **
	Summer 2013	30.3	-0.05640	0.0765 .

2.4.2 Effects of site and season on pooled GOI transcript abundance

Fragments originating from Acer 24 exhibited greater transcript abundance across all GOI than those originating from Birthday reef ($p = 0.010$). This indicated a potential effect of site of origin on gene expression. Additional support for the site of origin influencing a fragments response was provided by principal coordinate analysis (PCoA) of the Manhattan distances of transcript abundances among the GOI (Figure 15). Samples formed clusters dependent on site of origin rather than the alternate transplant site. The greatest dissimilarity between treatments was between fragments originating from Acer 24 and transplanted to Birthday reef and those originating from Birthday reef and transplanted back to Birthday reef. The site a fragment was transplanted to did not affect transcript abundance when GOI were collectively analyzed.

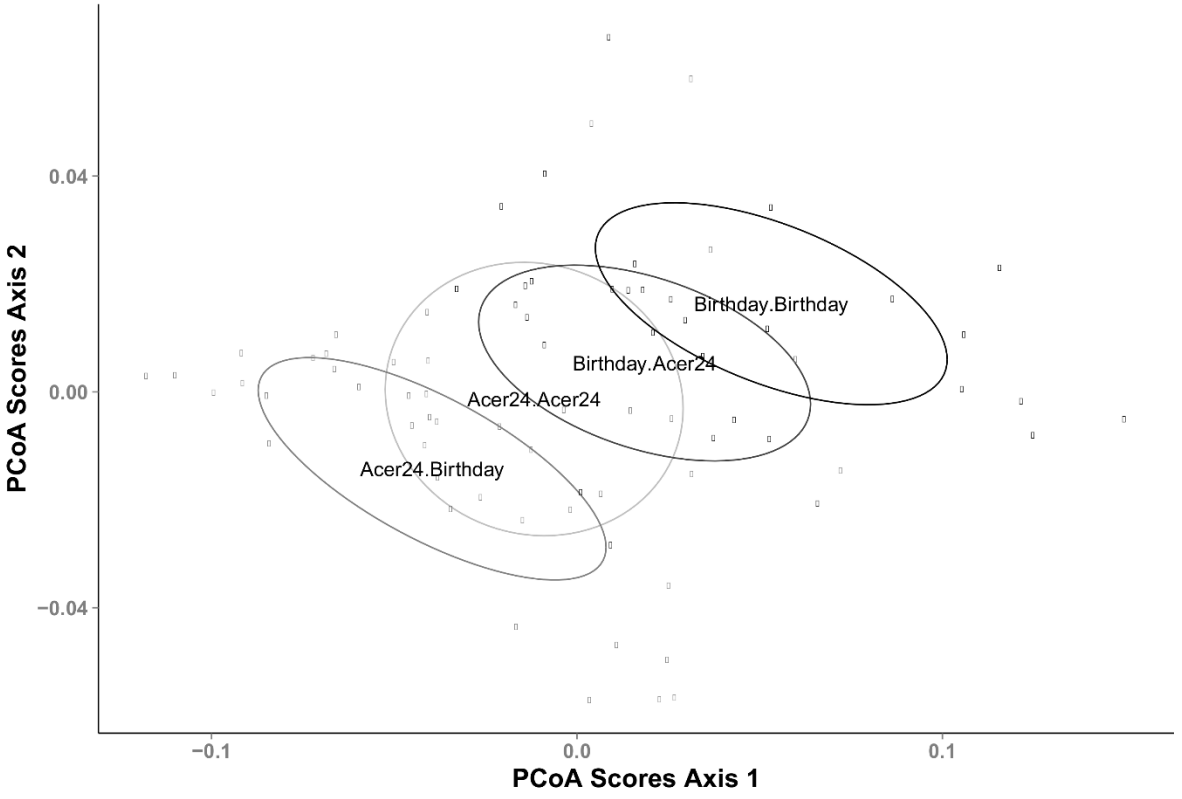


Figure 15: Principal Coordinate Analysis Results Indicating a Collection Site-Dependent Effect on Transcript Abundance. Manhattan distances of transcript abundances were used to create the distance matrix. Axis 1 and 2 are plotted. The labels represent the mean of axis 1 and axis 2 for each group. Grey points indicate corals collected from Acer 24 reef and black points represent those collected from Birthday reef.

A significant linear relationship was observed between the slope of SWT change associated with the 8 days preceding sampling and the second PCoA axis ($p = 0.005$) while the temperature at the time of sampling was not correlated to either axis (Table 2). This observation indicates that SWT change contributed to the difference in expression between transplant treatments along axis 2 (6%), however a large majority of the variance (62%) was not associated with SWT change during the preceding week. Transcript abundance differed between the winter and summer of 2013 for both the collection site ($p = 0.012$) and transplantation site ($p = 0.008$) models. Transcript abundances were lower during the summer of 2013 than the winter of 2013.

2.4.3 Gene specific responses of three genes of interest

2.4.3.1 TRAF3 expression

Porites astreoides fragments transplanted to Acer 24 reef displayed significantly greater transcript abundance than fragments transplanted to Birthday reef ($p = 0.01$; Figure 16, Table 4). This difference was driven by increased transcript abundances during the summers of 2012 and 2013 at Acer 24 reef compared to Birthday reef ($p < 0.001$ for both comparisons; Figure 16, Table 4). During the winter of 2012 TRAF3 gene expression was similar between the two reefs and reached comparable levels to those observed during each summer. During the Acer 24 sampling periods, transcript abundances observed during the winter were less than those quantified the following summer, indicating a potential effect of season on TRAF3 expression at Acer 24. This pattern was not observed at Birthday reef. Instead, a significant difference in transcript abundance was only evident between the winter of 2012 and the summer of 2013 ($p < 0.001$; Figure 16, Table 4).

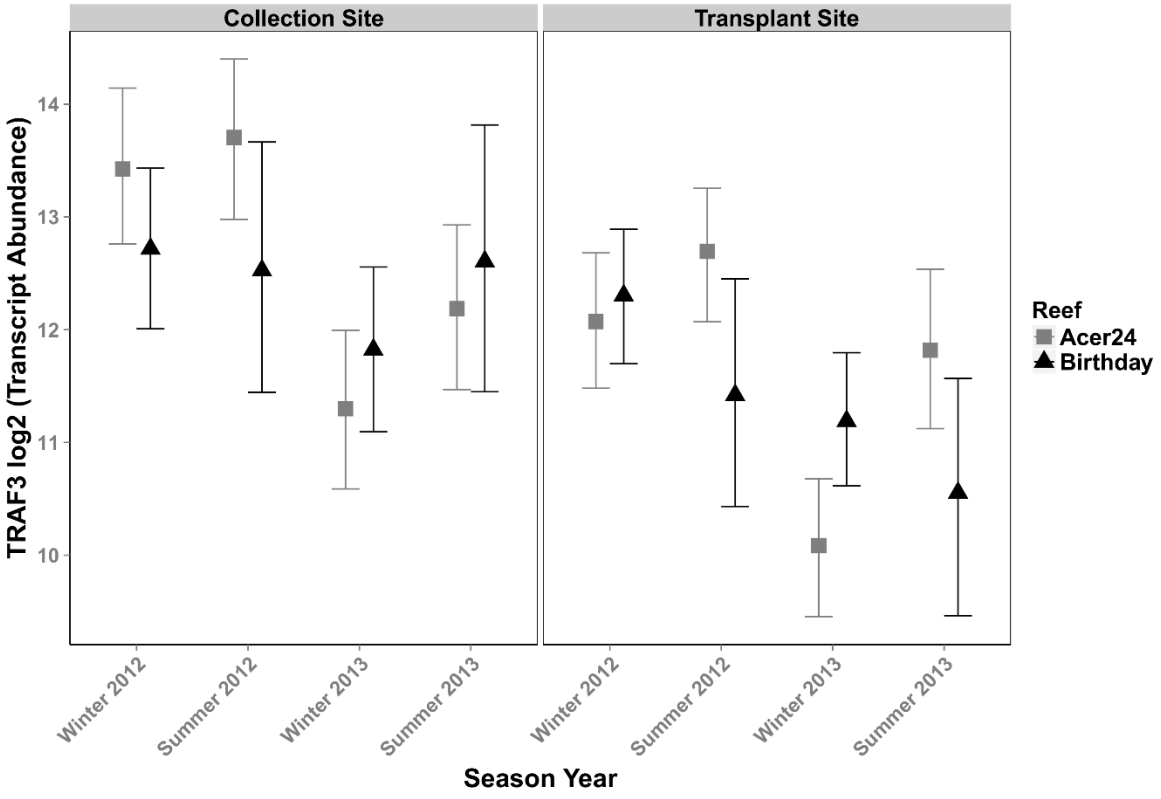


Figure 16: The log2 Scaled Abundances of TRAF3 Transcripts from Corals Reciprocally Transplanted Between an Inshore Site (Birthday Reef) and an Offshore Site (Acer 24 Reef). The left panel displays fragments grouped by the site of origin independent of the site transplanted to and the right panel displays fragments grouped by the site transplanted to independent of the site of origin. Samples were collected for analysis during the winter (February) and summer (September) of 2012 and 2013. Points represent the posterior means following MCMC generalized linear mixed modeling. Error bars indicate 95 % credible intervals defined by the model.

Table 4: Two-Way Factorial Mixed Effects Model for Transplant Site-Dependent and Seasonal Effects on Transcript Abundance of *Porites astreoides* Following Reciprocal Transplantation Between an Inshore Patch Reef (Birthday Reef) and Offshore Bank Reef (Acer 24 Reef). The results for the three genes of interest adenylate cyclase associated protein 2 (ACAP2), eukaryotic initiation factor 3 subunit H (EIF3H), and TNF receptor associated factor 3 (TRAF3) are presented. Comparisons have been made to transcript abundances of samples transplanted to Acer 24 reef and sampled during the winter of 2013.

GOI	Comparison	Posterior Mean	Lower 95% CI	Upper 95% CI	pMCMC
ACAP2	Birthday	-0.435	-0.937	0.029	0.086
	Winter 2012	-0.03757	-0.49681	0.44522	0.926
	Summer 2012	0.019	-0.372	0.500	0.938

Table 4 Cont'd

	Summer 2013	-0.14493	-0.61418	0.36624	0.576
	Birthday: Winter 2012	0.49994	-0.21072	1.17273	0.132
	Birthday: Summer 2012	0.40865	-0.24205	1.04486	0.210
	Birthday: Summer 2013	-0.30511	-0.86000	0.25533	0.312
EIF3H	Birthday	-0.208	-0.637	-0.173	0.342
	Winter 2012	-0.08121	-0.50590	0.24429	0.700
	Summer 2012	0.34064	-0.02892	0.74230	0.076 .
	Summer 2013	-0.14893	-0.59086	0.29031	0.508
	Birthday: Winter 2012	0.06488	-0.42012	0.68499	0.828
	Birthday: Summer 2012	-0.30511	-0.86000	0.25533	0.312
	Birthday: Summer 2013	1.00556	0.43073	1.65886	<0.001 ***
TRAF3	Birthday	0.76547	0.15549	1.40063	0.010 *
	Winter 2012	1.37740	0.73687	2.03280	<0.001 ***
	Summer 2012	1.80851	1.19198	2.48163	<0.001 ***
	Summer 2013	1.20155	0.50582	1.96071	<0.001 ***
	Birthday: Winter 2012	-0.60505	-1.45238	0.40027	0.182
	Birthday: Summer 2012	-1.21753	-2.07043	-0.28946	0.008 **
	Birthday: Summer 2013	-1.81851	-2.85665	-0.86877	<0.001 ***

Although site of origin did not impact the expression of TRAF3 (Table 5), differences between sampling periods were observed (Figure 16). The transcript abundance observed during the winter of 2013 was significantly lower than the expression observed during both the winter and summer of 2012 ($p < 0.001$ for each comparison). During a given sampling period site of origin did not affect TRAF3 expression, unlike that observed for the site a coral was transplanted to.

Table 5: Two-Way Factorial Mixed Effects Model for Collection Site-Dependent and Seasonal Effects on Transcript Abundance of *Porites astreoides* Following Reciprocal Transplantation Between an Inshore Patch Reef (Birthday Reef) and Offshore Bank Reef (Acer 24 Reef). The results for the three genes of interest adenylate cyclase associated protein 2 (ACAP2), eukaryotic initiation factor 3 subunit H (EIF3H), and TNF receptor associated factor 3 (TRAF3) are presented. Comparisons have been made to transcript abundances of samples transplanted to Acer 24 reef and sampled during the winter of 2013.

GOI	Comparison	Posterior Mean	Lower 95% CI	Upper 95% CI	pMCMC
ACAP2	Birthday	-1.147154	-1.670249	-0.666332	<0.001 ***
	Winter 2012	-0.013090	-0.446707	0.396193	0.958
	Summer 2012	0.277053	-0.157765	0.713639	0.200
	Summer 2013	0.119924	-0.364999	0.552681	0.618
	Birthday: Winter 2012	0.394370	-0.243777	1.033281	0.234
	Birthday: Summer 2012	-0.193949	-0.824670	0.472759	0.582
	Birthday: Summer 2013	0.351856	-0.372941	1.128164	0.374
EIF3H	Birthday	-0.586846	-1.032692	-0.112198	0.018 *
	Winter 2012	-0.266735	-0.671877	0.103723	0.184
	Summer 2012	-0.002953	-0.389189	0.347585	1.000
	Summer 2013	0.082943	-0.337972	0.471331	0.678
	Birthday: Winter 2012	0.488955	-0.150934	1.031597	0.108

Table 5 Cont'd

	Birthday: Summer 2012	0.460195	-0.172337	0.984013	0.124
	Birthday: Summer 2013	0.839239	0.196500	1.522053	0.022 *
TRAF3	Birthday	0.362722	-0.420370	1.282007	0.394
	Winter 2012	1.473018	0.761331	2.084224	<0.001 ***
	Summer 2012	1.666734	0.975813	2.272533	<0.001 ***
	Summer 2013	0.614901	-0.094805	1.261963	0.086 .
	Birthday: Winter 2012	-0.852657	-1.759411	0.160360	0.086 .
	Birthday: Summer 2012	-0.985686	-1.978128	-0.025191	0.054 .
	Birthday: Summer 2013	-0.930772	-1.904549	0.089161	0.072 .

2.4.3.2 eIF3H expression

The expression of eIF3H was significantly greater among corals originating from Acer 24 compared to Birthday reef site ($p = 0.018$; Table 5). This result was apparent despite the similarity in expression of eIF3H observed for all but one of the sampling periods, winter 2013 ($p < 0.022$; Figure 17, Table 5). The site a coral fragment was transplanted to significantly affected the expression of eIF3H during summer months but not winter months (Figure 17). During the summer of 2012 expression was greater for corals transplanted to Acer 24 compared to Birthday reef ($p < 0.001$) but this significant trend was reversed the following summer ($p < 0.001$).

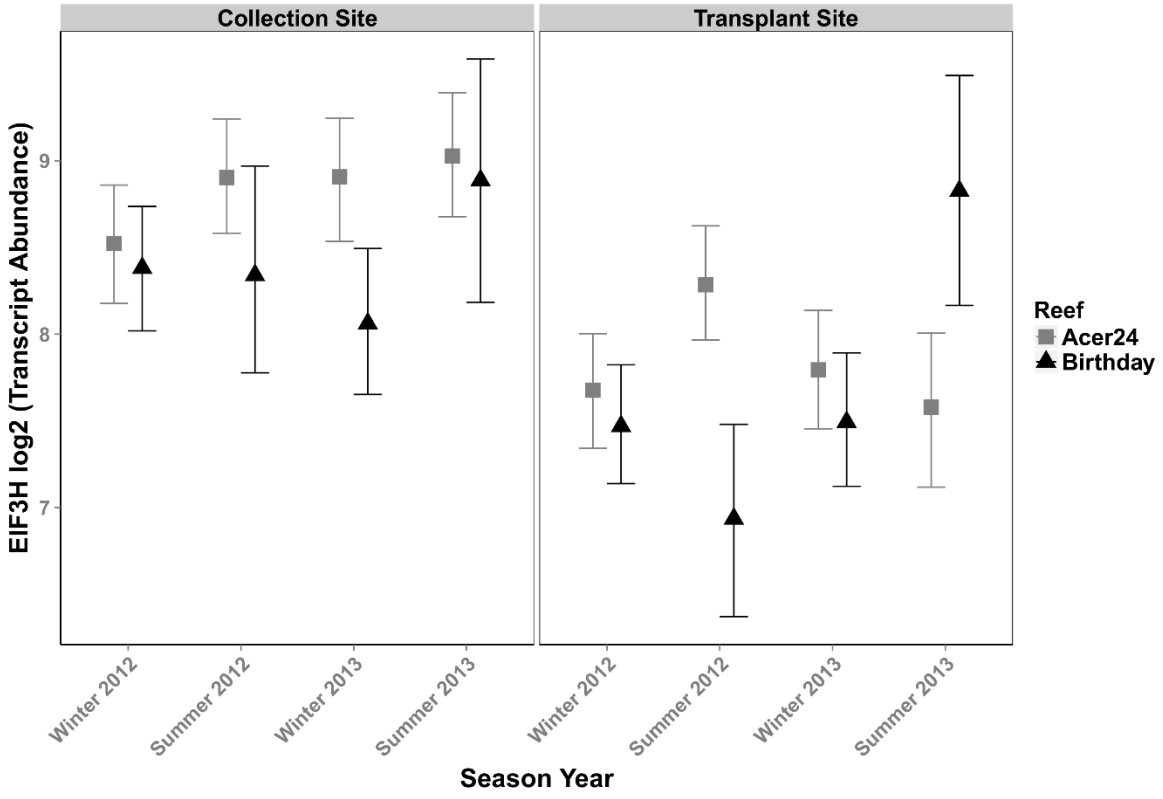


Figure 17: The log2 Scaled Abundances of eIF3H Transcripts from Corals Reciprocally Transplanted Between an Inshore Site (Birthday Reef) and an Offshore Site (Acer 24 Reef). The left panel displays fragments grouped by the site of origin independent of the site transplanted to and the right panel displays fragments grouped by the site transplanted to independent of the site of origin. Samples were collected for analysis during the winter (February) and summer (September) of 2012 and 2013. Points represent the posterior means following MCMC generalized linear mixed modeling. Error bars indicate 95 % credible intervals defined by the model.

2.4.3.3 ACAP2 expression

Transcript abundances of ACAP2 were significantly affected by site of origin ($p = 0.000$; Table 5). Corals from Acer 24 displayed greater ACAP2 expression for each sampling period compared to corals from Birthday reef (Figure 18). This site of origin-dependent effect on transcript abundance was not influenced by sampling period. Additionally, the site that a coral fragment was transplanted to did not affect ACAP2 transcript abundance.

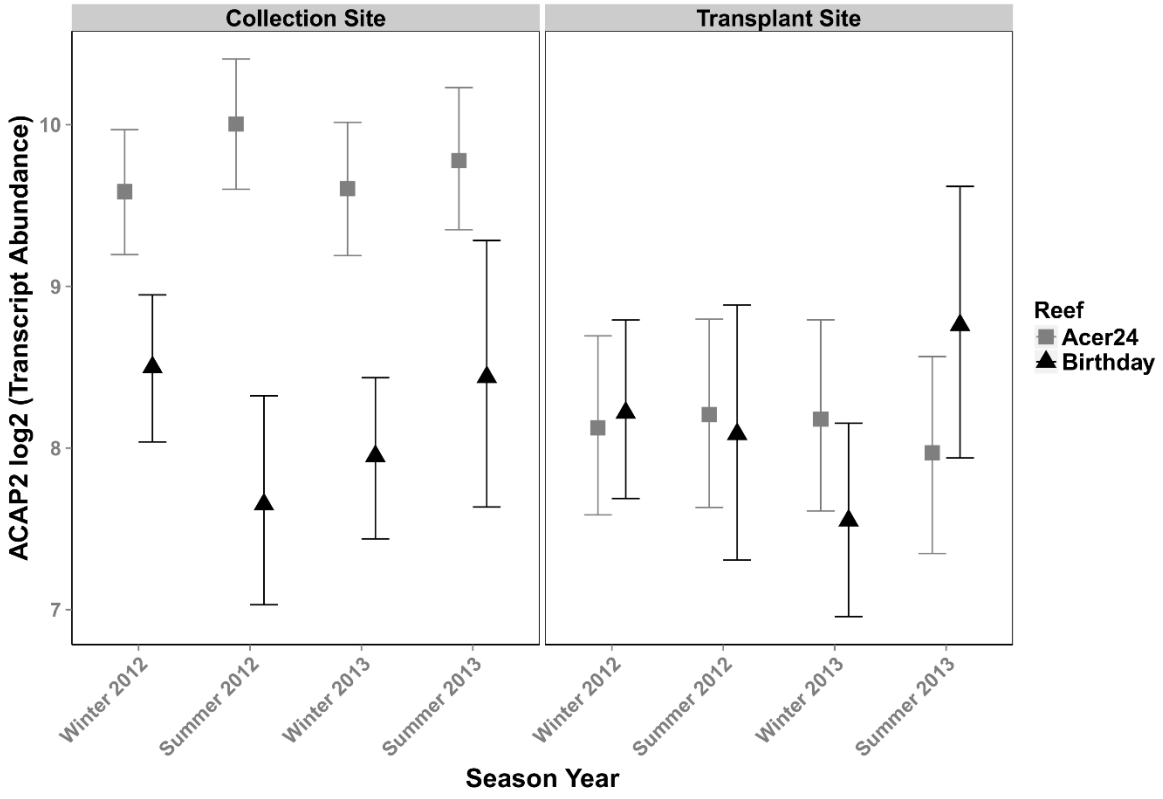


Figure 18: The log2 Scaled Abundances of ACAP2 Transcripts from Corals Reciprocally Transplanted Between an Inshore Site (Birthday Reef) and an Offshore Site (Acer 24 Reef). The left panel displays fragments grouped by the site of origin independent of the site transplanted to and the right panel displays fragments grouped by the site transplanted to independent of the site of origin. Samples were collected for analysis during the winter (February) and summer (September) of 2012 and 2013. Points represent the posterior means following MCMC generalized linear mixed modeling. Error bars indicate 95 % credible intervals defined by the model.

2.4.4 Summary of factors affecting host gene expression in *P. astreoides*

Distinct patterns of transcript abundance were identified for each of the three genes of interest (GOI). The expression of TRAF3 differed between winter and summer but only for samples transplanted to Acer 24. Significant transplant site-dependent effects were apparent in the expression of eIF3H during summer while a site of origin-dependent effect was observed for ACAP2. We also observed that the direction and magnitude of the slope associated with the previous week of temperature change was a

significant factor affecting gene expression while the previous days SWT during a given sampling event was not.

2.5 Discussion

Along the FRT corals inhabiting offshore bank reefs display decreased growth and abundance (e.g. fitness) compared to corals inhabiting inshore patch reefs (Haslun et al., 2015). Although elevated SWTs are generally associated with increased coral stress, higher SWTs and increased temperature variation are characteristic of inshore patch reefs to a greater extent than in the offshore (Soto et al., 2011). An alternative explanation for decreased offshore coral abundance is that SWT variation affects the activation of the immune system by influencing the level of biotic stress experienced by coral inhabitants. Increased temperature variability will control pathogen prevalence while decreased variability presents less stressful growth conditions (Harvell et al., 2002; Price and Sowers, 2004). I determined if the expression of genes associated with the activation of the immune system were environment-dependent and if adaptive responses to environment-dependent biotic stress had occurred. We identified increased activation of the immune system among all corals transplanted to the offshore site as well as an adaptive response to activation of the immune system in corals originating from the offshore site. The role of each gene examined will be discussed in turn to describe how SWT indirectly influences biotic stress level contributing to the observed differences in growth and abundance between the inshore and offshore sites.

2.5.1 Activation of host coral immune pathways: TNF receptor associated factor 3 expression

The protein TNF receptor associated factor 3 regulates the activation of the innate immune response along the MyD88 dependent and TRIF dependent pathways (Bagchi et al., 2007; Häcker et al., 2011). Following activation of TLRs by PAMPs, TRAF3 activates the production of effector molecules comprising the aforementioned pathways of the immune system, resulting in inflammatory responses (Häcker et al., 2011). Therefore, expression of TRAF3 reflects the host's recognition of and response to bacterial and viral molecular patterns in the surrounding environment. My results indicate that it is not the site of

origin that controls TRAF3 expression but rather the environmental conditions a coral host is currently exposed to. For this particular gene, a population dependent difference would be unlikely because toll-like receptors are conserved across animal phyla and activation occurs following the recognition of general rather than specific PAMPs (i.e. all bacterial lipopolysaccharides vs. species specific variants). The general and conserved nature of this pathway likely constrains selection on TLRs. The effect of local environment on TRAF3 expression was observed insofar as *Porites astreoides* transplanted to the offshore site displayed greater TRAF3 expression than those transplanted inshore in summer ($p < 0.001$). Increased SWT is often directly linked to responsive physiological changes in corals. Contrary to this generalization, increased temperatures during the summer did not co-occur with increased TRAF3 activation. Instead, mean daily temperatures at the inshore site were on average 1°C less than those of the offshore site. While the second principal coordinate axis, which delineated differences in gene expression among treatments, displayed a significant relationship with temperature variation the week prior to sample collection ($p < 0.005$, $R^2 = 0.136$), separation between treatment groups along this axis was minimal. Greater variation between transplants was identified along the first principal coordinate and a relationship with SWT was not observed (Figure 14). Therefore environmental factors other than SWT must contribute to variation in TRAF3 expression along this primary axis. During the winter, however, increased TRAF3 expression ($p > 0.000$) was observed following a period of increasing SWT whereas the lowest TRAF3 expression was observed following a period of decreasing SWT (Table 3). Thus cooling SWT during the winter, which reflect the inshore site SWT, was associated with less TRAF3 expression whereas warming SWTs during the winter enhanced TRAF3 expression, a characteristic of the offshore site.

The direct link between TRAF3 expression and the recognition of microbial derived molecular patterns through TLRs indicates that biotic stress may be an additional contributing factor in this environment-

dependent effect. However, during winter periods, high SWT undoubtedly contributes to the level of biotic stress experienced (Strychar and Sammarco, 2010; Strychar, 2014). Bacterial abundance decreases during the winter but shorter and warmer winter seasons diminish this effect (Harvell et al., 2002). Because warmer SWTs increase the metabolic and growth rates of microorganisms (Price and Sowers, 2004) many diseases affecting marine macro-organisms increase in prevalence including those affecting corals (Bruno et al., 2007; Cerrano et al., 2000; Sussman et al., 2003). Not surprisingly, seasonal variation in temperature (i.e. winter *vs.* summer) has been identified as one of the most prominent factors controlling the rate of microbial growth (Fuhrman et al., 2015; Gilbert et al., 2012; Jiang and Paul, 1994). Therefore during warmer periods (summer), an increased abundance of foreign substances capable of binding TLRs is expected as a result of increase proliferation and pathogenicity of microorganisms. Conversely, cooler periods are expected to result in decreased biotic stress and subsequent TRAF3 expression. We identified both of these trends in TRAF3 expression: decreasing winter temperatures limited TRAF3 expression *vs* warm periods consistent with the greatest levels of TRAF3 expression. Moreover, on an annual basis, temperature variation is greater at the offshore compared to the inshore site. Therefore according to the hypothesis laid out by Harvell et al. (2002) and evidence supplied by others (Cerrano et al., 2000; Price and Sowers, 2004; Sussman et al., 2003), however, the offshore sites warmer winter SWTs, fails to explain the accumulation of TLR compatible molecular patterns and the resulting activation of the immune system in the offshore relative to the inshore. Additionally, exposure of the coral fragments from this study to increased temperature (32°C) and lipopolysaccharide (5 μg mL^{-1}) indicated that corals originating from the offshore site activated the immune system to a greater degree than the inshore site (Haslun et al., in review). Corals transplanted to the offshore site likely encounter more immune activating compounds and therefore displayed significant upregulation of TRAF3 relative to a control treatment (28°C). Corals originating from the inshore site displayed expression similar to the control and significantly lower than that of corals with an

offshore origin. Therefore corals that had experienced increased immune system activation inherent to the offshore site upregulated TRAF3, whilst those from the inshore site did not activate this response as greatly. Upregulation likely reflects increased pathogen exposure from the local environment (i.e. offshore).

The differential TRAF3 expression I observed in corals transplanted to inshore and offshore sites indicates local differences in the degree of immune system activation. Moreover, our results indicate that the degree of immune activation is linked to SWT variation of a site. Lower winter SWT decreased immune system activation while the milder temperature regime of the offshore site increased activation. These temperature changes are undoubtedly linked to the abundance of PAMPs capable of activating the immune response although other abiotic factors such as irradiance and nutrients not quantified here likely contribute as well. Identification of the concentrations of immune system stimulating compounds present at inshore and offshore reefs is required to fully support this conclusion, however, increased expression of the immune system following exposure to the synergistic effect of increased temperature and LPS has been observed in offshore *P. astreoides* relative to inshore, indicating increased exposure to PAMPs offshore. Climate warming is expected to increase winter minimum temperatures along coastal regions like the FRT and result in greater inshore activation of the immune system. This will further exacerbating the currently observed activation of the immune system in the offshore environment.

2.5.2 Cellular stress response: eukaryotic translation initiation factor 3, subunit H (eIF3H)

Eukaryotic translation initiation factor 3, subunit H (eIF3H) is a component of the translation initiation complex formed by eIFs and therefore contributes to the synthesis rather than degradation of proteins (Zhang et al., 2008). This gene is upregulated in *P. astreoides* during periods of stress (Matz et al., 2013).

Upregulation of eIFs results in increased protein production that can counteract intracellular stress following metabolic dysfunction (Muñoz and Castellano, 2012) as observed in yeast (Singh et al., 2013). Increased expression of eIF3H is therefore an indication of stress.

Although we observed differential expression of eIF3H among corals transplanted to inshore and offshore sites during the summer, a consistent site-dependent effect on expression between summers was not observed. In fact, during 2012, eIF3H expression was greatest in corals transplanted to the inshore site, whilst during the following year corals transplanted to the offshore site displayed the greatest expression. Offshore SWT varied between 29°C and 30°C prior to sampling for gene expression during the summer of 2012 while SWT at the inshore site increased from 28°C to 32°C during the same period (Figure 14). Inshore transplanted corals experiencing a greater rate of SWT increase as well as higher SWTs would therefore be expected to upregulate eIF3H to counteract thermal stress. However, upregulation was only observed in corals at the offshore site. Hence, we observed significant differences in the expression of eIF3H between sites, but our experimental design did not reveal any temperature related stresses in 2012. Similarly, temperature dependent effects on eIF3h were not evident in the summer of 2013. Based upon the similar SWTs and SWT variation at both sites prior to sampling , we anticipated corals from both sites to display similar expressions or that corals transplanted to the inshore site would be less impacted due to the larger variation in annual temperatures that these corals are accustomed to. Instead, corals from the inshore site displayed greater expression of eIF3H compared to the offshore site contradicting our expectations.

Several factors may have contributed to the unexpected expression patterns of eIF3H. First, the temperature stress experienced during and prior to sampling may not have been significant enough to elicit a response driven by temperature. Temperatures prior to sampling did not reach the maximum

observed during either 2012 or 2013 (Figure 14). Second, eIF3H is likely to be activated by other stressors (e.g. irradiance) in addition to temperature. Therefore insightful applications of this gene may be more appropriate in laboratory settings, rather than in field where environmental conditions cannot be controlled.

2.5.3 Adaptive response to immune system activation: adenylate cyclase associated protein 2 (ACAP2)

Through interactions with activated Ras proteins, adenylate cyclase associated proteins (ACAPs) regulate the synthesis of cyclic adenosine monophosphate (cAMP) by adenylate cyclase (AC) (Shima et al., 2000). When synthesized after Ras coupled activation (Gibbs and Marshall, 1989), cAMP acts as a potent regulator of the inflammatory response (Serezani et al., 2008). Therefore ACAP2 reduces over-activation of the innate immune system.

Porites astreoides colonies originating from the offshore site expressed significantly greater levels of ACAP2 in all sampling periods relative to those from the inshore site. This result was observed independent of transplant location, indicating that corals inhabiting the offshore site may be locally adapted to this environment. Local adaptation requires very limited gene flow between populations along with intense selection on the variation of a phenotype (Kawecki and Ebert, 2004). The broad dispersive reproduction strategy used by corals in addition to the long lived nature of reef building corals has long been thought of as a barrier to local adaptation in the host animal. Wide dispersal facilitates consistent low-level gene flow between metapopulations and serves to increase diversity (Sammarco and Andrews, 1989, 1988). Although phenotypic diversity is necessary to produce local adaptation, diversity can also decrease the effect of selection pressure on a particular phenotype (Sanford and Kelly, 2011). For instance, in our study significant site of origin-dependent differences in

ACAP2 expression suggest local adaptation. Supporting our assumptions, Kenkel et al. (2015) observed very little gene flow between inshore and offshore populations of *P. astreoides* inhabiting the FRT.

Local adaptations confer fitness advantages to an organism that is confronted with a hostile environment. We have previously established, based on expression of TRAF3, that the offshore environment results in increased activation of the coral host's immune response relative to the inshore. Therefore our results indicate that the offshore environment exposes corals to increased biotic stress. Acute and chronic inflammatory responses act to the detriment of the host by decreasing fitness. For example, inoculation of the mealworm beetle (*Tenebrio molitor*) with non-lethal levels of bacteria has been shown to decrease longevity (Moret and Siva-Jothy, 2003). Moreover, individuals of *T. molitor* that produce elevated levels of melanin, a critical molecule in the invertebrate innate immune response, have decreased longevity even without external stimulation (Armitage et al., 2003). Thus reducing overactive or continuously activated immune responses may confer an advantage to the host. TRAF3 expression (i.e. an inflammatory response) was greatest among coral fragments transplanted offshore, which provides compelling evidence for an adaptive response to immune system stimulation. Organisms must allocate resources to all cellular processes from a finite supply, however, immune responses come with a resource cost (Lochmiller and Deerenberg, 2000). It is therefore common to observe tradeoffs (i.e. costs), in which the expression of an adaptive trait increases while the expression of another trait or traits decreases. Tradeoffs resulting from adaptive responses to the innate immune system are more commonly detected in higher level traits such as survival, growth, longevity, and fecundity (Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996) because of the difficulties inherent to observing the interactions between lower level traits. Coral species inhabiting the offshore reefs of the FRT, including *P. astreoides*, display both a decreased mean colony size as well as decreased abundance relative to the populations inhabiting inshore reefs (Haslun et al., 2015). Moreover, colony

size is correlated with increased fecundity in *P. astreoides* (Chornesky and Peters, 1987). *Porites astreoides* colonies originating from the offshore sites in our study averaged 7.34 cm in diameter while those inhabiting the inshore site were 11.44 cm in diameter (Haslun et al., 2015). A decrease in the rate of skeletal linear extension (i.e. growth) and decreased fecundity are likely to be a high level fitness tradeoff associated with diverting resources to both an active immune response and counteraction of that immune response. Similarly, *P. astreoides* colonies inhabiting an adjacent offshore site have been shown to display decreased growth following exposure to stress relative to corals collected from an inshore reef (Kenkel et al., 2013a).

My results indicate that the activation of the immune response has affected population dynamics of *P. astreoides*. The response of the host to activate the immune system at offshore sites likely resulted in an opposing adaptive response and decreased host growth rate and fecundity. To reiterate, I observed decreased colony sizes at the offshore site that is consistent with this observation. Although there is no doubt that climate related changes in abiotic factors (e.g. SWT) act to the detriment of corals and may also contribute to local adaptation, the interaction of abiotic and biotic stressors is important and should be considered in future studies. A detailed understanding of the contribution of the coral host's immune response, be it innate or other, may be especially important along reefs that have been drastically impacted and continue to be impacted by disease, such as the FRT (Porter et al., 2001).

2.6 Conclusion

My study shows that activation of the immune system in *P. astreoides* differs between an adjacent inshore and offshore reef. Colonies that were transplanted offshore expressed TRAF3 more than colonies that were transplanted inshore. Activation of TRAF3 occurs following recognition of foreign substances by TLRs, and therefore increased expression is likely a result of increased stress brought on

from biotic sources (i.e. bacteria and viruses). *P. astreoides* originating from the offshore environment also displayed increased expression of ACAP2 independent of any transplantation, an indication of local adaptation to stress. Because increased expression of the immune system results in fitness tradeoffs, this particular adaptation may be an effort by corals to enhance survival by depressing activation of the immune response brought on by the offshore environment. *P. astreoides* inhabiting the offshore reef not only activated their immune pathways but also regulated this response by expressing ACAP2. While this likely enhances survival, it limits resource availability for other traits such as growth. Offshore bank reefs throughout the FRT currently exhibit decreased mean colony size and decreased abundance relative to the inshore and our study provides evidence indicative of a link between increased immune responses and fitness.

Increased immune system activation was associated with decreased SWT variation. Offshore sites displayed a milder temperature regime compared to inshore sites. Because lowest TRAF3 expression was observed during a period of decreasing winter SWTs, lower winter temperatures may decrease the activation of the immune system by limiting sources of biotic stress. As climate warming continues, winter low temperatures will likely increase placing biotic stress on inshore reefs and further exacerbate warming at offshore reefs. The resulting increase in immune system activation may place resource constraints on a coral's ability to resist abiotic stressors and result in more severe bleaching events than currently occurs.

CHAPTER 3

DIVERGENT RESPONSES OF *PORITES ASTREOIDES* POPULATIONS TO BACTERIAL ENDOTOXIN: POTENTIAL CONSEQUENCES OF IMMUNE SYSTEM ACTIVATION

3.1 Abstract

Diseases have greatly impacted coral reefs of the Florida Reef Tract (FRT) causing changes to reef community structure. These changes have been realized in the form of mass mortalities of particular species. While the direct effects of disease are important, indirect effects of bacterial related stress have received less attention in the literature despite documented links between immune system activation and fitness related traits in invertebrates. Currently the inshore patch reef zone of the FRT contains more coral cover and larger coral colonies than the offshore bank reef zone despite increased exposure to thermal stress at the inshore site. The response of *Porites astreoides* originated from an inshore reef (n = 6) and offshore reef (n = 6) to a control condition (28°C), elevated seawater temperature (SWT; 32°C), and the synergistic effects of elevated SWT and bacterial lipopolysaccharide (5 μg mL^{-1}) was evaluated. The expression of two genes affected by SWT (eukaryotic translation initiation factor 3, subunit H; heat shock factor protein 1) and two genes affected by pathogens (TNF receptor associated factor 3; calcium dependent protein kinase) was quantified with quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR). Two gene regulation strategies were identified, which reflected site of origin-dependent variation in either SWT or biotic stress. Offshore corals displayed increased expression across all 4 genes to elevated SWT and LPS but not elevated SWT alone compared to the control treatment. Inshore corals displayed increased expression to elevated SWT but not to the synergistic treatment, compared to the control. Offshore fragments of *P. astreoides* also displayed increased gene expression compared to inshore fragments at the control treatment. While these results indicate that *P. astreoides* may be a highly adaptable coral species, they also indicate the potential for adaption to a single stressor. Increased response to bacterial stress was associated

with decreased coral abundance and size indicating potential tradeoffs that constrain fitness related traits. The importance of synergism between sea water temperature and microbial related stress deserves increased attention as a driver of coral community dynamics.

3.2 Introduction

Diseases of terrestrial and aquatic species can drastically affect ecosystem function as a consequence of mass mortality, decreased size distribution, and range shifts (Crowl et al., 2008). Marine pathogens are recognized for their ability to restructure communities across habitats (Burge et al., 2014). Examples include Dermo and Multinucleate Sphere X diseases of oyster populations along the east coast of the United States (Powell et al., 2008), an unidentified disease of the spiny sea urchin *Diadema antillarum* in the Caribbean (Lessios et al., 1984), white pox of *Acropora spp.* coral throughout the Caribbean (Gladfelter, 1982). Novel diseases continue to emerge (Hewson et al., 2014).

The importance of interactions between the impacts of marine epizootics and climate is becoming increasingly recognized. Increases in the abundance of pathogenic planktonic bacteria (e.g. *Vibrio spp.*) in response to increased sea surface temperature has occurred in the southern North Sea over the past 50 years (Vezzulli et al., 2012). As climate shifts, diseases affecting reef building corals are also likely to increase and cause as much mortality as temperature induced bleaching (loss of symbiotic algae) in the next 20 years (Maynard et al., 2015). The primary causes of this outcome are changes in host susceptibility and behavior as well as changes in pathogen virulence and growth (Maynard et al., 2015). Caribbean reefs display the greatest frequency of infection and diversity of diseases relative to reefs worldwide (Garzón-Ferreira et al., 2001; Weil and Rogers, 2011). Many diseases are uncharacterized. Such diseases do not have an etiological agent but are important infectious agents in the Caribbean (Weil and Rogers, 2011).

The acroporiid corals, once the dominant coral cover in the region, are now functionally extinct along the reef tract as a result of the enteric pathogen *Serratia marcescens* (Gladfelter, 1982). The prolific grazing sea urchin, *Diadema antillarum*, was similarly extirpated from this region by an unknown

pathogen (Lessios et al., 1984). This resulted in increased algal growth that further decreased coral cover. At present approximately 70 % of coral cover in the Florida Keys consists of inshore patch reefs rather than the offshore bank reefs that dominated historically. Additionally, offshore bank reef coral communities display decreased colony size and decreased species richness, indicative of a more detrimental environment than currently found inshore (Lirman and Fong, 2007). Site-dependent differences in the response of the host to detrimental levels of abiotic stress do not appear to correlate well with patterns of growth (Manzello et al., 2015) and community structure (Haslun et al., 2015). Because disease proliferation is dependent upon host-pathogen interactions, both host and pathogen characteristics may be affected by the environment changing those interactions (Burge et al., 2014). In marine habitats, temperature (Maynard et al., 2015; Ward et al., 2007) and nutrient regime (Vega Thurber et al., 2014) are hypothesized to be the primary agents of reef decline. However increased seawater temperature and elevated nutrient concentrations are more strongly evident in inshore patch reefs (Lirman and Fong, 2007). Inshore sites typically experience a 1°C increase in mean maximum seawater temperature (SWT) during summer months and a decrease in mean minimum SWT by 1°C as well as increased turbidity and nutrients relative to the offshore. Instead, the synergistic effects of temperature stress and overexpression of defenses against microbial pathogens may be significant factors detrimentally affecting offshore corals to a greater extent than the inshore populations. Therefore including biotic stressors in the estimation of host responses to environmental change provides a better representation of stresses driving declines in coral cover, diversity, and colony size, than thermal stress alone.

The innate immune system is the primary defense of the coral metazoan host against biotic stressors. Continued activation of the innate immune system can, however, result in a compromised state that can exacerbate the effects of previously benign conditions due to resource over-allocation. In this study I

explore the effect of environmental history on the response of *Porites astreoides* to thermal and bacterial stress. A lipopolysaccharide (LPS) endotoxin from *Serratia marcescens*, a known coral pathogen, was applied to induce a response indicative of bacterial stress. We hypothesize that corals inhabiting offshore sites display a greater stress response to LPS than inshore communities. As previously noted, disease attributed to *S. marcescens* infection has impacted the offshore bank reef system more than the inshore patch reef system (Patterson et al., 2002). Therefore, we predict that previous or continued exposure to pathogens will result in greater upregulation of the stress response. Corals inhabiting offshore bank reefs are expected to display greater upregulation of genes compared to conspecifics inhabiting inshore habitats. Our results support this hypothesis and moreover indicate divergent responses of inshore and offshore populations of *Porites astreoides* to bacterial endotoxin and temperature.

3.3 Methods

3.3.1 Colony collection and maintenance

The two sites selected for this study are in the lower region of the Florida Keys National Marine Sanctuary: Birthday reef (24.57917' N, -81.49692' W) and Acer 24 reef (24.55268' N, -81.43741' W) (Figure 19). Birthday reef is representative of inshore patch reefs while Acer 24 reef is representative of offshore bank reef environments. Both sites contain populations of *P. astreoides* that were sampled with minimal impact to the population (sampling affecting < 1 % of the population) (Erich Bartels pers. comm.). Transplant experiments at each site were conducted at a depth of approximately 6 m. Therefore, differences in light level as a function of depth was not a factor affecting the responses of coral colonies. Differences in coral cover and colony size between Acer24 and Birthday reef has been previously reported (Haslun et al., 2015 in review) and follow a trend of increased coral cover and colony size with decreasing distance from shore.

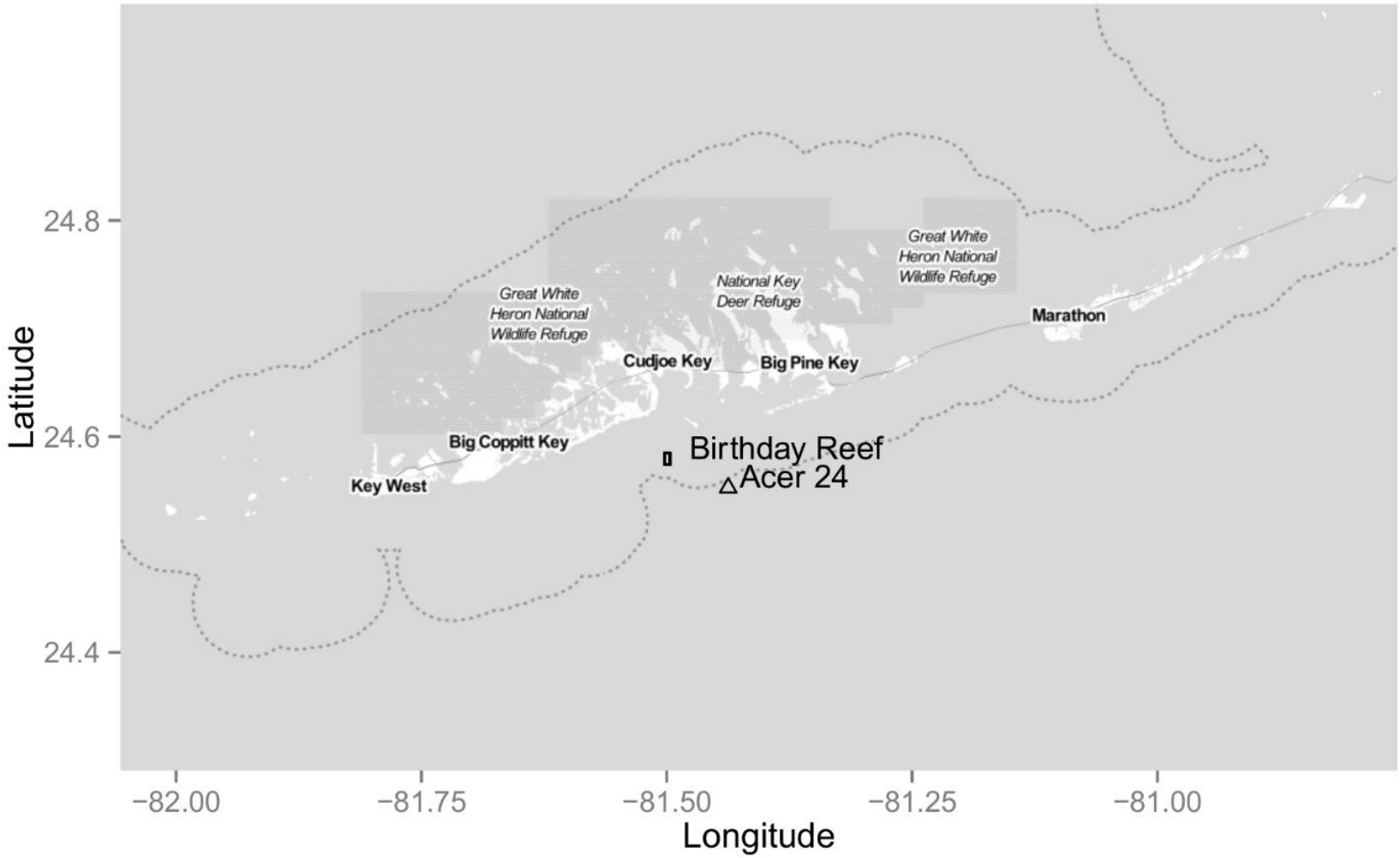


Figure 19: The Inshore Patch Reef (Birthday Reef; 24.57917' N, -81.49692' W) and Offshore Bank Reef (Acer 24 Reef; 24.55268' N, -81.43741' W) Sampling Sites are Pictured Above

Fragments of *Porites astreoides* colonies (n = 6), 16 x 16 cm, were collected with a cold chisel and mallet by divers using SCUBA (NOAA National Marine Sanctuaries Permit # FKNMS-2011-10). Fragments were transferred to Mote Marine Laboratories Tropical Research Laboratory in coolers filled with site derived water and upon arrival sectioned in half to provide samples for a companion study. The fragment halves were allowed to equilibrate for three days in raceways supplied with a constant flow of oceanic seawater and protected by shade cloth. Sampled were secured to concrete:aragonite (1:3) disks (radius 6 cm) with All Fix Epoxy (Cir-Cut, Lafayette Hill, PA) and equilibrated for two days in the raceway prior to being transplanted in the field. *Porites* fragments were affixed to submerged concrete cinder blocks with All Fix Epoxy (NOAA National Marine Sanctuaries Permit # FKNMS-2011-10) at their site of origin or at the companion site, either Birthday reef or Acer 24.

The random effects associated with the spatial distribution of sampled colonies within the benthic habitat were reduced by collection and subsequent transplantation back to the site of origin on a clean concrete substrate. Settlement position can impact the abiotic and biotic regime experienced by corals due to habitat heterogeneity, light levels, hydrodynamic differences, and interspecific interactions. Transplanting coral fragments back to the site of origin upon a known substrate at the exact same depth served to decrease potential differences associated with settlement dependent factors. Further, by allowing these coral fragments to remain at the site of origin for two years all fragments experienced very similar environmental conditions at a given site. Further details regarding sampling and transplantation can be retrieved from a previous manuscript (Haslun et al., 2015 in review).

After the two-year period of transplantation each colony was collected and returned to the Mote Marine Laboratory Tropical Research Station and returned to a raceway. Colonies were then allowed to recover from collection for two days at which point each fragment was cut with brick saw into nine 2.54

x 2.54 cm fragments. Sterile seawater was used as a lubricant to decrease heat associated with the cutting wheel. Each set of fragments from was allowed to recover for at least 3 days in the seawater raceway prior to experimentation.

3.3.2 Laboratory experiment

Two treatments and a control were used to evaluate the effect of collection site on the response of *Porites astreoides* to temperature and a bacterial endotoxin. The control environment of 28°C was chosen because it reflects the temperature during the transition from summer to winter when temperatures at these two sites is equivalent. The first of the two treatments simulated a temperature of 32°C. During the two-year field transplantation period this was the highest one-day mean temperature observed. Therefore we expected that it would reflect a realistic but stressful condition. The second treatment was chosen to evaluate the synergistic effect of high temperature (32°C) and exposure to a bacterial endotoxin. Lipopolysaccharide from *Serratia marcescens* ATCC 21639 (Sigma-Aldrich; St. Louis, MO) was applied at a final concentration of 5 μg mL^{-1}; a level previously chosen to evaluate endotoxin impacts on corals (Palmer et al., 2011). A bacterial endotoxin treatment at 28°C was not conducted for three reasons. First and foremost, sectioning each coral fragment into 9 samples limited the number of treatments possible while preserving an adequate sample size at the individual level (n = 3). Second, minimizing the environmental impact of additional coral collection was a concern in this region. Lastly, the aim of this study was to understand site dependent responses to the synergistic effects of temperature and LPS stress rather than the response to LPS across a range of temperature stress.

Experiments were carried out in acrylic boxes (5 x 5 x 18 cm) constructed from 0.030 cm thick acrylic sheet. Acrylic boxes contained 250 mL of sterile artificial seawater and each box separated one coral

fragment from another assuring an independent response of each fragment to the experimental treatment. Groups of fifteen boxes were placed in 37.85 L glass aquariums filled with sterile seawater, which served as temperature incubation chambers. Control and treatment temperatures were maintained with a 150 W adjustable aquarium heater (Eheim; Dollard-Des-Ormeaux, Quebec). A submersible Aquaclear 10 aquarium powerhead (Hagen; Mansfield, MA) served to circulate water and maintain a homogenous thermal profile.

Each aquarium containing 15 acrylic boxes was covered by shade cloth throughout the course of an experiment resulting in a maximum photosynthetically active radiation of 150 µmol photons $m^{-2}s^{-1}$. This level was similar to that experienced at each reef and is a level known to prevent photoinhibition of reaction centers in *Symbiodinium spp.* dinoflaggelate symbionts. Each experiment lasted for 8 h beginning at 9:00 AM and concluding at 5:00 PM the following day. Colony fragments in acrylic boxes were randomly distributed within an aquarium for a given treatment (n = 3) to avoid bias associated with variation of conditions within an aquarium. Following 8 h treatment, each section of a fragment was immediately frozen in liquid nitrogen (LN_2) and stored at -80°C until processing for quantitative reverse transcription PCR.

3.3.3 Sample processing

Coral fragments fixed with LN_2 were first pulverized into smaller sections using a hardened steel dounce-style homogenizer chilled with liquid nitrogen to retain sample integrity. Excess skeletal fragments were removed with forceps and then the remaining portion ground using a ceramic mortar and pestle. In order to prevent RNA degradation during processing, fragments free of extraneous debris, were crushed in a shallow pool of LN_2. The powder was then transferred to a microcentrifuge tube and stored at -80°C prior to RNA isolation. Mortar and pestles were cleaned with Alconox (Alconox; White Plains, NY),

rinsed three times with de-ionized water, RNase treated (RNase zap: Sigma-Aldrich, St. Louis, MO), and then rinsed with ultra-pure deionized water (E-Pure System; Thermo Fisher Scientific Inc.) before each subsequent sample was processed.

3.3.4 RNA isolation

RNA was isolated from sample powder (110 mg) using a mixture of guanidine thiocyanate and phenol in a monophase solution (TRI Reagent: Sigma-Aldrich, St. Louis, MO). TRI Reagent (1 mL) was added to the sample and aspirated to aid in cell disruption prior to incubation (10 min, room temperature). The sample was centrifuged (10,000 rcf, 10 min, 4°C). The supernatant was transferred to a new tube and a solution of 0.8 M sodium citrate:1.2 M sodium chloride added (250 µL). The sample was shaken vigorously for 5 sec. Isopropanol (neat, 250 µL) was added and tube shaken vigorously for 15 sec. Samples were incubated (10 min, room temperature) to precipitate RNA and then centrifuged (10,000 rcf, 10 min, 4°C). After decanting the supernatant, the RNA pellet was washed with ethanol (1 mL, 75%) and centrifuged (7,500 rcf, 5 min, 4°C). This step was carried out twice. The extracted RNA was dissolved in RNase and DNase-free water (100 µL) and integrity determined with a Caliper Lab Chip GX. RNA quality scores (RQS) greater than 6 were deemed of sufficient quality for two-step reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) (Fleige and Pfaffl 2006). RNA extractions yielded approximately 2 µg of RNA (110 mg sample^{-1}).

3.3.5 Two-step qRT-PCR

Isolated RNA (300 ng) was treated with 1 unit of DNase 1 (Life Technologies; Grand Island, NY) in accordance with the manufacturer's recommendations. The sample (260 ng) was reverse transcribed with the Superscript III first strand synthesis supermix (Life Technologies; Grand Island, NY) in 96-well plates on an Eppendorf Mastercycler (Eppendorf; Hauppauge, NY). The thermal profile was as follows:

10 min,25°C; 30 min,50°C; 5 min, 85°C. After denaturing the enzyme at 85°C, RNase H was added (1 µL) to each well and incubated (37°C, 5 min) to degrade the remaining template RNA.

To each cDNA product (20 µL), 7.5 M ammonium acetate (Sigma Aldrich; St. Louis, MO) was added (6 µL) followed -20°C isopropanol (neat, 50 µL). This solution was inverted 10 times to mix the contents and then chilled (-80°C, 1 h) to precipitate cDNA. Samples were centrifuged (18,000 rcf, 15 min, room temperature) to pellet the cDNA. The precipitation reagents were decanted and pellet washed twice with 75% ethanol (1 mL). Washed cDNA was centrifuged (18,000 rcf, 10 min, room temperature). After the second wash the pellet was allowed to air dry at room temperature for 10-15 min. The pellet was re-suspended in ultra-pure water (104 µL) to yield a final cDNA concentration of 2.5 ng μL^{-1}. This cDNA was stored at -20°C for no more than 1 week prior to qRT-PCR.

3.3.6 qRT-PCR primer validation

Sequences of transcripts of interest were obtained from the *Porites astreoides* SymBioSys database (http://sequoia.ucmerced.edu/SymBioSys/). Primers were created with Primer3 software applying selection criteria previously outlined (Haslun et al. 2015 in review). The top scoring primer pair was selected for qRT-PCR validation. Primer validation was performed by creating a standard curve relating the cycle of quantitation (Cq) to the log concentration of cDNA along a 2-fold serial dilution; 5 ng μL^{-1}, 2.5 ng μL^{-1}, 1.25 ng μL^{-1}, 0.625 ng μL^{-1}, and 0.3715 ng μL^{-1} of cDNA. Primer pairs amplifying a single product according to dissociation curves, displaying a highly linear relationship ($R^2 > 0.99$) and adequate amplification efficiency ($3.0 < E < 3.6$) were considered valid for analysis (Table 6). The genes of interest (GOI) included 2 genes previously utilized in a sister study (Haslun et al. 2015); Eukaryotic initiation factor 3 subunit H (eIF3H), TNF receptor-associated factor 3 (TRAF3). Two additional genes of interest were also included; Calcium calmodulin dependent protein kinase (CDPK) and Heat shock factor protein

1 (HSFP1). We included two housekeeping genes, 60S ribosomal protein L11 (RPL11), and Cathepsin L (CATL) as utilized in previous work on gene expression with this species (Kenkel et al., 2011; Haslun et al. 2015 in review).

Table 6: The amplification efficiency and primer sequences of each gene of interest and control gene investigated in this study.

Genes of Interest	Abbreviation	Primer Sequence (5'-3')	Efficiency
Calcium calmodulin dependent protein kinase	CDPK	F: TCAAGCATAAGTGGGTGCAG R: ATAGCCAACATTCCGCCTTT	1.91
Eukaryotic initiation factor 3, subunit H	EIF3H	F: TTGATTGATACCAGCCCACA R: ACAAACTGCTTTGCTTTCCC	1.97
Heat shock factor protein 1	HSFP1	F: CTGCTTTGCCAGATGATGAC R: GGGCTGTGATGTTGAAGGA	1.95
TNF receptor-associated factor 3	TRAF3	F: GTCTGGCTCCTCCCATCTTT R: GCCTCCAGCATTCTAACCTG	2.03
Control Genes			
60S ribosomal protein L11	RPL11	F: TTTCAAGCCCTTCTCCAAGA R: GACCCGTGCTGCTAAAGTTC	1.94
Cathepsin L	CATL	F: GGAAGGATTACTGGCTGGTC R: GGATAGATGGCGTTTGTGG	2

Real-time PCR reactions were conducted in 10 µL total volume (1 µL template cDNA, 5 µL Power SYBR green master mix (Life Technologies; Grand Island, NY), 1.5 µL primer pair (final concentration 250 nM), and 2.5 µL DNase and RNase-free water). Amplification and detection of transcripts was carried out on an Applied Biosystems 7900HT real-time PCR system in 384-well plate format following the manufacturer's recommendations. To prevent potential run to run variation and the need for inter-run calibration, all samples were analyzed in duplicate on a single 384-well plate for each gene of interest (Derveaux et al., 2010).

3.3.7 qRT-PCR analysis

A Bayesian model-based approach (Matz et al., 2013) was applied to Cq values based upon previous gene expression studies with *Porites astreoides* (Matz et al., 2013). This method converts Cq values to molecular counts creating a linear rather than exponential relationship for the response variable. We employed a 2-way factorial design including collection site (inshore patch reef and offshore bank reef) and treatment as factors. Equation 1 outlines the structure of the model in the MCMCglmm package (Matz, 2013). The variable "*gene*" indicates accounts for the mean expression associated with each gene, "*CollectionSite"* represents the habitat a fragment was collected from, and "*Treatment*" represents the level of expression associated with a given factor level (*i.e.* control, temperature, temperature + biotic stress). Conditions in brackets indicate random error terms and include the sample specific error, the gene specific sample error, and the gene specific error in the order presented in each model.

Equation 1:

$$\text{count} = gene + gene{:}\,CollectionSite + gene{:}\,Treatment + gene{:}\,CollectionSite{:}\,Treatment + [sample] + [gene{:}\,sample] + [gene{:}\,residual]$$

Much like traditional delta delta Cq methodology (Livak and Schmittgen, 2001) we chose to include reference genes in this model to control for variation associated with the experimental conditions. This adjustment is not necessary in a Bayesian model-based approach, but provides more precise estimates of transcript abundance across treatments.

3.4 Results

3.4.1 Control condition (28°C)

Porites astreoides sections experiencing the control environment (28°C) displayed the lowest transcript abundance across all genes of interest (GOI) at each site. Comparison of transcript abundance between sites indicated that expression significantly differed for eIF3H (Figure 20), HSFP1 (Figure 21), and TRAF3 (Figure 22) ($p < 0.0$). Mean gene expression in fragments originating from the offshore site (Acer 24) was 3 times greater than that of conspecifics from the inshore site (Birthday Reef). Although control levels of CDPK (Figure 23) did not significantly differ between sites the means followed this trend.

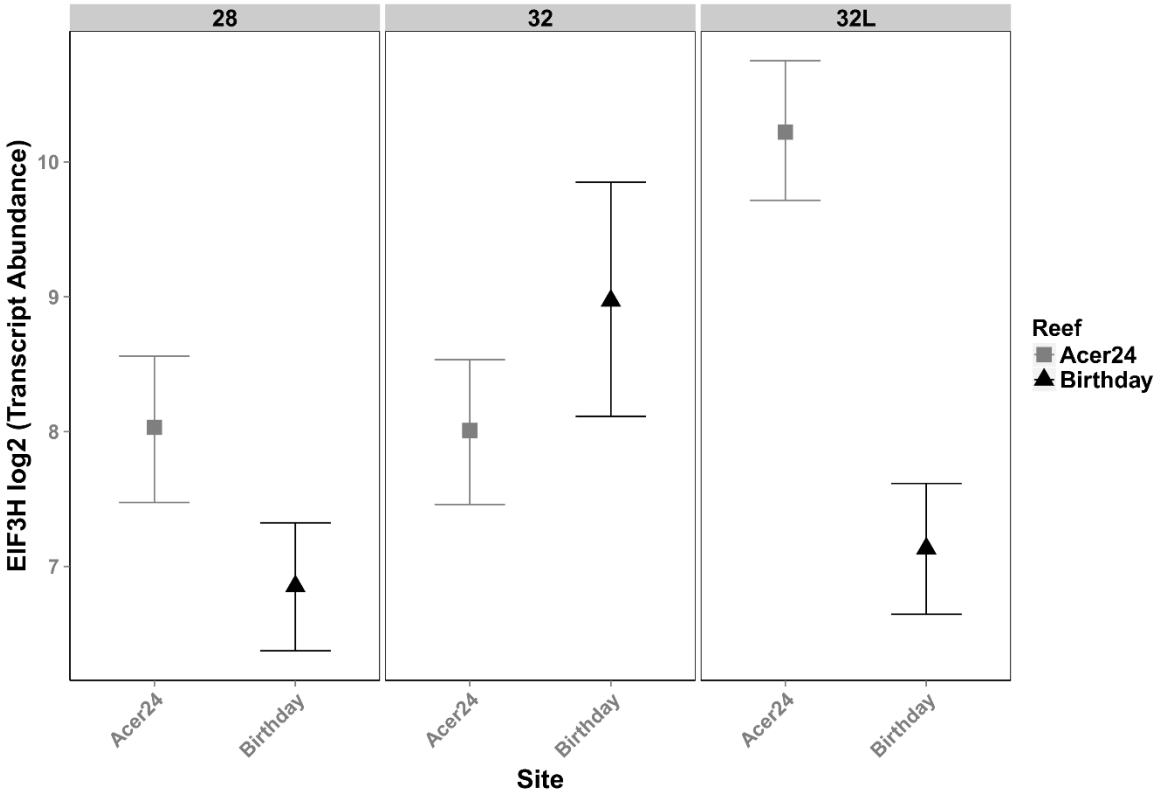


Figure 20: eIF3H Transcript Abundances Associated With *Porites astreoides* Fragment Origination at an Inshore or Offshore Reef Site Following 8 h Incubation With One of Three Treatments; 28°C (Control) (28) , 32°C (32) , and 32°C + Lipopolysaccharide (32L) of *Serratia marsescens*. Points indicate

Figure 20 Cont'd

posterior means and error bars indicate 95% credible intervals obtained from Bayesian generalized linear mixed modeling.

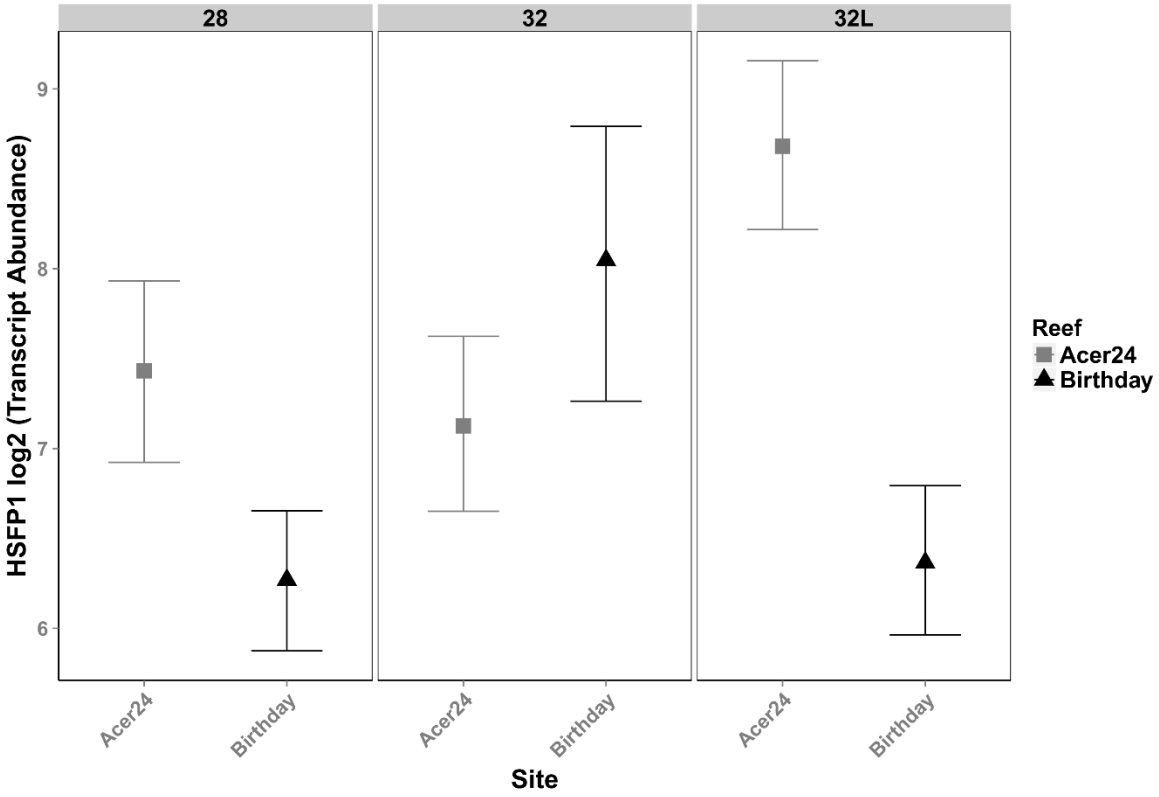


Figure 21: HSFP1 Transcript Abundances Associated With *Porites astreoides* Fragment Origination at an Inshore or Offshore Reef Site Following 8 h Incubation With One of Three Treatments; 28°C (Control) (28) , 32°C (32) , and 32°C + Lipopolysaccharide (32L) of *Serratia marsescens*. Points indicate posterior means and error bars indicate 95% credible intervals obtained from Bayesian generalized linear mixed modeling.

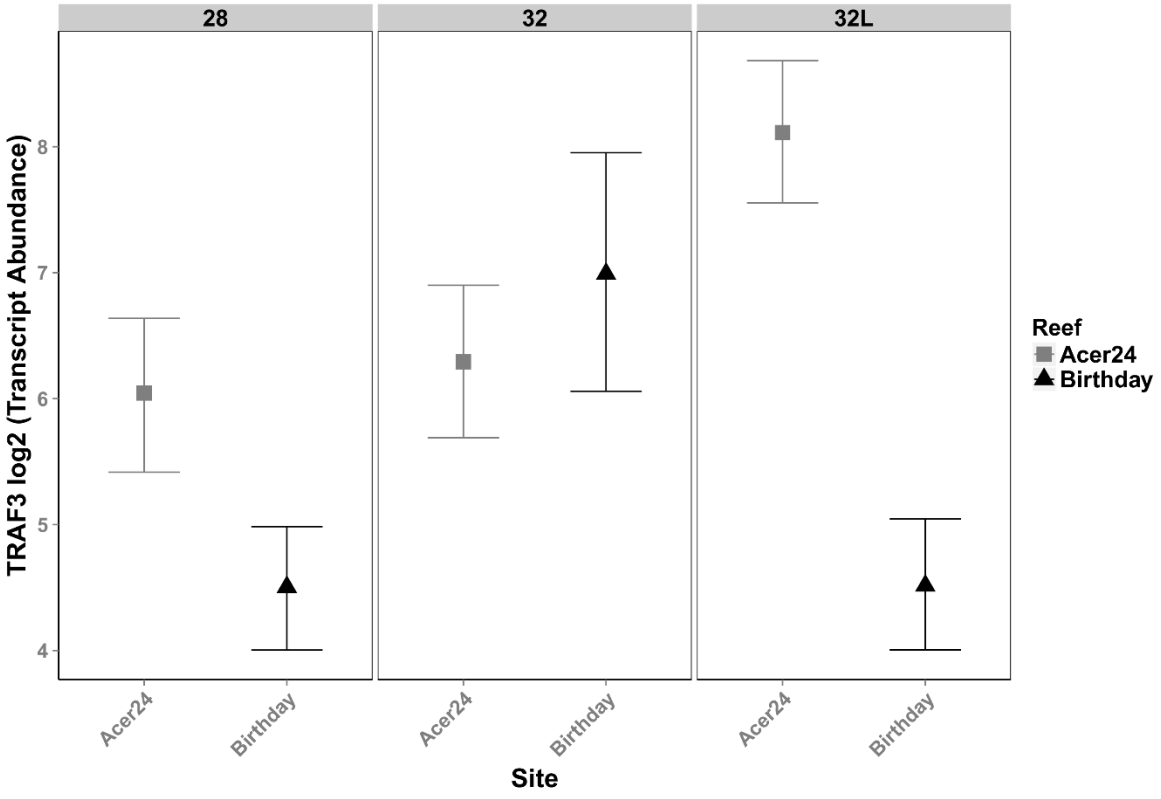


Figure 22: TRAF3 Transcript Abundances Associated With *Porites astreoides* Fragment Origination at an Inshore or Offshore Reef Site Following 8 h Incubation With One of Three Treatments; 28°C (Control) (28), 32°C (32), and 32°C + Lipopolysaccharide (32L) of *Serratia marsescens*. Points indicate posterior means and error bars indicate 95% credible intervals obtained from Bayesian generalized linear mixed modeling.

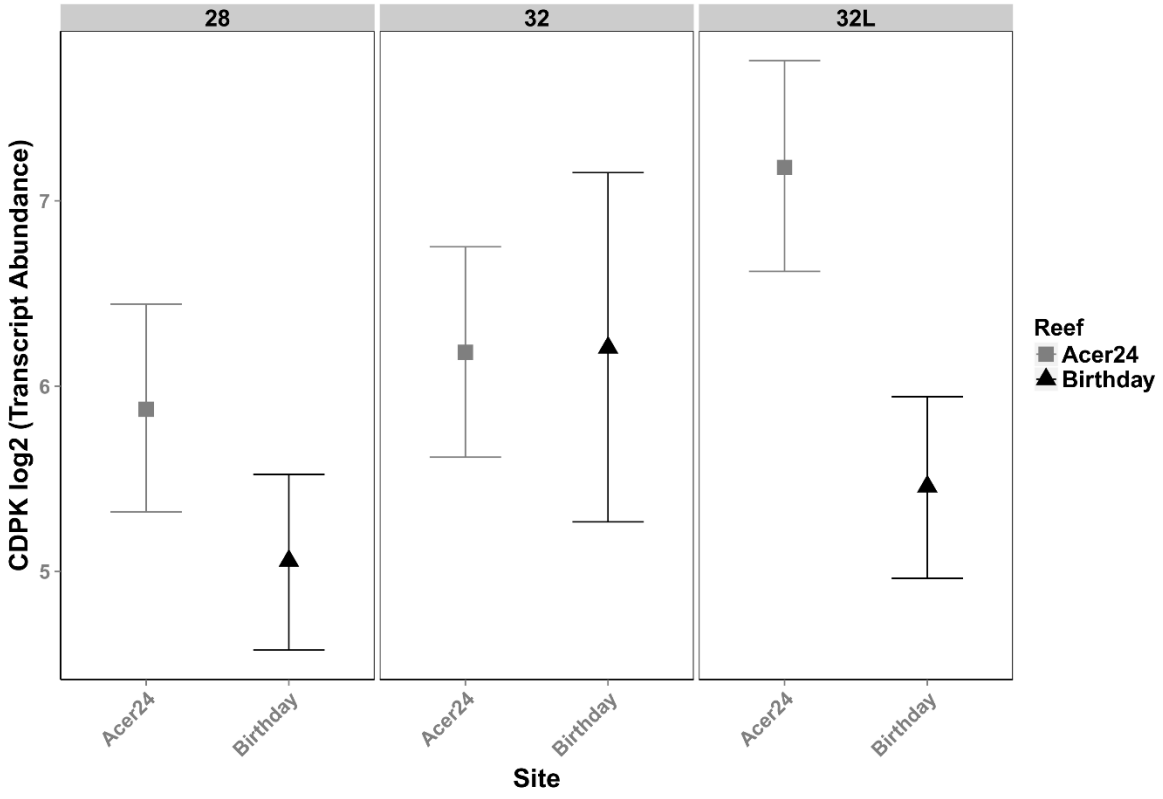


Figure 23: CDPK Transcript Abundances Associated With *Porites astreoides* Fragment Origination at an Inshore or Offshore Reef Site Following 8 h Incubation With One of Three Treatments; 28°C (Control) (28), 32°C (32), and 32°C + Lipopolysaccharide (32L) of *Serratia marsescens*. Points indicate posterior means and error bars indicate 95% credible intervals obtained from Bayesian generalized linear mixed modeling.

3.4.2 Increased temperature treatment (32°C)

Inshore and offshore fragments incubated at 32°C for 8 h did not display a significant difference in transcript abundance across the 4 genes of interest. When compared to the control treatment, fragments collected from the inshore reef displayed a significant upregulation of all genes except for CDPK. HSFP1 was upregulated 3 times that of the control, eIF3H 4 times that of the control, and TRAF3 5 times that of the control. Offshore fragments did not display significant changes in expression compared to the control treatment.

3.4.3 Synergistic effect of temperature and lipopolysaccharide (32°C + 5 µg mL^{-1} LPS)

Porites astreoides fragments from the offshore reef displayed greater transcript abundance for all GOIs then inshore conspecifics that were exposed to the synergistic effects of increased temperature and LPS stress. This difference was 1.75 times greater for CDPK expression, 4 times greater for eIF3H expression, 3 times greater for HSFP1 expression, and 3 times greater for TRAF3 expression.

Exposing offshore fragments to the synergistic stress of increased temperature and LPS resulted in the upregulation of all GOIs relative to both the control and elevated temperature treatment. Conversely, inshore fragments downregulated gene expression when confronted with the synergistic stress of elevated temperature and exposure to LPS relative to elevated temperature alone. Gene expression of the inshore fragments was comparable to the basal levels associated with the control condition.

3.5 Discussion

Previous observations of *Porites astreoides* populations inhabiting an offshore bank reef indicate a smaller mean colony size, decreased abundance, and increased constitutive expression of adenylate cyclase associated protein 2 (ACAP2), a gene associated with the innate immune response, compared to conspecifics inhabiting the inshore patch reef environment (Haslun et al., 2015). Because thermal stress from decreased variation in SWTs at offshore bank reefs is unlikely to play a role in the adaptation of gene expression (Haslun et al., 2015 in review) in this region I hypothesized that biotic stress plays a more prominent role in shaping the observed differences in phenotypes and coral communities. We established that *P. astreoides* colonies inhabiting an inshore and offshore reef of the lower FRT display divergent responses to brief thermal stress in a laboratory setting (8 h). We also determined that these two populations of *P. astreoides* display a comparably greater divergence in their response to the combination of thermal stress and biotic stress from bacterial lipopolysaccharide of a known coral pathogen (*Serratia marcescens*). These results indicate that non-lethal biotic stress may be underrepresented in the literature as a stressor capable of structuring communities especially when the effects of climate change simultaneously considered.

Eukaryotic and prokaryotic organisms affect changes in gene expression constitutively or responsively to environmental changes (Kussell and Leibler, 2005). Constitutive changes in expression are maintained over long periods of time (adaptation) while responsive changes are transient and dependent upon the occurrence of a particular stressor (Geisel, 2011). The organisms that upregulate a particular gene or suite of genes tend to experience increased fitness (e.g. growth and reproduction) compared to those that do not change regulation within a given environment. We observed that coral fragments collected from the offshore habitat displayed a 2-fold increase in expression of all GOI compared to conspecifics collected from the inshore habitat at the control condition (28°C). At increased temperature (32°C) *P.*

astreoides fragments displayed similar levels of expression independent of the collection site. However, at an elevated temperature, *P. astreoides* collected from the inshore site significantly upregulated gene expression relative to the control treatment across all GOI while fragments collected from the offshore site maintained their level of gene expression relative to the control. This result suggests that corals inhabiting offshore sites constitutively upregulate genes during short-term exposure to thermal stress while corals inhabiting inshore sites employ a responsive gene regulatory strategy. The observed difference in response strategy to temperature may reflect differences in the variability in SWT associated with each site. As stated previously, inshore patch reefs are exposed to a wider variation in SWT and therefore responsive regulation of gene expression may be an important component to fitness. In yeast, however, increased levels of gene expression are associated with significant energy costs (Wagner, 2007, 2005). A responsive gene expression strategy by *P. astreoides* to the wide variation in thermal stress, characteristic of inshore patch reefs, may decrease the impact of energy costs on fitness due to constant gene regulation. Thermal preconditioning experiments with corals support this view. Laboratory preconditioning with mild thermal stress has been observed to result in acquired resistance to severe thermal stress in the corals *Acropora millepora* (Bellantuono et al., 2012) and *Acropora aspera* (Middlebrook and Hoegh-Guldberg, 2008). Further, our previous research has shown that the coral *Montastraea cavernosa* displays an increased susceptibility to thermal stress in environments with lowered exposure to non-lethal thermal stress (Haslun et al., 2011). Responsive expression to temperature is also common in other benthic metazoan marine species. Following a prolonged period of exposure to elevated temperatures abalone (Li et al., 2012) and the purple sea urchin *Stronglyocentrotus purpuratus* (Osovitz and Hofmann, 2005) display responsive increases in the expression of hsp70, a chaperonin protein that decreases the effects of thermal stress.

Recent evidence from common garden experiments with juvenile *P. astreoides* from inshore and offshore reefs shows that the inshore population displays increased growth rates under thermal stress compared to offshore corals (Kenkel, Setta, and Matz, 2015). Although the authors conclude that a proportion the observed variation in fitness is attributable to maternal effects, the species appears to maintain population level genetic variation that provides the necessary material for adaptation to environmental variation. Although it is early to conclude that these adaptations are definitively heritable, our previous research reciprocally transplanting *P. astreoides* between the inshore and offshore reefs provides evidence of an occurrence of local adaptation in the expression of ACAP2 (Haslun et al., 2015 in review). The present study begins to elucidate the relative contributions of two environmental factors, temperature and LPS, on the phenotypic value (P) and variation (V_E) in four GOI of *P. astreoides*. The phenotypic value, in this case the mean gene expression, is defined as the sum of genotype driven and environmentally driven expression of the observed phenotype. We did not determine the genotype of the individuals used in this study and therefore comment on the effect of the environment only. It should be noted however that the variation associated with a given individuals expression of a gene was included in the model as a random effect. Moreover, as previously indicated (Haslun et al., in review; Kenkel, Setta, and Matz, 2015) these two regions display differences in traits that indicate distinct populations and therefore genetic differences are likely. Phenotypic variation (V_E) related to the environment is defined as the additive effect of the general environment (V_{Eg}), gene by environment interaction (V_{GxE}) and the microenvironment (V_{Es}) on the total phenotypic variation (Equation 1) (Byers and State, 2008). The contribution of the microenvironment was limited by the experimental design and thus assumed to be negligible.

Equation 1:

$$V_E = V_{Eg} + V_{GxE} + V_{Es}$$

Corals that experienced the offshore site environment displayed minimal differences in phenotypic value as well as phenotypic variation to both thermal treatments. This result suggests that variation in phenotypic value, the mean transcript abundance, is likely dependent upon genetic variation rather than environmental variation because there is limited change across the temperature range. Therefore, future adaptation to thermal stress may be constrained for the host in offshore populations of *P. astreoides.* An alternative explanation to the observed gene expression is that limited acclimation to increased thermal stress occurred as a result of the narrower thermal range characteristic of offshore sites. When combined with the short period of thermal stress used in this study (8 h), the experiment may not have been carried out across a long enough time span to produce an observable response in the expression of the GOI. In many instances corals spanning a reef display an observable response only after a number of degree heating weeks; weeks with prolonged temperature stress above the mean monthly maximum SWT (Gleason and Strong, 1995). However, decreased acclimation appears to be an unlikely explanation given that offshore corals displayed increased levels of expression at 28°C compared to the expression of inshore corals, which experience a wider range of temperatures. Therefore offshore corals maintained an increased level of expression in all GOI at all times. This strategy could limit resources needed to confront other stressors and contribute to decreased fitness. Although our data indicates that temperature has an impact on corals inhabiting these two regions, the emergence of disease as a driver of ecosystem change is closely linked to the effect of warming climates on microbe pathogenicity and host disease resistance (Burge et al., 2014). Therefore understanding the effect of this synergistic stress is a critical component to understanding future community dynamics. The strength of the link between disease and environment is affected by the host's sensitivity to environmental change. Homeothermic organisms are less sensitive to environmental change and therefore the disease dynamics are more impacted by the response of the pathogen to environmental change (Harvell et al., 2009). Conversely, ectotherms are more sensitive to climate warming thus there

is a strong link between host resistance to disease and environmental change. Corals are ectotherms that are highly sensitive to minor changes in SWT and are therefore an example of an animal in which the link between host resistance and warming is particularly important (Hoegh-Guldberg et al., 2007). Increased SWTs cause dysfunction in the photosynthetic apparatus of the algal symbiont resulting in the production of free radicals and potential expulsion of the symbionts from host (bleaching) along several different pathways (Lesser, 1997). As up to 98 % of the carbon required by the host is translocated from the symbiont (Muscatine et al., 1981), bleaching reduces the resources available to the host to respond to concurrent or later periods of stress from a biotic source. The effect of bleaching on disease susceptibility has observed. Following recovery from a severe thermal bleaching event in 2005 the coral population of the US Virgin Islands was decimated by an epizootic of lethal white plague disease. Fifty percent of the total coral population was lost (Miller et al., 2009). The authors concluded that the loss of resources to bleaching resulted in a compromised state to defend against such a disease. This example demonstrates how an abiotic stressor can decrease the host's ability to defend against pathogen exposure despite an apparent healthy state.

To my knowledge the contrasting link between a corals' stress level and climate change has not been observed. Invertebrates rely upon the highly conserved innate immune system to defend against foreign microorganisms. The response to foreign microorganisms is rapid and although invertebrates lack a specific response to a particular microorganism or antigen (adaptive immunity) a non-specific form of memory is recognized, called trained immunity (Netea et al., 2011). Trained immunity is defined as "a heightened response to a secondary infection that can be exerted toward the same microorganism and a different one (cross-protection)" (Netea et al., 2011). Empirical evidence of trained immunity has been reported across invertebrate phyla including meal worm beetles (Moret and Siva-Jothy, 2003), *Drosophila melanogaster* (Pham et al., 2007), sponges (Hildemann et al., 1980), and reef building corals

(Vollmer and Kline, 2008). Vollmer and Kline (2008), who identified trained immunity in corals, also identified a genotype effect on disease resistance in *Acropora cervicornis.* Therefore, not only does trained immunity occur in invertebrates but there is also selection for more efficient defense strategies with repeated exposure. Similarly, our previous research identified the presence of site-dependent selection pressure on *P. astreoides* inhabiting offshore reefs that is capable of producing local adaptation in the constitutive expression of the ACAP2 gene (Haslun et al., 2015 in review). This particular protein affects the production of cyclic adenosine monophosphate, an important second messenger effector molecule in apoptotic cascades, and produces the pro-inflammatory molecule NF-kB, both of which are important components of the immune response. *Porites astreoides* has also been shown to display levels of melanin production significantly greater than other common coral species following exposure to LPS (Palmer et al., 2011), and therefore may be particularly sensitive to biotic stress. Melanin is a conserved pigment that scavenges free radicals through the pro-phenoloxidase pathway preventing oxidative damage in animals (Grimaldi et al., 2012). In Cnidarians, melanin is expressed by innate immune system specific cells (amoebocytes) following exposure to pathogens and climate related stress (Couch et al., 2008). In this study I determined if the response of *P. astreoides* to LPS was affected by the previous environmental history in an inshore patch reef and offshore bank reef environment of the FRT. We combined this immune stimulator with increased thermal stress (32°C) because of the documented link between increased disease development and expansion across coral colonies (Cervino et al., 2004) and across reefs (Ben-Haim and Rosenberg, 2002) during warming periods. We observed divergent responses in *P. astreoides* fragments to this combined stress dependent upon their site of collection. The phenotypic value of corals collected from the offshore reef was greater following the 8 h treatment than corals collected from the inshore patch reef, which displayed phenotypic values similar to those observed in the control treatment. The site-dependent effect observed here indicates that *P. astreoides* fragments that had experienced the offshore

environment displayed a responsive strategy to the combined stressor while fragments inhabiting the inshore site displayed a limited response. These strategies likely reflect a fragments previous exposure to similar stressors. Our previous work identified that *P. astreoides* inhabiting the offshore site displayed seasonal expression of TRAF3, a gene tightly linked with the host's response to microbial insult, while those inhabiting the inshore site displayed a consistent level of expression (Haslun et al., 2015 in review). Therefore we infer that the factors contributing to the immune response of the offshore corals are more variable. Increased environmental variation leads to a responsive expression strategy, as was observed in the high temperature treatment for corals inhabiting the inshore site. This result may also indicate a limited potential for the coral host to adapt to bacterial related stress at inshore sites.

In order to summarize the response strategies of the two *P. astreoides* populations across all treatment levels we applied an additive linear model to the phenotype values (mean gene expression). Two different adaptive response strategies to bacterial LPS and thermal stress were evident for *P. astreoides* (Figure 4). Colonies inhabiting the inshore site (1) display decreased expression levels in the GOI studied and employ a responsive strategy to thermal stress but a constitutive strategy to LPS and thermal stress. In contrast, (2) colonies inhabiting the offshore site display increased expression to all treatments and display a responsive strategy to LPS exposure. The response planes produced in figure 24 include an estimate for the expression to LPS exposure at 28°C in order to produce a complete relationship between the treatment factors. We determined the estimate for each population by calculating the average expression between the control treatment and the combined stress treatment. Because inshore corals utilize a responsive gene regulation strategy to temperature but not LPS exposure, the estimate of gene expression was similar to the mean of control and combined stress treatment. Corals collected from the offshore site displayed a responsive strategy to LPS but not to thermal stress,

therefore the estimate of gene expression when exposed to LPS at 28°C fell between that of the combined stress treatment and control treatment. The proposed relationship, when combined with the known abundance and size distribution of the inshore and offshore reefs, show that responsive expression to thermal stress is correlated to increased growth and abundance while responsive expression to bacterial LPS is correlated with decreased growth and abundance. Therefore responsive activation of the immune system or its' over activation may confer fitness costs to *P. astreoides*. The general increased activation of genes may also contribute to decreased phenotypic values of fitness related traits as previously indicated for growth.

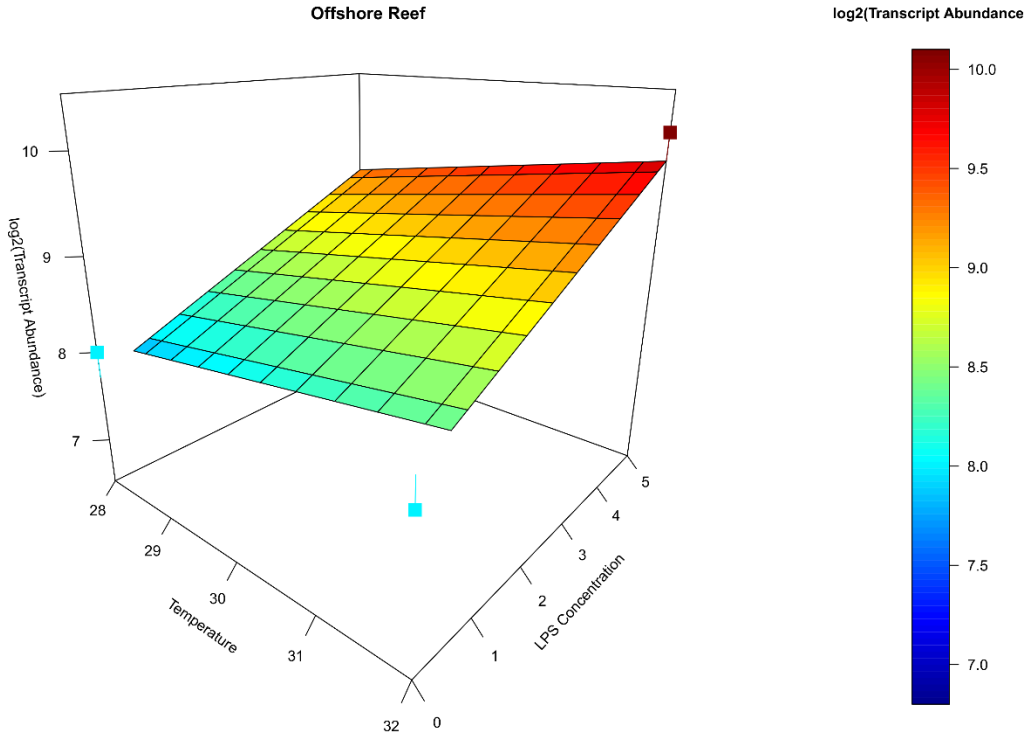
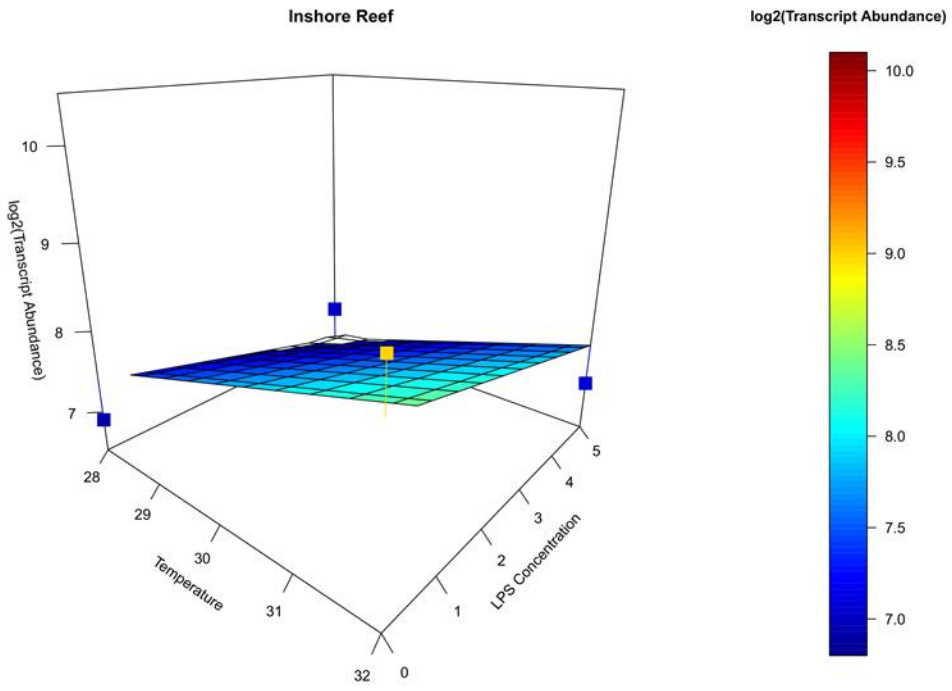


Figure 24: The Mean Gene Expression Response Strategies to Temperature and Lipopolysaccharide of *Porites astreoides* Inhabiting Inshore Patch Reefs and Offshore Bank Reefs of the Florida Reef Tract. Each three-dimensional surface represents a 10 x 10 matrix of predicted values from a linear model. The color of each point represents the mean transcript abundance identified in this study. Offshore corals

Figure 24 (Cont'd)

upregulate based on bacterial stress while inshore corals upregulate in response to temperature. Offshore corals also display increased expression compared to inshore corals.



Organisms that undergo adaptation to environmental change experience accompanying tradeoffs in the expression of other traits as a result of altered resource allocation. These tradeoffs are more likely observed in higher level traits such as growth, reproduction, and longevity because of the difficult nature of detecting gene regulation interaction. Adaptations to increased biotic stress or an increased range of biotic stress could potentially produce such an effect. Tradeoffs between immune defense and fitness have been observed in other invertebrates. Bacterial challenge of the immune system of *Tenebrio molitor,* the mealworm beetle, was found to significantly decrease individual longevity (Armitage et al., 2003). *Tenebrio molitor* also displays two color morphotypes, tan and black. The

darkness of this color is directly related to the production of melanin, an important component of arthropod and coral immune defense. Black morphotypes, which constitutively increase the production of this defense molecule, displayed decreased longevity (Armitage et al., 2003). Therefore fitness tradeoffs related to the production of defense molecules can even be produced without microbial attack. Our study indicates that offshore fragments of *P. astreoides* display both constitutive and responsive upregulation of genes following exposure to LPS while when exposed to the same treatment, inshore fragments maintain control and lower levels of expression. Therefore relative to the meal worm beetle, offshore corals display similar expression characteristics of the immune system and the tradeoffs associated with such a response should also be observed. We have described several sources of evidence corroborating such a fitness tradeoff and therefore biotic stress and its connection with SWT at inshore and offshore reefs requires increased attention.

3.6 Conclusion

This study found evidence indicating that biotic stress is an important driver of host adaptation in and thus community dynamics in the lower region of the FRT. Moreover, previous studies analyzing fitness of *P. astreoides* in this region links fitness tradeoffs with increased immune responses that we identify in *P. astreoides*. Based on our findings we predict that offshore populations of *P. astreoides* will continue to experience a chronic level of biotic stress that places constraints on their ability to resist other stressors. This population also displays a limited range of gene expression in response to thermal stress indicating constraints on future adaptation to this stressor. With continued climate warming marine microbial communities, particularly thermodependent pathogenic bacteria, are expected to increase in abundance (Bally and Garrabou, 2007) placing greater stress on offshore populations. Although the inshore population's response to temperature confers increased growth, the limited variation in response to LPS that we identified indicates decreased genetic variation for adaptation to biotic stress. If microbial communities continue to transition, inshore microbial communities may generate disease outbreaks that this population is unable to respond to. In the face of continued global warming the importance of investigating climate related effects on coastal marine microbial communities cannot be stressed enough. Although the immune system of *P. astreoides* displays adaptation to biotic stress, there is a fitness cost associated with its activation that leaves these animals in a weakened condition and less prepared to confront an inevitable increase in thermal stress.

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WORKS CITED

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