AN ABSTRACT OF THE THESIS OF

Viktor W. Brandtneris for the degree of Master of Science in Marine and Environmental Sciences presented on 18 March 2015

Title: An examination of seasonal variability in energy content among reef habitats

Abstract approved:

Tyler B. Smith

Energetic responses of zooxanthellate reef corals along depth gradients have relevance to the thermal stress refugia potential of mesophotic coral ecosystems (MCE). Previous observations suggested that MCE in the Caribbean are thermally buffered during the warmest parts of the year and occur within or just below the chlorophyll maximum, suggesting abundant trophic resources. However, it is not know if mesophotic corals can maintain constant energy needs throughout the year with changing environmental and biological conditions (e.g., thermal stress, reproduction). The energetic content of tissues from the stony coral species *Orbicella faveolata* and *Agaricia lamarcki* was measured on the southern insular shelf of St. Thomas, US Virgin Islands (USVI) over five periods from April 2013 to April 2014. Three sites for each species, at depths of 6m, 25m, 38m and 63m, were selected to capture energetic differences across the full vertical range of coral habitats in the USVI. Mesophotic colonies of *O. faveolata* exhibited a significant reduction in energetic content during the month of September 2013 compared to mid-depth and shallow colonies ($p=0.032$), whereas *A. lamarcki* experienced similar energetic variability, but with a significant reduction in energy content that occurred in July 2013 for colonies at sites deeper than 25m ($p=0.014$). The results of calorimetric analyses indicate that *O. faveolata* may be at risk during late summer stress events, possibly due to the timing of reproductive activities. The low-point of *A. lamarcki* energy content, which may also coincide with reproduction, occurs prior to seasonal thermal stress events, thus favoring this species in mesophotic habitats.

An examination of seasonal variability in energy content among reef habitats

by

Viktor W. Brandtneris

A THESIS

submitted to the

UNIVERSITY OF THE VIRGIN ISLANDS

in partial fulfillment of the requirements for the degree of

Master of Science

Presented 18 March 2015

Commencement May 2015

Master of Science thesis of Viktor W. Brandtneris

Presented on 18 March 2015

APPROVED:

Tyler B. Smith, Ph.D.

Marilyn E. Brandt, Ph.D.

Peter W. Glynn, Ph.D.

Marilyn E. Brandt, Ph.D., Director, MMES Program

Sandra Romano, Ph.D., Dean, College of Science and Mathematics

I understand that my thesis will become part of the permanent collection of the University of the Virgin Islands Library. My signature below authorizes release of my thesis to any reader upon request.

Viktor W. Brandtneris

ACKNOWLEDGEMENTS

I would like to thank R. Brewer, K. Brown, I. Byrne, R. Ennis, L. Henderson, D. Holstein, S. Kadison, M. Kammann, J. Kisabeth, R. Nemeth, S. Prosterman, A. Sabine, R. Sjoken, A. Tagini and V. Wright for field and laboratory assistance as well as data management. Funding was provided by the Black Coral Penalty Fund, VI EPSCoR (NSF#0814417) and the Lana Vento Charitable Trust. No funding agency participated in the design or interpretation of the research.

CONTRIBUTION OF AUTHORS

Dr. Tyler B. Smith provided invaluable assistance developing the experimental design and carrying out sampling excursions. Additionally, he was instrumental in organizing funding and interpreting results. This work would not have been possible without his help.

Dr. Marilyn E Brandt assisted with developing the experimental design and statistical analyses of the results.

Dr. Peter W. Glynn assisted with developing the experimental design and interpretation of the results.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction

Dramatic changes in the physical parameters of the ocean are predicted to increase mortality of corals and organisms associated with coral reefs (Baker et al. 2008; Munday et al. 2008; Glynn 2011). A majority of the studies undertaken to elucidate the effects of increased temperature and acidification on corals have produced disturbing narratives on the future of reefs. Recently a somewhat more positive outlook on the future of coral reefs has been espoused by those investigating deep, light-dependent coral habitats—referred to as mesophotic coral ecosystems (MCE). Hinderstein et al. (2010) defines MCE as ecosystems dominated by phototrophic corals between 30m and the depth at which light in the water column is too low to sustain photoautotrophy. Mesophotic coral systems can extend to depths greater than 150m depending on location (Kahng et al. 2010). Deeper water may provide a protective buffer for corals against increased temperature, storm-induced wave action and UV radiation (Glynn 1996; Riegl and Piller 2003; Gleason et al. 2006). The "deep reef refugia" hypothesis suggests that MCE sheltered from increased temperature and wave action have the potential to support healthy coral that can provide larvae for the repopulation of degraded shallow water coral ecosystems (Bongaerts et al. 2010; van Oppen et al. 2011).

As shallow water habitats decay it is increasingly necessary that workers begin to explore the factors that define mesophotic reef systems—both biotic and abiotic. Depth generalist coral species inhabiting both shallow and mesophotic reefs may experience widely variable conditions dependent on location and season. Often located far offshore, MCE may experience unique thermal, light, salinity and sedimentation regimes compared to their shallow, nearshore counterparts (Lesser et al. 2009; Kahng et al. 2010). Though refuge from increasing temperatures is the regular focus of MCE study, it is probable that reefs at mesophotic depths are afforded additional benefits that will bolster their survival. Hydrodynamic conditions vary greatly with depth—MCE are protected from strong wave action and storm-induced scouring. Tranquil conditions at depth provide conditions better suited to fragile

species and passive suspension feeders (Kahng et al. 2010). Conversely, increased sedimentation at mesophotic depths can have adverse impacts on the ability of species to recruit and grow (Aponte and Ballantine 2001)—especially on horizontally oriented bank reefs.

Coral Energetics and Refuges

The potential for MCE to serve as coral refugia in the face of climate change rests largely on the ability of corals beyond 30m to persist through increasingly prevalent stress events. It has been shown that coral colony energy content can play an important role in the ability of corals to survive and recover from intense thermal bleaching events (Yamashiro et al. 2005). Anthony et al. (2007) showed that energy content—in this case lipid content—could be used to accurately predict survivorship of laboratory colonies exposed to differing levels of temperature, light and sedimentation. Not only does energy content at time of bleaching greatly influence the survivorship of corals, heterotrophic plasticity also has been shown to influence increased resilience in at least one coral species subjected to thermally induced bleaching (Grottoli et al. 2006). Corals that are able to supplement reduced autotrophic energy production by means of suspension feeding on particulate matter may be more likely to survive prolonged bleaching events. Heterotrophic plasticity, however, is based not only on the coral species in question, but varies greatly through both space and time, depending on the presence of coral food sources in the water column.

Several different techniques can be used to measure the energy content of corals. Along with lipid content and isotopic signatures, basic measures of tissue biomass and zooxanthellae density prior to bleaching have been shown to influence recovery after thermal stress (Thornhill et al. 2011). Another technique not often utilized in modern reef study is coral calorimetry. First applied to corals by Richmond (1981), calorimetry is a direct measure of the total energy contained within a coral holobiont. The reductive nature of this methodology provides a single measure of

energy content that can be easily compared through space and time as well as across species. The technique is limited, however, in that the energy content measured is that of the overall pool of energy in a colony, and does not provide information on the sources of incoming energy (i.e., phototrophy versus autotrophy) or causes of energy loss. The energy available for growth and reproduction is masked within the overall measure of energetic content.

This study assesses whether calorimetry can be used to track seasonal changes in adult coral energetic status and measures seasonal energy changes across the depth ranges of two threatened Caribbean scleractinian corals, *Orbicella faveolata* and *Agaricia lamarcki*. Five coral collections were made between April 2013 and April 2014 across three depths representing the primary habitat range for each species – 6 to 38m for *O. faveolata* and 25 to 63m for *A. lamarcki*. Calorimetric values at each depth were compared to seasonal measures of environmental characteristics to describe the relationship between depth, light, heterotrophic potential and the energy content of both species.

Materials and Methods

Site Selection

Sampling locations were chosen to encompass the primary depth range for each species (Fig. 1). Colonies of *O. faveolata* were sampled at sites in approximately 6, 25, and 38m of depth, while *A. lamarcki* was sampled at 25, 38, and 63m depth (Table 1). Initial shallow samples of *O. faveolata* taken on May 1, 2013 were from an offshore site at Buck Island where colony density was found to be very low. Therefore subsequent shallow *O. faveolata* sampling was conducted at another offshore island, Flat Cay—deemed analogous to Buck Island due to similar environmental histories and distances from shore. All sites sampled are included in the annual Territorial Coral Reef Monitoring Program (TCRMP) for the US Virgin

Islands, providing consistent historic datasets for temperature and coral quality (Smith et al. 2012).

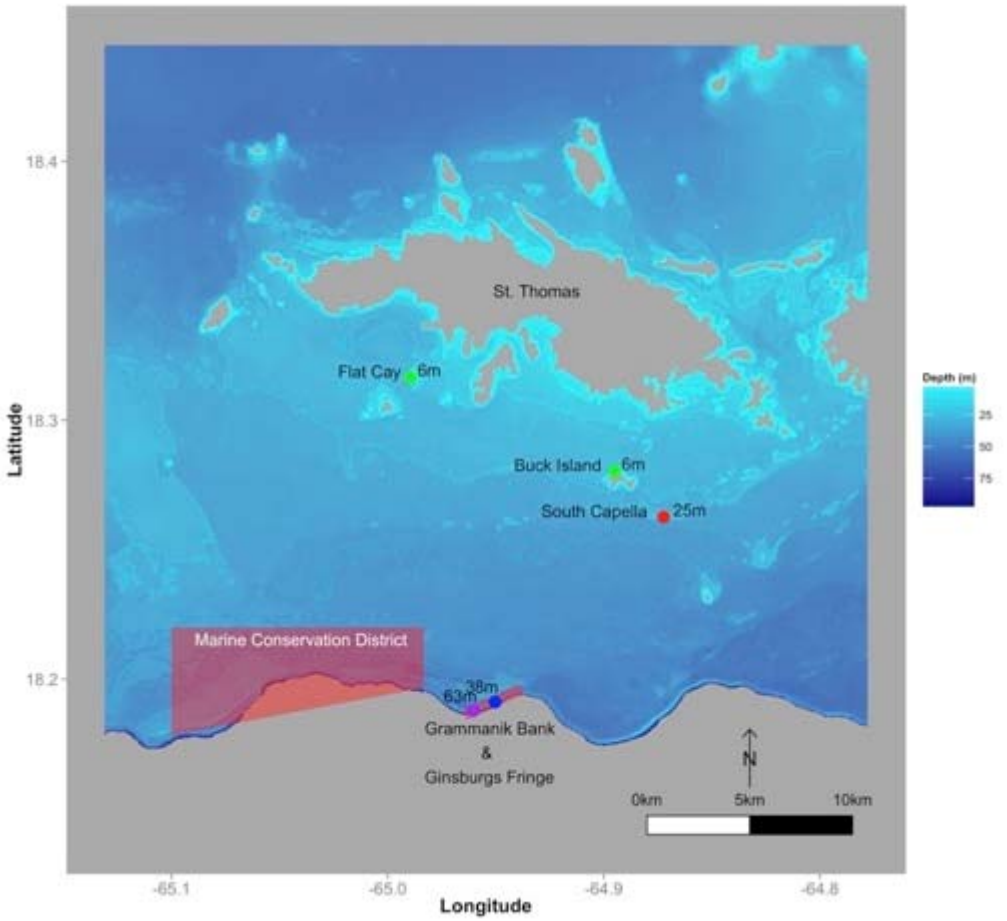

Figure 1 – Sampling locations on insular shelf south of St. Thomas, USVI. Major offshore Marine Protected Areas indicated in dark blue. Known shallow and mesophotic coral habitats indicated in pink and light blue respectively.

Coral Collection

Coral samples were collected over five periods between April 2013 and April 2014 at approximately two-month intervals (Table 1). Divers haphazardly sampled seven 15-30cm^2 replicate sections of each species separated by five fin-kicks while maintaining consistent depth at each site—producing a total of 105 samples for *O. faveolata* and 101 for *A. lamarcki* (Table 1). Colonies were not resampled during multiple collection periods. Hammer and chisel were used to collect from the tops of

O. faveolata colonies and the colony edges of *A. lamarcki* with a minimum radius of 25cm. Colonies of *A. lamarcki* were not sampled from the center as this caused fracturing of the entire colony; however attempts were made to include as much of the central portion of the colony as possible. Lastly, divers recorded the collection depths, and length, width and height of each sampled colony. At the surface, samples were transferred without seawater to pre-labeled whirl-packs and placed on ice for transport back to the laboratory. Time constraints related to post-processing limited sampling to two sites per field day—concurrent samplings were carried out no more than seven days apart.

Site	Depth (m)	Species	Dates	N
Buck Island	4-7	*O. faveolata*	1-May-13	7
Flat Cay	4-10	*O. faveolata*	11-Jul-13, 13-Sep-13, 19-Nov-13, 2-Apr-14	28
South Capella	23-28	*O. faveolata/A. lamarcki*	1-May-13, 11-Jul-13, 13-Sep-13, 19-Nov-13, 2-Apr-14	35/35
Grammanik Bank	36-40	*O. faveolata/A. lamarcki*	26-Apr-13. 5-Jul-13, 18-Sep-13, 14-Nov-13, 4-Apr-14	35/32
Ginsburg's Fringe	60-67	*A. lamarcki*	26-Apr-13. 5-Jul-13, 18-Sep-13, 14-Nov-13, 4-Apr-14	34

Table 1 – Sampling sites with depth, species sampled, sampling dates and sample sizes.

Calorimetry

Coral samples were denuded with an airbrush according to the methods of Szmant and Gassman (1990) using ultra-pure 18mOHM water. The blastate was homogenized and immediately frozen and stored at -20°C. Later, samples were partially thawed and transferred to lyophilization tubes before being re-frozen at -80°C for two hours. Samples were then freeze-dried for 24-36hrs at 220mbar and -105°C. Drying times were dependent on sample size and density—larger samples required longer drying times and in some cases re-freezing and a second round of lyophilization. Drying was deemed complete when samples could be easily powdered

using a scapula without the presence of ice or liquid water. Powdered coral samples were stored in centrifuge tubes in a dehumidified cabinet set to 10% humidity.

Calorimetric analyses were carried out using a semi-microbomb calorimeter (Model 6725, Parr Instrument Company, Illinois, USA). Powdered coral samples weighing 8-24mg were pelletized and combined with a purified mineral oil spike of known energy density for combustion. Due to variable humidity in the laboratory it was difficult to consistently re-hydrate samples. The mineral oil spike ensured complete combustion of the coral powder and slowed the burn to an acceptable rate. Samples were loaded into the prepared microbomb and pressurized to 30atm with medical grade pure oxygen. Calorimetric analysis requires fifteen minutes per run and each sample was analyzed at least twice. Traditionally, relative standard deviation (RSD) between two or more calorimetry runs is used to ensure the accuracy of the final energetic content (Golley 1961). If the first two runs did not achieve an acceptable RSD, the sample was rerun until either an acceptable RSD was achieved or the sample was depleted.

Carbonate rich organisms present a unique problem in calorimetry due to the reduced combustion of calcium carbonate. Samples with >20% carbonate require a correction of 0.586 J/g carbonate (Paine 1966). 6-38mg of each sample was burned for 4 hours at 500C to ascertain carbonate percentage. In all cases, carbonate proportions were greater than 20% and required correction.

Environmental Characterization

Continuous *in situ* records of temperature were recorded with sensors affixed to the substrate (Hobo Water Temperature Pro v2 U22, Onset Computer Corporation, Massachusetts, USA). Paired instruments at each site and at the coral sampling depths provided continuous temperature records at fifteen-minute intervals over the course of the study. Additionally, vertical profiles of water column temperature and chlorophyll were created within one month of coral sampling dates using a Seabird 25

Conductivity-Temperature-Depth multi-sensor (Seabird Scientific, Washington, USA) equipped with an EcoFLNT fluorometer (Wetlabs, Oregon, USA). Water column cross sections were sampled just offshore from the 38m and 63m sites to encompass the entire sampling depth range.

Analysis

Environmental records were summarized and compared with visual inspection. Plotted benthic temperature records were condensed to daily means for each site. In addition, the potential thermal stress experienced for a given site was calculated as the Degree Heating Week metric (DHW; NOAA 2006). Site-specific DHW calculations were based on derived bleaching thresholds for Flat Cay, South Capella, and Grammanik Bank (Smith, in review). No specific bleaching threshold is available for the deepest site, Ginsburgs Fringe (63m). A hypothetical bleaching threshold of 28.4°C was developed based on a relationship of bleaching threshold with depth from 24 sites of the Territorial Coral Reef Monitoring Program (Bleaching Threshold = 30.03°C - 0.0256431°C * Depth in meters).

The change in energy content over the sampling periods was tested separately for *O. faveolata* and *A. lamarcki*. The independent nature of individual coral samples through time allowed for the application of a two-way ANOVA. Sampling Period and Site were used as factors and Tukey's HSD post-hoc analysis was used to compare means when significant effects of the main factors were found. Graphical interpretation of thermal histories was deemed sufficient as high sample sizes for each site made significant results extremely likely regardless of trends.

Results

Calorimetry

Sufficient sample quantity was collected for calorimetric analyses in 74 colonies of *O. faveolata* and 80 colonies of *A. lamarcki.* The energetic content of *O. faveolata* showed stability over time at 6m, varying by only 10.1% (Fig. 2). In contrast, both the mid-depth and mesophotic sites exhibited considerable variability, 25m colonies varied by 20.5% and 38m colonies by 27.8% throughout the sampling period. Two-way ANOVA analysis resulted in a significant interaction between Site and Sampling Period ($p = 0.032$) (Table 3). Tukey's HSD post-hoc analysis of the interaction indicated that the September 38m data point was significantly lower than a number of other data points, including four of five shallow sampling periods (Figure 5). The energy density of individual *O. faveolata* colonies varied two-fold, from a minimum of 7.995 J mg^{-1} ash-free dry weight (AFDW) at the 38m site to a high of 15.859 J mg^{-1} AFDW at the shallowest 6m site—with an overall mean of 12.402 $\pm$ 0.205 J mg^{-1} AFDW ($\pm$ SE).

Shallow colonies of *A. lamarcki* (25m) exhibited greater stability through time relative to deeper samples, varying by only 5.8% (Fig. 2). Conversely, the 38m and 63m sites varied over the sampling periods by 33.3% and 36.1%, respectively, and had a very similar pattern over time. Two-way ANOVA analysis indicated a significant interaction between Sampling Period and Site ($p = 0.014$) (Table 2). Tukey's HSD post-hoc analysis of the interaction effect indicated that energetic content of *A. lamarcki* at both mesophotic sites in July, 2013 were significantly less than the 63m site in April, 2013 and the 38m site in April 2014. The energy density of individual *A. lamarcki* colonies varied two-fold, from a minimum of 8.035 J mg^{-1} AFDW to a maximum of 15.514 J mg^{-1} AFDW, with both extremes occurring at the 63m site. Mean energetic content was 12.346 $\pm$ 0.189 J mg^{-1} AFDW ($\pm$ SE).

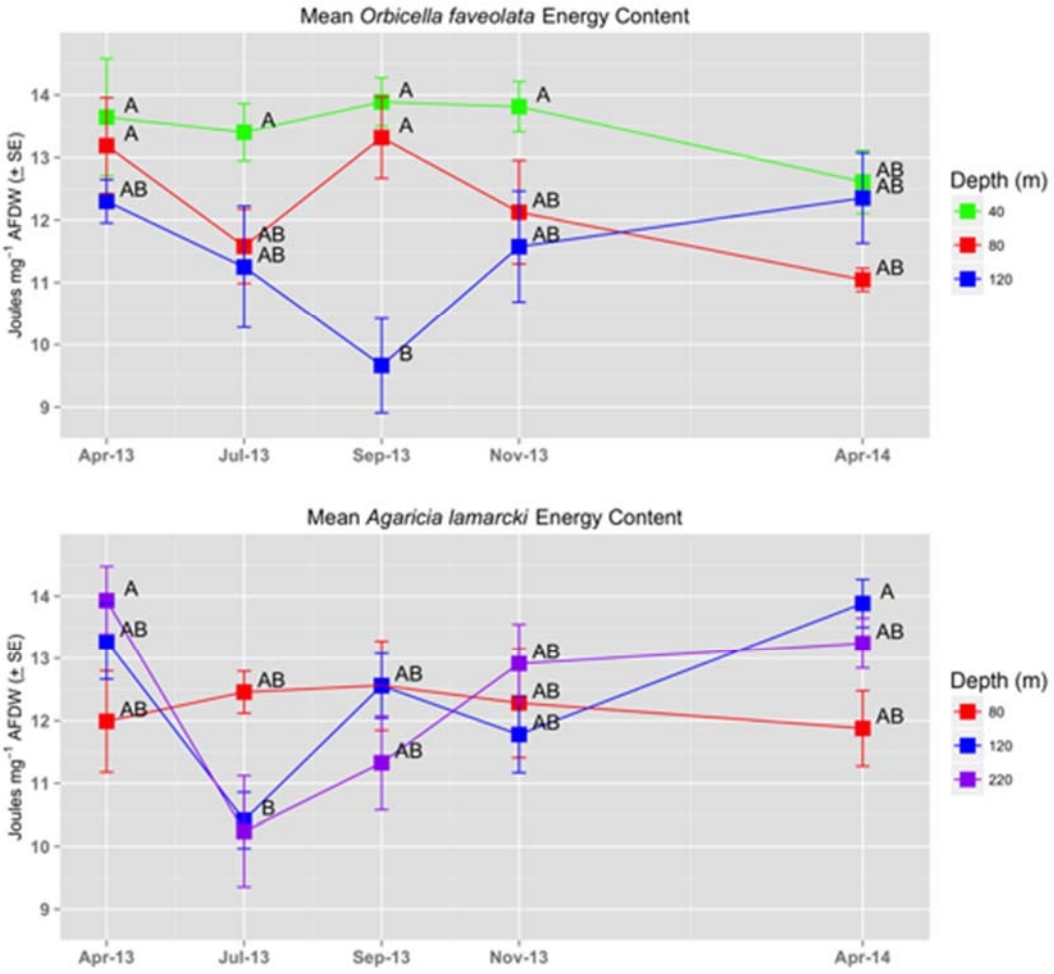

Figure 2 - Mean caloric content of (A) *Orbicella faveolata* and (B) *Agaricia lamarcki* subsamples between April 2013 and 2014 from three site and depth levels. Letters adjacent to means values indicate results of a Tukeys HSD post-hoc analysis of the overall interaction between site and sampling period.

Species	Site p (F_{df})	Period p (F_{df})	Site*Period p (F_{df})
Orbicella faveolata	0.00003 ($12.43_{2,59}$)	0.287 ($1.28_{4,59}$)	0.032 ($2.30_{8,59}$)
Agaricia lamarcki	0.722 ($0.33_{2,65}$)	0.003 ($4.38_{4,65}$)	0.014 ($2.65_{8,65}$)

Table 2 – Results of Two-Way ANOVA analyses comparing the energy content of coral tissue in *Orbicella faveolata* and *Agaricia lamarcki* across sites and sampling periods.

Environmental Characterization

Temperature trends showed considerable variability both within and between study sites (Fig. 3). Flat Cay (6m) and South Capella (25m) exhibited similar temporal trends for both 2013 and 2014. South Capella, however, experienced reduced thermal peaks when compared to Flat Cay, between the third and fourth sampling events. In 2012, prior to coral colony sampling, South Capella accumulated about 3 DHW of thermal stress, whereas Flat Cay showed almost no thermal stress. The pattern was reversed in 2013, when Flat Cay accumulated about 3 DHW during project sampling and South Capella experienced almost no thermal stress. During this period, temperatures at Flat Cay peaked to roughly 0.5°C higher than at South Capella. However, bleaching at Flat Cay in October was mild (9.8% prevalence) and not very different from other non-bleaching years (10.6% prevalence; mean of years 2009, 2011, and 2012 during the thermal maximum; data from the TCRMP).

The mesophotic sites at Grammanik Bank (38m) and Ginsburgs Fringe (63m) showed greater diel temperature variability than their shallow counterparts as well as reduced temperatures throughout both sampling years. In the year prior to sampling (2012), there was over 4 DHW of thermal stress recorded at Grammanik Bank, and this resulted in moderate bleaching (34.8% prevalence) compared to other non-bleaching years (12.0% prevalence, mean of years 2009, 2010, and 2011 during the thermal maximum; data from the TCRMP). Over the period of coral sampling in 2013 and 2014 there was little or no thermal stress recorded at Grammanik Bank or Ginsburgs Fringe.

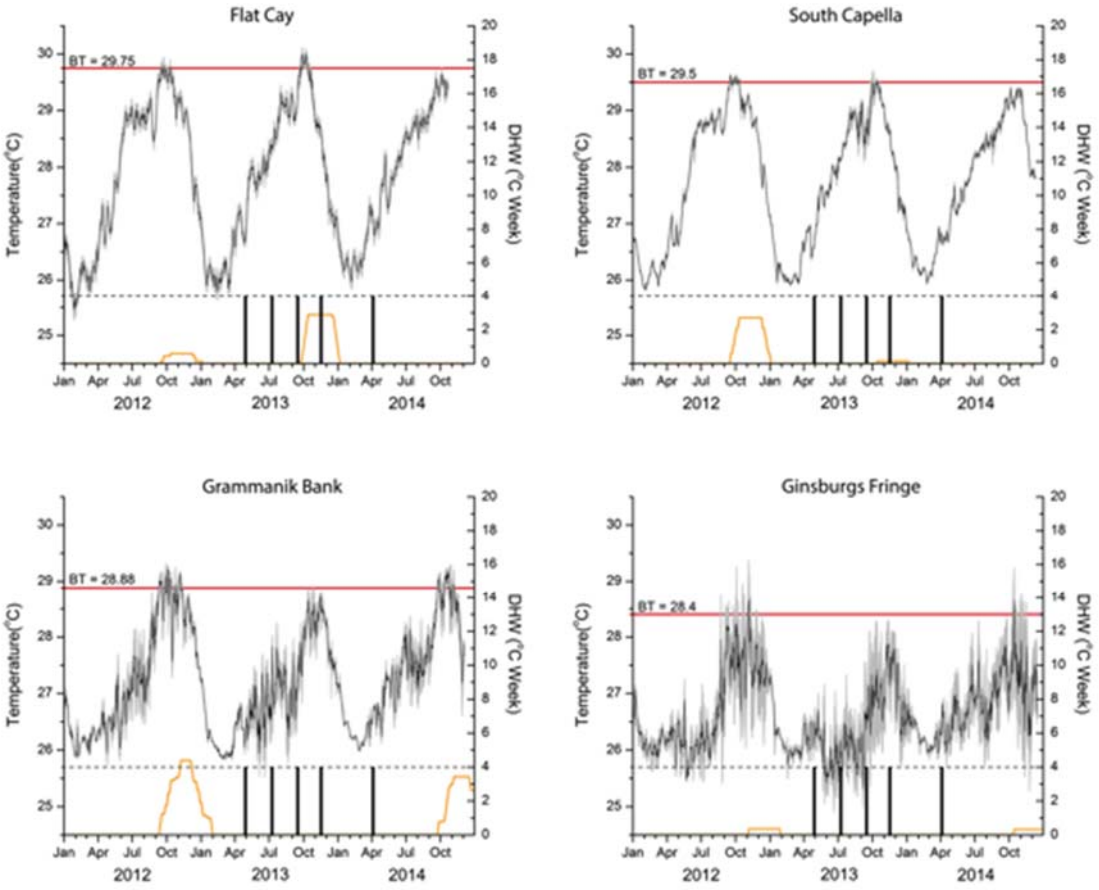

Figure 3 - Mean daily temperature and diel standard deviation (gray shading around mean line). Red line indicates bleaching threshold (BT) as calculated for each site and the BT value (°C) indicated. Yellow lines are calculated degree heating week (DHW) accumulation. Hatched black line indicates the 4 DHW level, suggested as the thermal stress level where bleaching is initiated in coral communities. Vertical black lines denote sampling periods.

Seasonal changes in water column stratification can be identified in vertical profiles (Fig. 4). During the early parts of both 2013 and 2014, the water column was well mixed to 60m depth, indicated by a consistent thermal regime and low variability in chlorophyll levels. A weak thermocline was evident at 35m in May 2013. As the summer of 2013 progressed, temperatures increased across all depths, but more abruptly shallower than 30m. During July and September thermoclines were present, resulting in a temperature range of 2°C across the sampling depth range. The November 2013 cast showed a return to the well mixed regime measured in both

spring samples; however, there were increased temperatures deeper than 30m compared to earlier in the year, most notably at depths exceeding 55m.

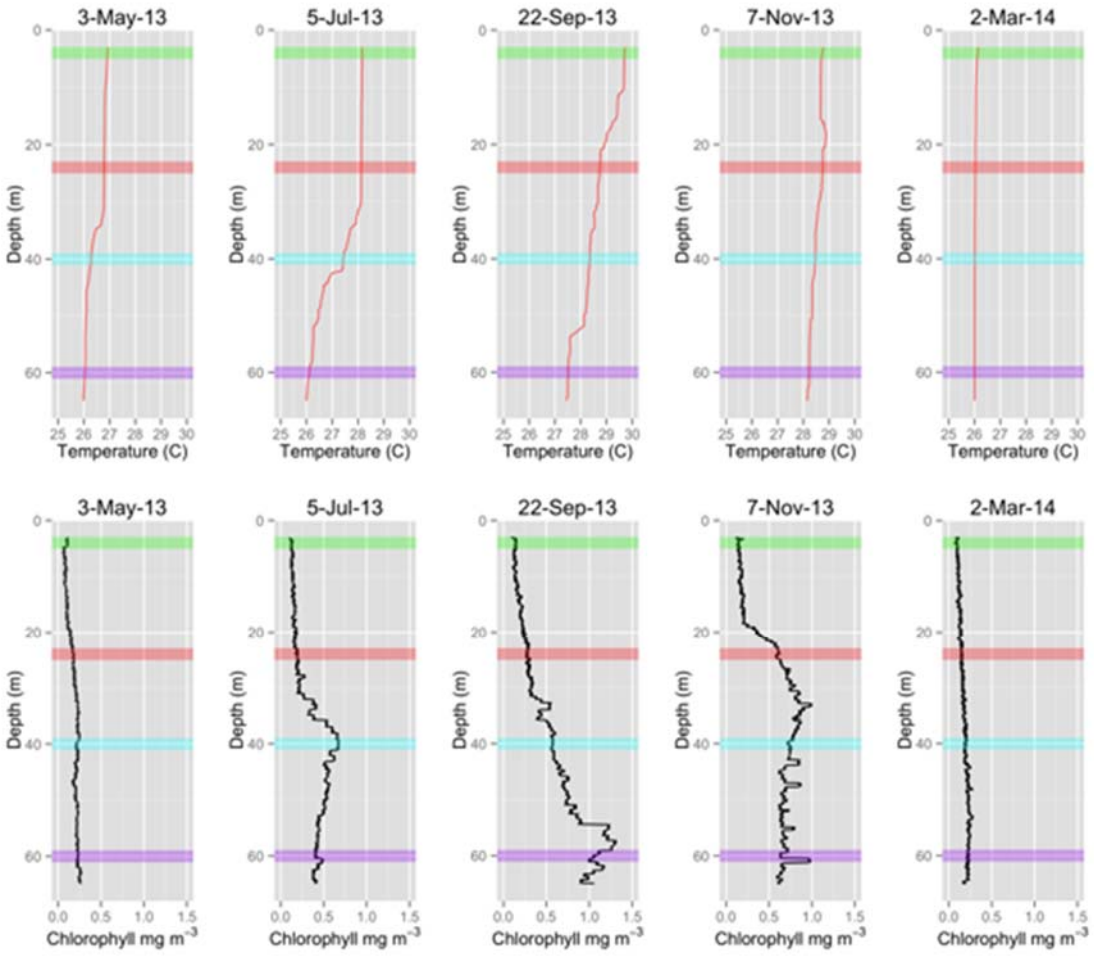

Figure 4 - Water column temperature and chlorophyll measurements concurrent with each sampling event. Horizontal colored bars correspond to sampling depths.

Variations in chlorophyll values exhibited similar trends to temperature. The spring casts showed low chlorophyll concentrations that were consistent across depths. During July and September, chlorophyll levels increased with maxima occurring at major thermocline depths. The November cast exhibited relatively consistent and high chlorophyll levels across the entire depth range below 20m. The chlorophyll maximum in July occurred at the Grammanik Bank sampling site, and the September maximum encompassed Ginsburgs Fringe. Benthic recording of chlorophyll at these

two sites in October and November 2013, between vertical profile sampling, showed that the Grammanik Bank typically had higher and more variable chlorophyll values than Ginsburgs Fringe (Fig. 5; $Mean_{Grammanik} = 0.430 \pm 0.180$ S.D., $Mean_{Ginsburgs\ Fringe} = 0.171 \pm 0.126$ S.D).

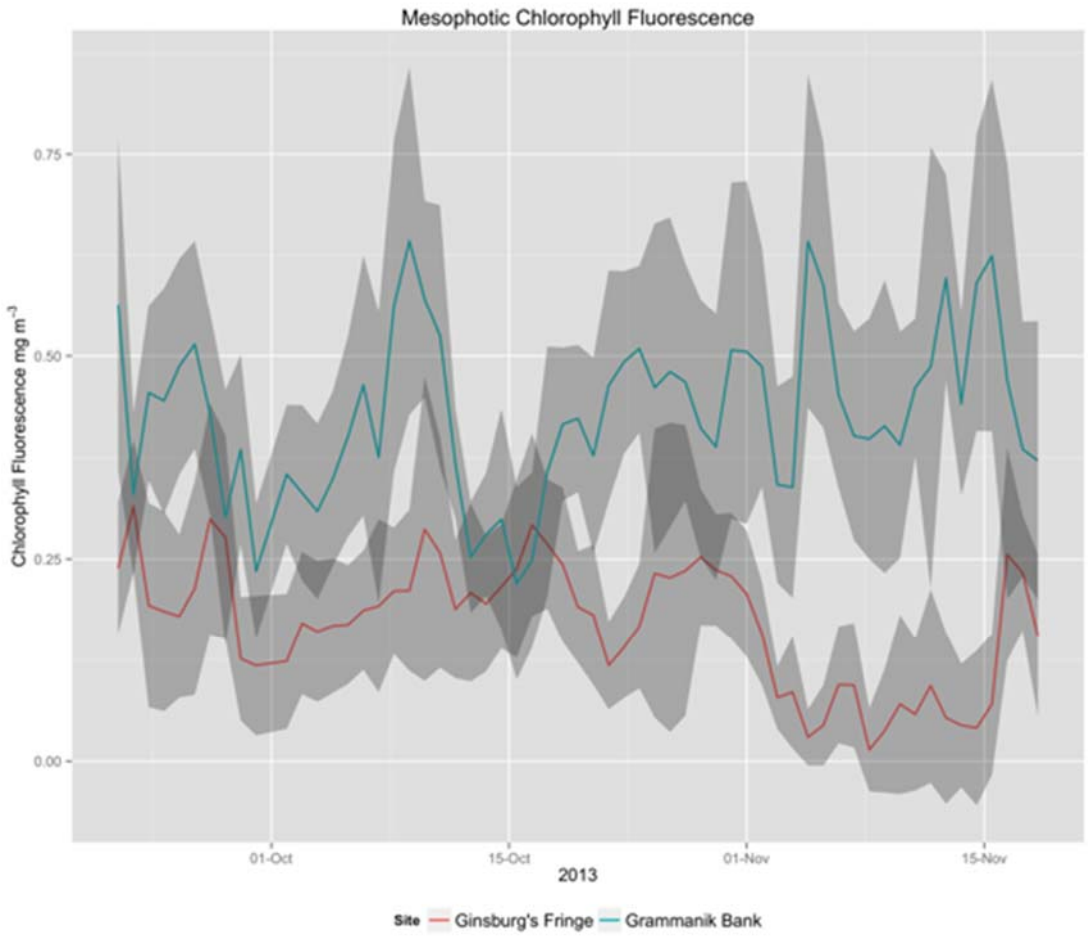

Figure 5 - Mean daily Chlorophyll concentration at Grammanik Bank and Ginsburgs Fringe from 9/21/13 to 11/19/13. Shaded regions represent daily standard deviation.

Discussion

Shallow vs. Mesophotic

Perhaps the most striking pattern revealed was the increasing variability of energy content in coral tissues with depth. The shallowest sites for both species exhibited far less change in energetic content through time than deep sites. The near-constant energy levels in shallow water corals compared to the highly variable deep corals indicate that there are habitat-specific processes driving coral energy content. Furthermore, timing differences between *O. faveolata* and *A. lamarcki* energy variation across sampling periods, but within the same sites, suggests species-specific responses to environmental variation. Energetic variability across depth is likely brought about by species-specific physiological responses to environmental variation. Better understanding the interplay between habitat variability and physiological responses will help to elucidate the refuge potential of MCE. The main mechanisms that likely contribute to depth-specific energetic responses among the corals sampled are reproduction, respiration, and autotrophy vs. heterotrophy.

Reproduction

Perhaps the most influential energetic activity corals undertake is that of sexual reproduction. The production of gametes and larvae requires considerable energy investment on the part of the coral. Richmond (1987) showed that colonies of *Pocillopora damicornis*, a brooding species in the region he investigated, invest between 2 and 20% of their total energetic content into larvae production during each month of reproduction. He went on to suggest that *P. damicornis* were investing 1-10 times the calories into reproduction as they were into growth. Assuming that the energy demands of reproduction rival those of tissue growth and maintenance, the influence of reproduction on the overall energy content of corals cannot be discounted.

The reproductive strategies of *O. faveolata* and *A. lamarcki* are different and may be very influential in the resilience of both species to future stress events. While reproduction was not measured directly in our study corals, we have inferred reproduction based on literature. *Orbicella* spp. are broadcast spawning species that release egg and sperm bundles that break up and fertilize in the water column. Reproduction in *O. faveolata* is expected in either August or September, just prior to the third sampling period in 2013 (Szmant et al. 1997). The energetic drop exhibited by the 38m colonies in September 2013 is likely to be a direct result of gamete release.

There are two factors that explain the opposing energetic trends between shallow and mesophotic *O. faveolata* during the reproductive period. First, it is probable that increased net productivity in shallow colonies allows for greater support of reproductive activities, thereby reducing or eliminating any energetic drop in shallow colonies during the reproductive season. Second, it has been shown that gametogenesis in *O. faveolata* is delayed, but more rapid once initiated, in mesophotic colonies relative to shallow colonies (Holstein 2013). Not only that, Holstein also showed that mesophotic colonies were hyper-fecund, producing greater numbers of gametes than shallow colonies. It is likely, therefore, that mesophotic *O. faveolata* experience a compressed period of strong reproductive activity, incurring the same or greater energy costs as shallow colonies over a much shorter period of time. The September drop in energetic content for mesophotic colonies is likely a result of intense gamete production followed by spawning. The lack of energetic drop in shallow colonies is probably a result of prolonged and less intense gametogenesis that is mostly or fully supported by photosynthesis.

In contrast to *O. faveolata*, *A. lamarcki* is a brooding species that undergoes internal fertilization and releases fully competent larvae during planulation. The timing of reproduction in *A. lamarcki* is unknown, but it has been suggested that planulation may occur during the spring alongside other deep-living Caribbean agariciids (van Moorsel 1982). The energetic minimum exhibited by mesophotic colonies in July 2013 supports the assertion that *A. lamarcki* are reproducing in the

first half of the year. The disparity between shallow and mesophotic energetics during reproduction is likely attributable again to differences in photosynthetic net productivity. Shallow colonies experiencing higher light levels may be capable of supporting reproduction without marked losses of energy while mesophotic colonies are not.

The timing of reproduction influences the extent to which energy content is affected in both species. Mesophotic *O. faveolata* and *A. lamarcki* both experienced similar energetic drops during their reproductive periods; however, since *O. faveolata* spawns in the fall, it may be at greater risk of disturbance in future stress events. Thermal stress across all sampling sites generally begins in the second half of September and continues through January (Figure 2). *O. faveolata* experience their energetic minimum in September, at the beginning of the thermal stress season. If algal symbionts are thermally stressed and this leads to a reduction in photosynthetic subsidies, such as occurs during bleaching events, then mesophotic corals may be more susceptible to mortality. Conversely, mesophotic *A. lamarcki* have considerably more time for energetic recovery following a July energetic minimum—with colonies exhibiting greater energetic content in September than *O. faveolata*.

Respiration, Photosynthesis, and Trophodynamics

Along with reproduction, variability in the environmental conditions on shallow vs. mesophotic reefs likely affects energy content of coral colonies. The effects of light on coral respiration are well documented. As light attenuates with depth, corals are forced to acclimate. Colonies exhibit flattened, plating growth forms intended to better capture light and reduced photosynthesis to respiration ratios (P/R ratio; Todd 2008; Lesser et al. 2010). Temperature variability can also affect the productivity of corals; increased temperatures can aid skeletal growth in corals, however benefits of increased temperature are lost as the bleaching threshold is reached (Langdon and Atkinson 2005). In addition, highly variable temperatures can

incur energetic costs due to investment in pre-emptive protection strategies such as the production of heat shock proteins (Hennige et al. 2010).

The stability of energy content in shallow corals in this study suggests that colonies at those depths may be maintaining positive net productivity throughout the year. High light levels and low short-term thermal variability at the shallowest sites may assist in maintaining more constant energy levels. Conversely, mesophotic colonies of both species are likely to receive less light and experience greater short-term thermal ranges. Both *O. faveolata* and *A. lamarcki* exhibit many of the morphological adaptations described by Todd (2008) with increasing depth, such as vertically compressed growth forms and denser skeletons.

Confounding the roles of respiration and photosynthesis in the energy content of mesophotic corals is an increased potential for heterotrophic feeding. Heterotrophic feeding can provide a coral with supplemental energy not acquired through photosynthesis—and has been shown to provide resilience during thermal bleaching events (Grottoli et al. 2006). The measured seasonal increase in chlorophyll on MCE likely indicates an increase in heterotrophic food sources (Leichter and Genovese 2006)—an assertion held true in the Bahamas when Lesser et al. (2010) found decreased $\delta^{13}C$ values among MCE colonies of *Montastraea cavernosa*. Without measures of P/R ratios and isotopic signatures it is difficult to directly correlate energetic changes shown here with small-scale coral physiological activities. A fruitful area of study in the future would be to measure these factors simultaneously in an attempt to link calorimetric measurements with coral physiological characteristics.

Synthesis

Reproductive biology and seasonal water column dynamics appear to be major drivers of the energy content of both *O. faveolata* and *A. lamarcki*—and therefore potential predictors of deep refugia. Shallow colonies exhibited the least variability in energy content through time, likely due to increased P/R ratios. Another possible

explanation for the low variability exhibited by shallow colonies versus mid and deep colonies has to do with historic seasonal dynamics. Specifically, the history of bleaching and coral mortality at 6m is considerably different than at the other two sites. Thermal stress events affected shallow water corals in 2005 and 2010 (Smith et al. 2013), but the effect of thermal stress declined with depth (Smith, in review). As such, it is possible that differential mortality has occurred between sites. It may be that the only surviving shallow colonies are those that had the most efficient energy maintenance regimes going into previous stress events, and therefore they showed constant energy content over the sampled year.

Calorimetry

Given the sporadic use of calorimetry to assess the energetic content of corals, it is important to evaluate it as a research tool. The main drawback to coral calorimetry is the extensive amount of time required to produce energetic values. The time required to go from *in situ* coral colony to energetic content value was approximately three hours per colony sampled. This includes the time requirements for post-processing, freeze-drying and running of the calorimeter. However, where time is not limited, coral calorimetry has proved to be successful at deriving habitat-specific colony energy dynamics. These dynamics could have important implications for the susceptibility of colonies to mortality in the face of thermal and other sources of stress (Anthony et al. 2007). Coral calorimetry is a valuable tool for investigating the fitness of corals through time and has great potential to further the collective understanding of coral reef response to stress events.

Conclusion

MCE as Refugia

The responses of *O. faveolata* and *A. lamarcki* to mesophotic conditions suggest differing refuge potential for each species. *O. faveolata* appears to be better adapted for shallow water living, and mesophotic colonies—though prevalent—may be at risk of future disturbance. Corals incur large energy costs during reproduction (Richmond 1987) and deep-living colonies appear to require a considerably longer recovery period than shallow colonies. Though mesophotic *O. faveolata* living south of St. Thomas may be protected from shallow water thermal stress, however, the timing of reproduction and the subsequent energetic minimum occurring in September places these corals at considerable risk. In contrast, spring brooding and subsequent energetic minimum exhibited by *A. lamarcki* colonies suggests they may be better suited for deep conditions. Colonies have enough time in less stressful, early summer conditions to regain energy content lost to reproduction. As such, *A. lamarcki* colonies living at or beyond 40m not only benefit from thermal protection attributed to mesophotic reefs, but also have a life history that allows them greater energy stores during the most stressful time of year.

The idea of mesophotic refugia—intuitively logical—is far more complicated than often presented. *O. faveolata* is far less likely to experience a refuge effect from mesophotic reefs than *A. lamarcki*. The timing of reproductive activities plays a large role in this conclusion, but it is also likely that energy acquisition methods are important for the recovery and maintenance of energy in these species. More work is needed not only to understand the driving forces behind oceanographic parameters on MCE, but also the life histories of deep living species.

References

Anthony KRN, Connolly SR, Hoegh-Guldberg O (2007) Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime. Limnol Oceanogr 52(2):716-726

Aponte NE, Ballantine DL (2001) Depth distribution of algal species on the deep insular fore reef at Lee Stocking Island, Bahamas. Deep Sea Res I 48:2185-2194

Armstrong RA, Singh H, Torres J, Nemeth R, Can A, Roman C, Eustice R, Riggs L, Garcı´a-Moliner G (2006) Characterizing the deep insular shelf coral reef habitat of the Hind Bank marine conservation district (U.S. Virgin Islands) using the SeaBED autonomous underwater vehicle. Cont Shelf Res 26:194–205

Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. Coral Reefs 29:309-327

Gleason DF, Edmunds PJ, Gates RD (2006) Ultraviolet radiation effects on the behavior and recruitment of larvae from the reef coral *Porites astreoides*. Mar Biol 148:503-512

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Change Biol 2:495-509

Golley FB (1961) Energy values of ecological materials. Ecology 42:581-584

Goreau TF, Wells JW (1967) The shallow-water scleractinia of Jamaica: revised list of species and their vertical distribution range. Bull Mar Sci 17:442-453

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186-1189

Hennige SJ, Smith DJ, Walsh SJ, McGinley MP, Warner ME, Suggett DJ (2010) Acclimation and adaptation of scleractinian coral communities along environmental gradients within an Indonesian reef system. J Exp Mar Biol Ecol 391:143-152

Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appledoorn R (2010) Theme section on "Mesophotic Coral Ecosystems: Characterization, Ecology, and Management". Coral Reefs 29:247-251

Holstein DM (2013) Vertical connectivity in mesophotic coral ecosystems. PhD Thesis, University of Miami, 204pp.

Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255-275

Langdon C, Atkinson MJ (2005) Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J Geophys Res 110:C09S7

Leichter JJ, Genovese SJ (2006) Intermittent upwelling and subsidized growth of the scleractinian coral *Madracis mirabilis* on the deep fore-reef of Discovery Bay, Jamaica. Marine Ecology Progress Series 316:95-103

Lesser MP, Slattery M, Leichter JJ (2009) Ecology of mesophotic coral reefs. J Exp Mar Biol Ecol 375:1-8

Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A (2010) Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 91:990-1003

Locker SD, Armstrong RA, Battista TA, Rooney JJ, Sherman C, Zawada DG (2010) Geomorphology of mesophotic coral ecosystems: current perspectives on morphology, distribution and mapping strategies. Coral Reefs 29:329-345

Moore Jr. CH, Graham EA, Land LS (1976) Sediment transport and dispersal across the deep fore-reef and island slope (-55m to 305m) Discovery Bay, Jamaica. J Sediment Petrol 46:174-187

van Moorsel GWNM (1982) Different reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). In: Gomez E (ed.) Proc. Fourth Int. Coral Reef Symp., Manila

NOAA (2006) Tropical Ocean Coral Bleaching Indices. National Oceanic and Atmospheric Administration, Silver Springs, Maryland. http://www.osdpd.noaa.gov/PSB/EPS/CB_indices/coral_bleaching_indices.html

van Oppen MJH, Bongaerts P, Underwood JN, Peplow LM, Cooper TF (2011) The role of deep reefs in shallow reef recovery: an assessment of vertical connectivity in a brooding coral from west and east Australia. Mol Ecol doi: 10.1111/j.1365-294X.2011.05050.x

Paine RT (1966) Endothermy in bomb calorimetry. Limnol Oceanogr 11(1):126-129

Richmond RH (1981) Energetic considerations in the dispersal of *Pocillopora damicornis* planulae. In: Gomez E (ed.) Proc. Fourth Int. Coral Reef Symp., Manila

Richmond RH (1987) Energetic relationships and biogeographical differences among fecundity, growth and reproduction in the reef coral *Pocillopora damicornis*. Bull Mar Sci 41:594-604

Riegl B, Piller WE (2003) Possible refugia for reefs in times of environmental stress. Int J Earth Sci 92:520-531

Smith TB, Blondeau J, Nemeth RS, Pittman SJ, Calnan JM, Kadison E, Gass J (2010) Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, U.S. Virgin Islands. Coral Reefs 29:289-308

Smith TB, Kadison E, Henderson L, Gyory J, Brandt ME, Calnan JM, Kammann M, Wright V, Nemeth RS, Rothenberger JP (2012) The United States Virgin Islands Territorial Coral Reef Monitoring Program. 2011 Annual Report. University of the Virgin Islands, United States Virgin Islands 247pp

Swart PK, Saied A, Lamb K (2005) Temporal and spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ of coral tissue and zooxanthellae in *Montastraea faveolata* collected from the Florida reef tract. Limnol Oceanogr 504:1049-1058

Szmant AM, Gassman NJ (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8:217-224

Szmant AM, Weil E, Miller MW, Colón DE (1997) Hybridization within the species complex of the scleractinian coral *Montastraea annularis*. Mar Biol 129:561-572

Todd PA (2008) Morphological plasticity in scleractinian corals. Biol Rev 83:315-337

Thornhill DJ, Rotjan RD, Todd BD ,Chilcoat GC, Iglesias-Prieto R, Kemp DW, LaJeunesse TC, McCabe Reynolds J, Schmidt GW, Shannon T, Warner ME, Fitt WK (2011) A connection between colony biomass and death in Caribbean reef-building corals. PLoS One 6(12): e29535. doi:10.1371/journal.pone.0029535

Yamashiro H, Oku H, Onaga K (2005) Effect of bleaching on lipid content and composition of Okinawan corals. Fisheries Sci 71:448-453