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Growth and recovery of three Caribbean scleractinian coral species following the severe thermally-mediated bleaching event of 2005

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Growth and recovery of three Caribbean scleractinian coral species following the severe thermally-mediated bleaching event of 2005

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Marine Biology by Benjamin Paul Neal

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2013
The Dissertation of Benjamin Paul Neal is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2013
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Human impacts on marine systems
Coral bleaching and recovery
Coral symbiont diversity
ABSTRACT OF THE DISSERTATION

Growth and recovery of three Caribbean scleractinian coral species following the severe thermally-mediated bleaching event of 2005

by

Benjamin Paul Neal

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2013

B. Greg Mitchell, Co-Chair

Richard D. Norris, Co-Chair

Coral reefs have been affected in the past few decades by a number of anthropogenic environmental stresses, and have suffered unprecedented declines in areal extent, live coral cover, and individual colony health. One primary factor affecting reefs has been increased thermal stress from higher water temperatures. Thermal stress is a principal environmental trigger, and increases the severity and extent of coral bleaching, a physiological response where the symbiotic
zooxanthellae in the corals are expelled. Coral bleaching is stressful to corals, and can result in rapid total or partial coral colony mortality. Furthermore, long-term impacts from these stress events are possible even after colony recovery. Post-disturbance recovery trajectories are not well defined for different scleractinian species, and are difficult to determine, given the extremely slow growth rates for scleractinian corals. Differential recovery trajectories following thermal disturbance could result in a severely altered coral community makeup over decadal time scales, affecting ecosystem sustainability and decreasing the economic services rendered by these systems. In 2005 much of the Caribbean Basin was subjected to a very intense coral bleaching event. Aspects of this bleaching event and the effects on the coral community in Bocas del Toro, Panama are investigated in this dissertation. Specifically I investigate the hydrographic and oceanographic structure leading to the thermal event, the long-term impact on coral survivorship and growth as determined through analysis of changes in the planar area of live coral tissue for individual colonies, and the effect of the disturbance event on the intracellular diversity of clades of the dinoflagellate of the genus *Symbiodinium*, which are obligate coral symbionts. This thesis provides an integrated long-term perspective of the oceanographic, organismal, and molecular realms as they relate to the community of hard corals in this location and provides an indication of how these reef communities might respond to forecasted future global increases in the severity and occurrence of thermal stress events.
CHAPTER 1: Introduction
INTRODUCTION

“Some experts look at global warming and increased world temperature as having a critical tipping point that is causing a crash in coral reef health around the world. And there's no question that it this a factor, but it has been preceded by the loss of coral resilience and degradation . . .”

Sylvia Earle, PBS NewsHour Interview, December 14th, 2004.

Corals are capable of creating largest biogenic structures on the planet, forming reefs easily visible from space through millennia of accretion. This vast scale and complexity of the reef ecosystem belies the fact that nearly all of this structure is created by the individual actions of small polyps, a dichotomy commented on by Charles Darwin during his voyage on the Beagle "We feel surprise when travellers tell us of the vast dimensions of the Pyramids and other great ruins, but how utterly insignificant are the greatest of these, when compared to these mountains of stone accumulated by the agency of various minute and tender animals!" (Darwin, 1889). We now recognize that these tender polyps, so powerful in aggregate, also host whole ecological worlds within them in the forms of communities of symbiotic algae. These endosymbionts live within a protected intracellular ecosystem, inside the very tissues of the coral animals, an environment rich in inorganic nutrients and free of predators. In return they provide the corals with the energetic products needed to fuel their
prodigious feats of construction. Healthy coral reefs are thus a study of scale within scale, consisting of healthy endosymbiont communities living inside polyps, which themselves form colonies, which form reefs, which themselves are potentially joined across oceanic basin scales, to form a globally encircling belt of tropical jewels, hosting and supporting the vast diversity of reef residents, including humans, who themselves rely on the reef for sustenance.

Coral reefs are recognized as being among the world’s most productive and diverse ecosystems (Reaka-Kudla 1997), although now are in decline worldwide (Pandolfi et al. 2003; Hoegh-Guldberg et al. 2007; Knowlton and Jackson 2008). Live coral cover is being lost at an alarming rate; Gardner et al. (2003) estimated that on average, live coral cover had declined by 80% in the Caribbean in 30 years. Recent activities of man are clearly contributing to the ecological factors behind the decline of reefs (Burke et al. 2011). These include overgrowth by fleshy macroalgae, whether due to algal growth stimulation from nutrient pollution and/or a reduction in herbivory due to overfishing (Hughes 1994, Lapointe 1997, McCook 1999, McCook et al. 2001, McClanahan et al. 2003, Smith et al. 2006); abrupt smothering or a slow reduction in fitness due to sedimentation (Dodge and Vaisnys 1977, Rogers 1990, Reigl and Branch 1995, Fabricius and Wolanski 2000); direct damage due to destructive fishing practices (McManus et al. 1997), irresponsible tourism (Barker and Roberts 2004), and boat damage (Saphier and Hoffmann 2005); and recent expansions in coral diseases (Harvell et al. 1999, Bruno et al. 2007). However, potentially the most
alarming is coral bleaching, during which corals lose the symbiotic dinoflagellate zooxanthellae algae that provide most of their food (Muscatine et al 1983; Brown 1997).

Unusually high thermal stress is one of a number of factors that can contribute to coral bleaching, and has been identified as the primary causative factor in most large-scale bleaching events (Bruno et al. 2007). Coral bleaching appears to be increasing in frequency and geographic extent as the climate warms (Hoegh-Guldberg 1999, Hughes et al. 2003), and some major bleaching episodes have led to mass mortality; for instance after a mass bleaching event in 1998, almost all *Agaricia spp.* corals died in the Belize lagoon, which had been the dominant species (Aronson et al. 2002). Interestingly, prior to *Agaricia* dominance, *Acropora spp.* were dominant in the lagoon, but were wiped out by disease in the 1980’s (Aronson and Precht 1997). While coral bleaching is an obvious disturbance, clearly visual to an observer underwater, and takes place over a discrete and fairly short period of time (weeks to a few months) the processes that both precede and follow coral bleaching are perhaps more important for understanding the long-term ecological impacts to this ecosystem. Adaptive mechanisms may exist that allow the coral to regain health in an altered environment (Baker et al. 2004), even if the disturbance conditions persist. On the other hand, there may be slow, long-term damaging effects from bleaching that accelerate the overall decline of corals, even after the acute signs of bleaching have ameliorated (Baker et al. 2008). On a global scale, we are just becoming aware of the
extent and impact that large-scale environmental alteration will are having on throughout the tropics on corals, in particular the effects of ocean acidification and the warming of surface waters associated with global climate change.

This dissertation seeks to examine the factors contributing to the 2005 record episode of warm water event and the effects from the subsequent coral bleaching event in Bocas del Toro, Panama across these multiple scales. Recognizing that the coral holobiont is affected by both large and small-scale processes, this bleaching event was examined on these scales: 1) a local oceanographic scale, limited to the western Caribbean and focusing particularly on the thermal dynamics of the enclosed Bahia Almirante embayment, in Bocas del Toro, Panama, and the area just offshore, which appears to have been the source of the anomalously warm water causing the bleaching event; 2) the colony scale, tracking individual coral colony growth and mortality for six years following the acute disturbance event of 2005; and 3) the intracellular scale, using genetic techniques to investigate endosymbiotic algae community dynamics through time of the endosymbiotic algae that live within the coral tissues and contribute much of their metabolic needs. The primary aim of this variety of disciplinary approaches is to move across the organizational scales that form a reef, to gain a more integrated understanding of how thermal stress and coral bleaching processes work in this area.

*Coral bleaching*
Coral bleaching is a generalized term for a response to stress in which the coral’s symbiotic zooxanthellae are expelled or consumed (Fitt et al. 2001). Coral bleaching occurs as a response to stress, and has been shown to occur in response to low salinities (Goreau 1964), cold temperatures (Muscatine et al. 1991) and high and low light (Glynn 1996), and other factors but on a large scale the most common cause for bleaching is thermal stress (Fitt et al. 2001). Thermal stress causes the zooxanthellae photosystem II to breakdown and produce reactive oxygen species (Lesser 1997, Downs et al. 2002). The coral host must then expel its zooxanthellae to avoid cellular damage by oxidative stress.

Corals are found in locations around the world that experience a wide range of mean temperatures, and some species have ranges that span a vast difference in thermal regimes (Hughes et al. 2003). The corals in each location appear to be adapted to their local thermal regimes, and a 1°C increase above the mean maximum monthly temperature will often result in bleaching (Hoegh-Guldberg 1999). However, there are many reasons that this thermal tolerance threshold is probably not static. Different clades of zooxanthellae have been shown to have different levels of thermal tolerance (Rowan et al. 1997, Berkelmans and van Oppen 2006, Garren et al. 2006) and corals may be able to shift or shuffle their symbionts as one way to resist future bleaching (Baker et al. 2004, Rowan 2004, Berkelmans and van Oppen 2006). Beyond this, other physiological and environmental effects beyond temperature can affect a coral’s ability to withstand or recover from bleaching. Rodrigues and Grottoli (2007) showed
that corals with higher lipid content before bleaching survived better than those with lower original lipid stores, Salih et al. (2000) found that fluorescent proteins in corals are photoprotective and can increase coral resistance to bleaching during heat stress, and there may be other as yet unrecognized physiological responses as well (Hughes et al. 2003). There is also some evidence that local stress such as poor water quality can reduce the thermal tolerance of corals (Wooldridge et al. 2009).

Because it appears that there is no simple thermal threshold at which corals bleach, it is important to compare how corals respond to subtle changes in thermal and other forms of stress, and how the history of thermal exposure for a given colony or area might affect bleaching response. To quantify thermal stress experienced by corals, several approaches have been taken. These include calculating “hotspots,” or locations in which the sea surface temperature (SST) exceeds 1°C above long-term average high temperatures (Goreau and Hayes 1994), “degree-heating-weeks,” or rolling 12-week windows that sum up all hotspot values (Strong et al. 1997), “degree-heating-months,” which draws from monthly SST data (Lough 2000), and “max-3d,” or the highest 3-day summertime SST (Berkelmans et al. 2004). High spatial and temporal resolution SST data (Berkelmans et al. 2004, Weeks et al. 2008) and reef topography data (Wooldridge and Done 2004) can more accurately predict bleaching than coarser-scale SST data, but these high resolution data are not available over long time periods. Therefore, in this dissertation work, I use a variety of records to first look at the etiology and stratified structure of the thermal stress event of 2005.
(Chapter 3) and compare the extent of bleaching observed over two different thermal stress events with similar levels of total thermal stress (Chapter 4).

**Resistance and resilience**

Coral resistance and resilience are ecological concepts that can have different meanings and implications under different applications (West and Salm 2003, Bellwood et al. 2004). I use resistance primarily to describe how an individual coral colony, or a group of conspecifics with a similar characteristic such as depth of prior bleaching history, may have differing responses to a similar level of thermal stress at different times and over time following that exposure. I use the term resilience to describe the ability of a coral animal to recover back to positive growth once a stress is removed, but on an ecosystem scale, resilience can also refer to the ability of a coral reef to remain coral-dominated (Bellwood et al. 2004, Knowlton 2004). These two processes are related, in that if the growth rate of individual corals are negatively affected, there will likely be a negative effect on the ability of that species to occupy space in the very competitive benthic environment. This may not be true in many ecological situations, if there is strong new recruitment and rapid growth of new individuals. An example of this would be in a forested environment, where post-fire succession may play a much larger role in determining community makeup than post-fire growth of the surviving individuals. However, this is generally not the case for coral reefs, where new settlement is very low, and individuals are long-lived.
Settlement and recruitment success following stress events like coral bleaching could also affect the future community makeup of the reef, but those topics are beyond the scope of this dissertation. Within the scope of looking at the effects of acute environmental thermal stress directly on coral resistance and resilience, the effects can be generally split into two groups: those that cause degradation directly from the acute and recent events in question, which in this case is bleaching but could be also disease outbreaks, explosions of predators, or damage from specific physical disturbance events such as storms, and those that cause reef resistance and resilience to be chronically reduced over time, possibly paving the way for these acute shocks to cause longer-term widespread damage over many years. In the first example, reefs are currently suffering from new and lethal stressors in the form of coral disease and bleaching (Aronson et al. 2003, Aronson et al. 2005, Aronson and Precht 2006). Conversely, under the second situation, as thermal stress events increase in extent and severity, coupled with an increase in attendant human-induced stressors like fishing and land-based pollution increase over time, reef resistance and resilience may be in a state of change, such that continued acute stress events may either send corals into sudden decline or, conversely, may over time create a community that is less subject to perturbation (Scheffer et al. 2001, Pandolfi et al. 2003, Hughes et al. 2003, Knowlton 2004, Mumby et al. 2007, Knowlton and Jackson 2008).

Within ecological theory there is support for the concept that some levels of disturbance may have positive effects on particular species within an ecosystem. There
are two examples of this that apply to this study of individual colony growth and species persistence on coral reefs. First, the Intermediate Disturbance Hypothesis (Connell 1978) states that species diversity is enhanced under some levels of disturbance. Intermediate levels of physical disturbance on coral reefs can result in decreased competitive advantage for the most dominant successional hard coral cover species, and in the case of this study, may result in lasting decreases in both size and extent of some species that are the result not of physiological impacts on those individual colonies or species, but rather from conferring an advantage on other competitive species (Aronson and Precht 1995). Second, in contrast to attributing only negative impacts from thermal disturbance, it must be recognized that there is the possibility that positive benefits may also be conferred on some individuals or species. The Adaptive Bleaching Hypothesis (Buddemeier 1993) states that coral may be an adaptive mechanism, and that after bleaching individuals or species may be able to select for either a more productive or thermally tolerant symbiont community, and thus be better suited for what were previously regarded as thermally stressful conditions, should they persist or recur. This dichotomy of possible long-terms stress responses, which on one hand can decrease the ability of corals to persist and thrive and on the other may possibly confers an adaptive advantage, as measured by changes in coral growth rates or by alterations in the symbiont makeup, is the central theme of this investigation.
Chapter 2: Improving estimations of two-dimensional area of coral colonies from underwater photographs

Aims and Objectives:
In chapter 2, I develop and test simple tools and methods for obtaining measurements of planar area from underwater photographs, using an adjustable monopod support and a semi-automated image segmentation processing system. The primary aim was to measure and detect variations in annual growth of individual coral colonies with minimal impact to the coral colonies.

Chapter 3: When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama

Aims and Objectives:
In Chapter 3, I investigate the fine-scale hydrologic structure of the high water temperature events that caused the bleaching in the autumn of 2005. Water temperature data from 1999 to 2011 from in situ instruments at three depths were used to calculate thermal stress on a coral reef in Bahia Almirante, Bocas del Toro, Panama, which was compared to satellite surface temperature data and thermal stress calculations for the same area and time period from the National Oceanic and Atmospheric Administration Coral Reef Watch Satellite Bleaching Alert system.
Chapter 4: Bleaching response, growth rates and mortality of three reef-building Caribbean corals over six years following the elevated water temperatures of 2005 and 2010

**Aims and Objectives:**

In chapter 4, I investigate the bleaching response, mortality, and recovery of three Caribbean scleractinian species (*Orbicella franksi*, *Siderastrea siderea*, and *Stephanocoenia michelini*) in Bocas del Toro, Panama over six years following the bleaching event of autumn 2005, for the purpose of predicting possible changes in the reef benthic community makeup resulting from thermal disturbance. These species-specific stress response differences have implications for the relative future persistence and dominance of these species, possibly leading to an alteration of the coral community composition of the reef over decadal or longer time scales.

Chapter 5: Long-term symbiont diversity of two reef-building Caribbean corals following bleaching disturbance

**Aims and Objectives:**

In chapter 5, I quantify changes in the community of photosynthetic dinoflagellate algal symbionts in the genus *Symbiodinium* that live within the tissues of two common reef-building coral species in Bocas del Toro, Panama. Differences in
the clade-specific ecological function of various *Symbiodinium* clades under different taxa-host relationships may confer benefits to the coral host, as corals may adapt to increasing temperatures by changing their dominant symbiont type. The long-term sustainability of the coral ecosystem may depend on the nature of this adaptive response.

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CHAPTER 2: Improving estimations of two-dimensional area of coral colonies from underwater photographs
ABSTRACT

The growth and mortality of individual scleractinian coral colonies is a core process underlying coral reef vitality and longevity. Measurements of coral live-tissue surface area are a key metric for monitoring this process. However, underwater, non-invasive, in situ, true three dimensional surface area measurements are not currently practical for large numbers of colonies. Photographically-projected, two-dimensional planar area measurements of individual colonies provide a practical, inexpensive and non-invasive proxy for true surface area, and this is thus a commonly used technique. However, significant methodological errors are often present with this method, limiting reliability and accuracy. Image processing can also often be prohibitively time-intensive. This chapter presents improvements in both hardware and software tools to increase speed and methodological accuracy for projected planar area estimations of coral colonies. Photo acquisition was improved through use of an affordable hand-carried monopod support with integrated size references, and analysis of photographs was standardized and accelerated through the use of a semi-automated graphical analysis tool. Sources and magnitudes of potential errors in measuring planar area were evaluated. Inter-operator error, intra-operator error, differences in image resolution, and inherent analytical process error were all found to be non-significant, while the significant sources of variation in repeat measurements were found to be: 1) consistency of orientation (pitch and yaw) of the linear reference; 2) horizontal alignment of the linear length reference in reference to the desired measurement plane; 3) maintaining consistent vertical aspect of photograph relative to
the subject; and 4) operator identification of bleached coral tissue. After addressing these sources of error, the coefficient of variation for repeated measurements of coral planar area in realistic ocean field conditions was reduced to 2.3%. This level of variance validates this method as a rapid, inexpensive field technique for measuring relative sizes of individual coral colonies for time-series growth monitoring, evaluating the extent of coral bleaching or disease impacts on individual colonies, and describing community size structure.

**Introduction**

Measuring size and growth dynamics of individual coral colonies over time is essential for understanding community and population structure and dynamics on coral reefs (Bak and Meesters 1998), for monitoring overall reef health (Edinger et al. 2000), and for determining disturbance impacts (Edmunds and Elahi 2007). The survival and mortality trajectories of individual colonies have been shown to be closely related to changes in environmental conditions, including water temperature, turbidity, organic carbon and nitrogen load, and other aquatic contaminants (Pandolfi et al. 2005; Carpenter et al. 2008; Cohen and Holcomb 2009), and evaluating the effects of these variables depends on having suitable field methods for evaluating colony size changes. Methods for measuring coral growth or size include hand measurements of the longest axis of the colony (Miller et al. 2000); determining surface area by covering with aluminum foil (Marsh 1970) or wax coating (Stimson
and Kinzie 1991); surface area determination through spectrophotometer readings of dye coated samples (Hoegh-Guldberg 1988); measuring displacement of buoyant weight in water (Spencer Davies 1989); measurements of linear extension over time (Bongiorni et al. 2003); three-dimensional representation of mass through extrapolation of two-dimensional photographs (Holmes 2008), deriving surface area from computer tomography (CT) scans (Laforsch et al. 2008; Naumann et al. 2009b); measuring calcification rates through extractive coring and X-ray or CT scan analysis of the cores (De'ath et al. 2009); extrapolating growth rates from point count methods of large transects (Francini-Filho et al. 2008; Dumas et al. 2009); and two-dimensional measurements of projected planar area from photographs (Rahav et al. 1991).

These techniques can be divided into those requiring the colony to be removed from the environment and those that can be performed in situ, with greater accuracy and repeatability generally available from the former, but at a cost of ease of execution and risk to the colony of perturbation. A recent methods review for estimations of true surface area identified X-ray CT scanning as the preferred method to provide the highest resolution for true three-dimensional surface area estimates (Veal et al. 2010), but this is a solution which requires specialized and expensive instruments that cannot be taken to the field, and necessitates the removal and often the destruction of the sample. These factors limit the practical application of these methods for field studies. Hand measurements of linear extension or longest colony axis is a much simpler metric, but this method is highly subject to both operator and notation error, and the
uni-dimensional metrics obtained are often not closely correlated with two-dimensional or three-dimensional metrics (Naumann et al. 2009b). There remains a need for simple, replicable, non-invasive, *in situ* coral colony growth measurements requiring a minimum of equipment, which can be used for reliable, cost-effective data gathering and ecosystem monitoring (Hill and Wilkinson 2004).

Measurements of two-dimensional planar area from projected underwater photographs are a practical solution to this need, as hand-held underwater photography while snorkeling or using SCUBA is an a simple and non-invasive method for monitoring coral ecosystems (Hill and Wilkinson 2004). Determining planar area from the photographs of individual colonies is a seemingly simple method to derive live tissue areal extent (Holmes 2008). However, significant potential error sources exist in both acquiring and processing the images, leading to highly inaccurate estimates of colony sizes (Naumann et al. 2009a). Potential sources of error in the acquisition of benthic photographs addressed during this experiment include changes in the both horizontal and vertical aspect of the camera relative to the subject, not using or incorrectly placing a standard length reference in the photographs, and poor quality of the images due to movement or being too far from the benthos. Similarly, processing error sources were identified to include intra- and inter-operator methodological error and inaccurate estimation of the area from the identified polygons in the photographs due to the software used. This analysis of coral photographs has been performed using a variety of software programs, including Coral Point Count (Kohler and Gill 2006),
Sigma-Scan (Leujak and Ormond 2007), ImageJ (Lirman et al. 2007), VidAna (Gonzalez-Rivero et al. 2012), and Photoshop (Shaish et al. 2008). In aggregate, both photographic and processing error sources can present a significant impediment to deriving consistent planar area data for coral colonies from projected photographs, and also for comparison and analysis of this data across space and time. This error can easily exceed annual growth for many non-branching scleractinian corals (Naumann et al. 2009a), rendering the data of little value for tracking inter-annual changes.

Coral imagery for scientific use can be acquired in a variety of ways, including taking hand-held images taken while free swimming, often using stills from continuous video, towing a photographer on an underwater sled (Kenyon et al. 2006), towing camera gear remotely (Zawada et al. 2008), using a stable rectangular-bottomed photo framer sitting directly on the benthos (Edmunds 2002), extracting still images from video (Lirman et al. 2007), or using a monopod or bipod support as described herein. Of these methods, the most common for coral surveys while diving is to use a photo framer, but there can be difficulty reliably setting a large rectangular-bottomed framer vertically on the benthos in areas of high rugosity or slope, as are common for many coral reef field study sites. This makes imaging both large and small corals more difficult, as the range of framing the image vertically is fixed. There is also the potential for a framer to have deleterious impact on the coral being observed, and some research permits for protected areas (such as the Florida Keys National Marine Sanctuary) consequently now do not allow equipment to touch the
bottom. Furthermore, the need for a framer introduces additional hardware expense and increased handling effort and time underwater, making it inappropriate for many community-based or other remote field research projects hoping to gather data on a large number of subjects with a minimum number of dives. A cost effective and practical alternative is to take hand-held, free-swimming (on SCUBA), *in situ* digital underwater photographs. Here, I focus on improving the accuracy of data acquired using these methods, as opposed to using a photographic framer, due to these practical imaging and site concerns.

The primary goal of these experiments was to examine the magnitude of error incurred from different sources when measuring planar area of individual coral colonies using a monopod imaging system coupled with semi-automated image processing. I describe changes made to improve both precision and accuracy of the method, and quantify the magnitude of error that can be expected when making planar area measurements in realistic reef field conditions. The hardware solution focuses only on using a lightweight monopod support, as this is more practical to deploy in many complex coral environments than other photographic methods. The software presented however is appropriate for use in analyzing images acquired in any fashion, including monopods, bipods, framers, other fixed camera mount, or video stills, and is optimized with features specific to deriving planar area for individual coral colonies. Given the relative speed and accuracy of this method, it is presented as a method appropriate for the estimation of annual growth or disturbance-related mortality in
hard corals, as demonstrated in Chapter 4 of this dissertation. With minimal equipment needs, it is also presented as a method applicable for wider use in community-based monitoring or other research efforts with limited time and funding seeking to make rapid but reasonably accurate and replicable measurement of coral colony size with minimal cost. The two-dimensional measurements obtained can be compared directly, or can for some species be used to extrapolate estimated three-dimensional surface area (Holmes et al. 2008).

**Materials and methods**

The system presented and tested here has three primary components: 1) a simple, lightweight monopod camera support; 2) a consumer underwater camera; and 3) semi-automated image processing software.

*Monopod camera support*

The camera support consists of a primary vertical member with an attached adjustable standard length reference and a horizontal crossbar mounting an underwater camera (Figure 2.1). The monopod system is designed to be lightweight, for ease of deployment, and also to allow easy adjustments by hand while underwater (Figure 2.2). The central support is a 150cm length of 1.25 inch aluminum round rod, cut in half and threaded for ease of transport. Two sections of sliding aluminum tube are placed on this rod, the lower one perpendicularly supporting the length reference (an aluminum commercial Amphibico ACWB0711 underwater white balance and color
chart), and the upper one attaching the camera-supporting crossbar. Both tubes are tapped for oversized thumb-screws with silicon-tipped ends, enabling fast but secure underwater adjustments. The top tube is perpendicularly attached to a section of aluminum square tube through which slides the camera-support arm. This piece is also tapped for thumb-screws to fix its position. Strobes were mounted with consideration to not restricting the sliding motion of the camera. A Solidworks manufacturing schematic of all parts of the monopod is available at:

http://vision.ucsd.edu/content/coral-colony-segmentation-and-area-measurement-tools.

**Image acquisition**

Photographs were taken with either a 10 megapixel Canon Powershot G11 digital camera, with a commercial Ikelite underwater housing and a flat front lens port, or a 21.1 megapixel Canon 5D Mark II with a Sea and Sea underwater housing, a 17-40 mm lens, and a dome port. Illumination was provided with either dual Ikelite DS-51 or DS-151 substrobes with diffusers with TTL strobe control enabled. Images were obtained in both CR2 RAW and JPG formats using ISO 200, on exposure control mode with exposure compensation set as needed between 0 and -2.0 to prevent overexposure, as judged by histogram readings of each individual photograph. Most underwater camera housings capable of being securely mounted to a tripod are adaptable to the monopod hardware.
Image analysis

Image analysis and area measurement are done with a semi-automated Matlab-based program based on the algorithm GeoStar (Gulshan et al. 2010) producing graphical as well as numerical area output for each segmented layer (Figure 2.3). Segmentation is semi-automatic, with a contour automatically generated after placing strokes inside and outside of the designated area, and subsequently adjusted by hand. Layers are user-defined (e.g. live, bleached, and partially bleached tissue), non-overlapping, and edge-snapping (improving operator speed when creating multiple layers). Bleached areas are delineated automatically with an adjustable white-saturation detection tool. Planar area is calculated by operator annotation of the length reference, correcting if needed for optical (lens or refraction) distortions in the image (Treibitz et al. 2012). These distortions are largest in image edges, and so pragmatically can be minimized by using the adjustable capability of the monopod to position both subject and length reference as close to the frame center as possible.

Program and operating manual are available on: http://vision.ucsd.edu/content/coral-colony-segmentation-and-area-measurement-tools.

System evaluation, improvements, and statistical analysis

To assess replicability and accuracy of the combined monopod and software systems four independent repeated-measures tests were performed. Test #1 established system accuracy with the length reference fixed on the monopod and vertically adjusted by diver buoyancy. Two Siderastrea siderea colonies were photographed
independently ~150 times, in 4 meters of calm water in Bocas del Toro, Panama. Tests #2 and #3 incorporated hardware improvements, using standardized targets (a sphere and hemisphere of known size) in both controlled pool conditions (to assess method-induced variance) and natural ocean conditions with significant water movement from a 1-2m swell (to assess field-induced variance). Test #2 added a lower slide for adjusting length reference height, enabling it to be individually set for each colony. Test #3 improved vertical aspect control with two bubble levels mounted to control side-to-side and fore-and-aft alignment. Targets were photographed both horizontally and vertically, to simulate measurement of both horizontally extending (massive or plating) and vertically extending (branching) coral morphologies. Test #4 returned to field conditions and natural subjects to quantify error expected in realistic field conditions; 23 colonies of mixed species were independently photographed 14 times each in 3-6 m of water with a 0.5 – 0.75 m swell in varying natural light conditions at Great Guana Cay, Bahamas, and independently measured for planar area. For each repeated–measure data set the coefficient of variation (CV) was calculated for each parameter (live area and bleached area for natural coral targets, and target area only for artificial targets), by dividing the mean by the Standard Deviation (SD), expressed as a percentage.

Inter-operator error was estimated by having two independent operators process all images from Test #1 (n=157). Intra-operator error was estimated for both operators through blind reanalysis of a randomly chosen subset of images.
subsequently compared to earlier measurements by the same operator (n=14).

Variance between camera systems was evaluated by shooting duplicate photosets in Test #3 with our two cameras (n=36). Resolution differences between RAW and JPG formats were determined by repeating segmentation and measurement for both file types of a randomly assigned subset of Test #1 (n=18). Systemic processing error was estimated by repeated blind re-analysis of a single image (n=12). Means and standard deviations for all above comparative datasets were compared for significance of difference using two-tailed Student’s t-tests (α=0.05).

**Results**

*Observed measurement variance for repeated measure experiments*

The first two experiments reported here (both part of Test #1) used a naïve measurement setup, with a randomly chosen natural target with an unknown correct area solution. For Colony 1 from the Panamanian photoset, the mean live pigmented coral tissue for all measurements from all photos (n=154) was 299.16 cm$^2$ with a maximum observation of 351.83 cm$^2$ and a minimum observation of 221.63 cm$^2$, and a standard deviation of 27.47, yielding a Coefficient of Variation (CV) of 9.18%. There was no bleached tissue area on Colony 1.

For Colony 2 from the Panamanian photoset the mean live pigmented coral tissue for all measurements from all photos (n=160) was 202.52 cm$^2$ with a maximum observation of 265.89 cm$^2$ and a minimum observation of 126.87 cm$^2$, and a standard
deviation of 20.81, yielding a CV of 10.27%. The mean bleached coral tissue area for all photos of Colony 2 (n=160) was 10.42 cm$^2$ with a maximum observation of 14.38 cm$^2$ and a minimum observation of 6.51 cm$^2$, and a standard deviation of 1.44, yielding a CV of 22.12%.

At this stage the monopod support was modified slightly from the previous experiment with a slider to allow for easier vertical adjustment of the length reference as well as for enabling grounding of the end of the monopod firmly on the substrate when framing the shot, enhancing reliability and stability of the relative planar placement of the linear length reference card. Repeated observations of the spherical target in natural ocean conditions (6 meters depth, sandy bottom, with strong wave surge and current) were made at a variety of aspect angles. These observations had a mean of 229.23 cm$^2$ for all photos (n=16), and a standard deviation of 9.26 cm$^2$, yielding a CV of 3.09%.

To address the issue of aspect-induced error, the monopod at this stage was further modified with the addition of dual bubble levels to allow for enhanced visual vertical control. Repeated underwater independent measures of the hemispherical target were made by hand in a horizontal position, demonstrating a mean of 183.71 cm$^2$ (n=14), and a standard deviation of 2.58 cm$^2$, yielding a CV of 1.4%. This was the first experiment for which the measurements were compared to a known correct answer; the hemispherical target when viewed from the side had a measured actual
planar area of 183.047 cm², giving an actual mean measurement error, if using the mean of all photos, of 0.36%. The mean of a random selection of any three images from the photoset in all model cases (n=20) varied less than 0.58% from the known area value of the target. However, accurate measurements of known area at various angles between 0° and 90° were not attained (error from expected values was -1.8 to 8.9%), even though the variation for sets of repeated measurements at a number of chosen angles was similar to the range described above.

For the final test in field conditions, (repeat observations of 23 mixed-species colonies in a transect in the Bahamas) the mean CV for all within-colony variance was 2.26% (n=227), with a maximum within-colony mean variance figure of 3.75% and a minimum of 0.99%. Only live pigmented coral tissue was measured for all colonies, as no bleaching was present in these subjects. The maximum measured individual colony size in the transects for was 1048.11 cm², and the minimum was 44.98 cm², with four genera represented.

_Evaluation of intra- and inter-operator error_

There was no significant difference ($t_{0.05,152} = 0.295, p = 0.768$) in measurements of live pigmented tissue area for Colony 1 between Operator 1 (mean area = 298.5 cm² ± 1SD 27.23 cm²; n=77) and Operator 2 (mean area = 299.81.5 cm² ± 1SD 27.86 cm²; n=77). Maximum and minimum observations were 344.91 cm² and
351.83 cm² and 221.63 cm² and 225.7 cm² for both operators, respectively. There was no bleached coral tissue area on Colony 1.

There was no significant difference ($t_{0.05,158} = 0.812, p = 0.418$) in measurements of live pigmented tissue area for Colony 2 between Operator 1 (mean area = 201.16 cm² ± 1SD 20.16 cm²; n=80) and Operator 2 (mean area = 203.88 cm² ± 1SD 21.45 cm²; n=80). Maximum and minimum observations were 265.81 cm² and 283.14 cm² and 137.55 cm² and 126.87 cm² for both operators, respectively.

There was no significant difference ($t_{0.05,158} = 0.177, p = 0.86$) in measurements of bleached coral tissue area for Colony 2 between Operator 1 (mean area = 10.44 cm² ± 1SD 1.3 cm²; n=80) and Operator 2 (mean area = 10.40 cm² ± 1SD 1.57 cm²; n=80). Maximum and minimum observations were 13.09 cm² and 14.38 cm² and 6.66 cm² and 6.51 cm² for both operators, respectively.

**Methodological error**

There was no significant difference ($t_{0.05,26} = 0.651, p = 0.521$) in intra-operator measurements of live pigmented coral tissue area for Colony 1 shown for a repeated analysis of a randomly chosen subset for either Operator 1 (First set mean area = 303.52 cm² and second set mean area 302.27 cm²; n=14), ($t_{0.05,26} = 0.909, p = 0.372$), or Operator 2 (First set mean area = 305.25 cm² and second set mean area 304.37 cm²; n=14).
There was no significant difference ($t_{0.05,18} = 0.286, p = 0.77$) from using either the RAW image files or the JPG image files for measurements of coral area, using images of Colony 9 from the Bahamian photoset (RAW file mean area = 535.11 cm$^2$ ± 1SD 9.28 cm$^2$; n =10), (JPG file mean area = 536.17 cm$^2$ ± 1SD 8.84 cm$^2$; n =10).

There was also no significant difference found in mean error between images taken with the two camera systems, with three trained operators each analyzing twelve images from each camera system. The mean coefficient of variation for the 10 megapixel image set was 4.01% (± 0.82), and for the 21.1 megapixel image set was 3.69% (±0.77).

**Discussion**

These experiments and improvements began after noting significant unexplained variance in the measured planar areas from initial photos taken on SCUBA without a fixed benthic framer for the camera (i.e. free-swimming with no monopod). This unexplained variance rendered data derived from these individual observations unreliable, as the error potentially exceeded the expected annual planar area growth signal for small massive-type coral colonies typical for the area where this time series was located (estimated at a maximum of approximately 10% per year, given an estimated linear growth rate of 0.5 cm yr$^{-1}$ for *Siderastrea siderea*, a common species in these images). This methodological variance was deemed excessive, considering that the aim was to track inter-annual colony changes. The purpose of the
set of experiments reported here was to reduce the variance of the planar projection photography method for measurement of coral colony areas to a level appropriate for replicable, rapid field assessments of coral colonies.

The first photoset of individual coral colony images taken by these authors exhibited unexplained variance ranging from 2.18% to 22.12% for various cord-length and planar area parameters of the same colony. With the introduction of a rigid monopod support with robust, 90° attachments for both the linear length reference and the camera housing (thus making the lens plane and the CCD plane of the camera itself parallel to the plane of the length reference) that ensured a perpendicular alignment of these two elements, the mean CV for repeated-measures of projected planar area was reduced to 3.75%. These supports were engineered with silicon-tipped hand screws to be easily adjustable in underwater conditions, so that the length reference could be fixed at any point to be in the same plane as the maximal planar extent of the colony even while firmly grounding the foot of the monopod. Vertical aspect control of the monopod support was then improved by the addition of dual horizontal bubble levels for both pitch and yaw, which further reduced the independent repeated-measures CV in still water conditions to 1.4%. Further practice with the standardized target under ideal underwater pool conditions shooting horizontally reduced the independent repeated measures CV measures to 0.28%, with an actual mean measurement error (compared to the known planar area of the standardized target) of 0.36%, when using multiple photos, with no single
measurement in the set varying by more than 1.6% from the known planar area. This was the minimum achievable inherent methodological error for hand-held photography, and is deemed precise enough for measurements of growth in coral colonies on less than an annual basis, and possibly even monthly for fast growing species. A final field test was then performed in realistic reef conditions, with an ocean swell and a highly rugose coral benthos. This test had a repeated-measures CV of 2.26%, or approximately one quarter of the annual growth expected for the typical small hard coral colonies in our original study. Note that this error is what would be expected from taking only single images of each subject for each observation period, and could likely be reduced further in actual data gathering by the practice of photographing and measuring each subject repeatedly for each individual observation time period (for example at least three times on the day of the observations, if this extra effort is allowed by the time limitations of the given investigation), and calculating the planar area individually for each image, and then using a mean of this set of measurements as the single annual observation data point. Camera differences in both image size and digital format, inter-operator error, intra-operator error, and software processing error were all also examined and shown to not be significant sources of the measurement variation. Image acquisition time depended on the field site, with up to 60 corals sampled on a standard dive at ~10m. Processing time for the images varied from two minutes to a half hour, depending on the complexity of the fragmentation and/or bleaching patterns present.
The initial goal of reducing the planar area measurement error for coral colonies to an acceptable level for a rapid assessment was thus achieved (Figure 2.4). By constructing and deploying the simple alignment tools demonstrated here, with a standard commercial digital camera with a matched housing and lens, and by using the semi-automated image processing system we present, making this measurement is shown to be reasonably accurate and precise, to be replicable across operators and time, and able to be performed with a minimum of cost and labor. Two-dimensional, planar-projection photography can thus be employed as a simple, rapid, and inexpensive method for accurately assessing a number of coral growth or community parameters, with applications including intra and inter-annual time series observations for individual colony growth and mortality, quantitatively monitoring and measuring the extent of bleaching and disease impacts in individual colonies of a larger population, and describing community size structure of small to medium sized coral colonies for a given area (Figure 2.5). With only simple hand-carried equipment, this method is useful for remote sites, single-operator studies such as student work, and community-based monitoring efforts operating under restrictions of time and money.

Planar area as a parameter has also been shown to often not translate directly to surface area across species or colony sizes (Naumann et al. 2009a). True surface area is in many cases a more meaningful description of coral biomass for species with significant three-dimensionality. However, attaining accurate three-dimensional representations is a much more difficult photography and image interpretation
problem, and measuring this parameter directly usually involves impact to the living coral. Therefore planar area is offered here as a more attainable measure, but not as a replacement. Development of a species-specific and size-specific conversion parameter for estimating 3D surface area from accurate planar area for a given study can in some cases allow for a surface area proxy to be calculated (Holmes 2008).

It is not necessary to use an expensive camera system for this measurement. Rather, in place of purchasing a costly large format single-lens reflex camera, a focus by the operator on careful control of the alignment factors described above, along with careful attention to supplemental light and correct photographic exposure and focus, will result in images from which reasonably accurate data can be derived that will yield insights into differing coral growth, survival, and mortality trends across time and space. Both the custom fabrication of the monopod apparatus as well as the purchase the camera and underwater housing should be within financial limits for most student, community or local groups.

However, it is critical that the mechanical parallel alignment of linear length reference and the camera plane be strictly maintained for all photos. It is also critical that the linear length reference is correctly and securely aligned relative to the maximum planar extent of the coral colony. The mechanism for doing this must also be able to be easily and relatively precisely manipulated in diving conditions, as this adjustment of length reference height must be done individually for each photo. The
maintenance of verticality of the monopod for the observation was also found to be a factor, as determined by an easily used physical protractor or bubble level system. If this image framing system is to be used for reliably replicating images at known angles, for example for images taken of colonies on vertical wall surfaces, then a more accurate inclinometer must be utilized for consistency.

This system was found to be most accurate in measurements of live, healthy, fully pigmented coral tissue. Measurements of bleached coral tissue exhibited much higher variation in these experiments than did the live area measurements. This is likely from both actual differences in the presentation of the bleached area under differing lighting and water quality conditions, as well as the lack of a specific spectral definition of bleached tissue, which was determined subjectively by each operator. The semi-automated processing tool for detecting bleached areas did rapidly select designated areas based on relative white-saturation values of those areas, but it must be noted that this software feature is equipped with a sliding intensity scale, and thus, while being automated, is equally subjective as human outlining. In practice, this feature was often not as robust in the operation of selecting bleached areas as desired, but it did provide a very rapid rough assessment of the extent of bleaching for an outlined colony in a photo, which could then be adjusted with hand corrections to the outline. There is also a natural spectrum of color in both healthy and bleached coral tissue, and thus deciding on an absolute spectral definition for the bleached parameter was found to be not possible, and achieving consistency within a research group on the
definition of bleached coral area through discussion, examination of results, and familiarity with the species of concern, is paramount for achieving ecologically useful results. Practically, all photo-processing operators for a project must agree on what is defined as live or bleached coral tissue for that investigation. Team discussion, analysis tuning, using species specific definitions of bleached area, and consistency of operators from year to year will greatly contribute to extracting meaningful bleaching information describing long-term size changes and bleaching conditions on a given reef.
Figure 2.1: Camera support setup for planar area analysis. A) Monopod with camera and strobes on the top arm and length reference and color correction card attached to lower sliding support, and b) imaging a small colony while on SCUBA in a patchy coral reef. Photo in 2-1.B by Mary Alice Coffroth.
Figure 2.2: Simplified schematic of the monopod camera support device, showing important construction details.
Figure 2.3: Image segmentation examples using this method, with perpendicularly fixed size reference held by the monopod (not in image frame) showing: a) horizontal image of a juvenile *Acropora cervicornis* colony from a restoration nursery, b) same image segmented for measurement of linear extension, c) vertical image of a tagged *Stephanocoenia michelini*, d) same image segmented for area measurement of healthy and bleached tissue, and e) graphical user interface of semi-automated segmentation program.
Figure 2.4: A) Mean and variance from repeated independent free-swimming underwater measurements of a single natural colony of unknown true size with: 1) the length reference fixed to the monopod (mean=299.16 cm$^2$, SD=17.47, CV= 5.84%, n=72) and 2) the length reference placed directly on the benthos next to the colony (mean= 307.46 cm$^2$, SD= 24.75, CV= 8.05%, n=76). Note reduced variance with the size reference fixed on the monopod (i.e. due to being fixed parallel to the camera lens plane), and size overestimation from placing size reference on the benthos, (i.e. below the measurement plane of greatest areal extent). B) Means and variance for repeated independent underwater measurements of an artificial hemispherical target when: 1) free swimming with monopod (mean=359.8 cm$^2$, SD=11.01, CV= 3.03%, n=48); 2) grounding the monopod on the substrate and using the sliding adjustable reference (mean=367.9 cm$^2$, SD= 6.9, CV=1.8%, n=36); 3) with bubble levels to control vertical aspect (mean=366.9 cm$^2$, SD=1.03 , CV=0.28%, n=42). Dotted line indicates actual planar area of target when viewed from directly above (366.4 cm$^2$). Free-swimming measurement shows underestimation from tendency to misplace the length reference above the intended plane when free-floating.
Figure 2.5: Example of colony size data obtained with these methods, showing recovery from bleaching disturbance over eight years, with segmented live tissue from 2005, 2007, 2009, and 2013. Color differences are from varying water properties and camera/lens differences, and do not affect area calculations.
References:


document the status of coral reef communities. Environmental Monitoring and Assessment 125:59-73


CHAPTER 3: When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama
When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama

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Abstract Coral reefs are increasingly affected by high-temperature stress events and associated bleaching. Monitoring and predicting these events have largely utilized sea surface temperature data, due to the convenience of using large-scale remotely sensed satellite measurements. However, coral bleaching has been observed to vary in severity throughout the water column, and variations in coral thermal stress across depths have not yet been well investigated. In this study, in situ water temperature data from 1999 to 2011 from three depths were used to calculate thermal stress on a coral reef in Bahia Almirante, Bocas del Toro, Panama, which was compared to satellite surface temperature data and thermal stress calculations for the same area and time period from the National Oceanic and Atmospheric Administration Coral Reef Watch Satellite Bleaching Alert System. The results show similar total cumulative annual thermal stress for both the surface and depth-stratified data, but with a striking difference in the distribution of that stress among the depth strata during different high-temperature events, with the greatest thermal stress unusually recorded at the deepest measured depth during the most severe bleaching event in 2005. Temperature records indicate that a strong density-driven temperature inversion may have formed in this location in that year, contributing to the persistence and intensity of bleaching disturbance at depth. These results indicate that depth may not provide a stress refuge from high water temperature events in some situations, and in this case, the water properties at depth appear to have contributed to greater coral bleaching at depth compared to near-surface locations. This case study demonstrates the importance of incorporating depth-stratified temperature monitoring and small-scale oceanographic and hydrologic data for understanding and predicting local reef responses to elevated water temperature events.

Keywords Temperature stress · Bleaching · Depth stratification · Caribbean

Introduction

Globally, coral reefs have been subject to increasing episodes of coral bleaching, caused largely by periods of elevated ocean water temperatures over the past two decades (Glynn and D’Croz 1990; Knowlton 2001; Hughes et al. 2003). In 2005, much of the coral reef habitat in the Caribbean basin was affected by a record-breaking warm water-mediated mass-bleaching event (Eakin et al. 2010), causing significant mortality and an attendant loss of ecologically important structural complexity (Alvarez-Filip et al. 2009). Such acute bleaching events are predicted to increase in both frequency and geographic scale in future years (McWilliams et al. 2005). Therefore, an improved understanding of the causes, effects, and variations of
thermal stress on corals is essential for management and conservation of reefs (Marshall and Schusterberg 2006).

Spatial and temporal patterns of sea surface temperature (SST) conditions and the consequent thermal stress on coral reefs have been well described (Pandolfi et al. 2005; Maima et al. 2011), and monitoring efforts have primarily focused on these surface temperature trends. For example, the decision support system (DSS) operated by the United States’ National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch (CRW) is the most extensive program providing near-real-time coral bleaching thermal stress monitoring information (Liu et al. 2005, 2008), but is based on satellite SST, and thus reflects only near-surface temperature conditions with a spatial resolution of 0.5° by 0.5° (approximately 50 km by 50 km cells) and a twice-weekly temporal resolution.

In normally stratified conditions, most physical stressors that originate at the sea surface, such as thermal stress caused by high SST, photic stress from visible or ultraviolet radiation, and physical stress from surface waves, can be expected to attenuate with depth (Brown 1997; Glynn 1996). Colonies at greater depths, if protected from disturbance, may serve as potential recruitment sources (Hughes and Tupper 2000; Mumby et al. 2011), and this recruitment has been proposed as a resilience mechanism supporting future recovery and resettlement of disturbed corals in shallower waters that have suffered major stress (Hoey-Guldberg et al. 2007). However, depth may not always provide this function, as corals in different depth strata in reef environments can be subject to thermal conditions different from those at the surface due to local circulation patterns (Leichter et al. 2006). Further, colonies living at deeper depths may also be differently acclimated and potentially more sensitive to temperature changes of smaller magnitude (Oliver and Palumbi 2011; Cuerl et al. 2012). Our study area provides a case study for investigating the structure and impacts of depth-dependent thermal dynamics on reef environments, as it is an area of extensive coral development that experiences large variation in both temperature and salinity (Kaufmann and Thompson 2005).

During the widespread 2005 Caribbean mass-bleaching event, one of the authors (D. Kline) noted strikingly increased coral bleaching extent at 10 m depth compared to bleaching observed at 1–3 m (Fig. 1), counterproving expectations that the impacts of thermal stress would be greater near the surface. This observation was supported by measurements of bleaching extent through planar area analysis of photographs of individual colonies, showing bleaching extent per colony increasing with depth for two species (Stenanthus siderea and Stephanocea michelini; D. Kline, personal observation). To investigate the eology of this unusual vertical distribution of bleaching severity, depth-stratified temperature data were used to calculate thermal stress indices through time and across depth. Specifically, the potential impact of localized, depth-specific temperature forcing was investigated by comparing the NOAA CRW satellite SST-based coral bleaching thermal stress index for this area to similar indices calculated from in situ temperature loggers from three depths. Given projected increases in severity and frequency of bleaching events (Moynard et al. 2009), understanding the

![Image](image_url)
impacts of patterns in thermal stress throughout the water column will be critical for making spatially accurate predictions about the extent and severity of bleaching.

Materials and methods

Two separate sea temperature time series were used to calculate coral thermal stress indices near Bocas del Toro, Panama: (1) NOAA CRW satellite SST from the closest cell to the area; and (2) stratified in situ seawater temperature measurements from the Smithsonian Tropical Research Institute (STRI) Physical Monitoring Program. The former is a single record, while the latter consists of three independent records from different depths at one location. All four datasets were analyzed identically to produce directly comparable calculated stress metrics. The NOAA CRW 50-km satellite cell and record will hereafter be referred to as the offshore site and record, and the three STRI datasets will collectively be referred to as the inshore site and record.

The NOAA CRW SST record is from a single satellite data cell of 0.5° × 0.5° spatial resolution (approximately 2,500 km²) centered at 10°00’ north/82°00’ west (Fig. 2). This is the cell closest to Colon Island, Bocas del Toro. In the absence of other data, this would be the most applicable cell for predicting local coral bleaching thermal stress. However, the large size of the cells and the necessary exclusion of cells containing terrestrial surface due to potential contamination of the satellite’s temperature measurement have the consequence of excluding direct coverage of actual coral habitat, most of which lies within the embayment; rather, this nearest reference cell contains only open ocean water. This dataset is a twice-weekly whole-cell mean SST time series, derived from nighttime measurements by advanced very high resolution radiometers (AVHRRs) on NOAA’s Polar Operational Environmental Satellites (POES). Only nighttime SSTs were used to reduce the influence of daily warming from surface solar heating and to avoid potential contamination from solar glare (Liu et al. 2008). Nighttime SST compares favorably with in situ temperature at 1 m depth (Gleason and Strong 1995).

Unlike the offshore surface record from NOAA CRW, which does not include any coral area and thus may not accurately represent actual ecosystem conditions, the inshore depth-stratified site was located on a patchy coral reef (Guzman and Gaspar 2001). Three HOBO Stow-Away TidbiT and HOBO Water Temperature Pro V2 instruments (Onset Computer Corporation, Bourne, MA, USA) were placed near the bottom along the reef slope at 4, 10, and 20 m deep. The site is located to the northwest of the STRI station on the western shore of Colon Island, in the embayment of Bahia Almirante, Panama, at 9°20.8’N/82°15.7’W, known locally as Samsan Point. This location is a long-term coral and sea grass physical and biological monitoring site for STRI (Kaufmann and Thompson 2005) and was also a Caribbean Coastal Marine Productivity Program (CARICOMP) monitoring site (Guzman et al. 2005). Temperature data were collected hourly from 1999 to present, and sensors were changed and calibrated biannually immediately prior to deployment in a water bath at 21 °C against a traceable thermometer, with data adjusted post-deployment. Accuracy of the data is ±0.25 °C (Kaufmann and Thompson 2005). The hourly record used for this analysis is continuous from March 28, 1999 to September 28, 2011. Nighttime-only data (between 2400 and 0800 hours local time) were extracted to match the nighttime constraint of the offshore satellite record.

The four temperature time series were processed with the same algorithm used to calculate NOAA CRW’s operational thermal stress monitoring products (Liu et al. 2008). Thus, direct evaluation of differences could be made across depths and between the two sites. First, a long-term temperature reference baseline (historical climatology) was calculated for each of the three in situ temperature records. This baseline is comprised of 12 monthly values, each the mean of all monthly nighttime temperature means for that month across the reference period. The single largest value
in this set was then selected as the climatological maximum monthly mean sea temperature (MMM-ST), which was used as the base value for calculating temperature anomalies specific to the location or depth of each of the datasets. MMM-ST is thus the highest mean monthly condition to which the corals in that location have been subjected (Liu et al. 2004). The historical MMM-ST value thus varies for each site and depth, but remains static for that site or depth for all subsequent analysis. For the offshore site, the MMM-ST was provided by NOAA CRW, as originally derived from reprocessed satellite SST data from the multi-channel SST dataset (MCSST) at the Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami, for the period 1985–1993, with 1991–1992 excluded to avoid aerosol contamination of the satellite measurements from the eruption of Mt. Pinatubo (Glesson and Strong 1995). The MMM-ST for the inshore temperature record was derived from a reference period consisting of the first six calendar years of these records (March 28, 1999 through December 31, 2004).

The climatology time period for the three inshore records thus does not correspond to the climatology time period for the surface site due to time limitations in the former time series. To assess any possible difference in climatologies introduced by using two different baseline time periods, differences in regional temperature anomalies specific to these two periods were compared to a mean of the monthly NOAA Caribbean Temperature Index (CAR) (Penland and Matrosova 1998) for both reference time periods (i.e., the 108 months from 1985 to 1993 and the 57 months from April 1999 through January 2004). The later period had a positive anomaly difference of 0.14 °C when compared to the former time period. This difference indicates that the earlier period was slightly cooler across the basin, suggesting that using the later time period as reference climatology for the inshore sites may have resulted in a slightly more conservative calculation of thermal stress for the inshore site compared to offshore. However, this potential difference was deemed to be small enough not to affect the analysis, and no correction was made for the difference in climatology time periods.

Two primary coral bleaching thermal stress indices were calculated, following NOAA CRW's nomenclature: (1) the twice-weekly Coral Bleaching HotSpot and (2) the 3-month cumulative coral bleaching Degree Heating Weeks (DHW). The HotSpot product is an anomaly metric reflecting the instantaneous occurrence and intensity of thermal stress conducive to coral bleaching, defined as the positive difference between the measured nighttime daily temperature record and the long-term maximum monthly mean sea temperature climatology (MMM-ST) (Liu et al. 2004). In our analysis, the HotSpot record is a continuous daily product for the three inshore study site records and is a twice-weekly product for the offshore NOAA CRW satellite cell, corresponding to the dates of the twice-weekly satellite SST record. HotSpot values of 1.0 °C or greater are considered sufficient to cause thermal stress to corals.

DHW is a running sum, calculated for each date by adding HotSpot values equal to or greater than 1.0 °C for the previous 12 weeks, including the reference date, consistent with NOAA CRW methodology. This cumulative parameter is designed to account for the impact of chronic thermal stress, which has been shown to have significant correlation with observed intensity of coral bleaching (Liu et al. 2005; McClanahan et al. 2007), as compared to the HotSpot metric, which reflects only instantaneous conditions. DHW are expressed in units of °C-weeks, with values of 4 °C-weeks or above indicating the onset of potentially damaging coral bleaching, and values of 8 °C-weeks or above indicating risk of widespread bleaching and coral mortality.

A second high-resolution satellite SST record was used to investigate basin-scale hydrologic conditions that could have contributed to bleaching events in Bahía Almirante during the period 1998-2011. AVHRR Pathfinder version 5.2 (PFV5.2) SST data were obtained for the Caribbean Basin at 4-km resolution from the Group for High Resolution Satellite Sea Surface Temperature (GHRSSST) and the NOAA National Oceanographic Data Center (NODC) (Casey et al. 2010). Aggregated monthly SST values were generated from these data for January 1, 1998-December 31, 2011 for two rectangular polygons, the first is a coastal area close to the mouth of Bahía Almirante representing nearshore water that could be carried into the bay, and the second is an area in the open southern Caribbean Sea, representing offshore water for comparison. The first box is from 80.8°W to 82.3°W, and from 8.7°N to 9.4°N, and the second is from 75°W to 78°W, and from 14°N to 15°N. These two areas are hereafter referred to as the Bocas del Toro nearshore SST reference area and the Caribbean Sea SST mid-basin reference area.

Vertical temperature differences reflecting water column stratification through time at the inshore site were calculated by taking the difference between 4 and 10 m nighttime temperatures, and between 4 and 20 m, with positive values indicating inverted temperature conditions (i.e., warmer water at depth). Local rainfall over the period 2000-2012 was investigated to determine whether unusual rainfall contributed to stratification within Bahía Almirante. A monthly record of rainfall was compiled from two records collected at the STRI laboratory. The first was a daily record from a 15-minute electronic automated sensor, and the second was a backup daily record from a manually collected tipping rain gauge. The electronic record was used for the majority of the compilation, with the exception of three short
(<10 days each) missing periods, which were augmented by the backup record. Rainfall was compiled into mean daily rainfall for all months, which was tested for correlation with the monthly inversion magnitude using Pearson's product-moment correlation coefficient test. Correlations were also investigated with the temperature record projected forward and backward 6 months at monthly intervals to reveal possible time-lagged correlations.

**Results**

Comparison of the four temperature records is limited to the continuous period from June 1, 2001 to October 15, 2011 (Fig. 3a-d). Nearly all of the annual temperature profiles across the 11-year time frame show two mid-year peaks, characteristic of the tropics, from the passage of the sun directly overhead twice a year (Rich et al. 1993) in March and September.
April and September. Daily nighttime mean temperatures for all sites and years ranged from a high of 31.26 °C on July 22, 2005 at 20 m at the inshore site to a low of 25.6 °C on March 30, 2007 at the offshore surface site. Collectively, mean monthly conditions from January to March were very similar across all sites and at all depths (Fig. 3c), ranging in January from a low of 27.3 °C at the offshore site to a high of 27.44 °C at 4 m at the inshore site, differing by only 0.14 °C. Maxima, minima, and seasonal temperature trends were generally in agreement with earlier reports for this embayment (Kaufmann and Thompson 2005).

During the peak insolation season from April to October, in contrast to the thermal similarity seen across all sites and depths for January to March, both daily and monthly mean nighttime temperatures were consistently higher for the inshore sites than for the offshore site and generally remained elevated over the offshore signal through November. Differences in the annual mean temperature for the entire 13-year period for all sites reflect these higher inshore temperature levels during this warmer time, ranging from 28.11 °C for the offshore site to 28.64, 28.66, and 28.71 °C for the 4, 10, and 20 m sites, respectively (Table 1). Maximum daily mean values reveal that the inshore sites reached 1.06–1.19 °C warmer than the maximum daily mean recorded offshore, with the highest maxima in the shallowest strata, as expected from solar heating (Table 1).

Maximum monthly means were also higher inshore than offshore, ranging from 0.87 to 1.16 °C warmer (Table 1). However, the inshore monthly means reveal an important difference in structure from the daily means, with the deepest depth reaching the warmest levels, as opposed to the surface. The maximum monthly mean at the 20 m depth is 0.05 °C warmer than the 10 m depth and is warmer than the 4 m depth by 0.24 °C. This difference between maximum daily and monthly means indicates that a persistent reverse thermal gradient occurs during some points of the year, despite the occasionally greater thermal input at the surface demonstrated by the higher maximum daily mean. Quarterly means were computed from the monthly means, and both sites and all depths have the highest quarterly peak in the September–November period and are lowest in the December–February period. The deepest depth inshore exhibits the warmest quarterly mean temperatures in every quarter except March–May, when the profile is reversed, with the water column getting cooler with depth, likely due to surface heating from increased direct solar radiation. This depth also reaches the highest quarterly mean of any site or depth, at 29.44 °C during the September–November period.

The historical climatologies differed among sites primarily in that the inshore sites collectively experienced greater seasonal differences compared to the offshore site (Table 2). The largest difference between inshore and offshore was in October when the difference between the coolest and warmest means of monthly sea temperature values increased to a difference of 1.33 °C, with the offshore site the least warm of the four at 28.5 °C (note that this nevertheless was still the single warmest value in the offshore climatology) as compared to the 10 m depth with the warmest October climatology of 29.83 °C. Differences between inshore and offshore decreased rapidly in November and December as the inshore sites cooled, however, and remained small through February, with the inshore sites showing historical temperatures similar to offshore, differing in January by only 0.15 °C, with the warmest value for this month at 4 m at the inshore site (27.45 °C), and the offshore surface site the coldest of the four sites (27.3 °C). Climatologies for all four time series demonstrated the characteristic mid-year dip in sea temperatures, with July and August lower than the 2 months preceding and the 3 months following. The three inshore climatologies were similar in this seasonal pattern, but displayed temporal and magnitude differences over the summer that illustrate differences between them in rate of thermal increase and retention of that energy. The 20-m site reached the first annual peak in June, as did the other 2 months, but was 0.27 °C lower. However, in the following month, during the mid-summer reduction in solar angle, the climatology for the two shallower depths both fell below that of the 20 m depth. This pattern was repeated in the second peak event, in October, with the deepest depth retaining more heat for the following 2 months. These climatological values indicate that temperature at depth is higher than that at the surface on a regular basis during warm periods of the year and that this inversion difference can peak weeks after maximum temperatures are recorded at shallower depths. The MMM-ST values from which anomalies are calculated were also considerably

Table 1: Temperature ranges and means for all sites and depths; nighttime only for inshore sites (0000–0800 hrs), °C.

<table>
<thead>
<tr>
<th>Location</th>
<th>Offshore</th>
<th>Inshore 4 m</th>
<th>Inshore 10 m</th>
<th>Inshore 20 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum daily</td>
<td>32.00</td>
<td>31.39</td>
<td>31.31</td>
<td>31.26</td>
</tr>
<tr>
<td>Minimum daily</td>
<td>25.60</td>
<td>25.69</td>
<td>25.83</td>
<td>25.80</td>
</tr>
<tr>
<td>Maximum monthly</td>
<td>29.80</td>
<td>30.67</td>
<td>30.91</td>
<td>30.96</td>
</tr>
<tr>
<td>Minimum monthly</td>
<td>26.18</td>
<td>26.62</td>
<td>26.57</td>
<td>26.52</td>
</tr>
<tr>
<td>Mean 2000–2010</td>
<td>28.11</td>
<td>28.64</td>
<td>28.66</td>
<td>28.71</td>
</tr>
<tr>
<td>Dec–Feb mean 2000–2010</td>
<td>27.23</td>
<td>27.45</td>
<td>27.48</td>
<td>27.66</td>
</tr>
</tbody>
</table>
higher for the inshore sites, reflecting these higher monthly means, varying from 28.50 °C in the offshore site to a mean of 29.70 °C for the three inshore locations.

In the offshore site, the HotSpots rose above the 1 °C threshold most notably in 2005 and 2010 (Fig. 4a). In the inshore site, the HotSpot intensity at the 10 m depth was greatest in 2010, whereas at the 20 m depth, the HotSpots rose above the 1 °C threshold only in 2005 (Fig. 4b-d). There was a general decrease in most years from 4 to 20 m in both HotSpot magnitude and persistence, with the exception of 2005. Across both sites and at all depths, many of the years showed a two-peaked pattern in HotSpot occurrence. This was similar to the distribution of temperature maxima, with peaks in June and October. The June peak was commonly lower than the October peak, with 2005 notable as the only year where the earlier mid-year peak in HotSpot intensity was much greater than the later peak, indicating an unusually large temperature anomaly earlier that year. Initial onset of HotSpot > 1.0 °C for the inshore records was similar for both 2005 and 2010 (June 13 and June 2, respectively) and differed primarily in the pattern of maximal level.

The calculated DHW stress parameter for the surface offshore site was highest in 2005 and 2010, in both years rising above the NOAA CRW defined threshold of 8 °C-weeks associated with widespread bleaching and coral mortality risk (Fig. 4e). NOAA CRW issued bleaching alerts for this area in both of those years. In 2009, the DHW for this site also approached near the 4 °C-week threshold identified by NOAA for onset of significant bleaching conditions, but did not rise to the 8 °C-week threshold. DHW for the depth-stratified sites collectively matched this pattern, being highest in 2005 and 2010, but showed sharply different distribution of stress levels across depth strata between these 2 years. In 2005, the stress was predominately limited to the deepest 20-m stratum, while in 2010, elevated stress levels were seen only at the upper two strata. Temporally, DHW at the two shallower sites (10 and 4 m) were at all years very similar to each other in temporal pattern, with the 10-m site slightly higher in magnitude in two of the three highest heating events of 2005, 2007, and 2010. There was a consistent temporal lag between occurrence of peak stress at the offshore surface site and occurrence at any of the inshore sites in both 2005 and 2010. Onset of maximum stress in 2005 at the offshore site was on July 23, compared to August 14 at the inshore site (at 20 m depth), and in 2010, maximum stress occurred September 6 at the offshore site and October 3 at the inshore site (at 4 m depth).

Average annual rainfall for the period March 2000 to September 2012 (Fig. 5a) was 3,435.4 mm year⁻¹ (n = 12, SD = 576), similar to an earlier report of mean rainfall of 3,277 mm year⁻¹ (n = 28, SD = 461 mm) for this area for 1972–2000 (Kaufmann and Thompson 2005). Monthly rainfall means were very stochastic (Fig. 5b); the lowest individual month was 29.46 cm month⁻¹ in February 2007, and the highest was 1,045.72 cm month⁻¹ in November 2008, during a record rainfall event on the Panamanian isthmus. Mean monthly rainfall (Fig. 5c) varied from a high of 467.27 cm month⁻¹ for November (n = 10, SD = 251.11) to a low of 152.92 cm month⁻¹ for September (n = 10, SD = 51.68).

Temperature differences among the inshore strata showed regular periods of both classical thermal structure development (temperature declining with depth) as well as temperature inversions (temperature increasing with depth). Occurrence of these thermal structures was irregular, but generally the first 6 months of the calendar year exhibited classical thermal structure development and the later 6 months exhibited periods of thermal inversion (Fig. 6). Monthly inversion strength was significantly but weakly correlated with monthly rainfall anomaly (r = 0.48, n = 139, p = 0.05). Time-lagging the rainfall by monthly intervals over 6 months both past and future did not reveal any significant correlations with inversion strength.

Analysis of the two sea surface reference areas from the AVHRR PAV5.2 satellite temperature record showed that the Caribbean Sea offshore SST reference area was nearly always cooler than the Bocas del Toro inshore SST reference area (Fig. 7). Over the period 1982–2011, the means for these two areas were and 27.296 and 28.089 °C, respectively, for a difference of 0.803 °C. In order to assess...
long-term change, the time series was split into two time periods, one from 1982 to 1997 (inclusive) and the other 1998–2011. For the inshore reference area, there was no significant difference between the early and late periods, with means for the early and late time periods of 28.036 and 28.139 °C, respectively ($p = 0.2628, F_{1,33} = 1.1216$), but for the open Caribbean Sea offshore reference area, the later time period was significantly warmer than the earlier period, with means for the early and late time periods of 28.04 and 28.15 °C, respectively ($p < 0.0001, F_{1,33} = 5.2553$).

**Discussion**

Calculations of thermal stress from both the offshore satellite data (NOAA CRW) and the inshore depth-stratified in situ data resulted in very similar overall levels of thermal stress (Fig. 4e). The years with highest stress corresponded with the authors’ observations of coral bleaching in this location, specifically 2005 and 2010. However, there were important differences in the stratification of this stress within the water column in the inshore site, with 2005
Fig. 6 Seasonal temperature inversions at the inshore sampling site. Black line indicates temperature difference between 20 and 4 m, and red line is difference between 10 and 4 m. Positive values indicate warmer water at depth. Gray shading indicates January–June, inclusive, and non-shaded areas indicate July–December, inclusive.

Fig. 5 Rainfall summaries for 2001–2012 at the Smithsonian Tropical Research Station at Bocas del Toro, ~4 km from the study site. a) Total annual rainfall (mm), with annual mean shown as solid black horizontal line, horizontal dashed lines indicating ±1 SD, and red asterisk indicating bleaching year; b) daily mean rainfall (mm) by month for the time period, with mean shown as solid black horizontal line, horizontal dashed lines indicating ±1 SD, and vertical red dotted lines indicating bleaching events; c) monthly rainfall means (mm), with mean for the period shown as a solid black horizontal line, horizontal dashed lines indicating ±1 SD, and error bars indicating ±1 SD for individual months (November error bar truncated for plotting).

experiencing maximum stress in the deepest stratum and 2010 exhibiting greater stress in the shallower strata. Furthermore, differences were also found in the timing of onset of thermal stress conditions between the two locations (defined as the date when DHW ≥ 4) with the inshore sites lagging behind the offshore site by roughly 3 weeks. These differences in distribution of stress by depth as well as timing of onset and disturbance duration are important for understanding the bleaching patterns observed in 2005 within this embayment, and also for predicting how other enclosed shallow reef environments experiencing stratified conditions could react to future elevated water temperature conditions. Augmenting the NOAA CRW satellite bleaching alert system with local depth-stratified monitoring could help reveal these disturbance dynamics.

This investigation started with the observation during the 2005 Caribbean warm water event that coral bleaching and mortality intensified with depth at our study site. This contradicted expectations, given that deeper strata were expected to experience dampened temperature fluctuation and reduced radiation stress due to the thermal mass and light attenuation properties of water. This dampening of environmental variation with depth is seen in the pattern of daily HotSpot results across most of the time period from 2005 to 2011, which generally showed a reduction in both intensity and duration of temperature excursions from the surface to the deepest record (Fig. 6a–d). However, this was strikingly not the case in the summer of 2005, when temperature, temperature anomaly, HotSpot, and thermal stress (DHW) reached their highest levels at the deepest strata in the temperature records (20 m). The peak temperatures and stress levels at 20 m were considerably higher than those experienced at 10 and 4 m. The offshore surface site also showed elevated thermal stress levels this year, prompting a bleaching alert from NOAA, but both shallower inshore depths (4 and 10 m) had only moderate stress levels. This concentration of high thermal stress in the deepest strata in 2005 was in contrast to 2010, the second major event with elevated water temperatures in the study period. In 2010, offshore waters again showed similar levels of significant thermal stress, exceeding the 8 °C-week threshold, but inshore stress levels did not manifest in the same pattern as 2005. Only the two upper
strata exhibited damaging levels of thermal stress, exceeding the 4°C-week threshold for onset of bleaching, while at 20 m, there was no stress condition in 2010.

We hypothesize that these differences in thermal stress at different depth strata result from density-driven water column stratification within the bay, which creates a temperature inversion and holds significantly warmer water at deeper levels. Temperature inversions are a previously described irregular feature in Bahía Almirante, occurring from April to December, persisting for durations of weeks (Kaufmann and Thompson 2005). In this study, temperature inversions between 20 and 4 m at the inshore location were present at some time in the second half of every calendar year for the decadal record 2002–2012 (Fig. 5). However, the extreme difference in temperature and stress between the three strata in 2005 indicates that an unusual synergy of forces may have increased the impact of the stratification event this year. We hypothesize that these conditions led to much higher and longer exposure to stressful temperatures for the deeper corals in 2005, contributing to increased bleaching response.

Rainfall was initially investigated as the primary driver of these inversions. Complex local dynamics affect water temperature and vertical mixing within Bahía Almirante, with solar radiation identified in one study as the dominant factor, followed by wind stress and rainfall (Kaufmann and Thompson 2005), with only a weak correlation between the variation in water temperatures and rainfall due to most of the rainfall coming at night, when surface water temperatures are already at their lowest diurnal levels, although rainfall was shown to strongly affect the formation of density-driven stratification layers. Our rainfall record closely agreed in both magnitude and seasonal pattern with this earlier report, with high rainfall across the year and high variability (Fig. 5c). The significant but weak association of monthly rainfall magnitude with monthly temperature inversion strength indicates that higher rainfall does contribute to inversion formation, but does not fully explain differences in the magnitude of inversion strength (temperature difference between 4 and 20 m). The existence of the inversion in every year of the decade demonstrates that higher than normal rainfall is not needed for inversion formation, and the lack of direct correlation between the years with most coral bleaching (2005 and 2010) and highest inversion strength further indicate that neither rainfall amount nor inversion strength fully explain the onset of bleaching conditions. Rather, bleaching at depth appears also to be a function of the temperature of selected water body layers, specifically the temperature in the deeper layers within the embayment and the maximum surface temperatures of the offshore waters, which appear to provide the source waters for advection to these layers.

The fact that the maximum temperature in the early part of the summer in 2005 at 20 m was higher than that
reached at 4 or 10 m at any point during the year indicates that this peak could not have been caused by vertical heat transfer from the surface at that location (i.e., from direct solar radiation at that site combined with wind or density-driven mixing), and therefore it appears that this water was horizontally advected to the 20 m location with thermal properties conserved from some other relatively near-by location, likely sea surface water just outside the bay close to the entrance. High-temperature, high-salinity water could enter the embayment through normal current and tidal water movement and be subducted by a fresher and cooler surface layer creating the described temperature inversion. Due to normal temporal gaps in the satellite record from cloud cover and satellite sampling frequency, it is not possible to follow individual packets of surface water entering the embayment on a daily or weekly basis. However, the monthly means do show increased temperature in localized areas of the near-shore waters along the Panama and Costa Rican Caribbean coastline in July 2005 and exhibit a thermal patchiness not evident in earlier years (Fig. 6).

Maximum SSTs for the two chosen reference areas from the AVHRR Pathfinder satellite temperature record are also correlated with the years of most extensive bleaching for the decade 2000-2010 (2005 and 2010). Only in these years does the maximum summer SST in the Caribbean Sea offshore reference area exceed that in the Bocas del Toro inshore reference area at the same time (Fig. 6). Furthermore, these are the only points after 2000 in this time period where the offshore surface temperature exceeds 31 °C. The correlation of these unusual conditions with the main periods of bleaching, along with the ubiquitous nature of temperature inversions in the summer months, indicates that the severity of the inversion and consequent bleaching event is driven mainly by incursions of warm offshore waters, which enter the embayment and subduct to form the deeper strata during these inversion events.

The difference in the magnitude of bleaching between the three inshore depth strata during the two primary warm water stress events may also reveal a depth-specific susceptibility to thermal disturbance. The 2010 DHW levels for the offshore site actually slightly exceeded the levels in 2005, even though the intensity of bleaching witnessed at the deepest stratum in 2005 greatly exceeded any bleaching seen in 2010 at any depth. If these two events are seen as parallel disturbances of comparable magnitudes but affecting different depth strata, this difference in intensity could be explained by an acclimatization hypothesis. The raw temperature record from 1999 shows only one brief period of temperatures >30 °C prior to 2005, whereas the records from the 4 and 10 m depths show repeated excursions >30 °C from 2005 to 2010. This difference in historical exposure of individual colonies and depth-specific communities may have amplified the reactions seen in 2005. Physiologically, corals experiencing smaller ranges of temperature variation have reduced tolerance to temperature stress (Gardner et al. 2005; Oliver and Palaniti 2011; Burritt et al. 2013). Therefore, if the community at 20 m had experienced many years of reduced temperature variations compared to the surface community, then small variations in the thermal pattern at this depth would represent an unusual pattern and could elicit an elevated physiological response. The bleaching observed in this location at depth is thus potentially a synergistic result of the early and persistent increase in temperature demonstrated in the stress calculations combined with reduced resistance to this type of disturbance for this depth. If this location continues in future years to exhibit the thermal conditions described here, an increasing mortality gradient could develop, with the reef dying from the bottom up, even if the temperature changes in the deeper strata are of a lesser magnitude compared to those at the surface. This likelihood of increased occurrence of thermal stress, given predictions of increasing water temperatures across the Caribbean, along with the amplified impact these conditions have on the deeper coral communities, warrants further investigation into the specific set of environmental variables that contribute to these inversions.

Temporal differences in the development of thermal stress conditions within each year were also seen between 2005 and 2010, with onset and peak of thermal stress at the inshore location occurring later than at the offshore location. In 2005, this delay in onset of peak stress conditions was 22 days, and in 2010 was 27 days. This time lag in onset was similar in both years, even though the regional-scale onset of stress conditions was very different in 2005 and 2010; in 2005, warm water anomalies appeared offshore earlier in the year, reaching 4 °C-weeks on the 25th of June, whereas in 2010, this was first recorded July 22. For the inshore site in 2005, the DHW rose above the 4 °C-week threshold for onset of bleaching conditions on 7 July, and in 2010, this level was exceeded over 2 months later, on September 16. These two dates fall on either side of the usual August reduction in sea temperatures caused by the decreased angle of the sun moving farther to the north, prior to the second peak in temperatures in September when the rays of the sun again strike more directly at this latitude. The year 2005 was one of only 2 years in the 1999-2011 period with the highest temperature for that year occurring in the first of these two warming periods and had the earliest peak of temperature of any year in the decade. Also, 2005 did have a normal mid-year temperature reduction (in fact a larger than normal mid-year decrease) but because the temperature peaked so much higher before this decrease, even this significant decline did not bring the temperature below the MMM-ST and thus did
not cause the accumulation of thermal stress. This early onset of stressed conditions on the reef in 2005 along with the lack of mid-summer temperature regime at 20 m effectively extended the duration of thermal stress in this year across many weeks. This unusually early and atypical chronic condition may have contributed to the eventual severity of bleaching seen later that year.

The temporal delay in the onset of thermal stress inshore, compared to offshore, is further circumstantial indication that inshore conditions are primarily driven by warm water inputs from offshore. The consistency of occurrence and length of this delay in maximum temperature onset supports the use of the NOAA CRW as a predictive indicator for onset of bleaching conditions in shallow reef areas. Furthermore, the correlation between maximum offshore summer surface temperatures and the years of greatest bleaching validates the use of this indicator for inshore bleaching. However, NOAA CRW products, providing a large-scale view of the background coral bleaching thermal stress condition, do not predict how this stress could stratify in response to local bathymetric, climatological, or hydrologic conditions, and are thus not able to predict local vertical distribution of this stress.

Here, we have demonstrated that thermal stress can manifest on the reef in a surprising fashion, increasing rather than reducing with depth under some stratified conditions. The historical lack of temperature variability at deeper locations also means that small increases in temperature at these depths can significantly increase calculated thermal stress, resulting in an increased risk of bleaching even when absolute temperature variation may not reach that experienced at the surface. Climate change could result in the deeper parts of a reef being increasingly subject to these smaller but potentially disproportionately damaging anomalies in water temperature. Our single observation of differential distribution of bleaching stress with depth in one location tentatively supports this idea, but more extensive investigation is warranted, as this effect will be limited to areas with physical and hydrologic conditions that support water column stratification. The relatively shallow depths involved in this work (up to 20 m) do not allow for direct comparison to the deep reef refugia hypothesis (DRRH) (Glynn 1996), which explicitly applies to mesophotic reef depths greater than 30 m, but this observation does provide a point for considering how offshore surface thermal changes could manifest as ecological impacts on deeper areas of an inshore reef, potentially with a complex and nonlinear increase in disturbance impact with depth. We conclude that monitoring and analysis of local depth-stratified temperature records would complement NOAA CRW’s large-scale coral bleaching thermal stress monitoring, particularly in years when bleaching is predicted. This additional monitoring could reveal bleaching risk not fully expressed at larger scales, and incorporating depth-stratified temperature monitoring and small-scale oceanographic and hydrologic factors will be important for accurately understanding observed patterns of bleaching response across the coral community and predicting response to future water temperature changes.

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References
Coral Reefs


CHAPTER 4: Bleaching response, growth rates and mortality of three reef-building Caribbean corals from 2005 to 2011 including the 2005 and 2010 elevated water temperature events
ABSTRACT

Coral bleaching has become more common and more severe in recent years, and is a significant factor in the worldwide decline of coral habitats. However, there is little information on the long-term demographics of reef-building hard corals following acute bleaching stress, following colony fates over a sufficient number of years post-disturbance to determine possible persistent effects associated with initial bleaching extent. In this chapter I investigate the bleaching response, mortality, and recovery of three Caribbean scleractinian species (*Orbicella franksi*, *Siderastrea siderea*, and *Stephanocoenia michelini*) in Bocas del Toro, Panama over six years following the bleaching event of autumn 2005. Significant differences between species were seen in initial bleaching extent as well as in growth rates following the acute initial bleaching event. These species-specific stress response differences have implications for relative future persistence and dominance of these species, possibly leading to an alteration of the coral community composition of the reef over decadal or longer time scales.
Introduction

Coral bleaching events associated with thermal stress have become more common in recent years (McWilliams, Cote et al. 2005; Carpenter, Abrar et al. 2008), and are forecast to increase in both occurrence and severity due to global warming (Walther, Post et al. 2002). There has been a large body of research describing various community aspects of coral bleaching (Baker, Glynn et al. 2008; Alvarez-Filip, Dulvy et al. 2009; Oliver and Palumbi 2011; Carilli, Donner et al. 2012) and the biological effects of coral bleaching, including reduced growth (Goreau and Macfarlane 1990; Baird and Marshall 2002; Cantin, Cohen et al. 2010), reduced reproduction (Szmant and Gassman 1990; Ward, Harrison et al. 2002; Baker, Glynn et al. 2008), increased mortality (Jokiel and Coles 1977; Miller, Waara et al. 2006), disease outbreaks (Miller, Waara et al. 2006; Rohwer 2006; Vega Thurber, Barott et al. 2008), and colony fragmentation (Meesters, Wesseling et al. ; Mumby 1999; Elahi and Edmunds 2007). However, there are few long-term demographic studies describing the effects of bleaching disturbance on the population biology of affected species and consequent implications for community composition, following recovery over a sufficient number of years to determine possible persistent growth effects from exposure to thermal stress (Baird and Marshall 2002). These studies are needed for making predictions of how the scleractinian community will respond on multi-year and decadal scales to predicted future environmental change.
To investigate the response and recovery of Caribbean corals from exposure to acute thermal stress and consequent bleaching, bleaching extent and colony size changes were observed for 178 individual tagged colonies of three coral species in Bocas del Toro, Panama over six years following the major warm-water event of 2005. This year was one of the warmest on record for much of the planet (Shein 2006), and there was widespread bleaching in the Caribbean basin (Eakin, Morgan et al. 2010). This analysis focuses on the effects of this thermal stress on three dominant reef-building hard corals in the Caribbean: *Obricella franksi* (previously known as *Montastrea franksi*), *Siderastrea siderea*, and *Stephanocoenia michelini*. During the 2005 event both bleached and non-bleached representatives of each species were selected and marked. Sampling for each of the three species. Individual colonies were repeatedly and non-invasively observed through underwater photography, and the images were analyzed for overall colony status, live, bleached, and partially bleached areal extent, which were used to derive long-term growth or decline rates. Photographs were taken at eight time points (three times in the 2005 bleaching year and approximately annually thereafter) over six years.

**Materials and methods**

*Study site and thermal stress conditions*

This study was conducted on a protected fringing reef near Punta Caracol on the western (leeward) side of Isla Colon, near Bocas del Toro, Panama (9.363°N, 82.282°W). The reef is located within the Bahia Almirante embayment, an area of
extensive but patchy coral cover, with coral development along a slope from the surface to 20m (Guzman, Barnes et al. 2005). Coral species diversity and cover in this protected location are high for the local area, and are typical for well-developed coral reefs in the western Caribbean (Guzman and Guevara 2001). The embayment is heavily influenced by both oceanic water input as well as high fresh water input (Collin, Huber et al. 2009), and water clarity is lower than many reef sites due to higher nutrient and chlorophyll concentrations, contributing to shallower reef development (Kaufmann and Thompson 2005). In 2005, the Caribbean basin was subjected to the highest water temperatures recorded to date for this area (Eakin, Morgan et al. 2010). Bleaching was extensive across the basin (Miller, Waara et al. 2006; Whelan, Miller et al. 2007), including our study site in the western region. Thermal stress conditions of lower magnitude were also experienced in 2010 (Guest, Baird et al. 2012).

Measurements of bleaching response and recovery

Groups of bleached and unbleached individual colonies of the three target species were selected, tagged and monitored on approximate annual intervals for six years. This photographic series was begun in October 2005 during the bleaching event. Colony photographs were analyzed for total live projected planar area (in units of cm²) of live, bleached, and partially bleached coral tissue. Colonies were selected along three depth transects (at ~<4m, 7-10m, and 10-13m) in the area of Punta Caracol, in the Bahia Almirante embayment near Bocas del Toro, Panama. Selected
colonies were marked with white PVC plastic tags with stamped numbers, fastened to dead areas of the substrate with stainless steel nails. These tags were removed and renewed twice over the course of the time series to ensure that the numbers were legible and that tags were not broken or lost. Effort was made to find a comparable number of representative samples of bleached and unbleached colonies for the three species in each transect, although it should be noted that the *S. michelini* population *in situ* was predominantly bleached, and the *S. siderea* population *in situ* was predominantly unbleached, requiring somewhat wider examination of the area to obtain representative populations for the two bleaching status groups. All colonies were revisited and photographs were obtained 6, 9, 22, 34, 46, 58, and 71 months after the initial observations in October, 2005 taken during the height of the bleaching event.

Images were taken with a variety of underwater cameras, with later years using a Canon 5D Mk II 23mp DSLR, with a Canon EF 17-40 mm lens with dome port and extender fitted to minimize radial distortion (Treibitz, Schechner et al. 2012). Underwater lighting was provided as needed with a variety of strobes. All photographs included a color and size reference in the image, and images were color corrected as needed and analyzed with a custom Matlab-based image segmentation tool (http://vision.ucsd.edu/content/coral-colony-segmentation-and-area-measurement-tools), resulting in planar area measurements of live coral tissue, dead area on the colony, and bleached and partially bleached tissue (as subsets of the live area...
measurement). Two size classes were defined for each species, large and small, in order to maintain adequate sample sizes for analysis, with the class division based on the mean size for each species in the sample set.

**Sample size and selection**

A total of 52 *O. franksi* colonies were tagged and photographed in 2005. 12 colonies were in 4 meters or less, 16 were in 7-10 meters depth, and 24 were in 10 meters or more. Colonies ranged in planar area from the smallest at 108 cm$^2$ to the largest at over 3400 cm$^2$, with a mean size of 457.5 cm$^2$ (± 63.4 SE). 32 colonies were classified as large, and 30 as small. In the case of this species only, some large individual colonies (over 1500 cm$^2$) were spatially sub-sampled for bleaching state, as measurement of these large colonies in their entirety was not practical with these photographic methods; bleaching extent and growth measurements for these colonies were all included in the large colony group dataset. 68 *S. micheleni* colonies were tagged and photographed in 2005. 13 colonies were in 4 meters or less, 24 were in 7-10 meters depth, and 31 were in 10 meters or more. Colonies ranged in planar area from 48 cm$^2$ to 403 cm$^2$, with a mean size of 239.9 cm$^2$ (± 18.8 SE). 25 colonies were classified as large, and 43 as small. The *S. siderea* dataset was comprised of 58 colonies, with 21 colonies in 4 meters or less, 21 in 7-10 meters depth, and 16 in 10-13 meters or more. Colonies ranged in planar area from 39 cm$^2$ to 1133 cm$^2$, with a mean size of 383.9 cm$^2$ (± 37.5 SE). 24 *S. siderea* colonies were classified as large, and 34 as small.
Statistical comparisons of bleaching severity, mortality and recovery

A variety of different approaches were used to evaluate the influence colony size, depth, and species on bleaching extent and long term growth rates, depending on the nature of the data (categorical or continuous) and the response of that species to stress.

Analysis is divided into three points in the time series, reflecting initial bleaching reaction, the response to that bleaching, and the recovery from disturbance. Initial acute bleaching response is described from a single series of photographic observations taken in late October and early November 2005. These photos were not all taken on the same day, due to practical limitations with locating, tagging, and imaging these numbers of colonies, but were all taken during the bleaching period, near the peak of water temperatures during the 2005 thermal stress event. The response period covers the time when the direct effects of the thermal stress event could be expected to be manifested in tissue loss (partial mortality), whole colony mortality, and pigment recovery. This period is hereafter referred to as the bleaching response period, and is defined as the first nine months following the disturbance. This period includes three observations, the first being the initial set of images, and those taken six and nine months later. The third analysis period covers a time when thermal conditions returned to long-term means, and there were no notable environmental variations. This period lasted four years, and includes four observations, taken 22, 34,
46, and 58 months after the initial disturbance. This period is hereafter referred to as the recovery period. There was an additional thermal stress event 60 months after the first event, of lesser disturbance magnitude. This was followed by a final observation taken 71 months after bleaching. This final set is treated separately in the discussion, as it is only a single data point. There was a wide range of responses in the first 22 months.

To evaluate within-species differences in bleaching and partial bleaching extent in 2005 between the large and small size groups ($\alpha = .05$), Student's t-tests were performed. As there was an a priori division in the selection of bleached and unbleached individuals, no statistical evaluation was made of differences between bleached and partially bleached areas for these groupings, although means are reported for confirmation that the unbleached sets had little visible effect from the thermal stress, and do represent a visibly distinct group of colonies.

Annual growth rates for the response and recovery periods are calculated in two ways, first by performing a linear regression on those time periods and annualizing the resultant monthly regression coefficient, and second by taking the difference in start and end points for the time periods, and expressing this as an annualized percent change.

*Ethics and permitting statement*
This work was conducted under the aegis of the Smithsonian Tropical Research Institute, with a scientific permit for working with protected species obtained from the Dirección de Áreas Protegidas de Vida y Silvestre, Autoridad Nacional de Ambiente, República de Panamá, in Balboa, Ancon, Panamá. No samples of protected or controlled resources were taken in the course of obtaining these photographs.

Results

*Initial bleaching response in 2005 by species*

There was a wide range of bleaching response by individual colonies, even among conspecifics in the same bleaching or size groups (Figure 4.1). This figure illustrates representative examples of this variance, and demonstrates both the range of dramatic bleaching differences possible between colonies as well as providing a visual reference of the occasionally subjective nature of the partial bleaching designation. The first three panels (Figure 4.1.1-4.1.3) show three distinctly different 2005 responses by three *O. franksi* colonies, but they all have similar long-term outcomes, specifically little or no partial colony mortality. For comparison, the final two panels show two badly bleached *S. micheleni* colonies, one of which recovered and the other of which suffered total colony mortality within 22 months. These illustrative examples in the context of this figure are not intended to be quantitative or representative of the full range of responses, but rather to demonstrate the possible differences in both initial bleaching and recovery that will be investigated in this analysis.
37 of the 52 *O. franksi* colonies exhibited visible bleaching in 2005, and 15 were in the unbleached group. This species exhibited patchy bleaching, with individual colonies commonly showing a mix of bleached and normally pigmented tissue. The bleached group had a mean bleaching extent per colony of 14.4% (± 2.4 SE) of live tissue, and a mean partial bleaching extent of 18.8% (± 1.8 SE) (Figure 4.2). The unbleached group had a mean bleaching extent of 0.7% (± 0.2 SE) and a mean partial bleaching extent of 1.6% (± 0.6 SE). These small amounts of bleached tissue in the unbleached group were commonly small spots of bleached and partially bleached tissue, usually on growth edges, which is normal for this species. The single most affected colony was 67.9% bleached, and several were <1% bleached. Colonies in the large group (n=32) had a mean bleached area of 11.5% (± 2.5 SE) of live tissue, with small colonies (n=20) having a bleached area of 8.9% (± 2.0 SE), a non-significant difference (*t* = .58, df = 50, *p* = 0.56). Partially bleached areas were also not significantly different (*t* = .76, df = 50, *p* = 0.45) between the two size groups, with large colonies having a mean of 15.1% (± 2.7 SE), and small colonies 12.4% (± 2.8 SE).

62 of the 68 *S. micheleti* colonies exhibited visible bleaching in 2005, and 6 were in the unbleached group. This species exhibited extensive whole-colony bleaching as well as some individual colonies showing a mix of bleached and normally pigmented tissue. Completely unaffected colonies were rare for this species in this bleaching event. The bleached group had a mean bleaching extent per colony of
38.3% (± 4.9 SE) of live tissue, and a mean partial bleaching extent of 19.4% (± 2.7 SE) (Figure 4.2). The unbleached group had a mean bleaching extent of 0.1% (± 0.05 SE) and a mean partial bleaching extent of 5.2% (± 1.4 SE). Several colonies were 100% affected (either bleached or partially bleached), and turf algal growth was visible on some colonies in these initial images, possibly indicating that these colonies had bleached earlier in the event and were already experiencing mortality over parts of their surface. Colonies in the large group (n=25) had a mean bleached area of 35.9% (± 6.1 SE) of live tissue, with small colonies (n=43) having a bleached area of 39.2% (± 5.9 SE), a non-significant difference ($t = 0.70$, df = 66, $p = 0.49$). Partially bleached areas were also not significantly different between the two size groups ($t = 0.56$, df = 66, $p = 0.59$), with large colonies having a mean of 20.6% (± 2.7 SE), and small colonies 12.4% (± 2.8 SE).

25 of the 58 S. siderea colonies exhibited visible bleaching in 2005, and 33 were unbleached. This species was generally much less severely bleached, and no colonies exhibited whole-colony bleaching. Colorations changes were more gradual and subtle than for the other two species, and generally occurred in larger patches over the surface of the colonies. The bleached group had a mean bleaching extent per colony of 13.1% (± 4.6 SE) of live tissue, and a mean partial bleaching extent of 12.8% (± 3.1 SE). The unbleached group had a mean bleaching extent of 0.2% (± 0.04 SE) and a mean partial bleaching extent of 0.9% (± 0.4 SE) (Figure 4.2). Unlike the other two species, S. siderea showed a significant difference in bleached tissue extent.
between the two size classes of the bleached group \((t = 5.7989, \text{df} = 56, p < 0.001)\); colonies in the large group \((n=24)\) had a mean bleached area of only 1.6% \((± 0.32 \text{ SE})\) of live tissue, with small colonies \((n=34)\) bleached 11.2% \((± 1.9 \text{ SE})\). Partially bleached areas were also significantly different between the two size groups \((t = 3.6830, \text{df} = 56, p = 0.0005)\), with large colonies having a mean of 2.6% \((± 0.5 \text{ SE})\), and small colonies 10.3% \((± 1.9 \text{ SE})\). It should be noted that for \(S. \text{siderea}\) our estimate of bleaching extent also explicitly applied only to the selected individuals in the visibly bleached group, and the extent of bleaching across the entire population would be considerably less than the amount reported here as we had to preferentially search for affected colonies for our sample, thus somewhat exaggerating the extent of bleaching in the sample population compared to the natural population.

**Tissue loss and mortality following bleaching**

The \(O. \text{franksi}\) bleached group \((n=37)\) lost an average of 26.2% \((± 5.4 \text{ SE})\) of live tissue area per colony in the first six months following the bleaching event (Figure 4.3). Tissue loss peaked 22 months after the disturbance, with colonies reduced by 35.0% \((± 7.8 \text{ SE})\) from their 2005 extent. The unbleached group did not lose tissue over this same 22-month period, gaining 2.8% \((\text{SE} ± 5.6)\) in live area. Three \(O. \text{franksi}\) colonies died completely in the first nine months following the 2005 thermal stress event, all of which were bleached in 2005, with an average bleaching area for this group of 18.5% \((± 5.2 \text{ SE})\). An additional 2 colonies died over the next 49 months, and 2 more died in the 13 months following the second thermal stress in 2010. As of
October 2011, 43 of the original 52 live colonies remained in the record, with 2 colonies lost for unknown reasons, presumably through tag shedding or overgrowth.

Bleached *S. micheleni* colonies (n=62) lost an average of 35.7% (± 4.7 SE) of live tissue area per colony in the first six months following the bleaching event, and tissue loss continued across the whole period, with colonies reduced at the final observation in 2011 by 56.9% (± 5.6 SE) from their 2005 extent (Figure 4.3). The unbleached group (n=6) also had extensive tissue loss, reaching the lowest point in month 46, with a 52.9% (SE ± 15.3) reduction in live area. *S. micheleni* had the highest colony mortality following the bleaching, with 16 of the 68 colonies dead in the first nine months. All but one of these colonies were bleached in 2005, with an average bleaching area for this group of 56.7% (± 25.1 SE). One additional colony died over the next 49 months, and 3 more died in the 13 months following the second thermal stress in 2010. As of October 2011, 41 of the original 68 live colonies remained in the record, with 5 colonies lost for unknown reasons.

*S. siderea* showed the least amount of tissue loss per colony, with the bleached colonies (n=25) losing an average of 11.2% (± 3.9 SE) of live tissue area per colony in the first six months following the bleaching event (Figure 4.3). However, live tissue area continued a slow but even decline across the whole period, with colonies reduced at the final observation in 2011 by 21.0% (± 3.9 SE) from their 2005 extent. The unbleached group (n=33) did not have significant initial tissue loss, losing <1% in the
first six months, but this group also showed a similar steady loss of tissue area over the next 66 months, losing 13.0% (SE ± 5.8) of live area. *S. siderea* had the lowest total colony mortality, with no colonies dying in the nine months following the bleaching, and only 1 colony dying over the whole time period. As of October 2011, 52 of the original 58 live colonies remained in the record, with 5 colonies lost through tag shedding or other unknown causes.

*Tissue pigmentation recovery from bleaching*

For bleached *O. franksi* colonies bleached tissue extent was reduced to <1% of live area by the second observation at 6 months, and did not exceed this threshold in any subsequent observations. Partially bleached area reduced more slowly, with colonies in the bleached group still showing 12.4% (SE ± 1.0) of their area as partially bleached after the third observation at nine months. In the unbleached control group neither mean bleaching nor mean partial bleaching exceeded 2.1% (± 0.5 SE) in any of the observations at any point in the time series.

For bleached *S. micheleni* colonies bleached tissue extent reduced from nearly 39.5% (± 5.0 SE) to 1.1% (± 0.1 SE) by the second observation at 6 months, but rose to 3.1% (± 0.4 SE) again in the second thermal event in 2011. A notable extent of partially bleached area was recorded for this species throughout the time series, dropping to a low of 5.1% 34 months after the bleaching event, but rising again in 2011 to 15.9% of live area. Colonies in the unbleached group showed an interesting
profile of increasing area of partially bleached tissue in observations subsequent to the initial event, roughly tripling in area by the 6th month to 15.5% (± 2.0 SE) and then declining thereafter. The unbleached group also had a slight increase of bleached area in 2011.

The initial mean bleached tissue extent of bleached S. siderea colonies was less than the other species, and also reduced more slowly, from nearly 13.1% (± 2.6 SE) in the initial observation to 3.0% (± 0.6 SE) by the third observation at 9 months. Bleached area in subsequent observations remained low (~1%), and stayed at that level through the second thermal event in 2011. Colonies in the unbleached group maintained low areas of both bleached and partially bleached area throughout the time series.

Long-term colony growth following bleaching

The period from month 22 to month 56 was unaffected by major water temperature anomalies, and it was assumed that there were no direct physiological impacts from the initial thermal stress event during this time. This time period is thus taken to be indicative of normal environmental conditions and potentially normal colony growth. Growth rates were calculated using two methods, first an annualized % change calculated from linear the monthly regression coefficients across multiple observations (columns 3 and 4 in Table 4.1), and second as annualized % mean change in individual colony planar size calculated from simple difference between
beginning and ending observations for the time periods (columns 5 and 6 in Table 1). There was close agreement between these two methods; we primarily use the results from the regression analysis for discussion.

Annualized mean colony growth for the unbleached *O. franksi* group in the recovery period was 1.9%, an increase from the essentially flat annualized growth rate of -0.53% in the first nine months following bleaching (Table 4.1). Maximum mean live tissue area loss for any observation point in the recovery period for this species was -3.4% (34 months after thermal stress), and by 71 months mean colony tissue change for this group was an increase of 1.1%. Variance in this group increased over time, as would be expected with a longitudinal study, but was not large in the initial years, indicating consistent initial growth and mortality response within the group. Given inherent methodological variance in measuring planar area from photographs and the absence in the record of some colonies in some years due to tag shedding or missed photographs, this result essentially indicates no net change in mean growth rate or net area for the entire time series for the unbleached *O. franksi* group. In contrast, the bleached group of *O. franksi* showed a notable return to positive growth in the recovery period, increasing in mean live area by an average annual rate of 2.23% during the period, after having decreased at an annualized growth rate of -40.3% for the first nine months.
In contrast to the flat response of the unbleached *O. franksi* colonies, the unbleached *S. micheleni* group declined in both the first nine months and in the recovery period (Table 1), although it must be noted that this group is too small (n=6) to be truly representative, and variance was high for all years. Nevertheless, it is apparent that for this species the unbleached group does not represent an unaffected group, as with *O. franksi*. Bleached *S. micheleni* colonies were quite different, ceasing to decline after initially losing large amounts of tissue (-56.6% annualized decline) and returning to a nearly flat growth (-0.05%) for the period from months 22-56.

For *S. siderea*, both bleached and unbleached groups of colonies slowly declined in live area across both time periods. After an initially larger loss, the bleached group reduced in area at a fairly similar rate (-1.66%) to the unbleached (-3.33%) group during the recovery time. This slow but steady decline characterized the response of this species, with neither the bleached or unbleached groups of *S. siderea* showing positive growth maintained over any three consecutive observation periods anytime in the time series.

Trends in actual live coral cover for the groups of selected colonies were different from the mean colony size change for the groups, due to sampling across a wide range of sizes and size-structured differences in both mortality and recovery by those groups (Table 4.2). Unbleached *O. franksi* colonies had little change in total live tissue coral cover over either the response or recovery periods, varying from 103.7%
to 98.99% of the 2005 cover for Months 9 and 58, respectively. Bleached *O. franksi* total coral cover decreased by 36.26% from Months 0 to 9, and decreased by 35.56% from Months 0 to 58, representing a 1.06% increase in live coral cover during the recovery period. In contrast to the stability of the unbleached *O. franksi* group, the unbleached *S. micheleni* colonies lost 9.33% in live coral cover during response period, and lost a further 20.08% live cover between Months 9 and 58. The bleached group of *S. micheleni* experienced cover loss in a reversed pattern of magnitude, compared to the unbleached group, with a loss of 37.51% in the response period, but a much reduced loss of 3.93% during the recovery period. Despite this reduction in area loss during the recovery period, this was still the single group with the largest total coral cover loss during the total observation period. *S. siderea* showed the least difference in live coral cover trends between the bleached and unbleached groups as well as the least difference between the response and recovery periods. Unbleached *S. siderea* declined 15.87% in the response period, and had a continued decline in total live area of 4.51% in the recovery period. Total cover of the bleached group also declined in both time periods, but with a larger total magnitude over the entire study period, despite a lower initial decline, losing 12.81% in the response period and 26.76% in the recovery period.

Growth and mortality response between the two periods varied by size for *O. franksi* and *S. micheleni* (Figure 4.4). In both cases smaller colonies had greater partial and total mortality than large colonies. *O. franksi* colonies experienced no total
mortality beyond 500 cm$^2$ during the response period, and larger colonies had proportionally smaller amounts of partial mortality, evidenced by displacement of the regression line below the identity line (the 1:1 line) for the bleached colony group for smaller colony sizes in Figure 3. Bleached colonies of all sizes in the recovery period tended closely to the identity line, indicating mean colony size maintenance for these 36 months, and the unbleached group in both the response and recovery period was above this line, indicating colony growth during these periods. The effect of size on mortality during the response period for *S. micheleni* was similar to that of *O. franksi* but more pronounced, with colonies below 400 cm$^2$ showing a wide variety of mortality responses up to and often including total colony death. Very few colonies, either bleached or unbleached, grew during this period. The mean growth trend in the recovery period fall close to the identity line, but with a wide variance of individual response, particularly for colonies below 200 cm$^2$. *S. siderea* colonies showed little departure from the identity line, and little variation with size. Mean growth for both bleached and unbleached groups was slightly below the identity line, indicating slow decrease in colony size for both groups in both periods.

*Response to the second thermal stress event in 2010*

There is only one observation point following the 2010 elevated water temperature event, taken in September 2011. The 2010 photographic observation and
sample collection does not represent the corals in a fully stressed condition, as the field period preceded the warmest period of the summer by about one month, and thus missed the most likely period of bleaching. Because of this issue, it is not possible to assess long-term effects from this second thermal stress event at this time, as there is only one data point after the event. However, this time series will continue and it is possible that this second disturbance may have a more noticeable effect over time. However, some initial effects were suggested by the single 2011 observation and these are detailed below, with the caveat that these are only preliminary indications.

*O. franksi* colonies did not show any strong effects in terms of either changes in coloration in 2010 or 2011 or decreases in colony size in 2011. There was a small increase in partial bleaching in 2011 that could indicate that bleaching did occur before the observation and was not observed, as the pigmentation recovery period would have been less than the amount of time that passed before the next observation. This small increase in partial bleaching could thus be a residual indicator of a bleaching event within the previous year, as was seen during the recovery from the 2005 event. Local reports do indicate that there was some bleaching in this year, but not at the level of 2005. *S. micheleni* colonies in both the group that bleached in 2005 and the initial unbleached group did exhibit a loss of live tissue in 2011, compared to 2010 areas. The 2005 bleached group also showed notably increased bleached and partially bleached areas in 2011, but the bleached area present was an order of magnitude less than that observed in 2005. However, partially bleached area for this
group in 2011 was similar in extent to that measured nine months after the 2005 event. Both the bleached and unbleached groups did show an increase in partial mortality in 2011, compared to 2010 sizes, which was similar to the first bleaching response period. This partial mortality indicates that bleaching may have taken place, and that pigment recovery had occurred by the time of the next observation eleven months later. Mean bleached area, partially bleached area, and annual growth rates for *S. siderastrea* colonies showed little change in this period, continuing a pattern of slow partial mortality or near maintenance of area. This lack of significant partial or total mortality in 2011 is similar to the initial resistance to damaging impacts shown by this species in the post-2005 bleaching response period.

**Discussion**

*Differences in initial bleaching response*

The initial disturbance effects related to the acute elevated water temperature event, as demonstrated by the relative extent of bleaching and partial bleaching per colony, varied widely by species. The bleached *S. micheli* group had the most extensive total areas in terms of both bleaching and partial bleaching, followed by the bleached *O. franksi* colonies. The bleached *S. siderastrea* colony group had the least amount of visible bleaching or partial bleaching of any bleached group of the three species.
Partial colony mortality in the nine months following the thermal disturbance for the bleached groups also varied widely by species, ranging from 12.81-37.51% and was closely correlated to the initial rate of bleaching in that species, suggesting that bleaching extent is a good predictive indicator for colony tissue loss. The largest amount of live tissue loss in the response period for bleached colonies was *S. micheleni*, followed by *O. franksi* and *S. siderea*. Whole colony mortality in the response period followed a similar species order pattern, with 25.8% of the *S. micheleni* dead within nine months, followed by 5.7% colony death for *O. franksi* and 0% for *S. siderea*.

These initial responses suggest that *S. siderea* has the greatest resistance to thermal stress, followed by *O. franksi* and *S. micheleni*. When interpreted within the context of the 9-month response period, this finding would seem to indicate that *S. siderea* gained a competitive advantage from this disturbance. However, over subsequent years it became apparent this was not a valid interpretation, as decline in live tissue area for this species continued, while other groups recovered to positive growth rates. This serves as a cautionary tale for extrapolating differences in initial bleaching extent for making estimations for long-term changes within coral ecosystems. This study suggests that observations under two years should be considered short-term responses, i.e. not long enough to reflect the complete disturbance impact and long-term changes to the ecosystem from this disturbance event. However, coral ecosystem studies with the goal of describing long-term impacts
often do not extend observations over this minimum time frame. For example, a study from the Australian Great Barrier Reef that quantified live area changes following bleaching over a self-described ‘long-term’ time period measured corals over eight months, similar in temporal scope only to the initial response period in this study (Baird and Marshall 2002).

*Differences in recovery following initial the bleaching period*

The precipitous declines in colony area shown in the nine-month bleaching response period for bleached colonies of *S. micheleni* and *O. franksi* were generally greatly reduced during the recovery period, and growth rates of the various groups showed little apparent variation across the sample set, varying for all six groups (three species and two bleaching groups) during this time between a gain of 2.23 and a loss of 3.41% (the *S. micheleni* unbleached group (n=6) had too few surviving members to be representative). In other words, all bleached and unbleached groups for all three species generally maintained their approximate extent of live coral cover over the 36-month recovery period, despite large differences in mortality in the initial period. This similarity of growth rates, and specifically the lack of further dramatic decline in live area in the bleached groups, indicates that acutely damaging effects of thermal stress on colony growth do not last past the initial bleaching response time period. This finding contradicted our expectation that there would be long term growth inhibition of bleached colonies, and suggests that recovery of coral colonies is generally possible following the direct mortality impacts of acute bleaching.
However, within this similarity of growth responses in the recovery period, there were differences between the species and groups. Interestingly all three species had a slightly higher growth rate during the recovery period for the bleached groups than for the unbleached groups. This difference between bleached and unbleached groups was largest for *S. micheleni* (difference of 1.99 in annual % growth rate) and *O. franksi* (difference of 2.85 in annual % growth rate). The unbleached *O. franksi* were also the only group to exhibit positive growth for any species during either of the two time periods, although growth for the bleached *S. micheleni* group was very close to neutral for this period. However, these differences noted in growth during the recovery period are subtle and are only over a time period of four years, and so must be presented with caution, but given the inherently slow growth rates of scleractinian corals, these differences may become more obvious over increasing time spans. One possible explanatory mechanism for the bleached colonies having greater growth rates may stem from an altered post-bleaching symbiont community composition for *S. micheleni* and *O. franksi*. These two species are known to repopulate after bleaching with symbionts of different clades, which have been associated with different growth rates in laboratory studies. Bleaching may thus be a precursor to greater individual colony growth rates, if symbionts are replaced with these more contributory species. This effect is known as the Adaptive Bleaching Hypothesis (ABH), and has been demonstrated in laboratory studies, but has not yet been proven showing differentiated
growth over a substantial (decadal) period of time in any long-term field studies
(Buddemeier and Fautin 1993; Buddemeier, Baker et al. 2004).

**Implications for long-term community population dynamics**

Total coral cover across the 58 month time period for all species declined by 30.48%, and mean annual growth in live area across all species and bleaching groups for the recovery period was -0.46%. These two summary statistics suggest that reef decline may be an ongoing long-term process in this area, and that this reef is gradually losing live area, and will be considered extirpated (below 2% of original area) in ~100 years. This alarming hypothesis is based on several factors. First, it is possible that this study did not observe the recovery of corals over a long enough time period to document complete ecosystem recovery following such a severe bleaching disturbance. If the study was continued over decades all of the coral species would return to assumed pre-disturbance positive growth rates. Second, it is possible that the chosen area for the study, being spatially very limited, does not represent the community trajectory for the larger region. Small-scale oceanographic dynamics of this area demonstrate unusual patterns of thermal heterogeneity (Neal, Condit et al. 2013), and the extent of bleaching disturbance could be localized, with mortality thus potentially exaggerated in the study area and not representative of regional trends. Lastly, and most significantly, the observations in this study could reflect a real background level of gradual reef decline, which growth rates returned to following the acute mortality of the bleaching event. Reef health is at risk and coral cover has been
declining in Panama in recent decades (Guzman 2003), in a similar fashion to the larger Caribbean basin (Alvarez-Filip 2009). These declines in coral health related to human activities have been taking place over long time scales, well beyond the approximately 40 year period most often thought of as the most significant period of anthropogenic reef mortality (Jackson, Kirby et al. 2001; Pandolfi, Bradbury et al. 2003). Specifically, caution must be used when evaluating the growth rates and recovery rates reported herein to recognize the implicit assumptions that: 1) reef health and growth were at normal long-term, healthy levels prior to the 2005 bleaching event; 2) that growth rates returned to these pre-disturbance levels following recovery from the stress event; and 3) that any differences seen between the response and recovery are directly related to the thermal stress event and not to other stressors which also had an impact on growth in the period 2005-2011.

These assumptions can only be true if conditions on the reef were not in a state of change, which may not be a valid assumption for the Caribbean for this period of time. In a similar but longer study, cover of *O. annularis* a species with similar growth dynamics and bleaching response to *O. franksi*, declined by 72% over a fifteen year period from 1988 to 1999 (the start of this study), but was statistically unchanged for the period 1999-2005 (Edmunds and Elahi 2007). In this same study, colony abundances and size structure also changed over this time period, with the 57% decline in colony number driven mainly by death and fission of large to medium colonies; fifty-year projections from these observations indicated a likelihood of
possible extirpation at this site in St. John, US Virgin Islands (Edmunds and Elahi 2007). Our report of declines in colony size and total live coral cover area by species for Bocas del Toro, Panama must be explicitly interpreted in light of this possible long-term trend for the Caribbean basin. Another long-term study of regional growth rates derived from x-ray analysis of density banding in coral cores taken from large *Montastrea favelolata* colonies in the Florida Keys showed that growth rates (using three metrics: coral extension, density and calcification) had not shown any significant decline over a 61 year period ending in 1996 (Helmle *et al* 2011), suggesting that growth rates for this coral have been largely unaffected by climatic changes up to that date. Placing the growth rates reported in the present study into this context must be done with caution, as the rate of change on the reef may be increasing, and these results are best interpreted primarily within the context of a response to a single acute disturbance.

*S. siderea* was the initially the most resistant species in our study to the effects of exposure to thermal stress, with the lowest levels of both bleaching and tissue loss during the bleaching response period. However, the continued slow but persistent decline of *S. siderea* across the recovery period, along with the similarity of decline exhibited in both the bleached and unbleached groups, suggests that this species may be influenced by different environmental or physiological drivers than the other two species. The unbleached group of this species, which had almost no initial tissue loss in the first nine months, was ~20% reduced in mean size six years later. This effect,
while fairly small on an annual basis, will result in extirpation of these colonies (reduction to <2% of original area) over some thirty years, considerably less than the expected life span of these corals. One possible explanation for this observation of slow decline in mean area is that fragmented colonies could have reduced growth of their residual remaining tissue after fission, as compared to the growth rates of non-fragmented, sexually derived juvenile colonies of similar size. Experimentally, small *S. siderea* colonies derived from sexual reproduction have been shown to have a 2.5 fold greater growth rate than small colonies derived from older, fragmented colonies (Elahi and Edmunds 2007). To varying degrees, many of our observed *S. siderea* colonies demonstrated fragmentation, in both the bleached and unbleached groups, with dead tissue areas often following a pattern of expanding through lesions on the colony surface or expanding in areas of lower relief, possibly indicating a process related to the collection of sediments or mucus from sediment removal. Population, tourism development, and forestry extractions are increasing in this area (Cusack and Dixon 2006), possibly degrading water quality through increased runoff and affecting long-term health of this species (Kuntz, Kline et al. 2005; Knowlton and Jackson 2008). In addition to the possible factors of colony fragmentation or local water quality, differences in size and depth of *S. siderea* colonies have been demonstrated to have a strong influence on colony growth, mortality, and bleaching, with *S. siderea* growth rates under normal conditions, with growth rates decreasing significantly with depth, ranging from 5.7 to 9.3 mm/yr at 6 to 15 m, to 2.7 to 4.2 mm/yr at 16 to 25 m (Huston 1985).
This study was all in water less than 10m, but turbidity and chlorophyll concentrations are high (Collin, Huber et al. 2009), reducing light and possibly creating light-limited conditions mimicking deeper water and affecting growth of this species. Furthermore, these slow-growing colonies may still be affected by previous stress events, such as the 1998 bleaching event (Goreau, McClanahan et al. 2000). Our observations began in 2005, some seven years after this last major thermal stress event, but this could still be affecting these corals. Finally, the size of the colonies selected for the sample may have an impact. In an earlier work, larger colonies of S. siderea were found to be more susceptible to damage and partial mortality following bleaching, measured by their proportion of dead surface area (Lewis 1997), and that those that were already affected by dark spot infections were more extensively bleached (Brandt 2009). I found the opposite: that smaller colonies had elevated bleaching rates, but there was no difference in partial or total colony mortality with size. This disparity in observed reactions indicates that bleaching susceptibility and growth responses of individual S. siderea colonies depends not only on the extent and severity of the stress event, but also on the environmental parameters and location of that colony, and on the corals’ current state of health, age and size. This raises the complex question of which factors contribute to corals’ resilience when they are exposed to a variety of environmental stressors. To summarize for this species, our observations do not follow growth for a long enough time period to positively indicate a real growth rate difference from the other colonies, but our initial observations do
indicate the possibility of a slow decline of this species at this time may not be directly related to the acute thermal stress event or direct bleaching impacts.
Figure 4.1: Initial bleaching and outcome 22 months after bleaching for several of the 168 tagged corals: Panel 1) *O. franksi* colony that bleached and then recovered completely; Panel 2) *O. franksi* colony that partially bleached, recovered, and then partially bleached in 2007 in a different part of the colony; Panel 3) *O. franksi* colony that never bleached despite living adjacent to several corals that bleached; Panel 4) *S. michelini* that severely bleached, recovered some pigment and then declined in size, and Panel 5) *S. michelini* colony that severely bleached and never recovered.
Figure 4.2: Mean bleaching and partial bleaching extent for all colonies in the bleached group each of the three species in autumn 2005, during the bleaching event. Normally pigmented area is represented in brown, partially bleached area in tan, and bleached area in white. Example images of single colonies for each species are shown below, with pigmentation data for that colony shown in the subset pie chart.
Figure 4.3: Mean live coral tissue area for unbleached (left) and bleached (right) colony groups for each of the three species, expressed as % remaining of the November 2005 live area (indicated by horizontal solid line). Error bars are SE for each annual set, and the vertical red dashed lines indicate the two thermal stress events at Months 0 and 60.
Figure 4.4: Individual change in size for colonies over the response and recovery time periods. Initial observation is on the X-axis, and the later observation is on the Y-axis. Solid diagonal line is a 1:1 response, indicating no change in colony size over that time period, with colony shrinkage falling below that line, and colony growth falling above. Solid blue circles are colonies that were unbleached in 2005, and open red circles are colonies that were bleached that year. Regression lines for the two groups are shown as dotted lines of the same colors.
Table 4.1: Mean colony growth rates for the response (between 0 and 9 months after bleaching) and recovery (between 22 and 58 months after bleaching) periods. Growth rates are calculated in two ways for comparison, first expressed as annualized % calculated from linear regression coefficients across multiple observations (columns 3 and 4), and as annualized % mean change in individual colony planar size calculated from difference between beginning and ending observations for the time periods (columns 5 and 6).

<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
<th>Annualized % Δ from</th>
<th>Annualized % Δ from</th>
<th>Mean annual % Δ in live area/colony:</th>
<th>Mean annual % Δ in live area/colony:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>regression:</td>
<td>regression:</td>
<td>Months 0-9</td>
<td>Months 22-58</td>
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<td></td>
<td></td>
<td>Months 0-9</td>
<td>Months 22-58</td>
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<td></td>
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<td>(r^2)</td>
<td>(r^2)</td>
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<tr>
<td><em>O. franksi</em></td>
<td>Unbleached</td>
<td>-0.53 (0.71)</td>
<td>1.90 (0.84)</td>
<td>-0.15</td>
<td>-0.92</td>
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<td>(n=15)</td>
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<tr>
<td><em>O. franksi</em></td>
<td>Bleached</td>
<td>-40.43 (0.95)</td>
<td>2.23 (0.62)</td>
<td>-40.26</td>
<td>1.93</td>
</tr>
<tr>
<td>(n=37)</td>
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<tr>
<td><em>S. michelini</em></td>
<td>Unbleached</td>
<td>-21.36 (0.77)</td>
<td>-2.77 (0.53)</td>
<td>-19.09</td>
<td>-1.85</td>
</tr>
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<td>(n=6)</td>
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<tr>
<td><em>S. michelini</em></td>
<td>Bleached</td>
<td>-59.22 (0.56)</td>
<td>0.79 (0.22)</td>
<td>-56.58</td>
<td>-0.05</td>
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<td>(n=62)</td>
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<tr>
<td><em>S. siderea</em></td>
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<td>-4.20 (0.79)</td>
<td>-3.41 (0.94)</td>
<td>-4.65</td>
<td>-3.33</td>
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<td><em>S. siderea</em></td>
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<td>-1.50 (0.64)</td>
<td>-21.18</td>
<td>-1.66</td>
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<td>(n=25)</td>
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Table 4.2: Total sample live coral cover extent areas and changes in total coral cover area by the ends of the response (between 0 and 9 months after bleaching) and recovery (between 22 and 58 months after bleaching) periods. Totals are shown for the total sample set, the unbleached colony group, and the bleached colony group for the three species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
<th>Total live area sampled in Nov 2005, cm²</th>
<th>Live area 9 months post-bleaching, cm²</th>
<th>% Live area 9 months post-bleaching/2005 area</th>
<th>Live area 58 months post-bleaching, cm²</th>
<th>% Live area 58 months post-bleaching/2005 area</th>
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<td><em>O. franksi</em></td>
<td>All</td>
<td>27699.51</td>
<td>19975.11</td>
<td>90.75</td>
<td>19849.09</td>
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<td>103.7</td>
<td>5743.75</td>
<td>98.99</td>
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<td>13957.23</td>
<td>63.74</td>
<td>14105.34</td>
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<td>16318.98</td>
<td>10198.8</td>
<td>62.49</td>
<td>9615.47</td>
<td>58.92</td>
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<td>1128.38</td>
<td>90.67</td>
<td>901.83</td>
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<td>60.17</td>
<td>8713.64</td>
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<td>84.13</td>
<td>11697.08</td>
<td>80.32</td>
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<tr>
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<td>Bleached</td>
<td>7708.49</td>
<td>6721.29</td>
<td>87.19</td>
<td>4922.41</td>
<td>63.86</td>
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References:


Huston M (1985) Variation in coral growth rates with depth at Discovery Bay, Jamaica. Coral Reefs 4:19-25


CHAPTER 5: Long-term symbiont diversity of two reef-building Caribbean corals following bleaching disturbance
ABSTRACT

Many species of tropical shallow-water scleratinian corals are obligate mutualistic hosts to a diverse group of photosynthetic dinoflagellate algal symbionts in the genus *Symbiodinium*. Corals can respond to environmental stress by expelling these symbionts, referred to as bleaching. Symbiont populations can recover within the coral tissues over time, but may be comprised of a different community composition than before the disturbance. Taxonomy of *Symbiodinium* is still in development, with eight phylogenetic clades (A-H) currently recognized within the *Symbiodinium* genus. Specific cladal and subcladal members within the *Symbiodinium* genus have been shown to exhibit differential host-specificity, ecological niche specialization and physiological responses to light, heat-stress and irradiance exposure, with Clade A generally described as fugitive opportunists, Clade C as heat-sensitive and Clade D as heat-resistant. Some coral species have been shown to host multiple clades, and to be able to shuffle their symbiont composition in response to changes in environmental conditions. Differences in the clade-specific ecological function of various *Symbiodinium* clades under different taxa-host relationships may confer an adaptive benefit to the coral host, as corals may adapt to increasing temperatures by changing their dominant symbiont types, with altered symbiosis dynamics in terms of transferred energy between host and symbiont. With high water temperature events forecast to increase, resulting in increasingly frequent global episodes of mass coral bleaching, it has been proposed that some species may be able to maintain these altered relationships over time in an apparent long-term response to thermal stress,
possibly conferring increased resistance to future thermal disturbance events. To investigate this possibility, symbiont types in tagged *Orbacella franksi* and *Stephanocoenia michelini* colonies in Punta Caracol, Panama were tracked during the major 2005 bleaching event and for seven subsequent years. Symbiont types were identified using fragment length variation in the hypervariable large subunit (LSU) region of Domain V of the 23S rDNA chloroplast gene. This study found that mutualistic associations between *Symbiodinium* and these two coral hosts were not stable but rather were highly variable between bleached and unbleached groups, were flexible over time, and varied between the species. These highly variable responses demonstrate the need to evaluate changes in coral symbiont communities relative to specific hosts over a sufficient period of time to evaluate the dynamics of these long-term pattern changes.

**Introduction**

Many species of tropical shallow-water scleratinian corals are obligate mutualistic hosts to a diverse group of photosynthetic dinoflagellate symbionts in the genus *Symbiodinium* (Trench 1979). This endosymbiotic relationship in many cases directly provides a significant portion of the metabolic synthate needed to sustain reef-building corals, as these symbionts translocate their photosynthetic products within the cell, and in return take advantage of the protected intracellular environment that provides them with enhanced levels of available inorganic nutrients from the metabolic waste products of the coral animal (Fitt et al. 1986).
Coral bleaching is the functional loss of these intracellular symbionts (commonly also known as zooxanthellae) either through expulsion or degradation of algal pigmentation within the symbiont cells (Glynn 1984). *Symbiodinium* cells give the coral the majority of its coloration (Douglas 2003), and with the loss or degradation of these cells, the coral animal takes on a white coloration, with the underlying white coral calcium carbonate skeleton visible through the thin and largely clear layer of live coral tissue. This process is thus commonly referred to as bleaching, and while it does not result in the direct immediate death of the coral host, which typically can also feed heterotrophically (Grottoli et al. 2006), it is associated with decreased metabolic function (Kayanne et al. 2005), increased rates of tissue loss (Loya et al. 2001), and possible total colony death (Glynn et al. 2001). Coral bleaching has become more common and more severe in recent years, and is a significant factor in the worldwide decline of coral habitats (Hoegh-Guldberg et al. 2007; Baker et al. 2008).

A number of different environmental changes can result in a disruption of the conditions needed for effective symbiosis, and can result in coral bleaching. The dominant bleaching stimulus is exposure to elevated water temperatures (Glynn and D'Croz 1990; Gates et al. 1992), but bleaching has also been associated with increased solar irradiance (Lesser 1996), changes of water column pH (Hoegh-Guldberg et al. 2007), bacterial and viral infection (Rosenberg et al. 2008), reduced availability of
Symbiont communities within scleractinian corals can recover when environmental conditions return to amenable conditions. This process can occur from acquisition of symbionts from the water column through phagocytosis or from an expansion of residual populations already within the coral host tissues (Lewis and Coffroth 2004). *Symbiodinium* can be among the most abundant eukaryotic microbes found in coral reef ecosystems in tropical oligotrophic marine environments, and can be a dominant constituent of the unicellular photosynthetic benthic community and therefore commonly available for acquisition from environment (Rowan 1998).

Within the genus *Symbiodinium* there is considerable taxonomic diversity, currently commonly divided into multiple groups called clades, each distinguished by a letter A-H. These phylogenic clades are recognized as being genetically distinct lineages exhibiting different ecological and biogeographic distributions (Stat et al. 2008). However, clade-level taxonomy is increasingly recognized as an over-simplified schema, and taxonomic revision of this genus is occurring at present, with the possibility that a number of these clades could be reclassified into distinct genera in the future (Sampayo et al. 2009). Clade and sub-clade divisions have been based on
ribosomal gene sequence data from the 23S region (Santos et al. 2003), mitochondrial gene coding for cytochrome oxidase 1 (cox1) (Takabayashi et al. 2004), ribosomal large sub-unit (LSU) coding in the D1-D5 domain (Wylezich et al. 2010), and differences in the internal transcribed spacer 2 region (ITS2) (LaJeunesse 2001).

Despite the current fluid taxonomy of *Symbiondinum* and the nomenclature change from clades to species, the clade schema still presents advantages when looking at community-wide responses to environmental disturbance, as the clades are associated with generalized functional differences (Stat et al. 2008). Specifically, clades can be divided into greater or lesser thermal tolerance, with clades A and D regarded as being thermally tolerant, and clades B and C regarded as thermally sensitive (Baker et al. 2004). These clades have also been suggested as being associated with different coral growth rates, hypothesized as related to differing rates of contribution to the host metabolism (Cantin et al. 2009). This contribution is inversely related to thermal tolerance, with clades A and D associated with reduced coral growth, and clades B and C associated with greater coral growth (Little et al. 2004). To conclude, clade-level taxonomy is possibly not monophyletic, but does represent accepted ecological grouping, specifically thermal tolerance and magnitude of metabolic symbiotic contribution to host growth. Clade level identifications will thus be used for this analysis, as the primary goal is to investigate long-term community assemblages based on dominant symbiont populations in natural
conditions following severe thermal stress and bleaching, and to associate these assemblages with long-term coral growth patterns.

Clade-level studies have suggested or demonstrated that there exists both variability in the thermal tolerance of distinct coral-\textit{Symbiodinium} symbioses and that in some cases environmental stress can raise the relative abundance of more tolerant symbioses (Cantin et al. 2009; Oliver and Palumbi 2009). This may be a significant mechanism by which coral reefs could adapt to future climate change and persistent ocean warming. However, it is not yet resolved how universal this adaptive process is in nature, whether these changes are predictable in response to specific thermal thresholds, how long the changes in clade association persist after the end of the thermal stress, if there is adaptive behavior for multiple stress events within a specific time period, or if these effects differ by clade (Stat and Gates 2010). To investigate these questions, the present study extracted genetic material from coral symbionts taken from groups of two common Caribbean reef-building scleractinians following exposure to thermal stress in 2005, and analyzed genetic diversity within this symbiont community to a clade level. Sampling was conducted approximately annually for seven years following the disturbance event, to track long-term changes in community makeup. Research questions focused on the following questions:

- Does the clade community for these two species change in response to changing environmental stress? Did it change after the 2005 bleaching event, or did it remain largely static through the post-disturbance period?
• Is the symbiotic clade community makeup largely the same between the two species? If not, what are the indications that symbionts may play different ecological roles in differing host species?

• Could the symbiotic clade community makeup have made a difference in susceptibility to bleaching when exposed to the same environmental (water temperature) conditions? Did the response of the clade community to the disturbance vary between bleached and unbleached groups of colonies within each of the two species, indicating that there is a clade/bleaching connection?

• Is there a stable or preferred state for the clade community for each species? If the clade community makeup changed after the 2005 disturbance, did it revert back to the initial community composition pattern over time, and if so, how much time did this reversion take?

• Can adaptive change take place, possibly leading to a different response later on when the same colonies are faced with similar environmental conditions? Was the response of the colonies and the clade communities to the second thermal stress event (in 2010) similar to the response in 2005?

Specific hypotheses developed from these questions were:

H1: *Orbacella franksi* (recently renamed, previously known as *Montastraea franksi*) and *Stephanocoenia michelini* are capable of altering their symbiont community compositions over time.
**H2**: Different symbiont communities will be associated with different susceptibilities to bleaching, for each of the two species of interest.

**H3**: If these two species show the ability to alter their symbiont community compositions over time, then they will show stability of these altered patterns over annual or decadal scales, indicating that there may be an alternate stable state for this symbiotic association. This hypothesis is in contradiction of the common ecological principle of deterministic succession following disturbance, where the community would revert back, given the passage of time without further disturbance, to a stable composition similar to the pre-disturbance community.

**H4**: If a long-term stable change is shown to be possible, as described in H3, these new associations will indicate, given assumptions of the differing ecological and mutualistic roles of the clades, that these changes may contribute to an adaptive strategy on the part of the hosts to reduce the future impacts on individual colonies from similar environmental disturbances.

**Materials and methods**

*Study site*

This study was conducted on a protected fringing reef near Punta Caracol on the western (leeward) side of Isla Colon, near Bocas del Toro, Panama (9.363°N, 82.282°W). The reef is located within the Bahia Almirante embayment, an area of
extensive but patchy coral cover, with coral development along a slope from the surface to 20m (Guzman et al. 2005). Coral species diversity and cover in this protected location are high for the local area, and are typical for well-developed coral reefs in the western Caribbean (Guzman and Guevara 2001). The embayment is heavily influenced by both oceanic water input and high fresh water input (Collin et al. 2009), and water clarity is lower than many reef sites due to higher nutrient and chlorophyll concentrations, limiting reef development to shallower areas (Kaufmann and Thompson 2005). Three 100m transects were laid out parallel to depth contours starting from 50m off the bayside shore of Isla Colon at depths of 2m, 5m and 9m respectively. On each transect, 100 coral colonies of seven species were permanently tagged with stiff polyvinylchloride (PVC) plastic tags attached with mild steel nails on dead areas of the coral skeleton (allowing for repeated sampling of the same colonies throughout the study). The analysis presented in this study examines symbiont clade diversity for two of the seven species tagged in this experimental transect.

**Thermal stress conditions during the study period**

In 2005, the Caribbean basin was subjected to the highest water temperatures ever recorded for this area, with regionally averaged temperatures the warmest in at least the previous 100 years. Water temperatures were clearly related to thermal conditions across the entire Northern Hemisphere, which experienced the hottest year on average in 2005 since the advent of reliable records in 1880. Thermal stress in the water column during the peak of the 2005 event exceeded any observed in the
Caribbean in the prior 20 years (Eakin et al. 2010), and coral bleaching was extensive across the basin (Miller et al. 2006; Whelan et al. 2007), including in Bocas del Toro, the site for this study, in the western region of the Caribbean Basin (see Chapter 3 of this dissertation). Temperatures in 2005 exceeded the previous 9 record years, all of which had been within the previous 15 years (Eakin et al. 2010). Thermal stress for corals in 2005 exceeded levels experienced in 1998, which previously held the record as the hottest year and most extensive record of massive coral losses through bleaching (Goreau et al. 2000; Aronson et al. 2002). Large areas of particularly warm surface waters developed to the east and south of the Caribbean sea entrances early in the summer of 2005, and the first thermal HotSpots (as specifically defined and designated by the National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch (CRW)) appeared in May, 2005 and rapidly expanded to cover the northern Caribbean, Gulf of Mexico and the mid-Atlantic by mid-August (Liu et al. 2008). These warm surface regions continued to expand and intensify until late October, after which tropical disturbances and reduced solar input from the onset of winter conditions cooled the waters to near normal by December. In Bahia Almirante, the transect area for this study, thermal stress peaked in late August, 2005, and was stratified in the water column, possibly indicating an offshore origin of the high-temperature water (Neal et al. 2013). Initial samples were taken during this focused stress period from three depths, with bleaching intensity increasing in the deeper strata. As a further indication of the anomalous nature of the greater Caribbean conditions associated with the thermal stress event at this time, it is notable that 2005
was also a major hurricane year, breaking the previous annual record with over 26 named storms, including 13 hurricanes within the basin, including Hurricane Dennis in July, striking Grenada, Cuba and Florida, Hurricane Emily two weeks later as the strongest hurricane to strike the Caribbean before August, and Hurricane Katrina in late August, the costliest storm ever to hit the United States (Beven et al. 2008). Thermal stress conditions of a lower magnitude were also experienced in this region in 2010 (Guest et al. 2012). The period of genetic sampling of coral symbionts as analyzed for this study overlaps both of these disturbance events.

Sample collection

The first sampling occurred in September 2005 during the mass-bleaching event, and the sample collection procedure was repeated 9 times over the following 89 months, roughly annually, but with increased frequency of sampling earlier in the time series. Subsequent sampling dates were November 2005, May 2006, August 2006, September 2007, September 2008, September 2009, September 2010, October 2011, and March 2013. Samples were analyzed for this report from two of the seven species tagged in the transect: *Orbacella franksi* (recently renamed, previously known as *Montastraea franksi*) and *Stephanocoenia michelini*. These species are known to be able to associate with a variety of *Symbiodinium* clades (Rowan and Knowlton 1995; Rowan 1998) and because these are two of the most ecologically significant reef-building hard corals in the Caribbean basin, and are both abundant species at the Punta Caracol study site. The field condition of each coral colony and each sample was
noted for each sampling point as one of three categories: Bleached, Not Bleached (>95% healthy tissue) or Partially Bleached. For bleached or partially bleached colonies, samples of both bleached and unbleached areas of the colony were generally collected, if present. Each colony was photographed and physically sampled in each of the sampling periods. Photographs were taken with a color reference card for further analysis of planar area and bleaching state (see Chapter 2 of this dissertation).

Tissue samples of live coral biomass were gathered directly from living colonies while underwater on SCUBA. These samples were collected in situ using 50 ml syringes with a blunt tip. The tip of the syringe was placed directly on the coral surface with the plunger fully depressed, and the plunger was slowly withdrawn in order to physically pull soft coral tissue from within the coralitte and interseptal spaces. Depending on the species, this procedure allowed for targeting of single polyps or small areas. The mixed seawater and tissue sample was then immediately filtered back through seawater-saturated 0.22 μm 13mm GFF filters held in individually labeled screw-in filter holders. These filters were held in a bag throughout the dive, transported to shore on ice, and were removed as quickly as practical after return and individually preserved in pre-loaded vials of EDTA-buffered, Dimethyl Sulfoxide (DMSO) salt-saturated buffer (20% DMSO, 0.25 M EDTA, pH 7.5) (Seutin et al. 1991). Samples were then transported and stored at room temperature.

This work, involving collection and international transport of species protected under both Panamanian and United States federal statues and variously listed under
Appendices I-III in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), was conducted under the aegis of the Smithsonian Tropical Research Institute, with a scientific permit for working with protected species obtained from the Direccion de Areas Protegidas de Vida y Silvestre, Autoridad Nacional de Ambiente, Republica de Panama, in Balboa, Ancon, Panama. All samples were obtained and exported within the restrictions of these permits and regulations.

**Genetic analysis methods**

Symbiont types within each coral sample were identified using fragment length variation in the hypervariable large subunit (LSU) region of the chloroplast 23S ribosomal DNA gene (henceforth referred to as cp23S-rDNA) (Santos et al. 2003). Filters were ground and DNA from each sample collected over the ten sampling periods was extracted and isolated using a modified chloroform/cold ethanol process (sensu Coffroth et al. (1992) based on the methods of Saghai-Maroo ef al. (1984)). Tissue and filter were ground together 300µl CTAB (hexadecyltrimethyl ammonium bromide) buffer [1.4 M NaCl, 20mM EDTA (ethylenediaminetetraacetic acid), 100 mM Tris-HCl pH 8.0, 2% CTAB and 0.2% 2-mercaptoethanol], and twice rinsed with a further 300µl CTAB, centrifuged at 500 rpm for 5 minutes, with supernatant discarded. The resulting pellet was suspended in another 300µl CTAB, vortexed for 30 seconds with glass beads, and mixed with 3.0µl of Proteinase K, and incubated at 65°C for 30 min. Following incubation, two suspensions and rinses were performed by adding 300µl of chloroform-isoamylalcohol (CIA - 24:1), with each sample vortexed
for 15 seconds with each addition, and centrifuged for 5 minutes at 12K RPM. In both rinses, the aqueous phase was withdrawn and transferred to a clean sample tube, and nuclear acids were precipitated through the addition of 1000µl of -20°C 95% ethanol (ETOH), inverting the sample several times to mix, and placing the sample in a -70°C environment for eight hours. Following precipitation, the resultant pellet was washed twice with -20°C 70% ETOH, dried for 20 minutes in a Speed-vac with the sample tube lids left open, and then re-suspended in 15µl TE buffer (10 Mm Tris, 1mM EDTA, with HCl added to adjust pH to 8.0, and dilution to 100 ml with double distilled water). DNA concentration in the extractions was then quantified on a 0.7% agarose gel and was diluted to 5-10ng/µl. This sample was then used directly for amplification of Domain V of the chloroplast 23S-rDNA region using the primer pair 23S1M13 (5’-CACGACGTGTAAAAACGACGG CTGTAACTATAACGGTCC-3’) and 23S2M1 (5’-GGATAACCATTTCCACACAG-GCCATCGTATTGA ACCCAGC-3’) (Santos et al. 2003). PCRs were performed in 50 µl volumes containing 10mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl2, 0.001% gelatin, 200 µM dNTPs, 100 pmol of each primer, 2 U Taq polymerase, and 50–70 ng of template DNA. Reactions were carried out in an automated thermocycler under the following conditions: initial denaturing period of 1min at 95°C, 35 cycles consisting of 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, with a final extension period of 7 minutes. The resulting amplicon was separated on a polyacrylamide gel under denaturing (7M urea) conditions and visualized on LI-COR’s NEN Global IR2 DNA Sequencer System. A multiple, mixed clade identification ladder was placed on the gel after every seven
samples, generally including at least five of the following clades, using amplicons from cultured isolates: A194, A198, B184, B211, C180, D206. The identification of single symbionts, and the profile of symbionts when multiple clades were present, were typically shown by strong single bands at the corresponding molecular size. These were sometimes accompanied by faint ‘background’ bands corresponding to different clades. Profiles with multiple bands were attributed to the presence of multiple symbiont types within the same sample, and were only recognized if they consistently appeared throughout repeats of genotyping tests. Bands were read as strong or weak, with most analysis relying only on repeated, strong banding present in multiple gel reads, due to the primary focus of this analysis on characterizing dominant clade community structure. All clade identifications were confirmed at least twice, from independent amplifications.

Data analysis

The first critical aspect to this analysis is to note that this type of cp23S-rDNA genotyping does not indicate abundance of the various clades within the samples. Thus the conclusions made in this study only take into account the presence of symbiont types, not the relative abundance of the symbionts when more than one type is present within the samples. Calculations were made on the basis of symbiont frequency within the samples of individual colonies at different points in time. When multiple symbiont types were found within a sample, each symbiont type was counted as 1, thus
discounting the effect of diversity, relative abundance and possible interactions between the different symbiont types.

Individual colony-level symbiont community results for the whole dataset were aggregated into two species groups (for interspecies comparison), and for each species in the dataset results were grouped into two groups based on bleaching status in 2005 (i.e. bleached or not bleached). Presence of specific symbiont types for any group are reported as % present within the sample for that group and sampling period. This enables direct comparison of results across species and years, which may have different sample sizes due to lost tags, dead colonies, or missed samples due to either practical field issues or processing problems (i.e. failed extraction, no amplification, or exhaustion of all extracted DNA without achieving matching repeated results).

Results

*Hypothesis 1: Symbiont community plasticity*

For *O. franksi* the hypothesis that this species can have an altered symbiont community makeup over time was supported, as diversity of clade types was seen both in single observation periods as well as through time (Figures 5.3 and 5.4). A total of 31 *O. franksi* colonies were sampled and successfully processed during the initial year of the study (2005), and no further colonies were added after this time; 28 of these colonies remained and were sampled in 2013. 19 of these colonies were bleached in 2005, and 12 were unaffected. For the dataset as a whole across the time series, nine
dominant (strong bands only) fragment types were identified as being present sometime in the time series, corresponding to seven commonly known clades and two fragment types with unknown clade assignment. Symbiont types D206, C180, and A198 were the three most dominant types, represented in >20% of the samples in 2005 (Table 5.1). There was considerable variation over time, with seven of these nine fragment types recorded exhibiting at least one observation point when none of the samples showed presence of these three dominant symbiont types. For the two clades that were present in at least one sample in every time point, these also showed variation in occurrence within the sampled colonies, with the most consistently present symbiont type of the set, D206, varying in presence from 50.0% to 88.2% of the colonies.

For *S. michelini* the hypothesis that this species can have an altered symbiont community makeup over time was supported, similar to *S. michelini*, with a diversity of clade types was seen in both many single observation periods as well as through time (Figure 5.5 and 5.6). A total of 35 *S. michelini* colonies were sampled and successfully processed during the initial year of the study (2005), and no further colonies were added after this time; mortality was higher for *S. michelini* then for *O. franksi*, and 22 of these colonies remained and were sampled in 2013. For the dataset as a whole across the time series, twelve dominant (strong bands only) fragment types were identified as being present sometime during the time series, corresponding to ten commonly known clades and two fragment types with unknown clade assignment.
Symbiont types D206 and A194 were the two most dominant types, represented in >20% of the samples in 2005 (Table 5.4). There was also considerable variation over time in this species, with ten of the twelve fragment types recorded exhibiting at least one observation point when none of the samples showed presence of these dominant symbiont types. For the two clades that were present in at least one sample in every time point, these also showed significant variation in occurrence within the sampled colonies, with the most consistently present symbiont type of the set, C180, varying in presence from 54.1% to 100.0% of the colonies.

**Hypothesis 2: Symbiont community association with bleaching susceptibility**

For *O. franksi* the hypothesis that bleaching in this species is related to the symbiont community present during times of thermal stress was supported (Figure 5.1). In 2005, the bleached group (Table 5.2) and the unbleached group (Table 5.3) showed differences in seven clades. Two of the largest differences were in clades D206 and C180. The former is a purportedly thermally tolerant clade, and the latter is a purportedly thermally sensitive clade. The unbleached group had a larger fraction of D206 and a smaller fraction of C180.

For *S. michelini* the hypothesis that bleaching in this species is related to the symbiont community present during times of thermal stress was not supported (Figure 5.2). At first it appears as if the two groups are different, with the bleached group harboring only clade C180. However, caution must be used in drawing this conclusion
because of the low number of unbleached colonies in the sample set, and because very little initial clade community diversity appears to have been present in these samples. Furthermore, C180 is believed to be a thermally sensitive clade, and finding it in only the unbleached group contradicts this belief. In 2005, the bleached group (n=62) (Table 5.5) and the unbleached group (n=6) (Table 5.6) showed differences in six other clades, but all of these were in much lower proportions. Thus the major difference is best characterized as one of total clade diversity, and not as much in proportional makeup. The finding that the bleached group has more clade community diversity then the unbleached group could also be a result of the lower sample size. It is important to note that this species had very extensive bleaching and significant long-term partial and total colony mortality (see Chapter 4 of this dissertation) and the exclusive existence of clade C180 in unbleached colonies may mean only that they have not exhibited visible bleaching signs yet. Rather than conclude that the profile of the unbleached group (i.e., harboring only clade C180) confers some bleaching protection, it seems more parsimonious that the entire population relied primarily on C180, and that the diversity seen in the bleached group was a secondary result of the disturbance. In other words, if this observation is taken as being representative of a point along a gradient of bleaching onset, then the pattern of harboring an exclusive population of C180 cannot be taken to be associated with bleaching resistance, as the colony could have been starting to bleach, or could have bleached later in the thermal stress event.
Hypothesis 3: Alternate stable states of the symbiont clade community

For *O. franksi* the hypothesis that this species is able to sustain long-term alternate associations of symbionts was supported. The main evidence for this is that clade A198 went from low levels in both the bleached and unbleached groups (0% and 11.1%, respectively) to a high (68.8% and 88.2%, respectively) and seemingly maintained presence by the end of the time series. A second indication of persistent change in the symbiont community was clade A194. This strain gradually declined from similar levels in the bleached and unbleached groups (25.0 and 22.2%, respectively) at the onset of the bleaching event to complete extirpation (0% in both groups for a number of years) in the later half of the time series. This strain, which has also been described as a bleaching opportunist, did not reappear in the second thermal stress event.

For *S. michelini* the hypothesis that this species is able to sustain long-term alternate associations of symbionts was not supported. The three most common clade types present in the initial month were also the three most dominant clades in month 91. Clade C180 was the most common symbiont, present in over half of the colonies at all time points. There was more variation in A198 and D206, but by the final observation they were very similar in proportion to the first sample. While this species is clearly able to shuffle symbiont clades, there does seem to be a preference in this location for a community based on C180 and A198, with occasional other members playing smaller roles. The only longer-term change seen in this species was for
fragment length 120 (clade unassigned), which was present in over half the colonies two months after the first heating event, but then declined rapidly back to 0% within two years. This strain reappeared in smaller proportion (8.3%) after the second heating event, but then disappeared again.

*Hypothesis 4: Advantages possibly associated with long-term adaptive change in the symbiont community*

For *O. franksi* the hypothesis that this species potentially gained an adaptive advantage from an altered long-term alternate community of symbionts was neither strongly supported nor rejected. The primary change to the symbiont community mentioned in Hypothesis 3 involved the adoption and persistence of clade A198 in the overall community makeup. This clade is associated with disturbance opportunities, but has not been strongly associated with thermal tolerance or bleaching resistance. In most previous studies, various clade D symbionts have been the main targets for investigation into thermal tolerance. The role of this persistently elevated level of clade A198 is thus not clearly defined through previous laboratory or field studies, but it is possible that it plays a role in bleaching resistance, resilience when stressed, or recovery of damaged areas and continued colony growth. This hypothesis thus cannot be supported or rejected through this study, but the altered community makeup for *O. franksi* that has been recorded presents an interesting topic for future investigation.
Hypothesis 4 could not be examined for *S. michelini*, due to the lack of evidence for this species forming alternate stable symbiont communities in response to thermal stress (i.e. rejection of Hypothesis 3). None of the symbiont types for this species changed in their relative fraction of presence in a way that indicated anything other than a short-term (less than 24 months) response to the acute stress event itself.

**Discussion**

*The importance and function of clade community changes*

The ability of the two coral species examined to shift their community of the endosymbiont genus *Symbiodinium* possibly confers upon them an adaptive advantage. These clade changes have been investigated for over twenty years, but our understanding of the complexity of these relationships is still preliminary. This study confirmed that both *Orbacella franksi* and *Stephanocoenia michelini* have the ability to alter these associations, and investigated the nature of those shifts. These two species were shown in Chapter 4 of this dissertation to have maintained close to neutral or positive growth for a number of years following acute thermal disturbance. It is possible that the ability of these two species to alter their symbiont community structure is one of the factors associated with maintaining the positive or neutral growth observed, and may be a factor in maintaining competitive benthic interactions of the coral reef ecosystem. As a field study, with no definitive control and with limited observation time, it is not possible to conclusively state if these shifted symbiont associations contributed to such advantages or disadvantages. However, this
study does provide possible observational field evidence that may be used for forming experimental hypotheses for further, more controlled investigations.

Differences between the two species

One of the key differences between the two species can be seen in the abundance of clade C180 in the overall populations of both species throughout the time series. One of the most striking aspects of the second bleaching event in 2010 is that *O. franksi* experienced a total rejection of this clade immediately following the second stress event. This complete rejection in 2005 was notably greater than the reduction of this clade for this species following the first event in 2005, which had much higher levels of damaging bleaching, and was also much greater than the rejection of this clade by *S. michelini* for these two time periods. This difference in rejection of this clade among coral species may play a role in the differential recovery of these two species following bleaching stress events.

A second primary difference was the ability of *O. franksi* to apparently maintain the altered symbiont community makeup over the 91 months of observation. The community present in this species at the end of this seven-year study was different from that present at the beginning, indicating the establishment of a persistent or potentially permanent alternate stable state. Given that individual colonies can exist for centuries, the observations period of less than a decade is not long enough to provide conclusive evidence of a permanent change, but does support the idea that
these changes are not seasonal, and can persist on the order of at least the several years that commonly may pass between thermal stress events. *S. michelini* did not show evidence of making this type of change. Different scleractinian species thus can respond differently, even among the already somewhat selective group of species that are able to alter their symbiont communities. The effect of these differences in differential growth on relative dominance in succession situations on the benthos cannot yet be positively discerned, given the relatively short observation span of this study (six years). However, given the slow growth and persistence of these two species, very small differences in success could have significant effects on the long-term population trajectories of these species. The differences in both the original symbiont community composition as well as differences in the response patterns to both stress and time demonstrate the need to approach questions of characterizing coral symbiont community dynamics from a species-specific perspective.

**Conclusion**

Both of the species examined here were able to change the community makeup of their endosymbiotic algal symbionts. These changes included patterns of change that were repeated in both thermal stress events, and one species (*O. franksi*) also demonstrated a possible long-term alteration of symbiont associations which may also give this species an adaptive advantage. The ability of these corals to manage the complex taxonomic variety of their intracellular symbiont communities potentially confers upon these species a change in their ability to react to changing environmental
conditions, particularly changes in water temperature, and to avoid bleaching and the associated effects of partial or total colony mortality. This ostensibly places them in the minority of scleractinian species that have been examined for this ability to date. However, as taxonomic division in the genus *Symbiodinium* rapidly continues to become more complex and better defined, it is possible that previously unrecognized community patterns will become known for many of these other coral host species. A better understanding of the intricacies of the effects of this change in symbiont community composition for a larger suite of scleractinian species will be essential for predicting how these species will react to anticipated global increases in coastal water temperatures, as an effect of global climate change. Differences in the ability of various coral species to remain healthy, reproduce, and grow in these altered conditions could drive changes in the overall community population of the reef. Given that the hard corals are the primary structure-creating organisms in the biogenic landscape of the reef, these changes are a potential threat to the very persistence of these ecosystems over both immediate and evolutionary time scales.
Figure 5.1: Occurrence of symbiont clade types for bleached and unbleached sample groups, as a percent of the total sample, for both bleached (n=19) and unbleached (n=12) groups of *O. franksi*, in 2005. Differences are seen primarily in D206 and C180. The former is a known thermally-tolerant clade, and the later is a known thermally-sensitive clade. All of the symbiont types on this plot for which there was no signal in 2005 were present at other sampling dates in the time series.
Figure 5.2: Occurrence of symbiont clade types for bleached and unbleached sample groups, as a percent of the total sample, for both bleached (n=62) and unbleached (n=6) groups of *S. michelini*, in 2005. Differences are seen primarily in B184 and A198, with C180 nearly ubiquitous in all samples. All of the symbiont types on this plot for which there was no signal in 2005 were present at other sampling dates in the time series.
Figure 5.3: Fraction of sample population containing symbiont types B184, C180, and B178 for *O. franksi* over the 91 months of the time series. These symbiont types are represented in blue, as they are all Clades B or C, which are typically regarded as heat sensitive.
Figure 5.4: Fraction of sample population containing symbiont types D206, A198, A194, B224, and B244 for *O. franksi* over the 91 months of the time series. The symbiont types represented in red are all Clades A or D, which are typically regarded as heat tolerant, and the types in gray, regardless of clade, are all of rare occurrence.
Figure 5.5: Fraction of sample population containing symbiont types B184, C180, and B178 for *S. michelini* over the 91 months of the time series. These symbiont types are represented in blue, as they are all Clades B or C, which are typically regarded as heat sensitive.
Figure 5.6: Proportional occurrence of symbiont types/fragment length D206, A198, 120, A194, and 170 for *S. michelini* over the 91 months of the time series. The symbiont types represented in red are those typically regarded as heat tolerant, and the types in gray, regardless of clade, are all of rare occurrence.
Table 5.1: Percent presence of primary *Symbiodinium* clades for all *Orbacella franksi* samples (bleached and unbleached) for all time periods. Time (in months) for sample collection shown above the table.

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<td>% of total sample with clade present</td>
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Table 5.2: Percent presence of primary *Symbiodinium* clades for bleached *Orbacella franksi* samples. Time (in months) for sample collection shown above the table.

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Table 5.3: Percent presence of primary *Symbiodinium* clades for unbleached *Orbacella franksi* samples. Time (in months) for sample collection shown above the table.

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Table 5.4: Percent presence of primary *Symbiodinium* clades for all *Stephanocoenia michelini* samples. Time (in months) for sample collection shown above the table.

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Table 5.5: Percent presence of primary *Symbiodinium* clades for bleached *Stephanocoenia michelini* samples. Time (in months) for sample collection shown above the table.

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Table 5.6: Percent presence of primary *Symbiodinium* clades for unbleached *Stephanocoenia michelini* samples. Time (in months) for sample collection shown above the table.

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Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742


CHAPTER 7: Summary of the Dissertation
SUMMARY OF THE DISSERTATION

This dissertation investigated the bleaching response, associated mortality and changes in growth, and community changes in intracellular symbiont type of two Caribbean scleractinian corals following stress caused by high water temperature. The response of coral ecosystems to elevated thermal conditions is a major part of predicting the future sustainability of coral ecosystems because thermal stress is predicted to increase in both occurrence and intensity under scenarios of global change (Hough-Guldberg and Bruno 2010) and is likely to be one of the primary anthropogenic stressors affecting the future health and possibly even the very existence of coral reefs as functioning ecosystems.

I found that coral bleaching was associated with increased subsequent partial and total colony mortality, with the areal extent of coral bleaching on individual colonies roughly correlated with extent of colony live tissue loss over subsequent next year. This was not a surprising finding, as coral bleaching as a precursor for short-term coral mortality has been previously well documented. However, following the growth response of both bleached and unbleached colonies over a time frame of several years following the disturbance has not been as well investigated, and this was one of the primary aims of this investigation. Tissue loss rates over the six years following the thermal stress event decreased, and some of the individual colonies and species groups showed a return to positive growth rates (i.e. an increase in live tissue area over time).
The groups of corals that showed this positive growth during the years after the initial disturbance event followed an unexpected pattern. The initial hypothesis was that the species with the most extensive bleaching, and the groups of bleached coral colonies within those conspecific sample sets, would experience decreased long-term growth, based on lasting organismal impacts from the bleaching disturbance. No evidence was found for this effect in the three species I studied. Among the three species, the species with the least amount of bleaching (S. siderea) had the lowest mean growth rate at the end of the observation period. Within the conspecific sample sets, the corals that showed bleaching in 2005 bleaching did show greater mortality initially, as described above, but growth rates of bleached and unbleached groups showed no significant difference during the period from 22 months to 72 months after the bleaching event. Prior bleaching history was thus not supported as a predictive indicator for current colony growth, assuming that there had been no additional bleaching or thermal stress in that area in the subsequent time period. Even more interesting, there was preliminary evidence that in some cases bleaching might even stimulate an increased growth rate. In all three species, the growth rate of the bleached corals during the period from 22 to 72 months after the stress event was greater than that for the previously bleached group. This observation must be taken as very tentative, as the changes in area are very small, the photographic measurements do have observation error, and the observation time frame (six years) is fairly short for long-lived corals. However, this observation does support the concept that there may
be physiological or organismal changes to some of the subjects that cause their growth to be possibly positively altered by bleaching disturbance.

One of the primary factors controlling coral health and growth is the obligate intracellular symbiosis they maintain with photosynthetic dinoflagellates of the genus *Symbiodinium*. I profiled the taxonomic diversity of the symbiont community of my subject colonies to investigate if variation in this community could be a contributing factor to both the initial bleaching as well as the surprising growth patterns seen in the years after the 2005 disturbance. My hypothesis was that these observed differences may have been related to the presence in those individual colonies of specific cladal or subcladal members within the *Symbiodinium* genus, some of which have been shown to exhibit differential host-specificity, ecological niche specialization and physiological responses to light, heat-stress and irradiance exposure. There are currently eight commonly recognized clades of *Symbiodinium*, with the nomenclature A-H, with my interest focusing on the most common clades A-D, with Clade A generally described as fugitive opportunists, Clade B as having mixed function, Clade C as heat-sensitive and Clade D as heat-resistant. These generalizations must be taken as simplifications of the true diversity that is currently being exposed in this genus, but are useful here for generalized discussion. For both of my species of interest, bleached colonies had a higher percentage of clades B and C, and both species showed a general increase in clades A and D following bleaching stress, for both bleached and unbleached groups. *S. michelini* clade community response and recovery following
this disturbance event did not show any evidence of adoption of a lasting change in community structure, but the *O. franksi* colonies did demonstrate such a change, seen primarily in a sustained increase in the presence of clade A198, expressed as a percentage of the colonies in the sample set that showed this symbiont type. This sustained change over the seven years of observation is possible evidence of a lasting response, possibly conferring an advantage to these corals. However, as with the observations of growth, it appears possible that changes in symbiont community structure are still taking place, and thus the time frame for the study might not have captured the full temporal range of dynamic change that occurs in post-disturbance symbiont ecology.

**Conservation implications**

This research indicates that the deleterious effect on corals from thermal stress may be a relatively short-term effect, specifically with reductions in growth rates lasting less than two years, suggesting that coral recovery past this period of initial direct impact associated with the disturbance is possible. The lack of evidence for lasting impacts on individual colonies implies that if the cause of the initial disturbance is reduced or eliminated, then coral ecosystem recovery should categorically follow. This resilience on the part of corals does have very positive conservation implications, but that optimism must be tempered by nature of the threat under discussion. Stated directly, there is no reason to believe at present that thermal stress events will do anything but increase in the near future.
The question for coral conservation then becomes one of rates: is the rate of return to positive growth on a time frame shorter than the occurrence rate of thermal stress events, and is the rate of positive growth in periods of no environmental disturbance enough to add enough live tissue to the overall community to make up for the direct tissue mortality resulting from the acute stress periods? Taken in this light, there is little reason for optimism in my results. At the growth rates calculated from changes in live coral cover for my sample set of *O. franksi*, this species will recover to pre-2005 levels in 23 years, *S. michelini* will recover to this level in 109 years, and *S. siderea* will decline in area indefinitely, reaching less than 5% of the 2005 cover within 75 years. Bleaching events are forecast to become at least a decadal event, and, under the most pessimistic scenario, will occur annually.

Without a sustained reduction in emission of greenhouse gasses, there is no reason to assume that temperature levels in coastal tropical seas will remain at current levels, much less reduce. These regions will likely be subject to higher maximum levels, to more sustained periods above the bleaching threshold, and, as demonstrated in Chapter 2, possibly unforeseen oceanographic mixing and distribution patterns that may exacerbate the impacts of this increased thermal stress even further for selected strata or locations. Marine protected areas, reductions in overfishing, control of anthropogenic nutrient releases, and conservation of areas from direct impacts such as coral mining or removal are all important local factors for coral conservation, but
these must be placed in the context of global thermal increases and changes in ocean chemistry that may transcend the benefits of local conservation.

**Future work**

Individual coral colony response to environmental conditions is the result of a complex interaction of multiple factors. Thermal acclimatization (Carilli *et al* 2012), symbiont community (LaJeunesse *et al* 2004), species (Marshall and Baird 2001; Fabricus 2011), colony stress history (Castillo and Helmuth 2005), and ontogenetic stage (Vermeij and Sandin 2008) are all colony-specific factors, while environmental factors affecting coral growth include the presence of chemical and nutrient pollution (Pandolfi *et al* 2005), algae density (Smith *et al* 2006), the community of fish (particularly grazers) (Fox and Bellwood 2007), water temperatures (Fitt *et al* 2001) and ocean acidification (Hoegh-Guldberg *et al* 2010). In this study I have looked at growth and symbiont community diversity as two separate factors, with the implication that they are closely linked. However, it must be recognized that the changes in individual colony size over time recorded in this study could result from this myriad of other factors, and that the effects of some of these variables might be altered by the changes in other factors, and that the impacts may be non-linear under these differing conditions. In future studies I would like to include some of these additional factors in the analysis. This time series is a good start, and provides a basis for years of future work, assuming that a representative selection of these tagged corals remains alive, but establishing causative relationships between growth and
environmental variables will require a broader approach to integrating these additional important variables that may be contributing to the differences seen in colony state.

Two-dimensional planar area as a measure of coral growth was not wholly satisfactory, and I would like to continue investigating other methods of assessing coral colony size. Most corals are at least somewhat three-dimensional in shape, and many of them have significant vertical development. Changes in both upward and downward aspects were under-represented in my analysis of planar area, and non-invasive in situ three-dimensional methods would increase the sensitivity of coral size data. Creating electronic wire-frame models from multiple photographs shows great promise for meeting this need. I have begun working with architectural software interests to use their systems underwater to create such models. When these models are overlain with color, other analyses of coral health are also possible, such as areas and extent of partial bleaching, and identifying differential growth areas around the colony. I expect this work to continue in the coming years in association with the company Autodesk, Inc, maker of AutoCAD and other visualization software.

Finally, I would like to begin trying to associate coral symbiont community diversity patterns with likely past thermal stress exposure and history for those colonies. In other words, in place of looking at current growth in a given year as being directly related to symbiont type present in that year or just the previous annual period, perhaps the symbiont type at a current observation may be of use as an indicator of
specific events many years in the past that were not directly observed. Symbiont pattern is of course highly variable, as shown by numerous previous studies as well as by my dissertation research, and this would greatly complicate this interpretation, but the lasting presence of symbiont type A198 in *O. franksi* indicates that this signal may last on time frames approaching a decade. Using specific clade presence as an indicator has been demonstrated for other symbiont types, and may be possible for some of the types in these samples.

The association of current symbiont community makeup with past stress events also will help in addressing the largest avenue for future work - the question of whether lasting adaptive change is possible on the part of the corals in response to bleaching. A much deeper investigation into the roles of the symbionts in these samples is needed. In many cases, clade change patterns in this dataset were not as expected from generalized expectations, particularly for Clade A, highlighting the inadequacy of inter-cladal generalizations and the importance of uncovering taxon-specific physiological differences within clades to accurately understand and predict the responses of corals to environmental change. This work has only scratched the surface of these issues, but did reveal two possibly intriguing responses that appeared to show a difference in response over time, a key principle underlying the idea that adaptive change is possible. The first goal is continued monitoring, to demonstrate if these changes are in fact lasting and sustained, and the second goal will be to associate
these symbiont changes with changes in physiology, behavior, gene expression, changes in future response to environmental stress, or colony growth.

References


