FUNCTIONAL AND GENETIC DIVERSITY OF SYMBIOTIC DINOFLAGELLATES

(GENUS: Symbiodinium) IN REEF-BUILDING CORALS

by

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(Under the Direction of WILLIAM K. FITT AND GREGORY W. SCHMIDT)

ABSTRACT

The relationship between reef-building corals and symbiotic dinoflagellates (genus Symbiodinium) enable corals to exist in oligotrophic tropical and subtropical oceans. Symbiodinium enhance coral calcification rates and provide greater than 90% of the coral’s metabolic requirements via photosynthetically fixed carbon. In return, corals provide Symbiodinium with a nutrient-rich place to reside. Symbiodinium are highly diverse and can occur in symbiosis with mollusks, protists, and cnidarians including reef-building corals. This dissertation studies the ecology and physiology of genetically diverse Symbiodinium.

A novel technique for sampling coral was developed and used throughout these works enabling hundreds of samples to be taken from a single coral whilst causing minimal damage to the coral. Using this technique, Symbiodinium community structure of the Caribbean coral Montastraea faveolata was documented and indicated geographic specificity and local irradiance driven zonation patterns. To investigate coral-Symbiodinium physiology in “high-light” and “low-light” regions of M. faveolata physiological parameters were measured examining Symbiodinium community structure, photobiology, and calcification rates. Results showed genetically different Symbiodinium displayed differential “high-light” or “low-light” photo-physiological and photoacclimation responses indicating niche specialization within M. faveolata, which ultimately influences coral physiology.

Finally, corals response to two separate environmental stresses was studied. Community structure and physiology of Symbiodinium associated with M. faveolata was studied during a coral bleaching event in Puerto Morelos, Mexico. A post-bleaching shift in Symbiodinium communities was documented and thermally tolerant Symbiodinium ITS-2 types A3 and D1a were predominant. These results indicate functional differences between genetically distinct Symbiodinium populations that may facilitate competitive exclusion of thermally sensitive Symbiodinium types resulting in the abundance of tolerant opportunistic Symbiodinium.

During the winter of 2010 abnormal cold temperature in the upper Florida Keys resulted in mass mortality of many inshore reef-building corals. Following this event, the physiological effects of low temperature on three common reef-building corals (Montastraea faveolata, Porites astreoides, Siderastrea siderea) were experimentally investigated. This study documented species-specific physiological responses indicating different coral and/or zooxanthellae cold tolerances. Visual surveys of inshore reefs corroborated experimental results, with S. siderea
being minimally affected by the cold-water anomaly whereas *M. faveolata* and *P. astreoides* experienced approximately 100% mortality.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Coral reefs are among the most biologically diverse and productive ecosystems on Earth (Odum and Odum 1955; Connell 1978). Coral reef ecosystems have been estimated to provide 375 billion dollars a year in ecosystem services including coastline protection from storms, habitat for fisheries, and substantial tourism revenue (Costanza et al. 1997; Moberg and Folke 1999; Worm et al. 2006; Mumby and Steneck 2008; Hughes et al. 2010). Structural and trophic foundations for coral reef ecosystems are dependent upon the obligate mutualism between reef-building corals and their dinoflagellate symbionts (genus *Symbiodinium*). The relationship between reef-building corals and *Symbiodinium* enables coral to exist in oligotrophic tropical and sub-tropical oceans. *Symbiodinium* enhance coral calcification and provide greater than 90% of the coral’s metabolic requirements *via* photosynthetically fixed carbon, which is translocated to the coral as carbohydrates. Corals provide *Symbiodinium* with a predator free, nutrient-rich place to reside (Muscatine 1967; Muscatine and Porter 1977; Gattuso et al. 1999; Yellowlees et al. 2008; Colombo-Pallotta et al. 2010).

Coral reefs have declined worldwide over the last several decades (Porter and Meier 1992; Hoegh-Guldberg 1999; Gardner et al. 2003; Bruno and Selig 2007) and emphasis has been placed on studying the ecological, physiological, cellular and biochemical effects of increased seawater temperatures that result in coral bleaching (e.g., Porter et al. 1989; Gates et al. 1992; Fitt et al. 1993; Gleason and Wellington 1993; Warner et al. 1996; Lesser 1997; Rowan et al. 1997; Warner et al. 1999; Fitt et al. 2000; van Woesik 2001; Berkelmans and van Oppen 2006;
Tropical and sub-tropical corals are typically found in shallow, clear waters and are among the most efficient light collectors on the planet; therefore, *Symbiodinium* can be exposed to dangerously high irradiance and UV radiation (Gleason and Wellington 1993; Enriquez et al. 2005; Teran et al. 2010). Abnormally high sea temperature and/or irradiance can breakdown the symbiotic relationship between coral and *Symbiodinium* (Glynn 1996; Brown 1997; Warner et al. 1999; Fitt et al. 2001; Weis 2008). To tolerate high-light environments, corals and *Symbiodinium* have photoprotective and photo-repair mechanisms (Iglesias-Prieto and Trench 1997; Warner et al. 1999; Banaszak et al. 2000; Brown et al. 2000; Banaszak et al. 2006; Dove et al. 2006; Warner and Berry-Lowe 2006; Reynolds et al. 2008; Hennige et al. 2009; Takahashi et al. 2009). *Symbiodinium* photoacclimate to different irradiance values by adjusting pigmentation and the number of reaction centers for light harvesting to maximize or minimize the amount of absorbed and transferred excitation energy (irradiance) (Coles and Jokiel 1978; Falkowski and Dubinsky 1981; Dubinsky et al. 1984; Porter et al. 1984; Iglesias-Prieto and Trench 1994,1997; Hennige et al. 2009; Hennige et al. 2010).

Differential thermal and irradiance tolerances have been identified in genetically distinct *Symbiodinium* (Rowan and Knowlton 1995; LaJeunesse 2002; Baker 2003; Ulstrup and Van Oppen 2003; Iglesias-Prieto et al. 2004; Sampayo et al. 2007), which were once classified as the pandemic species of *Symbiodinium microadriaticum*. *Symbiodinium* are now widely recognized as a highly diverse group of dinoflagellates belonging to nine clades (A-I) (Freudenthal 1962; Schoenberg and Trench 1980; Rowan and Powers 1991; Trench 1997; Rowan 1998; LaJeunesse 2001; Coffroth and Santos 2005; LaJeunesse 2005; LaJeunesse et al. 2010; Pochon and Gates 2010). It is clear that *Symbiodinium* are not only genetically diverse but also have physiological,
biochemical, and cellular differences that may allow them to occupy a particular niche. Physiological studies using cultured *Symbiodinium* have demonstrated differences in photosynthetic rates, thermal tolerance (to increased and decreased temperatures), photodamage repair, photo-protection strategies, cell size, and photosynthetic membrane structure among genetically distinct *Symbiodinium* types (Iglesias-Prieto et al. 1992; Warner et al. 1999; Banaszak et al. 2000; LaJeunesse 2001; Tchernov et al. 2004; Warner and Berry-Lowe 2006; Reynolds et al. 2008; Takahashi et al. 2008; Thornhill et al. 2008; Takahashi et al. 2009).

Numerous ecological surveys have focused on *Symbiodinium* biogeography, revealing vast genetic diversity including “generalist” and “specialist” phylotypes of *Symbiodinium* (Loh et al. 2001; LaJeunesse 2002; LaJeunesse et al. 2003; Santos et al. 2003; Van Oppen et al. 2005; Thornhill et al. 2009; Finney et al. 2010; LaJeunesse et al. 2010). Niche partitioning between distinct *Symbiodinium* phylotypes has been documented revealing environmental specialists, especially with regard to high and low light habitats (Rowan and Knowlton 1995; Toller et al. 2001; LaJeunesse 2002; Ulstrup and Van Oppen 2003; Garren et al. 2006; Sampayo et al. 2007). Additionally, population level genetic markers (micro-satellites) have documented high degrees of *Symbiodinium* population structure and endemism both in Caribbean reef-building corals and gorgonians (Santos et al. 2003; Pettay and LaJeunesse 2007; Kirk et al. 2009; Thornhill et al. 2009). While much insight has been gained on the degree of coral-zooxanthellae specificity and/or flexibility, this is still a topic of current debate (see Goulet 2006; Baker and Romanski 2007; Goulet 2007).

This dissertation investigates the diversity of *Symbiodinium* associating with the reef-building coral *Montastraea faveolata* and its affect on coral physiology utilizing a novel microsampling technique described in chapter two. This technique enables multiple samples to
be taken from a coral while causing minimal damage to the coral host (Kemp et al. 2008). Using microsampling, chapter three investigates biogeography and within-colony community structure of *Symbiodinium* associating with *M. faveolata* from four different geographic regions in the Caribbean. Chapter three corroborates previous finding of *M. faveolata* ability to simultaneously associate with diverse assemblages of *Symbiodinium* from clades A, B, C, and D. In addition, geographic endemism and fine-scale specificity in phylotypes (sub-cladal) where documented at each region indicating long-term ecological and evolutionary processes. Using described *Symbiodinium* community structure from chapter three, chapter four investigates physiological differences between high-light and low-light niches of *M. faveolata*. Chapter four shows that genetically different symbiont types display differential high-light or low-light photo-physiological and photoacclimation responses indicating *Symbiodinium* niche specialization within *M. faveolata* which ultimately influences coral physiology.

Chapters five and six examine the effect two separate environmental stressors have on coral-alga physiology and how genetically different *Symbiodinium* may be affected by the stress. In chapter five, community structure and physiology of *Symbiodinium* associated with *M. faveolata* is studied during a coral bleaching event in Puerto Morelos, Mexico. Using microsampling methods accompanied with *in situ* pulse amplitude modulated fluorometry (PAM) we monitored the effect coral bleaching has on *Symbiodinium* physiology community structure. I describe community shifts and suggest possible mechanisms that may influence competitive exclusion of thermally sensitive *Symbiodinium* ITS-2 types. Chapter six was unplanned and was the result of a cold-water anomaly that resulted in widespread catastrophic coral mortality of inshore coral reefs in the upper Florida Keys. This chapter combines ecological surveys with controlled experiments to investigate the effect of cold-water stress on three species of corals and
their endosymbiotic dinoflagellates. This study documents species-specific physiological responses, indicating different coral and/or zooxanthellae cold tolerances. These results corroborate with works of Mayer (1914) and I suggest potential physiological mechanisms that could influence cold-water susceptibility of coral-\textit{Symbiodinium} symbiosis.

Combining ecological observations with molecular and physiological methods I investigate coral-\textit{Symbiodinium} symbiosis in reef-building corals. I hypothesized that niche specialization would influence \textit{Symbiodinium} distribution within colonies of \textit{M. faveolata}. Additionally, I hypothesized that thermal adaptations of \textit{Symbiodinium} would result in differential coral physiology and potential mortality. The results of these studies indicate functional differences between genetically distinct \textit{Symbiodinium} and highlight the ecological importance of understanding the physiology of these symbiotic dinoflagellates.
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CHAPTER 2

A MICROSAMPLING METHOD FOR GENOTYPING CORAL SYMBIONTS


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ABSTRACT

Genotypic characterization of Symbiodinium symbionts in hard corals has routinely involved coring, or the removal of branches or a piece of the coral colony. These methods can potentially underestimate complexity of the Symbiodinium community structure and may produce lesions. This study demonstrates that microscale sampling of individual coral polyps provided sufficient DNA for identifying zooxanthellae clades by RFLP analyses, and subclades through the use of PCR amplification of the ITS-2 region of rDNA and denaturing-gradient gel electrophoresis (DGGE). Using this technique it was possible to detect distinct ITS-2 types of Symbiodinium from two or three adjacent coral polyps. These methods can be used to intensely sample coral-symbiont population/communities while causing minimal damage. The effectiveness and fine scale capabilities of these methods were demonstrated by sampling and identifying phylotypes of Symbiodinium clades A, B, and C that co-reside within a single Montastraea faveolata colony.

Key Words: Symbiodinium, Zooxanthellae, Microhabitat, Coral Sampling, Symbiosis, Genotyping
INTRODUCTION

The presence of photosynthetic endosymbionts commonly referred to as zooxanthellae contribute substantially to the productivity, survival, and success of coral reefs (Muscatine and Porter 1977). In the last 25 years, significant advances have been made in understanding the genetic diversity of these algae. Zooxanthellae are primarily represented by the genus *Symbiodinium* whose cladal and subcladal members exhibit differential host-specificity, ecological niche specialization, and temperature stress sensitivity (Rowan and Powers 1991; Iglesias-Prieto et al. 1992, 2004; Rowan and Knowlton 1995; Rowan et al. 1997; Warner et al. 1999; 2006; LaJeunesse and Trench 2000; LaJeunesse 2002; Rowan 2004; Tchernov et al. 2004; Goulet et al. 2005). With projected sea temperature increase, genetically diverse zooxanthellae may play an important ecological role in the survival of corals (Little et al. 2004; Berkelmans and van Oppen 2006; Ulstrup et al. 2006).

To date, genotypic surveys of coral symbionts have employed removal of branches, use of hammers and chisels, or coring in order to acquire coral tissue for DNA analyses. These methods provide abundant material for extractions of *Symbiodinium* DNA, but can inflict considerable damage to the coral colony that may increase susceptibility to diseases or initiate other necrotic processes (Mascarelli and Bunkley-Williams 1999; Henry and Hart 2005; Aeby and Santavy 2006).

This study describes a coral-zooxanthellae sampling protocol that enables zooxanthellae genotype identification from minimal amounts of coral tissue, and demonstrates the fine scale capabilities of these techniques on a common Caribbean coral *Montastrea faveolata*, known to simultaneously associate with diverse assemblages of *Symbiodinium* (Rowan and Knowlton 1995).
MATERIALS AND METHODS

Coral tissue (containing zooxanthellae) was collected from *M. faveolata* using a sterile 3 cc syringe that was tipped with a 16 gauge needle which was blunted (for safety reasons) with a file or grinding wheel. Typically, the needle was inserted and rotated to thoroughly penetrate a coral polyp and samples were withdrawn with pre-labeled 3 ml syringes by gentle suction (Figure 1). One to three separate coral polyps (depending on species) were extracted from the intact colony (Figure 2) resulting in approximately, 1.5-2.0 mg ash free dry weight (AFDW) of coral-zooxanthellae tissue. This amount of polyp mass can be highly variable due to seasonal variations in coral tissue and zooxanthellae densities (Fitt et al. 2000). During polyp extraction, surrounding seawater and coral mucus was inadvertently collected. Therefore, the possibility that some free-living *Symbiodinium* and microbial symbionts may be incorporated into the samples cannot be ruled out, as is the case with all bulk sampling methods.

Damage to the colony was difficult to see macroscopically since the calcified skeleton was not exposed. Moreover, healing usually occurred rapidly (weeks to months) by somatic proliferation from adjacent host tissues.

Syringes containing the samples were placed in coolers containing seawater, to maintain the ambient temperature, and then transferred to the laboratory to be processed further. Each sample was expelled from the syringe to pre-labeled 1.5 or 2.0 ml microcentrifuge tubes, centrifuged (~5,000 g) for 1-2 minutes to pellet zooxanthellae released from the coincidentally disrupted coral tissue. The supernatant was decanted and either 80% ethanol alcohol or DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride; Seutin et al. 1991) was added for DNA preservation. Symbiont DNA was extracted subsequently with the protocols prescribed for the Wizard DNA preparation kit (Promega) and refined for LaJeunesse
et al. 2003. The extracted DNA was dried, resuspended in 100 μl ddH₂O, and quantified using a Nano Drop ND-1000 spectrophotometer.

PCR-based fingerprinting techniques were used to identify Symbiodinium from the collected samples. Restriction fragment length polymorphisms (RFLPs) analysis were conducted by amplifying the small subunit (18S) rDNA with dinoflagellate biased primers “ss5” and “ss3z” (Rowan and Powers 1991), digested with Taq I restriction endonuclease, then separated in a 2% 1X TAE agarose gel to generate the RFLPs. RFLPs were compared to cultured standards for cladal identification. Denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 region (ITS-2) of the nuclear ribosomal RNA, using GC-rich clamp primers were done with the primers “ITSintfor2” and the “ITS2Clamp” following the protocols of LaJeunesse et al. 2003.

RESULTS AND CONCLUSIONS

The microsampling technique allowed the collection and analysis of numerous coral-zooxanthellae samples from precise positions on a single coral colony, while causing minimal damage to the colony itself (Figure 2). In this study, the yield of DNA varied between 45.01 and 90.58 ng μl⁻¹. DNA concentrations could be highly variable due to nucleic acid extraction efficiency between samples and/or unequal sample amount of host-symbionts that vary because of symbiont density and/or polyp size and vigor. The sampling protocol used here employed sampling two to three coral polyps, used the same sized needle and syringe, and removed ~1.5-2.0 mg of AFDW of coral tissue per sample. Because of the significant biological and mechanical variation, quantitative analyses, although possible would need to be used with caution.
All the DGGE banding profiles patterns were compared directly to previously sequenced standards for symbiont identification (Figure 3). In every case, RFLP cladal identifications coincided directly with DGGE genotypes (data not shown). Because of the greater specificity that DGGE allows, only ITS-2 genotypes are reported (Figure 3). There are no apparent reasons why this microsampling technique could not be used with alternative *Symbiodinium* identification applications such as microsatellite analyses (e.g., Santos et al 2003), single-stranded conformation polymorphisms (SSCP) (e.g., Little et al 2004), or quantitative real time PCR (qPCR) (e.g., Ulstrup and van Oppen 2003).

*Symbiodinium* discrimination by molecular genetics has revealed distinct host-symbiont populations and community structure (LaJeunesse 2002; Baker 2003; Thornhill et al. 2006). Niche partitioning between distinct *Symbiodinium* phylotypes has also been documented to a limited extent, revealing environmental specialists, particularly with respect to high and low light habitats (Rowan and Knowlton 1995; Toller et al. 2001; LaJeunesse 2002; Ulstrup and van Oppen 2003; Garren et al. 2006; Sampayo et al. 2007). Additionally, there have been numerous ecological surveys focusing on *Symbiodinium* biogeography (Loh et al. 2001; LaJeunesse 2002; LaJeunesse et al. 2003; Santos et al. 2003; van Oppen et al. 2005). While much insight has been gained on the degree of coral-zooxanthellae specificity and/or flexibility that occurs, this is still a topic of debate (see Goulet 2006; 2007 and Baker and Romanski 2007). A major deficiency, however, in many of these studies has been reliance on bulk sampling methods that do not spatially resolve coral polyp heterogeneity with respect to their most prevalent symbiont. Such crude methodologies may account for much of the confusion, for certain host species, as to whether some symbiont phylotypes are dominant while minor symbionts prevail or co-inhabit cryptic niches. Alternative conclusions from bulk coral sampling might equally be interpreted as
competitive/differential proliferation stemming from symbiont segregation among adjacent polyps as a result microhabitat specialization. As illustrated in Figure 3, some coral colonies have heterogeneous populations of *Symbiodinium* that are spatially distributed on a micro-scale (as determined by PCR-DGGE of ITS-2 rDNA from syringe samples of adjacent coral polyps). Such spatial distribution and coral-symbiont complexity would not be resolved by analysis of large coral pieces.

To date, the technique demonstrated in this study has also been used successfully to identify *Symbiodinium* from other Caribbean hard corals (*Montastraea annularis, Montastraea franski, Montastraea cavernosa, Siderastrea siderea, Porites asteroides and Stephanocoenia intersepta*). The only alterations to the methodologies have involved variations of the needle gauge size. Smaller needles (18-26 gauge) tended to work better for smaller polyped corals, but clogged more frequently, it is better, therefore, to use as large gauge needles as possible. The method can also potentially be extended to molecular certification of host identities and their possible genetic variation, along with the identification of additional microbial symbionts and pathogens that are also found to associate with corals (Knowlton and Rohwer 2003).
REFERENCES


Fig. 2.1. Application of the syringe technique on a *Montastraea faveolata* colony. Typically, two to three polyps were sampled. The rope was marked every 10 cm and used as a transect for sampling.
Fig. 2.2. a) *Montastraea faveolata* colony before using the syringe technique. b) Same area showing minimal damage after sampling using the syringe technique to sample the coral. Highlighted area is where three polyps were sampled.
Fig. 2.3. The distribution of *Symbiodinium* genotypes (ITS2 region) from *Montastraea faveolata* from Carrie Bow Cay, Belize. a) PCR-DGGE fingerprint profiles of the ITS2 region showing the genotypes involved. The gel profile is presented as a reverse image. The first two gel lanes are markers with common ITS2 PCR-DGGE profiles of *Symbiodinium* found in the Caribbean. Non-labeled, minor shadow bands in lanes NW50, N20, NW20 N50 and E20 are presumably heteroduplexes or intragenomic variants and, due to their consistent co-occurrence, provide an additional fingerprint for phylotype B17. The top of each lane indicates compass direction and the distance (cm) from the top of the colony where syringe samples of 2-3 neighboring polyps were taken every 10 cm. Below the gel subclade designated symbiont phylotypes are assigned; capital letters indicate clades, and numbers represent ITS2 type (LaJeunesse 2002). b) Microhabitat distribution of *Symbiodinium* genotypes (ITS2 region) in *M. faveolata* (2m depth).
CHAPTER 3

GEOGRAPHIC AND NICHE SPECIFICITY IN SYMBIODINIUM COMMUNITY STRUCTURE ASSOCIATED WITH MONTASTRAEA FAVEOLATA IN THE CARIBBEAN

2 Kemp, D.W., Thornhill, D.J., Schmidt, G.W., and W.K. Fitt. To be submitted to Coral Reefs
ABSTRACT

Some colonies of the common Caribbean reef-building coral *Montastraea faveolata* have been found to harbor multiple phylotypes of endosymbiotic dinoflagellates (genus *Symbiodinium*). Here, we use a minimally destructive sampling procedure to expansively collect coral tissue from fixed transects from four distinct regions in the Caribbean (Carrie Bow Cay, Belize; Puerto Morelos, Mexico; Lee Stocking Island, Bahamas; Florida Keys, USA). Community structure of *Symbiodinium* within *M. faveolata* samples was examined using denaturing gradient gel electrophoresis (PCR-DGGE) fingerprint analysis of the rDNA Internal Transcribed Spacer 2 (ITS-2) region. Intracolony zonation patterns of *Symbiodinium* were found to correlate with light availability indicating distinct capacities for photoacclimation among *Symbiodinium* clades. Geographic comparison of host-symbiont associations further revealed regional specificity of *Symbiodinium* from clades A, B, C. Three distinct ITS-2 types were identified for Clade C, unique to the Florida Keys, Bahamas, or Mesoamerican reefs (Belize and Mexico). Clade A was detected in Mesoamerican, but not the Florida Keys or Bahamas. Likewise, clade B17 was unique to Mesoamerica while B1 was found in reefs of the Florida Keys, Bahamas in addition to Mesoamerica. The occurrence of geographical and environmental genetic specificity and within-colony stability are considered as consequences of long-term ecological and evolutionary adaptations.

**Key Words:** Niche Specificity, *Symbiodinium, Montastraea faveolata*, Coral Reefs, Coral Microsampling, Caribbean, Symbiosis
INTRODUCTION

The relationship between reef-building corals and endosymbiotic dinoflagellates (genus *Symbiodinium*) enables coral to exist in oligotrophic tropical and sub-tropical oceans. *Symbiodinium* can provide greater than 90% of the coral’s metabolic requirements via photosynthetically fixed carbon translocated to the coral as carbohydrates (Muscatine 1967; Muscatine and Porter 1977; Gattuso et al. 1999; Yellowlees et al. 2008). The once believed pandemic species of *Symbiodinium microadriaticum* is now widely recognized as a highly diverse group of dinoflagellates belonging to nine clades (A-I) (Freudenthal 1962; Schoenberg and Trench 1980; Rowan and Powers 1991; Trench 1997; Rowan 1998; LaJeunesse 2001; Coffroth and Santos 2005; LaJeunesse 2005; LaJeunesse et al. 2010a; Pochon and Gates 2010). Members of these nine clades have different cellular components and biochemical processes, which result in varied physiological responses to environmental stress (Iglesias-Prieto et al. 1992; Warner et al. 1999; Tchernov et al. 2004; Reynolds et al. 2008; Takahashi et al. 2008; Weis 2008; Takahashi et al. 2009). Further molecular evidence indicates a preponderance of highly specific and stable host-symbiont genetic combinations, reflecting long-term ecological and evolutionary influences on the composition of the holobiont systems (Santos et al. 2004; LaJeunesse et al. 2005; Smith et al. 2009; Thornhill et al. 2009; LaJeunesse et al. 2010a).

Tropical and sub-tropical corals are typically found in shallow, clear waters and are among the most efficient light collectors on the planet; therefore, *Symbiodinium* can be exposed to dangerously high irradiance and UV radiation (Gleason and Wellington 1993; Enriquez et al. 2005; Teran et al. 2010). Differential thermal and irradiance tolerances have been identified in genetically distinct *Symbiodinium* and can be a basis of niche partitioning (Rowan and Knowlton 1995; LaJeunesse 2002; Baker 2003; Ulstrup and Van Oppen 2003; Iglesias-Prieto et al. 2004;
Sampayo et al. 2007). Abnormally high sea temperature and/or irradiance can trigger breakdown
the symbiotic relationship between coral and *Symbiodinium* (Glynn 1996; Brown 1997; Warner
et al. 1999; Fitt et al. 2001; Weis 2008). When tolerant of high-light environments, corals and
*Symbiodinium* have photoprotective and photorepair mechanisms (Iglesias-Prieto and Trench
1997; Warner et al. 1999; Banaszak et al. 2000; Brown et al. 2000; Banaszak et al. 2006; Dove et
al. 2006; Warner and Berry-Lowe 2006; Reynolds et al. 2008; Hennige et al. 2009; Takahashi et
al. 2009). *Symbiodinium* can also photoacclimate to different irradiance values by adjusting
pigmentation and the abundance of reaction centers or light harvesting complexes (Coles and

Since the widespread die-off of *Acropora* spp. in the Caribbean, *M. faveolata* has become
the most abundant reef-building coral throughout this region. *M. faveolata* can inhabit reef
habitats ranging in depth from intertidal zones to 80 m (Goreau and Wells 1967; Reed 1985).
Most corals to date have been found to associate with a single genetically dominant
*Symbiodinium*; *M. faveolata*, however, is commonly found to simultaneously host up to three
genetically distinct dominant communities of *Symbiodinium* (Rowan and Knowlton 1995; Toller
et al. 2001b; Garren et al. 2006; Kemp et al. 2006). *Symbiodinium* communities within *M.
faveolata* fluctuate spatially and temporally and this dynamism can alter coral physiology
(Brown et al. 1999; Fagoonee et al. 1999; Fitt et al. 2000; Warner et al. 2002; Warner et al.
2006). To date, no large-scale survey has been conducted to investigate the degree to which
*Symbiodinium* community structure within *M. faveolata* is conserved across geographic location
and habitat type. In this study we use micro-sampling techniques to investigate the genetic
diversity and community structure of *Symbiodinium* associating with *M. faveolata* in shallow (higher irradiance) and deep (lower irradiance) reefs at four geographic regions in the Caribbean.

**MATERIALS AND METHOD**

*Collection Sites*

Coral tissue samples were collected from four geographic regions in the Caribbean: Lee Stocking Island, Bahamas, Carrie Bow Cay, Belize, Puerto Morelos, Mexico, and the Florida Keys (Fig. 1). *Montastraea faveolata* colonies were collected from nearby shallow and deep reefs in each location to examine variation between habitats and among geographic regions.

Four colonies were sampled from the Bahamas, two each from North Norman Pond (NNP) (2-4m depth; 23.79.12°N, 76.1368°W) and South Perry Reef (SP) (12-15m depth; 23.7752°N, 76.0895°W) in 2006 and 2007 resulting in 65 total samples. In Belize, two colonies each were sampled from Carrie Bow Cay Lagoon (CBCL) (1-2m depth; 16.8016°N, 88.082786°W) and a deeper reef off Carrie Bow Cay Reef Crest (CBCR) (10-12m depth; 16.802117 N, 88.080308°W) in 2005 and 2006 resulting in 100 total samples. In Mexico, 197 *M. faveolata* tissue samples were collected from five colonies each at La Bocanna (LB) reef (2-4m depth; 20.5228°N, 86.5105°W), from 2006-2009 but, unfortunately, deeper reefs were not sampled.

*Montastraea faveolata* was sampled from three shallow reefs (< 4 m) in the upper Florida Keys from 2006-2008: two colonies at Turtle Reef (TR) (2-3m depth; 25.2944°N, 80.2191°W), four colonies at Admiral Patch Reef (ADM) (2m depth; 25.0446°N, 80.3945°W), and four colonies at Little Grecian Reef (LG) (2-4m depth; 25.1188°N, 80.3016°W). Additionally, two colonies from the deeper Alligator Reef (AG) (10-12m depth; 24.8424°N, 80.6244°W) were sampled in the middle Florida Keys, for a total of 164 samples from the Florida Keys.
**Micro-Sampling Procedure**

Using micro-sampling procedures of Kemp et al. (2008), *M. faveolata* coral tissue was collected in syringes along four transects laid from top to bottom of the colony at north, east, south, and west compass headings. Samples were collected from 2-3 polyps every 10 to 20 cm using 2cc syringes with 16 gauge needles resulting in 30-60 tissue collections per coral colony.

Upon returning to the laboratory, syringe samples were transferred into 2 mL Eppendorf tubes and centrifuged at (∼5,000g) for 2 min. Supernatants were decanted and either 80% ethanol alcohol or DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride) was added for DNA preservation (Seutin, 1991).

**Genetic Identification of Symbiodinium**

Genetic identities of the dominant *Symbiodinium* in each tissue sample were determined following the protocols of LaJeunesse et al. 2003. DNA was extracted using a modified Promega Wizard genomic DNA extraction protocol. Denaturing-gradient gel electrophoresis (DGGE) was used to analyze the ITS-2 region of nuclear ribosomal RNA genes (LaJeunesse 2002, LaJeunesse and Trench 2000). A touchdown thermal cycle described in LaJeunesse et al. 2003 was used for PCR amplification of the ITS-2 region using the forward primer “ITSintfor2” (5’-GAATTGCAGA ACTCCGTG-3’) and the reverse primer “ITS2CLAMP” (5’- CGCCCCGCCCCGCCCGCCGCGCCGCCGCCGGGATCTTAAGTTCAGCGGGGT’-3’), with a 39-bp GC clamp (italicized) (LaJeunesse and Trench 2000). Products were electrophoresed on 45-80% urea-formamide gradient denaturing gradient gels (100% consists of 7 M urea and 40% deionized formamide) for 10 h at 150 V at a constant temperature
of 60°C using a C.B.S. Scientific system (LaJeunesse 2002, Thornhill et al. 2010). Samples were run parallel to previously sequenced standards.

RESULTS

The syringe microsampling technique allowed us to precisely collect multiple coral tissue samples without significant damage to the coral colony (Kemp et al. 2008). Most colonies were inspected multiple times over a five-year period and no detrimental side effects were detected (Kemp, personal observation). Molecular identification of *Symbiodinium* revealed within colony zonation patterns at all sites. Additionally, geographic distinct *Symbiodinium* ITS-2 types were detected between the Bahamas, Florida Keys, and Mesoamerican reefs.

Shallow-water *Montastraea faveolata* colonies from Lee Stocking Island, Bahamas and the upper Florida Keys were found to have the lowest levels of *Symbiodinium* diversity as detected by DGGE analyses. 95% of the samples from North Norman Pond (NP) were found to associate with ITS2-type B1 *Symbiodinium* and only 5% of the samples were found to have only ITS-2 type C12. A small percentage (<5%) of samples contained *Symbiodinium* ITS-2 type D1a (Fig. 2a). Samples from the deeper reef of South Perry also to have greater abundances of *Symbiodinium* type C12. Overall, *Symbiodinium* type C12 were found in 68% of the samples from South Perry Reef (Fig 2b).

Similar to the Bahamas, low diversity of *Symbiodinium* is characteristic of shallow water *M. faveolata* colonies from the upper Florida Keys. 96% of the samples from upper Florida Keys also were found to associate with ITS2-type B1 *Symbiodinium* (Fig. 3a). However, coral colonies from Alligator Reef (AG) have more diverse *Symbiodinium* communities than do their
shallow water conspecifics. Hence, corals from AG were found to possess mixed Symbiodinium communities of ITS-2 types B1 and C3 (Fig. 3b).

Symbiodinium communities of M. faveolata from Mesoamerican reefs of Puerto Morelos, Mexico and Carrie Bow Cay, Belize have greater genetic diversity of ITS-2 types of Symbiodinium. Unlike M. faveolata colonies from the Florida Keys or the Bahamas, individual coral colonies from Mesoamerican reefs usually associate with three genetically dominant ITS-2 types of Symbiodinium (A3, B17 or B1, and C7) and, in very few instances, Symbiodinium type D1a was found in samples from Mexico (Figs. 4,5). In addition, intra-colonial zonation patterns are apparent with clade distributions that are largely coincident with the prevailing irradiance patterns at the coral’s surface. Generally, Symbiodinium ITS-2 type A3 were found in high irradiance areas on the coral (i.e. top of the colony), while type C7 were found in lower irradiance areas on the corals' lower surfaces. Symbiodinium type B17 was the most frequently detected Symbiodinium found in shallow water corals from Mexico (72% of all samples; Fig. 4a) and Belize (76% of all samples; Fig. 5a). Interestingly, Symbiodinium type B17 was not found in any of the deep-water corals. Instead, Symbiodinium ITS-2 type B1 were found in deep water colonies of M. faveolata from Mexico and Belize (Figs 4a, 5b).

**DISCUSSION**

Corals belonging to the Montastraea annularis “complex”, including M. faveolata, are often viewed as corals that have “flexible” symbiosis capabilities, that is, the ability to simultaneously host genetically distinct populations of Symbiodinium (Rowan and Knowlton 1995; Baker 2003; Baker and Romanski 2007). The ability of a colony of M. faveolata to simultaneously host up to four genetically distinct, dominant, Symbiodinium ITS-2 types is not
often seen among corals outside of the *M. annularis* “complex” (Rowan and Knowlton 1995; Rowan et al. 1997; Iglesias-Prieto et al. 2004; Berkelmans and van Oppen 2006; Kemp et al. 2008; Sampayo et al. 2008; LaJeunesse et al. 2010a; Lajeunesse et al. 2010b). The ecological and physiological advantages/disadvantages of hosting diverse symbiont assemblages are poorly understood. Significant progress in resolving this issue has been achieved with denaturing gradient gel electrophoresis (DGGE) to distinguish distinct ITS-2 phylotypes of *Symbiodinium* hosted by numerous cnidarians and other marine organisms (LaJeunesse and Trench 2000; LaJeunesse 2002; Kemp et al. 2006; Thornhill et al. 2006; Sampayo et al. 2007; Sampayo et al. 2009; Finney et al. 2010; LaJeunesse et al. 2010a). Using this technique, we herein documented geographic specificity between *M. faveolata* and *Symbiodinium* belonging to the clades A, B, C and in two cases very small proportions of the opportunistic type D1a was found. Our data also revealed intra-colonial zonation patterns, suggesting that *Symbiodinium* associating with *M. faveolata* occupy unique niches defined primarily by irradiance but possibly temperature clines as well.

*Symbiodinium* belonging to clade C were most prevalent in low-irradiance areas within shallow-water colonies and in colonies inhabiting deeper waters. These findings corroborate previous studies (Rowan and Knowlton 1995; Toller et al. 2001a; LaJeunesse 2002; Garren et al. 2006) but here we identify three different ITS-2 types of clade C and their according geographic distributions: ITS-2 type C3 from the Florida Keys, C12 from the Bahamas, and C7 from Mexico and Belize. Our data support the conjecture that clade C has undergone localized speciation throughout the Caribbean as previously documented in on the Great Barrier Reef and elsewhere (LaJeunesse et al. 2004; LaJeunesse 2005; Finney et al. 2010; LaJeunesse et al. 2010a)
Symbiodinium ITS-2 type B1 has been referred to as a “generalist” because of its occurrence in multiple host taxa and its occurrence in wide geographic and depth ranges (LaJeunesse 2002). We detected B1 at the majority of collection sites and throughout the entirety of the coral colonies. Interestingly, we did not find B1 in Mexico nor in shallow reefs in Belize. In these locations, B17 appears to occupy the niche otherwise held by B1. As a similar distribution disjunction, we detected clade A (ITS-2 type A3) Symbiodinium only in Mesoamerican reefs of Belize and Mexico; no clade A was identified in corals from the Bahamas or Florida Keys.

Clade A Symbiodinium have previously been found in regions of M. faveolata that endure high irradiance (Rowan and Knowlton 1995; Toller et al. 2001a; Garren et al. 2006; Rodriguez-Roman et al. 2006). Clade A Symbiodinium have greater photoacclimation capabilities, unique photosystem repair processes, and enhanced photoprotection pathways (Robison and Warner 2006; Reynolds et al. 2008; Takahashi et al. 2009) than do other clades. These adaptations to high-irradiance niches might account for intra-colonial zonation patterns relating to Symbiodinium ITS-2 type A3 found in M. faveolata from Mesoamerican coral colonies in this study, but do not explain the apparent absence of Symbiodinium type A3 in M. faveolata from the Bahamas or the Florida Keys. Our data suggest that A3, B17, and C7 associating with M. faveolata are restricted to Belize and Mexico and lend evidence for evolutionary processes of hosts and symbionts have occurred in the Mesoamerican barrier reef but not other Caribbean locations sampled in this study.

Coral species such as Acropora palmata, A. cervicornis, and Porites astreoides are found to primarily associate with clade A Symbiodinium in shallow water habitats throughout Caribbean. While species of the M. annularis “complex” have been reported to host clade A
*Symbiodinium* from reefs in the lower Caribbean (i.e. Panama, Belize, and Mexico), they are rarely found in *M. annularis* “complex” species in the Florida Keys and have never been documented in *Montastraea* spp. in the Bahamas (Rowan and Knowlton 1995; Toller et al. 2001a; LaJeunesse 2002; Garren et al. 2006; Rodriguez-Roman et al. 2006; Thornhill et al. 2006; Warner et al. 2006; Kemp et al. 2008; Baums et al. 2010). Plausible explanations for this geographic partitioning of clade A *Symbiodinium* found in *Montastraea* spp. in the Florida Keys and Bahamas could be that they are out-competed by *Symbiodinium* in clades B and C. Physiological studies and ecological surveys have confirmed that Clade A *Symbiodinium* have enhanced photoprotection and have been shown to have increased resilience to high temperature-induced coral bleaching (Rowan et al. 1997; Reynolds et al. 2008), but few studies to date have examined the effects of cold temperature have on coral-*Symbiodinium* associations.

Other ecological surveys done in the Dry Tortugas and the Florida Keys have documented severe coral mortality of *P. astreoides*, *A. palmata*, and *A. cervicornis* and other corals immediately following abnormal cold fronts causing seawater temperatures to drop below 16 °C (Davis 1982; Porter et al. 1982). Furthermore, early cold tolerance reconstruction studies conducted by Mayer (1914) found that *A. cervicornis*, *A. palmata*, and *P. astreoides*, (all corals we now know commonly associate with clade A *Symbiodinium*) were most sensitive to cold treatments. Recent experimental work (in prep) documents that *P. astreoides* with clade A *Symbiodinium* experienced greater amounts of photodamage and were the most negatively affected by decreases in temperatures than corals hosting *Symbiodinium* from clades B or C. Therefore, it is reasonable to conclude that *Symbiodinium* clade A are greatly stressed during the winter low temperatures that Florida and Bahamian reefs periodically experience and are
displaced during those episodes by competitive displacement by other more thermally flexible
Symbiodinium.

This study used microsampling of *M. faveolata* for fine scale comparisons of
*Symbiodinium* community structure at different spatial declinations. Our findings document
highly structured, intra-colony zonation patterns of genetically distinct *Symbiodinium* suggesting
niche diversification. Further investigation regarding within colony competition between
different *Symbiodinium* types is needed to better understand ecological zonation pattern
documented. Furthermore, results of this work document unique host-symbiont combinations in
the Florida Keys, Bahamas, Mexico and Belize as well as the strong population structure and
potentially dominant geographic endemism of *Symbiodinium* in the Caribbean.
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Fig. 3.1. Reef locations in the Caribbean investigated in this study.
Fig. 3.2. *Symbiodinium* (ITS-2 phylotypes) community structure in a *Montastraea faveolata* colony sampled along fixed transects from Lee Stocking Island, Bahamas. Pie graphs represent proportion of identified *Symbiodinium* from all sampled corals from the particular site

a) *Symbiodinium* distribution from North Normans Pond (2m depth) (n = 2 colonies; 37 samples)
b) *Symbiodinium* distribution from South Perry (12-14m depth) (n = 2 colonies; 28 samples).
Fig. 3.3. *Symbiodinium* (ITS-2 phylotypes) community structure in a *Montastraea faveolata* colony sampled along fixed transects from the Florida Keys, United States. Pie graphs represent proportion of identified *Symbiodinium* from all sampled corals at each region a) *Symbiodinium* distribution from the shallow reefs: Turtle Reef, Admiral Reef, and Little Grecian Reef (2-4m depth) (n = 10 colonies; 164 samples) b) *Symbiodinium* distribution from Alligator Reef (10-12m depth) (n = 2 colonies; 43 samples).
Fig. 3.4. *Symbiodinium* (ITS-2 phylotypes) community structure in a *Montastraea faveolata* colony sampled along fixed transects from the Puerto Morelos, Mexico. Pie graphs represent proportion of identified *Symbiodinium* from La Bocanna Reef (2-4m depth) (n = 5 colonies; 197 samples).
Fig. 3.5. *Symbiodinium* (ITS-2 phylotypes) community structure in a *Montastraea faveolata* colony sampled along fixed transects from Carrie Bow Cay, Belize. Pie graphs represent proportion of identified *Symbiodinium* from all sampled corals at each region a) *Symbiodinium* distribution from shallow Lagoon Reef (1-2m depth) (n = 2 colonies; 58 samples) b) *Symbiodinium* distribution from Reef Crest (10-12m depth) (n = 2 colonies; 42 samples).
CHAPTER 4

FUNCTIONAL DIVERSITY CORRESPONDS TO GENETICALLY DISTINCT
SYMBIODINIUM NICHE PARTITIONING IN MONTASTREA FAVEOLATA\(^3\)

ABSTRACT

The dominant Caribbean reef building coral *Montastraea faveolata* has the unique ability to host diverse assemblages of genetically distinct *Symbiodinium*. This makes *M. faveolata* an ideal species to examine the photo-physiology and calcification of different coral-symbiont associations. Using micro-sampling techniques we identified up to four genetically distinct *Symbiodinium*, representing four different clades, co-occurring within *M. faveolata* from the northern portion of the Mesoamerican barrier reef in Puerto Morelos, Mexico. Coral colonies were screened for symbiont diversity using denaturing gradient gel electrophoresis (DGGE) of the ITS-2 region of nrDNA and specific zones were chosen reflecting *Symbiodinium* diversity. Locally prevalent light fields on *M. faveolata* colonies best explained *Symbiodinium* zonation patterns. Within a coral colony, *Symbiodinium* type C7 was the dominant symbiont in low light areas, while types B17 and B1 were the dominant symbionts in high light areas. Coral samples were collected and a series of physiological parameters were measured examining *Symbiodinium* community structure and photobiology. Photo-physiological responses as determined by photosynthesis versus irradiance curves (*P* vs. *E*) revealed that *E*<sub>k</sub> was significantly lower among low light acclimated coral samples containing C7 *Symbiodinium*, while no significant difference was detected for *α* and *P*<sub>max</sub> for any type of *Symbiodinium*. Reflectance measurements converted into absorption spectra showed differences in photo-pigmentation, particularly the accessory pigments peridinin and chlorophyll *c*<sub>2</sub>, between top (high irradiance, A3, B1, B17 *Symbiodinium*) and bottom (low irradiance, C7 *Symbiodinium*). Calcification incubations revealed significantly higher calcification in the top compared with the bottom regions of coral colonies, corresponding to genetically distinct *Symbiodinium* communities. The results of this study showed genetically different symbiont types display differential high-light or low-light photo-physiological and photoacclimation responses indicating a high degree of *Symbiodinium* niche specialization within *M. faveolata* which ultimately influences coral physiology.

KEY WORDS: Coral Physiology, Photoacclimation, Coral Calcification, *Symbiodinium*, *Montastraea faveolata*, Photopigmentation
INTRODUCTION

Coral reefs are typically found in shallow warm tropical and subtropical oligotrophic waters. The symbiotic relationship between coral and endosymbiotic dinoflagellates (genus: *Symbiodinium*) facilitate coral prevalence in these environments and thus, are responsible for the trophic and structural complexity of coral reef ecosystems. Coral calcification is facilitated by photosynthetically fixed carbon translocated from *Symbiodinium* to the coral (Reviewed by: Gattuso et al. 1999). Optimally, intracellular *Symbiodinium* can provide greater than 90% of the metabolic carbon needed by corals (Muscatine 1967; Muscatine and Porter 1977). Recent coral bleaching events (i.e. loss of *Symbiodinium* and/or photosynthetic pigmentation) have resulted in dramatic loss of reef-building corals reiterating the importance of this obligate mutualistic symbiosis (Hughes 1994; Hoegh-Guldberg 1999; Hughes et al. 2003).

*Symbiodinium* are genetically diverse and are currently grouped into nine clades (A-I) (Pochon and Gates 2010). Within each clade, an enormous amount of diversity representing genetically distinct “types” has been described (LaJeunesse 2001; Coffroth and Santos 2005; LaJeunesse 2005). In addition, a high level of endemism and host-symbiont specificity has been detected using population level genetic markers (e.g. microsatellites) (Santos et al. 2004; Pettay and Lajeunesse 2007; Kirk et al. 2009; Pettay and Lajeunesse 2009; Thornhill et al. 2009; Finney et al. 2010). These findings suggest that long-term evolutionary and ecological processes have resulted in stable, highly productive symbiotic relationships.

Reef building corals are highly efficient light collectors (Enriquez et al. 2005; Teran et al. 2010). Corals achieve this by engineering CaCO₃ skeletons, which function as lambertian reflectors causing multiple scattering and homogenization of light. Irradiance levels on coral reefs are highly diverse, fluctuating spatially and temporally. *Symbiodinium* have been shown to
have differential photoacclimation capabilities and photoprotective mechanisms that can result in high and low irradiance specialists (Iglesias-Prieto and Trench 1994; Brown et al. 1999; Iglesias-Prieto et al. 2004; Robison and Warner 2006; Warner and Berry-Lowe 2006; Reynolds et al. 2008; Hennige et al. 2009; Hennige et al. 2010). In the Caribbean, corals found in shallow water, high irradiance habitats associate primarily with *Symbiodinium* belonging to clades A and B, while *Symbiodinium* belonging to clade C dominate deeper, lower irradiance habitats (LaJeunesse 2002).

High light and low light acclimated corals have been shown to have different physiology (Porter 1976; Wethey and Porter 1976; Dubinsky et al. 1984; Falkowski et al. 1984; McCloskey and Muscatine 1984; Porter et al. 1984; Anthony and Fabricius 2000). Photosynthetically fixed carbon generally decreases with irradiance, such that coral-*Symbiodinium* associations adapted to low light must have high photosynthetic efficiencies to stay phototrophic. Thus, it is paramount that the coral and *Symbiodinium* be acclimated to the specific niche they occupy for coral to receive sufficient photosynthetically derived carbon.

It is clear that *Symbiodinium* are not only genetically diverse but also have physiological, biochemical, and cellular differences that may allow them to occupy a particular niche. Physiological studies using cultured *Symbiodinium* have demonstrated differences in photosynthetic rates, thermal tolerance (to increased and decreased temperatures), photo-damage repair, photo-protection strategies, cell size, and photosynthetic membrane structure among genetically distinct *Symbiodinium* types (Iglesias-Prieto et al. 1992; Warner et al. 1999; Banaszak et al. 2000; LaJeunesse 2001; Tchernov et al. 2004; Warner and Berry-Lowe 2006; Reynolds et al. 2008; Takahashi et al. 2008; Thornhill et al. 2008; Takahashi et al. 2009). Several studies have reported that *M. faveolata* commonly coexists with multiple genetically
distinct types of Symbiodinium (Toller et al. 2001b; LaJeunesse 2002; Garren et al. 2006; Warner et al. 2006; Kemp et al. 2008). The purpose of our study was to investigate how genetically distinct types of Symbiodinium affect the physiology of M. faveolata. We examined Symbiodinium community structure, coral calcification rates, and photobiology of genetically diverse Symbiodinium coexisting within the same host colonies.

**MATERIALS AND METHODS**

*Site Location and Coral Collection*

Corals were collected using SCUBA in December 2008 from La Bocanna Reef, Puerto Morelos, Mexico (2-4m depth; 20.5228°N, 86.5105°W). Fragments of Montastraea faveolata (approximately 5-8 cm in diameter) were chiseled from the top (high irradiance) and the bottom (low irradiance) of each coral colony (n=6). These regions were pre-determined to represent different light niches and were found to associate with genetically different Symbiodinium communities (Kemp, unpublished data). Specimens were immediately transported back to National Autonomous University of Mexico, Puerto Morelos, Mexico (UNAM) and maintained in a temperature-controlled water table using continuously flowing natural seawater, under 50% natural sunlight regulated by multiple layers of neutral-density screens.

*Photosynthesis—Irradiance curves (P vs E curves)*

Photosynthetic rates as a function of irradiance (P-E curves) were measured using independent triplicate chambers (200 mL) equipped with Clark-type oxygen-electrodes (Hansatech Inc). Light was provided by 3 cold-white LED lamps and was attenuated using neutral density screens. Corals were sequentially exposed for 5 minutes to gradually increasing
irradiance intensities (0, 10, 27, 66, 106, 223, 567, 771 µmol photons m\(^{-2}\) s\(^{-1}\)) as measured by a LI-COR LI-250A light meter equipped with a LI-190 quantum sensor. A computerized analog/digital converter was used to record all \(P \text{ vs. } E\) oxygen data.

Mean dark respiration was measured prior to and after light exposure and net photosynthetic efficiency (\(\alpha\)) was calculated by fitting a linear regression to the first four data points of the P-E curve. Maximum net photosynthetic values (\(P_{\text{max}}\)) were calculated as the mean of the greatest two irradiances (no photoinhibition was detected) and saturation irradiance (\(E_k\)) corresponding to \(P_{\text{max}}\) was calculated by dividing \(P_{\text{max}}\) by \(\alpha\). Differences between the parameters \(\alpha\), \(P_{\text{max}}\), and \(E_k\) measured for top and bottom samples of each coral colony were analyzed with paired \(t\)-tests (\(\alpha \leq 0.05\)) in Sigma-Stat (Version 3.1).

**Coral Calcification Incubations**

Calcification rates were determined using the alkalinity anomaly principle based on the ratio of two equivalents of total alkalinity for each mole of CaCO\(_3\) precipitated (Smith & Kinsey 1978). Seawater for incubations was collected from the back-reef of Puerto Morelos and passed through a 0.45 µm Millipore filter. For each coral sample, a one-hour incubation was conducted in a 200 ml acrylic chamber equipped with internal submersible pumps to maintain water flow. A constant water temperature of 27°C was maintained inside the chambers using an external water jacket (Fisher Scientific, USA). A cold-white LED light source was placed above each chamber to provide a constant light intensity of 500 µmol photons m\(^{-2}\) s\(^{-1}\). Light intensity inside each chamber was measured using the light sensor of a Diving PAM (Heinz Walz, Germany) previously calibrated against a cosine-corrected sensor (Li-Cor). After the incubation period, the water was collected, re-filtered (0.2 µm Millipore filter) and stored at 4 °C. Alkalinity analyses
were performed within 24 hrs for all incubations as described in Colombo-Pallotta et al. (2010), using CRM seawater from Scripps Institution of Oceanography as a control. Rates of calcification were normalized to coral surface area, calculated using the aluminum foil technique described by Marsh (1970).

*Coral Reflectance/Absorption Measurements*

Reflectance spectra of coral samples were determined by measuring from 400 to 800 nm with 0.2 nm resolution using an Ocean Optics USB4000 spectrophotometer (Ocean Optics Inc., FL). Individual coral samples were submersed in a black-coated container filled with filtered seawater and illuminated by an incandescent light source placed 25 cm above the sample and a 400 µm fiber optic (Ocean Optics Inc., FL) placed 1 cm from the coral surface at a 45° angle. Reflectance was expressed as the ratio of radiance measured from the coral surface relative to the radiance obtained from a bleached non-living coral skeleton. All data were normalized using the 800 nm reflectance of the skeleton. Absorbance was calculated from reflectance spectra using the equation log (1/Reflectance) as described by Enriquez et al. (2005).

*Coral Processing and Symbiodinium Quantification*

Upon completion of all physiological measurements, coral tissue was removed using a Waterpik™ and filtered seawater (0.45 µm). The resulting slurry containing coral tissue and symbiotic algae was homogenized for approximately 10 seconds using a Tissue Tearror (BioSpec Products™). Aliquots (1 ml) were taken from homogenized slurry and preserved with formalin for *Symbiodinium* density quantification. *Symbiodinium* density was quantified microscopically via 8 replicate haemocytometer counts and normalized to coral surface area.
Symbiodinium DNA Extraction, PCR, and Denature Gradient Electrophoresis

Zooxanthellae pellets were preserved using DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride (Seutin et al. 1991). Genetic identity of Symbiodinium associated with each coral sample was determined following the protocols of LaJeunesse et al. (2003). DNA was extracted using a modified Promega Wizard genomic DNA extraction protocol. Denaturing-gradient gel electrophoresis (DGGE) was used to analyze the ITS-2 region of nuclear ribosomal RNA genes (LaJeunesse 2002, LaJeunesse and Trench 2000). A touchdown thermal cycle was used for PCR amplification of the ITS-2 region using the forward primer “ITSintfor2” (5’-GAATTGCAGA ACTCCGTG-3’) and the reverse primer “ITS2CLAMP” (5’- CGCCCGCCGC GCCCCGCGCC CGTCCCGCC CGCCCGCCGGGATCCATA TGCTTAAGTT CAGCGGGT’-3’) (LaJeunesse et al. 2003), with a 39-bp GC clamp (italicized) (LaJeunesse and Trench 2000). Products were electrophoresed on 45-80% urea-formamide gradient denaturing gradient gels (100% consists of 7 M urea and 40% deionized formamide) for 10 h at 150 V with a constant temperature of 60°C using a C.B.S. Scientific system (LaJeunesse 2002, Thornhill et al. 2010). Samples were run parallel to previously sequenced standards for directed comparison.

RESULTS

Symbiotic Algae Density and Genetic Identity

Symbiotic algal densities were no different between the top (high irradiance) and bottom (low irradiance) regions of coral colonies (Fig. 1; paired t-test; p =0.089;), but were found to have genetically different dominant Symbiodinium communities (Table 1). Specifically, top samples were dominated by either Symbiodinium ITS-2 type B1, B17, or a mixture of B17 and
A3. Bottom samples were dominated by *Symbiodinium* ITS-2 type C7 that was found either as the single dominant *Symbiodinium* type or as mixtures with either B1 or B17 (Table 1).

*Reflectance Measurements and Absorption Spectra*

Reflectance measurements across PAR (400-700nm) were used to calculate absorption curves of each coral sample and revealed region specific trends. Coral samples from the bottom (low irradiance) regions always had greater absorption values than the top (high irradiance) samples (Fig. 2a). Closer analysis revealed that at 675 nm (the wavelength of maximum chlorophyll \(a\) absorption) bottom samples had 18% greater absorption than top samples. At 535 nm (the wavelength of maximum peridinin absorption) bottom samples had 27% greater absorption than the top samples and at 643nm (the wavelength of maximum chlorophyll \(c_2\)) bottom samples had 26% greater absorption than top samples. These results indicate that bottom samples had greater amounts of photosynthetic pigments than samples from the top region of the coral. Additionally, differences absorption spectra between top and bottom coral samples were calculated and plotted (Fig. 2b). This analysis revealed greater photopigment absorption in bottom samples, including chlorophyll \(a\) (675 nm peak) as well as the accessory pigments peridinin (535 nm peak) and chlorophyll \(c_2\) (643 nm peak) (Fig. 2b).

*Coral Net Photosynthesis vs. Irradiance Curves (P-E Curves)*

To further investigate photosynthetic response as a function of irradiance, \(P \text{ vs. } E\) curves were conducted on samples from the top and bottom regions of *M. faveolata*. Photosynthetic parameters \(\alpha\), \(P_{\text{max}}\), and \(E_k\) were calculated from generated net P-E curves (Fig. 3). No significant differences in \(\alpha\) or \(P_{\text{max}}\) were found between the top and bottom coral regions (Fig.
3a,b). Alternatively, samples from the bottom regions had significantly lower photosynthesis saturating irradiances ($E_k$) compared to samples from the top regions of corals (Fig. 3c; paired $t$-test; $p<0.05$).

**Coral Calcification Rates**

Calcification rates from top and bottom regions of *Montastrea faveolata* were experimentally measured. Samples from top regions of the coral were found to have significantly (paired $t$-test, $p<0.05$) greater calcification rates (34%) than samples from bottom regions (Fig. 4).

**DISCUSSION**

Correlating physiological differences with genetic diversity of *Symbiodinium* is of fundamental importance for coral reef ecology. The common Caribbean reef-building species of the *Montastraea annularis* “complex”, *M. annularis*, *M. franksi*, and *M. faveolata*, have all been documented to associate with polymorphic populations of *Symbiodinium* (Rowan and Knowlton 1995; Toller et al. 2001b; LaJeunesse 2002; Garren et al. 2006; Kemp et al. 2008). The ability of *Montastraea* corals to host diverse *Symbiodinium* communities within a single host colony provided a unique opportunity to study the eco-physiology of diverse coral-algal symbioses.

The zonation patterns of *Symbiodinium* within the reef-building coral *M. faveolata* were found to be similar to those described by Rowan and Knowlton (1995). More specifically, the top horizontal regions (high irradiance) of the coral were dominated by *Symbiodinium* ITS-2 types B1, B17 and A3, while bottom vertical regions (low irradiance) were dominated by type C7. No *Symbiodinium* type C7 were found in any of the top samples and no *Symbiodinium* type
A3 were found in any of the bottom samples, indicating irradiance driven zonation patterns. Alternatively, type B *Symbiodinium* have been documented in both high and low irradiance niches within *M. annularis*, *M. faveolata* and *M. franksi* and are often the dominant type of *Symbiodinium* found in shallow water *M. annularis* species “complex” corals (Rowan and Knowlton 1995; Garren et al. 2006; Baums et al. 2010). The diverse assemblages of algal symbionts in *M. faveolata* have been shown to have different susceptibility to increased temperature. Symbiont community structure can temporarily shift in the genetically dominant *Symbiodinium* types following coral bleaching episodes (Rowan et al. 1997; Toller et al. 2001a; Thornhill et al. 2006).

Our study shows that samples from the top of *M. faveolata* colonies have different photopigmentation than samples from the bottoms of the colony, correlating with photoacclimation to high or low level irradiance (Fig. 2). The ability of corals to photoacclimate has been documented by examining corals at different depths and microhabitats, as well as coral transplantation studies where coral colonies were moved to different light habitats (Falkowski and Dubinsky 1981; Dubinsky et al. 1984; Falkowski et al. 1984; Porter et al. 1984; Anthony and Hoegh-Guldberg 2003b,a; Hennige et al. 2010). In this study, absorption spectra revealed greater light absorption by samples from the bottom regions (low irradiance) than from samples from the top (high irradiance). Additionally, absorption differences in the dinoflagellate specific accessory pigment peridinin (535 nm) and chlorophyll c2 (643 nm) as well as chlorophyll a (675 nm) were greatest in bottom samples (Fig. 2b). Several studies have demonstrated different photoacclimation capabilities of genetically diverse *Symbiodinium* in culture (Chang et al. 1983; Iglesias-Prieto and Trench 1994; Hennige et al. 2009). The photoacclimation capacity of
different *Symbiodinium* may be under genetic constraint resulting niche diversification and may contribute to the ecological zonation patterns evident in *M. faveolata*.

Photo-physiological measurements using photosynthesis—irradiance curves (*P* vs. *E* curves) are commonly used to make inferences about photosynthetic efficiency (*α*), photosynthesis saturating irradiance (*E*k), and maximum photosynthetic rate (*P*max) (Jassby and Platt 1976; Falkowski and Raven 2007). Using *P* vs. *E* curves researchers have demonstrated that corals and their symbiotic dinoflagellates display specific responses to high and low irradiance environments (Wethey and Porter 1976; Porter et al. 1984; Anthony and Hoegh-Guldberg 2003a). The findings of this study did not find a statistical difference in *α* or *P*max, but coral samples from bottom portions of the coral had statistically lower photosynthesis saturating irradiance (*E*k) than top samples (Fig. 3c). Corals found in low irradiance environments, such as deep water and underwater caves, have been reported to have lower *E*k than conspecifics found in high irradiance (Porter et al. 1984; Anthony and Hoegh-Guldberg 2003a). Having lower *E*k is advantageous in light limited environments and could be a result of increased number and size photosynthetic units (PSUs) of low light acclimated *Symbiodinium*, as demonstrated by Iglesias-Prieto and Trench (1997) in cultured *Symbiodinium*.

Coral calcification has been shown to increase in the light and is enhanced by the presence of *Symbiodinium* (Goreau and Goreau 1959; Gattuso et al. 1999; Furla et al. 2000). Photosynthetically derived carbon is incorporated into the coral skeletal matrix; thus, differential maximum photosynthesis should proportionally affect calcification rates. This study revealed a statistical difference in *CaCO*3 deposition between top and bottom regions of the coral (Fig. 4). No statistical difference in *P*max was detected between top and bottom regions of the coral; therefore, *P*max cannot explain the physiological differences observed (Fig. 3b). Genetically
distinct *Symbiodinium* communities associating with each region could explain differences in coral calcification. Differences in coral growth have been detected in coral larval hosting genetically different *Symbiodinium* (Little et al. 2004; Cantin et al. 2009). Likewise, differential carbon transfer was detected between genetically different *Symbiodinium* and the sea anemone *Condylactis gigantea* (Loram et al. 2007). Therefore, intra-colonial differences in calcification, as demonstrated by our study, may be a result of differential inorganic carbon uptake and organic carbon transfer between genetically different *Symbiodinium* and their coral host.

In conclusion, the reef-building coral *M. faveolata* has the ability to simultaneously host multiple assemblages of genetically distinct *Symbiodinium*. These populations may have genetically conserved photoacclimation capabilities promoting niche diversification within a coral colony that results in ecological zonation patterns controlled by irradiance. *M. faveolata* is an excellent model organism to investigate physiological differences between genetically different *Symbiodinium* types, though caution should be used in generalizing physiological findings due to the vast genetic diversity of *Symbiodinium*. Our study is the first to document *Symbiodinium* specific coral calcification rates within the same coral colony. The specific mechanisms and potential genetic differences in inorganic carbon uptake, carbon fixation, and organic carbon transfer between symbiont and coral host are still unknown, but these biochemical processes are of paramount importance in understanding coral reef physiology and ecology.
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Table 4.1. Coral region and *Symbiodinium* ITS2-type(s) found within each sample as determined by PCR-DGGE (n=5 per region). Percentages represent the number of samples found to associate with identified *Symbiodinium* ITS2-types.

<table>
<thead>
<tr>
<th>Top (High Irradiance)</th>
<th>Bottom (Low Irradiance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 (40%)</td>
<td>B1,C7 (40%)</td>
</tr>
<tr>
<td>B17 (40%)</td>
<td>B17,C7 (20%)</td>
</tr>
<tr>
<td>B17,A3 (20%)</td>
<td>C7 (40%)</td>
</tr>
</tbody>
</table>
Fig. 4.1. Symbiotic algae cell density from top (high irradiance) and bottom (low irradiance) regions of *Montastraea faveolata* colonies. Cell density data expressed as means ±95% confidence intervals (n = 5).
Fig. 4.2. a) Estimate absorption spectra calculated from reflection measurements of whole coral samples. Data expressed as means ±95% confidence intervals (n = 5). b) Calculated top and bottom coral absorption differences (between 400—750nm) (n = 5).
Fig. 4.3. Photosynthesis parameters of top and bottom coral samples calculated from $P$ vs. $E$ curves. a) Initial slope of $P$ vs. $E$ curve ($\alpha$). b) Maximum photosynthetic capacity ($P_{\text{max}}$). c) Photosynthesis saturating irradiance ($E_k$). All data expressed as means ±95% confidence intervals (n = 5). *Significantly different between top and bottom coral regions (Paired $t$-test; $p < 0.05$).
Fig. 4.4. Calcification rates of top and bottom regions of *Montastraea faveolata* coral colonies. Data expressed as means ±95% confidence intervals (n = 5). *Significantly different between top and bottom coral regions (Paired t-test; p <0.05).
CHAPTER 5

COMMUNITY DYNAMICS AND PHYSIOLOGY OF *SYMBIODINIUM* BEFORE, DURING AND AFTER A BLEACHING EVENT

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ABSTRACT

Community structure and physiology of Symbiodinium associated with Montastraea faveolata was examined before, during and after a coral bleaching event in Puerto Morelos, Mexico using in situ pulse amplitude modulated fluorometry (PAM) followed by micro-sampling genotyping methods. To investigate within-colony variation of bleaching intensity, pigmented “non-bleached” portions of M. faveolata were compared to “bleached” portions of the same colony. Denaturing gradient gel electrophoresis (PCR-DGGE) fingerprint analysis of the rDNA internal transcribed spacer 2 (ITS-2) region revealed that prior to bleaching, ITS-2 phylotypes of Symbiodinium were B17, C7 and A3 in order of their overall prevalence. During the bleaching event of October, 2009 “non-bleached” regions on the coral were predominately populations of Symbiodinium ITS-2 type A3, while adjacent bleaching-prone patches had been populated with Symbiodinium ITS-2 types B17 or C7. During bleaching, maximum photosynthetic efficiency of the photosystem II (PSII) was found to be significantly lower and highly variable compared to previous “non-bleaching” summers. During recovery we document a symbiont community shift in M faveolata to predominantly Symbiodinium type A3 and D1a, both of which are known to have higher thermal tolerances. These results indicate functional differences between genetically distinct Symbiodinium populations that may facilitate competitive exclusion of thermally sensitive Symbiodinium types and result in the temporary abundance of tolerant, but perhaps opportunistic, Symbiodinium.

KEYWORDS: Coral Bleaching, Coral Reef, Symbiodinium, Montastraea faveolata, Competitive Exclusion, Micro-Sampling
INTRODUCTION

Increased sea temperatures in conjunction with high light are known to disrupt the symbiotic relationship between symbiotic dinoflagellates (genus: *Symbiodinium*) and their coral hosts, resulting in bleaching of many species. *Symbiodinium* photosynthetic productivity is greatly inhibited during bleaching, thereby decreasing the amount of photosynthetically derived carbon transferred to the coral host. Sustained (or Chronic) stress causes a reduction in coral tissue growth, skeletal calcification, and fecundity while increasing coral susceptibility to disease and mortality (Szmant and Gassman 1990; Fitt et al. 1993; Brown 1997; Hoegh-Guldberg 1999; Fitt et al. 2000; Baker et al. 2008; Brandt and McManus 2009; Cantin et al. 2010; Colombo-Pallotta et al. 2010). Corals are often able to “recover” from bleaching events and accompanying genetic shifts in *Symbiodinium* communities have been documented (Baker 2001; Berkelmans and van Oppen 2006; Jones et al. 2008; LaJeunesse et al. 2009). Understanding *Symbiodinium* physiology and community structure is of paramount importance for ecosystem wide analyses of global climate change scenarios as predicted by Intergovernmental Panel on Climate Change (IPCC).

Bleaching events have highlighted functional differences between genetically diverse *Symbiodinium* consisting of nine clades (A-I). Over the last decade significant research emphasis has been focused on delineating thermally tolerant and thermally sensitive coral-*Symbiodinium* associations (Rowan et al. 1997; Baker 2001,2003; (Iglesias-Prieto et al. 1992; Rowan et al. 1997; Tchernov et al. 2004; Berkelmans and van Oppen 2006; Robison and Warner 2006; Reynolds et al. 2008). An increased prevalence of corals hosting *Symbiodinium* clade D has been documented in regions that experienced extensive bleaching (Baker et al. 2004; Jones et al. 2008; Oliver and Palumbi 2009). Pacific corals with clade D *Symbiodinium* often have higher
thermal tolerance than conspecifics hosting other clades of *Symbiodinium* (Berkelmans and van Oppen 2006; Mieog et al. 2009; Oliver and Palumbi 2009). However, very low densities *Symbiodinium* clade D (< 1% total *Symbiodinium* community) has been documented in many coral taxa during periods when stress conditions are minimal (Mieog et al. 2007; LaJeunesse et al. 2009). Observations that the “background” *Symbiodinium* clade D only appears to prevail in corals following a bleaching event has invoked the hypothesis that corals may be selectively alternating their *Symbiodinium* communities to acclimatize to warmer temperatures (Buddemeier and Fautin 1993; Baker 2001; Baker et al. 2004). However, the hypothesis of rapid changes in coral–algal symbiosis to acclimatize to warmer temperatures is challenged by the high level *Symbiodinium* population structure and stability documented in both hard and soft corals (Santos et al. 2003; Pettay and LaJeunesse 2007; Kirk et al. 2009; Thornhill et al. 2009; LaJeunesse et al. 2010).

In contrast to tropical Pacific corals, clade A *Symbiodinium* are commonly found to associate with shallow-water hosts in the Caribbean, leading to the hypothesis that *Symbiodinium* belonging to clade A are high irradiance and high temperature specialists (LaJeunesse 2002). Physiological studies on cultured *Symbiodinium* isolates confirmed this hypothesis and have shown that most clade A *Symbiodinium* are high-light and temperature tolerant due to greater photoacclimation capabilities, unique photosystem repair processes, and enhanced photoprotective pathways (Robison and Warner 2006; Reynolds et al. 2008; Takahashi et al. 2009).

Most corals have been found to associate with a single genetically dominant *Symbiodinium. Montastraea faveolata*, however, has been found to simultaneously host up to three genetically distinct ITS-2 types of *Symbiodinium* (Rowan and Knowlton 1995; Toller et al.
Symbiodinium associated with *M. faveolata* exhibit high niche diversification within coral colonies influenced by localized irradiance patterns (Rowan and Knowlton 1995; Toller et al. 2001a; Garren et al. 2006). During coral bleaching events, differential “patchy” bleaching of *M. faveolata* has been documented (Rowan et al. 1997; Baker et al. 2008) and is correlated with distribution patterns of the genetically distinct *Symbiodinium* assemblages that exhibit differential thermal tolerance.

Following a bleaching event vacant but potentially nutrient-rich niches within the coral host may facilitate the establishment or proliferation of normally low abundant but opportunistic *Symbiodinium*. *Symbiodinium* recovery following bleaching events is not very well understood and very few studies have monitored genetic structure of *Symbiodinium* community after bleaching events. Two surveys of *Symbiodinium* community structure have documented the emergence of *Symbiodinium* D1a as an increasingly dominant symbiont type concurrent with coral bleaching (Thornhill et al. 2006; LaJeunesse et al. 2009). In the 2-3 years following bleaching events, the subsequent abundance of D1a was very low (undetectable by traditional methods) presumably due to competitive displacement by clade members that predominate during periods of reduced thermal stress (Thornhill et al. 2006; LaJeunesse et al. 2009).

Few studies have related functional differences of genetically distinct *Symbiodinium* to coral bleaching (but see Rowan and Knowlton 1997). Here, we report the effect of coral bleaching on the diverse *Symbiodinium* community of *M. faveolata* in Puerto Morelos, Mexico. Using micro-sampling techniques along fixed transects, we document a *Symbiodinium* community shift following a coral bleaching event. To investigate functional differences of genetically diverse *Symbiodinium* associating with *M. faveolata*, we examined intra-colonial
variability in *Symbiodinium* phylotypic identity, cell density, chlorophyll $a$, and maximum photosynthetic capacity of photosystem II (PSII).

**MATERIALS AND METHODS**

*Symbiodinium Quantification and Chlorophyll $a$ Determination*

Corals were collected using SCUBA in October 2009 from La Bocanna Reef, Puerto Morelos, Mexico (2-4m depth; 20.5228°N, 86.5105°W). One fragment (approximately 5-8 cm in diameter) was chiseled from a bleached and “non-bleached” region of *6 Montastraea faveolata* colonies. Coral tissue was removed with filtered seawater using a Waterpik™ (0.45 µm) (Johannes and Wiebe 1970). The resulting slurry, containing coral tissue and symbiotic algae, was homogenized for approximately 10 seconds using a Tissue Tearorr (BioSpec Products™). Aliquots (1 ml) were taken from homogenized slurry and preserved with formalin.

*Symbiodinium* density in preserved samples was quantified microscopically via 8 replicate haemocytometer counts and normalized to coral surface area (Marsh 1970).

Two replicate 15 ml subsamples of the coral slurry were centrifuged (1,500 rpm for 5 min) to pellet zooxanthellae, the supernatant discarded and the pellet frozen until further analysis. Chlorophyll $a$ was extracted using 100% acetone and light sonication for approximately 10 s using a Sonicator Model W-225 (Heat Systems-Ultrasoundics) at setting 4, then immediately placed in the dark at −20 °C for 24 h. Chlorophyll absorbance was measured with a Bio Rad SmartSpec™ 3000 spectrophotometer, calculated using the equations of (Jeffrey and Humphrey 1975), and normalized to coral surface area (Marsh 1970).

*Micro-Sampling Procedure and in situ PAM measurements*
Using micro-sampling procedures of Kemp et al. (2008), *M. faveolata* coral tissue was collected from four of the same corals sampled above, using syringes along four fixed transects laid from top to bottom of the colony at north, east, south, and west compass headings. In September 2006 (non-bleached), October 2009 (bleached) and December 2009 (recovery) *M. faveolata* colonies were sampled every 20 cm using 2cc syringes with 16 gauge needles. The maximum photosynthetic efficiency of PSII ($F_v/F_m; F_v = F_m - F_0$) was measured along the same transect points using saturation pulses from a pulse-amplitude modulated fluorometer (Diving PAM, Walz). To ensure relaxation of non-photosynthetic fluorescence quenching all dives were conducted at sunset.

Upon returning to the laboratory, syringe samples were transferred into 2 mL Eppendorf tubes and centrifuged at (∼5,000g) for 2 min. Supernatants were decanted and preserved either DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride) or 80% ethanol alcohol (Seutin et al. 1991).

*Genetic Identification of Symbiodinium*

Genetic identities of the dominant *Symbiodinium* in each tissue sample were determined following the protocols of LaJeunesse et al. (2003). DNA was extracted using a modified Promega Wizard genomic DNA extraction protocol and the ITS-2 region of nuclear ribosomal RNA genes was analyzed via denaturing-gradient gel electrophoresis (DGGE) (LaJeunesse 2002, LaJeunesse and Trench 2000). PCR amplification of the ITS-2 region was accomplished using a touchdown thermal cycle described in LaJeunesse et al. (2003) with the forward primer “ITSintfor2” (5’-GAATTGCAGA ACTCCGTG-3’) and the reverse primer “ITS2CLAMP” (5’-CGCCCGCCGC GCCCGCGCC CGTCCCGCCG CCCCCGCCG GGGATCCATA
TGCTTAAGTT CAGCGGGT’-3’), with a 39-bp GC clamp (italicized) (LaJeunesse and Trench 2000). Using a C.B.S. Scientific™ system, products were electrophoresed on 45-80% urea-formamide gradient denaturing gradient gels (100% consists of 7 M urea and 40% deionized formamide) for 10 h at 150 V at a constant temperature of 60° C (LaJeunesse 2002, Thornhill et al. 2010). Samples were run parallel to previously sequenced standards.

Reef Temperature Measurements

Seawater temperatures were recorded at depth on the reef using Onset HOBO Pro v2 underwater temperature loggers (sampled daily; resolution±0.2 °C). Temperature loggers were deployed in 2006 and were recovered annually for data upload.

Statistical Analysis

All statistical analyses were performed using Sigma-Stat (Version 3.1). All data sets satisfied assumptions of normality and were analyzed either by paired $t$-test, Chi-squared ($\chi^2$) or repeated measures of one-way ANOVA, using a significance level, $\alpha$, no greater than 0.05. For Chi-squared analysis we used the Symbiodinium community assemblage of September 2006 (pre-bleaching) as a null hypothesis of Symbiodinium community structure and tested for deviation from this assemblage.

RESULTS

Seawater Temperatures

During 2009, temperature loggers on La Boccanna reef recorded 106 days at or above 29.5 °C, of which 22 days reached 30 °C. Extensive coral bleaching was observed during the
Summer 2009 when peak temperatures reached 30.3 °C. In 2006, a year when no bleaching was observed, only 26 days registered 29.5 °C and only one of these days reached 30 °C (Fig. 1).

Zooxanthellae densities and chlorophyll a content

*Symbiodinium* density (cells cm\(^{-2}\)) was 8X higher and chlorophyll *a* (µg cm\(^{-2}\)) was 44X higher in “non-bleached” compared to “bleached” samples (paired *t*-test; *p* < 0.05) (Fig. 2).

Community of *Symbiodinium* Structure During Pre-Bleaching, Bleaching, and Recovery

Differences in *Symbiodinium* community structure associated *Montastraea faveolata* was observed between “pre-bleaching”, “bleaching”, and “recovery” events (*X\(^2\); *p* < 0.001) (Fig. 3). The most prevalent shift in *Symbiodinium* was observed between ITS-2 types A3, C7, and D1a. In September 2006 during non-bleaching conditions type A3 was found in 14% of all transect samples, C7 in 37%, and D1a was not detectable in any of the samples. During the bleaching event of October 2009 *Symbiodinium* type A3 was found in 38%, C7 in 6%, and D1a in 8% of the samples. During initial recovery in December 2009 *Symbiodinium* type A3 was found in 51% of the samples, C7 in 11%, and D1a in 14% (Fig. 3).

Intra-Colony PAM Fluorescence Measurements

Maximum photosynthetic efficiency of PSII, measured along transects from top to bottom of the colony, showed little variability among samples and colonies during pre-bleaching (September 2006) and recovery (December 2009) sampling periods. Mean maximum photosynthetic yields (*F\(_{\text{v}}\)/*F\(_{\text{m}}\)) for September was 0.583 ± 0.034 SD and for December was 0.642 ± 0.063 SD. This is in contrast to high within colony *F\(_{\text{v}}\)/*F\(_{\text{m}}\) variability documented during the
bleaching event of October 2009 when mean values were 0.486 ± 0.124 SD. This amount of 
$F_v/F_m$ variability documented in bleached corals was 3.5 times greater than the summer of 2006
and 2 times greater than during recovery. Repeated measures One-Way ANOVA revealed
significant differences between each time point sampled ($p < 0.001$)(Fig. 4).

DISCUSSION

In this study we document how normally stable, but niche-dependent genetically diverse,
*Symbiodinium* communities associating with *Montastraea faveolata* are significantly affected
during and after a coral bleaching event. Shifts in *Symbiodinium* abundance and prevalence
demonstrate differential susceptibility to bleaching stressors among *Symbiodinium* cladal types.
We describe within colony variability in physiological parameters including *Symbiodinium*
densities, levels of photosynthetic pigments, and photosynthetic efficiency during a coral
bleaching event. Our data suggest that enhanced photoprotective traits of ITS-2 type A3 and
D1a *Symbiodinium* facilitate niche displacement of ITS-2 types B17, and C7. Gause’s Law
states that two species that compete for the exact same resources cannot stably coexist. (Gause
1934,1937). Perhaps, within colony spatial/temporal heterogeneity and environmental
fluctuations facilitates diverse assemblages of endosymbiotic dinoflagellates associated with *M.
faveolata* (Hutchinson 1961).

Of the known nine clades of *Symbiodinium* (Pochon and Gates 2010) currently classified,
clades A and D have the greatest thermal tolerance in corals (Rowan et al. 1997; Baker et al.
2004; Rowan 2004; Berkelmans and van Oppen 2006; Baker et al. 2008; Reynolds et al. 2008;
Oliver and Palumbi 2009). Our findings that type A3 was the most abundant type of
*Symbiodinium* during bleaching concur with studies of Rowan et al. (1997) and are likely the
result of enhanced photoprotection demonstrated by Reynolds et al. (2008) *Symbiodinium* ITS-2 type D1a, a generalist throughout the Caribbean and Pacific, is commonly found in low densities (<1%) in many coral taxa and can proliferate during bleaching and recovery (Mieog et al. 2007; LaJeunesse et al. 2009). Pre-bleaching in September 2006, ITS-2 type D1a was not detectable by PCR-DGGE in any of our 75 samples micro-samples, but may have been low-density background abundances. In comparison, 14% of post-bleached samples contained type D1a. Similarly, we saw dramatic increases of *Symbiodinium* type A3 during bleaching and recovery from 14% (pre-bleaching) to 51% (post-bleaching) detection in samples.

Differential reduction in the maximal photosynthetic capacity of PSII has been correlated to shifts in *Symbiodinium* community structure by several studies that document the preponderance of PSII photoinhibition experienced by thermally susceptible *Symbiodinium* (Warner et al. 1996,1999; Smith et al. 2005; Warner et al. 2006). However, our observations highlight potential functional differences in maintenance or repair of PSII within genetically diverse types of *Symbiodinium*. We also document here the replacement of thermally sensitive *Symbiodinium* communities with thermally tolerant, presumably opportunistic, types of *Symbiodinium*. Therefore, it appears we document a trade-off between competitive ability and thermal tolerances of *Symbiodinium* ITS-2 types C7 and B17. Similarly, only during bleaching events are type ITS-2 type D1a is able to out-compete other types of *Symbiodinium* and take over the vacant niche.

In this study we found that “non-bleached” portions of coral had 8 times greater *Symbiodinium* cm^-2 than “bleached” regions on the same coral (Fig. 3). Therefore, thermally tolerant algae that presumably have enhanced photoprotection and/or cellular repair mechanisms may have a “head start” in niche repopulation. When corals are bleached, they still feed
heterotrophically that may result in high intra-colony dissolved nitrogen and phosphorus levels (Muscatine et al. 1989; Falkowski et al. 1993; Grottoli et al. 2006). Cell division rates have been estimated between 5 and 10 days (Wilkerson et al. 1988). Therefore repopulation of “bleached” areas of the coral can occur rapidly by *Symbiodinium* that have not suffered mortality or been expelled by the host. LaJeunesse et al. (2009) estimate that background populations of 1000–5000 *Symbiodinium* cm$^{-1}$ could reach densities of 1 million cells cm$^{-2}$ with 1–3 months. However, Thornhill et al. (2006) found that it took up to 4 years for *Montastraea* spp. *Symbiodinium* communities to recover from 1998 El Niño bleaching event.

Repopulation of *Symbiodinium* populations within severely bleached corals is paramount not only for nutritional requirements, but also for the absorption of harmful irradiance that may directly cause host damage and exacerbate photoinhibition of photosynthesis (Lesser and Farrell 2004; Rodriguez-Roman et al. 2006). Corals are among the most efficient light collectors on the planet by engineering CaCO$_3$ skeletons that function as lambertian reflectors enhancing multiple light scattering (Enriquez et al. 2005; Teran et al. 2010). This normally advantageous adaption backfires during bleaching events when *Symbiodinium* populations are drastically reduced and internal light fields increase as a result of reduced light absorbing pigmentation, potentially resulting in increased photoinhibition (Lesser and Farrell 2004; Rodriguez-Roman et al. 2006).

Little is known of the potential physiological consequences of hosting more thermally resilient types of *Symbiodinium* in Caribbean corals. In the Pacific coral *Acropora millepora*, a few studies have shown reduced photosynthetic performance, diminished translocated carbon, and reduced coral growth as a result of hosting thermally tolerant clade D *Symbiodinium* instead of type C2 (Little et al. 2004; Cantin et al. 2009; Jones and Berkelmans 2010). These studies, however, are from a single host taxa from one specific region on the Great Barrier Reef and
therefore caution should be used in generalizing these studies to corals worldwide. Additionally, because of the resolution limitations of *Symbiodinium* identification (single strand conformation polymorphism; SSCP) used in these studies it is unknown whether the detected clade D *Symbiodinium* are the same as ITS-2 type D1a found throughout the world (LaJeunesse et al. 2010).

The phylotype specific physiological differences resulting in competitive displacement and ultimately niche diversification of *Symbiodinium* within *M. faveolata* colonies is still incompletely resolved. Our results corroborate several previous studies that have documented and increase in opportunistic *Symbiodinium* with higher thermal tolerances such as ITS-2 types D1a and A3 following bleaching events (Rowan et al. 1997; Toller et al. 2001b; Thornhill et al. 2006; LaJeunesse et al. 2010). Thornhill et al. (2006) and LaJeunesse et al. (2010) sampled corals several years after a bleaching event and documented that *Symbiodinium* communities gradually shift back to pre-bleaching *Symbiodinium* community structure within 2–3 years when stress subsided. The gradual competitive displacement of thermally tolerant types of *Symbiodinium* during recovery suggests a trade-off between enhanced thermal tolerance, photoprotective traits and superior cellular repair mechanisms, and competitive ability under less stressful conditions. *Symbiodinium* may have subtle yet ecologically important physiological and cellular differences resulting in differential niche optimization. Such fundamental traits as carbon fixation efficiency, cellular proliferation, and nutrient utilization need further investigation to be able to better understand *Symbiodinium* community dynamics.
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Fig. 5.1. Temperature from data loggers placed on La Bocanna Reef, Puerto Morelos, Mexico (2–4 m depth) (Onset HOBO Pro v2; sampled every 4 h; resolution ± 0.2 °C). Arrow indicates sampling date during coral bleaching event.
Fig. 5.2. a) Regions of the *Montastraea faveolata* sampled for “non-bleached” and “bleached” analysis. One sample of each region was taken per coral \((n=6)\). b) *Symbiodinium* cell density from “non-bleached” and “bleached” sampled corals. Cell density data expressed as means ± 95\% confidence intervals \((n=6)\). c) Chlorophyll \(a\) content of corals per unit area from “non-bleached” and “bleached” regions of sampled corals \((n=6)\). *Significantly different between “non-bleached” and “bleached” regions of coral colony (Paired \(t\)-test \(p < 0.05\)).
Fig. 5.3. *Symbiodinium* (ITS-2 phylotypes) community structure in *M. faveolata* sampled along fixed transects from La Bocanna Reef, Puerto Morelos, Mexico. Samples were taken from top to bottom along north, east, south, and west transects of *M. faveolata* (n=4) using micro-sampling techniques. Identical coral colonies and transects were sampled in September 2006 (pre-bleaching), October 2009 (bleaching) and December 2009 (recovery). a,b,c Dissimilar letters denote statistical differences in *Symbiodinium* community structure between sampling periods ($\chi^2; p \leq 0.001$).
Fig. 5.4. Fixed transect along the same *Montastraea faveolata* colony sampled in a) September 2006, b) October 2009, and c) December 2009. Micro-samples were collected every 20cm for *Symbiodinium* identification, represented by colored symbols and *in situ* fluorescence measurements were taken directly adjacent to micro-samples. Vertical line graphs are $F_v/F_m$ measurements aligned along the coral to correlate *Symbiodinium* type with $F_v/F_m$ taken along fixed transects from top to bottom of the colony.
CHAPTER 6

CATASTROPHIC MORTALITY ON INSHORE CORAL REEFS OF THE FLORIDA KEYS DUE TO SEVERE LOW-TEMPERATURE STRESS

ABSTRACT

Coral reefs of the Florida Keys typically experience seasonal temperatures of 20–31 °C. Deviation outside of this range causes physiological impairment, potentially leading to colony death. In January and February 2010, two closely spaced cold fronts caused sudden and severe seawater temperature declines in the Florida Keys. Inshore coral reefs (e.g., Admiral Reef) experienced lower sustained temperatures (i.e., <12 °C) than those further offshore (e.g., Little Grecian Reef, minimum temperature = 17.2 °C). During February and March 2010, we surveyed Admiral Reef and documented a mass die-off of reef-building corals, whereas 12 km away, Little Grecian Reef did not exhibit coral mortality. Following this event, the physiological effects of low temperature stress on three common reef-building corals (*Montastraea faveolata*, *Porites astreoides*, *Siderastrea siderea*) were experimentally investigated over a range of temperatures that replicated the inshore cold-water anomaly (i.e., from 20 °C to 16 °C to 12 °C and back to 20 °C). Throughout the temperature modulation, coral respiration as well as endosymbiont gross photosynthesis and maximum PSII photosynthetic efficiency were measured. In addition to these physiological measurements, *Symbiodinium* genetic identity, cell densities and chlorophyll a content were quantified at the beginning and conclusion of the experiment. Although all corals were significantly affected at 12 °C, species-specific physiological responses were found indicating different coral and/or zooxanthellae cold tolerances. *Montastraea faveolata* and *P. astreoides* appeared to be most negatively impacted upon return to 20 °C, with significant reductions in gross photosynthesis and dark respiration. Under comparable temperature conditions, *S. siderea* recovered to pre-treatment rates of dark respiration and gross photosynthesis. Visual surveys of inshore reefs corroborated these results, with *S. siderea* being minimally affected by the cold-water anomaly whereas *M. faveolata* and *P. astreoides* experienced approximately 100% mortality.

Key Words: Cold Stress, Florida Keys, *Montastraea faveolata*, *Porites astreoides*, *Siderastrea siderea*, *Symbiodinium*
INTRODUCTION

Considerable attention has been given to worldwide coral reef decline over the last several years (Porter and Meier 1992; Hoegh-Guldberg 1999; Gardner et al. 2003; Bruno and Selig 2007) with major emphasis placed on the negative effects of increased seawater temperatures (Glynn 1991; Brown 1997; Hoegh-Guldberg 1999; Fitt et al. 2001; Weis 2008; Cantin et al. 2010) and ocean acidification (Kleypas et al. 1999; Feely et al. 2004; Hoegh-Guldberg et al. 2007; Anthony et al. 2008; Manzello et al. 2008). In recent decades, many studies have focused on the ecological, physiological, cellular and biochemical effects of increased seawater temperatures resulting in coral bleaching (e.g., Porter et al. 1989; Gates et al. 1992; Fitt et al. 1993; Gleason and Wellington 1993; Warner et al. 1996; Lesser 1997; Rowan et al. 1997; Warner et al. 1999; Fitt et al. 2000; van Woesik 2001; Berkelmans and van Oppen 2006; Grottoli et al. 2006; Thornhill et al. 2006a; Thornhill et al. 2006b; LaJeunesse et al. 2009; Thornhill et al. 2009) but the effect decreased temperatures have on coral-zooxanthellae biology has not been studied in much detail (but see Mayer 1914; Jokiel and Coles 1977; Porter et al. 1982; Saxby et al. 2003; Hoegh-Guldberg et al. 2005; Thornhill et al. 2008).

In the last decade, improved understanding of coral biology has illuminated the importance of numerous symbiotic taxa associating with corals. This symbiotic fauna includes diverse microbial communities residing externally in the coral surface mucus layer (Rohwer et al. 2001; Rohwer et al. 2002; Ritchie 2006; Dinsdale et al. 2008) endosymbiotic dinoflagellates in the genus Symbiodinium (commonly referred to as zooxanthellae), as well as cyanobacteria, apicomplexans, and fungi (Toller et al. 2002; Knowlton and Rohwer 2003). Symbiotic relationships between the coral host and its microbial partner(s) are often extremely sensitive to perturbations in the abiotic environment. Most notable is coral bleaching, which is the symbiotic
break down between corals and zooxanthellae during abnormal increases in temperature and/or irradiance (Glynn 1996; Brown 1997; Warner et al. 1999; Fitt et al. 2001; Weis 2008).

The minimum prolonged temperature for coral reef accretion is approximately 18 °C (Vaughan 1916; Kleypas et al. 1999). For instance, Crossland (1984) demonstrated that metabolic activity of a common Australian coral essentially ceased below 18 °C. This result is corroborated by the early experimental work in the Florida Keys by Mayer (1914,1915), which documented species-specific thermal susceptibility to short-term low temperatures and cessation of coral feeding below 16 °C. Low-temperature stress induces physiological responses similar to elevated-temperature, including loss of zooxanthellae cell density and chlorophyll a content, as well as photoinhibition (Saxby et al. 2003; Hoegh-Guldberg and Fine 2004). Furthermore, cold-water stress disrupts the coral-dinoflagellate symbioses, resulting in bleaching (Steen and Muscatine 1987; Hoegh-Guldberg and Fine 2004; Hoegh-Guldberg et al. 2005). To date, little attention has been given to examine the effect cold-water stress has on the physiology of corals with genetically different zooxanthellae.

Increased seawater temperature causes photodamage via photoinhibition of electron transport in photosystem II (PSII) in *Symbiodinium* (Warner et al. 1999). Such perturbation decreases metabolic performance and is a proximate cause of thermal bleaching in corals (Warner et al. 1999; Takahashi et al. 2004; Smith et al. 2005; Takahashi et al. 2008). As with high-temperature stress, zooxanthellae exposed to cold temperatures experience decreased maximum photosynthetic capacity of PSII, potentially leading to photoinhibition and photodamage (Saxby et al. 2003; Thornhill et al. 2008). Cellular mechanisms involved in cold-water photoinhibition of zooxanthellae are not well understood, but Thornhill et al. (2008) hypothesized that a loss of photosynthetic membrane fluidity decreases the photosynthetic
capacity of PSII. In early 2010, the Upper Florida Keys, U.S. experienced two abnormal cold fronts that reduced seawater temperatures below 12 °C on inshore coral reefs and sustained temperatures below 18 °C for approximately two weeks. Here we investigated the effect of cold-water stress on three species of corals and their endosymbiotic dinoflagellates. To accomplish this, we combined field observations and controlled experiments designed to simulate the 2010 cold anomaly that ultimately resulted in mass mortality of many reef-building corals.

MATERIALS AND METHODS

Reef Assessment and Temperature Measurements

Seawater temperatures were recorded at each site using Onset HOBO Pro v2 underwater temperature loggers (sampled every 4 h; resolution ± 0.2°C). Temperature loggers were deployed in 2005 and were recovered annually for data upload. In March of 2010, we surveyed six 10 m² linear transects over Admiral Reef in order to assess the percentage of living versus dead symbiotic cnidarian colonies. To insure a random sub-sampling of the reef, the starting location and compass bearing of each transect was determined using a random number generator. The numbers and species identities of living and dead colonies were assessed in 1-m² increments by three independent investigators. Accuracy of the identifications was further verified via photographic evidence. Colonies experiencing partial mortality (i.e., reduced living tissue area on their skeletons) were scored as living; as a result, the survey data represent a conservative underestimate of the impacts of the 2010 low-temperature anomaly.
Reef Locations

Three species of corals, including *Montastraea faveolata*, *Porites astreoides* and *Siderastrea siderea*, were collected in February 2010 from two reefs separated by approximately 12 km in the upper Florida Keys (Fig. 1). These reefs included Little Grecian Reef (LG), located on the outer reef crest (3–4 m depth; 24.84°N, 80.62°W), and Admiral Reef (ADM), an inshore patch reef (1–2 m depth, 25.04°N 80.30°W). For each coral species, two replicate fragments were collected from six coral colonies from Little Grecian Reef. Only colonies of *S. siderea* (*n* = 6) were sampled from Admiral Reef due to the recent and total mortality of other coral species at this location.

Coral Collection and Maintenance

Coral fragments approximately 5–8 cm in diameter were collected via S.C.U.B.A. using a hammer and chisel and immediately transported to the laboratory in a seawater-filled cooler. Specimens were maintained in a temperature-controlled recirculating water table using seawater under natural sunlight. Total system volume was approximately 160 l. Temperature control was accomplished via submersible heaters and a thermostat-controlled chiller unit (Pacific Coast CL-600 ¼ hp), supplemented with sealed bottles of ice as necessary. Photosynthetic photon flux density (PPFD) was measured using a LI-COR LI-250A light meter equipped with a LI-190 quantum sensor. PPFD was maintained at ≤750 µmol photons m⁻² s⁻¹ using multiple layers of neutral-density screens. Water was continuously filtered with a mechanical filter, a filter sock (100 µm) and a 10 W flow-through ultraviolet sterilizer (Lifegard Aquatics). Salinity was monitored periodically with a refractometer and maintained at 35 psu with additions of freshwater.
**Experimental Treatment**

To reconstruct the cold-water temperatures recorded on Admiral Reef from January 10\textsuperscript{th} thru January 15\textsuperscript{th} 2010. Corals were maintained at 20 °C for two days, then water temperatures were reduced to 16 °C (overnight) and kept constant for 24 h before reducing the temperature to 12 °C (overnight). Corals were maintained at 12 °C for 24 h before increasing the temperature back to 20 °C (overnight) for 24 h prior to processing. This experimental temperature profile was modeled after temperatures experienced on Admiral Reef over January 9–13, 2010 (Fig. 2a). Corals physiological response to each temperature was tested and, upon conclusion of the experiment, corals were processed for *Symbiodinium* genetic identity, cell densities, and chlorophyll *a* content.

**Coral Processing**

One of the two replicate pieces from each coral colony was processed prior to the experiment for baseline comparisons under ambient conditions. All coral tissue was removed using a Waterpik\textsuperscript{TM} and filtered seawater (0.45 µm) (Johannes and Wiebe 1970). The resulting slurry was homogenized for approximately 10 sec using a Tissue Tearor (BioSpec Products\textsuperscript{TM}) and sub-sampled for zooxanthellae density, zooxanthellae genetic identity, and chlorophyll *a* content. Zooxanthellae density was quantified microscopically via 8–10 replicate haemocytometer counts. Two replicate 15 ml subsamples of the coral slurry were centrifuged (1,500 rpm for 5 min) to pellet zooxanthellae, the supernatant discarded and the pellet frozen until further analysis. Chlorophyll *a* was extracted using 100% acetone and light sonication for approximately 10 s using a Sonicator Model W-225 (Heat Systems-Ultrasonics) at setting 4, then immediately placed in the dark at −20 °C for 24 h. Chlorophyll absorbance was measured using a
Bio Rad SmartSpec™ 3000 spectrophotometer and calculated using the equations of (Jeffrey and Humphrey 1975). Coral surface area was estimated using the aluminum foil method (Marsh 1970). Zooxanthellae pellets were preserved using DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride (Seutin et al. 1991).

**O₂ Measurements, Respirometry, and PAM Fluorometry**

All non-living areas of the coral fragments surrounding living coral tissue were encased in modeling clay. This was performed with care to avoid overlap with any live tissue. Fragments were placed in transparent custom-built acrylic chambers (approx. 315 ml) for respirometry using Clark-type oxygen electrodes (YSI 5100) in filtered natural seawater (0.45 µm). electrodes were calibrated against air-saturated filtered natural seawater. Chambers were placed in a water bath for temperature control. Chamber circulation was maintained with the integrated impeller of the oxygen electrode as well as a stir bar inside each chamber.

Photosynthetic measurements were taken using artificial lighting in the laboratory during the natural photoperiod (09:00 to 17:00) to control for photoperiodicity. The chambers were covered for 15 min to obtain linear respiratory rates in the dark and then illuminated at a photosynthesis-saturating irradiance of 400–450 µmol photons m⁻² s⁻¹ (Kemp, unpublished data) by a compact fluorescent lighting fixture (Coralife Aqualight, Oceanic Systems) for 21 min to measure photosynthetic oxygen evolution. The measurements of the first 6 min of illumination were discarded to focus on linear rate of photosynthesis. Oxygen flux was measured every 24 h at four separate treatment temperatures of 20 °C, 16 °C, and 12 °C before returning to 20 °C.

Using saturation pulses from a pulse-amplitude modulated fluorometer (Diving PAM, Walz), maximum photosynthetic efficiency of the PSII \( \frac{F_v}{F_m} \); \( F_v = F_m - F_0 \) of all coral
fragments was measured at each experimental temperature, at least two hours after sunset to ensure relaxation of non-photosynthetic fluorescence quenching.

**Zooxanthellae identification**

Genetic identity of *Symbiodinium* associated with each coral sample was determined following the protocols of LaJeunesse et al. (2003). DNA was extracted using a modified Promega Wizard genomic DNA extraction protocol following Thornhill et al. (2006a). Denaturing-gradient gel electrophoresis (DGGE) was used to analyze the ITS-2 region of nuclear ribosomal RNA genes (LaJeunesse and Trench 2000; LaJeunesse 2002) A touchdown thermal cycle described in LaJeunesse et al. (2003) was used for PCR amplification of the ITS-2 region using the forward primer “ITSintfor2” (5’-GAATTGCAGA ACTCCGTG - 3’) and the reverse primer “ITS2CLAMP” (5’- CGCCCGCCGC GCCCCGCGCC CGTCCCGCCG CCCCCGCCG GGGATCCATA TGCTTAAGTT CAGCGGGT - 3’), with a 39-bp GC clamp (italicized text) (LaJeunesse and Trench 2000; LaJeunesse 2002) Products were electrophoresed on 45–80% urea-formamide gradient denaturing gradient gels (100% consists of 7 M urea and 40% deionized formamide) for 10 h at 150 V at a constant temperature of 60 °C using a C.B.S. Scientific system (LaJeunesse 2002; Thornhill et al. 2010) Samples were run parallel to previously sequenced standards from identical corals (Thornhill et al. 2006a; Thornhill et al. 2006b)

**Statistical Analysis**

All statistical analyses were performed using Sigma-Stat (Version 3.1). Data sets satisfying assumptions of normality were analyzed by one-way repeated measures ANOVA or a
paired t-test using a significance level, $\alpha$, no greater than 0.05. Tukey post hoc multiple comparisons were performed whenever overall treatments effects were found. All data expressed in percentages were arcsine transformed. Only one data set (M. faveolata chlorophyll $a \ cm^{-2}$) failed normality test ($p < 0.05$), therefore, the Wilcoxon signed rank test was used to test for significant difference.

RESULTS

Seawater temperature and coral mortality

Admiral Reef sustained abnormally low temperatures ($< 18^\circ$C) for 11 days including 3 days $\leq 12 ^\circ$C (Fig. 2a). The lowest temperature recorded was at Admiral Reef during this event was $11.7 ^\circ$C (Fig. 2a). To put the abnormally low temperatures in context from 2007-2009 the minimum temperature recorded by our data loggers was $19.7 ^\circ$C and the mean winter temperature (Jan-Feb) from 2007-2009 was $23.3 ^\circ$C. The sustained low-temperatures at Admiral Reef likely stressed many common reef-building corals, resulting in mass mortality (Fig. 2b, Fig. 3). The most affected corals were *Porites astreoides*, *Acropora cervicornis*, *Favia fragum*, *Colpophyllia natans*, *Dendrogyra cylindrus*, and *Montastraea annularis*, with 100% mortality throughout our surveys. Other negatively impacted corals included *M. faveolata* (90% mortality), *Diploria strigosa* (66.7% mortality), *D. clivosa* (50% mortality), *M. cavernosa* (33.3% mortality), and the octocoral *Gorgonia ventalina* (16.7% mortality). By comparison, the cold water did not kill colonies of certain coral species, including *P. divaricata*, *Siderastrea siderea*, *Dichocoenia stokesii*, the hydrocoral *Millepora alcicornis*, octocoral *Pseudopterogorgia americana*, and the zoanthid *Palythoa caribaeorum* (Fig. 2b, Fig. 3).
Compared to the inshore Admiral Reef, offshore Little Grecian Reef did not experience the same severity of cold-water stress. Little Grecian Reef experienced temperature <18 °C for several hours, hitting a minimum temperature of 17.2 °C (Fig. 2a). Corals on Little Grecian Reef showed no visible signs of stress related to the anomaly and no subsequent coral mortality was observed (Kemp and Fitt, personal observation).

**Zooxanthellae densities and chlorophyll content**

To further investigate the impacts of the cold-water stress on three species of reef-building corals, we conducted an experiment that replicated the most extreme three days of the low-temperature event at Admiral Reef (Fig. 2a). Fragments of *M. faveolata*, *P. astreoides*, and *S. siderea* were subjected to a temperature series that began at 20 °C and decreased first to 16 °C, then to 12 °C, and finally returned to 20 °C over a total of four days. Before and after the experiment, *Symbiodinium* genotype, cell density and chlorophyll *a* content were assessed. All post-experiment corals experienced a significant loss (paired t-test; *p* < 0.05) in zooxanthellae compared to pre-experiment corals, with the exception of *Porites astreoides* (Fig. 4; *p* = 0.065). All post experiment corals had significant loss of chlorophyll *a* content cm⁻² (Fig. 5a; paired t-test; *p* < 0.05), although, only *P. astreoides* showed significant loss in chlorophyll *a* cell⁻¹, explaining why we were able to detected significant loss in chlorophyll *a* when there were no significant loss in zooxanthellae. (Fig. 5b; paired t-test; *p* < 0.01). Interestingly, *Siderastrea siderea* samples from the offshore reef showed an increase in chlorophyll *a* content cell⁻¹ (Fig. 5b; paired t-test; *p* < 0.05).
Zooxanthellae Genetic Identity

Genetic identity of *Symbiodinium* determined by DGGE of the ITS-2 nrDNA revealed similar results to previous studies of the same coral species and reefs (Thornhill et al. 2006a,b, 2009), indicating stable coral-dinoflagellate associations (Table 1). Within the detection limits of DGGE (Thornhill et al. 2006b; LaJeunesse et al. 2009) all coral replicates harbored one dominant ITS-2 type of *Symbiodinium*. Each coral species associated with its own *Symbiodinium* ITS-2 type(s) and no overlap was observed between coral species (Table 1). Within a coral species, all replicates contained identical types of zooxanthellae, with the exception *S. siderea* (Inshore), which four replicates were found to have *Symbiodinium* ITS-2 type C3 and two replicates had *Symbiodinium* ITS-2 type B5a. Within a coral colony, no mixed communities of *Symbiodinium* were detected and there were no detectable shifts in *Symbiodinium* community identity following cold stress (Table 1).

Coral-Zooxanthellae Gross Photosynthesis and Dark Respiration

Coral-zooxanthellae oxygen flux is summarized in Figs. 6–7. For this section all statistical analyses were assessed via Tukey post-hoc multiple comparisons unless otherwise noted. After the initial temperature decline to 16 °C, the physiological effects of temperature decline were species and collection location specific. *Montastraea faveolata* (Offshore; \( p = 0.714 \)) and *S. siderea* (Offshore) (\( p = 0.369 \)) did not show a significant decrease in gross photosynthesis at 16 °C. Only *P. astreoides* (Offshore; \( p < 0.001 \)) and *S. siderea* (Inshore; \( p < 0.05 \)) had significant decreases in gross photosynthesis at 16 °C (Fig. 6a). Upon reaching 12 °C, all corals experienced a significant decrease in gross photosynthesis at 12 °C (Fig. 6a; \( p < 0.001 \)).
To analyze overall cold treatment effect, reduction in gross photosynthesis, and photosynthesis recovery potential, coral-zooxanthellae oxygen flux was measured again at 20 °C after the cold-stress treatment. *Porites astreoides* (Offshore), *M. faveolata* (Offshore) and *S. siderea* (Inshore) were found to have significantly different reductions in gross photosynthesis compared to *S. siderea* (Offshore) (Fig. 6; Tukey post hoc multiple comparison of arcsine-transformed data; \( p < 0.05 \)). *S. siderea* (Offshore) were the only corals found to recover to pre cold-stress gross photosynthesis levels. All other corals were found to have reductions in gross photosynthesis post cold-stress (Fig. 6b).

Dark respiration of *M. faveolata* (Offshore; \( p = 0.497 \)) and *S. siderea* (Offshore; \( p = 0.618 \)) were not significantly affected at 16 °C (Fig. 7a). Similar to gross photosynthesis results, all corals were found to have significant reduction in dark respiration at 12°C (Fig. 7a; \( p < 0.01 \)). Comparisons between species showed a significant difference in dark respiration after the cold-stress treatment between *P. astreoides* (Offshore) and *S. siderea* (Offshore) (Fig. 7b; Tukey post-hoc multiple comparison of arcsine-transformed data; \( p < 0.05 \)). As with gross photosynthesis measurements, only *S. siderea* (Offshore) recovered to pre cold-stress dark respiration rates. All other corals were found to have reductions in dark respiration post cold-stress (Fig 7b).

*Coral-Zooxanthellae PAM Fluorescence*

Maximum quantum efficiency of PSII (\( F_v/F_m \)) of all corals was significantly reduced as experimental temperatures decreased \( (p < 0.01) \) with the exception \( F_v/F_m \) of *M. faveolata* (Offshore) \( (p = 0.119) \) and *P. astreoides* (Offshore) from 20 °C to 16 °C \( (p = 0.221) \) (Fig. 8a). Both inshore and offshore *S. siderea* and offshore *M. faveolata* showed significant increases in \( F_v/F_m \) at the end of the experiment when temperatures were returned to 20 °C (Fig. 8a; \( p < 0.05 \)).
The exception was *P. astreoides*, which did not show a significant difference in $F_v/F_m$ when temperatures changed from 12°C to 20°C, reflecting chronic and potentially irreversible photoinhibition. Additionally, *P. astreoides* experienced the greatest loss in $F_v/F_m$ over the course of the experiment and the percent loss in $F_v/F_m$ was found to be significantly different from both *S. siderea* inshore and offshore samples (Fig. 8b; Tukey post-hoc multiple comparison of arcsine-transformed data; $p < 0.05$).

**DISCUSSION**

Species-specific cold-water susceptibility and survivorship was observed on inshore reefs (e.g., Admiral Reef) located in the upper Florida Keys. Following cold-water stress, reef surveys of Admiral Reef showed catastrophic mortality of many common reef-building corals including *Montastraea faveolata* and *Porites astreoides*, but not *Siderastrea siderea*. Cold-water physiological experiments confirmed that *S. siderea* (Offshore) and their zooxanthellae showed a high level of resilience to initial drops in temperatures and exhibited the greatest recovery in dark respiration and gross photosynthesis after experimental cold-water treatments.

Unusually severe cold-water climatic events in the Florida Keys and Dry Tortugas resulting in considerable damage to inshore reefs have been reported during the winters of 1969–70, 1976–77 and 1980–81 (Hudson et al. 1976; Davis 1982; Porter et al. 1982). These events have played an important role in the community structure of inshore reef corals of the Florida Keys. For example, Porter et al. (1982) reported 96% coral mortality in shallow (<2 m) reefs in the Dry Tortugas following the winter of 1976–77. Similarly, Davis et al. (1982) reported numerous dead corals from inshore reefs of the upper Florida Keys including *M. annularis*, *Agaricia agaricites*, *P. astreoides* and *Acropora cervicornis* following the winter of 1980–1981.
Consistent with our findings, no reports of cold-water mortality of *S. siderea* from the Florida Keys have been reported. This cold-water resilience of *S. siderea* is not entirely unexpected, as *S. siderea* has been reported in benthic surveys off of North Carolina where winter bottom temperatures remain below 16 °C for three months per year and can be as low as 10.6 °C (Macintyr and Pilkey 1969).

Early coral physiological experiments (Mayer 1914) in the Dry Tortugas found that *S. siderea* tolerated 11.7 °C without apparent injury and survived temperatures as low as 5.1°C. This cold-water resilience starkly contrasts to the cold-water sensitivity of *P. astreoides*, where temperatures below 15.3°C were lethal (Mayer 1914). Mayer (1914) found that *M. annularis* (reported as *Orbicella annularis*) could endure 16 °C without apparent injury and could survive temperatures as low as 14.1 °C. It is difficult to know which species of the *M. annularis* complex (now recognized as three closely-related species: *M. annularis*, *M. faveolata*, *M. franksi* (Knowlton et al. 1992)) Mayer (1914) used for his experiments; therefore, we will avoid direct comparisons to his results.

Our experimental results of *P. astreoides* and *S. siderea* (Offshore) support the findings of Mayer (1914). We found *P. astreoides* to be the most affected by low temperatures and to have the greatest loss in $F_v/F_m$, indicating that *Symbiodinium* associated with *P. astreoides* experienced the greatest amount of photodamage. Interestingly, we found physiological differences between *S. siderea* inshore and offshore samples. A possible explanation for these differences is inshore *S. siderea* samples had experienced cold-water stress several weeks prior to our experiment (unlike offshore samples) and, as a result, may have already suffered reduced physiology.
A second potential contributor to differences between inshore and offshore *S. siderea* is symbiosis with physiologically and genetically distinct *Symbiodinium* spp. Inshore *S. siderea* harbored either *Symbiodinium* ITS-2 type B5a (2 colonies) or C3 (4 colonies), while offshore *S. siderea* samples only had *Symbiodinium* ITS-2 type B5a. Using cultured *Symbiodinium*, Thornhill et al. (2008) compared the photo-physiology of *Symbiodinium* (ITS-2 type B2) found in temperate corals to *Symbiodinium* (ITS-2 types A3, B1, C2) found in tropical hosts. After cold treatment of 10 °C for 14 days, *Symbiodinium* type B2 was able to rapidly recover photosynthetic capacity and was thereby more able to rebound from cold-water stress compared to *Symbiodinium* from tropical areas. This apparent ability of *Symbiodinium* type B2 to recover from cold-water episodes seemingly is a characteristic shared by *Symbiodinium* type B5a found in *S. siderea* from our study.

Maintenance of membrane fluidity is a major characteristic required for sustained metabolic function and photosynthesis of low-temperature adapted organisms (Routaboul et al. 2000; Los and Murata 2004). High percentages of polyunsaturated fatty acids have been documented in many low-temperature adapted phototrophic organisms including sea ice diatoms, dinoflagellates, and green algae (reviewed by Morgan-Kiss et al. 2006). Therefore, it is likely that some *Symbiodinium*, such as phylotypes from *S. siderea*, may express similar biochemical signatures to maintain photosynthetic capacity at low temperatures. Conversely, saturation of fatty acids of photosynthetic membrane lipids has correlated with high-temperature tolerance of a subset of *Symbiodinium* (Tchernov et al. 2004). Whether unique lipid composition is also coincidental with low-temperature tolerance of certain *Symbiodinium* species remains to be determined.
Cold-water stress has similar negative effects on coral-zooxanthellae physiology as warm-water stressors. This and previous studies have shown that severe cold-water temperatures result in the reduction in zooxanthellae density, chlorophyll $a$ levels and photosynthetic capacity, but cellular and biochemical processes should not be assumed to be the same as in warm-water bleaching events. Low temperatures additionally have been shown to reduce the feeding capability and calcification rates of corals (Mayer 1915; Crossland 1984). Because corals form complex endo- and ecto-symbioses with a diverse assemblage of taxa, it is difficult to distinguish the exact point(s) of physiological perturbation. Although this study demonstrates that low temperatures significantly reduce zooxanthellae physiology, host cellular damage as a result of cold-water stress should not be discounted (Watson and Morris 1987; Gates et al. 1992).

Nevertheless, the 2010 winter cold-water perturbation was a significant stressor to inshore coral reefs of the upper Florida Keys, causing mortality of numerous corals, including some $M. faveolata$ colonies estimated to be $\geq$200 years old (Hudson 1981).

This study demonstrates species-specific cold-water tolerance, physiology, and recovery potential for different coral-zooxanthellae symbioses. Combined reduction in zooxanthellae, chlorophyll $a$ content, and photosynthetic capacity as a result of cold-water stress ultimately result in a reduction of photosynthetically fixed and translocated carbon to the coral, compromising important metabolic processes such as seasonal accumulation of coral lipid reserves (Muscatine 1967; Fitt et al. 1993; Fitt et al. 2000). As shown by Fitt et al. (2000), Florida Keys corals normally have the greatest density of zooxanthellae and greatest amount of host tissue biomass during winter months. If corals’ seasonal physiological acclimatization is disrupted by an abnormal cold-water event, this additional stress may not have only severe short-
term effects (coral mortality) but may also contribute to reduced physiology of surviving species during warmer months, potentially reducing the corals’ resilience to warm-water stressors.
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Table 6.1. Coral samples and their identified *Symbiodinium* ITS-2 “type” used for cold-stress experiment, collected from Admiral Reef (Inshore) and Little Grecian Reef (Offshore).

<table>
<thead>
<tr>
<th>Coral</th>
<th>Symbiont ITS-2 type (Pre-experiment)</th>
<th>Symbiont ITS-2 type (Post-experiment)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Siderastrea siderea</em> (Inshore)</td>
<td>C3 (n=4); B5a (n=2)</td>
<td>C3 (n=4); B5a (n=2)</td>
</tr>
<tr>
<td><em>Siderastrea siderea</em> (Offshore)</td>
<td>B5a (n=6)</td>
<td>B5a (n=6)</td>
</tr>
<tr>
<td><em>Montastraea faveolata</em> (Offshore)</td>
<td>B1 (n=6)</td>
<td>B1 (n=6)</td>
</tr>
<tr>
<td><em>Porites astreoides</em> (Offshore)</td>
<td>A4a (n=6)</td>
<td>A4a (n=6)</td>
</tr>
</tbody>
</table>
Fig 6.1. Reef locations in the upper Florida Keys (U.S.) investigated in this study.
Figure 6.2. a) Temperature from data loggers placed on Admiral Reef (Inshore reef) and Little Grecian (Offshore reef) (Onset HOBO Pro v2; sampled every 4 h; resolution ± 0.2 °C).
b) Percent mortality vs. survivorship of common symbiotic cnidarians as sampled over 60 m² of random transects on Admiral Reef.
Fig 6.3. Photographs of coral colonies from Admiral Reef before and after the cold water anomaly. Photographs were taken in May 2009 (“Before”) and February 2010 (“After”). a,b) Montastraea faveolata c,d) Porites astreoides e,f) Siderastrea siderea. February 2010 photographs (panels b, d) of M. faveolata and P. astreoides show dead colonies while S. siderea colonies (panel f) remained alive. Pigmentation of dead M. faveolata (panel b) is due to overgrowth of the coral skeleton by cyanobacteria and filamentous algae.
Fig 6.4. Zooxanthellae cell density from pre-experiment and post-experiment sampled corals. Cell density data expressed as means ± 95% confidence intervals (n=6). *Significantly different between pre- and post-experiment (Paired t-test $p < 0.05$)
Figure 6.5. a) Chlorophyll $a$ content of corals per unit area from pre-experiment and post-experiment sampled corals. b) Chlorophyll $a$ content of zooxanthellae per cell between pre-experiment and post-experiment. All chlorophyll $a$ data expressed as means ± 95% confidence intervals (n = 6). *Significantly different between pre- and post-experiment (Paired t-test $p < 0.05$).
Figure 6.6. a) Gross photosynthetic O$_2$ fluxes as a function of temperature. All O$_2$ data expressed as means ± 95% confidence intervals ($n = 6$). Like letters denote within species means that are significantly indistinguishable from one another; different letters indicate significant differences (Tukey post hoc multiple comparison $p < 0.05$). b) Change in gross photosynthesis at 20°C post cold-water treatments. Data shown as percent difference between pre- and post-experiment. Like letters denote no significant difference among species (paired t-test of arcsine-transformed data; $p < 0.05$).
Figure 6.7. a) Coral O$_2$ fluxes in the dark as a function of temperature. All consumed O$_2$ data expressed as means ± 95% confidence intervals (n = 6). Like letters denote within species means that are not significantly different from one another (Tukey post hoc multiple comparison $p < 0.05$). b) Change in consumed O$_2$ at 20 °C following cold-water treatment. Data shown as percent difference between pre- and post-experiment. Like letters denote no significant difference among species (paired t-test of arcsine-transformed data; $p < 0.05$).
Figure 6.8. a) Maximum photochemical efficiency of PSII (Fv/Fm) of corals as a function of temperature throughout the 4-day experiment. Data are presented as means ± 95% confidence intervals (n = 6). b) Change in maximal photochemical efficiency of PSII (Fv/Fm) at 20°C following cold-water treatments. Data shown as percent difference between pre- and post-experiment. Like letters denote no significant difference among species (Tukey post hoc multiple comparison of arcsine-transformed data; p < 0.05).
The success of reef-building corals is due to the endosymbiotic dinoflagellates that provide significant amounts of metabolic carbon and enhance calcification. This dissertation investigates the diversity of *Symbiodinium* associating with reef-building corals and how diverse *Symbiodinium* may affect coral physiology. The majority of these chapters concentrated primarily on the dominant Caribbean reef-building coral *Montastraea faveolata* due to the fact that this coral has been found to associate with highly predictable, genetically diverse communities of *Symbiodinium*. These works progress from documenting geographic and within-colony zonation patterns, identifying the functional diversity of genetically diverse *Symbiodinium*, and the investigation of differential physiological patterns as a result of warm-water coral bleaching and a cold-water stress event.

Chapters two and three are related to each other. Chapter two describes a novel technique for sampling coral that I developed and used throughout these works enabling me to take hundreds of samples from a single coral over time whilst causing minimal damage. Chapter three uses microsampling to examine within-colony distributions of *Symbiodinium* and found community structure correlated with light availability resulting distinct zonation patterns. Geographic comparison of host-symbiont associations revealed regional specificity in *Symbiodinium* from clades A, B, C. Three distinct ITS-2 types were identified for Clade C, unique to the Florida Keys, Bahamas, and Mesoamerican reefs (Belize and Mexico). Clade A
was detected in Mesoamerican, but not the Florida Keys or Bahamas. Likewise, B17 was unique to Mesoamerica while B1 was found in reefs of the Florida Keys, Bahamas in addition to Mesoamerica. Geographic genetic specificity and within colony stability indicate long-term ecological and evolutionary processes.

Chapter four used microsampling techniques to screen for Symbiodinium diversity of *M.faveolata* in Puerto Morelos, Mexico. Observed *Symbiodinium* zonation patterns were best explained by locally prevalent light fields on *M.faveolata* colonies. Within a coral colony, *Symbiodinium* type C7 was the dominant symbiont in low light areas, while types B17 and B1 were the dominant symbiont in high light areas. Coral samples were collected and a series of physiological parameters were measured examining how *Symbiodinium* community structure affects coral calcification and photobiology. The results of this study showed genetically different symbiont types display differential high-light or low-light photo-physiological and photoacclimation responses indicating a high degree of *Symbiodinium* niche specialization within *M.faveolata* which ultimately influences coral physiology.

Chapters five and six examined the effect two separate environmental stressors have on coral-alga physiology and how genetically different *Symbiodinium* may be affected by the stress. In chapter five, community structure and physiology of *Symbiodinium* associated with *M.faveolata* was studied during a coral bleaching event in Puerto Morelos, Mexico. Using microsampling methods accompanied with *in situ* pulse amplitude modulated fluorometry (PAM) we monitored the effect coral bleaching had on *Symbiodinium* physiology and community structure. Within-colony differential bleaching was observed in *M.faveolata* colonies. Additionally, we investigated within colony variability associated with coral bleaching. Pigmented “non-bleached” portions of *M.faveolata* were compared to “bleached”
portions of the same colony. Not surprisingly, we found significantly less *Symbiodinium* and chlorophyll *a* cm⁻² in “bleached” regions. During bleaching, maximum photosynthetic efficiency of the photosystem II (PSII) was found to be significantly lower and highly variable compared to the “non-bleaching” September 2006. We monitored these colonies during recovery and documented a symbiont community shift in *M. faveolata* to *Symbiodinium* type A3 and D1a, ITS-2 types known to have high thermal tolerances. The results of this chapter indicate functional differences between genetically distinct *Symbiodinium* populations that may facilitate competitive exclusion of thermally sensitive *Symbiodinium* types and result in the prevalence of tolerant opportunistic *Symbiodinium*.

Chapter six investigated coral physiology during cold-water stress in the upper Florida Keys. During February and March 2010, we surveyed Admiral Reef and documented a mass die-off of reef-building corals, whereas 12 km away, Little Grecian Reef did not exhibit coral mortality. Following this event, the physiological effects of low temperature stress on three common reef-building corals (*M. faveolata*, *Porites astreoides*, *Siderastrea siderea*) were experimentally investigated over a range of temperatures that simulated the inshore cold-water anomaly. This study documents species-specific physiological responses that were found indicating different coral and/or zooxanthellae cold tolerances. *M. faveolata* and *P. astreoides* appeared to be most negatively impacted upon return to 20 °C, with significant reductions in gross photosynthesis and dark respiration. Under comparable temperature conditions, *S. siderea* recovered to pre-treatment rates of dark respiration and gross photosynthesis. Visual surveys of inshore reefs corroborated these results, with *S. siderea* being minimally affected by the cold-water anomaly whereas *M. faveolata* and *P. astreoides* experienced approximately 100% mortality.
This dissertation uses ecological observations accompanied with molecular and physiological methods to investigate coral-\textit{Symbiodinium} symbiosis. The results of these studies indicate the ecological importance of understanding the physiology of genetically diverse \textit{Symbiodinium}. As coral reefs continue to decline at accelerating rates, it is paramount to continue studying the ecological and evolutionary processes of \textit{Symbiodinium}. Though, \textit{Symbiodinium} are only one part of a complex symbiotic assemblage of the coral “holobiont” they can greatly influence coral physiology and knowledge of functional and genetic diversity should be implemented into conservation efforts.