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Intraspecific Genetic Variability in Temperature Tolerance in the Coral Pocillopora damicornis: Effects on Growth, Photosynthesis and Survival

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INTRASPECIFIC GENETIC VARIABILITY IN TEMPERATURE TOLERANCE IN THE CORAL *POCILLOPORA DAMICORNIS*: EFFECTS ON GROWTH, PHOTOSYNTHESIS AND SURVIVAL

By

Phillip Gillette

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Intraspecific Genetic Variability in Temperature Tolerance in the Coral *Pocillopora damicornis*: Effects on Growth, Photosynthesis and Survival (December 2012)

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Coral reef ecosystems worldwide are in decline as a result of climate change and other anthropogenic impacts. Prolonged exposure to extreme temperatures are predicted to occur more frequently in the future, resulting in coral bleaching and/or mortality. Different coral colonies within a species often show variation in both bleaching and mortality when exposed to temperature stress, but whether this is a result of genetic differences in the coral host, or its algal symbiont communities, is not clear. To distinguish these influences, the effect of different temperatures (20, 26, 30 and 32^{o}C) on four different genotypes of *Pocillopora damicornis* (identified using microsatellites) containing different *Symbiodinium* communities (identified using quantitative PCR), was investigated. Corals grew most rapidly at control temperatures (26^{o}C, $p<0.001$), but this effect varied in strength across genotypes. Extreme temperatures had a negative effect on growth in three of the four genotypes ($p<0.016$). There was differential mortality in the highest temperature (32^{o}C), with genotypes 1 and 3 showing the earliest mortality on day 52, while genotypes 2 and 4 experienced total

mortality by day 66. In the 30°C treatment, mortality occurred on day 80, with genotype 4 having the highest mortality (100%), followed by genotype 2 (67%), 1 (44%) and 3 (7%). Genotype 3 was the only coral to show mortality at the cold temperature (20°C), with 7% of colonies having total mortality and 78% of colonies having at least some partial mortality. Quantitative PCR analysis of the algal symbionts (*Symbiodinium* spp.) in these corals revealed genotypes 1, 2 and 4 were initially dominated by *Symbiodinium* C1b-c, while genotype 3 was dominated by *Symbiodinium* D1. However, when exposed to 30°C, all genotypes became dominated by D1 by day 72. All corals experienced a decrease in symbionts at 30°C. However, the density of C1b-c symbionts decreased by an average of 98% across genotypes 1, 2 and 4 when the temperature was raised to 30°C, suggesting that the shift to D-dominance is most likely due to the expulsion of C1b-c, with D1 symbionts in genotype 3 decreasing by an average of 61% at this temperature. Exposure to cold temperatures resulted in a large increase in densities of clade C symbionts, with genotype 4 having a 350% increase in the number of symbionts per host cell. Conversely, cold temperatures caused an 80% decrease in D1 symbionts in genotype 3, compared to initial levels. Photochemical efficiency of symbionts also varied across temperatures and coral host genotype. Fv/Fm values for the clade C-hosting genotypes were similar at all temperatures except at 20°C, where genotype 1 values were significantly lower than those of genotypes 2 and 4 at 20°C and 26°C. Genotypes 2 and 4 showed no photochemical response to cold temperatures relative to the control. Clade C symbionts had a strong negative response to high temperatures, with Fv/Fm values significantly lower compared to controls. Fv/Fm

values recovered at 30^{o}C for C1b-c symbionts towards the end of the experiment, likely due to the expulsion of C1b-c symbionts and the resulting dominance by D1. At high temperatures, D1 symbionts in genotype 3 had similar Fv/Fm values as the control, but lower values at 20^{o}C. Together, these data suggest that variability within coral genotypes plays a significant role in thermal tolerance. This variation is further influenced by the algal symbiont community, with complex interactions occurring between the host genotype and symbiont identity. The data presented in this study supports the growing volume of scientific literature that suggests that coral host genotype is an important component in the coral holobiont's thermal tolerance. These data also show that, while D1 symbionts are more tolerant of high temperatures, corals hosting these symbionts may not survive indefinitely if the host genotype is itself thermally sensitive. This information may help restoration efforts designed to increase the resilience of coral reefs to climate change, by identifying coral genotypes and host-symbiont combinations best suited to the prevailing thermal environment.

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Chapter 1: Introduction

The thermal limits and temperature tolerance of reef-building corals

Coral reefs occur in tropical waters worldwide, and are essential habitats, not only for reef-associated organisms, but also for the people who depend on them. Coral reefs support nearly a third of all fish species, and these fish account for over 10% of global fish catch, with tens of millions of people relying directly on coral reefs for survival (Moberg and Folke 1999). In spite of this, reefs worldwide are in serious decline, being negatively affected by a number of stressors (Hughes et al. 2003), perhaps the most pressing of which are the combined effects of increasing temperatures and decreasing aragonite saturation state associated with climate change (Marshall and Schuttenberg 2006, Hoegh-Guldberg et al. 2007). The Intergovernmental Panel on Climate Change has reported a rise in temperature of 0.74^{o}C during the 20th century, and conservative models predict a further rise of 1.8-4.0^{o}C in the coming century (IPCC 2007). Because tropical reef building corals live near their upper thermal limits, thermal anomalies that increase temperatures above normal summer maxima can create significant challenges for their survival. The temperature generally considered optimal for coral growth occurs at 26-27^{o}C (Bosscher 1992). Even small deviations from this narrow temperature range can result in decreased calcification and increased stress, with effects becoming more severe at the extremes. Severe or prolonged temperature stress can eventually lead to cessation of calcification, bleaching, and mortality (Glynn 1993, Davy et al. 2012).

Cold water limits the growth and survival of reef-building corals at higher latitudes outside the tropics. The worldwide distribution of coral reefs is typically associated with the 18^{o}C minimum thermal isotherm, *i.e.,* significant coral reef development does not occur in waters which routinely experience seasonal temperature minima <18^{o}C for sustained periods (Kleypas et al. 2001). Early experiments by Mayor (1914, 1915) showed that corals no longer fed at 16^{o}C, and complete coral mortality occurred below 14^{o}C. However, coral response to low temperatures is variable. One study in the Arabian Gulf showed species-specific mortality after a cold water event, with *Acropora pharaonis* and *Platygyra daedalea* showing severe mortality at temperatures as low as 13^{o}C, while *Porites compressa* showed little long-term damage (Coles and Fadlallah 1991). Recent cold temperatures in Florida during the winter of 2010 resulted in significant species-specific coral mortality. The species-specific mortality trends seen during this event were often the opposite of those experienced during warm water anomalies, with corals that had resisted bleaching succumbing to cold water stress (Lirman et al. 2011). Some scleractinian corals survive in waters off North Carolina, where temperatures can be as low as 10.5^{o}C, but these corals are solitary, typically azooxanthellate, and do not form reefs (Macintyre and Pilkey 1969).

Cold water has also been shown to result in coral bleaching (Hoegh-Guldberg et al. 2005). LaJeunesse et al. (2010) showed significant bleaching in *Pocillopora* in the Gulf of California in 2008 due to unusually low temperatures. Cold temperatures have been considered by some researchers to be potentially more harmful than high

temperatures in reef development (Jokiel and Coles, 1977), and the 2010 Florida cold-water anomaly caused mortality than was 1-2 orders of magnitude higher than that experienced following warm water anomalies (Lirman et al. 2011). However, cold tolerance in corals is not generally well understood, and most studies investigating thermal anomalies have focused on elevated temperatures (Lirman et al. 2011).

Rising ocean temperatures have been referred to as one of the most pressing issues facing the survival of coral reefs in the near future (Hoegh-Guldberg et al. 2007). Most tropical coral reefs worldwide occur close to the upper threshold of their thermal tolerance (Jokiel and Coles, 1990; Glynn, 1993; Brown, 1997; Hoegh-Guldberg, 1999). Corals are therefore very susceptible to even small increases in temperature over the average summer maximum (Jokiel and Coles 1990). One of the most visible impacts of increased ocean temperature is coral bleaching. Short-term exposure of 3-4^{o}C above the summer maximum temperatures, or long-term exposure of only 1-2^{o}C, has been shown to lead to bleaching (Jokiel and Coles, 1990). While bleaching most often occurs due to high thermal stress, a number of other factors can also cause bleaching, or act synergistically to increase its severity. These secondary factors include low temperatures, bacterial pathogens, high light levels, environmental pollutants, and extreme salinity fluctuations (Glynn, 1993; Kerswell and Jones, 2003). In addition to causing bleaching, temperature extremes (both high and low) have been shown to strongly influence calcification rates in corals, with extreme temperatures resulting in marked reductions in calcification (Marshall and Clode, 2004; Al-Horani, 2005; Clausen and Roth, 1975; Jokiel and Coles, 1977).

Reef-building corals are meta-organisms comprising the coral host, its algal symbionts, and its associated bacterial and viral communities. These different partners all contribute to the survival and physiological properties of the coral "holobiont", and in some cases, can be critical in understanding coral response to environmental stress. For example, corals exposed to temperature extremes typically expel their algal symbionts (*Symbiodinium* spp.) during episodes of coral "bleaching", and these symbionts are key in understanding this response, since they are the primary target of thermal stress. Algal symbionts (commonly referred to as "zooxanthellae") typically translocate 90% of their photosynthates to their coral hosts, which use them as a principal source of carbon (Sumich 1996). Therefore, the loss of zooxanthellae in corals is considered one of the biggest threats to coral reefs worldwide (Marshall and Schuttenberg 2006, Hoegh-Guldberg et al. 2007).

The loss of algal symbionts during bleaching can often lead to mortality of the host coral (Glynn 1993). These symbionts provide a significant source of carbon to corals in oligotrophic tropical oceans (Muscatine and Porter 1977), and have been shown to translocate sufficient carbon to meet 90% of the coral host's respiratory needs (Muscatine and Porter 1977, Falkowski et al. 1984, Muscatine et al. 1984). The genus *Symbiodinium* consists of (at least) 9 distinct clades (Baker 2003, Coffroth and Santos 2005, Pochon & Gates 2010). In scleractinian corals the most typical symbionts are members of clades A, B, C and D. There is significant inter- and intra-cladal physiological variation among different *Symbiodinium* clades, but clade D has drawn special attention because it contains several member that are thermotolerant. Although many corals

often associate with only one symbiont type (Goulet 2006), there is evidence that many corals species can display plasticity in relation to which clade they associate with, with some species hosting multiple clades simultaneously (Baker 2003, Baker & Romanski 2007, Silverstein et al. 2012).

It has been hypothesized that corals under stressful environmental conditions can shuffle the proportion of different symbionts between dominant and background types, or even change symbionts types altogether by acquiring new strains from the environment (Buddemeier and Fautin 1993, Baker 2004). The ability of corals to obtain exogenous symbionts in the field is still being debated, however (Coffroth and Santos 2005, Coffroth et al. 2006), as well as the ontogenetic timing of the ability to take up algae into the host cells (Little et al. 2004).

Very little is known concerning the intraspecific variation in the response of a coral species exposed to thermal stress, although the concept of heat-tolerant coral genotypes has been proposed (Jokiel and Coles 1990, Edmunds 1994, Brown 1997, Hughes et al. 2003, Weiss 2010). During the 1987 Caribbean bleaching event, the bleaching pattern of *Montastraea annularis* colonies were aggregated, with patches of bleached corals occurring together, suggesting bleaching patterns were the result of intraspecific variation in temperature response among genotypes of *Montastraea*.

Pocillopora damicornis in the tropical eastern Pacific has also showed genotype-dependent responses in bleaching when exposed to high temperatures. D'Croz and Mate (2004) found that *Pocillopora damicornis* from the Gulf of Panama and the Gulf of

Chiriqui bleached to different degrees, with genotypes from the Gulf of Panama exhibiting more susceptibility to thermal stress. It was also found that, within the Gulf of Chiriqui population, there were difference in response among genotypes, suggesting genetic factors, as well as environmental history, were responsible for the variability. However, as the authors acknowledged, there were no genotypes that were common to both locations, and they were unable to identiyy the *Symbiodinium* contained by these hosts, both of which are factors which may have contributed to the differences observed between the two regions.

In a study of *Acropora* on the Great Barrier Reef, Berkelmans and van Oppen (2006) suggested that observed differences in thermal tolerance were entirely dependent on symbiont type, and that host genotypic differences had little or no effect on the thermal tolerance of the coral holobiont. After a cold-water bleaching event in the eastern Pacific, LaJeunesse et al. (2010) found that symbiont type alone could not account for all the differences in bleaching observed, and also suggested that host genotypic variation was important in determining thermal tolerance (LaJeunesse et al. 2010).

The purpose of the current study is to investigate differences in temperature-tolerance among different coral genotypes. Four different genotypes of *Pocillopora damicornis*, identified using microsatellite markers, were exposed to four experimental temperatures (20, 26, 30 and 32^{o}C) under controlled laboratory conditions for a period of 8 weeks. Growth was measured at the end of the experiment, and photochemical

efficiency was measured every other week. *Symbiodinium* communities were identified and quantified at the start and end of the experiment using quantitative real-time PCR (qPCR). All four genotypes were originally collected from the Gulf of Panama, and had been maintained in an experimental facility at a constant 26^{o}C for 5 years, thus minimizing the impact of environmental history on thermal tolerance.

Because the coral holobiont comprises a number of linked genomes, impacts on one member of the holobiont may also affect other members of the holobiont. The current study investigates the effect of thermotolerance on different coral genotypes by measuring the effect of temperature on both the coral host and the *Symbiodinium*. Chapter 2 discusses the effect of host genotype on growth and mortality under different temperature treatments. Chapter 3 discusses the effect of temperature on symbiont community structure, density, and photochemical efficiency. Chapter 4 synthesizes the results of Chapters 2 and 3, and discusses the interaction between host genotype and algal symbiont identity, and its effect on coral thermotolerance.

Chapter 2: Influence of coral genotype on growth and mortality under different temperature treatments

To assess host genotypic effects on thermal tolerance, four different genotypes of *Pocillopora damicornis* were exposed to four different temperatures (20, 26, 30 and 32^{o}C) for eight weeks. Growth was measured at the start and end of the experiment using an optical micrometer, and mortality was determined at 2-week intervals. There was strong genotypic variability in coral growth at 26^{o}C, with genotypes 2 and 4 growing most rapidly, followed by genotype 1 and then genotype 3. Exposure to all experimental temperatures resulted in strong reductions in growth in all genotypes. There was 100% mortality by week 6 in the highest temperature (32^{o}C), and partial mortality of some genets in the 20^{o}C and 30^{o}C treatments by week 8. Together, these data suggest that coral genotypes vary in their thermal tolerance and growth rates, even after the influence of environmental history has been removed.

2.1 Materials and methods

2.1.1 Experimental set-up

Experiments were undertaken in 5 identical semi-recirculating systems exposed to natural solar irradiance (shaded by 30% using neutral density screen) at the University of Miami's Coral Resource Facility. Each system consisted of paired recirculating fiberglass tanks mounted vertically (one on top of the other, see Figure 1). The lower sump tank (182 x 69 x 40cm, 500 liter capacity) was used to regulate

temperature, while the upper tank (182 x 69 x 28cm, 350 liter capacity) was used as the experimental incubation tank.

Temperature regulation in each system was accomplished using a combination of heaters and heat exchangers. Each sump contained a CLEPCO (Cleveland Process Corp.) titanium submersible heater and a TiTech titanium heat exchanger for cooling, which worked together to maintain the desired temperature. The heater and heat exchanger were controlled using an Omega CN-7800 controller, which either activated the heater via an Omega Solid-State Relay (SSR), or opened a solenoid valve, allowing chilled (18^{o}C) water to pass through the heat exchanger. Daily temperature data were recorded manually from each tank's digital readout every morning (Figure 2).

A MagDrive MD1200 pump was used to circulate water through the experimental system and heat exchanger. Approximately half of the water (~220 liters per hour) was diverted through the heat exchanger, while the remaining water (~220 lph) was sent to the top tank. A constant flow (approximately 0.2 L/min) of filtered (5 micron spun filter) Bear Cut water was supplied to each system as makeup water. Tanks were exposed to shaded natural sunlight, measured hourly using a LI-COR LI1000 datalogger and LI-COR LI-190 quantum sensor, and reported as the mean mol/m^2/wk (Figure 3).

Corals were fed twice per week. Feeding consisted of placing corals into small styrofoam coolers underwater, with care taken not to expose the corals to air. Coolers were supplied with Rio 50 submersible powerheads for circulation, and 1 tablespoon of

Zeigler Larval Diet (100-150 micron) was dissolved into the water and allowed to circulate for 1 hour, after which corals were returned to the treatment tanks.

Figure 1: Experimental systems in use at the University of Miami. The top tank in each system is for experimental incubations, while the lower tank is the temperature regulation sump.

2.1.2 Collection and maintenance of experimental animals

Colonies of *Pocillopora damicornis* were collected from a depth of ~4 m by Peter Glynn (University of Miami) from the Saboga Island reef, in the Pearl Islands, Panama, on 21 March 2005. Saboga Island is located in the Gulf of Panama, which is subject to seasonal wind-induced upwelling. During upwelling, temperatures can fall below 20^{o}C, driving annual temperature variation of >10^{o}C (D'Croz and Mate, 2004). To maximize

the chances of collecting different genotypes, six colonies were collected from different areas of the Saboga Island reef. Colonies were exported to Miami, and clonal lines propagated from them in the indoor recirculating Coral Resource system at the University of Miami. They were successfully propagated for 5 years under artificial light (90 mol/m^2/week) at a temperature of 26^{o}C for 5 years before beginning the experiment.

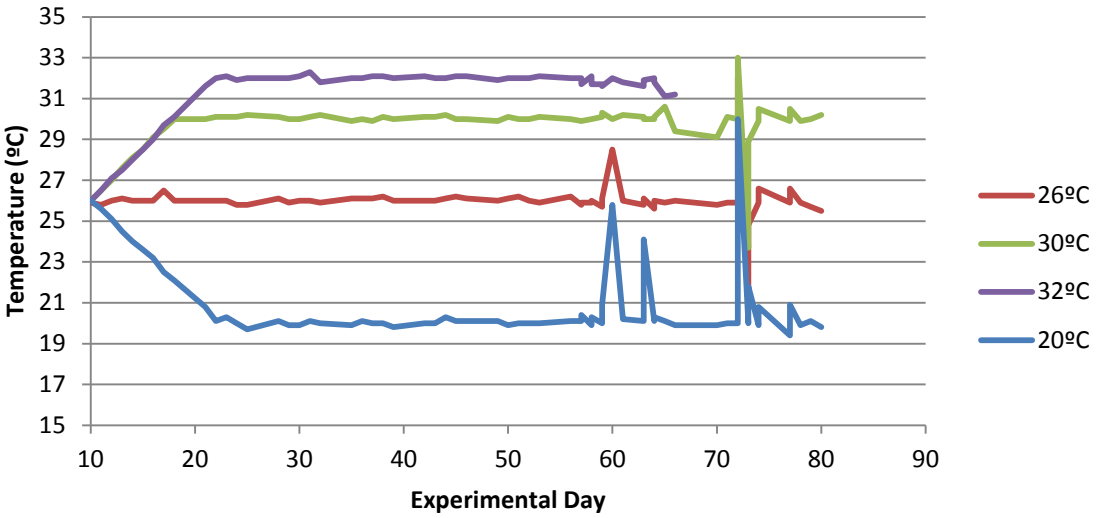

Figure 2: Temperature data plotted against experimental day.

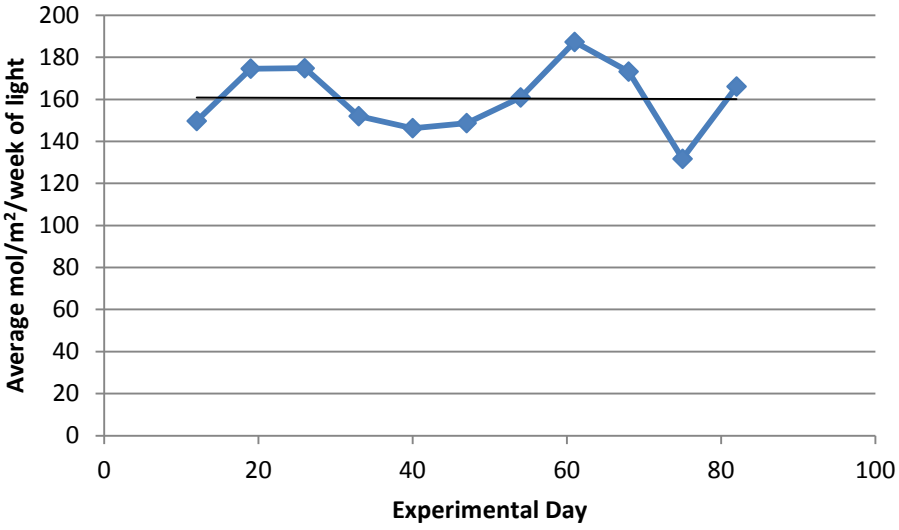

Figure 3: Light levels reaching coral tanks, measured over the course of the experimental period and reported as the average mol/m^2/week. Light levels were stable throughout the experiment (y=-0.0119x + 161.04, p=0.85).

2.1.3 Genotyping of experimental animals

The 6 experimental colonies (labeled in the UM Coral Resource as Pda-PAN-6, Pda-PAN-8, Pda-PAN-9, Pda-PAN-10, Pda-PAN-11 and Pda-PAN-12) were genotyped by Becky Hersch (University of Miami) using 8 microsatellite markers (Starger et al. 2008). The eight markers used were Pd-145, Pd-192, Pd-4, Pd-402, Pd-6B11, Pd-7F8, PV2 and PV6.

2.1.4 Experimental design

To investigate the response of different genotypes of *Pocillopora damicornis* to thermal stress, corals were exposed to 4 temperature treatments: 20°C, 26°C, 30°C and 32°C. Nine replicates of each of the six prospective *Pocillopora damicornis* genotypes

were used in each of the four temperature treatments (N=216 total). Each replicate consisted of a single non-bifurcating branch (1.0-1.5 cm height). Three coral fragments were glued to each PVC measurement sled, with care taken to randomize replicates and genotypes between sleds in each treatment. On May 31 2010 (day 0), sleds with mounted corals were placed in experimental tanks at 26^{o}C and allowed to acclimate to ambient light and tank conditions until 14 June 2010 (day 14). On 6 June 2010 (day 6), initial micrometer measurements were taken on all sleds. Beginning on 14 June (day 14), temperatures in the 32^{o}C and 20^{o}C tanks were increased at a rate of 0.5^{o}C/day, and on 17 June (day 17) temperature was increased at the same rate in the 30^{o}C tank. All treatments reached target temperatures simultaneously on 25 June (day 25). The experiment ended on 19 August 2010 (day 80). However, because the micrometer was not available on this day, corals were removed from the water and final micrometer measurements were taken on the dead coral skeleton on 21 October 2010.

2.1.5 Measurement of growth and mortality

Growth measurements were taken using an optical micrometer. Coral branches were glued (using cyanoacrylate gel) to gray PVC sleds. Each sled (10cm x 3.8cm) contained a stainless steel reference pin centered 5mm from one end. Each sled was placed on an optical micrometer to measure the height of corals on the sled. The micrometer measures linear extension by emitting a high-intensity green LED across a coral, creating a shadow which is recorded by a linear CCD receiver, precisely measuring the height of a coral (Bielmyer et al. 2010). Measurements were taken at the start and

the end of each experiment. Growth data was analyzed as percent growth to account for any differences resulting from the varying starting sizes of fragments. Mortality data, recorded as alive, dead or exhibiting partial mortality, were taken every 2 weeks. Growth data were compared statistically using ANOVA and Proc-GLM run in SAS (1990). Multiple-pairwise comparisons were Bonferonni corrected, with a critical p-value of $p>0.016$ (Sokal and Rolf 1987).

2.2 Results

2.2.1 Host genotyping

Microsatellite analysis revealed that 3 of the original 6 colonies (Pda-PAN-9, Pda-PAN-11 and PDA-PAN-12) had identical alleles at all 8 microsatellite loci and were likely members of the same genotype ('Genotype 3'). Pda-PAN-6 ('Genotype 1'), 8 ('Genotype 2') and 10 ('Genotype 4') were all distinct for at least 1 of the loci tested (Table 1).

	Locus								
Colony	**145**	**192**	**4**	**402**	**6B11**	**7F8**	**PV2**	**PV6**	**Genotype**
Pda-PAN-6	168	203/212	199	193					1
Pda-PAN-8	165	212	201/205	195					2
Pda-PAN-9	165	203	199	195	215/218	193/196	131/142	205/211	3
Pda-PAN-10	165	209/212	201/205	195					4
Pda-PAN-11	165	203	199	195	215/218	193/196	131/142	205/211	3
Pda-PAN-12	165	203	199	195	215/218	193/196	131/142	205/211	3

Table 1: Microsatellite analysis revealed that 3 of the original 6 colonies had identical alleles at all 8 microsatellite loci and were likely members of the same genotype, genotype 3. Genotype 1, genotype 2 and genotype 4 were all distinct for at least 1 of the loci tested.

2.2.2 Experimental conditions

Two temperature anomalies (on days 60 and 72) were recorded during the experiment. The day 60 anomaly, resulting from a chiller failure, produced a short temperature spike of +5^{o}C in the 20^{o}C treatment and +3.5^{o}C in the 26^{o}C treatment, but lasted no more than an hour. The day 72 anomaly, resulting from a solenoid power-supply failure, produced temperature spikes of +10, +6 and +3^{o}C in the 20, 26 and 30^{o}C treatments, respectively. To solve this problem, the solenoid valves were removed, allowing cold water to flow freely through the heat exchangers, exposing all treatments to cool temperatures (~22^{o}C) until day 73, at which point temperatures were stabilized.

2.2.3 Growth of experimental colonies

A 2-way ANOVA of the effect of temperature and host colony on the growth of genetically identical colonies (Pan-9, Pan-11 and Pan-12, collectively referred to as "Genotype 3") found no significant differences between these colonies at each temperature ($p=0.680$) and no interactions between colony and temperature ($p=0.398$). Growth data for these three colonies were therefore pooled.

There was a statistically significant effect of both temperature and coral genotype on growth ($p<0.001$) (Figures 4 and 5), as well as a significant interaction between these two factors ($p<0.001$). Corals in the 26^{o}C control treatment showed significantly higher growth in genotypes 1, 2 and 4 than the 20^{o}C, 30^{o}C and 32^{o}C

treatments ($p<0.001$) (Figure 4). In the control (26°C) treatment, genotypes 2 and 4 displayed the highest growth (+12.5% and +14.7%, respectively), and were not statistically different from each other. Genotype 1 grew slightly less (+5.7%, $p<0.001$), and genotype 3 was the slowest growing genotype (+2.4%, $p= 0.0138$, Figure 5). At the three experimental temperatures (20°C 30°C and 32°C) there was very little growth (<2%) and no differences between genotypes. There was no difference in growth for genotype 3 across any temperature.

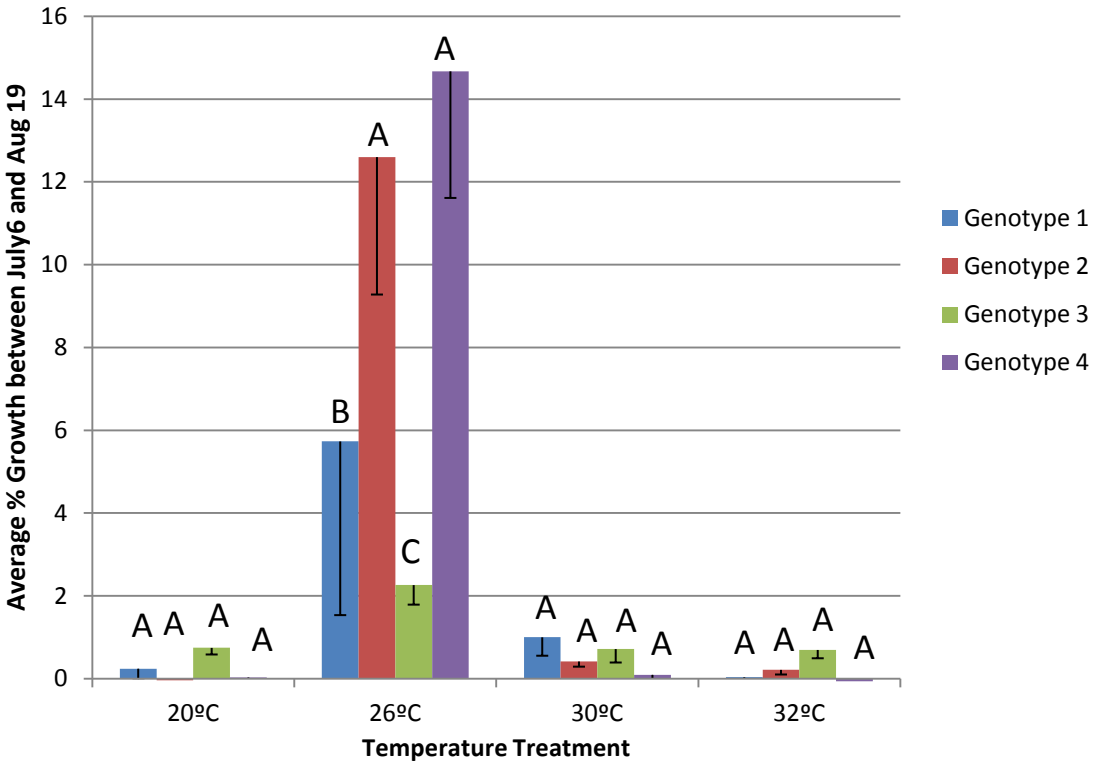

Figure 4: Mean growth of genotypes 1-4 as a function of temperature. Genotypes did not differ from one another in their growth rate at 20°C, 30°C and 32°C, and growth rates at these experimental temperatures did not differ from one another. At 26°C, growth rates varied by genotype (2=4>3>1, all $p<0.0166$).

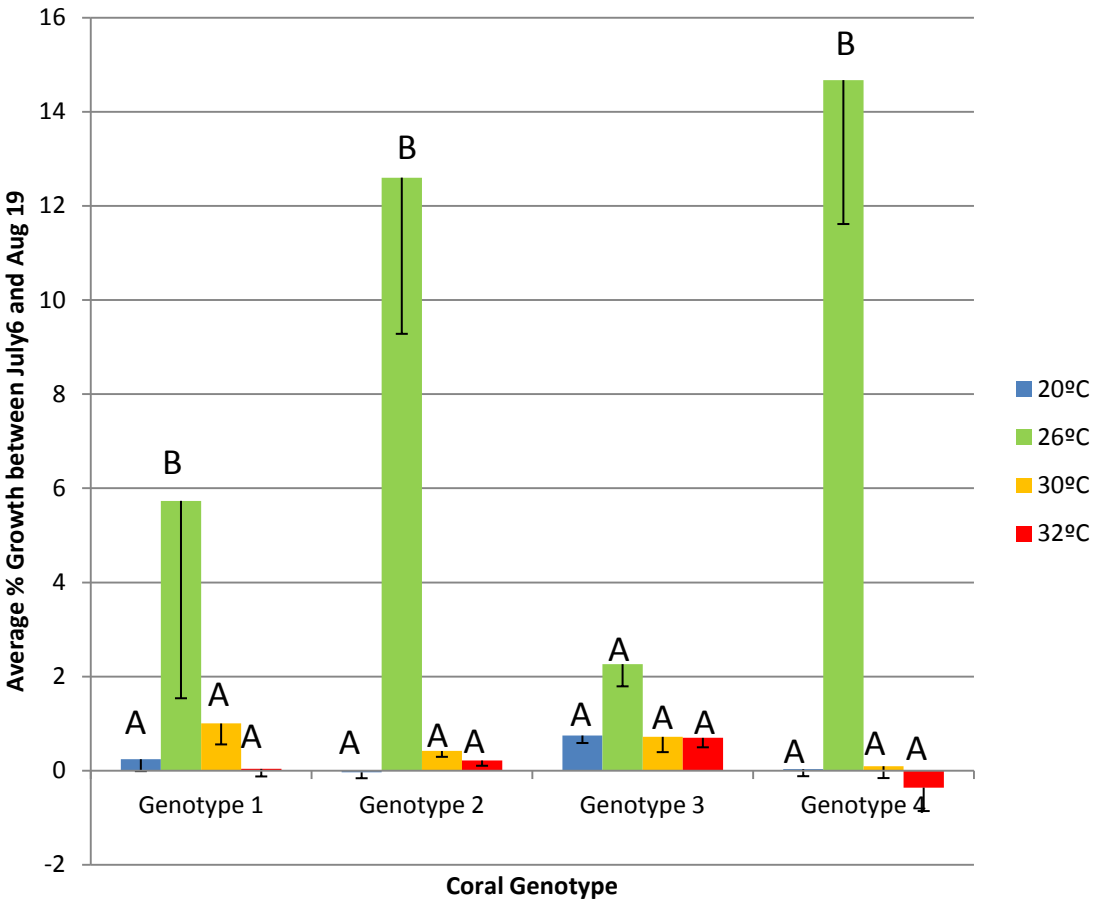

Figure 5: Mean percent growth of genets 1-4 at each temperature, grouped by temperature to highlight differences between temperature treatments for each genotype. At 26°C, growth rates varied by genotype (2=4>3>1, all $p<0.0166$).

2.2.4 Mortality of experimental colonies

Mortality was recorded visually every 2 weeks prior to IPAM measurements. Total mortality was determined by complete loss of tissue, while partial mortality was the loss of tissue over a portion of the fragment. With strongly bleached colonies, tissue loss was most easily observed by the presence of a thin layer of very fine filamentous algae covering the skeleton. There was no mortality recorded in the control treatment

for the entire experiment (80 days). In the 32°C treatment, coral mortality was observed on day 52, with genotype 3 showing 100% mortality and genotype 1 showing 89%. By day 66, all corals in the 32°C treatment had died, regardless of genotype. By day 80, one member (7%) of genotype 3 at 20°C had died, and some members of genotype 1 (44%), genotype 2 (67%), genotype 3 (7%) and genotype 4 (100%) at 30°C had died. Partial mortality was only observed on day 80, and was scored simply as whether or not partial mortality had occurred, not as a quantitative estimate. In the 20°C treatment, only genotype 3 showed partial mortality, (78% of fragments). No corals showed any partial mortality in the control treatment. At 30°C, 22% of genotype 1, 33%, of genotype 2, and 11% of genotype 3 had partial mortality. Table 2 shows total and partial total mortality data for all genotypes and temperatures.

2.3 Discussion

2.3.1 Effect of coral genotype on growth

Different genotypes of *P. damicornis* varied in their growth rates at the control temperature (26°C). Genotypes 2 and 4 grew significantly faster than either genotype 1 or 3, with genotype 3 showing the lowest growth at 26°C. Corals at the three experimental temperatures (20, 30 or 32°C) tended to grow very slowly, and there was no overall difference in growth rates between these three temperatures for any of the coral genotypes. Similarly there was no difference among coral genotypes at these experimental temperatures. As observed in previous studies, extreme temperatures

resulted in decreased growth rates (Clausen and Roth 1975, Jokiel and Coles 1977, Marshall and Clode 2004, Al-Horani 2005).

The dramatic decrease in growth rate at the extreme temperatures suggests corals were very stressed by exposure to these temperatures after >5 years of being maintained at control temperatures (26^{o}C). Several studies have suggested that the thermal history of a coral may significantly influence the ability of the coral to adapt to temperature fluctuations (Hughes et al. 2003, D'Croz and Mate 2004). For instance, a coral from an upwelling area may be able to handle colder temperatures, while corals from a shallow reef flat may handle higher temperatures than a conspecific from a deeper reef. All four genotypes of *Pocillopora* used in this experiment had similar thermal histories before collection, as they all came from the same reef in Panama. Furthermore, these corals were held and propagated at the University of Miami Coral Resource Facility for five years prior to experimentation under a constant temperature of 25±1^{o}C. Therefore, in this study, we are able to reduce the variable of thermal history and the effect it may have on the coral's thermotolerance, and show that corals with the same environmental history responded differently to temperature stress.

Although not statistically significant, genotype 3 appeared to have the highest growth at both 20^{o}C and 32^{o}C. In fact, there were almost no discernible differences in growth at 20, 30 or 32^{o}C for genotype 3 (Figure 4), and no significant differences across all four temperatures. This suggests that temperature had little to no effect on growth in genotype 3, resulting in a wide range of thermal tolerance in which optimal growth

occurs. Genotypes 2 and 4, and to a lesser degree genotype 1, had very significant declines in growth at the experimental temperatures, but showed the highest growth at 26^{o}C. This suggests a strong sensitivity to temperature, and a very narrow thermal window for optimal growth. These data bolster the idea of heat-tolerant coral genets, as proposed in the literature (Jokiel and Coles 1990, Edmunds 1994, Brown 1997, Hughes et al. 2003, Weiss 2010).

The control treatment showed no mortality during the experiment. No mortality was observed in the 20 and 30^{o}C treatments until day 80, where the partial mortality observed was most likely the result of a failure in the temperature regulation system on day 72 (see Figure 2). It should be noted however, that although all three treatments increased in temperature to >30^{o}C, the control still did not record any mortality. It may be that, unlike the experimental corals, the control corals were not under prolonged stress and were therefore able to temporarily handle the stress of the day 72 temperature increase. The corals in the 20 and 30^{o}C treatments, however, had been stressed for 62 days, and the temperature anomaly may have exhausted the compensatory mechanisms of these already-stressed corals.

Patterns of mortality at 30^{o}C were the opposite of those observed in the growth data*, i.e.,* genotypes which grew quickly in the control temperatures tended to die more readily in the experimental temperatures. Genotypes 1 and 3 were the first to show mortality at 32^{o}C, but genotype 3 was the faster growing genotype at that temperature. Similarly at 20^{o}C, genotype 3 was the only genotype to show mortality on day 80 (85%

cumulative mortality), although this genotype again had the highest non-significant growth. At 30^{o}C, genotypes 2 and 4 had the highest mortality on day 80, with genotype 3 exhibiting little mortality. This trend is opposite that observed at 32^{o}C, and may be either the direct result of the day 72 temperature anomaly, or a host genotypic influence. The idea that a sharp increase in temperature such as that observed on day 72 can result in a different pattern of mortality may indicate there is a difference in the ability of these coral genotypes to react to a rapid, acute stress vs. a slow, chronic temperature stress.

Temperature	Genotype	Day 52 % Total Mortality	Day 66 % Total Mortality	Day 80 % Total Mortality	Day 80 % Partial Mortality
20°C	1	0	0	0	0
20°C	2	0	0	0	0
20°C	3	0	0	7	78
20°C	4	0	0	0	0
26°C	1	0	0	0	0
26°C	2	0	0	0	0
26°C	3	0	0	0	0
26°C	4	0	0	0	0
30°C	1	0	0	44	22
30°C	2	0	0	67	33
30°C	3	0	0	7	11
30°C	4	0	0	100	0
32°C	1	89	100	-	-
32°C	2	0	100	-	-
32°C	3	100	100	-	-
32°C	4	0	100	-	-

Table 2: Mean per cent mortality (total and partial) for each genotype at each temperature.

Chapter 3: Effect of high and low temperature extremes on the photosynthesis, density and community composition of algal symbionts (*Symbiodinium* spp.) in *Pocillopora damicornis*

Colonies of the Indo-Pacific reef coral *Pocillopora damicornis* that had been maintained at 26^{o}C for 5 years, and which hosted *Symbiodinium* C1b-c and/or D1, were exposed to four temperature treatments (20, 26, 30 and 32^{o}C) for eight weeks. Photochemical efficiency (Fv/Fm) was monitored every two weeks using chlorophyll fluorometry, and symbiont density and community composition was compared at the start and end of the experiment. At 30^{o}C and 32^{o}C, corals dominated by C1b-c showed significant declines in Fv/Fm, while corals with D1 showed no such decline. At 20^{o}C, these patterns were reversed, with corals containing D1 symbionts showing greater declines in Fv/Fm than C1b-c corals. Corals at 30^{o}C that were initially dominated by C1b-c shifted their community composition in favor of D1, while corals at 20^{o}C maintained the community composition but with large changes in density. Corals at 32^{o}C had all died by day 66. These results revealed clear interactions between *Symbiodinium* community structure, symbiont density and photochemical efficiency under different temperature treatments, with D1 being strongly favored under high thermal stress. However, these data also show under low temperatures, C1b-c was the favored symbiont type, with both higher density and higher photochemical efficiency.

3.1 Background

Reef-building corals are meta-organisms comprising the coral host, its algal symbionts, and its associated bacterial and viral communities. These different partners all contribute to the survival and physiological properties of the coral "holobiont", and in some cases, can be critical in understanding coral response to environmental stress. For example, corals exposed to temperature extremes typically expel their algal symbionts (*Symbiodinium* spp.) during episodes of coral "bleaching", and these symbionts are key in understanding this response, since they are the primary target of thermal stress. Algal symbionts (commonly referred to as "zooxanthellae") typically translocate 90% of their photosynthates to their coral hosts, which use them as a principal source of carbon (Sumich 1996). Therefore, the loss of zooxanthellae in corals is considered one of the biggest threats to coral reefs worldwide (Marshall and Schuttenberg 2006, Hoegh-Guldberg et al. 2007).

The genus *Symbiodinium* is highly diverse, and comprises (at least) 9 clades (A-I). The clades most commonly found in scleractinian corals are A through D, with significant diversity found among subclades ("types") within these four clades (Baker 2003, Coffroth and Santos 2005, Jones et al. 2008). In general, clade D symbionts are considered to be more thermal tolerant, while clade C symbionts are adapted to more moderate temperatures (Rowan 2004, Baker et al. 2004, Glynn et al. 2001). Many coral species are thought to be flexible in the types of *Symbiodinium* they harbor (Baker et al. 2004), with juvenile corals showing little specificity in type when first infected (Little et al. 2004). Many corals have been observed containing multiple types of symbionts

within a single colony at varying proportions (Rowan et al. 1997, Ulstrup and van Oppen 2003). This allows for the shuffling of symbiont proportions based on environmental conditions. It has been suggested that corals that are dominated by thermally sensitive symbionts, such as those found in clade C, may be able to expel these symbionts during bleaching, allowing a low-density background symbiont, such as a thermally-tolerant clade D, to increase in density (Baker 2001, Baker 2004, Rowan 2004, Little et al. 2004). That corals might undergo shuffling of symbionts in response to environmental conditions was first framed as the Adaptive Bleaching Hypothesis by Buddemeier and Fautin (1993). There have been several studies in the field in which corals were observed to change symbionts after a bleaching event (Baker 2003, Baker et al. 2004, Berkelmans and van Oppen 2006, Jones et al. 2008). Berkelmans and van Oppen (2006) found that corals which shuffled symbiont types from C to D were able to tolerate temperatures approximately 1-1.5^{o}C higher. Corals may also be able to switch dominant symbiont types by acquiring novel strains from the environment after bleaching (Baker 2003), although direct evidence is lacking (Berkelmans and van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2010). However, the degree to which corals undergo shuffling vs. switching of symbionts is still an area of intense research (Jones et al. 2008, LaJeunesse et al. 2010). Baker et al. (2004) found that reefs which experienced very high temperatures had a higher proportion of clade D vs. clade C symbionts than similar reefs in areas with more moderate high temperatures, suggesting a shift within corals from C dominance towards D dominance after repeated thermal stress. The authors suggest this shift is in fact an adaptive mechanism for corals

to increase their resistance to bleaching events. In contrast, other recent research has suggested that the symbiont-coral relationship is in fact stable long-term. LaJeunesse et al. (2010) states that there is little evidence that corals readily take up novel strains of symbionts in the field. While they acknowledge corals under severe stress may obtain unusual symbiont communities, they argue that these are short-term associations and the original symbionts return to dominance after recovery. The shifts towards D dominance observed in many reefs, they argue, is the result of selective mortality of corals with a stable C dominant relationship (low thermal tolerant symbiont), which thereby increases the proportion of corals surviving that already harbored clade D (LaJeunesse et al. 2010). Jones et al. (2008) found in fact that 37% of the observed shift to D dominance at their study site was the result of differential mortality of clade C hosting corals. However, 42% of the remaining corals had shifted from C to D, allowing the authors to propose the role of shuffling symbionts may be more important than differential mortality.

There have been numerous studies which show that there is differential coral bleaching in the field based on the type of symbiont harbored by corals. In particular, corals of various species harboring clade C symbionts were shown to bleach, or otherwise show thermal stress, in response to high temperatures in the eastern Pacific (Baker et al. 2004, LaJeunesse et al. 2010), Guam (Rowan 2004), the Great Barrier Reef (Jones et al. 2008) and many other locations. These studies also showed that corals containing clade D symbionts experienced less bleaching and photosystem stress. Algal symbionts are therefore key in understanding differences in thermotolerance, although

the degree of importance is still debated (Berkelmans and van Oppen 2006, LaJeunesse et al. 2010).

3.1.1 Measurement of coral photosynthesis using chlorophyll fluorescence

Pulse Amplitude Modulated (PAM) fluorometry is a powerful and commonly used method to determine the photosynthetic and photochemical properties of plants (Maxwell and Johnson 2000), as well as those of *Symbiodinum* in corals. Briefly, when light is absorbed by chlorophyll, energy can follow one of three pathways. It can be used to drive photosynthesis, it can be dissipated as heat energy, or it can be re-emitted as fluorescence. An increase in any of these processes will lead to a corresponding decrease in one or both of the other two processes. Therefore, by measuring the proportion of light entering the system that is emitted as fluorescence, it is possible to estimate photochemical quenching (energy going to photosynthesis) and non-photochemical quenching (energy going to heat dissipation). By measuring the minimal fluorescence of the system, F_0, and the maximum fluorescence , F_m, after a saturating light pulse, the equation $(F_m-F_0)/F_m$ can be used to measure F_v/F_m, or the maximum quantum yield of PSII, where $F_v = F_m-F_0$. In dark-adapted samples, F_v/F_m values are used as an indicator of photochemical efficiency, with low values indicating exposure to stress (Maxwell and Johnson, 2000). For light-adapted samples, the effective quantum yield, which is lower than the dark adapted maximum quantum yield, is given by the equation $\Phi_{PSII} = (F'_m-F)/F'_m$, where F'_m is the maximum fluorescence and F is the minimal fluorescence when light-adapted (Ralph and Gademann, 2005).

3.2 Materials and methods

3.2.1 Photochemical measurements

Chlorophyll fluorescence was measured using an IMAGING-PAM (I-PAM) *M-Series* MAXI Version (Walz, Effeltrich, Germany) with ImagingWin software. I-PAM data were recorded on 10 June 2010 (pre-ramping) and 27 June 2010 (post ramping), and then every two weeks until 19 August 2010 (day 80). For each I-PAM measurement, corals were moved from the outdoor experimental tanks to a dark indoor room. Light-adapted measurements were taken immediately, after which corals were held indoors in darkness for 15 minutes before the dark-adapted measurements were taken.

Values of Fv/Fm were compared statistically using ANOVA and Proc-GLM run in SAS (1990). Data were grouped by temperature treatment and then grouped by genotype (reported in Chapter 4), and multiple-pairwise comparisons were Bonferonni corrected, with a critical p-value of $p>0.016$ (Sokal and Rolf 1987) used to test for significance.

3.2.2 Symbiont identity, density and community composition

Each temperature treatment was randomly assigned 6 replicates of each genotype to act as sacrificial colonies for DNA sampling. These sacrificial colonies were not used in growth or IPAM data collection. Three colonies of each genotype at each temperature were sampled on day 10 and three on day 72. Small branch tips (~1cm)

were clipped from each colony, and tissue samples were preserved in 1% SDS in DNAB. DNA extraction used a modified organic extraction protocol.

Quantitative PCR (qPCR) was used to measure the density and community structure of *Symbiodinium* in representative corals exposed to experimental treatments. PCR procedures were undertaken by Ross Cunning (University of Miami). An actin-based qPCR procedure optimized for *P. damicornis* was used to identify and quantify algal symbionts to the level of clade (Cunning and Baker 2012), and Denaturing Gradient Gel Electrophoresis (DGGE) of the internal transcribed spacer-2 (ITS-2) region of *Symbiodinium* ribosomal DNA (rDNA) was used to identify symbionts to the level of symbiont "type" (LaJeunesse 2001). *Symbiodinium* ITS-2 rDNA was amplified using the primers 'ITSintfor2' and 'ITS2clamp', and amplification products were separated by DGGE (35-75% gradient) using a CBS scientific system. Dominant bands on the gel were excised, re-amplified, and sequenced using BigDye Terminator v3.1 cycle sequencing kit and an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA, USA).

Actin sequences were obtained from samples of *P. damicornis* and its particular clade C and D symbionts. Actin genes were amplified using universal actin forward primer 2 and universal actin reverse primer. PCR was carried out in 25 µL reactions consisting of 0.5 µM forward and reverse primers, 0.2 µM each deoxynucleotide (Bioline, Boston, MA, USA), 2.0 µM $MgCl_2$, 1x Green GoTaq® Flexi Buffer, and 1 U GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA). Reaction conditions consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of

denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 74°C for 1 min. PCR products were cloned using the pGEM®-T Easy Vector System (Promega, Madison, WI, USA), and inserts were amplified using M13 primers and sequenced as above (Cunning and Baker 2012).

All qPCR reactions were performed using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA). Reaction volumes were 20 µL, with 10 µL Taqman® Genotyping Master Mix and 1 µL genomic DNA template. The *P. damicornis* assay included 100 nM forward primer (PdActF), 200 nM reverse primer (PdActR), and 100 nM Taqman probe (PdActProbe). The multiplexed *Symbiodinium* clades C and D assay included 50 nM clade C forward primer (CActF), 75 nM clade C reverse primer (CActR), 100 nM clade C probe (CActProbe), 50 nM clade D forward primer (DActF), 75 nM clade D reverse primer (DActR), and 100 nM clade D probe (DActProbe). Thermal cycling conditions consisted of initial incubation at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds and annealing/extension at 60°C for 1 minute. Cycle threshold (C_T) values were calculated by the StepOnePlus™ software package using automatic baseline calculations and a set fluorescence threshold of ΔR_n=0.01 (Cunning and Baker 2012).

Positive amplifications were counted only when both technical replicates produced C_T values <40 and there was no amplification in no-template control reactions. C_T values were adjusted for differences in fluorescence intensity among the three different reporter dyes associated with the Taqman® MGB probes. These differences

were calculated from average C_T values produced by standard curves of copy number standards ranging from 10^8 to 10^2, which revealed that the *P. damicornis* assay (NED dye) and clade C assay (VIC dye) produced C_T values 4.48±0.12 and 2.68±0.14 (SE) cycles higher than the the clade D assay (FAM dye), respectively. Symbiont to host cell ratios were then calculated from adjusted C_T values by using the formula: $2^{(C_{T(host)} - C_{T(symbiont)})}$, and then dividing by the symbiont to host ploidy ratio (1/2), DNA extraction efficiency ratio (1.21), and target locus copy number ratio (3 for clade C, 1/3 for clade D) (Cunning and Baker 2012).

3.3 Results

3.3.1 Symbiont density and community structure

At the start of the experiment (Day 0), genotypes 1, 2 and 4 contained 100% C1b-c *Symbiodinium*. No clade D was detected in these genotypes, except in one replicate of genotype 2 that was initially hosting 0.75% clade D1. Genotype 3 was initially dominated (~98.5%) by D1 *Symbiodinium* (mean ~1.5% clade C). For the purpose of this chapter, all genotypes initially dominated by clade C1b-c (genotypes 1,2 and 4) will be clumped together and referred to as clade C corals, while genotype three (initially clade D1 dominated) will be referred to as clade D corals. Where they occur, symbiont differences between the three clade C genotypes will be discussed in Chapter 4.

The clade D1 corals showed a significantly higher ($p<0.001$) cellular *Symbiodinium* density of 0.022 symbionts per host cell when averaged over all

temperatures on day 0, compared to the clade C1b-c corals, which had an average of 0.007 symbionts per host cell (Figure 6). By day 72, the density of symbionts in the 20^{o}C treatment had increased by nearly 200% in the clade C corals and decreased in the clade D corals by 80% (t-test, $p<0.001$) (Figures 7 and 8). There were no data for the 32^{o}C treatment on day 72 since all genotypes had died in this treatment by day 66. In the 26^{o}C treatment, both clades experienced very small increases in symbiont density (Figure 8). There were differences in symbiont densities between the three clade C corals at 26^{o}C, and these differences will be discussed further in Chapter 4. The corals in the 30^{o}C treatment all experienced reductions in symbiont densities, but this effect was much stronger (reduction of 98.7%) for the initially clade C corals when compared to initially clade D corals (60%) (t-test, $p<0.001$) (Figure 8).

Over the course of the experiment, there was a significant shift in symbiont communities in the 30^{o}C treatments, which became increasingly dominated by clade D *Symbiodinium* in all corals. The corals which were initially clade C showed a shift to >98% dominance of type D symbionts. However, these corals were all severely bleached. The density of clade D symbionts in the clade C corals on day 72 was 1.25% of the total initial density measured on day 0. In the 20^{o}C and 26^{o}C temperature treatments there was no shift in symbiont communities. The initially clade D corals remained D dominated on day 72, while the clade C corals continued to be C dominated, with negligible (<0.1%) amounts of clade D observed.

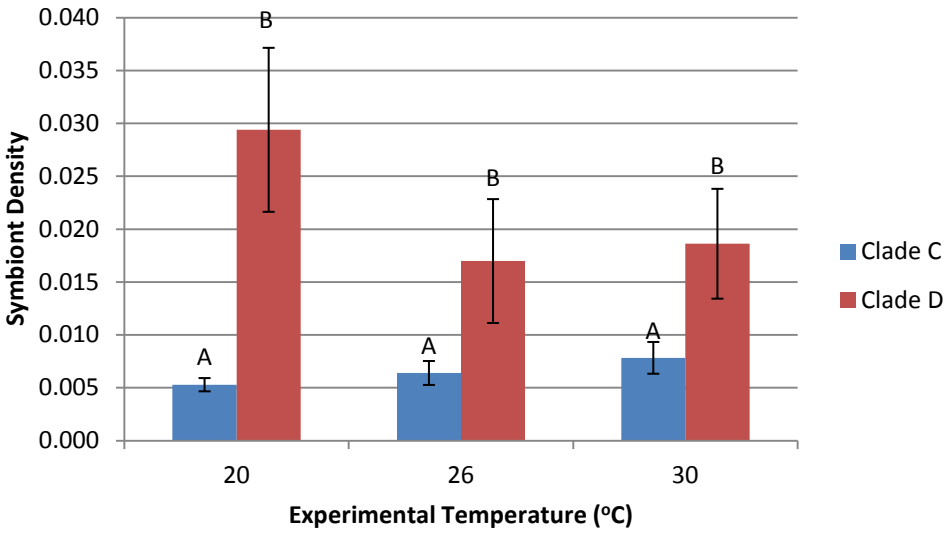

Figure 6: Initial symbiont densities (expressed as symbiont to host cell ratio) in *Pocillopora damicornis* on experimental day 0. Clade D corals had significantly higher symbiont densities in all temperature treatments (t-test, P<0.05).

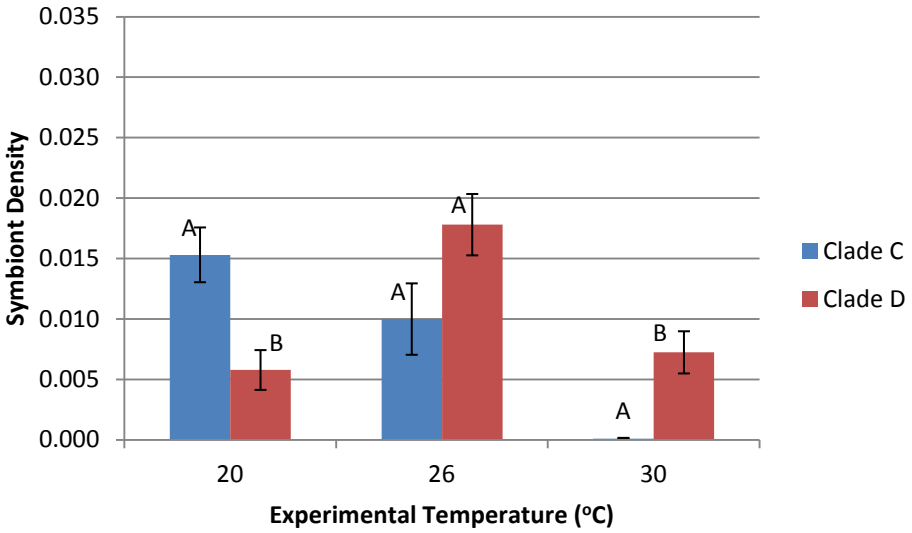

Figure 7: Final symbiont densities (expressed as symbiont to host cell ratio) in *Pocillopora damicornis* on experimental day 72. Significant differences in density at each temperature indicated by letter (t-test, P<0.05).

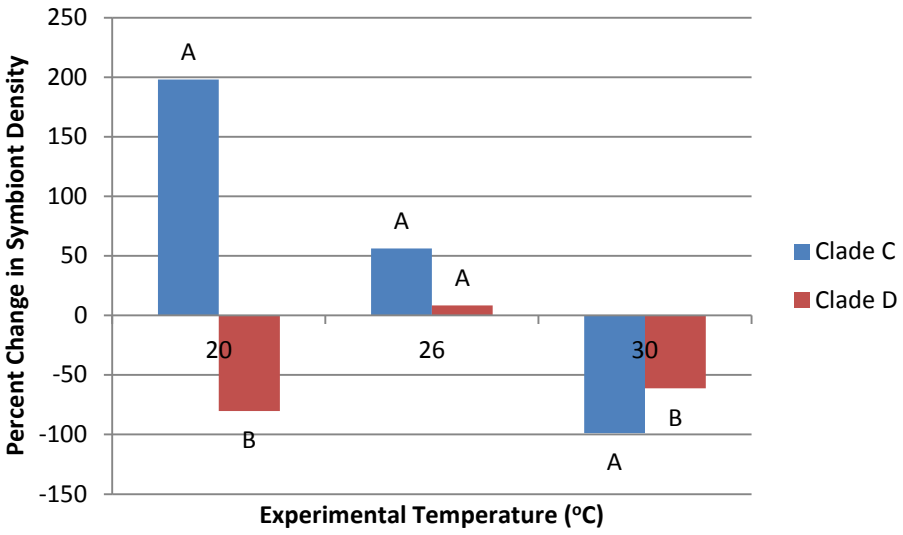

Figure 8: Comparison of percent change in symbiont density between day 0 and day 72. Letters indicate significant differences (T-test, $p<0.05$) in symbiont density between genotypes at each temperature treatment.

3.3.2 Temperature effects on photochemical efficiency

At 20°C, the clade D corals experienced a significant reduction in Fv/Fm by the end of the ramping period (day 27). Fv/Fm values then remained stable for all genotypes until day 66, when all corals showed similar reductions in Fv/Fm (Figure 9).

At control temperatures (26°C), the ramping period resulted in a drop in photochemical efficiency. The drop seen during ramping is most likely a result of continued photoacclimation to the higher light levels of the shadehouse. After the ramping period ended (day 27), there was an increasing trend in Fv/Fm over the remainder of the experiment, with Fv/Fm values differing marginally among the all corals. However, the differences that were observed among corals were not consistent

across time points, and by the end of the experiment (day 80) corals hosting clade C and D symbionts were similar (Figure 9).

At 30^{o}C, the clade C corals showed declines in Fv/Fm over the course of the experiment, until day 52 when two of the three clade C-containing genotypes began to recover. By day 80, all surviving clade C corals had fully recovered (see Chapter 2 for mortality data). The initially clade D dominated corals remained unaffected throughout the experiment (Figure 9).

At 32^{o}C, the clade C corals all showed reductions in Fv/Fm, while the clade D corals remained unaffected. However, by day 52, all corals in one of the Clade C genotypes and all the clade D genotype had died. The remaining clade C corals showed signs of recovery by day 52, but by day 66 they had also all died (Figure 9).

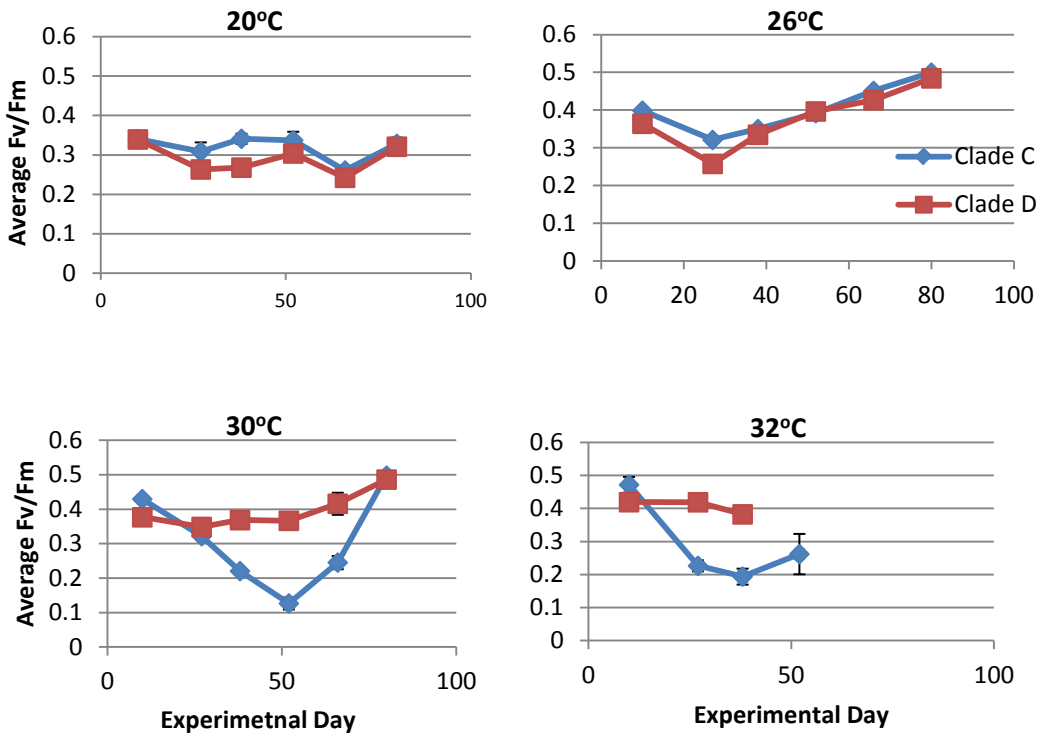

Figure 9: Average photochemical efficiency (Fv/Fm) for all clade C corals (genotypes 1,2 and 4) and all clade D corals (genotype 3) for all four temperatures over the course of the 80-day experiment. Clade C corals are represented by the blue diamonds and clade D corals are represented by the red squares.

3.4 Discussion

This experiment exposed 4 different coral genotypes containing different algal symbiont communities to different temperature treatments, and revealed clear interactions between *Symbiodinium* community structure, symbiont density and photochemical efficiency under these treatments. Three different coral genotypes contained similar algal symbiont communities, collectively referred to as clade C corals,

and these corals provided an opportunity to assess the effect of coral genotype in determining response.

3.4.1 Reduction in symbiont density at high temperature results in shifts to favor clade D

At high temperatures (30^{o}C), corals initially clade D dominated showed higher Fv/Fm than those dominated by clade C symbionts. However, by day 72, these clade C corals had lost most of their C symbionts, and the remaining symbionts were dominated by clade D. This may explain why low Fv/Fm values for the clade C corals rebounded late in the experiment (day 66 and 80) and eventually reached the same values as those corals that started with clade D symbionts. The recovery of Fv/Fm values around day 66 probably reflects the transition from clade C- to D-dominance. Similarly, in the 32^{o}C treatment, clade D corals had the highest Fv/Fm values by day 27, as is expected with a clade D dominated coral. Two of the three clade C corals begin to increase slightly by day 52 when exposed to 32^{o}C temperatures, possibly due to a dominant symbiont switch to D as seen in the 30^{o}C treatment. Complete mortality across all genotypes in this temperature treatment before symbionts could be typed out prevents more robust conclusions from being drawn; however, it is surprising that the initially clade D dominant corals did not survive longer than hosting clade C symbionts in the 32^{o}C treatment. The reason behind this is unknown, although it may be the result of the host genotype being unable to cope with such high temperatures.

3.4.2. Clade D symbionts perform well at high temperatures, but poorly at low temperatures

Corals which started with clade D symbionts remained dominated by clade D symbionts at all temperatures throughout the course of the experiment. Figure 9 clearly shows that clade D symbionts in the two high temperature treatments, 30^{o}C and 32^{o}C, did not differ significantly from the control treatment with respect to photochemical efficiency, indicating that these symbionts perform equally well under high thermal stress. The fact that all three C-dominated genotypes had switched to D-dominance in the 30^{o}C treatment also shows that clade D symbionts are preferred under hot conditions. However, when the temperature was lowered to 20^{o}C, clade D corals showed a significant reduction in Fv/Fm compared to the control (Figure 9), as well as a significant reduction in symbionts density over time (Figure 8). These data support the idea that clade D symbionts are hardy and thrive at higher temperatures, but are inefficient at lower temperatures.

Chapter 4: Interactions between host genotype and algal symbiont community determine effect of temperature stress on coral growth, bleaching and survivorship

Coral reef ecosystems worldwide are in decline as a result of climate change and other anthropogenic impacts. Prolonged exposure to extreme high (>32^{o}C) and low (<20^{o}C) temperatures typically leads to coral bleaching and/or mortality. Different coral colonies often show variation in their response, but whether this is a result of genetic differences in the coral host or its algal symbiont communities is not clear. To distinguish these influences, the effect of different temperatures (20, 26, 30 and 32^{o}C) on four different genotypes of *Pocillopora damicornis* (identified using microsatellites) containing different *Symbiodinium* communities (identified using quantitative PCR), was investigated. Corals grew most rapidly at control temperatures (26^{o}C, $p<0.001$), but varied across genotypes. There was 100% mortality by week 6 in the highest temperature (32^{o}C), and partial mortality of some genotypes in the 20^{o}C and 30^{o}C treatments by week 8. Quantitative PCR analysis of the algal symbionts (*Symbiodinium* spp.) in these corals revealed genotypes 1, 2 and 4 were initially dominated by clade C, while genotype 3 was dominated by clade D. However, when exposed to 30^{o}C, all genotypes were dominated by clade D by day 72. Photochemical efficiency of symbionts also varied across temperatures and coral host genotype. Together, these data suggest that coral genotypes vary in their thermal tolerance and growth rates, and that this variation is further influenced by the algal symbiont community. This information may help restoration efforts designed to increase the resilience of coral reefs to climate

change by identifying coral genotypes best suited to the prevailing thermal environment.

4.1 Background

Nine replicates of four genotypes of *Pocillopora damicornis* were exposed to one of four temperature regimes, ranging from 20^{o}C to 32^{o}C, for eight weeks. Effects on the coral host were measured by growth of the coral nubbins and by mortality (see Chapter 2). Effects on the symbiotic algae (*Symbiodinium* spp.) were analyzed using a Pulse Amplitude Modulated Fluorometer to measure photochemical efficiency (Fv/Fm) in the symbionts, and by measuring the density and community structure of the algal symbionts using qPCR (see Chapter 3). This chapter synthesizes the results of Chapters 2 and 3, and discusses the interacting role of coral host genotype and symbiont community dynamics, and the effect this interaction has on the holobiont's thermal tolerance.

4.2 Methods

Refer to Chapter 2 for methods on growth and mortality measurements and Chapter 3 for methods on photochemical measurements.

4.3 Results

4.3.1 Growth Results

See Chapter 2 for growth results

4.3.2 Symbiont efficiency and community structure results

See Chapter 3 for symbiont data and results

4.3.3 Symbiont-coral genotype interaction results

Genotype 1 (*Symbiodinium* C1b-c) showed a strong reduction in Fv/Fm when exposed to cold temperatures, with the 20^{o}C treatment resulting in significantly lower Fv/Fm values than the control. Fv/Fm values at 20^{o}C were similar to those measured at both high temperature treatments (Figure 10). Symbionts in genotypes 2 and 4, also C1b-c corals, behaved differently at cold temperatures, showing relatively little sensitivity to the low temperature treatment (Figure 11). The 20^{o}C treatment resulted in Fv/Fm values in these two genotypes that were not different from the control until day 66, at which point they were depressed slightly below those of the control. Genotype 1 however experienced a significant reduction in Fv/Fm until day 66 (Figure 11). There were no statistical differences between genotypes 2 and 4 at each time period in the 20^{o}C treatment, but genotype 1 data did not match with the other clade C genotypes until day 66. The three clade C-dominated genotypes all behaved similarly at 26^{o}C, 30^{o}C and 32^{o}C, with both high temperature treatments resulting in significant reductions in Fv/Fm (Figure 11).

Genotype 3, the D1-dominated coral genotype, exhibited a different trend over time than the other genotypes. For the first 28 days, the highest average Fv/Fm values were found in the 30 and 32^{o}C treatments. From day 38 on, the 30^{o}C treatment Fv/Fm values did not differ from those of the control. The low temperature treatment remained significantly depressed throughout the experiment (Figure 11).

While all three clade C coral genotypes had similar symbiont densities within temperature treatments on day 0 (Figure 12), the final densities on day 80 showed differences between the three genotypes (Figure 13). The percent change in symbiont density was different between the clade C dominated corals at 20^{o}C (Figure 14), although all experienced an increase. Genotype 4 had the statistically largest increase in symbionts (352%), followed by genotypes 1 (146%) and 2 (91%). Genotype 3, the clade D genotype, experienced a 61% decrease in symbionts at 20^{o}C. In the 26^{o}C treatment, there was an increase in symbiont density in genotypes 2(91%) and 4 (28%) (Figure 14). At 30ºC, all genotypes experienced a similar reduction in symbiont density. However, there was a switch to D dominance in genotypes 1, 2 and 4, as mentioned in Chapter 3, with genotype 1 becoming 99.5% D, genotype 2 becoming 89.1% D and genotype 4 becoming 100% clade D.

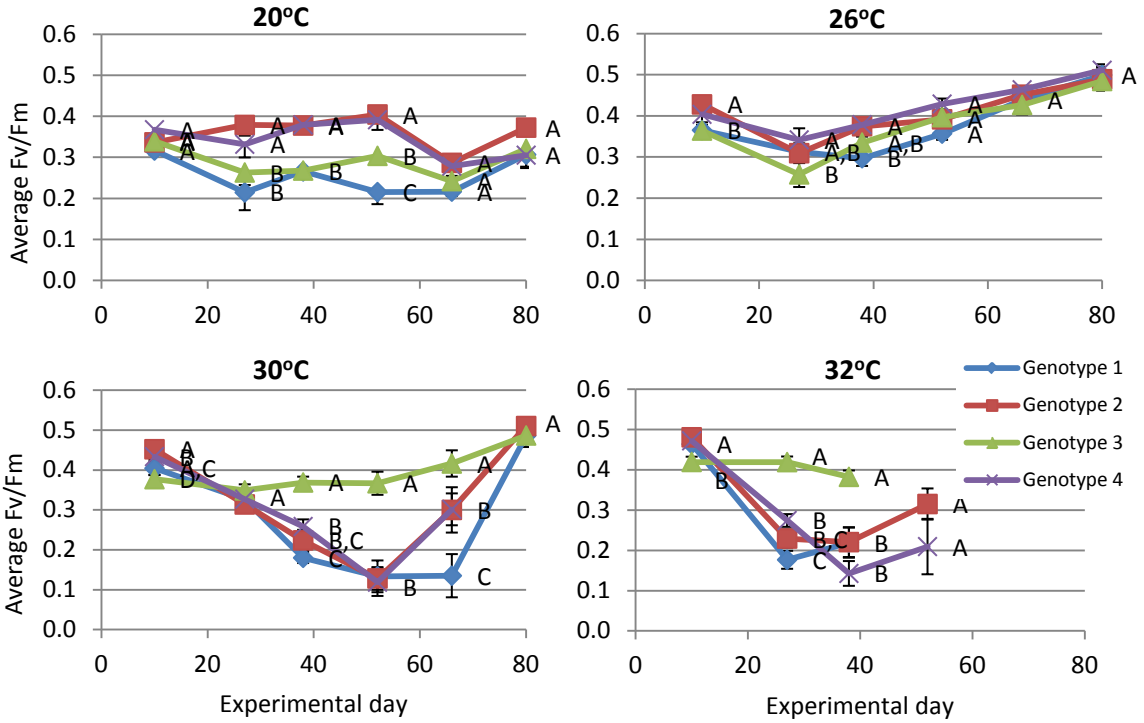

Figure 10: Effect of temperature on photochemical efficiency (Fv/Fm) of *Symbiodinium* in different coral genotypes during an 80-day experiment. Statistically significant differences (ANOVA, $p<0.0016$) are denoted by letter, with each temperature treatment independent of the others.

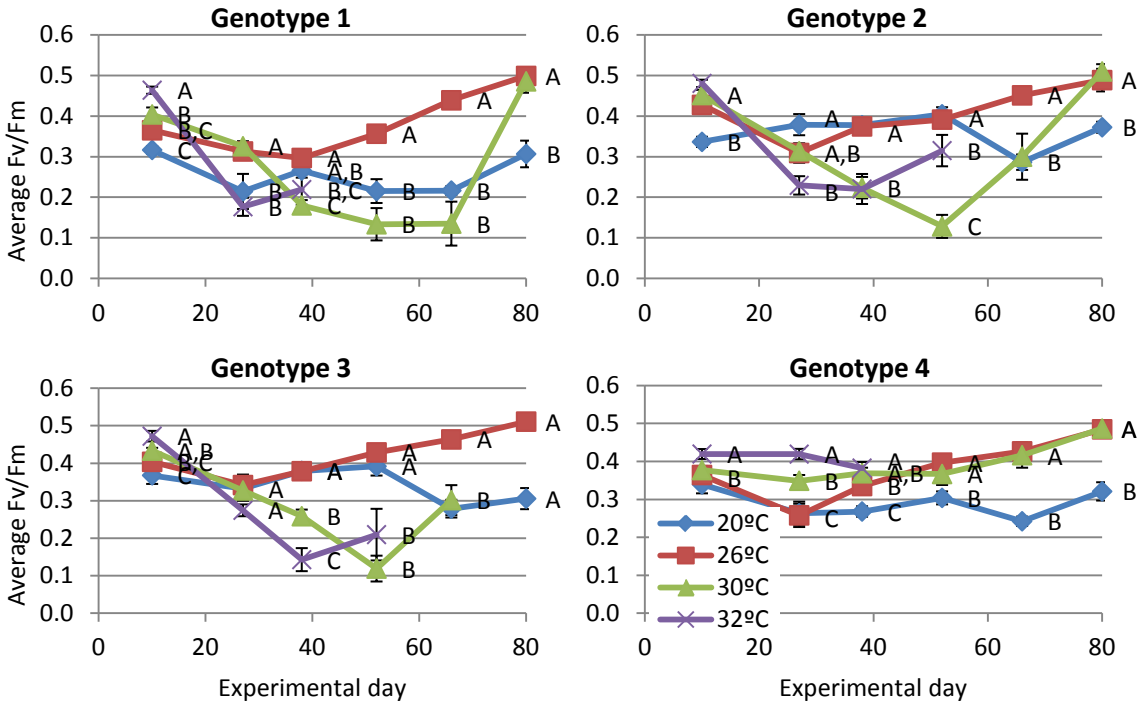

Figure 11: Effect of temperature on photochemical efficiency (Fv/Fm, plotted on the vertical axis) for all temperature treatments over the course of the 80-day experiment. Statistically significant differences (ANOVA, $p<0.0016$) are denoted by letter, with each temperature treatment independent of the others.

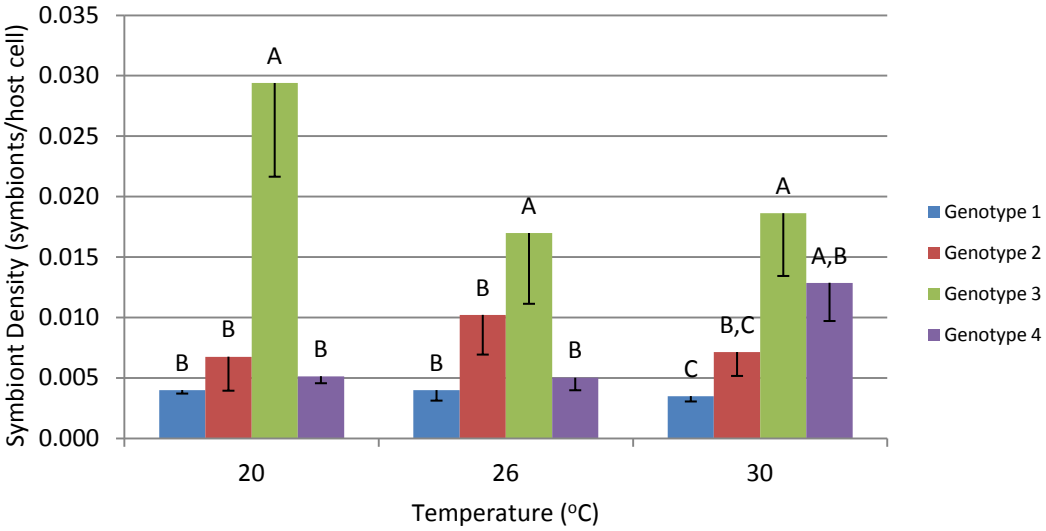

Figure 12: Initial symbiont densities (expressed as symbiont to host cell ratio) in *Pocillopora damicornis* on experimental day 0. Statistically significant differences (ANOVA and Holm-Sidak method $p<0.05$) are denoted by letter, with each temperature treatment independent of the others.

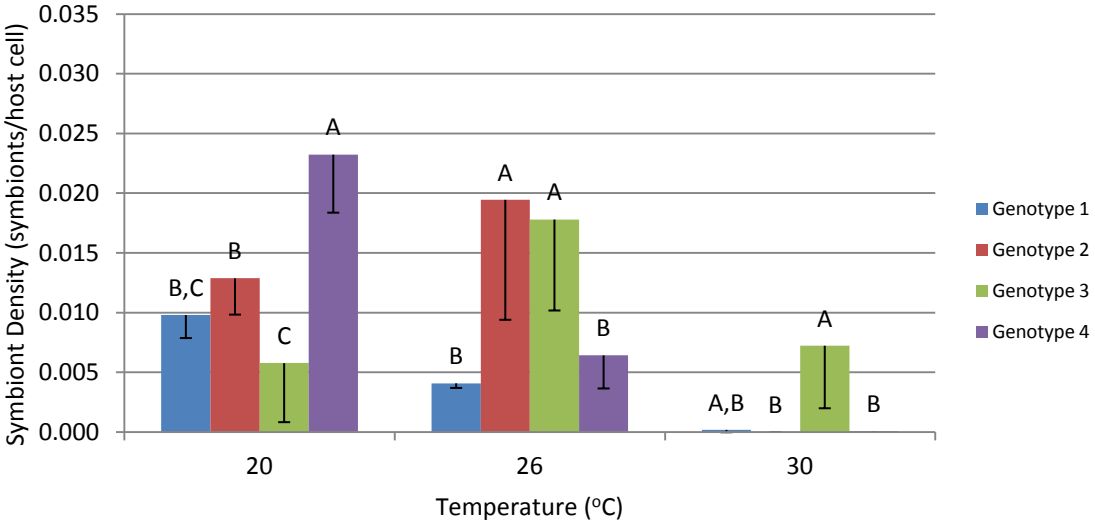

Figure 13: Final symbiont densities (symbionts/host cell) on experimental day 72. Statistically significant differences (ANOVA and Holm-Sidak method $p<0.05$) are denoted by letter, with each temperature treatment independent of the others.

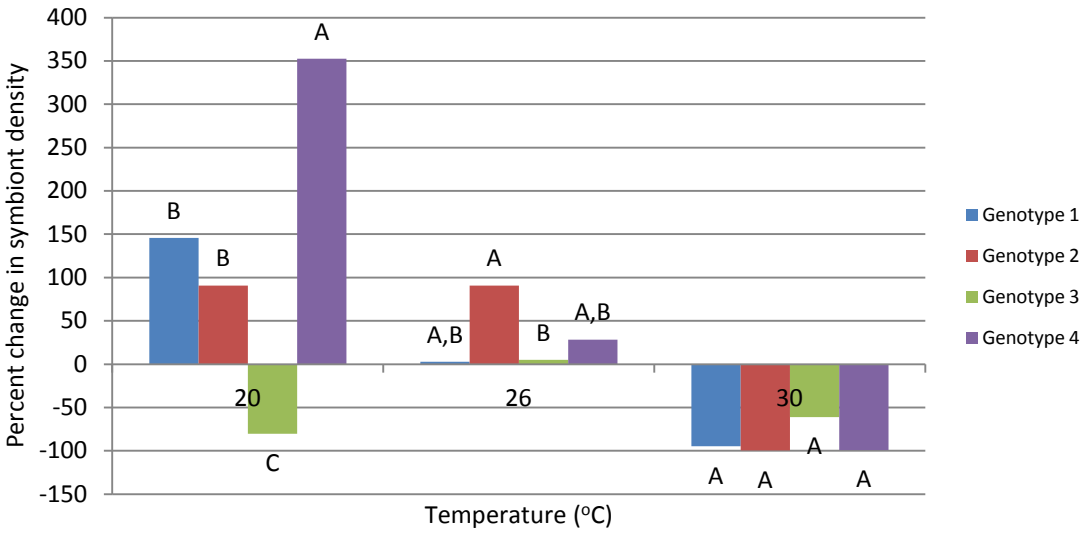

Figure 14: Comparison of percent change in symbiont density between day 0 and day 72. Letters indicate significant differences (ANOVA and Holm-Sidak method $p<0.05$) in symbiont density between genotypes at each temperature treatment.

4.4 Conclusions and discussion

Because the coral holobiont comprises of a number of symbioses, experiments focused solely on the host, or on just the symbionts, ignores a major source of possible experimental variability, since the host and symbionts may each respond differently or synergistically to an experimental manipulation. An impact to either component of the symbiosis may result in effects on the holobiont. The current study investigates the effect of thermotolerance in conspecific coral genotypes by measuring the effects on both the coral host and the *Symbiodinium*. All four genotypes of *Pocillopora damicornis* used in this experiment had been collected from the same reef in Panama and common gardened under similar environmental conditions (26°C in laboratory conditions for five

years before the start of the experiment). Therefore, thermal history is unlikely to have been a factor in this experiment.

4.4.1 Genotypic effects on coral growth

Growth data showed that at the control temperature of 26^{o}C, there was a strong genotype-based influence on growth within the same species of coral. Genotypes 2 and 4 showed equally high growth, while genotype 1 grew significantly less than the other two clade C1b-c genotypes ($p<0.016$). Genotype 3, hosting D1 *Symbiodinium*, grew the least at the control temperature ($p<0.016$). It has been suggested in the literature that clade D symbionts, while more thermally tolerant, have the tradeoff of being less efficient at producing excess nutrients for the coral host, and therefore less of a benefit at moderate temperatures (Little et al. 2004, Cantin et al. 2009). The data in this experiment back this assertion. However, genotype 1 had the same symbionts, C1b-c, as both genotypes 2 and 4. The observed difference in growth between these three genotypes suggests that the host genotype played a significant role. This study indicates that the coral host genotype does influence growth rate, with host genotype resulting in as much as a six-fold difference in growth rate between colonies with the same algal symbionts.

4.4.2 Genotypic effects on coral mortality

The first genotypes to show mortality in the 32^{o}C treatment were genotypes 1 (89%) and 3 (100%). This is somewhat counter-intuitive since genotype 3 hosted clade D1 symbionts, had the highest Fv/Fm values at this temperature, and had the highest

survival in the 30°C treatment. Also, genotype 1, which hosted C1b-c symbionts, would not be expected to behave more similarly to genotype 3 than the other C1b-c corals at 32ºC. Unfortunately, there is no symbiont density or community data for this temperature treatment, so it is difficult to speculate on why this pattern was observed. Regardless of the mechanism, this situation highlights the importance of looking at the coral host and symbionts simultaneously, and not just one or the other. At 32°C, genotype 1 began to experience mortality on day 52, earlier than both genotypes 2 and 4. Mortality in the 30°C treatment revealed different patterns than those observed in the 32°C treatment. At 30°C, genotype 4 showed the highest total mortality (100%), followed by genotype 2 (67%), genotype 1 (44%) and genotype 3 (7%). When partial mortality is taken into account, genotype 2 had 100% cumulative mortality, genotype 1 had 66%, and genotype 3 only experienced 18% cumulative mortality (Table 2). Genotypes 2 and 4, which had the latest onset of mortality in the 32°C treatments, showed the most (and presumably earliest) mortality in the 30°C treatment, while genotype 1 had better survival than genotypes 2 and 4 (opposite of the trend observed in the 32°C treatment). Although it is impossible to know for sure, the most likely source of mortality in the 30°C treatment was the temperature anomaly on day 72, since no mortality was observed before this event. The fact that genotype 4 showed high mortality in 30°C but survived longer in 32°C, and the opposite trend occurred in genotype 1, hints that there may be differences in the coral host's response to chronic high temperatures versus acute temperature anomalies, or in the rate at which temperatures change. The day 72 temperature increase was very sharp, with

temperatures increasing to 30°C, 32°C and 33°C for the three treatments (20°C, 26°C and 30°C respectively) in a period of fewer than 6 hours. The data suggest genotype 4 may be able to better acclimatize with the slow increase to 32°C than the quick jump experienced on day 72. Further study is needed to investigate the effect that the rate of change in temperatures has on coral mortality.

The only genotype to show mortality in the 20ºC treatment was genotype 3. On day 80, there was 7% total mortality and 78% partial mortality, for a cumulative mortality of 85%, despite the fact this genotype showed the highest, although non-significant, growth at 20°C. However, looking at the symbiont photochemical data (Figure 10), the clade D1 zooxanthellae were doing better at this temperature than the clade C1b-c associated genotype 1. One possible explanation, as mentioned earlier, would be the acute stress of the day 72 temperature anomaly. However, the fact that this coral was dominated by clade D symbionts, which only showed a small stress response photosynthetically when compared to the control (Figure 11), and still responded poorly suggests the possibility that the coral host genotype was incapable of handling either the low temperatures or the acute stress of the temperature anomaly.

It has been suggested in the literature that there is a tradeoff between growth and stress tolerance in corals, in particular relating to the type of symbiont hosted in the coral (Little et al. 2004, Cantin et al. 2009). These data support this hypothesis, with the more thermotolerant genotype 3 exhibiting the lowest growth at control temperature compared to genotypes 1, 2 and 4, but relatively higher growth at thermal extremes.

However, as discussed in Cantin et al. (2009), these tradeoffs are usually considered to be a result of an association with different symbiont types and the affects of stress on the photosynthetic performance of these symbionts. Genotype 3, which was found to be hosting clade D symbionts, exhibited the expected tradeoff of low growth but high stress tolerance (Cantin et al. 2009). However, genotypes 1, 2 and 4 all associated with clade C symbionts, but showed differences in growth rates and thermal tolerance, especially with respect to mortality. These differences cannot be attributed to symbiont differences, and therefore suggest there are also tradeoffs between different coral genotypes.

4.4.3 Host genotypic effects on algal photochemistry

The Fv/Fm data show that genotype 2 and genotype 4 behave similarly, never having values that are statistically different from one another. These two genotypes had the highest Fv/Fm values over the course of the experiment at 20^{o}C, which may seem contradictory to the fact these genotypes had the worst growth at 20^{o}C. Interestingly, the density of symbionts (clade C1b-c) actually went up in genotypes 1, 2 and 4 by the end of the experiment in this treatment. One possible explanation is that the photosynthetic products resulting from this increased efficiency (Fv/Fm), which would normally be exported to the coral, in this case were used by the zooxanthellae for reproduction, thereby increasing the density of algae but not helping the coral put energy towards its own growth.

Analyzing the Fv/Fm data with respect to each genotype, the relative degree of stress in each treatment on clade C-containing genotypes (1,2 and 4) is $32^{o}C=30^{o}C>20^{o}C>26^{o}C$ (control). The relative stress tolerance of each clade C dominated genotype was the same for $30^{o}C$ and $32^{o}C$, but at $20^{o}C$ genotype 1 was more sensitive than genotypes 2 and 4 (Figure 11). The fact that these three genotypes all had the same symbionts suggests the observed sensitivity to cold water in genotype 1 is possibly the result of stress on the coral host and not directly the symbionts.

The clade D-dominated genotype 3 behaved very differently than the other genets at all three experimental temperatures. Because genotype 3 was D1 dominant from the start, it cannot be compared to genotypes 1,2 and 4 from the standpoint of analyzing genotype-dependent differences. However, it does create a valuable comparison between clade C and clade D corals exposed to thermal stress under laboratory conditions. Corals dominated by clade D showed significant advantages at extreme temperatures, with slightly higher growth and photochemical efficiency at high temperatures (Figure 10). Cold temperatures did result in a depression of Fv/Fm values and mortality in D1 corals.

4.4.4 Variation in response between different host genotypes containing similar symbionts

Overall, exposure to high temperatures affected the C1b-c coral genotypes more negatively than exposure to low temperatures with respect to the symbionts (both in photochemical efficiency and density the symbionts). However, when looking at the

growth data, cold temperatures depressed growth slightly more than at 30°C. In the 30°C treatment, the response of the three genotypes (1, 2 and 4) that hosted only clade C symbionts at the start of the experiment was similar until day 66, when symbionts in genotypes 2 and 4 began to recover when compared to genotype 1. This may reflect differences in the capacity of different coral host genotypes to deal with stress as opposed to the symbionts, as the total number and type of symbionts were not different between them when measured on day 72 ($p>0.9$). Interestingly, when exposed to low temperatures (20°C), the clade C symbionts in genotype 1 behaved more similarly, with respect to Fv/Fm, to the clade D symbionts of genotype 3 rather than to those of the C-dominated genotypes 2 and 4. The symbiont densities in all three of these C-dominated genotypes increased at 20°C, while in genotype 3 the densities decreased as a result of thermal stress. The increase in symbiont density in genotypes two and four may explain the higher Fv/Fm values compared to genotype three (Figure 14), but doesn't explain why genotype 1 behaves more like genotype 3 with respect to Fv/Fm. There is most likely a host effect on genotype 1 depressing the symbiont's processes at 20°C.

4.4.5 Comparisons with the current literature

The data presented in this study support earlier reports in the literature that genotypic variation in corals may play an important role in bleaching susceptibility. Edmunds (1994), in an attempt to explain the patchy distribution of bleaching observed on reefs, used a nearest-neighbor analysis to compare the distribution of genetically similar

Montastraea faveolata ramets (as determined by connected dead skeleton) and the distribution of bleached ramets to determine that the probability that bleached ramets were in the same genet was higher than expected by chance. Edmunds also undertook a manipulative experiment using *Porites porites* that showed there was a difference in the rate of symbiont loss among different genets (different genotypes determined by a >15m spatial separation). Brown suggested in 1997 that differential bleaching to high temperatures and irradiance in Thailand depended on genetic variability in both the host and zooxanthellae, with a clone-specific bleaching response based on the spatial proximity of bleached corals. Barshis et al. (2010) conducted a reciprocal tansplant experiment between fore- and back reefs with *Porites lobata*, and determined that genetic variability in the coral host was responsible for differences in protein production in response to temperature and light change. In their study, there was no genetic difference in symbionts between the two sites, suggesting all differences were the result of the coral host.

Berkelmans and van Oppen (2006) state that the type of symbionts associated with a coral is the dominant factor affecting that coral's ability to acclimate to elevated temperatures. Similar to the data presented by D'Croz and Mate (2004), I found that there was indeed an effect from the coral host genotype on its thermal tolerance. Building upon their results, I was able to compare different *Pocillopora* genotypes and different symbiont types. When comparing clade C1b-c corals with D1 corals, symbiont type did in fact play a pivotal role in the ability of a coral to survive high and low temperatures in the current study. However, the data presented here also strongly

suggest that the coral host genotype does have an important effect on the thermal tolerance of coral holobiont. Different coral genotypes associating with the same clade C1b-c symbiont expressed different photochemical efficiencies, symbiont densities and growth. While symbiont type is undoubtedly a very important factor, host genotype is also an important driver of thermal tolerance. Whether the measured importance is a result of the host influence over its symbionts or the hosts' direct ability to cope with temperature stress needs further investigation.

The data presented in the current study support the Adaptive Bleaching Hypothesis (Buddemeier and Fautin 1993, Baker et al. 2004, Rowan 2004, Little et al. 2004, Berkelmans and van Oppen 2006, Jones et al. 2008). When corals in this study were exposed to high temperatures, a distinct shift in symbiont types was observed. Corals which had initially hosted C1b-c symbionts were found at the end of the study to be clade D1 dominant. In fact, on day 0, only one replicate of the nine clade C corals analyzed had any detectable D1 symbionts (0.75% of the total symbionts in one replicate). By day 80, these corals were dominated (>98%) by D1 symbionts. The degree to which this shift in the symbiont community is due to shuffling of existing algae, or the acquisition of exogenous symbionts from the environment, is unknown. Because D1 was detected in one replicate clade C coral, it is probable that the other corals had very low background levels of D1 as well, but the levels were below the detectable limit of the qPCR analysis used. However, environmental acquisition ('switching') of D1 by corals previously hosting only C1b-c cannot be discounted.

In contrast to the data obtained in this experiment, LaJeunesse et al. (2010) found that clade D1 harboring *Pocillopora* in the eastern Pacific were more tolerant of temperatures below 20^{o}C than C1b-c associated corals. They observed bleaching in 100% of C1b-c colonies surveyed, with significantly reduced symbiont densities. Fifty-six percent of C1b-c colonies they surveyed showed partial or total mortality. Only one coral with D1 was observed to have any mortality or bleaching in their study. These trends are essentially opposite to those observed in the current study, where clade C1b-c associated corals had higher Fv/Fm values and higher symbiont densities than the clade D1 genotype at 20^{o}C. My study showed there was a differential response between the three genets hosting clade C1b-c. Although my study only looked at one clade D coral, genetic variation is also expected in clade D1-hosting corals in the field, and differential mortality in previous cold-water events may have influenced the results observed by LaJeunesse. The corals used in my study had been maintained under constant 26^{o}C laboratory conditions for 5 years before experimentation, with the idea of reducing the role of recent environmental history. However, these corals were originally collected from the Gulf of Panama, and upwelling zone where temperatures can routinely drop below 20^{o}C during upwelling events (D'Croz and Mate 2004). It has been suggested that the environmental history of a coral's environment can influence the thermal tolerance by selecting for resistant genotypes of corals, symbionts or both (D'Croz and Mate 2004, Coles and Jokiel 1978, Jokiel and Coles 1997). Therefore, the long-term environmental history in the upwelling area of the Gulf of Panama, where the study corals were originally collected, may have selected for C1b-c hosting corals which

are more tolerant of cold water. It is difficult to directly compare field corals from the Gulf of California to corals collected from the Gulf of Panama. The data presented here also suggests that the rate at which temperatures change may also have a major effect on a coral's thermal tolerance, and that not all genotypes respond the same to rates of change. The LaJeunesse et al. (2010) study did not directly investigate the genotypic diversity of *Pocillopora* sampled, although they do point out that not all colonies with C1b-c responded the same, and suggest this may be the result of genotypic variation within the corals.

The data presented show a significant increase in symbiont densities for the clade C1b-c hosting genotypes at 20^{o}C, especially in genotype 4. Genotype 4 had a nearly 350% increase in the ratio of symbiont to host cells, significantly higher than the increases in genotypes 1 and 2. In fact, this is the only time that genotype 4 differed significantly from genotype 2 in any of the data presented. It was speculated earlier in this chapter that the overall increase in symbiont densities, taken with the relatively stable photochemical efficiencies at low temperatures may suggest an overproduction of photosynthates promoting symbiont growth. Recent work by Cunning and Baker (2012) shows that symbiont to host cell ratios, obtained via qPCR, actually may increase at high temperatures just before bleaching. The authors speculate that one explanation for the increase in this ratio is the result of a seasonal reduction in host cell numbers. The current study similarly shows an increase in the symbiont to host cell ratio when the coral is exposed to thermal stress, although in this case it is a cold stress. Cunning and Baker also report a larger increase in symbiont density in corals hosting clade D1 in

warm water, which is the opposite of the trend observed at 20^{o}C, where clade D1 decreased in density and C1b-c increased.

It has been suggested by Abrego et al. (2008) that there are complex and variable interactions that can occur between the coral host and symbionts. They found that juvenile *Acropora tenuis* hosting clade C1 actually responded better to high light and temperature than those hosting clade D. Interactions between host and symbiont factors, such as the production of protective enzymes and other cellular mechanisms, may elicit differing responses to thermal stress (Abrego et al. 2008). The ontogenetic stage of corals may also influence the efficacy of a particular host-symbiont interaction. While the specific symbiont types found in the *A. tenuis* study are not found in *Pocillopora* from the eastern Pacific, the suggested host-symbiont interactions may explain some differences between my study and the results of LaJeunesse et al. (2010).

In addition to genetic variability in temperature tolerance, a study by Volmer and Kline (2008) found that genetic variability also underlined natural disease resistance to white band disease (WBD). It was found that 6% of *Acropora cervicornis* genets surveyed (3 out of 49) were found to be completely resistant to WBD in both the field and aggressive transmission experiments. Interestingly, they found that there was a wide range of phenotypic variation in the susceptibility of surveyed *A. cervicornis* to WBD, with some genets being highly susceptible and some genets being completely resistant. It is likely that a similar range of genotypic and phenotypic variation may be present in thermal tolerance as well, as is hinted at in my current study with genotype 1 vs.

genotype 2 and 4. Vollmer and Kline also predict that corals carrying the WBD-resistant gene will have a selective advantage, and these genotypes should accumulate locally over time via asexual fragmentation. The same logic could apply to *Pocillopora*, or any coral, with a selective advantage towards thermal tolerance. This could be an effective strategy for localized recovery of corals after disease outbreak (Vollmer and Kline 2008) or bleaching, although more aggressive methods would be required for large-scale recovery.

4.5 Implications

The ability of coral reefs in the future to naturally recover from wide-scale bleaching, which is predicted to increase in frequency and magnitude, will be dependent upon the ability of coral populations to naturally select for thermally tolerant host genotypes, as well as shuffle more thermally tolerant symbiont types.

As coral reefs worldwide are declining, efforts are being made to transplant corals to damaged reefs for restoration efforts. As climate change continues to occur, and bleaching events become more frequent, it will become critical to use thermally tolerant corals for restoration work. This study has demonstrated that corals vary in their thermal tolerance, both as a result of coral genotype and algal symbiont community. Restoration efforts in the future should focus on using coral genotypes that have a wide thermal tolerance, such as genotypes 1 or 3 in this experiment, for reefs that are likely to experience thermal stress, while sheltered reefs with more constant temperatures should use corals, such as genotypes 2 and 4, which will grow quickly in

these constant environments. Vollmer and Kline (2008) suggest that future genetic surveys might be able to identify corals with genes for disease resistance. If the genes behind thermal tolerance are discovered, similar surveys may allow for the selection of thermally tolerant corals as well as, or in addition to, disease resistant corals.

4.6 Future Work

While the current study suggested there is in fact a host genotype affect on temperature tolerances, the number of genotypes studied was not enough to definitively say there is a strong role. Future work needs to increase the number of genotypes investigated to provide a more definitive analysis of the role of coral host genotypes on thermal tolerance. Ideally, the use of many genotypes from a wide geographic range would be important, and if possible find genotypic overlap in these regions. This study also demonstrated the importance of investigating the role of chronic temperature stress versus acute temperature stress, and the effect these two very different stressors may have on a coral's ability to adapt to climate change. There has been much conjecture in the literature concerning the likelihood of shifted symbiont communities returning to a pre-bleaching composition (Baker et al. 2004, Berkelmans and van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2010). Unfortunately in this study, I was unable to allow for a recovery phase, as the corals needed to be preserved for growth analysis. A recovery phase would be very important in future work to help determine if in fact stressed symbiont communities do return to pre-stress

compositions, and would help assess the long-term stability of these communities (LaJeunesse et al. 2010).

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Appendix I – Preliminary temperature ramping rate and its effect on growth and photosynthesis

Background

It has been well documented that rapid increases in temperature can lead to coral bleaching and mortality (Jokiel and Coles 1990, Glynn 1993). The duration as well as the degree of temperature change is important in the severity of bleaching (Jokiel and Coles 1990). The current study was conducted to determine how the rate of temperature change effected the growth and photosynthetic efficiency of *Pocillopora damicornis.* The data gathered from this study was used to determine the temperature ramping rate in the main genotype-specific thermal tolerance study.

Methods

The ramping study was conducted in the experimental system described in Chapter 2. Four different rates of temperature increase were used; 0.2^{o}C/day, 0.5^{o}C/day, 1.0^{o}C/day (as well as a control of 0.0^{o}C/day). All four treatments began at 26^{o}C, and were ramped up to 32^{o}C at the given rate. A total of 24 fragments of *Pocillopora damicornis* were used, with each temperature treatment consisting of 2 sleds with 3 corals per sled. Coral were photographed every 7 days from April 9 2010 to May 6 2010. Coral height was measured from these photographs using Image-J software. Growth data was analyzed using a one-way ANOVA with Tukey post-hoc test.

An IPAM was used to measure photosynthetic efficiency (F_v/F_m) every 7 days over the same period (see Chapter 3 for I-PAM methods).

Results

Growth

Figure 15 shows the average net changes in growth for the four temperature ramping rates. Photographs for the 1.0^{o}C/d treatment were lost for day 7, so the data start on day 14 for this treatment. On day 14, the 0.5^{o}C and 1.0^{o}C treatment showed significantly higher growth than the control (ANOVA, p=0.026 and p=0.012, respectively). However, by day 27 the 0.2^{o}C/d treatment showed significantly more growth than the 0.5^{o}C and 1.0^{o}C/d treatments (p=0.016 and 0.005, respectively), but was not different from the control (p=0.2).

Photochemical efficiency

The Fv/Fm values for the 1.0^{o}C per day treatment were significantly reduced from control values ($p<0.05$). The 0.2 and 0.5^{o}C values did not vary at the end of the experiment on day 27, although they were significantly higher than those from the 1.0^{o}C treatment and significantly lower than those from the control treatment ($p<0.05$).The day 0 measurements for the 1.0^{o}C treatment were lost.

Discussion

There was very little difference observed in growth over the 27 day experiment, most likely due to the short experimental period and low replication. The 0.2^{o}C and control treatments showed similar growth rates, that were slightly higher than the other two temperature treatments. However, the Fv/Fm data did show clear differences between treatments. While the replication was low for this preliminary study, the data suggest that there is very little difference between increasing temperatures at 0.2^{o}C or 0.5^{o}C per day up to 32^{o}C. However, an increase of 1.0^{o}C per day resulted in significantly reduced photochemical efficiency, indicating this rate was stressful on the coral holobiont. Because of the similarity between 0.2 and 0.5^{o}C/day, the rate of 0.5^{o}C/day was chosen for the main experiment.

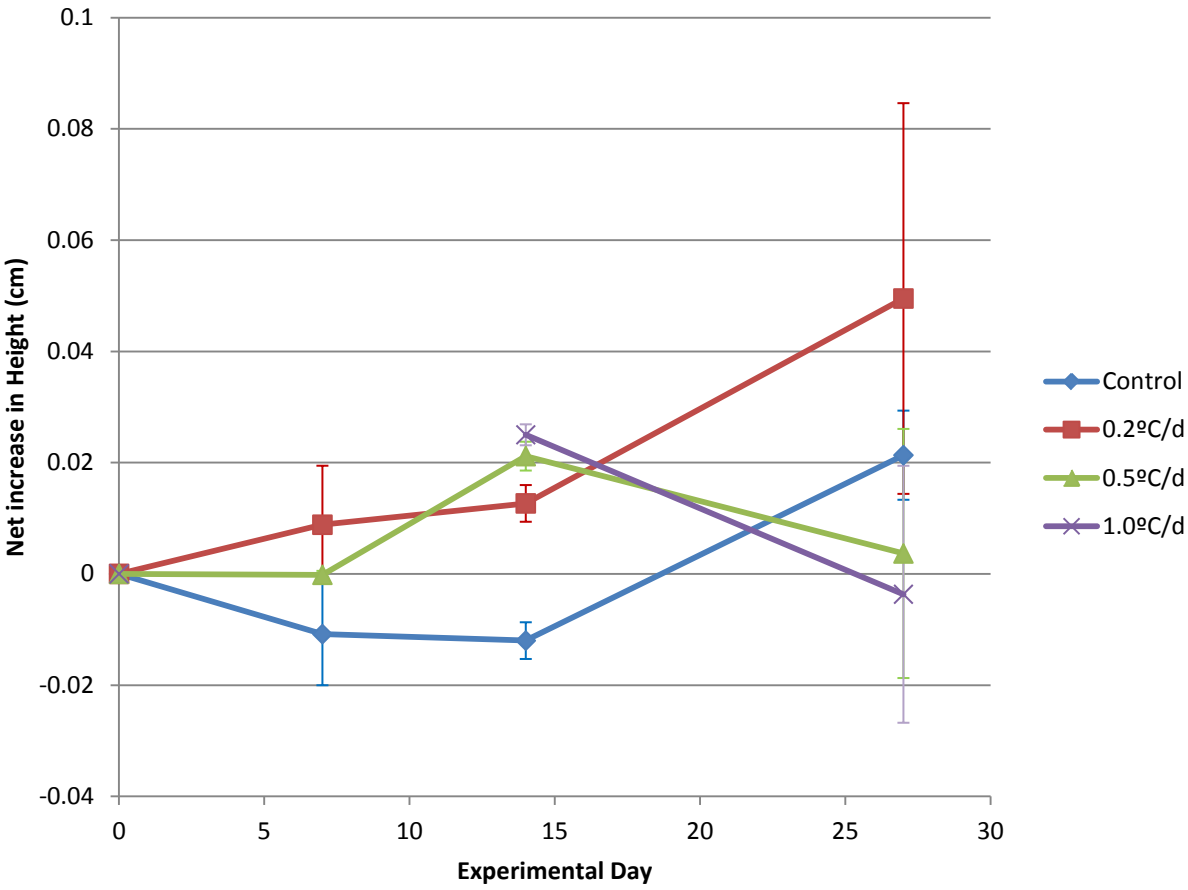

Figure 15: Growth (expressed as net increase in height in cm) for corals exposed to different rates of temperature increase.

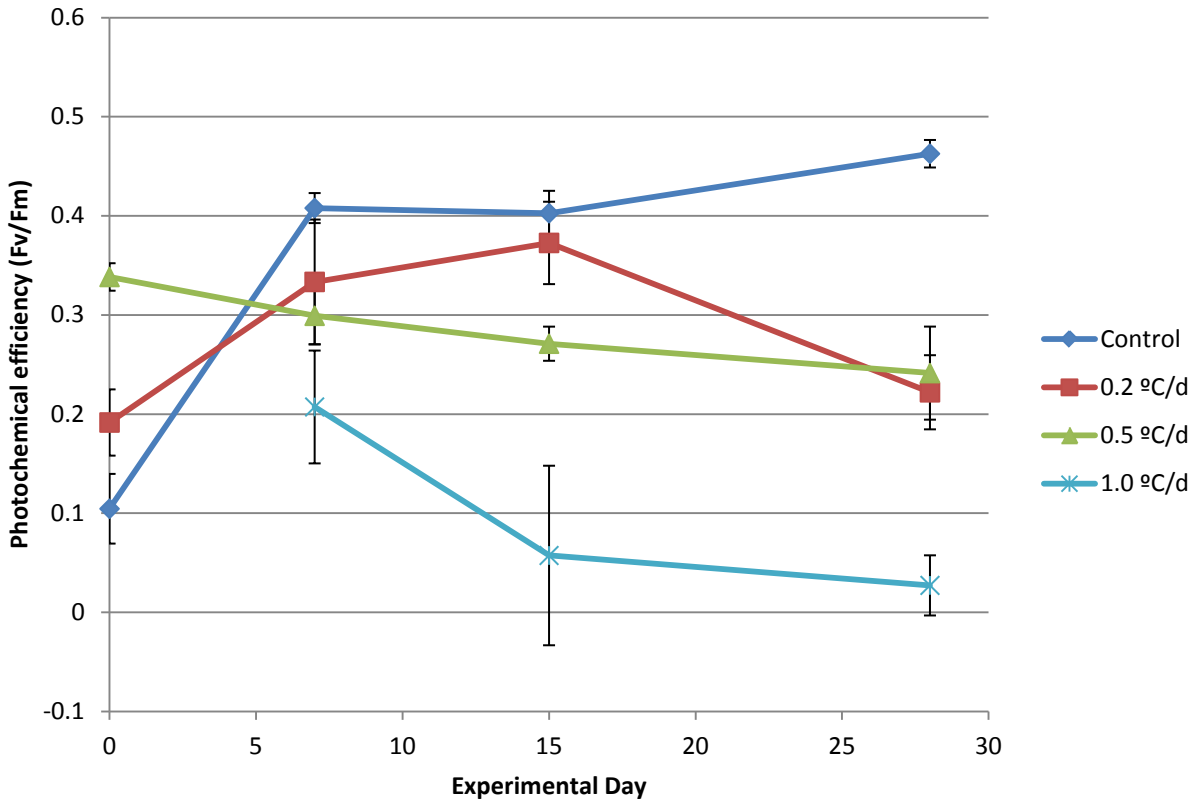

Figure 16: Photochemical efficiency (Fv/Fm) over the 27-day temperature ramping experiment.

Appendix II – A photographic method for the non-destructive estimation of coral *Symbiodinium* density

Background

Prolonged exposure to thermal stress in corals has been shown to often result in coral bleaching, or the loss of *Symbiodinium* (Jokiel and Coles 1990, Glynn 1993). Having knowledge about the density of zooxanthellae in experimental corals is fundamental in understanding the dynamics of any thermal-tolerance experiments on corals. However, for studies using small fragments of corals, direct sampling of tissue for cell counts is not feasible due to the high percentage of tissue that must be removed. The following protocol describes a system for non-destructively estimating the density of *Symbiodinium* in a coral by fitting the color value of the coral to a developed color-density curve.

Methods

Corals were fragmented so that uniform-colored pieces were used in this analysis. Photographs were taken of experimental fragments of *Acropora cervicornis* together with a color standard and 18% gray card. Each coral fragment was measured for width and thickness using calipers, and a surface area was calculated. Tissue was then blasted off each branch using filtered seawater and collected. The volume of the blastate was brought up to 15mL using filtered seawater, and the solution was then homogenized using a Dounce-type glass homogenizer until smooth. Three replicate 100μL samples were removed from each branch homogenate, to which 100μL of Lugols

solution was added. A haemocytometer was then used to conduct 3 cell counts per sample, resulting in 9 counts per branch. The cell counts were then used to estimate the *Symbiodinium* density per given surface area in the original coral fragment. The color value (measured in red, green, and blue) and gray scale value (% black) of each coral fragment was determined using Adobe Photoshop (see Figure 17). Each color/gray value was plotted against *Symbiodinium* density to obtain an equation from which *Symbiodinium* density could be estimated. A linear regression analysis was used to determine statistical significance.

Eight branches of *Acropora cervicornis* (see Figure 17), each of varying color, were used to test the above methodology. Symbiodinium community types were determined to be the same between all Acropora genotypes in the collection using denaturing gradient gel electrophoresis (DGGE).

Results

The data show a strong linear relationship between the color value and symbiont density. When analyzing the red, green and blue colors, the graphs showed statistically significant negative linear relationships ($p<0.001$ for all three), with R^2 values of 0.737, 0.703 and 0.584 respectively (see Figures 18-20). The black value obtained from the gray scale image showed a significant positive linear relationship ($p<0.001$), with an R^2 of 0.697 (see Figure 21).

Discussion

There was a strong linear relationship between the color value obtained and the measured symbiont density for each coral fragment. The relationship for the grayscale values was positive, while the trend each color value was negative. This result is most likely due to the fact that the grayscale value is recorded as a percent black, as opposed to saturation for the RGB color scale. Regardless, all four regression equations appear to provide a fairly accurate estimate of total symbiont density in *Acropora cervicornis*, without the need for destructive tissue sampling for direct symbiont counts. These methods could be of use for future experiments using this species.

Figure 17: Adobe Photoshop was used to determine the RGB color values and grayscale values of 4 points on a fragment of *Acropora cervicornis* using the color dropper tool.

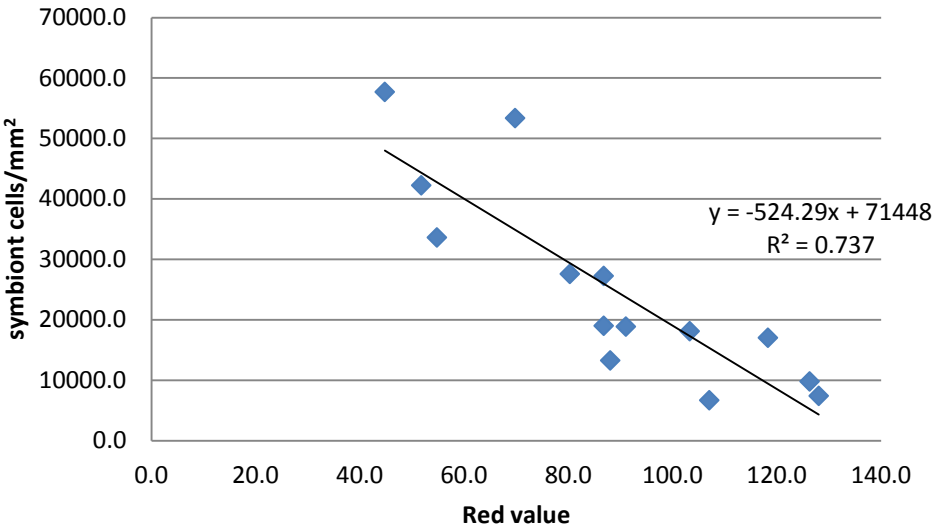

Figure 18: The RGB red value of a coral fragment regressed against the measured symbiont density measured by cell counts using a haemocytometer. There is a strong negative linear relationship ($p<0.001$) between the red value and symbiont density.

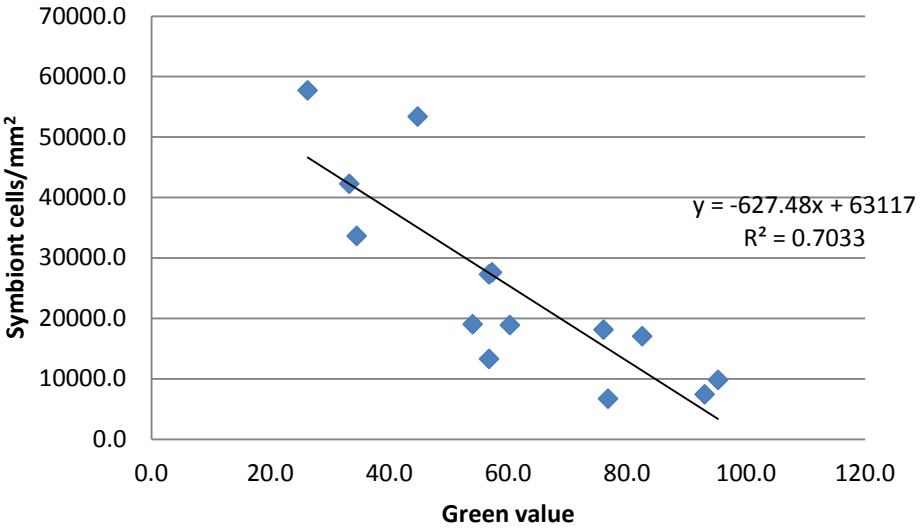

Figure 19: The RGB green value of a coral fragment is regressed against the measured symbiont density measured by cell counts using a haemocytometer. There is a strong negative linear relationship ($p<0.001$) between the green value and symbiont density.

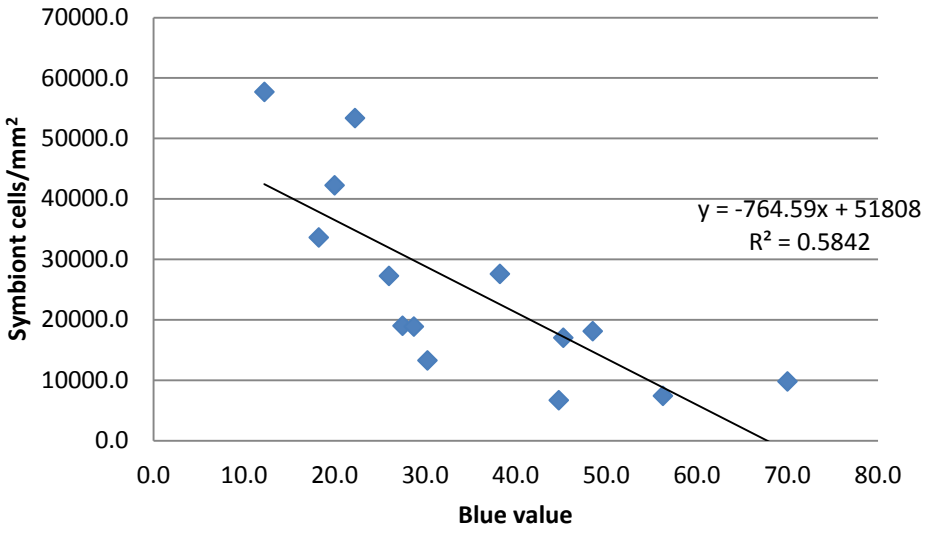

Figure 20: The RGB blue value of a coral fragment is regressed against the measured symbiont density measured by cell counts using a haemocytometer. There is a strong negative linear relationship ($p<0.001$) between the blue value and symbiont density.

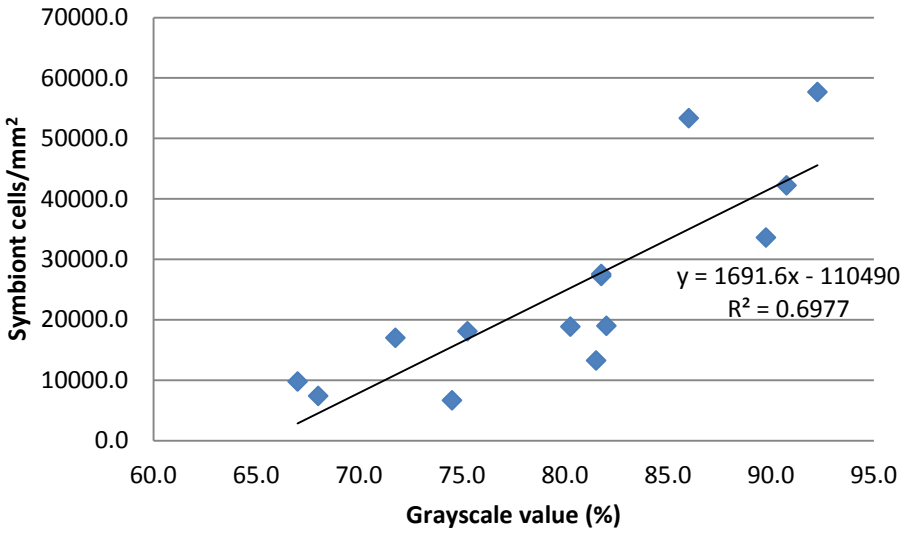

Figure 21: The grayscale value of a coral fragment is regressed against the measured symbiont density measured by cell counts using a haemocytometer. There is a strong positive linear relationship ($p<0.001$) between the grayscale value and symbiont density.