Spring 2016

Symbiont type and photophysiology of Acropora loripes and Platygyra daedalea under future scenarios of rising ocean temperatures and pCO2

Lauren Howe-Kerr

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Symbiont type and photophysiology of *Acropora loriipes* and *Platygyra daedalea* under future scenarios of rising ocean temperatures and pCO$_2$

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Submitted in partial fulfillment of the requirements for Australia: Rainforest, Reef and Cultural Ecology, SIT Study Abroad, Spring 2016
ABSTRACT

Coral cover is declining at an alarming rate, and it is estimated that 60% of reefs worldwide may be lost by 2030. Elevated seawater temperatures and ocean acidification are contributing to an increase in the frequency and severity of bleaching events. These events disrupt the symbiosis between corals and their photosynthetic dinoflagellates (*Symbiodinium* spp). Relatively little is known about the ability of corals to acclimatize to changing environmental conditions or whether the rate of climate change is too fast for corals to keep up, limiting the accuracy of future predictions for reef resilience. However, the ability of some coral species to acclimatize to elevated seawater temperatures has been linked to *Symbiodinium* composition and the flexibility of these associations. This study compares *Symbiodinium* communities and the rates of photosynthesis of two coral species, *Acropora loripes* and *Platygyra daedalea*, under three conditions of water temperature and pCO$_2$: ambient, predicted levels for 2050, and predicted levels for 2100. Pulse amplitude modulated (PAM) fluorometry, which measures chlorophyll fluorescence, was used as an indicator of photosynthetic rate. Photosynthetic rates of *A. loripes* and *P. daedalea* differed significantly, and PAM yields were reduced in the 2050 and 2100 treatments, suggesting a stress response. The majority of genotypes of both coral species hosted clade C-type *Symbiodinium* suggesting that sub-type differences or host physiology may be involved in species-specific differences in photophysiology. Sequencing-based methods for *Symbiodinium* typing would help distinguish these possibilities and increase understanding of the influence of *Symbiodinium* type on photosynthetic rates of corals under changing environmental conditions.

*Key words:* PAM fluorometry, *A. loripes, P. daedalea*, acclimatization, climate change
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Acknowledgements

Thank you to Dr. Line Bay and Dr. Carly Kenkel for advising me and providing me with the opportunity to work at AIMS. It was an honor to get to work with both of you, and I have learned so much in the past five weeks.

I am grateful that I was able to work with those involved in the Evolution 21 project and the chance to work at the Australian National SeaSimulator facility at AIMS. Specifically, thanks to Dr. Heidi Luter and Andrew Wallace for their help and support during the past month. I am also appreciative that the Evolution 21 project financed my commuter car to and from AIMS.

I would like to thank Tony Cummings and Jack Grant for their guidance throughout the entire ISP process. Additional thanks to Jenny Zhang for her encouragement and support during our month at AIMS.

I would also like to thank Dr. Adrienne Simmoes-Correa at Rice University for sparking my interest in coral reef ecology and specifically coral physiology. Thank you for helping me brainstorm ISP ideas before even leaving for Australia, providing me suggestions for contacts, and helping me establish my specific project. Your enthusiasm and support from half way around the world have been a tremendous help!
INTRODUCTION

Coral reefs are one of the most biodiverse and productive ecosystems in the world (Perry et al. 2013; Hoegh-Guldberg et al. 2007; Alvarez-Filip et al. 2009) yet they are also one of the most threatened. Since the 1970’s, there has been approximately an 80% decrease in coral cover in the Caribbean (Perry et al. 2013) and an estimated 50% decline in Western Pacific reefs (Bruno & Selig 2007). Anthropogenic climate change is expected to accelerate the rate of coral decline since elevated sea water temperatures and ocean acidification stress the symbiotic relationship between corals and their endosymbiotic dinoflagellates (*Symbiodinium* spp). Coral acclimatization to rising temperatures has been linked to *Symbiodinium* type, and while there is evidence that some corals can shift *Symbiodinium* composition to increase thermal tolerance (Berkelmans & van Oppen, 2006), little is known about the extent to which this is possible across species or if the rate at which climate change is progressing exceeds the rate at which corals can keep up (Hughes et al. 2003; Guest et al. 2012). This study compares the photophysiology of reef-building corals under different predicted future scenarios for rises in seawater temperatures and ocean acidification to contribute to our understanding of coral acclimatization and future reef persistence.

1.1 Thermal Stress and Ocean Acidification

Increases in atmospheric CO₂ concentrations induce global warming, resulting in elevated seawater temperatures. These elevated water temperatures can cause corals to bleach. Coral bleaching occurs when corals expel their endosymbiotic dinoflagellates (*Symbiodinium* spp.), and without these photosynthetic pigments, corals lose their color (Brown 1997). Although the white coral tissue is still alive when bleaching occurs, stress of high intensity
and/or duration prevents corals from regaining their *Symbiodinium*, resulting in partial or complete coral mortality (Eakin 2010; Hoegh-Guldberg et al. 2007). Even if corals are able to survive mild thermal stress-induced bleaching, their rate of growth decreases and they become more susceptible to disease (Hoegh-Guldberg et al. 2007). Global ocean temperatures have risen by over 0.7°C in the past 25 years (Hoegh-Guldberg et al. 2011) and are expected to rise by 3°C by 2100 (Collins et al. 2014). Bleaching events are increasing in frequency and severity as global temperatures continue to rise (Dove et al. 2013; Hoegh-Guldberg et al. 2007; Eakin et al. 2010). The third mass bleaching event is currently underway (NOAA 2016), making it an even more crucial time to study the potential of corals to acclimatize to changing ocean conditions.

Increases in atmospheric CO$_2$ pose additional challenges to coral reefs by causing ocean acidification. When CO$_2$ mixes with water, carbonic acid is formed, which then breaks down into bicarbonate ions and protons. These protons combine with carbonate to form more bicarbonate, thus reducing the carbonate that is available to scleractinian corals and other reef building organisms to form their skeletons (Hoegh-Guldberg et al. 2010). As a result, coral calcification rates are declining and many reefs, especially in the Caribbean, are currently or predicted to soon be in accretionary stasis- where net growth no longer exceeds net erosion (Perry et al. 2013). In addition to its more widely known effects on calcification rates, ocean acidification may also play a role in coral bleaching. Ocean acidification, especially when acting synergistically with elevated water temperatures, is thought to cause bleaching by impacting the photoprotective mechanisms in the photosystems of *Symbiodinium* and may lower the bleaching threshold of corals (Anthony et al. 2008).
1.2 Coral symbiosis and acclimatization

The responses of corals to stressors like elevated temperatures and ocean acidification are influenced in part by *Symbiodinium* composition. Algae in the genus *Symbiodinium* are highly diverse, with eight clades (A-H) and multiple genetic sub-types within each clade (Little et al. 2004; Berkelmans & van Oppen 2006). Coral associations with different clade types have been shown to have varying sensitivities to light and heat stress (Iglesias-Prieto et al. 2004; Robinson & Warner 2006). For example, corals hosting clade D *Symbiodinium* are more thermally tolerant than those hosting clade C (Berkelmans & van Oppen 2006). Some associations are more flexible than others, and some corals harbor more than one *Symbiodinium* type at the same time, which could allow for switches in the relative abundance of certain types (Berkelmans & van Oppen, 2006; Jones et al. 2008). The flexibility of these coral-*Symbiodinium* associations is vital to the potential of corals to acclimatize to changing environmental conditions.

Acclimatization refers to non-genetic responses to environmental changes, as opposed to genetic adaptation (Gates & Edmonds 1999). Acclimatization can occur much more quickly than adaptation, and is therefore more relevant in terms of the potential for corals to survive bleaching events. Studies in the Indo-Pacific indicate there is a possibility of coral acclimatization to warmer temperatures. Guest et al. (2012) found that reefs that were more exposed to variable temperatures in the past were more resistant to bleaching, even some of the genera that are most susceptible to bleaching, like *Acropora*. There is also evidence of changes in *Symbiodinium* community composition and diversity following bleaching events, where the holobiont, the host coral plus all of its symbionts, shifts to a higher proportion of thermally tolerant *Symbiodinium* types (Jones et al. 2008). Berkelmans & van Oppen (2006) showed that
Corals can increase their thermal tolerance by approximately 1-1.5°C when their dominant *Symbiodinium* type shifts from C to D. However, even if corals are able to acclimate to elevated temperatures, they will also have to deal with the compounding effects of ocean acidification in order to have the potential to resist future bleaching.

1.3 Specific aims

Photosynthetic rates reflect the condition of the *Symbiodinium* in corals and can therefore be used as one parameter for monitoring coral health and responses to stressors like elevated water temperatures and pCO$_2$. When corals bleach, there is non-reversible damage to the photosystems of the associated *Symbiodinium*, and this damage is reflected in the lowered photosynthetic rates of the coral colony. Photosynthetic rates can also vary to reflect reversible photo-stress to *Symbiodinium*, such as when *Symbiodinium* divert photons away from the photosystems to prevent damage from high light intensities (Fitt et al. 2001). In this study, we compared the photosynthetic rates of *Acropora loriipes* and *Platygyra daedalea* under ambient temperature and pCO$_2$ conditions, predicted conditions for 2050, and predicted conditions for 2100. *Symbiodinium* composition of the different genotypes were also examined, and together were used to make predictions about the ability of these corals to acclimate in the short-term to changes in environmental conditions predicted as a result of climate change.

Pulse amplitude modulated (PAM) fluorometry measures chlorophyll fluorescence and was used in this study to compare photosynthetic rates. PAM readings are commonly used to detect early signs of stress in the photosystems of *Symbiodinium* (Fitt et al. 2001). We expected to record lower PAM readings for the corals exposed to the conditions predicted for 2100 since these conditions are closer to the thermal threshold of the corals, where the photoinhibition of
*Symbiodinium* and bleaching are more likely. Additionally, we expected to see differences in responses to treatments based on genotype and *Symbiodinium* composition since genotypes with more thermally tolerant *Symbiodinium* types will likely have higher PAM readings.
Methods

2.1 Study site and experimental design

All data was collected as part of the Evolution 21 project at the Australian Institute of Marine Science (AIMS) in Townsville, QLD. 15 colonies (~genotypes) of two species, *A. loripes* and *P. daedalea*, were collected from Davies Reef (18°49.816’, 147°37.888’) (Figure 1) from 16-22 February 2016 under the Great Barrier Reef Marine Park Authority permits G12/35236.1 and G14/37318.1. Each colony was split into three fragments, and fragments were placed in shaded holding tanks with 0.2 μM filtered flow-through seawater (FSW, 27°C, 150 μM PAR). At the Australian National Sea Simulator facility, a system of nine ~1500 L outdoor tanks (3 per treatment) was set up with the following three treatments: ambient water temperatures and pCO2 (+0.0°C, 380 μatm pCO₂), predicted water conditions for 2050 (+1.0°C, 685 μatm pCO₂), and predicted water conditions for 2100 (+2.0°C, 940 μatm pCO₂). The three coral fragments of each of the 15 genotypes of *A. loripes* and *P. daedalea* were distributed randomly throughout the tanks of each treatment (n=5 genotypes per species per tank, Figure 2). Temperatures were not elevated above the known thermal tolerance of the coral species because the intention was not to kill the corals but study their responses under stress. Ambient water conditions and seasonal fluctuations in salinity and water temperature were based off of the conditions present at Davies Reef. Corals were exposed to the natural light/dark cycle, and the corals in each tank were fed *Artemia* naupli at a density of 5 naupli/ml daily. Tanks were cleaned daily.
Figure 1. Davies Reef location. All of the coral samples were collected from Davies Reef, Great Barrier Reef (18°49.816’, 147°37.888’), circled in red above, in March 2016.
Figure 2. Layout of corals, treatments, and tanks at the Australian National SeaSimulator facility at the Australian Institute of Marine Science. The first number in the coral shape refers to genotype and the decimal refers to replicate number.
2.2 Study species

*A. loripes* and *P. daedalea* are common scleractinian corals in the Indo-Pacific. *A. loripes* is a branching coral in the family Acroporidae that can grow as bushes or plates and is most commonly found on upper reef slopes (Figure 3a, Veron 2000). *P. daedalea* is a massive meandroid or submeandroid coral in the family Faviidae (Figure 3b) and is especially prevalent on back reef margins (Veron 2000). It is also found in the Persian Gulf, where temperatures reach 36°C (Mostafavi et al. 2007), making it a good candidate for studying acclimatization to elevated temperature conditions resulting from climate change.

![Figure 3. Study species. Acropora loripes (a) and Platygyra daedalea (b).](image)

2.3 PAM fluorometry

A Mini-Pulse amplitude modulated (PAM) fluorometer (Walz, Germany) was used to measure photosynthetic rates of each coral fragment (n=3) twice per week for four consecutive weeks in April 2016. PAM measures chlorophyll fluorescence, an indicator of photosynthetic energy conversion, by using pulses of multiple types of light to measure effective quantum yield.
of photosystem II (EQY) in *Symbiodinium* (Coad 2014). The Mini-PAM was set to factory settings save that measuring intensity was set to 12 and gain to 5. Measurements were always taken between 11am and 2pm to coincide with solar maximum (approximately 12:15pm). The order of the tanks used to PAM the corals was randomly selected for each survey period to avoid any effect of light availability due to time of day on yield measurements.

2.4 *Light meters*

Light meters were set up in a grid pattern in each tank (Figure 4) to measure light variation within tanks, as photosynthetically active radiation (PAR) levels may contribute to variation in photosynthetic rates. Tanks were measured sequentially, and meters were left for approximately 48 hours in each tank. A permanent light meter was also installed in each tank (Figure 4), and used to obtain daily light integral (DLI) and PAR data from the dates corresponding to the dates when PAM recordings were taken.

*Figure 4. Layout of light meters in coral tanks.* Blue circles represent light meters that were left in each tank for 48 hours to measure light variation within tanks, and the black circle represents the permanent light meter that was established in each tank to compare light levels between tanks.
2.5 DNA extraction, lsu PCR, and restriction enzyme digests

DNA from each coral genotype was extracted using Wayne’s Method (Lundgren et al. 2013). Pellets were resuspended in 30ml of 10 mM Tris (pH=9) and stored at -20°C. DNA was diluted to 50 ng/µl for A. loripes and 20 ng/µl for P. daedalea with MilliQ water prior to amplification. Primers from the lsu rRNA region were used to target Symbiodinium types since the DNA was a mixture of host and symbiont DNA, and PCR was completed as described in Palstra (2000) and van Oppen et al (2001). 1µl of DNA sample was combined with 9µl of lsu master mix (6.35µl MilliQ water, 2µl 5x buffer, 0.5µl 10µM lsu primer, and 0.15µl Taq, Bioline). 35 cycles of amplification were completed as follows: 20 sec. at 95°C, 30 sec. at 60°C, 90 sec. at 72°C. Yield and quality of PCR product was examined by running 2µl of PCR product on 1% agarose TBE gels stained with ethidium bromide (110V, 180mA, 40 min).

The restriction enzyme Taq1 was then used to digest the PCR product. 5µl of PCR product was combined with 5µl of digest mix (3.85µl MillióQ water, 1µl 10x buffer, and 0.15µl of Taq1, New England Biolabs) and was digested for 2 hours at 65°C. Restriction digests were examined by running 2µl of the digested PCR product on 1% agarose TBE gels stained with ethidium bromide (110V, 180mA, 40 min) (Figure 5).
2.6 Data analysis

All statistical analyses was completed with R statistical software (Version 0.99.473) (R Development Core Team 2008). A linear mixed model was used to determine the effect of treatment and species on PAM yields using the lme command of the nlme package (Pinheiro et al. 2013). Date and genotype were included as random effects. Wald tests were used to calculate significance of fixed factors, and Tukey’s post hoc tests were used to calculate significance among factor levels when warranted.

An ANOVA was used to test if there was an effect of tank on DLI. Regression analysis was used to determine whether there was a correlation between PAR variation (standard deviation) within tanks and PAM yield variation within tanks. Symbiodinium clades were identified through comparison of restriction enzyme digests of lsu PCR gels to visualize banding patterns described in Palstra (2000) (Figure 5).

Figure 5. Restriction enzyme digest of lsu PCR product using Taq1. Columns 1 and 14 are DNA ladders. C1, C2, and D refer to the Symbiodinium types and their corresponding banding pattern that was used for identification in this study. Figure taken from Palstra 2000.
RESULTS

3.1 PAM fluorometry

Five rounds of PAM were completed on April 7, 13, 18, 22, and 26 (Figure 6). On average, PAM yields in *P. daedalea* were greater than yields in *A. loripes* by 0.016 (Wald, $f=12.166$, $p<0.001$) (Figure 7). Treatment also had a significant effect on yield (Wald, $f=7.236$, $p<0.001$) (Figure 7), but treatment was not significant on an individual factor level (Tukey A-M: $z=1.804$, $p=0.17$; M-A: $z=1.004$, $p=0.56$; M-H: $z=0.8$, $p=0.7$).

![Figure 6](image_url) **Figure 6.** Mean PAM yields over time for *A. loripes* and *P. daedalea*. Dates from 1-5 represent the following dates, respectively: 7 April, 13 April, 18 April, 22 April, and 26 April 2016. For the treatments, A indicates ambient conditions of temperature and pCO$_2$ (blue line), H indicates predicted conditions for 2100 (red line), and M indicates predicted conditions for 2050 (green line). Error bars indicate standard error.
Figure 7. Mean effective quantum yield of A. loripes and P. daedalea by treatment. For the treatments, A indicates ambient conditions of temperature and pCO$_2$, M indicates predicted conditions for 2050, and H indicates predicted conditions for 2100. Error bars indicate standard error.

3.2 Light data

Tank did not have a significant effect on DLI (ANOVA, $f=2.45$, $p=0.121$). On a factor level, the DLI between multiple pairs of tanks differed significantly with a greatest average difference of 1.6 between tanks 7 and 9 (Appendix A) (Figure 8). In regards to light variation within tanks, there is a tendency for a negative relationship between variation of mean PAR levels and variation of mean PAM yields for P. daedalea ($R^2=0.42$, $f=5.03$, $p=0.06$), but no trend between variation of mean PAR levels and variation of mean PAM yields for A. loripes ($R^2<0.01$, $f=0.03$, $p=0.88$) (Figure 9).
Figure 8. Mean daily light integral by tank. Average daily light integral (DLI) was calculated based on days prior to the days that PAM fluorometry was conducted in April 2016 (6, 12, 21, 25). Error bars indicate standard error.

Figure 9. Standard deviation of average photosynthetically active radiation (PAR) levels compared to standard deviation of coral PAM yields. *P. daedalea* yields are plotted to the left ($R^2=0.42, f=5.03, p=0.06$) and *A. loripes* to the right ($R^2<0.01, f=0.03, p=0.88$). PAR data was collected in April 2016.
3.3 PCR, restriction enzyme digest, and gel analysis

DNA was extracted from 19 genotypes of *A. loripes* and 17 genotypes of *P. daedalea*. Lsu PCR successfully amplified DNA for all 19 *A. loripes* genotypes (Figure 10) and for 15 of the 17 *P. daedalea* genotypes (Figure 12). Restriction enzyme digest by Taq1 revealed that type C was the most dominant *Symbiodinium* clade in all of the *A. loripes* genotypes (Figure 11, Table 1). Type C was also the most dominant *Symbiodinium* clade in *P. daedalea* genotypes, but there were two different type C banding patterns, and genotype 3 also hosts clade D-type *Symbiodinium* (Figure 13, Table 1).

![Figure 10. Agarose gel results from lsu PCR of A. loripes genotypes. Numbers above wells refer to the A. loripes genotype. L indicates ladder (Quick-Load, 1kb, 100pb, New England BioLabs) and 500 and 1000bp are labeled on the ladders. B indicates a blank run, with MilliQ water, to serve as a control. A, b, and c indicate samples that were run on separate gels. Approximate band length could not be determined in (b).](image-url)
Figure 11. Agarose gel results from restriction enzyme digest of *A. loripes* lsu PCR product. Numbers above wells refer to the *A. loripes* genotype. L indicates ladder (Quick-Load, 1kb, 100pb, New England BioLabs) and 500 and 1000bp are labeled on the ladders. A, b, and c indicate samples that were run on separate gels. Approximate band length could not be determined in (b).

Figure 12. Agarose gel results from lsu PCR of *P. daedalea* genotypes. Numbers above wells refer to the *P. daedalea* genotype. L indicates ladder (Quick-Load, 1kb, 100pb, New England BioLabs) and 500 and 1000bp are labeled on the ladders. B indicates a blank run, with MilliQ water, to serve as a control.
Figure 13. Agarose gel results from *lsu*-PCR of *P. daedalea* genotypes. 1-17 refers to different *P. daedalea* genotypes. L indicates ladder (Quick-Load, 1kb, 100pb, New England BioLabs). B indicates a blank run, with MilliQ water, to serve as a control.

Table 1. Dominant symbiont type for each genotype of *A. loripes* and *P. daedalea*. Dashes indicate that there was no data available for that genotype. Asterisks indicate a potentially different type of clade C *Symbiodinium*.

<table>
<thead>
<tr>
<th>Genotype</th>
<th><em>A. loripes</em></th>
<th><em>P. daedalea</em></th>
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<tr>
<td>1</td>
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DISCUSSION

Photosynthetic rates of corals under different scenarios of elevated water temperatures and pCO$_2$ were compared, and we expected these elevated conditions to stress the symbiosis between coral and *Symbiodinium*, resulting in lowered photosynthetic rates. Consistent with this expectation, lower PAM yields were measured in the corals exposed to conditions predicted for 2050 and 2100, even within the relatively short time span that corals were exposed to the different treatments (Figure 7). As PAM yields also differed between species, I hypothesized that variation in *Symbiodinium* type may have been a factor. However, most coral genotypes hosted the same dominant *Symbiodinium* clade, therefore observed differences in the photophysiology of corals may be attributable to sub-cladal variation in *Symbiodinium* types or host species-specific physiology. Alternatively, light variation between and within tanks may also have been partially responsible for variation in PAM yields.

The efficiency of photosystem II of *in hospite Symbiodinium* differed between coral species and in response to treatment, as reflected in the PAM yields of the corals. Corals in ambient water temperature and pCO$_2$ had the highest photosynthetic yields, and yields decreased as the treatment intensity increased (Figure 7). This was expected as high temperatures stress the coral-algae symbiosis by damaging the photosystems of *Symbiodinium* (Fitt et al. 2001), so the lowered PAM yields of corals in the elevated temperature and pCO$_2$ treatments is likely an initial stress response. However, physiological responses to treatments may differ long term since measurements for this study were completed within the first two months of corals being exposed to different treatments. Moderately elevated pCO$_2$ levels have been found to cause increased productivity in some coral species (Castillo et al. 2014), but this pattern was not observed in the present experiment. Corals in the moderately increased pCO$_2$ and temperature tanks still had
slightly lower photosynthetic rates than those in the ambient treatment tanks (Figure 7). The synergistic effects of elevated temperature and pCO$_2$ may have cancelled out any increase in productivity that would have resulted from moderately elevated pCO$_2$ alone. Though elevated pCO$_2$ may not be as significant of a stressor as temperatures to some coral species (Castillo et al. 2014), the relative effects of pCO$_2$ and temperature cannot be determined since they were confounded within the present experimental design.

Restriction enzyme digest of symbiont lsu rRNA for *A. loripes* and *P. daedalea* revealed slightly different *Symbiodinium* community profiles. *A. loripes* hosted what appeared to be a single type of clade C *Symbiodinium* (Figure 11). *P. daedalea* also predominantly hosted C-type *Symbiodinium*, but there appeared to be two separate clade C sub-types (Figure 13). In addition, one genotype of *P. daedalea* also hosts clade D *Symbiodinium* (genotype 3, Figure 13), which was never observed in *A. loripes*. Variation in *Symbiodinium* types can significantly influence thermal tolerance of the coral holobiont (Berkelmans & van Oppen 2006). The observed variation in *P. daedalea* symbiont communities suggests the greater potential for this species to acclimatize to changing environmental conditions by switching dominant *Symbiodinium* types, which may partially explain its uniformly elevated photosynthetic yields in the present experiment (Figure 7). Fisher and Dove (2011) found that *P. daedalea*, along with *Acropora millepora*, *Acropora aspera* and *Acropora formosa* were generally dominated by type C3 *Symbiodinium* near Heron Island, Great Barrier Reef. Seven common reef-building corals were examined, some that were dominated by type C3 and some by C15. Type C3 was found to be more susceptible to thermal stress than type C15, providing evidence of variation in responses to heat stress within a single *Symbiodinium* clade. Consistent with the present results, Tonk et al. (2013) found that *P. daedalea* can host type C3 in addition to other C3 variants (C3h and
C3_Fisher). A 2007 study in the Persian Gulf found clade D *Symbiodinium* in all of the 8 coral species studied, including *P. daedalea*, and this dominance may be explained in part by the high temperature variation characteristic of the area (Mostafavi et al. 2007). *A. loripes* has only been reported to associate with type C3 thus far (Tonk et al. 2013). If *A. loripes* only forms associations with C3 while *P. daedalea* can host more types, this supports our assertion that *P. daedalea* may be more tolerant to the elevated temperature and pCO$_2$ conditions since this species may have greater potential for symbiont shuffling and flexibility of associations. Longer term studies comparing changes in *Symbiodinium* community composition in *P. daedalea* and *A. loripes* over time are necessary to support this hypothesis.

Variation in *Symbiodinium* types may not be the only explanation of differences in photophysiology between species. Host genotype is also known to influence bleaching thresholds (Abrego et al. 2008). Slower growing massive corals, such as *P. daedalea*, with high metabolic rates are thought to have a better chance at acclimatizing to changing environmental conditions than fast growing branching species with low metabolic rates such as those in the genus *Acropora* (Gates & Edmonds 2008). If we assume that both *P. daedalea* and *A. loripes* were dominated by the same type of clade C *Symbiodinium*, host effects may be at play. There is emerging evidence that stress responses of corals to heat and light are influenced not only by *Symbiodinium* type, but by also by host responses and interactions between host and symbiont. Protective mechanisms of the host coral, such as the production of certain enzymes and fluorescent pigments, can influence the holobiont’s stress responses (Abrego et al. 2008). Host-symbiont interactions maybe have influenced the differences in photosynthetic rates between *A. loripes* and *P. daedalea* in this experiment, though specific mechanisms of host responses need to be further explored.
A primary limitation to this study was that *Symbiodinium* clades could not be definitively narrowed to specific types. More specific information about *Symbiondinium* types of each genotype would contribute to a clearer understanding of why there was an effect of species on photosynthetic rates. Knowledge of the specific *Symbiondinium* sub-types would also allow us to compare the effect of treatment and dominant *Symbiondinium* type on photosynthetic rates. There is also the potential that the *P. daedalea* PCR samples were contaminated since faint bands were present in the blank control run (Figure 12). However, no bands were present in wells 5 and 6, suggesting that not all of the samples could have been contaminated.

Yet another alternative explanation for variation in photobiology between species could be environmental variance across treatment tanks. Other studies have shown that light environment can impact photosynthetic function of *Symbiodinium*. When corals are exposed to strong light levels, photoinhibition can occur, and there is reduced activity of photosystem II (Fitt et al. 2001; Murata et al. 2006). Seasonal changes in light intensity influence *Symbiodinium* densities and coral biomass, likely because higher PAR levels, especially when combined with elevated temperatures, reduce the photosynthetic rates of *Symbiodinium* (Fitt et al 2001). On a smaller scale, microhabitat light differences may similarly impact *Symbiodinium* photosynthetic rates, with corals in more exposed microhabitats having lowered photosynthetic rates to prevent damage to photosystem II. In the present experiment, DLI did not vary significantly among tanks, but there were some significant differences between tanks on a factor level (Figure 8). I hypothesized that these differences may be influencing PAM yields, and the effect of treatment may be stronger if DLI was more consistent among tanks. There was a marginally significant relationship between high variation in light and high variation in photosynthetic rates in *P. daedalea* (Figure 9). However, the pattern did not conform to expectations as greater
photosynthetic variance was associated with reduced light variance across tanks. In addition, this pattern was not observed with *A. loripes* (Figure 9), which suggests that differences in photosynthetic yields among species and treatments was not a factor of varying environmental conditions.

**CONCLUSION**

The observed decreases in photosynthetic rates of *A. loripes* and *P. daedalea* likely represent stress responses of the corals to elevated conditions of water temperature and pCO$_2$, with the most extreme responses seen in the conditions predicted for the end of the century. *P. daedalea* had higher photosynthetic rates than *A. loripes*. Since the genotypes of both species were dominated by clade C *Symbiodinium*, species differences may be due to differences in specific types of clade C *Symbiodinium*, host factors, or a combination of the two. Future research into the stress responses of corals associated with different *Symbiondiminium* types will further our knowledge of the likelihood of coral acclimatization to the changing environmental conditions predicted as a result of climate change and which corals may be most successful.
LITERATURE CITED


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Appendix A
Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = DL1 ~ tank, data = DL1dat)
Linear Hypotheses:

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|----------|
| 2 - 1 == 0 | 1.3620 | 0.2408 | 5.656 | <0.01 *** |
| 3 - 1 == 0 | 0.5120 | 0.2408 | 2.126 | 0.4636 |
| 4 - 1 == 0 | 1.1520 | 0.2408 | 4.784 | <0.01 *** |
| 5 - 1 == 0 | 0.6660 | 0.2408 | 2.766 | 0.1429 |
| 6 - 1 == 0 | -0.0520 | 0.2408 | -0.216 | 1.0000 |
| 7 - 1 == 0 | -0.1680 | 0.2408 | -0.698 | 0.9987 |
| 8 - 1 == 0 | 1.3220 | 0.2408 | 5.490 | <0.01 *** |
| 9 - 1 == 0 | 1.4360 | 0.2408 | 5.963 | <0.01 *** |
| 3 - 2 == 0 | -0.8500 | 0.2408 | -3.530 | 0.0190 * |
| 4 - 2 == 0 | -0.2100 | 0.2408 | -0.872 | 0.9938 |
| 5 - 2 == 0 | -0.6960 | 0.2408 | -2.890 | 0.1067 |
| 6 - 2 == 0 | -1.4140 | 0.2408 | -5.872 | <0.01 *** |
| 7 - 2 == 0 | -1.5300 | 0.2408 | -6.354 | <0.01 *** |
| 8 - 2 == 0 | -0.0400 | 0.2408 | -0.166 | 1.0000 |
| 9 - 2 == 0 | 0.0740 | 0.2408 | 0.307 | 1.0000 |
| 4 - 3 == 0 | 0.6400 | 0.2408 | 2.658 | 0.1797 |
| 5 - 3 == 0 | 0.1540 | 0.2408 | 0.640 | 0.9993 |
| 6 - 3 == 0 | -0.5640 | 0.2408 | -2.342 | 0.3297 |
| 7 - 3 == 0 | -0.6800 | 0.2408 | -2.824 | 0.1247 |
| 8 - 3 == 0 | 0.8100 | 0.2408 | 3.364 | 0.0307 * |
| 9 - 3 == 0 | 0.9240 | 0.2408 | 3.837 | <0.01 ** |
| 5 - 4 == 0 | -0.4860 | 0.2408 | -2.018 | 0.5357 |
| 6 - 4 == 0 | -1.2040 | 0.2408 | -5.000 | <0.01 *** |
| 7 - 4 == 0 | -1.3200 | 0.2408 | -5.482 | <0.01 *** |
| 8 - 4 == 0 | 0.1700 | 0.2408 | 0.706 | 0.9986 |
| 9 - 4 == 0 | 0.2840 | 0.2408 | 1.179 | 0.9587 |
| 6 - 5 == 0 | -0.7180 | 0.2408 | -2.982 | 0.0847 . |
| 7 - 5 == 0 | -0.8340 | 0.2408 | -3.463 | 0.0230 * |
| 8 - 5 == 0 | 0.6560 | 0.2408 | 2.724 | 0.1553 |
| 9 - 5 == 0 | 0.7700 | 0.2408 | 3.198 | 0.0490 * |
| 7 - 6 == 0 | -0.1160 | 0.2408 | -0.482 | 0.9999 |
| 8 - 6 == 0 | 1.3740 | 0.2408 | 5.706 | <0.01 *** |
| 9 - 6 == 0 | 1.4880 | 0.2408 | 6.179 | <0.01 *** |
| 8 - 7 == 0 | 1.4900 | 0.2408 | 6.187 | <0.01 *** |
| 9 - 7 == 0 | 1.6040 | 0.2408 | 6.661 | <0.01 *** |
| 9 - 8 == 0 | 0.1140 | 0.2408 | 0.473 | 0.9999 |

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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)