INVESTIGATING THE EFFECTS OF HIGH LIGHT VERSUS LOW LIGHT IN TWO DIFFERENT TYPES OF *SYMBIODINIUM* WITHIN THE REEF-BUILDING CORAL, *ACROPORA MILLEPORA*

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science Marine Science with Distinction

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ABSTRACT

Coral reefs are invaluable ecosystems upon which a massive number of coastal organisms rely, including human communities. The reefs themselves are composed primarily of colonial organisms, and the vast majority of their energetic budget is provided by glucose-rich photosynthate derived from their endosymbiotic dinoflagellates (genus *Symbiodinium*). Here, two different types of *Symbiodinium* were investigated, *Symbiodinium* C1 and D3, within the same species of Indo-Pacific coral, *Acropora millepora*, which was acclimated to either high- (1000 μmol quanta m^{-2} s^{-1}) or low-light (100 μmol quanta m^{-2} s^{-1}) conditions. Samples were analyzed by means of traditional coral metrics, biochemical composition, and targeted gene expression in order to gauge differences in photoacclimation mechanisms between the two symbiont types, as well as to better understand each alga's respective role in holobiont health and photobiology. The coral harboring the D3 symbiont had higher levels of protein and carbohydrate, as well as increased expression for genes that encode an intracellular carbonic anhydrase, GAPDH, and glutamine synthetase, all of which were higher under the high-light treatment. In contrast, extracellular carbonic anhydrase expression was greater in the coral hosting the C1 symbiont, under high-light-acclimation. Average chlorophyll a concentration was greater in *Symbiodinium* C1 than D3, and the photosynthetic and respiratory data suggest that the coral hosting *Symbiodinium* D3 may have a higher respiratory demand. These findings, in conjunction with previous studies, provide a continued groundwork for understanding

both symbiont diversity and the role that members of *Symbiodinium* play in the health of their host.

Chapter 1

INTRODUCTION

1.1 Coral Reefs

Coral reefs serve as a source of shelter and nutrients, as well as a central location of biodiversity in tropical locations. Humans benefit from coral reefs both directly and indirectly through the construction of successful fisheries, a natural coastal barrier to storm damage, and the inherent attractiveness of the reefs that supports substantial ecotourism (Molina-Dominguez et al., 2007; Graham and Nash, 2013). Environmental and anthropogenic pressures alike continue to threaten reefs on a global scale. Calcification by healthy scleractinian corals is integral for the continual growth and maintenance of reefs, but such high rates of skeletal accretion are energetically costly for corals. For most scleractinian corals, much of the required energy for growth comes from glucose-rich photosynthate derived from symbiotic dinoflagellate algae (genus *Symbiodinium*) living within their gastrodermal cells (Muscatine et al., 1984; 1989; Muscatine and Kaplan, 1994), and calcification rates tend to correlate with photosynthetic rates of the symbionts. The dinoflagellates benefit from nutrients originating from the nitrogenous waste of the host, as well as protection from predators in a region within the euphotic zone for optimal photosynthetic activity (Figure 1). Importantly, these symbiotic dinoflagellates represent highly diverse taxa with complex associations with many host species (Coffroth and Santos, 2005). Understanding how symbiont diversity affects the holobiont's (i.e. the host coral and resident symbionts) overall physiology will be

critical in predicting the future of coral reefs as they face increasing global and regional environmental stressors.

Figure 1. A myriad of *Symbiodinium* (in greenish-brown) within the translucent tissues of coral polyps (http://ocean.si.edu/ocean-life-ecosystems/coral-reefs-0)

1.1.1 Symbiosis with the Endosymbiont *Symbiodinium spp.*

The genus *Symbiodinium* is a highly diverse group of dinoflagellates separated into nine clades (A-I), and contains hundreds of individual species as identified through analysis of the internal transcribed spacer 2 (ITS2) region of the ribosomal array (LaJeunesse 2001). Further, the use of microsatellite markers allow for even greater resolution into sub-species or clonal variants (Pettay and LaJeunesse, 2009). Our understanding of the genetic diversity of *Symbiodinium* has progressed much further than that of the physiological diversity. Importantly, the dinoflagellate-coral

symbiosis itself is highly diverse, with some symbiont types expressing host specificity, while others are found in multiple hosts (Sampayo et. al, 2009). Many of the associations can be highly variable, and are often associated with different environmental parameters such as depth, temperature, and coral life history. Likewise, symbiont distribution can exhibit distinct patterns by depth which in turn influences the vertical distribution of its host coral species (Iglesias-Prieto et al., 2004). Thermal tolerance is an important physiological aspect which has been a central focal point for understanding *Symbiodinium* diversity as it pertains to climate change and, in particular, to global warming (Fisher et al., 2012; Kemp et al., 2014). Less thermally tolerant symbiont types can undergo photo-damage during high-temperature events, triggering the host to expel the symbionts in a process known as coral bleaching, leading to potential tissue loss and/or mortality (Baker et al., 2008). However, thermally tolerant symbiont types are able to withstand high temperature stress, thereby reducing the susceptibility to coral mortality during these high temperature events. *Symbiodinium trenchii,* is a particularly well studied clade D symbiont, important for its thermal tolerance and global distribution (Ladner et al., 2012; LaJeunesse et al., 2014). Variants of *Symbiodinium trenchii* can be found within both Caribbean and Pacific coral species, making them a particularly important species for study. Several other clade D symbionts are thermally tolerant, thereby placing greater attention on the clade and its potential importance under future climate change scenarios {IPCC, 2013, #85156}. By understanding the characteristics, genetics, and distribution of these symbionts, marine scientists can better understand the changes likely to happen in the coming years, thereby creating a stronger knowledge base from which to aid reef systems and coastal ocean health.

Although cnidarian-algal symbioses are heavily studied, the endosymbiont also exhibits a mutualistic symbiosis in protists, poriferans, and molluscs (Coffroth and Santos, 2005). These associations further highlight the importance of *Symbiodinium spp.* as a significant contributor in the health of many organisms living in coastal and shallow-water ecosystems, especially in the context of global climate change and commonly-encountered, thoroughly-explored stressors such as thermal events and ocean acidification (Bertucci et al., 2011; Leggat et al., 2002).

1.2 Photoacclimation

Light has been intensely studied for years, both as critical to the productivity of corals and as a possible environmental stress factor that must be dealt with on a daily basis. Because light is such a dynamic resource in shallow waters, *Symbiodinium* must constantly adjust and reacclimatize to optimize photosynthetic activity (Hennige et al., 2008). Photoacclimation is central to coral reef biology due not only to the implications that varying acclimation strategies have on the host coral's resources, but also as it relates to a corals ability to withstand additional environmental stress.

The effect of light on coral reefs is one of the oldest aspects of coral reef research, with its positive effect on calcification rates being noted as early as the mid-20th century (Chalker and Taylor, 1975). Coral calcification rates at peak day-light have been shown to increase as much as three times when compared with calcification rates at night (Moya et al., 2006). The increased light the holobiionts experience during the day allows the dinoflagellates to photosynthesize at a higher rate, thereby providing the host with higher levels of glucose-rich photosynthate to drive several metabolic processes (Dubinsky and Stambler, 2009; Kuguru et al., 2010). When considering the variation in PSII reaction centers that coincides with changes in light

intensity, high light yields a lower number of reaction centers, while low light increases that number, paralleling the changes in chlorophyll content within symbionts (Moore et al., 2006). This balance between light-harvesting compounds and photosynthetic reaction centers is indicative of traditional photoacclimation methods in marine phytoplankton. By decreasing the amount of chlorophyll under high light, the symbionts are able to efficiently photosynthesize without the need for extra pigments, creating space in the holobiont's energetic budget for other processes (Roth, 2014). Temperature plays a compounding role in that the PSII reaction center goes under intense stress when exposed to a high temperature anomaly. Its failure causes inhibited photosynthesis, which in turn leads to a physiological collapse of the holobiont (Warner et al., 1999). This is just one of many factors that contribute to the dynamic nature of light utilization by coral reefs.

The reasoning behind the absence of a great number of light-harvesting compounds under high light becomes even more evident when considering the exposure that *Symbiodinium* encounters within the translucent tissue of a host coral. The inherent skeletal structure of the coral's polyp network serves as a scattering base for light, providing the endosymbionts with light exposure from nearly every angle (Enriquez et al., 2005). This maximizes the light utilization capabilities of the symbiont, and consequently the holobiont, which in turn reduces the need for more light-harvesting complexes, as the light level recorded inside the coral is higher than the ambient level (Enriquez et al., 2005).

This present experiment sought to understand the photoacclimation effects on biochemical composition and metabolism in a host coral species and its symbionts using both *Symbiodinium* C1 and the thermally-tolerant *Symbiodinium* D3. To clearly

see the effect that the two different symbionts have on the same host, a wide range of holobiont factors were measured for which the experiment of growing corals under two different light levels was assessed. The host coral, *A. millepora,* is a common Indo-Pacific species that has been well studied, thereby making it an ideal model system for this study (Kortschak et al., 2003; Miller et al., 2000). A multi-tiered approach was used involving analyses at the molecular, cellular, and organismal level in order to understand the effects of housing the two distinct symbiont types on both the macro and micro level. Investigating how these two symbiont types adjust to a dynamic light environment will galvanize research into photoacclimation, not only as a new proxy by which to differentiate *Symbiodinium* subtypes, but also as a way to predict biochemical changes in host coral colonies as their respective familiar habitats continue to undergo change on a global scale.

Chapter 2

Methods and Materials

2.1 Experimental Setup

Colonies of *Acropora millepora* were collected in May of 2011 at a depth of five meters in northwest Fiji and housed in the Reef Systems Coral Farm (New Albany, OH) in an outdoor greenhouse, which utilized ambient light conditions and synthetic seawater. Two colonies, one housing *Symbiodinium C1* and a second with *Symbiodinium D3,* were each divided into ten fragments for a total of 20 fragments. The corals were acclimated to high light (1000 μmol quanta m^{-2} s^{-1}) within the greenhouse for roughly 16 months prior to the start of the experiment. During the experiment, which was carried out in October of 2012, all 20 fragments were housed within the same experimental system and were divided into two sections, with one section being heavily shaded to create a low light (100 μmol quanta m^{-2} s^{-1}) environment. Five corals of each host-symbiont combination were placed underneath the shaded area, with the other five fragments left at full exposure to the natural sunlight. All samples were left under experimental conditions for a total of 18 days. In order to minimize any tank effects resulting from the experimental conditions, corals were periodically moved within their low or high light sections. Temperature was kept constant throughout the experiment to minimize any possible temperature effects, and the corals experienced moderate water movement to prevent a stagnant environment. On day 18, all fragments were flash frozen in liquid nitrogen and stored at -80^{o}C for future analysis.

2.2 Traditional Coral Metrics

2.2.1 Photosynthesis and Respiration

On day 17, maximal photosynthetic rates and light acclimated dark respiration (R_L) were measured for all 10 fragments per host/symbiont combination via oxygen evolution and consumption with galvanic electrodes (Qubit systems) housed in clear acrylic chambers (350 mL). Chambers were surrounded by a water bath to maintain the control and experimental temperature. Constant circulation was provided by a stirbar in each chamber. Illumination was supplied by a customized 24 LED array (Cree Cool White XP-G R5). Photosynthesis (P_{net}) was recorded for 15-20 minutes, at eight different light intensities (20, 50, 80, 120, 250, 400, 800 μmol quanta m^{-2} s^{-1}) followed by a 10-minute dark incubation to record the light acclimated dark respiration (R_L). The photosynthesis to respiration ratio was calculated as ($Pmax_{gross}$)/(R_L) where $Pmax_{gross} = (Pmax_{net} - R_L)$. Pmax was recorded at 800 μmol quanta m^{-2} s^{-1}. Net photosynthesis at each light step, along with light acclimated dark respiration (R_L) was normalized to total surface area (cm^2) for each coral fragment (described below).

2.2.2 Sample Processing

To collect combined host and algal tissues from each individual coral fragment, tissue was removed using the water pick method described in Johannes and Wiebe (1970). The resulting slurry, containing both host and symbiont tissues, was collected and then stored separately at -80°C. Skeletal surface areas were calculated using methods established by Stimson and Kinzie (1991). Briefly, the newly-exposed skeletons of the fragments were weighed to establish a dry weight in grams. They were submerged into paraffin wax, allowed to dry, and weighed again. The difference

between these two weights was calculated for each of the samples and compared against a standard curve in order to quantify a surface area for the skeleton in mm^2. The standard curve was constructed utilizing wooden blocks of various sizes and known surface areas.

2.2.3 Cell Volume and Density

Algal cell density and volume were analyzed by fluorescence microscopy (light: 100, exposure: 45 ms, gain: 10.8 dB). The mixed holobiont samples were pipetted onto the two sides of three hemocytometers, totaling six hemocytometer counts. Under bright field conditions and 20x magnification, the microscope was focused onto random areas of the hemocytometer to alleviate bias in choosing areas of imaging. The light field was switched to a wavelength of 655 nm (cube GD655), and two images were taken for a total of 12 images for each sample. These images were loaded into an ImageJ script that, accounting for circularity and fluorescence parameters, processed all of the images per a known area to give cell densities, as well as mean cell volumes per sample.

2.2.4 Chlorophyll a

All chlorophyll a extractions were performed under indirect ambient light to prevent degradation. The remaining symbiont portion from each sample was centrifuged for five minutes at 7000 rpm. The samples were then broken in 1 mL of 90% methanol and glass beads for one minute in a bead-beater (BioSpec, Inc.). The samples were then incubated for two hours in the dark at -20°C. After freezing, samples were centrifuged again to remove cellular debris. Chlorophyll a was quantified in a 96-well plate (two technical replicates per sample) at wavelengths of

652, 665, and 750 nm in a FLUOstar Omega plate reader (BMG Labtech) (Jeffrey and Humphrey, 1975).

2.3 Biochemical Composition

2.3.1 Protein

Protein was quantified for the both the host and algal samples with the BCA protein protocol (Smith et al., 1985), with bovine serum albumin as the standard. Sample absorbance (540 nm) was read in a FLUOstar Omega plate reader (BMG Labtech).

2.3.2 Carbohydrates

For carbohydrate analysis, host and symbiont samples were homogenized with a bead-beater for 1.5 minutes before being extracted via the sulfuric acid/phenol protocol, using glucose as a standard as established by Dubois et al. (1956). The FLUOstar Omega plate reader (BMG Labtech) measured the absorbance of the samples at 485 nm.

2.4 Targeted Gene Expression

2.4.1 Gene Selection

The genes targeted in host and symbiont were chosen due to their respective roles in metabolism, including carbon metabolism (glyceraldehyde-3-phosphatedehydrogenase), carbon acquisition (intracellular and extracellular carbonic anhydrase), nitrogen metabolism (glutamine synthetase), and glycogenolysis (glycogen phosphatase). Additionally, two housekeeping genes commonly used in conjunction with cnidarian holobiont genetics (ribosomal protein S7, elongation factor

1-alpha) were examined in order to normalize the findings of the target genes (Seneca et al., 2010).

2.4.2 RNA Extraction

RNA was extracted from the samples and thoroughly purified using the TRIzol reagent (Invitrogen) and the Aurum Total RNA Mini-Kit (Bio-Rad). These samples were qualified and quantified using spectrophotometry (NanoDrop 2000, Thermo Scientific). For each sample, 200 ng of RNA went into each cDNA reaction using a high capacity cDNA Reverse Transcription Kit (Applied Biosystems).

2.4.3 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

All qRT-PCR reactions were done in 96-well plates covered with optical film. The reactions were monitored using an ABI Prism 7500 Sequence Detection System (Applied Biosystems), as well as a SensiMix real time detection system with 2X SYBR HI-ROX Mastermix (BIOLINE). Each well housed a 10-µL reaction containing 0.2 µL of 1:10 diluted cDNA and 0.8 of a master mixture specific to the target gene in question. This mixture was comprised of 430 µL of 2X SYBR HI-ROX Mastermix (BIOLINE), 43 µL each of the forward and reverse primers for the target gene (refer to Table 1 for sequences), and 172 µL of nuclease-free water. Within each plate, all eight standards were run in triplicate while samples were run in duplicate. Additionally, there were six wells of no template control (standard sans RNA transcriptase) that were included in every run.

Table 1. Forward and reverse primer sequences, efficiencies, and GenBank accession, citation, or contig ID numbers for the target genes.

Gene ID	Forward (5'-3')	Reverse (5'-3')	Efficiency	Accession number, citation or contig ID number
A. millepora				
GAPDH	ACCATCCATGCTTACACTGCGACA	AGGAATCACCTTTCCCACAGCCTT	100.5	EZ026309.1
CA-IN	GGCAAAGAAATACAAGTTCGAGC	TGTGTCTCGCAATCCCAATG	106.2	EU863783.1
CA-EX	TCGGTGAAGATTGGAGTTACAG	AGTTGGTCAAGGTGAAGCTC	103.5	EU863782.1
RP-s7	AGCAAAGGAGGTTGATGTGG	GACGGGTCTGGATCTTTTGA	107.7	Seneca 2010
EF1-a	TGGCTTTTGTACCTATCTCTGG	TTGTCCAGTGCGTCGATAAG	107.7	GO003400
GluS	ACCTTCCAAGCAGAAGGCTGCAAT	TCCAGGCTTGTGGTCGAAGTTGAA	99.7	DY579366.1
GlyP	TGGATGGAGCCAACGTGGAAATGA	TTGCCCTGAGCGCATTCACTTCTT	99.7	EZ031953.1

2.5 Statistical Analysis

All data sets were tested for homoscedasticity using the Bartlett test, and subsequently were tested for normality using the Shapiro-Wilk test. Statistical analyses were performed using R with statistical significance set at $p \leq 0.05$. Two-way analysis of variance (ANOVA) was utilized, using the Kruskal-Wallis test if rules for normality and homoscedasticity were violated. Post-hoc testing was done using the Tukey-LSD test.

Chapter 3

Results

3.1 Traditional Coral Metrics

3.1.1 Photosynthesis and Respiration

Five facets of the gas exchange data were analyzed: a low-light photosynthetic productivity step (20 μmol quanta m^{-2} s^{-1}), a high-light photosynthetic productivity step (800 μmol quanta m^{-2} s^{-1}), respiration, the photosynthesis-respiration (P:R) ratio, and light-enhanced dark respiration (LEDR). At the low-light productivity step, there was a significant light effect on the two symbiont strains ($p = 0.0007$) in that only low-light conditions yielded positive photosynthetic rates; however, there was no noticeable effect at the high-light level to differentiate the two (Figure 2).

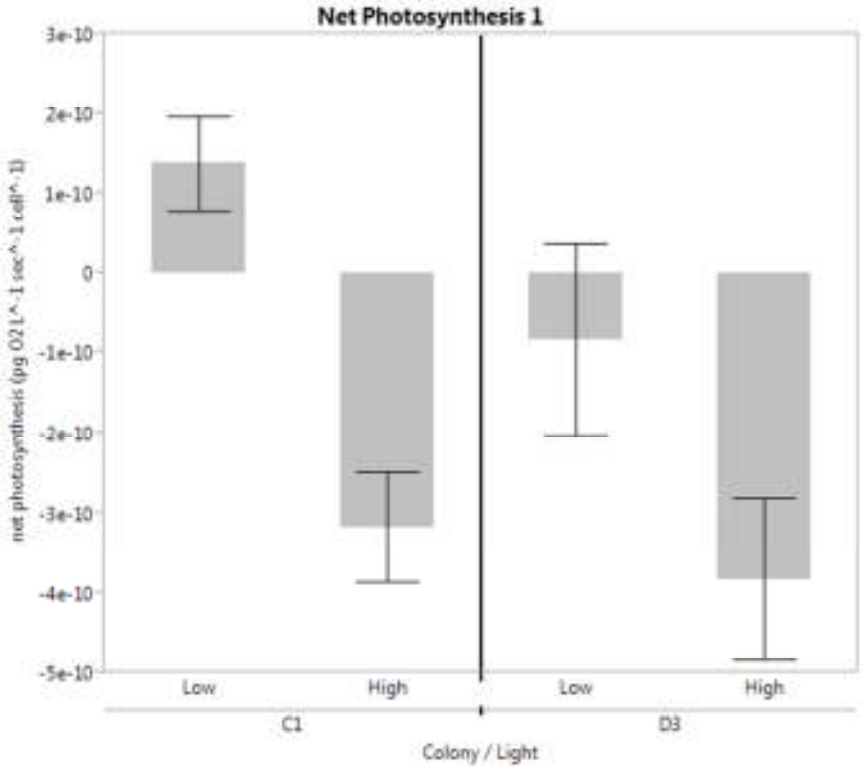

Figure 2. Net photosynthesis normalized to number of cells for low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

There was no effect on the respiration patterns between the two symbiont types, but there was a noticeable light effect on the P:R ratio. Although not statistically significant ($p = 0.07$), the P:R appeared to decrease under high-light conditions (Figure 3). Further investigation is needed to determine the true significance of light on the P:R between these two symbiont types.

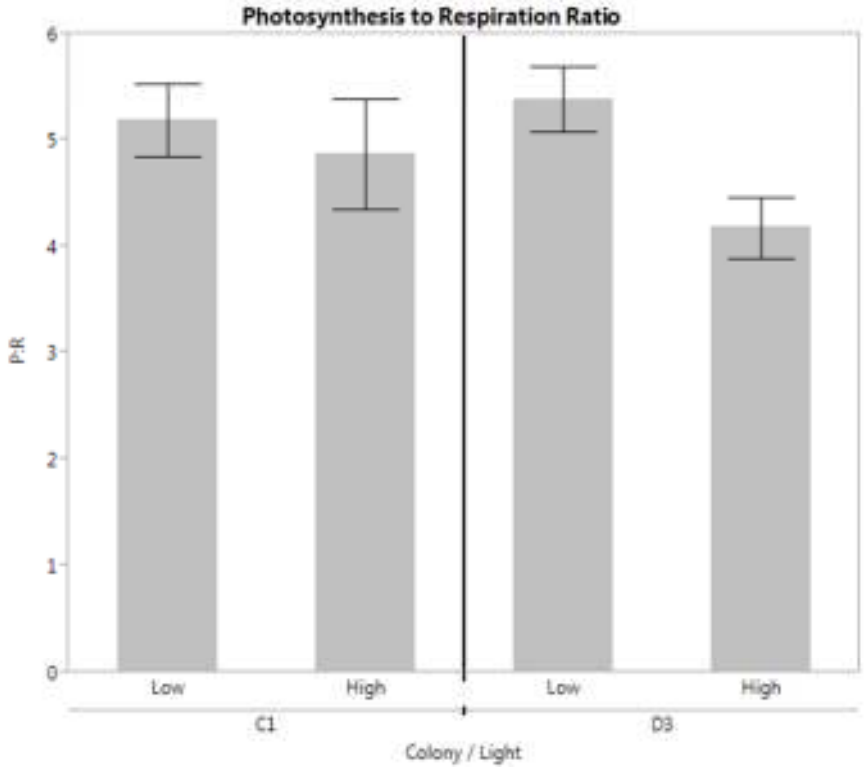

Figure 3. P:R ratios for low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

Additionally, there was a significant light effect ($p = 0.020$) on the light-enhanced dark respiration (Figure 4). Higher light levels produced LEDR rates of a greater magnitude than low-light conditions.

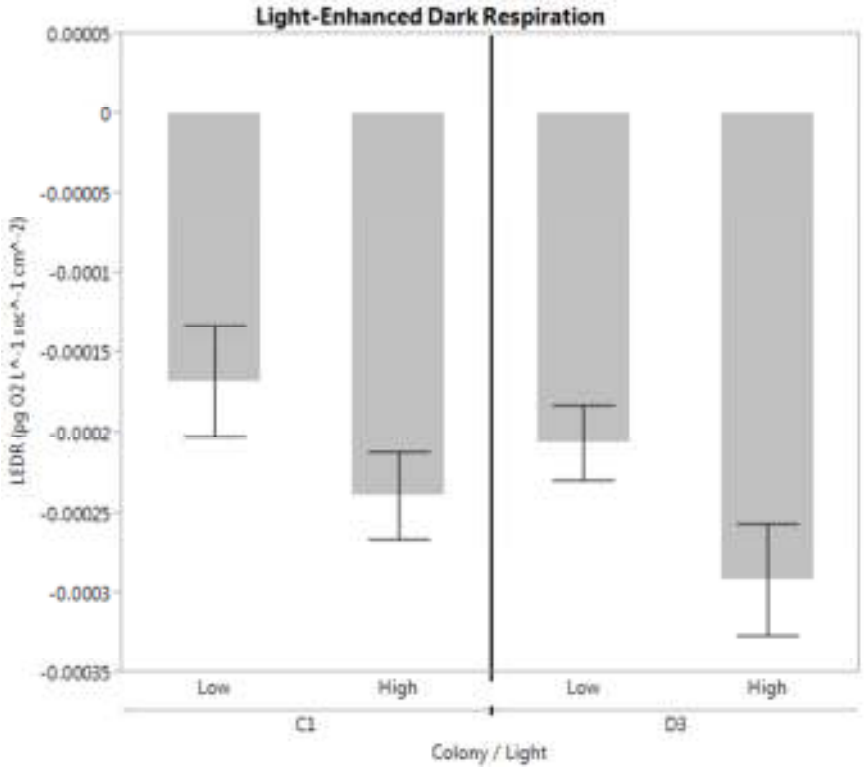

Figure 4. Light-enhanced dark respiration (LEDR) normalized to area for both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

3.1.2 Cell Volume and Density

In regards to cell volume, there was no noticeable light effect between the high (1000 µmol quanta m^{-2} s^{-1}) and low light (100 µmol quanta m^{-2} s^{-1}) levels. The data suggested there was a symbiont type effect and that the mean algal cell volume was greater in symbiont C1 than symbiont D3; however these data were found to not be statistically significant. There was no significant effect from either light level or symbiont type on the cell densities of *Symbiodinium* C1 and D3.

3.1.3 Chlorophyll a

Both light conditions (p = 0.033) and symbiont type (0.041) had a significant effect on the chlorophyll a concentrations of the C1 and D3 symbionts (Figure 5). The lower light intensity of 100 μmol quanta m^{-2} s^{-1} yielded a higher chlorophyll a concentration in both symbiont types. At both high and low light, symbiont type C1 had greater concentrations of chlorophyll a than did symbiont type D3.

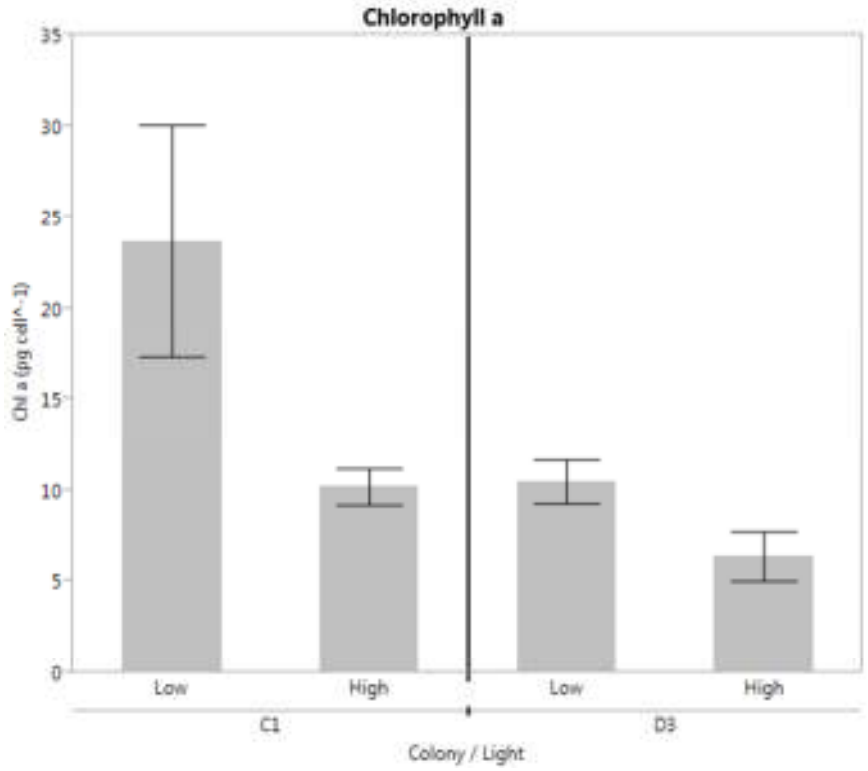

Figure 5. Chlorophyll a concentrations normalized to number of cells for both low-light and high-light acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

3.2 Biochemical Composition

Animal protein exhibited a strong interactive effect, as indicated by the Kruskal-Wallis test ($p = 0.029$). In the coral hosting symbiont type C1, protein concentrations decreased under the low-light conditions ($p = 0.044$) (Figure 6). Additionally, there was a significant colony effect on the host protein concentrations ($p = 0.050$) with host samples housing symbiont type D3 having higher protein levels than those housing symbiont type C1 (Figure 6). Symbiont protein concentrations showed no significant changes between either symbiont types or light levels.

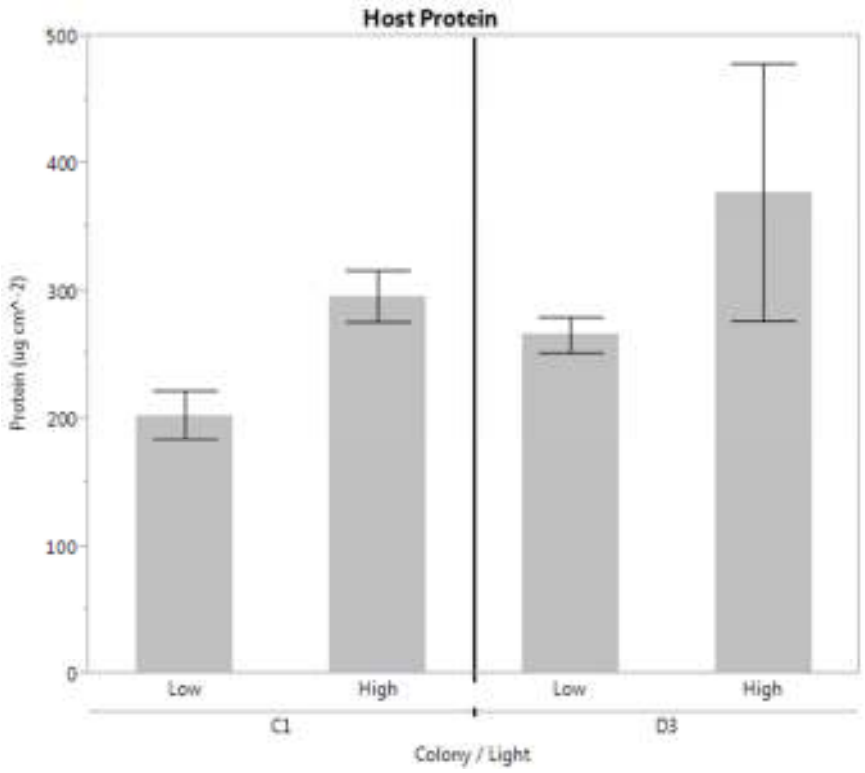

Figure 6. Host coral protein concentrations normalized to area for both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

The differences in host carbohydrate concentration contrasted significantly by both symbiont type ($p = 0.004$), and light intensity ($p < 0.0001$) (Figure 7). Low-light conditions yielded much lower carbohydrate concentrations in both the animal and symbiont when compared with the high-light conditions. Similar to the protein concentrations, the mean carbohydrate concentration in the host coral harboring the D3 symbiont was significantly higher than that of the colony with C1.

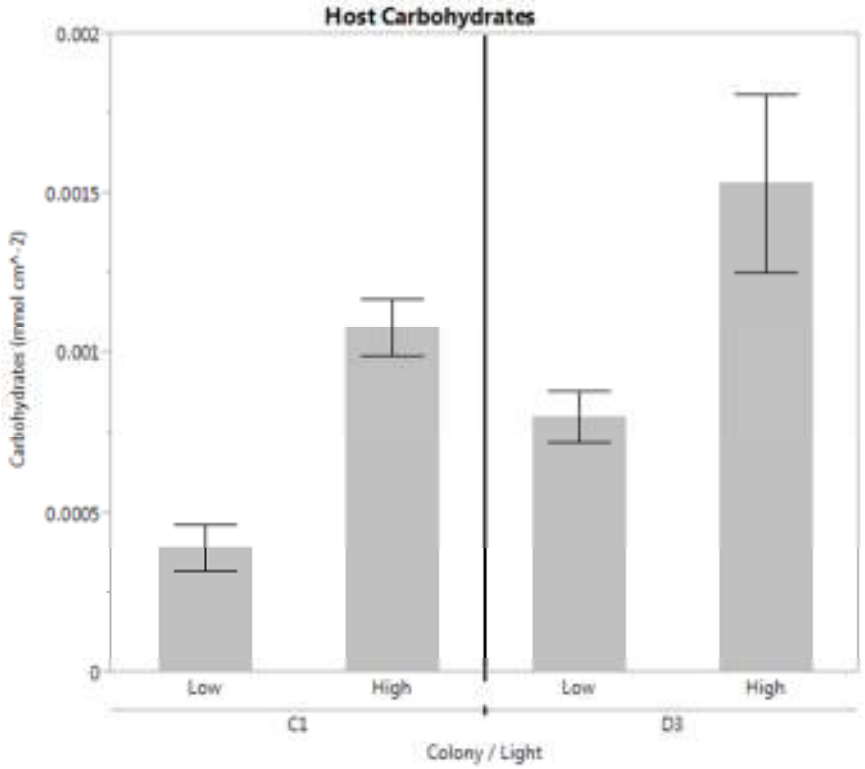

Figure 7. Host coral carbohydrate concentrations normalized to area for both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

Regarding carbohydrate levels within the algal symbionts, there was a noticeable difference between light conditions. Although not statistically significant ($p = 0.06$), carbohydrate levels within both symbiont types appeared to be higher under high-light conditions similar to the host (Figure 8).

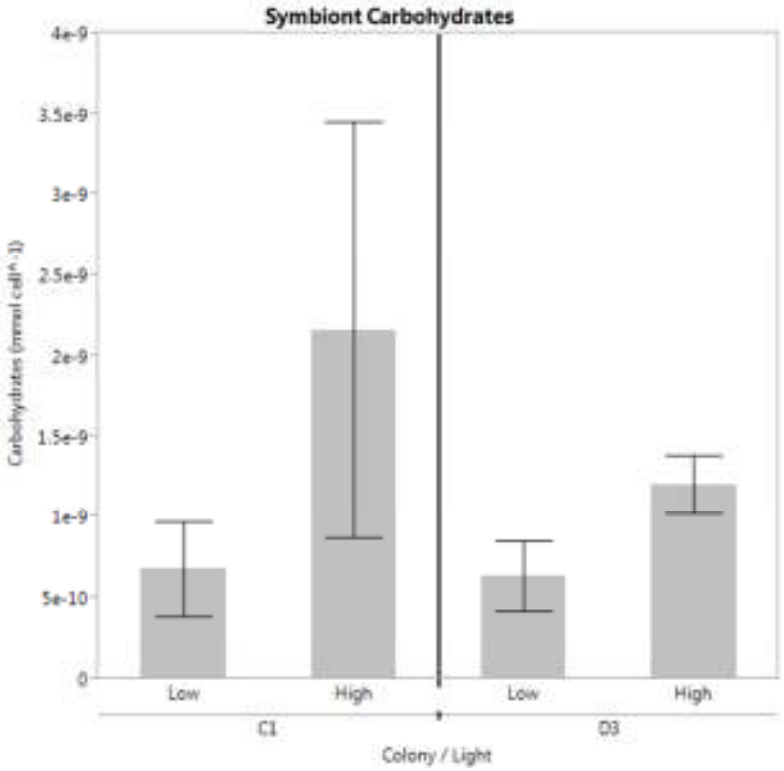

Figure 8. Algal symbiont carbohydrate concentrations normalized to number of cells for both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

3.3 Targeted Gene Expression

Quantified expression of the intracellular carbonic anhydrase showed significant difference between symbiont types ($p = 0.005$), but not between light levels

(Figure 9). The expression of intracellular carbonic anhydrase was higher at both light levels in host fragments containing symbiont D3 than it was in symbiont C1.

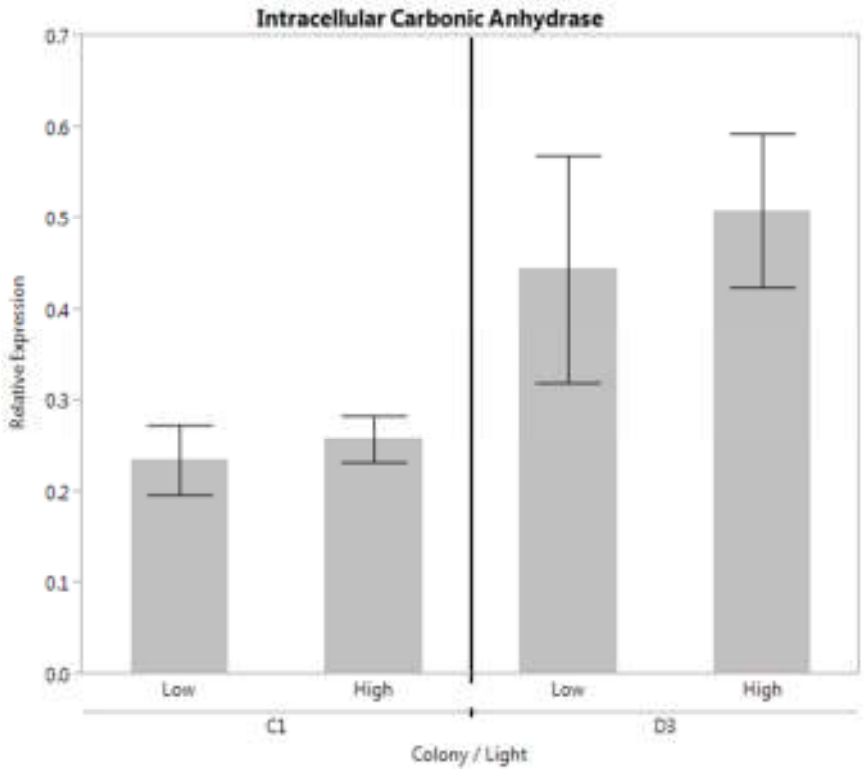

Figure 9. Relative expression of intracellular carbonic anhydrase (InCA) in both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

Extracellular carbonic anhydrase expression showed a noticeable, but not significant, difference between symbiont types ($p = 0.057$), as well as a significant interactive difference ($p = 0.029$) (Figure 10). The expression of extracellular carbonic anhydrase was greater in C1 than in D3, but the fragments housing symbiont C1 under high-light

conditions had much greater levels of expression than their low-light counterparts or either of the two D3 light levels.

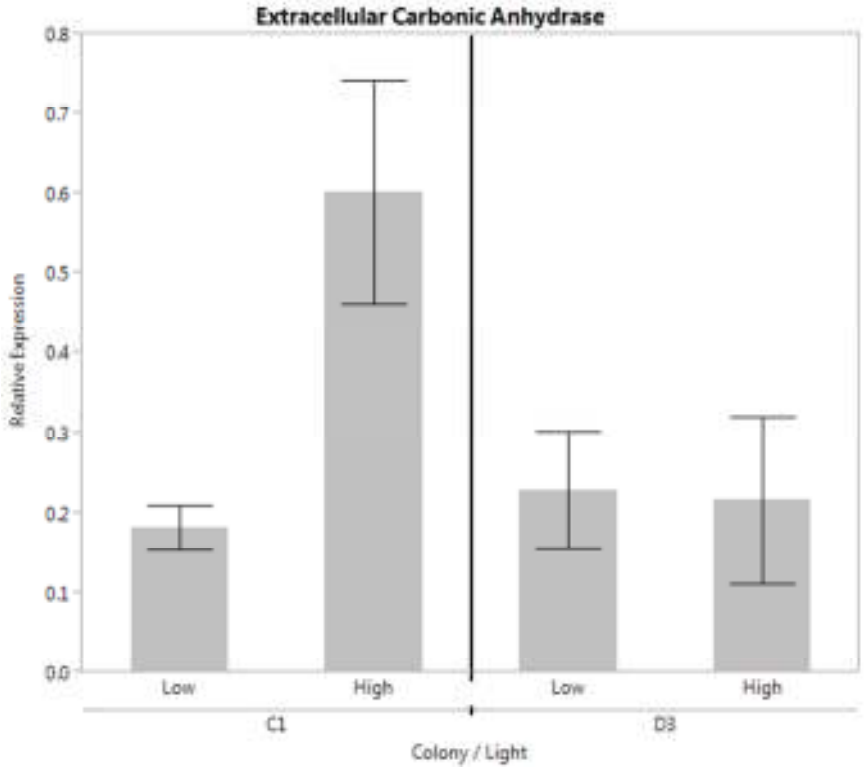

Figure 10. Relative expression of extracellular carbonic anhydrase (ExCA) in both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

The expression of glyceraldehyde-3-phosphate dehydrogenase exhibited light ($p = 0.004$), symbiont type ($p = 0.03$), and interactive ($p = 0.04$) differences (Figure 11). The mean expression was higher in symbiont type D3 than in C1, and there was increased expression at high-light levels, albeit solely in symbiont D3. Light had no

significant effect on the expression of glyceraldehyde-3-phosphate dehydrogenase in symbiont type C1.

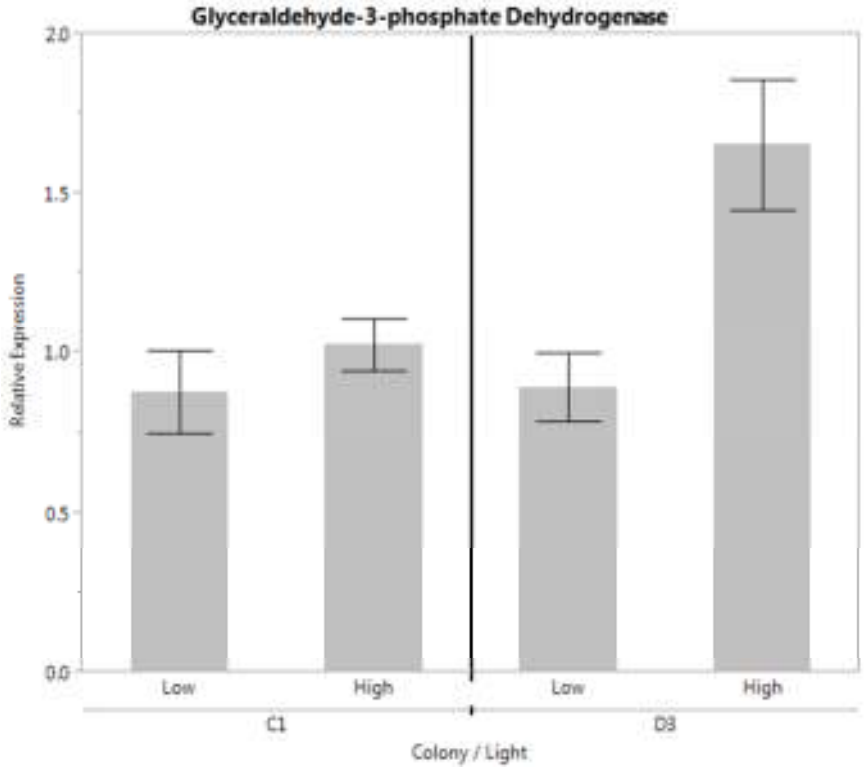

Figure 11. Relative expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

Similarly, glutamine synthetase exhibited significant symbiont type ($p = 0.005$) and light ($p < 0.0001$) effects (Figure 12). Once again, the relative expression of the gene was higher in symbiont type D3, and the light-induced increase was at the high-light level of D3. There was no significant light effect in symbiont type C1. Additionally,

there was no significant effect from either light or symbiont type regarding glycogen phosphatase.

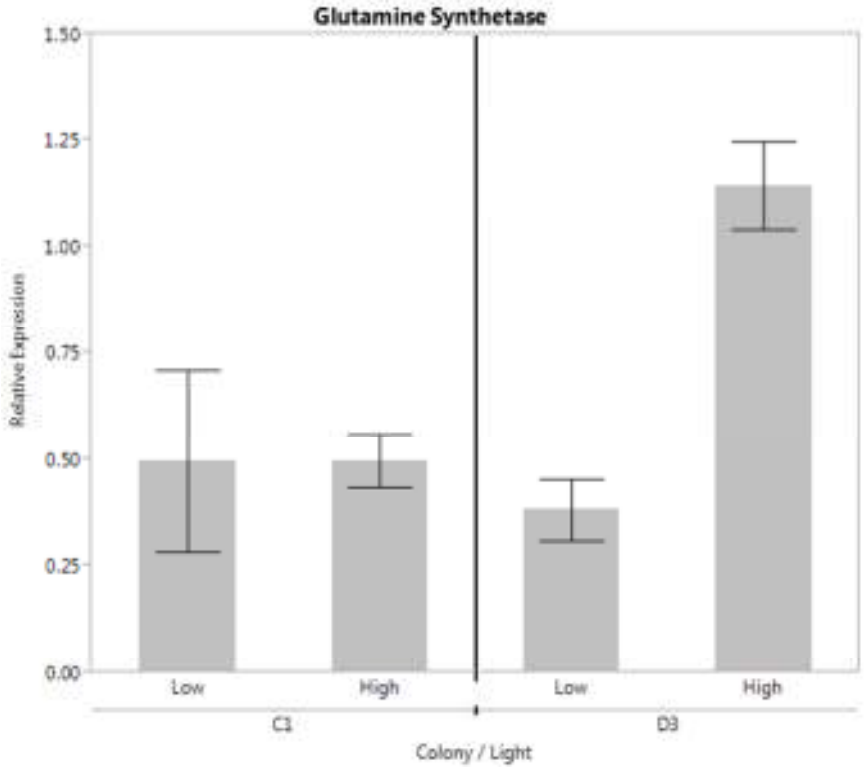

Figure 12. Relative expression of glutamine synthetase in both low-light and high-light-acclimated corals harboring C1 and D3 symbionts (n=5 ±SE)

Chapter 4

Discussion

The primary goal of this project was to investigate photoacclimation in two different types of *Symbiodinium* within the same host coral species. Using these data, the respective roles that these endosymbiotic dinoflagellates play in holobiont health and photophysiology could then be explored.

4.1 Photosynthetic Capabilities

Under high-light conditions, the concentration of light-harvesting pigments such as chlorophyll typically decreases alongside the concentration of photosynthetic reaction centers (Kuguru et al., 2010). As expected, the inverse of this also holds true (Levy et al., 2003). In a biological case of supply and demand, the need for higher levels of light-harvesting pigments increases in low light because the greater number of pigments allows the organism to collect as much of the minimal light as possible. The organism's increased light-capturing ability promotes more reaction centers, such as PSII, in order to rapidly utilize and convert the copious amount of light to which the pigments are being exposed. In samples acclimated to the lower of the two light levels, there was a positive photosynthetic response, while those same low-light samples exhibited higher concentrations of chlorophyll a than their high-light counterparts. These findings are in direct agreement with those reported in the literature; however, symbiont C1 contained significantly greater chlorophyll a concentrations than the D3 symbiont, indicating that the C1 symbiont may have greater flexibility in altering

light-harvesting pigment levels under low-light conditions relative to the D symbiont. Much work has been done delineating the different drivers and mechanisms of symbiont diversity (Coffroth and Santos, 2005; Sampayo et al., 2009; LaJeunesse et al., 2003), but the primary emphasis has been on molecular analysis and genotypic variations as opposed to phenotypic differences such as chlorophyll a concentrations. At 800 μmol quanta m^{-2} s^{-1}, there were no significant changes based on light acclimation or symbiont type. In the low-light photosynthetic step, samples acclimated to high-light conditions could detect the minimal light levels, but they were producing less oxygen at this level than the amount they were respiring: that is, the O_2 production rate was less than the respiration rate. As the intensity of the light curve increased, those acclimated to high-light began to photosynthesize efficiently as the light intensity matched their low-pigment requirements. One might expect the samples acclimated to low-light to have possibly been photoinhibited at the high point of the light curve, but, unlike the high-light samples, these samples were flexible enough to manage the temporary exposure to high light. An increased exposure time may have resulted in the holobiont exhibiting stress, but the low-light acclimated samples appeared to be more robust regarding brief shifts to higher light levels. Neither algal cell volume nor algal cell density exhibited any changes, indicating that the holobiont relied solely upon their preexisting symbionts to photoacclimate rather than attempt to acquire symbionts with more light-harvesting pigments or reaction centers through horizontal transmission (Suzuki et al., 2013). However, this lack of change is contrary to what was observed in some research which suggested that symbiont cell density should decrease with depth (Table 2, Battey and Porter, 1988).

Table 2. Relationship of symbiont ("zooxanthellae") cell density, chlorophyll a concentration, and protein weight in the coral *Montastraea annularis* at varying depths (Battey and Porter, 1988).

Depth (m)	10^6 zoox cm^{-2} (N = 6)	µg chl *a* cm^{-2} (N = 9)	µg Chl c_2 cm^{-2} (N = 9)	pg Chl *a* $cell^{-1}$ (N = 6)	pg chl c_2 $cell^{-1}$ (N = 6)	Chl *a*/c_2 (N = 6)	mg protein cm^{-2} (N = 6)
0.5	2.45 ± 0.96	2.72 ± 0.26	0.48 ± 0.19	1.21 ± 0.32	0.24 ± 0.15	5.67	0.97 ± 0.07
10	1.65 ± 0.36	3.78 ± 0.63	2.48 ± 1.15	2.43 ± 0.54	1.62 ± 1.10	1.52	1.04 ± 0.08
20	1.10 ± 0.48	4.20 ± 1.30	3.34 ± 2.45	3.32 ± 0.57	2.77 ± 1.79	1.26	0.78 ± 0.06
30	1.17 ± 0.46	4.47 ± 1.23	2.79 ± 2.66	3.28 ± 0.28	2.86 ± 2.71	1.60	0.68 ± 0.03
40	1.55 ± 0.35	5.06 ± 0.84	3.96 ± 0.83	3.06 ± 0.06	2.49 ± 0.30	1.28	0.54 ± 0.11
50	1.83 ± 0.05	5.95 ± 0.44	6.61 ± 2.67	2.62 ± 0.61	4.47 ± 0.86	0.90	0.37 ± 0.05

This trend, or lack thereof, is dissimilar to that seen with many species but mirrors the Caribbean Acroporid *Acropora cervicornis* (Fitt et al., 2000).

The initial dark respiration showed no significant differences, but the light-enhanced dark respiration (LEDR) showed a significant increase in respiration for those acclimated to high light, as has been shown in similar studies (Wangpraseurt et al., 2014; Crawley et al., 2010). Additionally, the P:R ratio had a noticeable, but not statistically significant, decrease under high-light conditions in symbiont D3. As a measurement of the trophic balance of oxygen produced and respiration, this finding could indicate that holobiont hosting symbiont type D3, although very thermally robust (Grottoli et al., 2014), has a higher respiratory demand than the holobiont hosting the C1 symbiont. Because the P:R is comparable between the two light treatments, the increased respiration at high-light could mean that the coral is garnering more translocated material from the algae and burning more of it in the higher light. Research suggests that the PSII reaction center is an early casualty of elevated temperature anomalies (Heckathorn et al., 1997; Warner et al., 1999), so one could infer that the D clade of *Symbiodinium* relies more heavily upon its thermal

tolerance to protect its internal reaction centers in order to allocate energy to functions other than photoacclimation.

4.2 Biochemical Composition and Genetic Drivers

There is a trend that flows throughout the majority of this study's findings regarding the biochemical composition of the holobiont: the host portion of the holobiont acclimated to the high-light conditions (1000 μmol quanta m^{-2} s^{-1}) generally exhibited increased genetic expression of target genes, as well as contained greater protein and carbohydrate concentrations. When contrasting these high-light acclimated samples against those acclimated to low-light, host protein, host carbohydrate, and algal carbohydrate concentrations were all greater in the high-light samples. As you can see in Figure 14, there have been documented trends of some deeper corals having less biomass when compared with the same species at a shallower depth (Fitt et al., 2000).

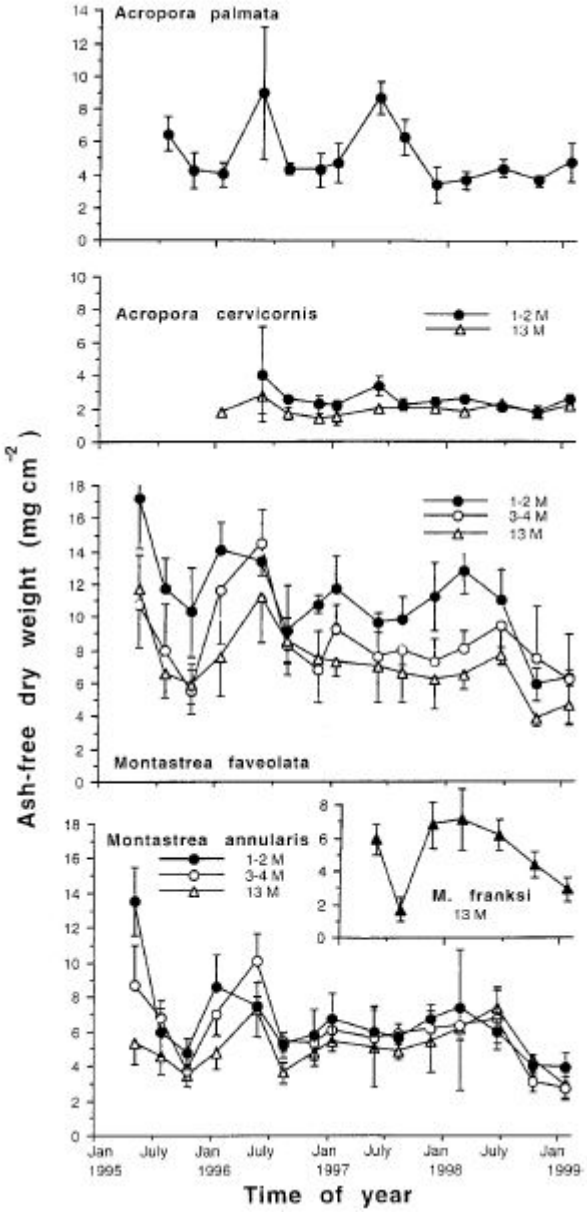

Figure 13. Ash-free dry weights of five species of coral as a function of depth (Fitt et al., 2000)

Under higher light conditions, photosynthetic productivity is inevitably going to be much greater than it would be in a darker light regime. As previously stated, an

increase in light availability coincides with a decrease in photosynthetic reaction centers due to a decreased need for utilizing every last photon of available light. The increased ambient light levels allow the symbionts to increase the rate of production of glucose-rich photosynthate derivatives (Yellowlees et al., 2008). Additionally, this translates to a greater amount of translocated carbon material from the algae to the host. Under low-light conditions, the symbiotic algae must also dedicate carbon to building more light-harvesting proteins automatically decreasing the amount of carbon available to the host. This increase in available energy as sugars clearly explains the marked increase in carbohydrate concentrations in both the host coral, *A. millepora*, and its associated symbionts. Once these glucose molecules transform through glycolysis and the citric acid cycle, they produce energy-rich substrates, such as ATP, GTP, NADH, etc., that can be used in any number of biological processes including protein synthesis (McEwen et al., 1963; Lai and Behar, 1993). This increase in primary productivity in high-light conditions and the subsequently increased rate of carbon fixation also explains the notable increase in glyceraldehyde-3-phosphate dehydrogenase (GAPDH), at least in symbiont type D3 (Figure 11). As a pivotal, rate-limiting enzyme within the Embden-Meyerhof glycolysis pathway (Romano and Conway, 1996), increased expression of GAPDH suggests that there might be an increase in glycolytic activity, continuing to support the idea that greater light intensity promotes greater primary productivity from the *Symbiodinium*. This in turn provides the coral with the necessary carbon and energy to devote to growth, reproduction, and other important processes. As previously stated, this trend of increased health alongside increased light is echoed by the concept of light-enhanced calcification (Marshall, 1996). Algal protein concentrations did not change between light

treatments, but this may be due to the host utilizing the newly-produced algal sugars before the symbiont itself is able to process them into proteins.

While the effect of light on primary productivity and its subsequent carbon fixation is well-documented, it was not the only difference noted within the data set. Host protein concentration, host carbohydrate concentration, intracellular carbonic anhydrase expression, GAPDH expression, and glutamine synthetase expression were all significantly greater in the coral harboring the symbiont D3 than in the symbiont C1 symbiosis. The high-light acclimated coral exhibited increased expression of the target genes within all five cases of D3, and these data suggest that coral housing symbiont type D3 is much more metabolically active than symbiont type C1 under high-light conditions. As noted earlier, the P:R ratios of the fragments hosting the D3 symbiont were seen as lower than that of fragments hosting the C1 alga at the higher light treatment, which could mean that this subset of *Symbiodinium*, which may have more energy available to it as a result of its inherent thermal tolerance, may use these energy stores to maximize photosynthetic productivity. The difference between the two types of holobionts is not very large, so this variation may just be driven by a higher respiration rate in the coral harboring the D3 symbiont. Glutamine synthetase is an enzyme important for nitrogen assimilation (Eisenberg et al., 2000), while the host's intracellular carbonic anhydrase (InCA) facilitates the interconversion of carbonic acid and carbon dioxide/water (Giordano et al., 2005; Lindskog, 1997). Importantly, CA facilitates the supply of aqueous CO_2 which is the ultimate substrate needed by the *Symbiodinium* for carbon fixation (Bertucci et al., 2011). The fact that these two enzymes also exhibited increased expression under these conditions further supports symbiont type D3 as the more metabolically active symbiont as compared to

C1. It should also be stated as a caveat that, although higher transcript levels may be indicative of an increase in enzymatic rate, this is not always the case.

The only enzyme that deviates from these trends is the host's extracellular carbonic anhydrase (ExCA). Although this enzyme has a role comparable to that of other carbonic anhydrases such as InCA, it only showed elevated expression when acclimated to high-light conditions and found within the host housing symbiont type C1. By definition, ExCA exists outside of the cell membranes, ensuring that the correct carbon species is being transported into the cell so that it may eventually make its way to the internal *Symbiodinium* (Brading et al., 2013). While nearly every other target gene exhibited greater levels of expression in D3, ExCA increased in the presence of C1. From this ressult, one could infer that an effect of high-light acclimation on a coral colony housing symbiont type C1 is the loading of carbon species into the cell as a way to maximize the presence of a limiting photosynthetic substrate.

Chapter 5

CONCLUSION

Results from this experiment revealed photoacclimation mechanisms common to both C1 and D3 symbionts of the genus *Symbiodinium*, as well as differences that could very well serve to aid in continued symbiont diversification. Findings regarding photosynthetic and respiratory capabilities, as well as chlorophyll a concentrations, were similar between the two symbiont types, but the data also suggested that perhaps the coral harboring the C1 symbiont was more flexible when manipulating light-harvesting pigments under low-light relative to the D3 holobiont to maximize photosynthetic productivity. Host protein concentration, host carbohydrate concentration, intracellular carbonic anhydrase expression, GAPDH expression, and glutamine synthetase expression were all greater in the coral harboring the D3 symbiont, and even more so in the D3 holobiont acclimated to the higher of the two light levels. This was likely due to increased light availability and subsequent increased symbiont productivity. ExCA was the only gene with an apparent surplus in expression in corals with C1 symbionts relative to those with D3. Future research is needed, not only to expand upon the questions raised within this experiment, but to place these data in the context of more C and D symbionts as well as symbionts outside of these two clades. Once the basic photoacclimation mechanisms of more specific symbiont types are established, differences among symbionts will become increasingly evident, leading to an increased understanding of diversity within the genus *Symbiodinium* and a better grasp of cnidarian-algal symbioses as a whole.

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