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HOST-SYMBIONT COMPATIBILITY IN CNIDARIAN-ALGAL MUTUALISMS

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ABSTRACT

The mutualistic relationships between corals and symbiotic algae (dinoflagellates) are threatened by global warming, thus impacting the viability of coral reef ecosystems. The precarious relationship between the symbiont and host is dependent on partner specificity and compatibility. The photosynthetic dinoflagellates in the genus *Symbiodinium microadriaticum* forms an exclusive mutualistic relationship with the upside-down jellyfish *Cassiopeia* despite the prevalence of other potentially competitive *Symbiodinium* species in the host's environment. Strobilation, a process of metamorphosis of the jellyfish, is a functional signal of the successful symbiotic infections. To better understand the partner compatibility of *Cassiopeia xamachana*, we tested the hypothesis that strobilation timing of the animals is dependent on the different symbiont species. Here we show that metamorphosis of the jellyfish was initiated by its native symbiont (*S. microadriaticum*), but strobilation was completed earlier by the non-native symbionts (*S. minutum* and *S. trenchii*). Symbiont cell densities per host increased over time and were highest for the native strain. This suggests that symbiont competition is important to specificity exhibited by these mutualistic symbioses in nature. Future studies to understand cell signaling would analyze host growth under direct competition between native and non-native symbiont strains, which would provide deeper insight into partner compatibility of the symbiont and its host. The ultimate understanding of partner compatibility between symbionts and hosts provide insights on the extent to which these symbioses may form new partnerships in response to climate change.

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Chapter 1

A Little Dinoflagellate Named *Symbiodinium*

Symbiodinium Biology

The dinoflagellate genus *Symbiodinium* contains numerous species, many of which are the endosymbionts for numerous marine animals (Blank and Trench 1986). These unicellular organisms typically form symbiotic relationships with cnidarians such as sea anemones, zoanthids, and various coral species (Douglas 2003). *Symbiodinium* are often referred to colloquially as "zooxanthellae" (Wakefield *et al.* 2000). The genus *Zooxanthellae* was coined in the late 1800s and for many decades, all symbiotic dinoflagellates were classified as *Zooxanthellae* (Blank and Trench 1986). Today, organisms originally classified as *Zooxanthellae* are part of several different genera, including *Symbiodinium* (Wakefield *et al.* 2000).

Symbiodinium are coccoidal, 6-15 um, and brown-yellowish (Blank and Trench 1986; LaJeunesse *et al*. 2005; Douglas 2003). This coloration, which is distinctive to many photosynthetic dinoflagellates, is attributed to the peridinins, types of carotenoids associated with the chlorophyll of the cells (Douglas 2003). The chloroplast is the most central organelle of *Symbiodinium*. They are located peripherally in the cell with several thylakoids (Wakefield *et al.* 2000). The pyrenoid, an important structure utilized for carbon fixation, is located within the chloroplast (Bose 1943; Wakefield *et al.* 2000). Other organelles typically found in *Symbiodinium* cells include mitochondria, vacuoles, fibrous structures, and a large granular "accumulation" body (Wakefield *et al.* 2000).

Symbiodinium alternate between two different stages: coccoid (non-motile) form and mastigote (motile) form (Trench and Thinh 1995). While in their hosts, the *Symbiodinium* are often in the coccoid form. These cells are round and contained by a continuous cell wall. However, as a mastigote, the *Symbiodinium* have two flagella which allows them to swim. The transition between each of these forms occurs during cell division. Clonal propagation through frequent mitotic events by *Symbiodinium* leads to their rapid proliferation (Trench and Blank 1987).

The *Symbiodinium* life cycle is largely dependent on exposure to light and nutrient availability. The cells have a natural circadian rhythm which is dependent on alternating light and dark periods (Fitt and Trench 1983). Light signals the *Symbiodinium* to continue with the cell cycle; nitrogen, phosphorous and other vital nutrients are used by dividing cells. Typically, the growth of the cell (in preparation for mitosis) occurs while light is present, but nuclear division occurs in the dark (Wang *et al*. 2008). The next light cycle initiates cytokinesis. In nature, the replication process is fully complete a few hours after dawn (Wang *et al*. 2008).

The transition between each of these forms occurs during the log-phase in which *Symbiodinium* proliferates rapidly (Trench and Blank 1987). Replication gives rise to the motile cells. (It is thought that the motile cells can infect their hosts easier.) Replication may result in doublet cells or tetrad cells; however, the exact process is still unknown (Fitt and Trench 1983). Subsequent to this, the daughter cells escape from the mother and swim away. After ecdysis occurs, in which the thecae and flagella are shed, the cells become non-motile (Wakefield *et al.* 2000).

Symbiodinium Diversity and Species Recognition

The genus *Symbiodinium* can be divided into many different clades, or groups, each of which contain several different *Symbiodinium* species. Currently 9 different clades exist; there are varying levels of *Symbiodinium* diversity within the clades (Baker 2003; LaJeunesse *et al*. 2012). The large variation between the species of *Symbiodinium* was characterized through a study of the mitochondrial cytochrome oxidase 1 gene. The use of cytochrome oxidase 1 to create a barcode baseline for this genus revealed that many species were originally reclassified in the past and presents the possibility that many are currently misclassified (Stern *et al.* 2010).

There are hundreds of different species of *Symbiodinium*; however most of these are undescribed. Many divergent and distinct lineages have been classified based off of differences in ribosomal DNA (Rowan 1998; Rowan and Powers 1992). The classification of the *Symbiodinium* species is difficult and is currently an ongoing process. The morphological species concept alone is also a poor method to differentiate species of *Symbiodinium* since they look very similar and they possess few visually distinct characteristics. One of the most ideal ways to define species is through the biological species concept. It was believed for many years that *Symbiodinium* do not sexually reproduce (Baker 2003). It was largely believed that prior to the advent of the 21st century (before many genomics studies on *Symbiodinium*) that the differences in cytogenic assays between various *Symbiodinium* represented different ploidy states and thus provided evidence that sexual reproduction could not be possible as it would result in too many chromosomal abnormalities (Blank and Trench 1985). LaJeunesse (2001) advocated that sexual reproduction occurred due to high levels of allele recombination found among cultured isolates and symbionts in the field. Today, a combination of morphological, phylogenetic, molecular,

biochemical, and host specificity data are used to formally classify species of these symbionts (LaJeunesse *et al*. 2012; LaJeunesse *et al*. 2014).

Chapter 2

Symbiodinium Species

Symbiodinium microadriaticum

S. microadriaticum was the first formally recognized species in the genus (Freudenthal 1962). The cell surfaces are smooth and the cell walls are relatively thin. The cells of *S. microadriaticum* are 8-14 um in length and 6-12 um in width. The number of chromosomes is approximately 98. *Symbiodinium microadriaticum* belongs to Clade A and is typically found in the Western Atlantic, Caribbean, and Red Sea regions (Lee *et al*. 2015). The primary host for *S. microadriaticum* is the cnidarian *Cassiopeia xamachana*, although it may also occur in symbioses with giant clams from the Red Sea (Pochon *et al.* 2006).

Symbiodinium microadriaticum is tolerant of a wide range of thermal and high light stress (Robison and Warner 2006). However, extreme elevated temperatures will inhibit photosynthesis in these symbionts (Bouchard and Yamasaki 2008).

Symbiodinium minutum

Symbiodinium minutum is a Clade B species; these species are typically found in the Western Atlantic and Caribbean regions (Pochon *et al.* 2006; Suggett *et al*. 2015). Clade B species hold symbiotic relationships with many cnidarian and coral hosts (Pochon *et al.* 2006). *S. minutum* primarily holds a symbiotic relationship with *Aiptasia pallida*, a sea anemone (Suggett *et al*. 2015). This symbiont is found in generally low concentrations in most waters around the world. *S. minutum* is a mutualistic species, but can be an opportunistic species for hosts which

underwent a bleaching process, like the coral *Pocillopora*. However, *S. minutum* is not particularly well adapted to temperature fluctuating environments. The microsatellite marker Sym15 and *cpS23* gene are useful to categorize the different species in Clade B (LaJeunesse *et al*. 2012). *S. minutum* is fairly divergent compared to other symbionts. This species has one the smallest genomes in comparison to other *Symbiodinium* (Shoguchi *et al*. 2013). The cells of *S. minutum* are 6-9 um in diameter when in the coccoid stage, distinctly the smallest amongst all other *Symbiodinium* species (LaJeunesse *et al*. 2012).

Symbiodinium trenchii

Symbiodinium trenchii is a Clade D species; these species are typically found in the warm waters of the Western Atlantic, Pacific, and Indian Ocean regions. Clade D species hold symbiotic relationships with several types of *Scleractinian* corals. Clade D *Symbiodinium* is a highly divergent lineage compared to the other *Symbiodinium* clades, but there is little genetic diversification among Clade D species (LaJeunesse *et al*. 2014). *S. trenchii* is a stress tolerant species. As a result, the symbioses of corals harboring this symbiont species remain intact during episodes of sea surface warming that would otherwise cause bleaching and mortality in animals with different symbionts (Pochon *et al*. 2006). Under certain situations, the unnatural presence of *S. trenchii* may reduce rates of calcification. This suggests that mal-adapted symbioses can have negative consequences (Pettay *et al*. 2015).

Symbiodinium necroappetens

Symbiodinium necroappetens is a newly recognized Clade A species. It is found in the Western Atlantic and Caribbean regions like *S. microadriaticum* (LaJeunesse *et al*. 2015). Even though both species have similar morphological structures, the cell volume for *S. necroappetens* is about double that of *S. microadriaticum* (LaJeunesse *et al*. 2015; Suggett *et al*. 2015). While both species have similar rDNA and cytochrome b sequences, large sequence differences in the *psbA* plastid gene indicate that the species are evolutionary divergent (LaJeunesse and Thornhill 2011; LaJeunesse *et al*. 2015). Further, *S. necroappetens* is less thermally tolerant than *S. microadriaticum*. Interestingly, unlike *S. microadriaticum* (which is a mutualistic species), *S. necroappetens* appears to be opportunistic species and normally found in diseased coral tissues or in colonies immediately recovering from severe stress events like bleaching (LaJeunesse *et al*. 2015).

Chapter 3

A Not So Little Jellyfish Named *Cassiopeia xamachana*

Cassiopeia xamachana is an upside-down jellyfish that typically occurs in shallow waters and are often found in mudflats near mangroves of the Caribbean and the Florida Keys (Hofmann *et al*. 1996; Niggl and Wild 2009; Holland *et al*. 2004). Mangrove habitats provide shelter and the degradation of plant matter which gives the jellyfish nutrients it needs to grow (Holland *et al*. 2004). *Cassiopeia* are invasive species; they overtook the waters of the Hawaiian Islands when the vessels of WWII arrived to those shores (Niggl and Wild 2009; Holland *et al*. 2004). Their proliferation has negative impacts on the economic development of the surrounding land (Niggl and Wild 2009).

Cassiopeia was once used as model organism to study nerve centers and regeneration. Many parts of the jellyfish are controlled by nerve centers located peripherally on the organism. These nerve centers are dispersed along the circumference of its bell, equidistant from each other. It is easy to remove the nerve centers without completely destroying the organism. Further, singular nerve centers can be removed to see its effect on one part of the body, while the other nerve centers can remain intact (Cary 1917). Also, this organism has a remarkable ability to regenerate portions of its nerve centers and its oral arms (Cary 1917; Bigelow 1900). While no longer used in these disciplines of biology, over the past several decades, they have been utilized in experiments to examine symbiotic relationships between cnidarians and *Symbiodinium*.

Cassiopeia xamachana Biology

Mature *Cassiopeia xamachana* is a mostly transparent organism with various shades of brown and blue. Normally there is an outer brownish ring and white bands below which extend in a radial direction. The pigmentation of the *C. xamachana* is primarily due to its symbiotic algae. The cells are the densest at the center of the bell around its oral arms. The organism has 8 oral arms which form four pairs in the shape of a Maltese cross (Figure 1) (Bigelow 1900). *Cassiopeia* is typically found upside down so its oral arms can extend upward to face the sun, presumably allowing the symbiont population greater exposure to light and enhanced photosynthesis. *C. xamachana* does not have a primary mouth; however, it has many secondary mouths on its oral arms (Santhanakrishnan *et al*. 2012). By pulsating, water is able to filter through its oral arms and the jellyfish is able to feed and flush away waste matter (Figure 2) (Santhanakrishnan *et al*. 2012; Hofmann *et al*. 1996).

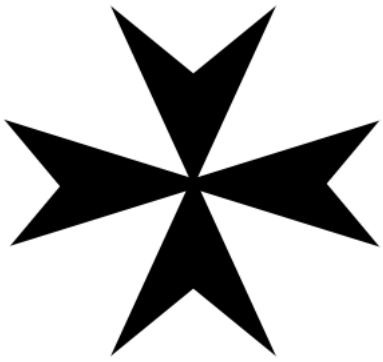

Figure 1: Maltese cross

The eight oral arms of Cassiopeia xamachana form a Maltese cross. The oral arms contain several secondary mouths which capture food, nutrients, and symbionts.

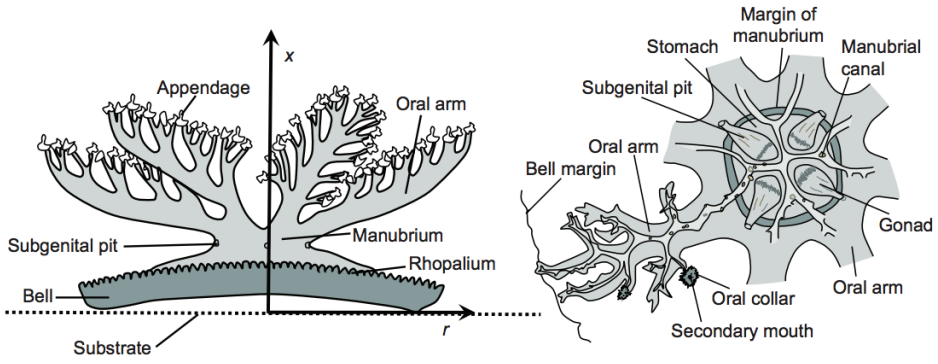

Figure 2: Morphology of *Cassiopeia xamachana*

The jellyfish lies upside down in shallow waters to allow its symbionts maximum sunlight to undergo photosynthesis. (Adapted from Santhanakrishnan *et al*. 2012)

C. xamachana alternates between sexual and asexual stages (Niggl and Wild 2009). The polyp stage, or scyphistoma, asexually reproduces when no symbionts are present. Buds at the base of the polyp form, detach, quickly settle and the metamorphose to form a new polyp. Infected polyps eventually develop, or strobilate, to form the medusa, the sexual motile phase of Scyphozoans (Ojimi *et al*. 2009). Metamorphosis begins in the oral region of the animal. Ephyra and younger medusa are relatively sessile. As the organism becomes older, it finds a mudflat in shallow waters to make its home and becomes mostly sedentary (Bigelow 1900; Holland *et al*. 2004). Slight suction of the bell against the mudflat prevents the organism from moving (Bigelow 1900). The medusa sexually reproduces to form more polyps which become infected horizontally and vertically (Figure 3) (Sachs and Wilcox 2006; Ojimi *et al*. 2009). The symbionts that infect the jellyfish are already present in the environment or have been expelled by juvenile and mature *Cassiopeia* (Ojimi *et al*. 2009).

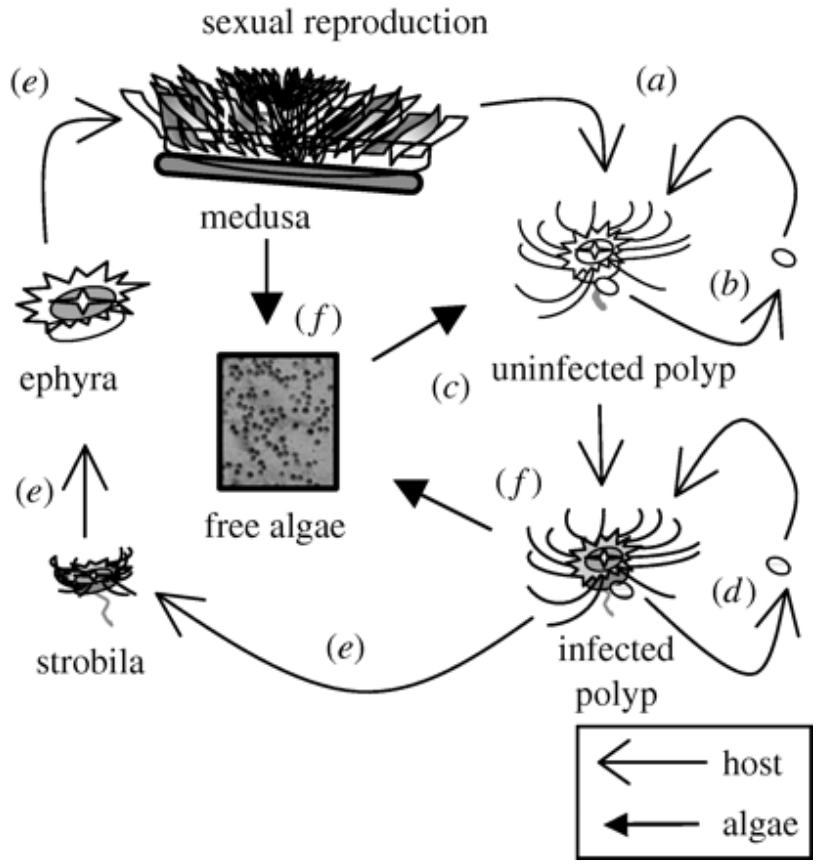

Figure 3: Life cycle of *Cassiopeia xamachana*

a. Medusa release larvae which develop into polyps b. New polyps bud off from uninfected polyps c. Free-living Symbiodinium have the opportunity to infect aposymbiotic polyps d. New polyps with Symbiodinium bud off from infected polyps e. Infected Cassiopeia undergo metamorphosis and eventually strobilate into medusa f. Infected Cassiopeia xamachana release their symbionts into the environment (Adapted from Sachs and Wilcox 2006)

Chapter 4

Symbiont-Host Relationship

Mutualistic Relationship between Symbionts and Hosts

Symbiodinium are typically found in symbiosis with many kinds of marine animals (Rowan 1998). They have been found to form mutualistic and possibly commensal relationships with their hosts (Lee *et al.* 2016). Most often, they form mutualistic relationships with cnidarians and reef building corals (Rowan 1998). Some of these hosts include anemones, zoanthids, scleractinian corals, and jellyfish (Baker 2003). Within corals and cnidarians, the *Symbiodinium* provide photosynthetic products, such as glycerol and glucose, to help the host with its growth, reproduction, and metabolic processes (Buck *et al*. 2002; Davy *et al*. 2012). In return, the symbionts receive protection and certain nutrients, like phosphorus, carbon, and nitrogen, from their hosts (Davy *et al*. 2012). Hosts are typically found in shallow waters so symbionts have greater exposure to the sun for photosynthesis (Holland *et al*. 2004). In fact, the photosynthetic efficiency of many of the *Symbiodinium* is dependent upon the location depth of their hosts. Hosts may form symbioses with more than one kind of symbiont, and are influenced by the external environment. However, most hosts exhibit remarkable specificity for particular symbiont taxa (Lesser *et al*. 2013). The fidelity of the symbiont-host relationship is dependent on the identity of both partners as well as the environment (Stat *et al*. 2008).

Symbiodinium uptake

Very little is understood about the partner specificity with symbionts and their hosts (Stat *et al*. 2008). *Symbiodinium* are obtained by their hosts either through vertical transmission of the host mother or horizontal transmission via phagocytosis (Blank and Trench 1985; Davy *et al*. 2012). It is unknown how the phagosome differentiates between food for ingestion and symbionts for symbiosis (Davy *et al.* 2012). Gruenberg and van der Goot (2006) and Davy *et al*. (2012) suggest particular signaling events take place between the symbiont and the host. The fact that *Symbiodinium* spp. probably secrete different signaling molecules indicates some type of host specificity (Markell and Wood-Charlson 2010). A symbiosome forms in the gastric cavity of the jellyfish when *Symbiodinium* are engulfed by the host cell. The symbiosome has host cell and algal cell origin (Davy *et al*. 2012; Rands *et al*. 1993). The porous membrane facilitates the transport of nutrients. While nutrient transport is mainly under control of the host, ATP-ase transport pumps on the symbiosome allows the symbiont plays a role in nutrient uptake as well (Rands *et al*. 1993). The density of the symbiont cells to the host cell is dependent on the nutrient availability. Both healthy and damaged symbiont cells are constantly expelled from the host, perhaps as a means to regulate the standing population of the symbiont (Davy *et al*. 2012; Fishman *et al*. 2008). When the host experiences starvation, the symbiont populations decline. Unquestionably, signaling is involved in the regulation of symbiosis between *Symbiodinium* and *Cassiopeia*. Yet, processes related to partner compatibility and regulation remain unknown (Davy *et al*. 2012).

Chapter 5

The Project

The *Symbiodinium-Cassiopeia* system can be used to understand the symbiont-host specificity relationship. Exploiting the fact that certain *Symbiodinium* can form symbiosis not commonly found in nature helps to elucidate this relationship (Stat *et al*. 2008). In this study, *C. xamachana* was infected with various *Symbiodinium* that were not known to be symbiotic or non-symbiotic to determine the polygenetic relationship of all known *Symbiodinium* spp. We show that not every *Symbiodinium* can form symbioses with *C. xamachana*. *C. xamachana* were also infected with its native symbiont (*S. microadriaticum*) and its non-native symbionts (*S. minutum, S. trenchii, S. necroappetens*) in isolation to understand the impact of symbionts on their hosts. We hypothesized that host development of the jellyfish would be impacted differently under symbioses of the homologous and non-homologous strains of *Symbiodinium*.

Chapter 6

Materials and Methods

Symbiodinium spp. can form symbioses with *C. xamachana*. A change in morphology, known as strobilation, indicates the success of a particular symbiont-*Cassiopeia* symbioses. Infections of *C. xamachana* with various *Symbiodinium* tests both the compatibility of the symbiont, and also the physiological performance of the association through the timing of the morphological changes of the jellyfish. Moreover, analyses of symbiont cell densities within the jellyfish can elucidate differences in the symbioses of different species of *Symbiodinium* with *C. xamachana*.

Growing *Symbiodinium* spp. and *Cassiopeia xamachana*

S. microadriaticum (CCMP 2464, A1) was isolated from *Cassiopeia xamachana* from Florida Keys, USA. *S. minutum* (rt-002(2), B1) was isolated from *Aiptasia pallida* from Florida Keys, USA. *S. trenchii* (CCMP 2556, D1a) was isolated from colonies in the Caribbean. *S. necroappetens* (CCMP 2469, A13) was isolated from the sea anemone *Condylactis gigantea* from the Caribbean. Cultures were grown in the ASP-8A culture media and transferred every couple of months. The cultures were kept in a 28°C incubator under a fluorescent light with 14 hour light and 10 hour dark cycles. The cultures were used after substantial growth after several weeks.

Cassiopeia xamachana polyps from Florida Keys, USA were obtained from the laboratory of Dr. Monica Medina. The aposymbiotic polyps were allowed to asexually reproduce for five years. Juvenile aposymbiotic polyps were always kept in the dark in a 28°C incubator.

The polyps were submerged in a 35ppm Millipore-filtered saline sea water solution. The sea water was replaced after feeding. The uninfected polyps were fed with EZ egg baby brine shrimp a couple of times a week. The dehydrated eggs of the brine shrimp were immersed in sea water and mixed with an air filter. The brine shrimp hatched after approximately 36 hours. Each polyp was fed about 5-10 brine shrimp using a transfer pipette (Diaz *et al*. 2013).

Cassiopeia xamachana **infections**

Polyps were collected a week prior to infection to acclimate them to their new environment. All polyps were submerged in 33-38 ppm filtered sea water. They were kept in a 27°C incubator under a fluorescent light with 14 hour light and 10 hour dark cycles. They were fed with brine shrimp twice prior to infection. The water was completely replaced a day after feeding. All polyps looked healthy prior to the *Symbiodinium* infections. The *C. xamachana* were infected in isolation with each of the *Symbiodinium* cultures: *S. microadriaticum*, *S. minutum*, *S. trenchii*, and *S. necroappetens*. Three polyps were placed in each of the six wells of the six welled, flat bottom, Corning ® Costar ® cell culture plates. Three plates were designated for each of the four cultures, for a total of 12 plates. Each set of three plates for a particular culture had n=54 polyps. A single plate with a total of about 20 polyps was designated as the negative control. These polyps were not infected with *Symbiodinium*.

Prior to infection, the *Symbiodinium* cultures were repeatedly spun down in microcentrifuge tubes for 5 minutes at 10,000 rpm. The pellets were collected and vortexed in the culture media. Cell density measurements were obtained using a standard hemocytometer. Total cells/ml were calculated using the following equation:

$$\textbf{Total cells/ml} = \textbf{Total cells counted} \times \frac{\textbf{dilution factor}}{\textbf{\# of squares}} \times \textbf{10,000 cells/ml}$$

The cells were not diluted. The number of symbiont cells was normalized to the lowest volume available. Final volumes added reflect the volume added to the 18 replicate wells. Volume was subsequently adjusted so there were about 10,000 cells per polyp because a larger number of cells per polyp leads to an overgrowth of symbionts in the wells (unpublished data) (Table 1). Symbiont solutions were added to each of the wells using clean pipette tips to prevent contamination. Polyps were immediately fed with brine shrimp to facilitate infection process.

***Symbiodinium* culture**	**Average cell counts**	**Total cells/ml**	**Lowest volume (ml)**	**Lowest cell number**	**Normalized to lowest volume (ml)**	**Volume to add (ul)**
S. microadriaticum	35	43750	1.363	59631.25	1.363	37.86
S. minutum	133	166250	-	-	0.359	9.96
S. trenchii	87.875	109844	-	-	0.543	15.08
S. necroappetens	138.25	172813	-	-	0.345	9.59

Table 1: Hemocytometer cell counts

Original cultures of Symbiodinium were normalized to approximately 10,000 cells/polyp

Stages of metamorphosis

Infected and uninfected *C. xamachana* were kept in the 27°C incubator under a fluorescent light with 14 hour light and 10 hour dark cycles. They were fed three times a week (MWF) and the water was replaced with fresh sea water twice a week subsequent to feeding. Clean transfer pipettes were used during every feeding and cleaning to prevent contamination. Every two-four days, the morphological changes of the jellyfish were scored (Figure 4). Jellyfish

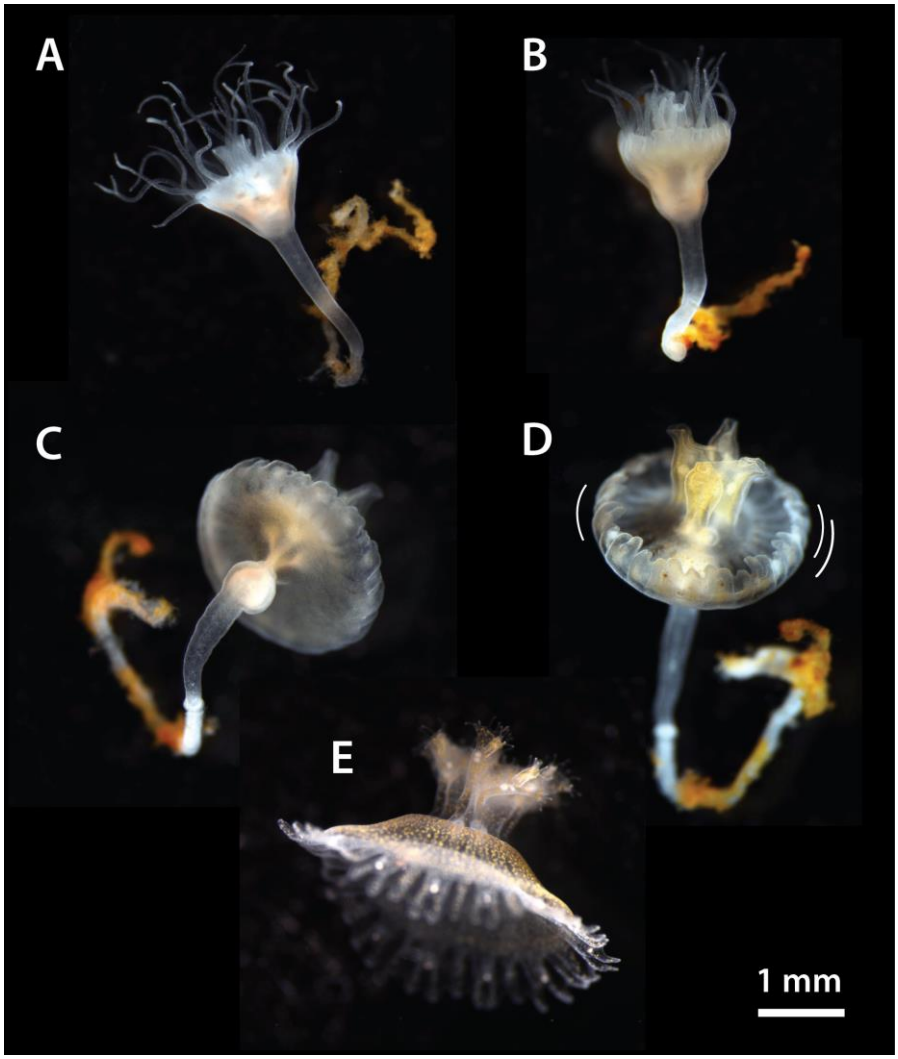

Figure 4: Different morphological stages of *Cassiopeia xamachana*

A. Stage 0: Aposymbiotic polyp B. Stage 1: Infected polyp; bowl shaped C. Stage 2: Infected strobila; outer ring formation D. Stage 3: Pulsating strobila E. Stage 4: Ephyra

Microscopy

In order to analyze the density of the symbionts, images were taken on the Keyence BZ-9000 Fluorescence Microscope (Live Cell) one week after strobilation and approximately 3 weeks after strobilation. Ephyra were placed upside down on slides and submerged in a solution of $MgCl_2$ to limit motion. Jellyfish were squashed using a coverslip. Images taken at 2x captured

the entire ephyra, including the bell diameter. A fluorescent image of the symbionts was overlaid on the brightfield images of the organisms to reveal density of infections. Three images per ephyra taken at 20x captured the clusters of symbiont cells within *C. xamachana*. These images were taken near the outer rim of the organism's bell. Imaged animals were preserved in a solution of DMSO for subsequent maceration.

Statistics

Metamorphosis patterns of the jellyfish were graphed as categorical variables for each treatment on R (See Code 1, Appendix). To analyze differences between developmental states, bootstrap analysis (n=1,000,000) was conducted under a beta distribution with a 95% confidence interval. A student's t-test was applied to test for significance ($p<0.05$) between being in stage x and not (See Code 2, Appendix). Random cell counts were taken of the symbiont clusters from the 20x images. An ANOVA was used to test for variance of means within the groups of the same symbiont and a student's t-test with unequal variances was used to test for difference of means within clusters of *S. microadriaticum* and *S. trenchii* cells.

Maceration of Ephyras

Almost all of the remaining ephyra (n=33 for *S. microadriaticum*, *S. minutum*, and *S. trenchii* and n=30 for *S. necroappetens*) were imaged under a light microscope to measure the bell sizes. Ephyra were placed upside down on trays and immersed in a solution of $MgCl_2$ to eliminate excess movement. Jellyfish were preserved in a solution of DMSO subsequent to imaging. Preserved ephyra were macerated using a buffer solution and glass beads. Volumes

were concentrated and normalized to each other. Cells of symbionts were counted on the hemocytometer and densities were calculated.

Chapter 7

Results

Morphological Changes of *C. xamachana*

The metamorphosis process of *C. xamachana* was different for each of the *Symbiodinium* treatments (Figure 5).

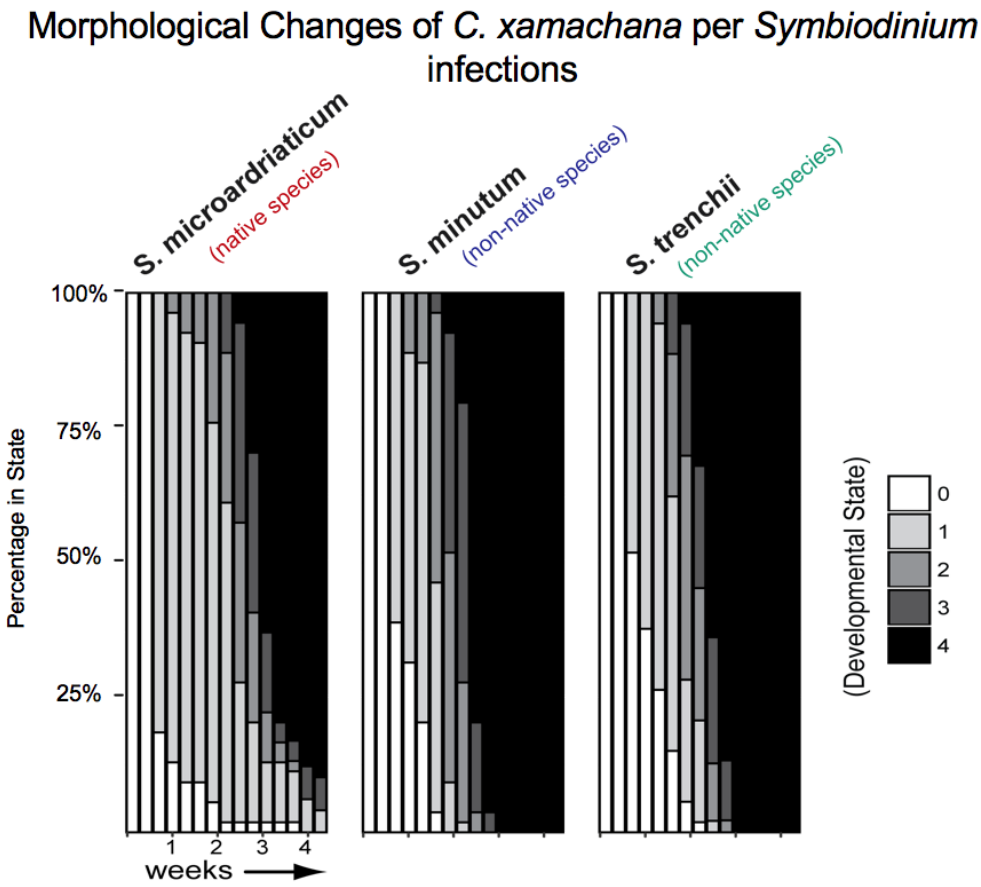

Figure 5: Metamorphosis of *C. xamachana* per *Symbiodinium* infections

Quantitative analysis of morphological changes of jellyfish infected with native and non-native Symbiodinium. Gradient of color represents progression of metamorphosis.

The metamorphosis process began earlier for the native strain, *S. microadriaticum*, as opposed to the non-native strains, *S. minutum* and *S. trenchii*. There was no significant difference between the two non-native strains in beginning the metamorphosis process. However, *S. minutum* reached the next three morphological stages faster than *S. trenchii*. Jellyfish infected with *S. minutum* first reached strobilation significantly faster than those with *S. trenchii*, which reached strobilation significantly faster than those with *S. microadriaticum*. All organisms infected with the two non-native strains, *S. minutum* and *S. trenchii*, completely strobilated before the *C. xamachana* infected with the native strain (Figure 6, Table 2).

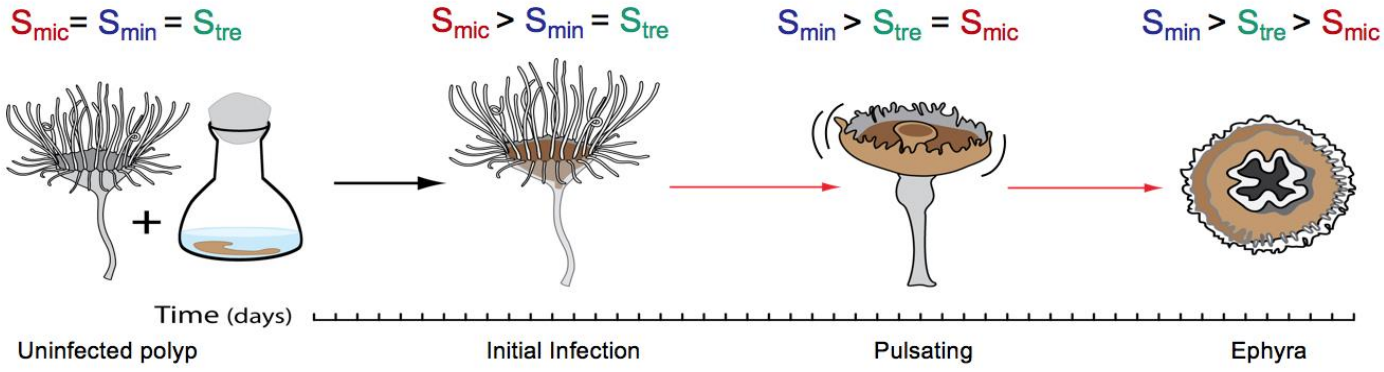

Figure 6: Rates of morphological change per symbiont

Metamorphosis began with the native species, but finished earlier with the non-native species. Smic (*S. microadriaticum*), Smin (*S. minutum*), Stre (*S. trenchii*)

p-values for comparison of infected *C. xamachana* in morphological stage			
Morphological Stage	***S. microadriaticum* vs *S. minutum***	***S. microadriaticum* vs *S. trenchii***	***S. minutum* vs *S. trenchii***
Bowl Shaped	<0.05 * (*S. microadriaticum*)	<0.001 * (*S. microadriaticum*)	0.09
Outer Ring Formation	0.10	0.19	<0.05 * (*S. minutum*)
Pulsating	<0.001 * (*S. minutum*)	0.07	<0.01 * (*S. minutum*)
Ephyra	<0.001 * (*S. minutum*)	<0.0001 * (*S. trenchii*)	<0.05 * (*S. minutum*)
Ephyra (all)	<0.001 * (*S. minutum*)	<0.0001 * (*S. trenchii*)	0.37

Table 2: Significance of *C. xamachana* metamorphosis

Bootstrap analysis to compare the significance of infected C. xamachana in a particular morphological stage. * indicates significance using a 95% confidence interval. Symbiont species with more jellyfish in particular stage is labelled in parenthesis. The first four stages represent when S. microadriaticum reached a particular stage. The last stage, Ephyra (all), represents when all of the organisms infected with the non-native strains reached strobilation.

Cell Densities of the Symbionts in Host Cells

The symbiont density within *C. xamachana* for the native strain, *S. microadriaticum*, and the non-native strains, *S. minutum* and *S. trenchii* varied. Images taken when the ephyras were approximately one week old for each *Symbiodinium* treatment revealed that *S. microadriaticum* was the densest, while *S. minutum* was the next densest and *S. trenchii* was the least dense. There was an average of 11.5 ± 0.8 (95% CI) *S. microadriaticum* cells per host cell and an average of 7.4 ± 0.7 (95% CI) *S. trenchii* cells per host cell (Figure 7). Images taken when the ephyras were a couple of weeks old (*S. minutum* and *S. trenchii* infected ephyras were a few days older than the *S. microadriaticum* infected ephyras) revealed the same trend of cell densities. Average number of symbiont cells per host cell were not counted.

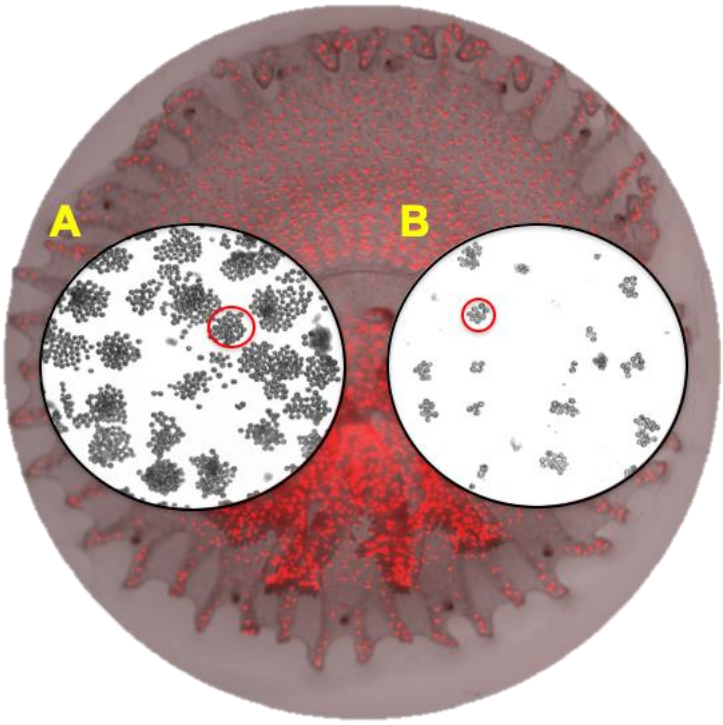

Figure 7: Symbiont cell densities comparisons

Jellyfish infected with the native strain had more symbiont cells per host cell than jellyfish infected with the non-native strains. A. S. microadriaticum cells; average 11.5 ± 0.8 (95% CI) symbiont cells per host cell B. S. trenchii cells; average 7.4 ± 0.7 (95% CI) symbiont cells per host cell

Chapter 8

Discussion

Symbiodinium are a diverse group of dinoflagellates (Manning and Gates 2008; Stat *et al.* 2008). The native symbiont for *Cassiopeia xamachana* is *S. microadriaticum,* despite the pervasiveness of other *Symbiodinium* in the waters (Pochon *et al*. 2006). We found that *C. xamachana* is capable of forming symbiotic relationships with other species including *S. minutum* and *S. trenchii,* both are atypical, or heterologous, symbionts (Pochon *et al.* 2006; Suggett *et al*. 2015; LaJeunesse *et al*. 2014). The morphological changes to reach strobilation begins earlier for *S. microadriaticum,* but unexpectedly, individuals infected with the non-native strains, *S. minutum* and *S. trenchii*, strobilated first. For all combinations, symbiont cell densities increased over time, but the symbiont per host cell densities were always greater in jellyfish infected with *S. microadriaticum* as compared to those infected with the non-homologous strains.

The relationships differ between *Cassiopeia* and various *Symbiodinium*

Free-living species of *Symbiodinium* may no longer be capable of forming stable symbioses (Lee *et al*. 2016). Attempts to infect *C. xamachana* with cultures of these various species failed (Data not shown). It would seem that throughout the evolution of this dinoflagellate group, some lineages have lost the capacity to form stable mutualisms (Figure 8). It can be inferred that the common ancestor of *Symbiodinium* was symbiotic, but over time certain members, such as Clade E and lineages of Clades A and F evolved to occupy other ecological niches. Much remains unknown about the ecology of free-living *Symbiodinium* (Manning and Gates 2008). Future genomic studies of these in comparison to symbiotic species

may provide insight into which genes are important in the formation and maintenance of these symbioses.

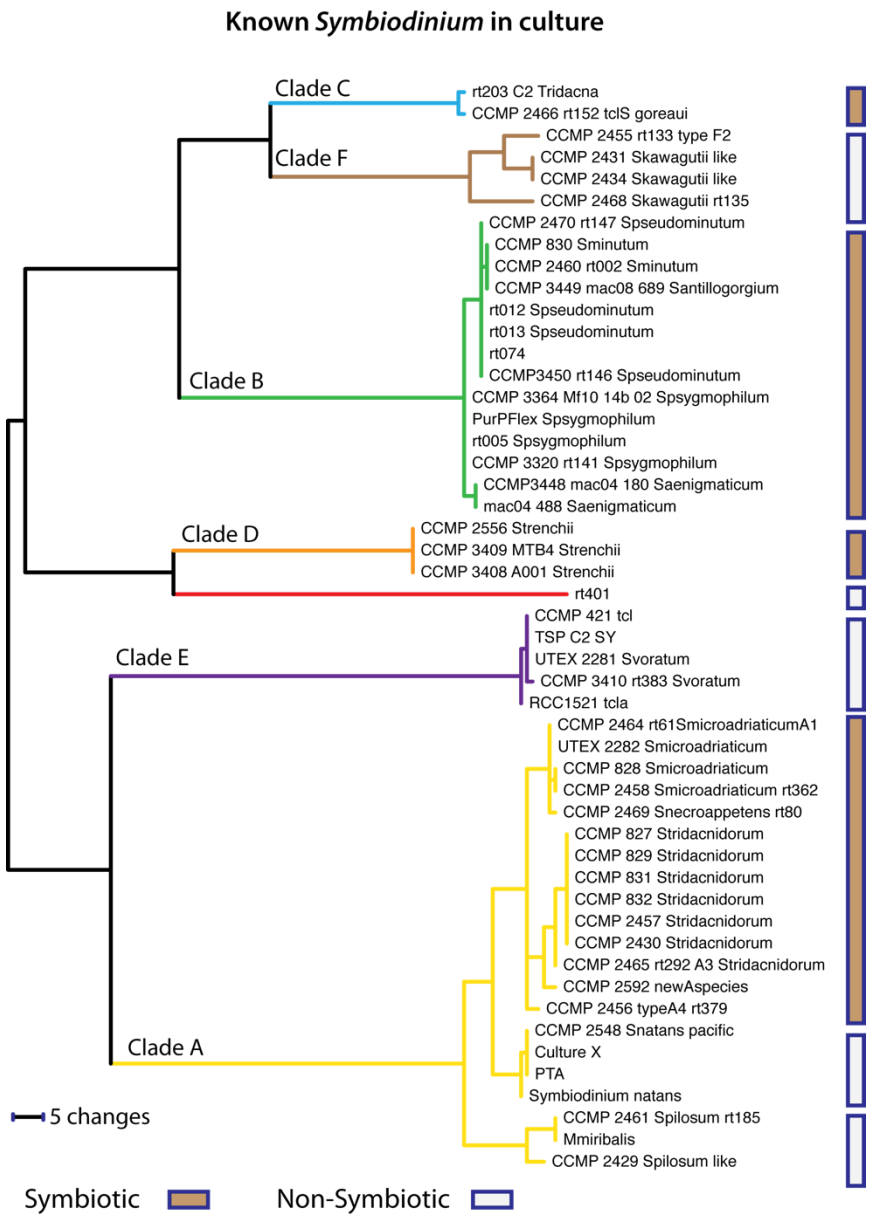

Figure 8: Phylogeny of all existing *Symbiodinium* spp.

The common ancestor of *Symbiodinium* was symbiotic. Not every *Symbiodinium* lineage is symbiotic.

Differences in the timing of ephyra metamorphosis

C. xamachana infected with homologous *S. microadriaticum* began the metamorphosis process earlier than *S. minutum* and *S. trenchii*. This was to be expected, however, subsequent to successful infections, the rate of metamorphosis was faster in *C. xamachana* infected with strains of non-homologous species. *S. minutum* was typically the fastest in reaching the next stage in comparison to the other cultures. Generally, jellyfish infected with *S. microadriaticum* were the slowest to undergo morphological changes. However, rates of morphological changes normalized between the non-homologous cultures when all organisms reached strobilation. This novel result shows clear differences in the physiological performance of particular host-symbiont pairings. Moreover, this begs the question, if *S. minutum* and *S. trenchii* can function well with this animal, why are these associations not typically found in nature? One possible explanation raises the possibility that competition among *Symbiodinium* drives aspects of the host-symbiont specificity found naturally. Some researchers presume that the host has full control over its symbionts (Sachs and Wilcox 2006). However, Sachs and Wilcox (2006) demonstrated that when *C. xamachana* was infected with various types of *Symbiodinium* spp., the more harmful algae would often be found to infect the aposymbiotic polyps faster, rather than the homologous *S. microadriaticum*. This implies that there is some combination of external and internal controls that effect which symbiont dominates the animal in nature.

The finding that the animals with the non-homologous symbionts reached strobilation before animals with homologous symbionts was unexpected, but may offer insight into partner specificity observed under natural conditions. In the field, there are many species of *Symbiodinium* spp. in the same environments as *C. xamachana*. And yet the jellyfish exhibits a predictable relationship with *S. microadriaticum* (Lee *et al*. 2015). In the Stat *et al*. (2008)

experiment, *Acropora*, a coral species, was found to hold a symbiotic relationship with a Clade A species after a stressful event. These animals typically harbor Clade C *Symbiodinium* spp. The atypical relationship between *Acropora* and this unidentified Clade A species underperformed as the symbiont translocated less carbon to the host, suggesting an unbalanced or maladapted relationship. In order to better understand what is occurring at the symbiont-host level during the period prior to strobilation, a follow-up competition experiment in which both the homologous and non-homologous strains were kept in the same environment as *C. xamachana* should be performed. A genetic analysis of the organisms during every stage they reach should be completed to look at potential changes in *Symbiodinium* spp. densities throughout time.

Differences in symbiont cell densities per host cell suggest some incompatibility

The number of homologous symbiont cells per host cells were greater than symbioses with non-homologous symbiont cells, but over time, the symbiont cell densities increased in both the homologous and heterologous combinations. It seems that the number of symbiont cells per host cell reaches a maximum value, and thus symbiont densities increase as host cells with symbionts increase in number. One of the important roles the symbiont cells play in the host is the translocation of carbon (Davy *et al.* 2012). The fact that the symbiont cell density per host cell was greater for *S. microadriaticum* suggests that the flux of total translocated carbon is greater in hosts with this species. From this observation, we may infer that the rate of growth for *C. xamachana* would be greater with *S. microadriaticum*. Experiments examining growth rates after strobilation constitutes a next logical step in assessing the physiological value of different *Symbiodinium*.

Challenges

Despite the successes of these experiments, there were several challenges. The infection experiment had to be repeated due to a hypersaline episode that killed all animals. In nature, the *C. xamachana* gets resources from its surrounding environment of mudflats and mangroves (Hofmann *et al*. 1996; Niggl and Wild 2009; Holland *et al.* 2004). As it is impossible to completely replicate this environment in the lab, keeping the nutrient levels, water salinity, and light controlled is important.

Furthermore, *S. necroappetens* is thought to be an opportunistic and free-living species (LaJeunesse *et al*. 2015). Due to this fact, it was not expected to hold symbiosis with *C. xamachana*. However, strobilation of these animals did occur. The animals were scored with some bias due to the perceived expected results. As a result, the data for this culture must be removed from the experimental analysis. In the future, it would be ideal to modify the stages because many morphological changes occur between the individual stages. For example, there is a large classification of polyps that are bowl shaped (stage 1). Adding more stages to the morphological scoring would make finding the rate of metamorphism more accurate.

Conclusionary Remarks

Understanding the compatibility between corals and *Symbiodinium* is critical in understanding how these symbioses may respond to climate change through partner recombination. While a high level of symbiont specificity exists for many coral, there is some flexibility in the system (LaJeunesse *et al*. 2010). As the climate continues to warm, many coral symbioses will degrade, resulting in coral death (Stat *et al*. 2008; Pratchett 2008). Coral

bleaching is the phenomenon in which the symbionts are lost from the coral, resulting in the exposure of its bare calcium carbonate white skeleton (Pratchett *et al*. 2008; Douglas 2003). Coral reefs are important to the world's biosphere. They provide shelter and nutrients to many aquatic animals, including over a quarter of all fish species. Apart from their ecological importance, many developing countries rely on coral reefs for economic gain from fish harvesting to tourism (Pratchett *et al*. 2008). It is predicted in the next several decades that almost all corals around the world will experience bleaching events as the global sea temperature rises (Brown and Dunne 2015).

Learning how to exploit the partner compatibility of particular hosts will be essential in saving these coral from coral bleaching. Many symbionts are heat-tolerant and could potentially be better symbionts for their aquatic hosts (Stat *et al*. 2008, LaJeunesse *et al*. 2014, Pochon 2006). Partner specificity plays a role in microbial interactions with their hosts. This includes symbiotic relationships where humans are the hosts with microbes such as *E. coli*. Developing the partner specificity model can hold broader impacts not only for aquatic ecosystems, but also for the health of humans (Mushegian and Ebert 2015).

Appendix

Codes in R

Code 1: Comparative Analysis of Metamorphosis of *Cassiopeia xamachana*

```
ggplot(Data, aes(x=Time, fill=as.factor(State)))+geom_bar(position="fill")+facet_grid(.~Sym)
```

Code 2: Significance of Morphological Changes

```
 BayesAB<-function(N,A,nA,B,nB)

      {pdf<-rbeta(N,A+1,nA+1)
      PDF<-pdf
      pdf<-pdf[order(pdf)]

      lowA<-pdf[as.integer(N*.05)]
      highA<-pdf[as.integer(N*.95)]
      print(lowA) print(highA)
      data.frame(pdf)->pdf
      pdf2<-rbeta(N,B+1,nB+1)
      PDF2<-pdf2
      pdf2<-pdf2[order(pdf2)]

      lowB<-pdf2[as.integer(N*.05)]
      highB<-pdf2[as.integer(N*.95)]
      print(lowB) print(highB)

      data.frame(pdf2)->pdf2

      pdf3<-PDF-PDF2
      diff<-sum((pdf3<0)*1)
      print(diff) print(diff/N)

      dis<-cbind(pdf,pdf2)
      dis<-melt(dis,measure.vars=c("pdf","pdf2"))

ggplot(dis,aes(x=value,fill=variable))+stat_density()}
```

BIBLIOGRAPHY

1. Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. Annual Review of Ecology, Evolution, and Systematics 34: 661–689
2. Bigelow RP (1900) The anatomy and development of *Cassiopea xamachana*. (Boston: Pub. by the Boston Society of Natural History)
3. Blank RJ, Trench RK (1985) Speciation and Symbiotic Dinoflagellates. Science 229: 656–658
4. Blank RJ, Trench RK (1986) Nomenclature of Endosymbiotic Dinoflagellates. Taxon 35: 286–294
5. Bose SR (1943) Role of Pyrenoids in Algae and of Vacuoles in Plastids of Higher and in Fungi. Botanical Gazette 104: 633–638
6. Bouchard JN, Yamasaki H (2008) Heat Stress Stimulates Nitric Oxide Production in *Symbiodinium microadriaticum*: A Possible Linkage between Nitric Oxide and the Coral Bleaching Phenomenon. Plant Cell Physiol 49: 641–652
7. Brown BR and Dunne RP (2015) Coral Bleaching: The Roles of Sea Temperature and Solar Radiation. In Diseases of Coral, CM Woodley and JW Porter, ed. (Hoboken: John Wiley & Sons, Inc.), 266-283
8. Buck BH, Rosenthal H, Saint-Paul U (2002) Effect of increased irradiance and thermal stress on the symbiosis of *Symbiodinium microadriaticum* and *Tridacna gigas*. Aquatic Living Resources 15: 107–117
9. Cary LR (1917) Studies on the physiology of the nervous system of *Cassiopea xamachana*. (Washington, DC)

10. Chang SS, Prézelin BB, Trench RK (1983) Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. Marine Biology 76: 219–229
11. Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. Microbiol Mol Biol Rev 76: 229-261
12. Diaz E, Zavalza M, Kelly S (2013) *Cassiopeia xamachana* Basic Manual. (University Park, PA)
13. Douglas AE (2003) Coral bleaching—how and why? Marine Pollution Bulletin 46: 385–392
14. Fishman Y, Zlotkin E, Sher D (2008) Expulsion of Symbiotic Algae during Feeding by the Green Hydra – a Mechanism for Regulating Symbiont Density? PLoS ONE 3:e2603 2601–2605
15. Fitt WK (1985) Effect of different strains of the zooxanthella *Symbiodinium microadriaticum* on growth and survival of their coelenterate and molluscan hosts. Proc 5th Int Coral Reef Congr Int 6:131-136
16. Fitt WK, Trench RK (1983) The Relation of Diel Patterns of Cell Division to Diel Patterns of Motility in the Symbiotic Dinoflagellate *Symbiodinium Microadriaticum* Freudenthal in Culture. New Phytologist 94: 421–432
17. Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a Zooxanthella: Taxonomy, Life Cycle, and Morphology. The Journal of Protozoology 9: 45–52
18. Gruenberg J, van der Goot FG (2006) Mechanisms of pathogen entry through the endosomal compartments. Nat Rev Mol Cell Biol 7: 495–504

19. Hofmann DK, Fitt WK, and Fleck J (1996) Checkpoints in the life-cycle of *Cassiopea* spp.: control of metagenesis and metamorphosis in a tropical jellyfish. Int. J. Dev. Biol. 40: 331–338

20. Holland BS, Dawson MN, Crow GL, and Hofmann DK (2004) Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Marine Biology 145: 1119–1128

21. LaJeunesse TC (2001) Investigating the Biodiversity, Ecology, and Phylogeny of Endosymbiotic Dinoflagellates in the Genus *Symbiodinium* Using the Its Region: In Search of a "Species" Level Marker. Journal of Phycology 37: 866–880

22. LaJeunesse TC, Thornhill DJ (2011) Improved Resolution of Reef-Coral Endosymbiont (*Symbiodinium*) Species Diversity, Ecology, and Evolution through *psbA* Non-Coding Region Genotyping. PLOS ONE *6*, e29013

23. LaJeunesse TC, Lambert G, Andersen RA, Coffroth MA, Galbraith DW (2005) *Symbiodinium* (pyrrhophyta) Genome Sizes (DNA Content) Are Smallest Among Dinoflagellates. Journal of Phycology 41: 880–886

24. LaJeunesse TC, Smith R, Walther M, Pinzón J, Pettay DT, McGinley M, Aschaffenburg M, Medina-Rosas P, Cupul-Magaña AL, Pérez AL, *et al*. (2010) Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. Proceedings of the Royal Society of London B: Biological Sciences 277: 2925–2934

25. LaJeunesse TC, Parkinson JE, Reimer JD (2012) A genetics-based description of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two dinoflagellates symbiotic with cnidaria. J Phycol 48: 1380–1391

26. LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) Clade D are different species. Phycologia 53: 305–319
27. LaJeunesse TC, Lee SY, Gil-Agudelo DL, Knowlton N, Jeong HJ (2015) *Symbiodinium necroappetens* sp. nov. (Dinophyceae): an opportunist "zooxanthella" found in bleached and diseased tissues of Caribbean reef corals. European Journal of Phycology 50: 223–238
28. Lee SY, Jeong HJ, Kang NS, Jang TY, Jang SH, LaJeunesse TC (2015) *Symbiodinium tridacnidorum* sp. nov., a dinoflagellate common to Indo-Pacific giant clams, and a revised morphological description of *Symbiodinium microadriaticum* Freudenthal, emended Trench & Blank. European Journal of Phycology 50: 155–172
29. Lee MJ, Jeong HJ, Jang SH, Lee SY, Kang NS, Lee, K.H., Kim, HS, Wham DC, LaJeunesse, TC (2016) Most Low-Abundance "Background" Symbiodinium spp. Are Transitory and Have Minimal Functional Significance for Symbiotic Corals. Microb Ecol 71: 771–783
30. Lesser MP, Stat M, Gates RD (2013) The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. Coral Reefs 32: 603–611
31. Manning, MM, Gates RD (2008) Diversity in populations of free-living *Symbiodinium* from a Caribbean and Pacific reef. Limnol. Oceanogr. 53: 1853–1861
32. Markell DA, Wood-Charlson EM (2010) Immunocytochemical evidence that symbiotic algae secrete potential recognition signal molecules in hospite. Mar Biol 157: 1105–1111
33. Mushegian AA, Ebert D (2016) Rethinking "mutualism" in diverse host-symbiont communities. BioEssays 38: 100–108

34. Niggl W, Wild C (2009) Spatial distribution of the upside-down jellyfish *Cassiopea* sp. within fringing coral reef environments of the Northern Red Sea: implications for its life cycle. Helgol Mar Res 64: 281–287

35. Ojimi MC, Isomura N, Hidaka M (2009) Telomerase activity is not related to life history stage in the jellyfish *Cassiopea* sp. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 152: 240–244

36. Pettay DT, Wham DC, Smith RT, Iglesias-Prieto R, LaJeunesse TC (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. PNAS 112: 7513–7518

37. Pochon X, Montoya-Burgos JI, Stadelmann B, Pawlowski J (2006) Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. Molecular Phylogenetics and Evolution 38: 20–30

38. Pratchett MS, Munday PL, Wilson SK, Graham NAJ, Cinner JE, Bellwood DR, Jones GP, Polunin NVC, McClanahan TR (2008) Effects of climate-induced coral bleaching on coral-reef fishes - ecological and economic consequences. Oceanography and Marine Biology 46: 251–296

39. Rands ML, Loughman BC, Douglas AE (1993) The Symbiotic Interface in an Alga-Invertebrate Symbiosis. Proceedings of the Royal Society of London B: Biological Sciences 253: 161–165

40. Robinson JD, Warner ME (2006) Differential Impacts of Photoacclimation and Thermal Stress on the Photobiology of Four Different Phylotypes of *Symbiodinium* (pyrrhophyta). Journal of Phycology 42: 568-579

41. Rowan R (1998) Review—Diversity and Ecology of Zooxanthellae on Coral Reefs. Journal of Phycology 34: 407–417

42. Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proc Natl Acad Sci USA 89: 3639–3643

43. Sachs JL, Wilcox TP (2006) A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. Proceedings of the Royal Society of London B: Biological Sciences 273: 425–429

44. Santhanakrishnan A, Dollinger M, Hamlet CL, Colin SP, Miller LA (2012) Flow structure and transport characteristics of feeding and exchange currents generated by upside-down *Cassiopea* jellyfish. Journal of Experimental Biology 215: 2369–2381

45. Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujiwara M, *et al*. (2013) Draft Assembly of the *Symbiodinium minutum* Nuclear Genome Reveals Dinoflagellate Gene Structure. Current Biology 23: 1399–1408

46. Stat M, Morris E, Gates RD (2008) Functional diversity in coral–dinoflagellate symbiosis. PNAS 105: 9256–9261

47. Stern RF, Horak A, Andrew RL, Coffroth MA, Andersen RA, Küpper FC, Jameson I, Hoppenrath M, Véron B, Kasai F, *et al*. (2010) Environmental Barcoding Reveals Massive Dinoflagellate Diversity in Marine Environments. PLOS ONE 5: e13991

48. Suggett DJ, Goyen S, Evenhuis C, Szabó M, Pettay DT, Warner ME, Ralph PJ (2015) Functional diversity of photobiological traits within the genus *Symbiodinium* appears to be governed by the interaction of cell size with cladal designation. New Phytol 208: 370–381

49. Trench RK, Blank RJ (1987) *Symbiodinium Microadriaticum* Freudenthal, *S. Goreauii* Sp. Nov., *S. Kawagutii* Sp. Nov. and *S. Pilosum* Sp. Nov.: Gymnodinioid Dinoflagellate Symbionts of Marine Invertebrates. Journal of Phycology 23: 469–481

50. Trench RK, Thinh L (1995) *Gymnodinium linucheae* sp. nov.: The dinoflagellate symbiont of the jellyfish *Linuche unguiculata*. European Journal of Phycology 30: 149–154

51. Wakefield TS, Farmer MA, Kempf SC (2000) Revised description of the fine structure of in situ "zooxanthellae" genus *Symbiodinium*. Biol Bull 199: 76-84

52. Wang LH, Liu YH, Ju YM, Hsiao YY, Fang LS, Chen CS (2008) Cell cycle propagation is driven by light–dark stimulation in a cultured symbiotic dinoflagellate isolated from corals. Coral Reefs 27: 823–835

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- Analyzed the timely implementation of palliative care in American and Dutch nursing homes
- Presented at the American Society of Aging Conference; Mar 2016
- Presented to American client Russ McDaid (President and CEO of the PA Healthcare Association); Apr 2016
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- Maintained ED cleanliness, transported patients to tests, interacted extensively with patients, assisted nurses in triage, and learned to read CT scans
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- Shadowed physicians and physician assistants as they treated patients

Global Medical Brigades…….May 2014
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- Set up a temporary clinical in rural Ghanaian village
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Biology Teaching Assistant…….....……Aug 2014 – Dec 2015
BIOL 110, BIOL 110H, BIOL 240

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Diversity and Academics Departments

- Served as liaison between student Scholars and Honors College administrators
- Invited motivational speaker Scott Fried, initiated dialogue concerning racial issues, and developed Table Talks Program facilitating conversation amongst students and staff

GLOBE First Year Representative……Oct 2013 – May 2014
Schreyer Honors College, Special Living Option

- Promoted ideas to encourage inclusiveness between first year students and executive board

Honors and Awards

Braddock Scholarship…….....Aug 2012 – May 2016
Dean's List…….....…..Aug 2012 – May 2016
Schreyer Academic Excellence Scholarship……..….Aug 2012 – May 2016
KAUST International Undergraduate Research Competition..…….……Jan 2016
Davey Memorial Scholarship……..……...…..Aug 2015
Hayes Memorial Scholarship……...……Aug 2015
Penn State Undergraduate Research Grant……Nov 2014, Nov 2015
Schreyer Ambassador Travel Grant……...….May 2014
Thompson Scholarship in Science……..…….....…….Aug 2014
NaNoWriMo Challenge Winner……..……...Nov 2012