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EFFECT OF TEMPERATURE STRESS ON COMPETITION IN ALGAL-CNIDARIAN SYMBIOSIS

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ABSTRACT

Marine heatwaves associated with climate change and subsequent mass coral-bleachings have prompted decades of increased interest in the vital symbiosis between reef-building corals and mutualistic dinoflagellates in the family Symbiodiniaceae (zooxanthellae). The upside down jellyfish *Cassiopea xamachana* is a useful model system for studying symbiosis establishment, and competition between their potential symbionts. Previous studies have looked at competition between symbiont species in this system, however, none have looked at competition under heat stress. In some corals, thermotolerant symbionts may be more successful under high heat than their less tolerant counterparts. We ran competition experiments between three symbiont species known to establish symbiosis with *C. xamachana*; the jellyfish's homologous symbiont *Symbiodinium microadriaticum,* and the heterologous symbionts *Breviolum minutum, as well as* the thermally tolerant *Durusdinium trenchii*. Each experiment paired species against one another, at different starting concentrations (25:75, 50:50, 75:25). The experiments were run under both control (27˚C) and heat-stressed (32˚C) conditions. Under control temperatures *S. mic.* and *D. trenchii* both competitively displaced *B. min.*, but when competing against one another there was less of a clear winner in either direction. Under heat-stress, the polyps that were given heterologous symbionts did poorly; only two ephyrae established symbiosis and all polyps appeared bleached, however the polyps that were given the homologous symbiont continued strobilating throughout the experiment. Under heat stress, *S. mic.* dominated both of the other species. These results suggest that partner specificity in *C. xamachana* is somewhat strong and although the system can harbor heterologous symbionts, these foreign symbionts do not help the animal survive during heat stress even if the symbionts themselves are thermotolerant.

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Chapter 1

Introduction

Cassiopea Xamachana

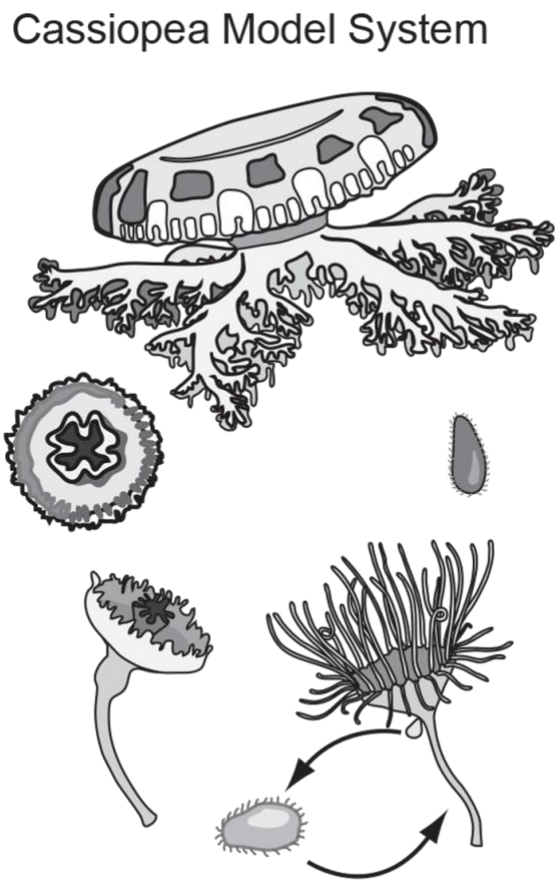


Figure 1: Diagram of *C. xamachana* life cycle (Trench).

The upside-down jellyfish, *Cassiopea xamachana*, is named for its unique behavior among medusae; unlike most scyphozoans, which are free-swimming during their medusa stage, *C. xamachana* spends much of its life resting on the seafloor with its four oral arms facing

upward, and its bell resting on the bottom; the reason for this behavior is to expose the dinoflagellate symbionts, which it harbors in its mesoglea tissue, to the sunlight from above (Medina 2021).

C. xamachana engages in a symbiotic relationship with the dinoflagellate *Symbiodinium microadriaticum* (S. mic), but has been found multiple times to be able to establish a symbiosis with other species of algae, one of which is the thermally tolerant species *Durusdinium trenchii*, as well as *Breviolum minutum* (Solanki 2016). *C. xamachana* is native to the Caribbean and the Gulf of Mexico, however other species of *Cassiopea* are found in tropical and subtropical regions worldwide (Medina 2021). It has two major life stages; the polyp or scyphistoma, and the medusa stage. It is dioecious and can reproduce sexually or asexually; in the case of sexual reproduction, a fertilized egg will develop into a free-swimming planula larva; in the case of asexual reproduction, the planula will bud off of a polyp; in both cases the planula will later settle on hard substrate and develop into a polyp. From here, the polyps, which do not inherit symbionts from the parent, will uptake them directly from the environment through ingestion and phagocytosis, in a manner that is nonspecific, however, *C. xamachana* most frequently maintains host-symbiont specificity in the wild with the dinoflagellate *Symbiodinium microadriaticum* (Colley and Trench 1983).

Following symbiont uptake, if a stable host-symbiont relationship is established, the polyp will begin to metamorphosize, which can take approximately 4-6 weeks, in which the animal develops from a sessile polyp into a free-swimming ephyra or miniature adult jellyfish, that will grow into a fully fledged medusa (Medina 2021). Despite the host-symbiont specificity seen between *C. xamachana* and *S. microadriaticum*, scyphistoma are able to develop symbioses

with non-homologous (heterologous) symbionts, such as those in the genera *Durusdinium* and *Breviolum* (Solanki, 2016) and (Winstead, 2019). In both Solanki and Winstead's research, successful establishment of symbiosis was measured by the initiation of strobilation, which is triggered only when a stable host-symbiont relationship has been formed.

C. xamachana is a widely used model system for studying cnidarians as well as the symbiotic relationship between Symbiodiniaceae and cnidarians, with obvious interest toward the implications to improve the understanding of these mutualisms in reef corals. *Cassiopea* is valued for its ease in culturing as opposed to most corals which present several difficulties when it comes to lab testing (Ohdera 2018). Moreover, *Cassiopea* is unique as a system because while strobilation in most scyphozoans is triggered by abiotic environmental factors, *Cassiopea* specifically begins metamorphosis when a symbiotic relationship has developed between the polyp and compatible dinoflagellate symbionts. The algae enters after being eaten and phagocytosed by the polyp, and successful symbiosis can only be established if the algae can successfully evade the polyps immune response; as such, not just any symbiont can find its way into the system (Ohdera 2018).

Symbiodiniaceae

Symbiodiniaceae, colloquially known as zooxanthellae, is a family of single-celled, eukaryotic, photosynthetic organisms which, though they have the ability to live as free-swimming phytoplankton, are very often found in association with marine invertebrates. They are most known for association with corals, but some species also associate with jellyfish, anemones, and clams; (LaJeunesse et. al. 2018) though, of course, due to the decline of coral

reefs during the past few decades, the majority of interest in symbiodiniaceae centers around their vital association with stony, reef-building corals.

Symbiodiniaceae form an endosymbiotic relationship with cnidarians where they live inside animal mesogleal cells; this is notably one of the only major endosymbiotic relationships involving two eukaryotes. Once thought to be a single genus *Symbiodinium*, the diverse family Symbiodiniaceae now consists of numerous genera, with a long evolutionary history (LaJeunesse et. al. 2018). Many can exist in two main morphological forms; the motile or mastigote phase in which they are free swimming in the water column, and the coccoid stage, in which the cell is not motile and is morphologically round. This is the form they will take when living within host tissue. Symbiodiniaceae usually colonize a host early in the host's life through being ingested through the mouth of the host, then phagocytosed so the symbiont cell can establish itself in the host mesoglea (Colley and Trench 1983). Instead of digesting its new algal symbiont, the coral will harbor the algae within its cells. Inside the cells, the symbiont is encased in a membranous symbiosome (Wong et. al. 2021). The mutualistic relationship between the two partners is as follows; the symbiont photosynthesizes, which produces food for the coral, and in return the symbiont receives nutrients in the form of cell waste products.

The relationship between these two partners becomes more complicated under conditions of heat stress; when average ocean temperatures rise a few degrees above the norm, cnidarian-algal symbiosis can undergo bleaching; that is, loss of symbionts resulting in animals looking white in color (Herrera et. al, 2021). Without their symbionts supplying them with nutrients, many corals will die off, which in mass bleaching events, can devastate entire reefs. However, some corals have been shown to be more resistant to bleaching than others; sometimes between

coral colonies of the same species (Hoadley et. al. 2019). In this study, corals from inshore habitats, which tended to be much warmer than offshore, all harbored the highly thermotolerant *D. trenchii* while offshore corals harbored less thermally tolerant symbionts. It has been thought that colonization by thermotolerant symbionts might help corals survive bleaching events and recover more quickly, however some host-symbiont associations are highly specific, and even a thermotolerant symbiont will not outcompete a native symbiont under heat stress (Gabay et. al. 2019). This work sought to test the influence of thermal stress on partner specificity and stability.

Chapter 2

Methods

Pre-Experiment

Growing Microalgae:

Cultures of *S. microadriaticum* (KB8), *B. minutum* (RT-002), and *D. trenchii* (CCMP 2556) were obtained from the culture library in Dr. Todd LaJeunesse's lab. Cultures were maintained in an incubator at 25˚C and transferred/fed with new ASP8A media periodically to grow them up until there was a sufficient quantity to run the experiment, then maintained at this quantity by periodically removing some culture and replenishing with media. To prevent cross-contamination of the media bottle, cultures were transferred into new, autoclaved Erlenmeyer flasks with media during each feeding. The algae was kept under fluorescent light on a schedule of 12 hours light and 12 hours dark.

Cassiopea Polyps:

Aposymbiotic *C. xamachana* polyps of the T1C strain were obtained from the lab of Dr. Monica Medina at Penn State University. Polyps were kept in an incubator under light of 880 lux/11 mmol. Salinity was kept at 30-35 ppt and monitored before each water change. Polyps were fed every other day with *Artemia* nauplii of the San Francisco strain and water in the wells was changed once a week. Polyps were acclimated to the incubator conditions two weeks prior to distribution into experimental wells, and distributed into 3 wells in each of 13 6 well plates two weeks prior to inoculation to allow the polyps to acclimate, with ~4 polyps distributed into each of 3 wells. As size of polyps naturally varies, each plate was given a variety of large and small

polyps. After the first week, if any polyps were dead or appeared unhealthy, they were removed and replaced with new polyps.

Culture Identification and Cell Counts

Prior to each inoculation, each culture was extracted and sequenced to ensure the correct species was being used. Cells were homogenized and counted and cell concentrations were obtained using a hemocytometer. Cell inoculations were performed with 10,000 cells being introduced into each well, in concentrations as illustrated below.

Competition Experiment:

3 competition experiments were run under normal conditions; a temperature of 25-27˚C, salinity of 30-35 ppt, and lighting of ~ 880 lux (11 umol) in 6-well culture plates. Different concentrations of symbionts were added to each plate, as illustrated in the chart below.

Table 1: Experimental Setup

Plate	Initial concentrations
1	100% *S. mic*
2	75% *S. mic* 25% *B. min*
3	50% *S. mic* 50% *B. Min*
4	25% *S. mic* 50% *B. min*

Plate	Initial concentrations
5	100% *B. min*
6	75% *B. min* 25% *D. trenchii*
7	50% *B. min* 25% *D. trenchii*
8	25% *B. min* 75% *D. trenchii*

Plate	Initial concentrations
9	100% *D. trenchii*
10	75% *D. trenchii* 25% *B. min*
11	50% *D. trenchii* 50% *B. min*
12	25% *D. trenchii* 75% *B. min*
13	No Symbionts

Plates 1, 5 and 9 were positive controls, which contained only one type of symbiont, *S. mic.*, *B. min.*, and *D. trenchii* respectively. Plate 13 was a negative control, containing no symbionts. Symbiont concentrations needed for each plate were calculated by taking and counting an aliquot of homogenized cell culture and then pipetting into each well the volume of cells needed for a total of 100,000 cells per well. For the competition experiments under normal temperatures, inoculation for plate 5 was performed on August 12, 2022 to ensure inoculation would not stress out the polyps, and all other plates were inoculated August 15, 2022.

Polyps were fed freshly hatched *Artemia* immediately after introducing symbionts in order to facilitate phagocytosis of the algal cells. After inoculation, all polyps were fed with *Artemia* twice weekly, with weekly water changes. The first water change following inoculation was done two days after algae was introduced to ensure that algae was eaten by the polyps.

Upon strobilation, ephyrae were collected and preserved in 0.5ml DMSO solution and stored at -20˚C for DNA extraction. The competition experiment was concluded on October 30, 2022.

Heat Stress + Competition Experiment:

The heat stress experiment was identical to the initial competition experiment, the only factor that was altered was the ambient temperature in the incubator, which was increased to 32˚C during the day to mimic the conditions of a coral reef undergoing heat stress. Night temperature was set to 31˚C. As in the prior experiment, aposymbiotic polyps of the T1C strain were obtained from the Medina lab, acclimated to the incubator conditions for two weeks in the dish they had been obtained in, transferred into 6 well plates in 10 mL wells, acclimated for two more weeks to the plates, before inoculation. One week following inoculation, the polyps were

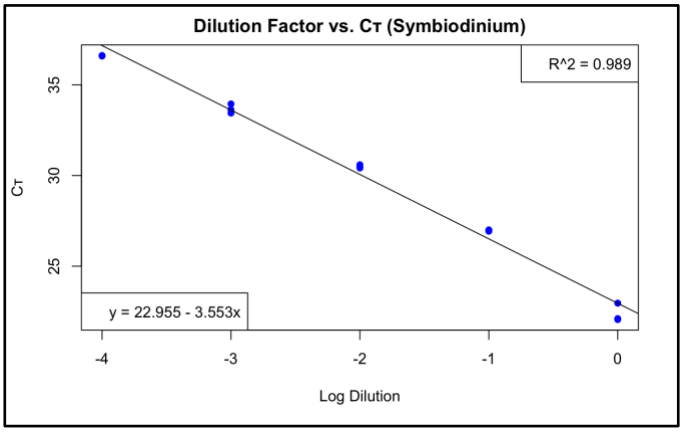
gradually exposed to heat stress, increasing the incubator temperature by 1˚C per day until temperatures reached 32˚C. For the temperature stress competition experiments, all inoculations were performed on October 31, 2022, and the experiment was concluded on December 16, 2022.

QPCR:

Genus specific primers for Durusdinium, Breviolum and Symbiodinium were used to do a quantitative analysis on the symbiont composition within each ephyra (Correa 2009).

To establish a standard curve, a DNA extraction of one million cells from each species of symbiont was performed in triplicate, and the DNA concentration of each extraction was measured using a qubit or nanodrop to determine stock concentration of DNA. The DNA extractions were then normalized to a concentration of 1 ng/uL, then serially diluted to 0.1, 0.01, 0.001, and 0.0001 concentrations which were used to create a standard curve with which to extrapolate unknown DNA concentrations via a semi-logarithmic regression.

A.



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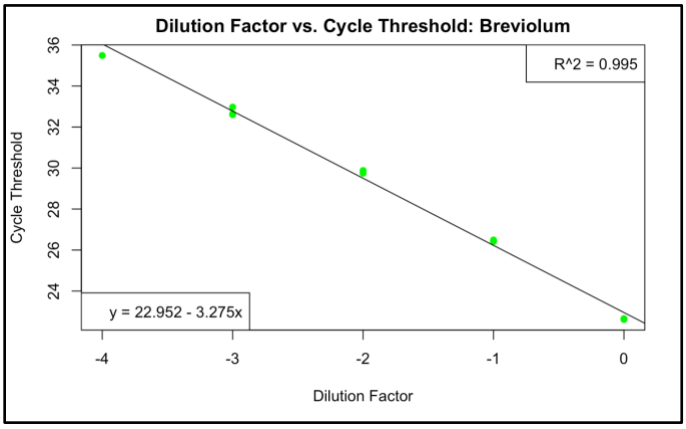

C.

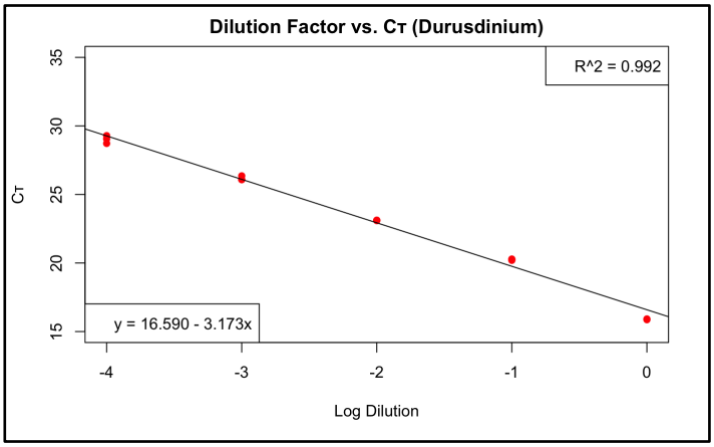

Figure 2: Standard curves for each symbiont species, used to quantify symbiont cell abundance in metamorphosed ephyrae.

Before running qPCR, normal PCR reactions were run using the genus-specific primers on all 9 samples to confirm genus-specificity. PCR and QPCR were both run with a 10 minute denaturing step at 95˚C, 35 cycles of 95˚C for 30 seconds, annealing at 60˚C for 30 seconds, then 72˚ for 30 seconds (Correa 2009). A nucleotide BLAST was also performed for each primer to ensure genus specificity.

Chapter 3

Results

Results of Competition Experiment

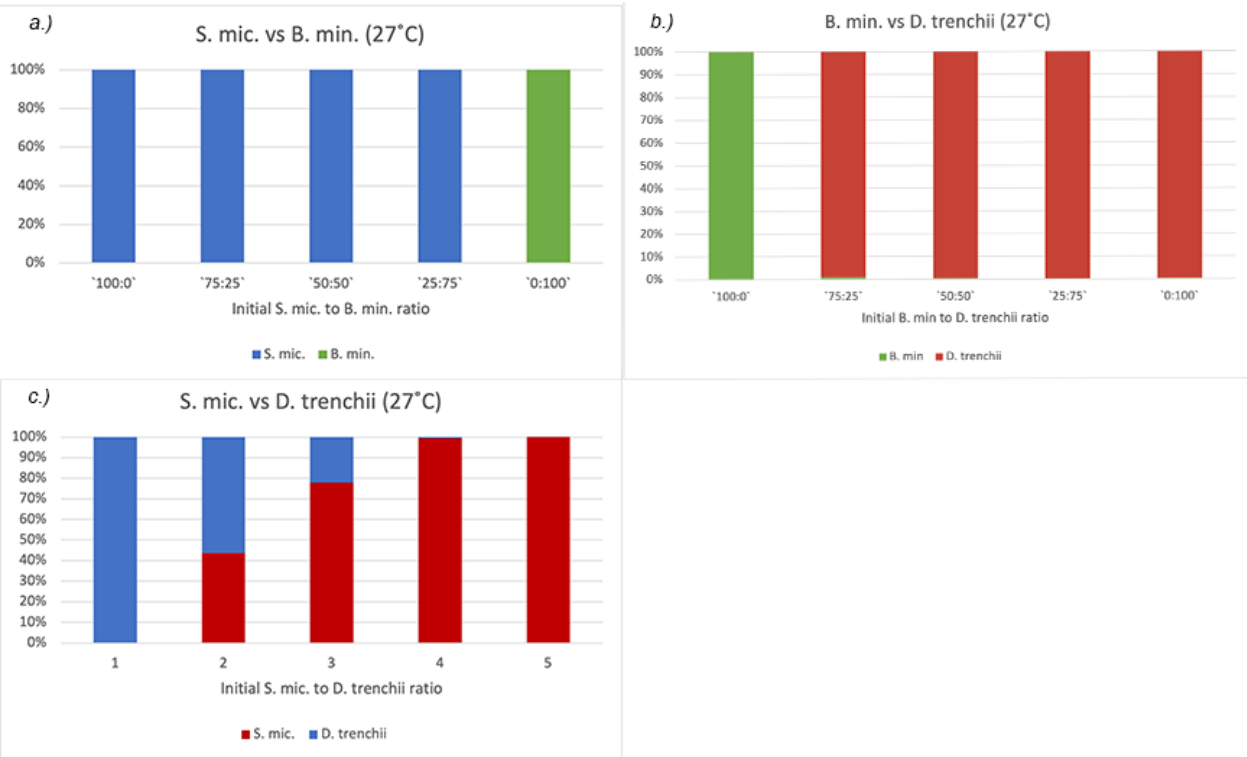

Figure 3: qPCR results of competition experiments between a.) *S. mic. vs B. min.*, b.) *S. mic*. vs *D. trenchii*,, and *c.) B. min.* vs *D. trenchii.* Relative abundance of each symbiont species was quantified using the standard curve regression line for each species.

Competition experiments under normal temperature conditions between these three species showed *B. min.* losing out to both *S. mic.* and *D. trenchii.* However, when *S. mic.* was placed in competition with *D. trenchii*, there was less of a clear takeover, with *D. trenchii* actually dominating over the homologous *S. mic.* in the 25% *S. mic* : 75% *D. trenchii* plate. Some plates yielded far fewer ephyrae than others, the full *S. mic.* plate yielded only two ephyrae, as did the 75:25 *S. mic.* vs *D. trenchii* and the 100% *B. min plate.*

To extrapolate the relative abundance of each symbiont species, equations from the standard curves for each symbiont species were used via a semi-logarithmic regression. To account for noise, no CT value over 33 was counted as a positive result.

Table 2: Significant Difference of Symbiont Quantities

Competition	p value
75% S. mic. vs 25% *B. min.*	2.328e-07*
50% S. mic. vs 50% *B. min.*	0.001853*
25% S. mic. vs 75% *B. min.*	3.996e-05*
75% B. min. vs 25% *D. trenchii*	6.425e-05*
50% B. min. vs 50% *D. trenchii*	0.007296*
25% B. min. vs 75% *D. trenchii*	0.0007414*
75% D. trenchii vs 25% *S. mic.*	0.07829
50% D. trenchii vs 50% *S. mic.*	0.001087*
25% D. trenchii vs 75% *S. mic.*	0.06678

* denotes significance at < 0.05 confidence

It was found that most competition trials showed a significant difference in abundance between the two symbiont species, except for the 75:25 and 25:75 *D. trenchii* vs *S. mic.* trials. In these, domination of one species over the other did not fully occur.

Results of Heat Stress Experiment

During the heat stressed experiments, in which polyps were held at 32˚C, the only competition experiments that yielded a statistically useful amount of ephyrae were the experiments that contained the homologous symbiont of *C. xamachana*, *S. microadriaticum.* The

heterologous competition yielded only two ephyrae throughout the duration of the experiment, and both of these animals strobilated early on in the experiment, before they had been exposed to heat stress for very long. At the end of the 8 week experiment, the polyps remaining in the heterologous competition plates appeared white and bleached under a dissecting microscope, while polyps in plates with *S. mic.* looked healthy and brown (visibly associated with symbionts). Both competition experiments containing *S. mic*. continued strobilation throughout the duration of the experiment.

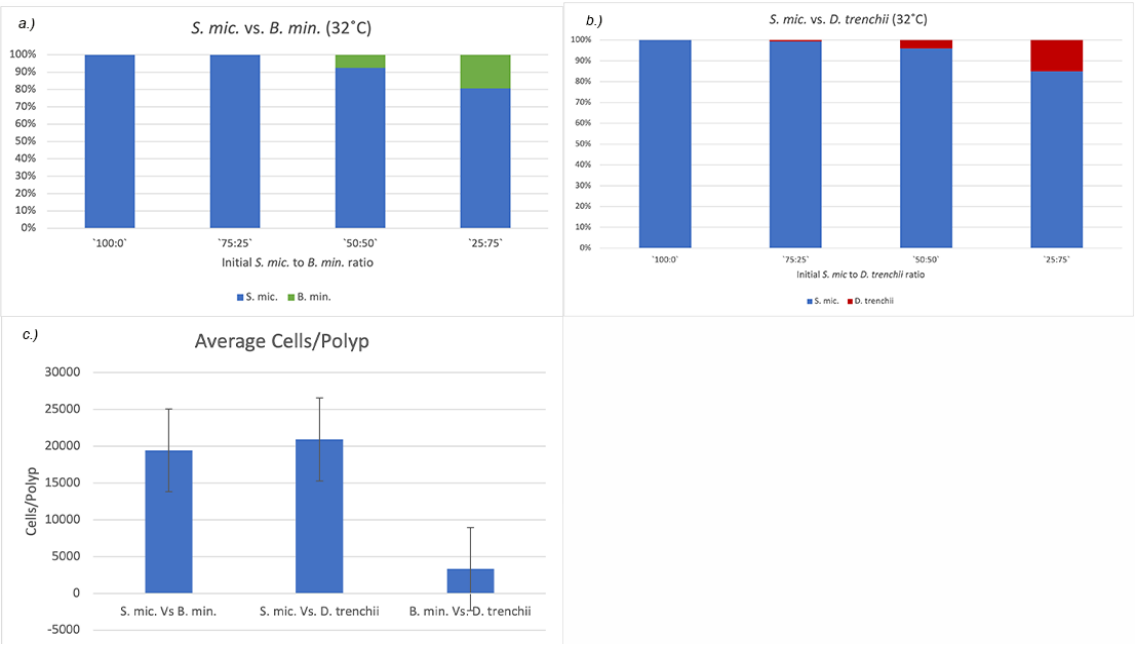

Figure 4: Results of competition experiments under heat stress (32˚C) A.) qPCR quantification of competition results between *S. mic.* and *B. min.*, and B.) between *S. mic.* and *D. trenchii.* The competition between *B. min.* and *D. trenchii* only yielded two ephyrae throughout the duration of the experiment: not enough to be statistically robust. C.) Cell counts for polyps from each of the three competitions.

Due to the lack of data for the heat stress + heterologous competition, n = 5 polyps from each treatment were collected and preserved in DMSO for imaging cell density using a Keyence BZ-9000E Fluorescence microscope. 3 polyps from the 50:50 competition for each competition

pair were gently squashed under a coverslip, taking care to minimize dissociation of host tissues, and imaged in both brightfield and fluorescence. One polyp from the 75:25 *S. mic*. to *B. min*. ratio was also imaged because the 50:50 polyps were a bit damaged from handling. Fluorescent images of symbionts were overlaid over brightfield images to show the distribution of symbionts within the animal tissue. Images were taken at 4x to capture the entire head of the polyp, where most symbiont cells tend to concentrate, to observe distribution of symbiont cells throughout the host tissues. A second set of images was taken at 10x, to observe cell density within each polyp. Some polyps were smaller than others and so were only imaged at 10x. To obtain more concrete data on cell abundance between these three experiments, imaged polyps were carefully removed from slides and placed in new tubes with uniform volumes of DMSO; host animals were macerated and samples homogenized, and cell counts were performed on a hemocytometer. From the images alone it was readily visible that the experiments containing *S. mic.* had a substantially higher cell density than those with only the heterologous symbionts. The heterologous competition polyps only presented with a sparse ring of symbionts around the edge of the polyp, appearing to be around the oral disk, however it is difficult to be certain as

preservation in DMSO caused the host tissues to become fragile and easily broken when being squashed for microscopy.

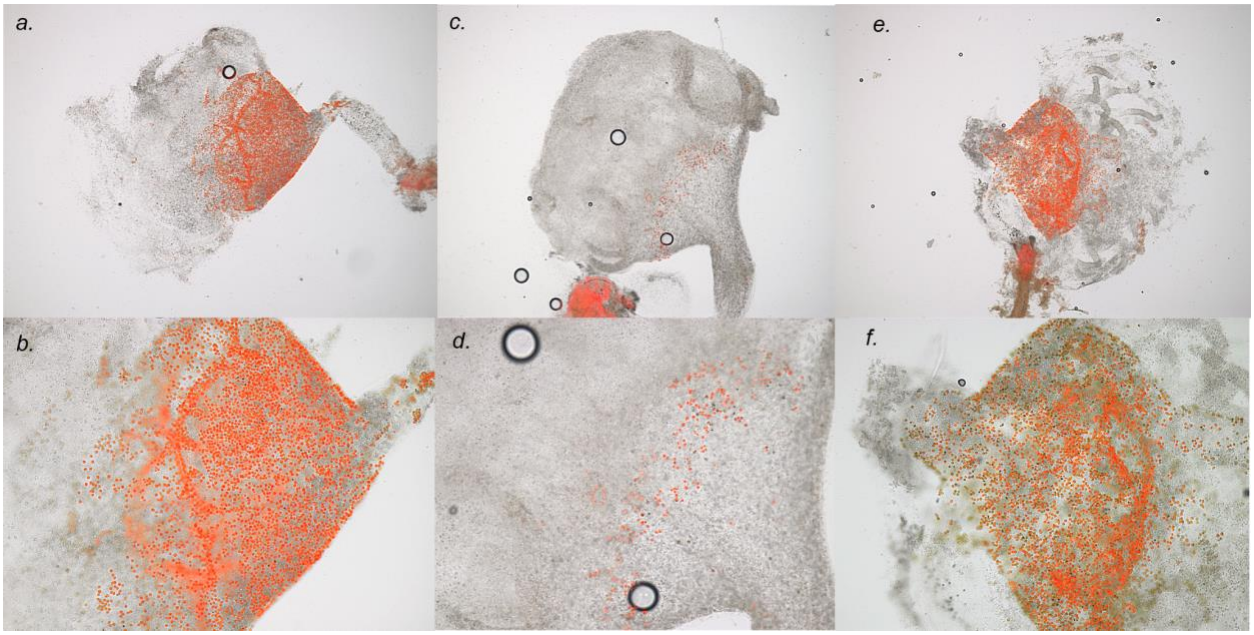

Figure 5: Fluorescent images of symbionts overlaid over brightfield images of polyps from the three competition groups at 32˚C. **a.)** and **b.)** *S. mic.* vs *B. min.*, **c.)** and **d.)** *B. min.* vs. *D. trenchii.*, **e.)** and **f.)** *S. mic.* vs *D. trenchii.* The top row is taken at 4x and the bottom row at 10x. The competition containing the native *S. mic.* has a visibly higher cell density than the competition between the two heterologous symbionts.

Table 3: Heat-Stress Cell Counts

Experiment	**Cell Count/polyp (average)**
S. mic. vs *B. min.*	19,444
B. min. vs. *D. trenchii*	3,333
S. mic. vs. *D. trenchii*	20,925

Table 4: Significance Differences Between Symbiont Abundances

	S. mic. vs *B. min.*	*B. min.* vs. *D. trenchii*	*S. mic.* vs. *D. trenchii*
S. mic. vs *B. min.*		0.01244*	0.7897
B. min. vs. *D. trenchii*	0.01244*		6.152e-06*
S. mic. vs. *D. trenchii*	0.7897	6.152e-06*	

* denotes significance at < 0.05 confidence

After imaging three polyps from each competition group, the polyps were removed from the slides and resuspended in 0.5 ul DMSO solution in 1.5ul microcentrifuge tubes. To homogenize these samples and dissociate the host tissue, samples were placed in a beadbeater for 2 minutes, without adding any glass beads. DMSO tends to make animal tissue fragile, and the beadbeater was able to agitate the samples sufficiently that host tissue broke apart, releasing the symbiont cells into solution.

Cell counts using a hemocytometer were performed on each sample in triplicate and the average cell counts were taken. To ensure that the cells being imaged were actually symbiont cells, three polyps from the negative control group were also homogenized and observed under a light microscope. It was found via T test that there was no significant difference in cell quantity between the *S. mic.* vs *B. min.* and the *S. mic.* vs *D. trenchii* competitions (p value > 0.5, 0.7897), but there was a significant difference in cell count between the two aforementioned groups and the heterologous competition *B. min.* vs *D. trenchii* (p values < 0.5, 0.01244 for *S. mic.* and *B. min*., and 6.152e-06 for *S. mic.* and *D. trenchii*).

Chapter 4

Discussion

The homologous symbiont *S. mic.* was overall the most successful under both control and heat stressed conditions, however it was most competitively dominant under heat stress as opposed to *B. min*. and *D. trenchii*. The thermotolerant *D. trenchii* was less successful under heat stress, where it was decisively defeated by *S. mic*, than under normal temperatures, where it held its own against *S. mic*. This may mean that, as suggested in Newkirk et. al. (2020), the system of *C. xamachana* is so optimized to harboring *S. mic* as its primary symbiont that higher thermotolerance of another, heterologous symbiont is irrelevant in terms of surviving heat-stressed conditions. Under conditions of stress, the system favors the native symbiont, whereas under normal conditions, the system may be more flexible and may accept non-native symbionts. This inter-partner specificity was also observed in Gabay et. al. (2019), within the brown anemone *Exaptasia pallida.* (2019).

The results of these experiments also suggest that symbiont competition is indeed happening. All things being equal, in the absence of interspecies competition, the faster growing symbiont should always win out. *B. minutum* grows considerably faster than both *D. trenchii* and *S. microadriaticum,* (Klueter et. al., 2017) so in the absence of other modes of competition, it should win out. However, as seen in this experiment, *B. minutum* was outcompeted by the two slower-growing species *S. mic*. and *D. trenchii*. Very little is known about the mechanisms by which competition between symbionts occurs, and further research might look into the intracellular and intercellular activity that drives symbiont competition.

These results also suggest that there may be an unseen cost to harboring non-native symbionts. In Solanki (2016), images of symbiont cell clusters were taken from week old ephyra infected with either *S. mic.*, *B. min.*, or *D. trenchii*, and it was found that ephyrae harboring *D. trenchii* had much smaller clusters when compared to cell clusters from polyps infected with *S. mic*. It is possible that the lower cell density within the animal and slower proliferation of *D. trenchii* in relation to *S. mic*. might be one cause for the reduced fitness under stress - animals could be getting less nutrients needed to combat stressful conditions.

Two polyps strobilated in the heterologous competition under heat stress, both within approximately the second week of heat stress (polyps were acclimated for a week following initial inoculation to allow animals to uptake the symbionts). Presumably the polyps experienced onset of symbiosis normally and persisted long enough for the two ephyrae to strobilate before temperature stress induced the bleaching phenotype. Performing cell counts on the un-strobilated polyps from the temperature stress group revealed that the two competitions involving the homologous *S. mic.* did not have a significantly different number of cells between the two of them ($p < 0.5$), but both had significantly higher cell counts than the heterologous competition group (*B. min.* vs. *D. trenchii.*). This further illustrates that polyps are less equipped to handle stressful conditions when harboring non native symbionts, even if the symbionts themselves are known to tolerate thermal stress.

Overall, DNA yields from 32˚C ephyra extractions were somewhat lower than those from 27˚C ephyrae but it is unsure whether this is a result of harboring a lower density of symbionts or problems with preservation or extraction.

Challenges

This project was not without challenges. Several inoculations failed early on in the experiments due to mass polyp deaths for unknown reasons. *Cassiopea* polyps can sometimes be rather hardy animals to work with, but other times they can be very temperamental. In working with this system it is important to ensure temperature, salinity, and light are being maintained at levels healthy for the animals, as at one point the incubator was malfunctioning and provided light for 24 hours rather than on a diurnal cycle, and this led to animal deaths. An additional note was that upon introducing polyps to a new environment, it is important to allow them around a day to acclimate before first feeding, and around a week to acclimate before changing water for the first time. For this experiment, to take extra care in keeping animals healthy, wells were "cycled" prior to addition of polyps by first introducing *Artemia* nauplii into the wells and allowing them to die in order to establish a bacterial community which was observed to be beneficial to polyps.

Careful technique minimized contamination while running qPCR, however one sample (100% *S. mic*) appeared with a possibly positive ID (a CT value of 32, still rather high, but worth investigating). Upon re-running this sample, the CT values fell back to 33 and below. Additionally, a few samples were not successfully extracted, namely one of the polyps from the *B. min.* vs *D. trenchii* experiment. When running this sample on nanodrop no DNA was detected. Fortunately, the 50:50 *B. min.* vs. *D. trenchii* experiment produced a few extra ephyrae which were able to be extracted.

Chapter 5

Further Research

A major question that was originally going to be addressed in this experiment was “are there direct fitness detriments to harboring a non-native symbiont in the long term?” Initially, a subset of ephyrae were meant to be maintained for an additional month following strobilation, however there was not a good way to keep the ephyrae reliably alive for long enough; as the animals grew larger it became harder to maintain them in the small culture plates they were in, and they were prone to dying unexpectedly, perhaps from poor oxygenation or not being fed frequently enough. However, an experiment on the long term fidelity of *C. xamachana* harboring non-native symbionts would provide valuable insight. In the wild, despite *D. trenchii*’s slower growth, it has been observed that similar species of corals that naturally harbor *D. trenchii* vs another species do not experience significant slowing of growth (Rivera et. al. 2022). However, considering *C. xamachana*’s observed partner specificity with *S. microadriaticum,* there may be observed detriments to harboring a different symbiont in the long-term. In Solanki (2019), high-res images were taken of week-old ephyrae infected with *S. mic.* and *D. trenchii,* in which *S. mic.* ephyrae had much larger symbiont cell clusters and greater cell density. It is possible that the lower symbiont density may influence nutrient acquisition on the part of the system.

Another possible study could look at recovery from bleached conditions - as seen via imaging polyps, although heat stressed polyps in the heterologous competition appeared very bleached and white, under the fluorescent scope it was clear they still harbored a small amount of symbionts. Presumably then, if returned to normal temperature conditions, the symbionts might

begin to proliferate again and induce strobilation. In Newkirk et. al. (2020), polyps were able to undergo complete bleaching and then reuptake symbionts once again, something that is not usually seen in adult jellyfish, so it is possible that polyps could also phagocytose algal cells from the environment following bleaching.

Chapter 6

Conclusion

The partner specificity between *C. xamachana* and *S. microadriaticum* is apparent. When competing against two other species of compatible symbionts under normal and stressful conditions, *S. mic*. competitively dominates despite the faster growth of *B. minutum* or the thermotolerance of *D. trenchii.* Introduced from the Indo-Pacific, *D. trenchii* is an invasive species in the Caribbean. When present in host corals, the corals are protected against bleaching. Thus, by minimizing coral bleaching, its proliferation in the Caribbean potentially raises the resilience of corals to climate change. (LaJeunesse et. al. 2009). However, studies such as the findings presented here point to the fact of partner specificity weighing out potential benefits of the introduction of a new symbiont, even when it is more physiologically adapted to the changing environment. In other words, even symbionts capable of tolerating various conditions may not increase fitness of a host cnidarian which has coevolved with its own specialized native symbiont.

Appendix

R code 1: Comparative Analyses of Symbiont Cell Counts Under Heat Stress

```
t.test(AB, AD, paired = FALSE)

t.test(AB, BD, paired = FALSE)

t.test(AD, BD, paired = FALSE)
```

R code 2: Comparative Analyses of Symbiont Cell Abundance

```
# Plate 2 75:25 AB

t.test(Abundances$A2, Abundances$B2, paired = TRUE)

# Plate 3 50:50 AB

t.test(Abundances$A3, Abundances$B3, paired = TRUE)

# Plate 4 25:75 AB

t.test(Abundances$A4, Abundances$B4, paired = TRUE)

# Plate 6 75:25 BD

t.test(Abundances$B6, Abundances$D6, paired = TRUE)

# Plate 7 50:50 BD

t.test(Abundances$B7, Abundances$D7, paired = TRUE)

# Plate 8 25:75 BD

t.test(Abundances$B8, Abundances$D8, paired = TRUE)

# Plate 10 75:25 DA

t.test(Abundances$D10, Abundances$A10, paired = TRUE)

# Plate 11 50:50 DA

t.test(Abundances$D11, Abundances$A11, paired = TRUE)
```

```
# Plate 12 25:75 DA

t.test(Abundances$D12, Abundances$A12, paired = TRUE)

# Plate 2 TEMP 75:25 AB

t.test(Abundances$AH2, Abundances$BH2, paired = TRUE)

# Plate 3 TEMP 50:50 AB

t.test(Abundances$AH3, Abundances$BH3, paired = TRUE)

# Plate 4 TEMP 25:75 AB

t.test(Abundances$AH4, Abundances$BH4, paired = TRUE)

# Plate 10 TEMP 75:25 DA

t.test(Abundances$DH10, Abundances$AH10, paired = TRUE)

# Plate 11 TEMP 75:25 DA

t.test(Abundances$DH11, Abundances$AH11, paired = TRUE)

# Plate 12 TEMP 75:25 DA

t.test(Abundances$DH12, Abundances$AH12, paired = TRUE)
```

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ACADEMIC VITA

Michael Hewitt

Education

The Pennsylvania State University, Schreyer Honors College
Graduation Year 2023
Bachelor of Science in Biology with Ecology Focus
Minor in Marine Science

Research Experience

Undergraduate Researcher/ Research Assistant at Pennsylvania State University, Symbiosis Evolution and Ecology Lab

- I currently work as an undergraduate researcher in the Symbiosis Evolution and Ecology Lab with Dr. Todd LaJeunesse. We primarily study symbiodiniaceae (algae) and the symbioses they form with cnidarians such as corals and jellyfish.
- **Symbiont Competition in *Cassiopea***
    - I am submitting my undergraduate thesis for the Schreyer Honors College, in which I am doing algal competition and heat stress experiments within the model system *Cassiopea xamachana*.
- **The influence of photosymbiosis in *Cassiopea xamachana* regenerative success**
    - I performed DNA extractions to identify symbiont species harbored in the jellyfish used in this experiment looking at regenerative abilities in *C. xamachana*.
- **High Molecular Weight DNA Extractions from Host Tissue**
    - I have helped develop protocols and run extractions of high molecular weight DNA from symbiont cells extracted from host corals.
- **Sequencing Symbiodiniaceae species**
    - I assisted in sequencing samples for a project focused on describing previously undescribed species of Symbiodiniaceae.

College Intern at Mote Marine Laboratory
Benthic Ecology Lab

- In the summer of 2021 I worked as an intern at the Mote Marine Laboratory in Sarasota, Florida. I worked in the Benthic Ecology Lab, primarily on *Karenia brevis* and red tide mitigation, as well as bayfront monitoring, shellfish restoration, and blue hole research. I also worked closely with the Red Tide Institute and the Ocean Acidification Lab.

Awards/Grants

2022 - Erickson Discovery Grant Recipient

Aug 2021 - May 2023 (expected) - Dean's List

2019 - 2023 - Schreyer Academic Excellence Scholarship

Involvement and Leadership

Penn State Marine Science Society

President (2022 - 2023)
Communications Officer (2022)
Events Chair (2021 - 2022)

- I am currently the President of the Penn State Marine Science Society, and have served on the Executive board for three years. We are a student organization focused on education, outreach, and conservation in marine science. In this position, I help plan and facilitate weekly meetings, preside over officer meetings, help with recruiting new members, fundraising, and planning events and trips.

Statespeare

Secretary (2021 - 2023)
Director (2023)
Technical Director (2021)

- I have served on the executive board for Statespeare, Penn State's Shakespeare organization dedicated to the study and performance of Shakespeare's works. I served as the Technical Director in 2021, and from 2021 to 2023 I have served as the club's secretary.
- In Spring of 2023 I was the lead director of our production of *Julius Caesar*. I have also performed in several of Statespeare's plays.

Penn State Renaissance Faire Club

Treasurer (2021)

- I was involved in the Penn State Renaissance Faire Club from 2019 until it's disbandment in 2021, in which time I briefly served as the club's treasurer.