7-24-2019

Timing and Potential Drivers of Symbiont Selection in the Early Life Stages of the Massive Starlet Coral Siderastrea siderea

Sarah G. Koerner
sk1310@mynsu.nova.edu

Thesis of
Sarah G. Koerner

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

M.S. Marine Biology

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

July 2019

Approved:
Thesis Committee

Major Professor: Joana Figueiredo, Ph.D.

Committee Member: Nicole Fogarty, Ph.D.

Committee Member: Andrew Baker, Ph.D.

HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

TIMING AND POTENTIAL DRIVERS OF SYMBIONT SELECTION IN THE EARLY LIFE STAGES OF THE MASSIVE STARLET CORAL *SIDERASTREA SIDEREA*

By:

Sarah G. Koerner

Submitted to the Faculty of
Halmos College of Natural Sciences and Oceanography
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

July 2019

Table of Contents

List of Figures

Abstract

The ability of corals to build reefs can be attributed to their relationship with single-celled algae of the family Symbiodiniaceae. Through the process of photosynthesis, these algae can provide their coral hosts with over 90% of their daily energy requirements. Most coral species acquire multiple species of symbionts from the surrounding water during their larval stage or immediately after settling. However, over time, the coral will select a dominant symbiont species that can depend on the local environment. Until this study, the size or age of the coral at which this transition from multiple Symbiodiniaceae species to one dominant species occurs has remained uncertain. Likewise, it was unclear whether the selection of Symbiodiniaceae species is influenced by the environment. The environmental conditions and symbiont composition of one hundred and eighteen juvenile *Siderastrea siderea* were assessed across four sites in Broward County, Florida. Presuming newly settled corals acquire multiple symbionts and then select just one dominant species, it was determined that the transition from multiple symbiont species to one dominant species in *Siderastrea siderea* occurs in the single polyp stage, between the time of settlement and approximately 4 to 6 months of age. The results also suggest that the selection of these dominant symbiont species is influenced by the environment, and that juveniles commonly select the same species as adults inhabiting similar environmental conditions. The selection of symbionts homologous to adult corals combined with environmental influences may be an early indicator of acclimatization in *Siderastrea siderea.*

Key Words: Symbiodiniaceae*,* environmental conditions, juveniles

Introduction

Scleractinian corals are the foundation for tropical coral reefs, one of the most diverse ecosystems on Earth (Odum and Odum 1955; Stoddart 1969; Hughes 1989). Coral reefs provide habitats and essential resources such as food and shelter to countless marine organisms (Cesar et al. 2003; Cole et al. 2008). Not only are reefs ecologically important, but they also contribute to the economies of numerous countries (Moberg and Folke 1999) in the form of fisheries and tourism. The high economic value of these activities often leads to over-exploitation: fish stocks become depleted and coral colonies become broken or damaged from boat anchors, which have deleterious impacts on the ecosystem (Moberg and Folke 1999; Jackson et al. 2001). In addition, coastal development increases chronic sedimentation and pollution through industrial runoff (Moberg and Folke 1999; Talbot and Wilkinson 2001). Reef structures protect coastlines from harsh wave action, that in turn impedes erosion and excess sedimentation (Talbot and Wilkinson 2001). The ability of scleractinian corals to build large reefs can be attributed to their relationship with dinoflagellates in the family Symbiodiniaceae (Odum and Odum 1955; Muscatine and Porter 1977). These single-celled algae perform photosynthesis in the gastrodermal tissue of the coral and translocate the photosynthetic byproduct (carbon) directly to the host (Trench 1979; Muscatine 1990). Corals benefit from this association by using carbon to build calcium carbonate skeletons (Lesser et al. 1994), in addition to receiving energy needed for growth and reproduction (Falkowski et al. 1984; Grottoli et al. 2006).

Corals can associate with multiple species of Symbiodiniaceae, but typically one dominant, and this dominance is thought to be established early in life. The family Symbiodiniaceae is composed of nine genera and each genus is composed of multiple species (LaJeunesse 2002; LaJeunesse et al. 2003; LaJeunesse et al. 2004; Pochon et al. 2006; LaJeunesse et al. 2018). There are six genera associated with scleractinian corals (Pochon et al. 2014; Stat et al. 2008; Baker 2003): *Symbiodinium, Breviolum, Cladocopium, Durisdinium, Fugacium,* and *Gerakladium* (formerly clades A – D, F and G respectively) (LaJeunesse et al. 2018). Adult scleractinian corals generally associate with multiple Symbiodiniaceae*,* but one species is often relatively more abundant within the coral tissue than others and thus termed as the dominant species (Rowan and Knowlton 1995; van Oppen 2001; LaJeunesse 2002; Goulet 2006; Mieog et al. 2007). The dominant algal symbiont species within the adult coral is often established early in life (Little et al. 2004; Gomez-Cabrera et al. 2007; Abrego et al. 2009). In

many coral species, symbionts are transmitted horizontally, i.e. the coral's eggs do not contain symbionts and the larvae or newly settled juveniles acquire symbionts from the water column and benthos (Babcock and Heyward 1986; Harrison and Wallace 1990; Baird et al. 2008). During these early life stages corals are quite promiscuous and each coral larva or juvenile will uptake different symbiont species (Cumbo et al. 2012) that are often reflective of the composition found within the local environment. Juvenile corals may maintain diverse algal symbiont communities (including symbionts in different genera) for many months (Little et al. 2004; Gomez-Cabrera et al. 2007) or even years (Abrego et al. 2009). However, over time, a dominant species of symbiont is established in the coral (Abrego et al. 2009). This association with the dominant symbiont species may be homologous or heterologous to the one found adult colonies (Coffroth et al. 2001). This means that the juvenile coral may choose a dominant symbiont species that is the same as local adult colonies (homologous), or a species that is different from the local adult colonies (heterologous). However, it is hypothesized that this selection is dependent on the environmental conditions the coral experiences (Little et al. 2004; Gomez-Cabrera et al. 2008; Abrego et al. 2009).

The depth, light, and temperature experienced by newly settled corals likely have an important role in the selection of a dominant symbiont because each species of symbiont provides different benefits under different environmental conditions (Rowan 2004; Berkelmans and van Oppen 2006). In the Caribbean, the symbiotic associations of scleractinian corals exhibit a depth and light distribution pattern. Some symbiont species are commonly found in corals in high-light/shallow water (0-6m), while others are more often found in corals inhabiting low-light/deeper water (6-14m) (Rowan and Knowlton 1995; Rowan et al. 1997; LaJeunesse 2002; Baker 2001, 2003). Symbiodiniaceae produce amino acid compounds that provide protection from damaging light irradiance (Neale et al. 1998; Banaszak et al. 2000), suggesting that protection from high light irradiance is an advantage for corals living under those conditions (LaJeunesse 2002). In deeper environments with low irradiance, Caribbean scleractinians commonly associate with different symbiont species that are more beneficial (e.g. sustain higher calcification rates) under low light (Rowan and Knowlton 1995; Baker 2001; LaJeunesse 2002). While in pristine coral reefs light and depth are inversely related, meaning as depth increases, light will decrease. Differences in turbidity between sites of the same depth may lead to a difference in light irradiance at those depths. This causes symbiont species commonly found in

deeper waters to appear more often in shallow, turbid areas. Symbiont diversity may also differ within an adult colony (Rowan et al. 1997; Kemp et al. 2015), particularly in mounding corals where the polyps with high light exposure have a different symbiont species than those on the sides or bottom of the colony that experience low light exposure (Reich et al. 2017). In terms of temperature, it was previously thought that symbiont species in the genus *Durisdinium* are the most thermally tolerant (Glynn et al. 2001; Baker et al. 2004; van Oppen et al. 2005; Jones et al. 2008); however, it has been recently determined that some species within other genera also possess an increased thermal tolerance (Cunning et al. 2015; Swain et al. 2017). This characterization of *Durisdinium* spp. was based on their abundance within coral colonies post-bleaching (Glynn et al. 2001; Baker et al. 2004; van Oppen et al. 2005; Jones et al. 2008) and within corals living in lagoons exposed to higher temperatures (Fabricius et al. 2004). A study by Rowan (2004) found that adult corals hosting *Durisdinium* spp. had higher photochemical efficiency and higher ratios of net photosynthesis compared to adult corals hosting species in the genus *Cladocopium*. A second study by Berkelmans and van Oppen (2006) determined adult corals that had shuffled their symbionts from *Cladocopium* C2 to a *Durisdinium* spp. post-bleaching also had higher photochemical efficiency in a heat-stressed environment. *Durisdinium* spp. were initially hypothesized to provide higher thermal tolerance than other genera but to promote less growth. However, a study by Cunning et al. (2015) found that corals hosting *Durisdinium* spp. grew slower than corals with *Cladocopium* spp. at 26°C and 27.5°C, but at 29°C *Durisdinium* spp. did not reduce coral growth. These findings were consistent with field observations and further support the idea that association with at least some *Durisdinium* spp. enhances the thermal tolerance of corals (Abrego et al. 2008). Although there are some tradeoffs associated with hosting *Durisdinium* spp. (e.g. decreased carbon translocation, decreased photochemical efficiency, and high metabolic costs; Abrego et al. 2008; Cunning et al. 2015) it is hypothesized that thermal tolerance and survival is enhanced at little cost to the coral host.

The selection of algal symbionts by juvenile scleractinian corals can also be species-specific, with many consistently selecting the same algal symbiont species that are dominant in the adults, regardless of their environmental conditions (Coffroth et al. 2001; Weis et al. 2001; Rodriguez-Lanetty et al. 2004). Multiple species of symbiont may be acquired initially, but the symbiont associated with the adult population generally dominates after days or months (Coffroth et al. 2001; Weis et al. 2001; Rodriguez-Lanetty et al. 2004; Cumbo et al. 2012).

Coffroth et al. (2001) found that newly settled polyps of the gorgonian octocorals *Plexaura kuna* and *Pseudoplexaura porosa* associated with multiple symbiont species but did not initially reflect the dominant abundance of *Breviolum* species observed in adults. Regardless of habitat (4m, 6m, or 17m depth reef), newly settled polyps naturally acquired symbionts belonging to the genera *Symbiodinium*, *Breviolum* and *Cladocopium*. Yet after 3 months, 77% of the polyps harbored *Breviolum* spp. (Coffroth et al. 2001). This observation was confirmed by a field survey of juvenile gorgonians (10 cm or less) where all juveniles also contained *Breviolum* spp. at 3 months of age (Coffroth et al. 2001). Similar studies by Weis et al. (2001) and Rodriguez-Lanetty et al. (2004) found that coral larvae of *Fungia scutaria* also associated with the same species of symbiont as the adults. Weis et al. (2001) observed no changes in symbiont population densities after 4 days, regardless of the symbiont species inoculated or the light environment (low vs. high light) in which larvae were incubated. Rodriguez-Lanetty et al. (2004) determined *Cladocopium* C1f was dominant within the larvae 24 hours after inoculation. This symbiont species was also found in adult *F. scutaria.* These studies suggest a selection process which occurs some time during the early life stages of coral, but also that this selection may be in favor of homologous symbionts.

In contrast, other studies found that juvenile *Acropora* acquire and maintain symbiont species different from those found in adult parent colonies. Studies of *A. tenuis* have shown that adults associate with *Cladocopium* C1 (Little et al. 2004), and sometimes *Cladocopium* C2 (van Oppen et al. 2001; Ulstrup and van Oppen 2003). Little et al. (2004) discovered that *Durisdinium* spp. and *Cladocopium* C1 were acquired by juveniles in the first month. However, by 5 months the relative abundance of those species changed with a 57% increase in *Durisdinium* spp. and a 100% decrease in *Cladocopium* C1. The dominance of *Durisdinium* spp. in early juveniles of *A. tenuis*, in comparison to the dominance of *Cladocopium* C1 in adults (Ulstrup and van Oppen 2003, van Oppen et al. 2001), suggests that the host may be actively selecting certain species to maximize symbiont effectiveness in accordance with the physiological demands (Little et al. 2004). Similarly, adult colonies of *A. longicyathus* had *Cladocopium* spp. (86.7%), *Symbiodinium* spp. (5.3%), or a mixture of both *Symbiodinium* spp. and *Cladocopium* spp. (8.0%) (Gomez-Cabrera et al. 2008). Oddly, all 10-day-old juveniles hosted *Symbiodinium* spp., while 83-day-old juveniles contained *Symbiodinium* spp., *Cladocopium* spp. and *Durisdinium* spp. even though development occurred in the same reef location. *Symbiodinium* spp. were

dominant in both 10 and 83-day-old juveniles (99% and 97% of all recruits, respectively), but *Durisdinium* spp. were also found in 31% of 83-day-old juveniles. Gomez-Cabrera et al. (2008) suggested that neither adult species association nor the location within the reef influenced the symbiont species acquired by juvenile corals, and the dominant symbiont was simply determined by the exposure to one symbiont or the other. However, these differences in species composition between juveniles of different ages may simply be a result of varying times of acquisition of symbionts from different genera. For example, the 10-day-old juveniles may have just begun to acquire symbionts while the 83- day-old juveniles may have had time to acquire all local species.

The aforementioned studies on symbiont acquisition and selection of algal symbionts by coral juveniles only monitored the symbiotic relationship up to a maximum of 7 months after settlement (Abrego et al. 2009). Abrego (2009) and colleagues analyzed juvenile *Acropora tenuis* and *A. millepora* and found that associations with *Cladocopium* C1 or *Cladocopium* C2 (*A. tenuis)* and *Cladocopium* C2 or *Durisdinium* (*A. millepora)* may not be established until 2.5–3.5 years in the life cycle. At first, most juvenile *Acropora* colonies were dominated by species that were heterologous to their adult populations. The proportion of *A. tenuis* juveniles dominated by *Cladocopium* C1 increased at 18 months, while *A. millepora* showed no change over the course of the study. They hypothesized that changing environmental conditions associated with vertical growth of juvenile colonies at 18 months caused *A. tenuis* juveniles to favor *Cladocopium* C1 over *Durisdinium* spp. This hypothesis further suggested that both species may be acquired simultaneously, and one symbiont species may remain at background levels (Mieog et al. 2007) until more favorable conditions enable it to out-compete the other symbiont. This would indicate that environmental conditions may be the driver for symbiont selection.

Understanding the timing and environmental drivers of symbiont selection in coral juveniles is imperative to predict how these organisms may acclimatize and persist through environmental changes. This study characterized the diversity of symbionts in newly settled massive starlet coral, *Siderastrea siderea*, in Southeast Florida and determined the size and age at which juveniles select a dominant species of Symbiodiniaceae. This study also determined how the selection of symbiont species is driven by the environmental factors present in the field.

Methodology

Study Species

The coral species *Siderastrea siderea* is an abundant reef builder in the Florida Reef Tract (FRT) (Moyer et al. 2003; Banks et al. 2008). Single polyp recruits are identified by a shallow corallite approximately 2-5mm in diameter, 44-50 septa per corallite, 3-5 synapticulate rings, a weak corallite wall, thin columella, and high calyx elevation (Figure 1A; Foster 1980). Adult colonies have a light brown color, mounding or encrusting shape, and small recessed polyps (Figure 1B) (Ellis and Solander 1786). The species reproduces annually by broadcast spawning gametes between September and November (St. Gelais 2010) and acquires Symbiodiniaceae through horizontal transmission from environmental pools. Although colonies are relatively small (typically <50cm diameter) (St. Gelais et al. 2016), all colony sizes from recruit to adult are widely accessible in the local reefs of Broward County (Moyer et al. 2003; Banks et al. 2008; Walker and Gilliam 2013, Harper 2017). This species was chosen for the study because it acquires symbionts through horizontal transmission, and juveniles are relatively abundant in Broward County. Such abundance allowed for specimen collection to be carried out with minimal risk to wild populations.

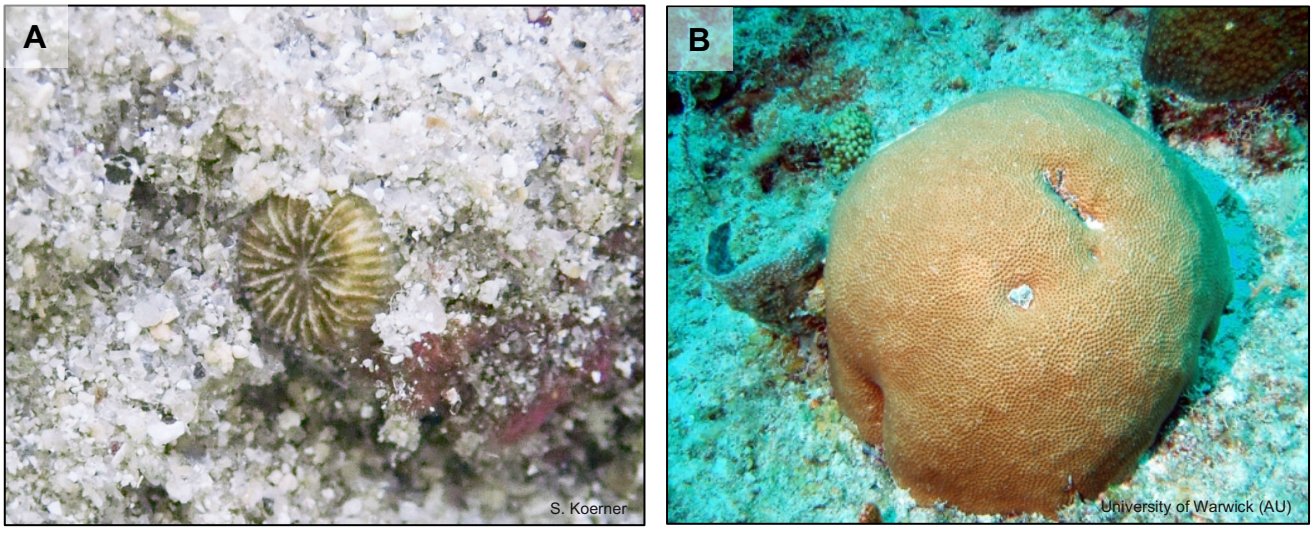

Figure 1: (A) Juvenile recruit and (B) adult colony of *Siderastrea siderea*

Study Location

Coral colonies were collected from four reef sites located along the FRT in Broward County, FL (Figure 2). Sites were chosen based on the relative abundance of juvenile *Siderastrea siderea* and variations in environmental conditions such as depth, light and turbidity. Each site represented three different reef types: Inner Reef (IR), Middle Reef (MR) and Outer Reef (OR) (Gilliam et al. 2013; Jones 2018). Site 1 (IR) was located 0.5 km from shore at 26° 08.878' N, 80° 05.772'W with maximum depth of 5.8 m. Site 2 (IR) was located 1.2 km from shore at 26° 08.963' N, 80° 05.364' W with max depth of 9.1 m. Site 3 (MR) was located 1,6 km from shore. at 26° 09.597' N, 80° 04.950' W with maximum depth of 17.1 m. Site 4 (OR) was located 2.2 km from shore at 26° 09.500'N, 80° 04.638' W with maximum depth of 19.8 m.

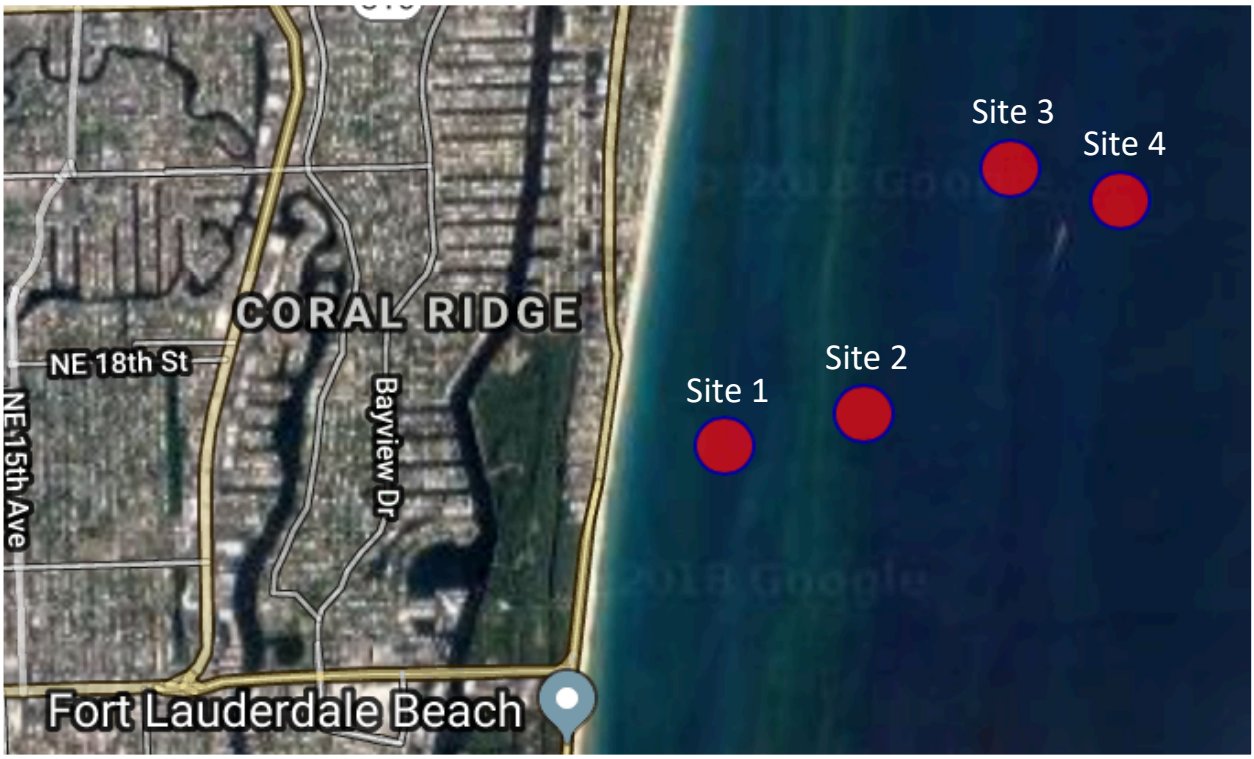

Figure 2: Study locations of four reef sites located in Broward County

Size at the Time of Dominant Symbiont Selection

Stratified sampling of 118 *Siderastrea siderea* juveniles ranging from one to twelve polyps in size was conducted. Six size classes were predetermined based on the number of polyps in a juvenile colony: 1 polyp, 2 polyps, 3 polyps, 4 to 6 polyps, 7 to 9 polyps, and 10 to 12 polyps. Five individuals from each size class (~30 samples total) were haphazardly selected on SCUBA at each of the four sites (Figure 2). Prior to sampling, the length (a) and width (b) of each recruit/colony was measured to the nearest millimeter using fractional calipers. Surface area of the recruit/colony was later calculated using the equation for the area an ellipse ($A = a/2 \times b/2 \times \pi$).

Environmental Conditions Affecting Dominant Symbiont Selection

Environmental conditions of temperature, turbidity, depth, sediment cover, algal cover, and coral cover were assessed for each of the 118 juvenile *S. siderea* sampled. Temperature was measured at the surface and at depth of each site using a YSI® Pro20 temperature probe. Water samples were also taken at depth of each site, and turbidity was later measured using a LaMotte® 2020we turbidimeter in Neplelometric Turbidity Units (NTU). For each coral sample, depth was measured using an Aqua Lung® i300C computer console. Sediment, algal, and coral cover was assessed by laying a 38 x 50 cm quadrat around each juvenile sampled. Using an Olympus® Tough TG-5, a photograph was then taken of the quadrat prior to sampling of the recruit or colony. These photographs were later analyzed using the Coral Point Count with Excel extensions® (CPCe) program to determine the percentage of benthos covered by sediment, algae, and coral.

Presence of Symbiont Species in Juvenile Tissues

The 118 coral juveniles sampled were collected for genetic analysis using a hammer and chisel, then placed in 0.38 L Whirl-pak® bags individually labelled with the site name and sample number. At the surface, the coral samples were transferred to a cooler and relocated to Nova Southeastern University's Oceanographic Campus (NSU-OC). Upon arrival, each sample was transferred into a 1.5-mL micro centrifuge tube containing DNA Buffer with 1%SDS (Rowan and Powers 1991). Samples were heated for 90 minutes in a 65°C water bath to stabilize

lysates at room temperature. The preserved samples were then transported to the University of Miami, FL for DNA extraction following protocols established in the laboratory of Dr. Andrew Baker (Baker and Cunning 2016). Presence of different symbiont species were assessed using quantitative PCR (qPCR) and an actin-based assay, supplemented by DGGE of ITS-2 rDNA to assess species diversity. Cultures of three symbiont species typically found in *S. siderea* were used as standards: *Cladocopium* C1, Cladocopium C3, and *Durisdinium* D1a. Fifty-six unknown bands from the six resultant electrophoresis gels were cut out and sent for Sanger sequencing to determine the genomic composition. Geneious Prime©, version 2019.1.1 was then used to reverse-complement, align, and manually remove the forward and reverse primers. These cleaned up sequences were then compared against a collection of annotated ITS-2 sequences (Hume 2019) for known symbiont species using the National Center for Biotechnology Information (NCBI) nucleotide BLAST® program. Annotation for the presence of symbiont species within each sample was completed by comparing visible bands from each sample to the known symbiont marker bands (*Cladocopium* C1, Cladocopium C3, and *Durisdinium* D1a standards) and the previously unknown symbiont bands (Figure 3).

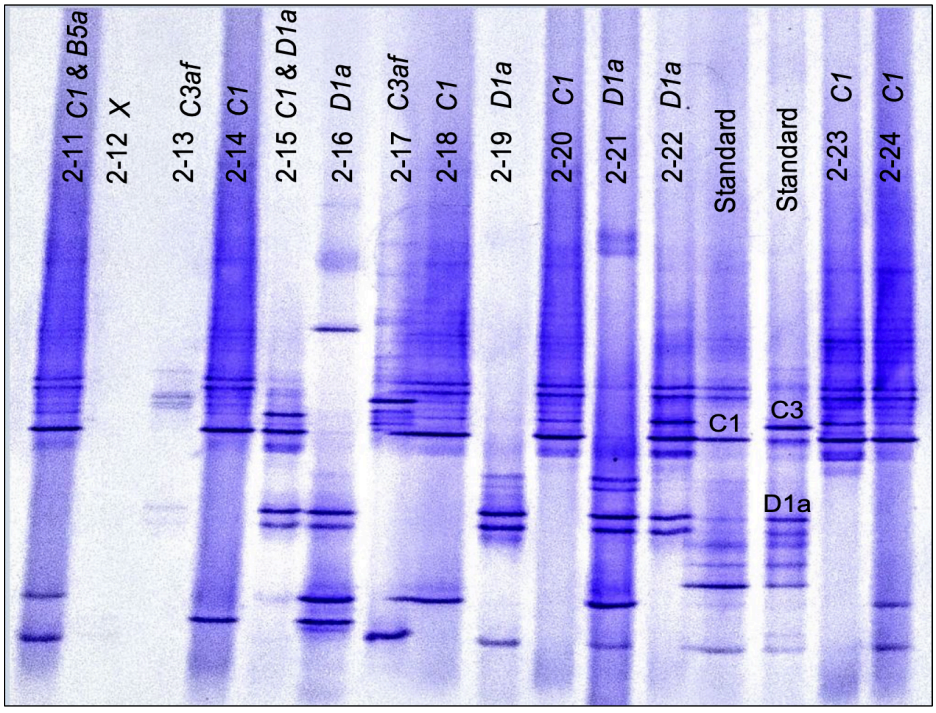

Figure 3: Electrophoresis gel 3 of 6 from DGGE analysis showing samples 11 - 24 from Site 2. Annotations of symbiont species within each sample can be seen in italic above the sample ID.

Statistical Analysis

To determine the size at which juvenile *Siderastrea siderea* select a dominant symbiont species, a simple regression was first used to describe the relationship between the number of polyps and surface area of juvenile corals. The data was mildly non-normal due to outliers, and a M-Regression was used to complete the analysis. To determine the association between coral size (number of polyps and surface area) and the number of symbiont species present in the tissue of juvenile corals, Frequency Analysis (contingency table) and Fischer exact tests were used. The number of polyps were divided into the six size classes previously determined: 1 polyp, 2 polyps, 3 polyps, 4 to 6 polyps, 7 to 9 polyps, and 10 to 12 polyps. The surface area was divided uniformly into four size classes: 1-35 mm^2, 36-71 mm^2, 72-107 mm^2, and 108-143 mm^2.

To assess the effects of environmental conditions (depth, sedimentation, and algal cover) on the dominant symbiont selected, simple linear regressions were used. Each condition was analyzed to determine if the dominant symbiont species changed as a result of depth, sedimentation or algal cover at each site. The effects of temperature, turbidity and coral cover on symbiont selection were not further analyzed due to the limited differences observed between sites. As the dominant symbiont was being assessed, data for *Cladocopium* C3f and *Cladocopium* C1ao was too small to conduct separate analyses and these species were combined with *Cladocopium* C3 and *Cladocopium* C1, respectively.

All analyses were performed in R Studio© with the R program©, version 3.5.3.

Results

Size at the Time of Selection of a Dominant Symbiont

There was a significant relationship between the number of polyps in a colony and the surface area of a colony ($p = 0.0001$, $R^2 = 0.759$) (Figure 4). There was no significant association between the number of symbionts present in the tissues of the juvenile corals ($n = 104$) and the number of polyps of the juvenile ($p = 0.549$) (Figure 5A) nor their surface area ($p = 0.952$) (Figure 5B). Most juveniles (70%), regardless of their size, contained only one symbiont species (Figures 5 and 6); only 17% containing two symbiont species and 2% (two individuals from site 1) containing three symbiont species (Figure 6). Fourteen of the initial 118 sampled juveniles were not used in the analysis because they produced very faint bands, likely due to low symbiont densities in their tissues.

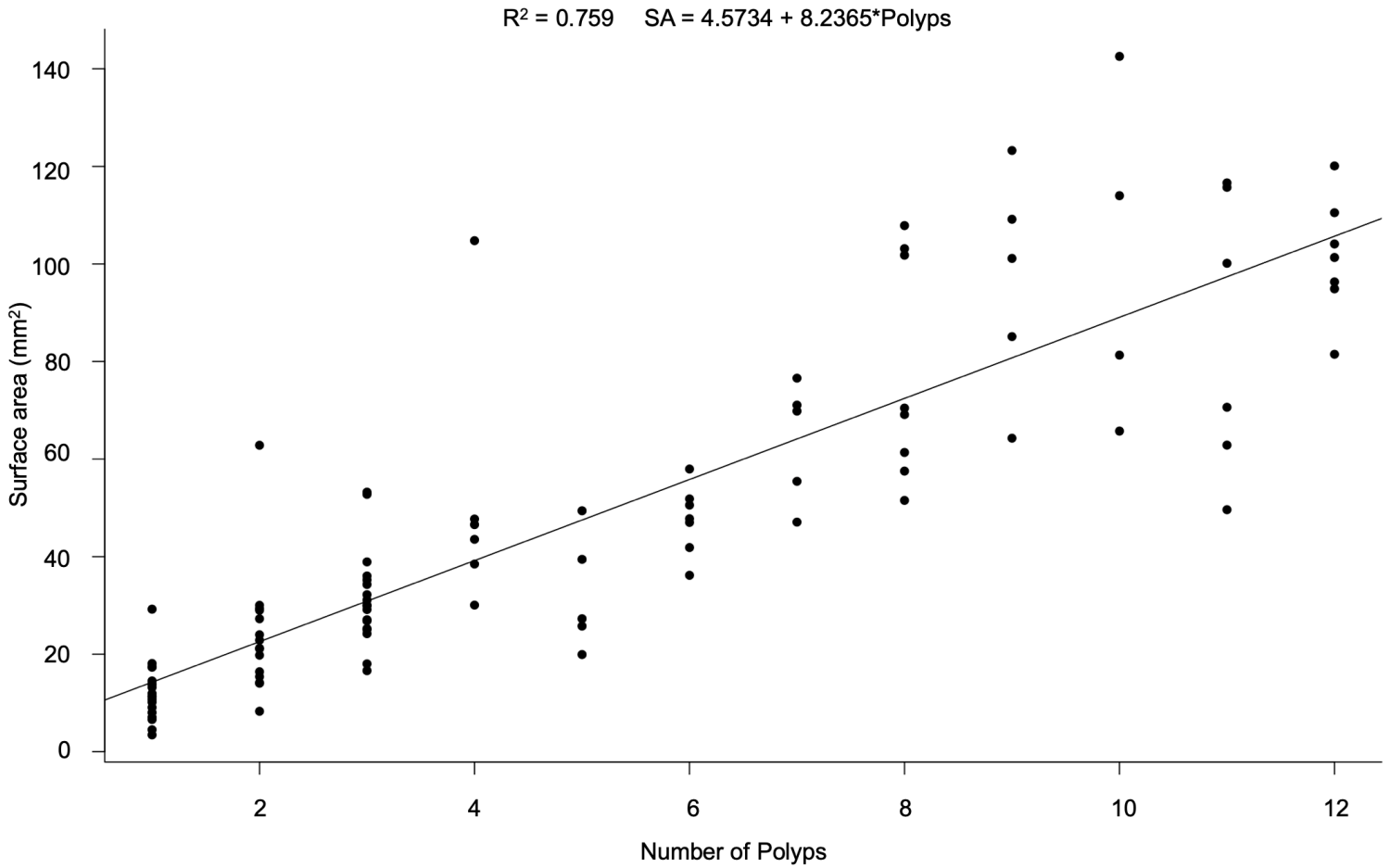

Figure 4: Relationship between the surface area of juvenile *S. siderea* and the number of polyps that make up the juvenile.

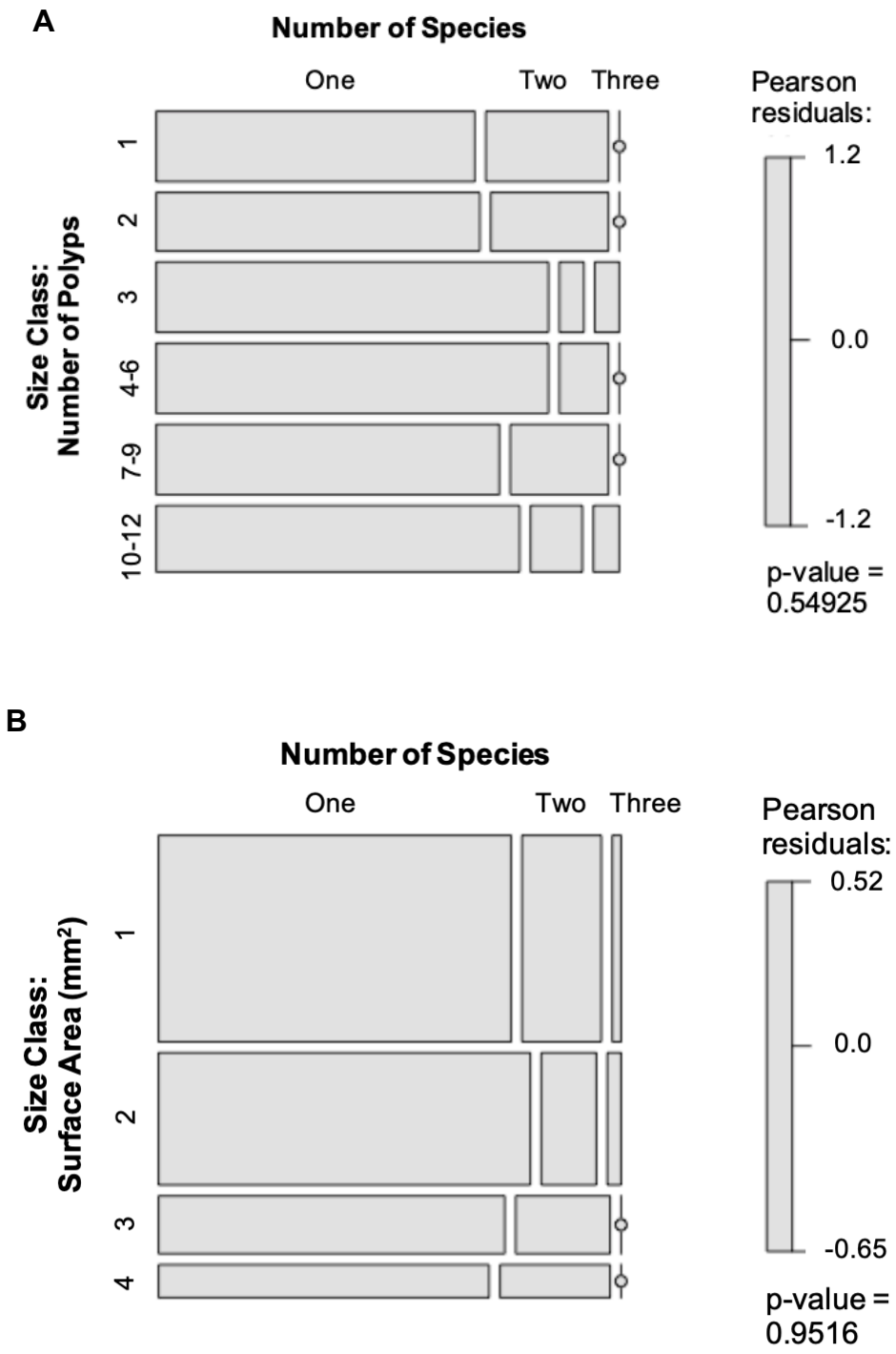

Figure 5: Structure plots of the association between size classes (number of polyps and surface area) and the number of symbiont species.

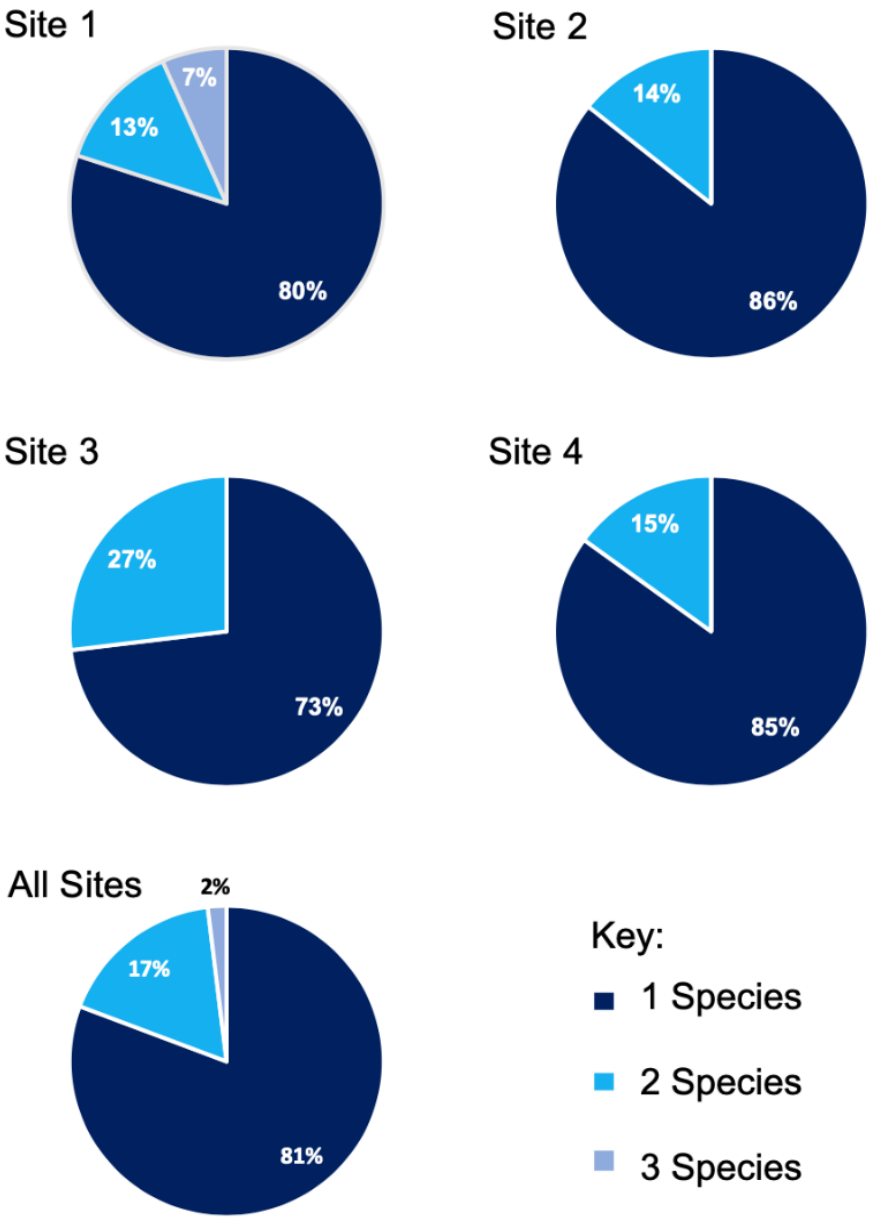

Figure 6: Number of symbiont species per sample as seen per location and across all sites combined.

Presence of Symbiodiniaceae Species in Juvenile Tissues

Most *Siderastrea siderea* juvenile samples (104 out of 118) yielded viable results indicating present symbiont species. Samples combined from the four sites contained symbiont species from three genera: *Breviolum* (40%)*, Cladocopium* (42%)*,* and *Durisdinium* (18%) (Figure 7A, C). The six symbiont species from these genera included *Breviolum* B5a (39%), *Cladocopium* C1 (26%), C1ao (1%), C3 (8%), and C3af (8%), and *Durisdinium* D1a (18%) (Figure 7B).

Siderastrea siderea samples from the shallower sites (Site 1 and 2), were dominated by *Breviolum* B5a (67% and 60% respectively, Figure 8). In the deeper sites, Site 3 and Site 4, *S. siderea* juveniles were dominated by *Cladosporium* C1 (36% and 46% respectively, Figure 8). *Durisdinium* D1a and *Cladocopium* C3af were found across all sites (Figure 8). *Durisdinium* D1a dominated 10% to 30% of the juveniles, while *Cladocopium* C3af dominated 4% to 11% of the juveniles (Figure 8). No juvenile from Site 4 was dominated by *Breviolum* B5a, and no juveniles from Site 2 were dominated by *Cladocopium* C1. *Cladocopium* C1ao was only found as a dominant species in some colonies at Site 4, but in a small amount, 4% (Figure 8).

Eighteen juveniles possessed secondary symbiont species (Figure 7C, D). *Durisdinium* was the most common secondary species (44%), followed by *Cladocopium* (39%) and *Breviolum* (17%, Figure 7C). *Cladocopium* C1ao was not present as a secondary symbiont (Figure 7D). While *Cladocopium* C1 and C3 were not present as dominant symbionts at Site 2, they were present as secondary species (Figure 8D and Figure 9). However, it is important to note that these appearances are derived from only two samples which had *Breviolum* B5a as a dominant symbiont (Figure 8).

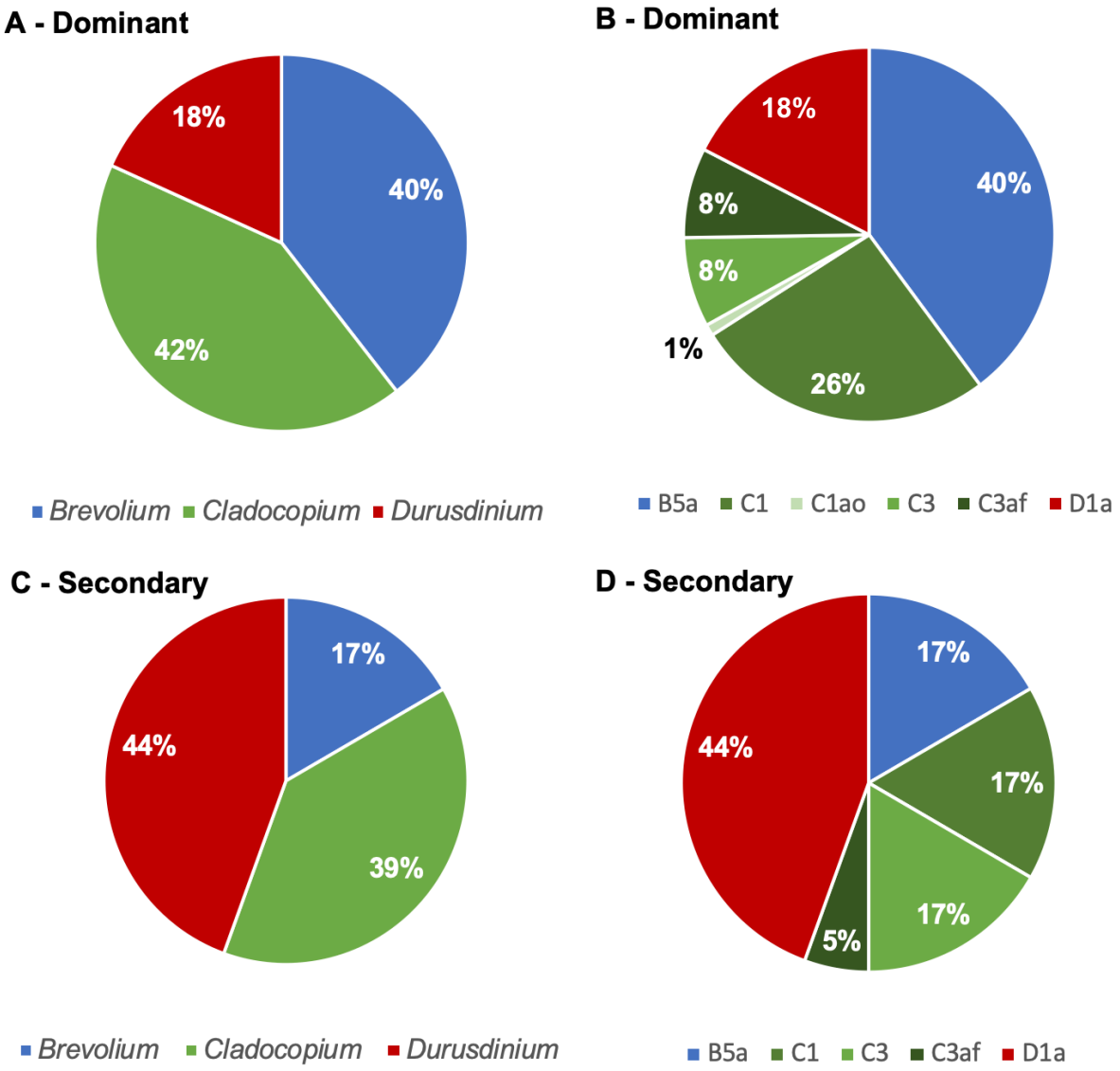

Figure 7: Diversity of dominant genera (A) and symbiont species (B), and secondary genera (C) and symbiont species (D) across all sites.

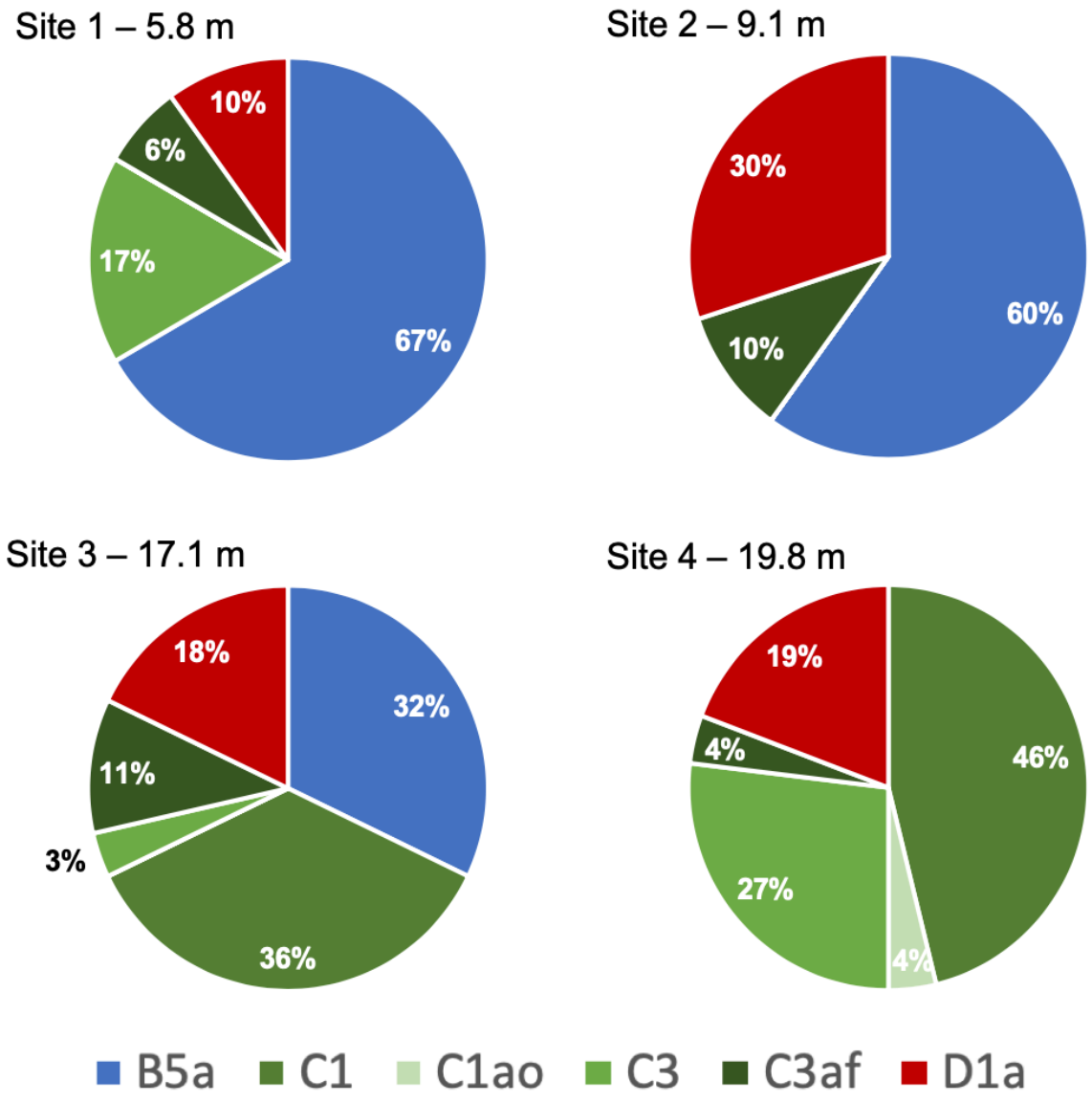

Figure 8: Diversity of dominant symbiont species for all sites.

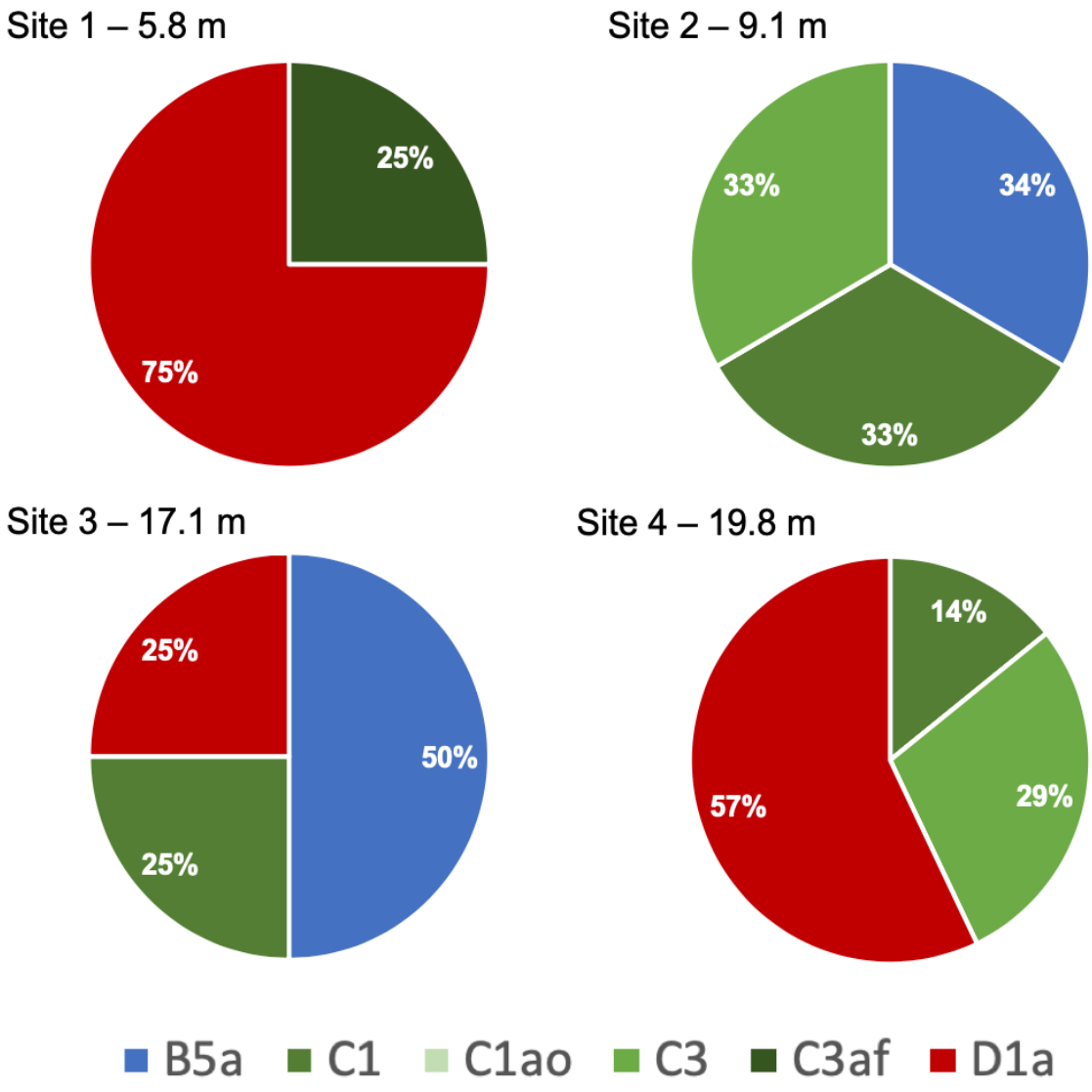

Figure 9: Diversity of secondary symbiont species for all sites.

Environmental Conditions Affecting Dominant Symbiont Selection

The shallow sites, Site 1 (5.8 m depth) and Site 2 (9.1 m depth), had a similar average sediment cover (54% $\pm$ 4.4 and 54% $\pm$ 4.7, respectively) but differed in average algal cover (39% $\pm$ 4.2 for Site 1 and 44% $\pm$ 4.5 for Site 2) (Figure 10). The deeper sites, Site 3 (17.1 m depth) and Site 4 (19.8 m depth), had respectively 49% $\pm$ 3.1 and 34% $\pm$ 5 average sediment cover and 43% $\pm$ 2.9 and 57% $\pm$ 5.1 average algal cover (Figure 10). Although temperature, turbidity and coral cover were measured, they were not included in the analysis due to the small variation between sites. Average temperature for Sites 1 through 4 were 24.4°C $\pm$ 0.065, 24.7°C $\pm$ 0.064, 24.9°C $\pm$ 0.102, and 25°C $\pm$ 0.018 respectively, turbidity was 0.156 NTU, 0.4 NTU, 0.184 NTU, and 0.256 NTU, and coral cover was 5% $\pm$ 1.9, 3% $\pm$ 1, 4% $\pm$ 1, and 7% $\pm$ 1.5.

The dominance of *Breviolum* B5a decreased significantly with depth ($p = 0.042$, $R^2 = 0.917$, Figure 11A) and increased with average sediment cover ($p = 0.045$, $R^2 = 0.911$, Figure 11B), but not with average algal cover ($p = 0.093$). The dominance of *Cladocopium* C3 also decreased significantly with average sediment cover ($p = 0.012$, $R^2 = 0.976$, Figure 11D) and increased significantly with average algal cover ($p = 0.022$, $R^2 = 0.957$, Figure 11C), but not with depth ($p = 0.130$). *Cladocopium* C1 and *Durisdinium* D1a did not change significantly with depth ($p = 0.142$ and 0.890, respectively), average sediment cover ($p = 0.148$ and 0.732, respectively), or average algal cover ($p = 0.327$ and 0.955, respectively). These results should however be interpreted with caution as they are based on the depth from each location.

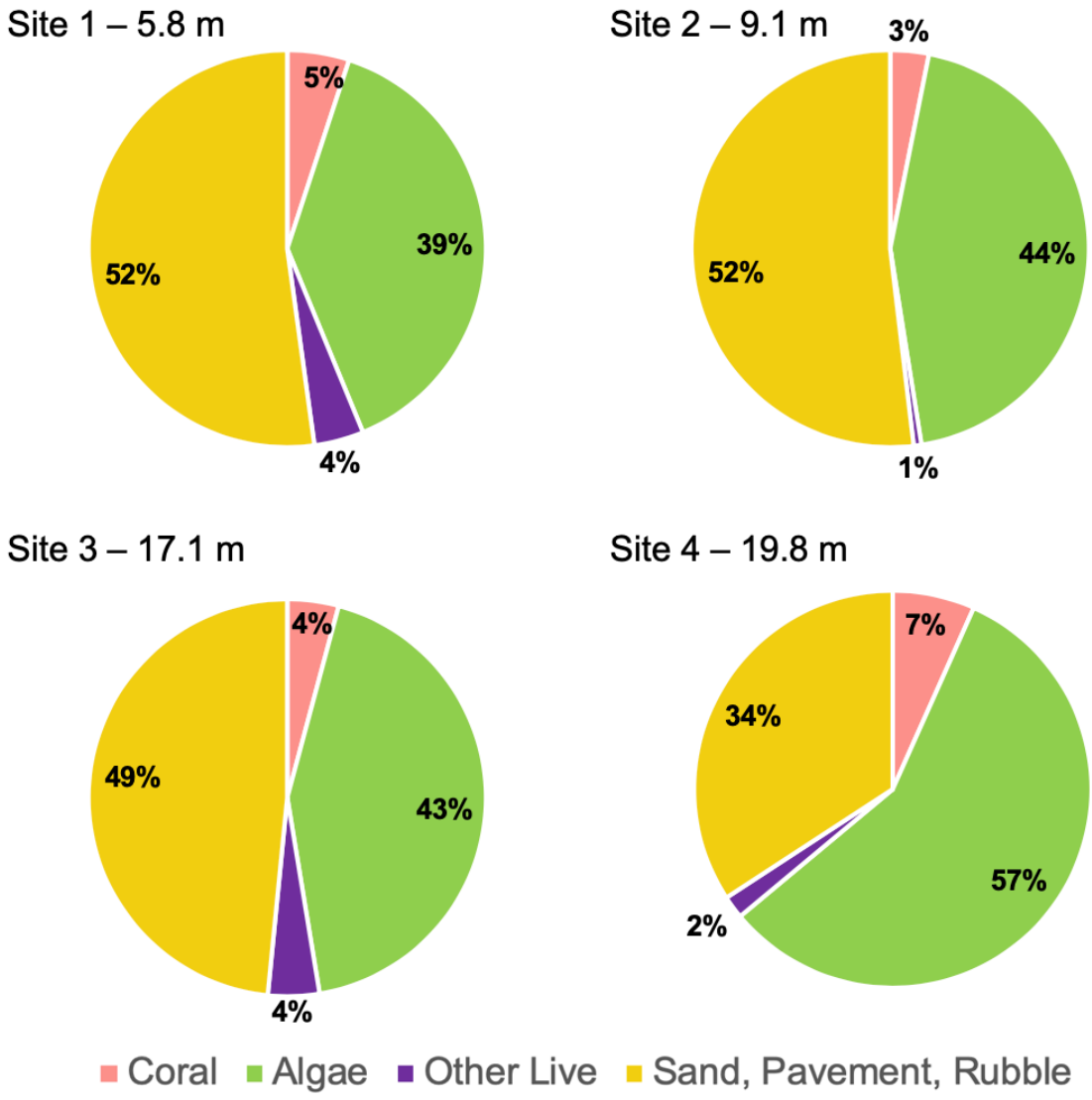

Figure 10: Average benthic cover across all sites.

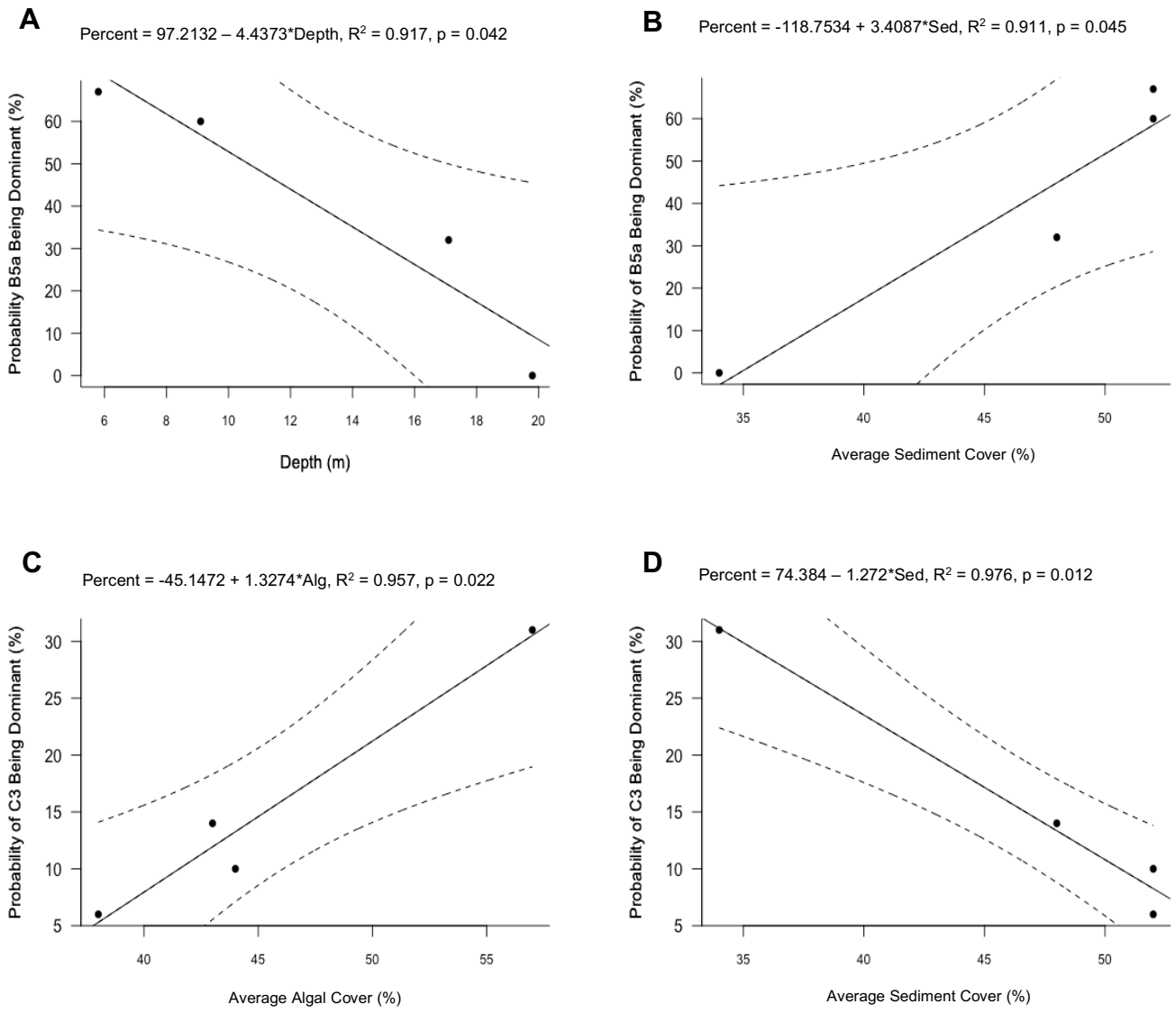

Figure 11: Dominant symbiont changes of *Breviolum* B5a (A and B) and *Cladocopium C3* (C and D) as a result of depth, average sediment cover and average algal cover.

Discussion

This study found that *Siderastrea siderea* juvenile corals can simultaneously associate with at least three species of Symbiodiniaceae. The results also suggest that symbiont selection takes place within the one-polyp stage, likely within the first 4 to 6 months after settlement. The presence of *Breviolum* B5a as dominant symbiont species decreases at greater depths and increases with average sediment cover, while the dominance of *Cladocopium* C3 decreases with average sediment cover and increases with average algal cover. Juvenile *Siderastrea siderea* corals in Broward County exhibit a similar association with symbiont species found in adult *S. siderea* that inhabit the Caribbean.

The lack of association between the size of a juvenile coral and the number of symbiont species it hosts, aligned with the fact that one-polyp corals often have a single Symbiodiniaceae species, suggests that symbiont selection occurs at the one-polyp stage within the first 4 to 6 months of recruitment. If coral larvae are promiscuous by indiscriminately acquiring multiple algal symbionts from the water column and benthos (Coffroth et al. 2001; Weis et al. 2001; Cumbo et al. 2012), then the transition towards a dominant symbiont species is occurring by the time a *Siderastrea siderea* recruit is one polyp in size. It is possible that symbiont diversity was more plentiful (greater than 3 species of Symbiodiniaceae) very early on in the one polyp size, and the selection period was missed in this study due to the timing of sampling, This could explain why most single polyp *S. siderea* already had one dominant symbiont. On the other hand, it could be hypothesized that single polyp *Siderastrea siderea* exhibit an immediate selection of a dominant symbiont instead of being promiscuous and indiscriminately acquiring symbionts. The dominant symbiont species juvenile *S. siderea* select may remain dominant in the coral until the 12-polyp stage and possibly throughout adulthood. However, evidence of this hypothesis has yet to be reported in literature. It is also probable that the time frame of this selection is 4 to 6 months after settlement because sampling began 4 to 6 months after the peak of annual spawning for this species (new moon October; St. Gelais 2010). This hypothesis is consistent with the fact that *S. siderea* are slow-growing, and only increase in diameter approximately 0.2 to 1 cm yr^{-1} (Huston 1985). Field studies by Harper (2017) also indicated that year old *S. siderea* recruits were measured between 1- 8 mm in size. Single polyp juveniles from this study were 1.9 - 6.1 mm. Therefore, selection is expected to occur early in life while the coral is of a smaller size. These results also agree with the age range found by Little et al. (2004) in other coral species.

Specifically, *Acropora tenuis* juveniles that were ~3 to 5 polyps in size began to adjust symbiont species abundance at four months old.

The symbiont species dominating juvenile *Siderastrea siderea* corals are partly dependent on depth, sediment, and algal cover. *Siderastrea siderea* sampled from Broward County associated with up to three symbiont species, with the most prevalent (the dominant) species varying between individuals and sites. As hypothesized, depth was shown to influence dominant symbiont selection. The probability of *Breviolum* B5a being the dominant symbiont decreased as depth increased (Figure 11A), which may be explained by the effects of climate change on Symbiodiniaceae during the Plio-Pleistocene. Five million years ago, Cladocopium C1 occurred in shallower habitats (~5 m) where they likely experienced temperature and light fluctuations. Thornhill et al. (2014) suggested that the success of *Breviolum* species in shallow Atlantic habitats during this time period (LaJeunesse 2002; Finney et al. 2010) may have led the genera to out-compete *Cladocopium* C1. *Breviolum* B6a has previously been found at depths ranging from 1 to 17m in the nearby Florida Keys (Thornhill et al. 2006, Correa et al. 2009; Bonthond et al. 2018). If *Breviolum* B5a is most successful in depth ranges up to 17 m, then *Cladocopium* C1 would likely be found at deeper depths in this area. This hypothesis is further supported in this study, as *Cladocopium* C1 was found to dominate at depths greater than 17 m (Figure 8).

It is unclear if the increased dominance of *Breviolum* B5a (Figure 11B) and decreased dominance of *Cladocopium* C3 with increasing average sediment cover (Figure 11D) is actually related to sediment cover, or if it is just an artifact that sediment cover changes with depth and distance from shore. In the Florida Reef Tract, average sediment cover is typically lower at deeper sites because they are located at greater distances from shore and thus less exposed to wave action (Banks et al. 2008). Therefore, it is possible that depth alone, not sediment cover, is driving the selection of *Breviolum* B5a and *Cladocopium* C3 as dominant symbionts in *S. siderea* of Broward County. However, it may be that *Cladocopium* C3 is not suited for environments with high turbidity or sediment cover, especially because it was not present as a dominant or background species in either of the shallow sites (Site 1 and 2) in Broward County. On the other hand, depth, turbidity and sediment cover are factors that are well correlated with light, which suggests that this selection pattern seen with depth is a reflection of the effect of light on algal symbiosis. The algal cover along the Florida Reef Tract is known to fluctuate seasonally and

across the different reef types (Banks et al. 2008, this study). The increased dominance of *Cladocopium* C3 with average algal cover found in this study is thus likely fortuitous and a result of low sampling.

While the selection of a particular symbiont as the dominant species is influenced by environmental factors, juvenile *Siderastrea siderea* are also exhibiting a selection that is in favor of symbionts homologous to their adult counterparts. In this study, it was found that juvenile *Siderastrea siderea* in Broward County, FL host *Breviolum* B5a, *Cladocopium* C1, C1ao, C3, C3af, and *Durisdinium* D1a (Figure 7B). The dominance of *Breviolum* B5a in juvenile *S. siderea* of Broward County (39%, Figure 7B) can easily be explained by the dominance of this symbiont in adult *S.* siderea in a shallow habitat of the nearby Florida Keys (Thornhill et al. 2006; Kemp et al. 2016). Adult colonies in the Caribbean have been documented to associate with *Breviolum* B5a, albeit less frequently (Thornhill et al. 2006; Kemp et al. 2016; Bonthond et al. 2018); while association with *Cladocopium* C3 (Thornhill et al. 2006; Thornhill et al. 2014; Kemp et al. 2016) and *Cladocopium* C1 (LaJeunesse 2002; Thornhill et al. 2014; Davies et al. 2018) are more frequent. In the Bahamas, Belize, Curacao, and St Croix, adult *S. siderea* associate with *Cladocopium* C3 and *Cladocopium* C1 (Thornhill et al. 2006; Thornhill et al. 2014; Davies et al. 2018). The similarity in symbiont preference among closer regions compared to the dissimilarity between different regions may reflect the variation in environmental conditions between regions and/or local co-adaptation of corals and Symbiodiniaceae species.

Although these *S. siderea* recruits are mostly dominated by symbionts homologous to their parents, 18% are dominated (Figure 7B) by (or have in the background; 44%, Figure 7C) a symbiont that is not typically dominant in this species: *Durisdinium* D1a (Figure 9). The presence of *Durisdinium* D1a in juvenile *S. siderea* is likely due the variability of the environment, where salinity, turbidity, and temperature fluctuations are common. Nonetheless, the presence of *Durisdinium* D1a may be an indicator that these coral species are enhancing their thermal tolerance in reflection of increased ocean temperatures. Mean sea surface temperatures have increased on average 0.13°C per decade over the past 100 years (NOAA 2019). Small deviations in ocean temperature result in the breakdown of the symbiotic relationship between Symbiodiniaceae and corals, a process known as bleaching which often leads corals to die (Jokiel and Coles 1977; Hoegh-Guldberg and Smith 1989; Glynn and D'Croz 1990; Brown et al. 1995; Glynn 1996). It is possible that *S. siderea* may be associating with *Durisdinium* D1a

because the thermal tolerance of *Breviolum* B5a, *Cladocopium* C3 and *Cladocopium* C1 are lower than that of *Durisdinium* D1a (Swain et al. 2017). Although hosting *Durisdinium* species comes with its disadvantages (e.g. decreased carbon translocation and high metabolic costs; Abrego et al. 2008; Cunning et al. 2015), corals hosting these species exhibit an increase in photochemical efficiency and net photosynthesis (Rowan 2004; Berkelmans and van Oppen 2006). Environmental changes, particularly increased sea-surface temperatures, are threatening the survival and existence of coral reef communities. If corals associated with *Durisdinium* species, even if only at background levels, this could allow for an increase in the relative abundance prior to or during a heat event or provide easier establishment of thermally-tolerant symbionts post-bleaching. An early association with a thermally-tolerant symbiont, can thus be a sign of acclimatization by juvenile *Siderastrea siderea.* If symbiont selection continues to be influenced by the environment, it may lead coral juveniles to increasingly establish permanent associations with thermally tolerant symbiont species. This could potentially prevent bleaching and thus provide corals with a greater resilience to extreme warming events. Further monitoring of symbiotic changes in reef-building corals such as *Siderastrea siderea* will provide insight into how coral populations are preparing to persist through a changing environment.

Acknowledgements

Siderastrea siderea colonies were collected under Broward County permit GL-WD1704-014 and Florida Fish and Wildlife Conservation permits SAL-17-1902-SRP and SAL-18-1902-SRP.

Funding for this research was provided by the Gumbo Limbo Nature Center Gordon Gilbert grant, the Broward Shell Club Shell scholarship, the Southern Florida Chapter of the Explorers Club grant, and the NSU President's Faculty Research and Development Grant.

I would like to thank my major advisor, Dr. Joana Figueiredo, and committee members Dr. Andrew Baker and Dr. Nicole Fogarty for their guidance. Thank you to Caroline Dennison and Olivia Williamson from the University of Miami for their help with the genetic analyses. I would also like to thank past and present members of the Marine Larval Ecology and Recruitment lab, Coral Reef Restoration, Assessment & Monitoring lab, and Reproduction and Evolutionary Ecology lab at NSU. Many thanks are owed to my parents Don and Paula, and boyfriend, Spencer, for their constant love, support, and encouragement. And Emma Brennan because without her, I would not have survived.

Literature Cited

Abrego D, Ulstrup K, Willis B, van Oppen M. 2008. Species–specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society of London B: Biological Sciences,* 275(1648): 2273-2282.

Abrego D, van Oppen M, Willis B. 2009. Onset of algal endosymbiont specificity varies among closely related species of *Acropora* corals during early ontogeny. *Molecular Ecology*, 18(16): 3532-3543.

Babcock R, Heyward A. 1986. Larval development of certain gamete-spawning scleractinian corals. *Coral Reefs*, 5(3): 111-116.

Baird A, Bhagooli R, Ralph P, Takahashi S. 2008. Coral bleaching: the role of the host. *Trends in Ecology and Evolution*, 24(1): 16-20.

Baker A, Cunning J. 2016. Bulk gDNA extraction from coral samples. *Protocols.io*, dx.doi.org/10.17504/protocols.io.dyq7vv

Baker A, Starger C, McClanahan T, Glynn P. 2004. Coral reefs: corals' adaptive response to climate change. *Nature*, 430(7001): 741.

Baker A. 2001. Ecosystems: reef corals bleach to survive change. *Nature*, 411(6839): 765-766.

Baker A. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, 34(1): 661-689.

Banaszak A, LaJeunesse T, Trench R. 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *Journal of Experimental Marine Biology and Ecology*, 249(2): 219-233.

Banks K, Riegl B, Richards V, Walker B, Helmle K, Jordan L, Phipps J, Shivji M, Spieler R, Dodge R. 2008. The reef tract of continental southeast Florida (Miami-Dade, Broward and Palm Beach counties, USA), in Coral Reefs of the USA. *Springer:* 175-220.

Berkelmans R, van Oppen M. 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1599): 2305-2312.

Bonthond G, Merselis D, Dougan K, Graff T, Todd W, Fourquerean J, Rodriguez-Lanetty M. 2018. Inter-domain microbial diversity within the coral holobiont *Sidereastrea siderea* from two depth habitats. *PeerJ,* 6: 4323.

Brown B, Le Tissier M, Bythell J. 1995. Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Marine Biology*, 122(4): 655-663.

Cesar H, Burke L, Pet-Soede L. 2003. The economics of worldwide coral reef degradation, in Cesar Environmental and Economics Consulting (CEEC). *CEEC*: 2-23.

Coffroth MA, Santos S, Goulet T. 2001. Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. *Marine Ecology Progress Series,* 222: 85-96.

Cole A, Pratchett M, Jones G. 2008. Diversity and functional importance of coral-feeding fishes on tropical coral reefs. *Fish and Fisheries,* 9(3): 286-307.

Correa A, Brant M, Smith T, Thornhill D, Baker A. 2009. *Symbiodinium* associations with diseased and healthy scleractininan corals. *Coral Reefs*, 28(2): 437-448.

Cumbo V, Baird A, van Oppen M. 2012. The promiscuous larvae: flexibility in the establishment of symbiosis in corals. *Coral Reefs*, 32(1): 111-120.

Cunning R, Gillette P, Capo T, Galvez K, Baker A. 2014. Growth tradeoffs associated with thermotolerant symbionts in the coral *Pocillopora damicornis* are lost in warmer oceans. *Coral Reefs*, 34(1): 155-160.

Davies S, Ries J, Marchetti A, Castillo K. 2018. Symbiondinium functional diveristy in the coral *Siderastrea siderea* is influenced by thermal stress and reef environment, but not ocean acidification. *Frontiers in Marine Science,* 5(150): 1-14.

Ellis J, Solander D. 1786. The Natural History of many curious and uncommon Zoophytes collected from various parts of the Globe. Systematically arranged and described by the late Daniel Solander. Benjamin White & Son, London: 1-206.

Fabricius K, Mieog J, Colin P, Idip D, van Oppen M. 2004. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology*, 13(8): 2445-2458.

Falkowski P, Dubinsky Z, Muscatine L, Porter J. 1984. Light and the bioenergetics of a symbiotic coral. *Bioscience*, 34(11): 705-709.

Finney J, Pettay D, Sampayo E, Warner M, Oxenford H, LaJeuness T. 2010. The relative significance of hos-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. *Microbial Ecology*, 60: 250-263.

Foster A. 1980. Environmental variation in skeletal morphology within the Caribbean reef corals *Montastrea annularis* and *Siderastrea siderea*. *Bulletin of Marine Science,* 30(3): 618-709.

Gilliam D, Brinkhuis V, Ruzicka R, Walton C. 2013. Southeast Florida coral reef evaluation and monitoring project 2012 year 10 final report. Florida DEP Report #RM085. Miami Beach, FL. P 53.

Glynn P, D'Croz L. 1990. Experimental evidence for high temperature stress as the cause of El-Nino-coincident coral mortality. *Coral Reefs*, 8(4): 181-191.

Glynn P, Mate J, Baker A, and Magnolia O Calderon. 2001. Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. *Bulletin of Marine Science,* 69(1): 79-109.

Glynn P. 1996. Coral reef bleaching: facts, hypotheses and implications. *Global Change Biology*, 2(6): 495-509.

Gomez-Cabrera M, Ortiz J, Loh W, Ward S, Hoegh-Gulberg O. 2007. Acquisition of symbiotic dinoflagellates (*Symbiodinium*) by juveniles of the coral *Acropora longicyathus*. *Coral Reefs*, 27(1): 219-226.

Goulet T. 2006. Most corals may not change their symbionts. *Marine Ecology Progress Series*, 321: 1-7.

Grottoli A, Rodrigues L, Palardy J. 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature*, 440(7088): 1186-1189.

Harper L. 2017. Variation in coral recruitment and juvenile distribution along the southeast Florida reef tract.. Master's thesis, Nova Southeastern University (FL), 89p.

Harrison P, Wallace C. 1990. Reproduction, dispersal and recruitment of scleractinian corals. *Ecosystems of the World*, 25: 133-207.

Hoegh-Guldberg O, Smith G. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Experimental Marine Biology and Ecology*, 129(3): 279-303.

Hughes T. 1989. Community structure and diversity of coral reefs: the role of history. *Ecology*, 70(1): 275-279.

Hume B. 2019. ITS-2 Annotated Sequences. *Github*, https://raw.githubusercontent.com/didillysquat/SymPortal_framework/master/symbiodiniumDB/refSeqDB.fa

Jackson J, Kirby M, Berger W, Bjorndal K, Botsford L, Bourque B, Bradbury R, Cooke R, Erlandson J, Estes J, Hughes T, Kidwell S, Lange C, Lenihan H, Pandolfi J, Peterson C, Steneck R, Tegner M, Warner R. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science,* 293(5530): 629-637.

Jokiel P, Coles S. 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals. *Marine Biology*, 43(3): 201-208.

Jones A, Berkelmans R, van Oppen M, Mieog J, Sinclair W. 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1641): 1359-1365.

Kemp D, Colella M, Bartlett L, Ruzicka R, Porter J, Fitt W. 2016. Life after cold death: reef coral and coral reef responses to the 2010 cold water anomaly in the Florida Keys. *Ecosphere* 7(6):1-17.

Kemp D, Thornhill D, Rotjan R, Iglesias-Prieto R, Fitt W, Schmidt G. 2015. Spatially distinct and regionally endemic *Symbiodinium* assemblages in the threatened Caribbean reef-building coral *Orbicella faveolata*. *Coral Reefs,* 34: 535-547.

LaJeunesse T, Loh W, Woesik R, Hoegh-Guldberg O, Schmidt G, Fitt W. 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnology and Oceanography,* 48(5): 2046-2054.

LaJeunesse T, Parkinson J, Gabrielson P, Jeong H, Reimer J, Voolstra C, Santos S. 2018. Systematic revision of Symbiondiniaceae highlights the antiquity and diveristy of coral endosymbionts. *Current Biology* 28: 2570 – 2580.

LaJeunesse T, Thornhill D, Cox E, Stanton F, Fitt E, Schmidt G. 2004. High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, 23(4): 596-603.

LaJeunesse T. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology*, 141(2): 387-400.

Lesser M, Weis V, Patterson M, and Jokiel P. 1994. Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora damicornis* (Linnaeus): diffusion barriers, inorganic carbon limitation, and biochemical plasticity. *Journal of Experimental Marine Biology and Ecology,* 178(2): 153-179.

Lewis J. 1997. Abundance, distribution and partial mortality of the massive coral *Siderastrea siderea* on degrading coral reefs at Barbados, West Indies. *Marine Pollution Bulletin,* 34: 622-627.

Little A, van Oppen M, Willis B. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science*, 304(5676): 1492-1494.

Mieog J, van Oppen M, Cantin N, Stam W, Olsen J. 2007. Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs*, 26(3): 449-457.

Moberg F, Folke C. 1999. Ecological goods and services of coral reef ecosystems. *Ecological Economics,* 29(2): 215-233.

Moyer R, Riegl B, Banks K, Dodge R. 2003. Spatial patterns and ecology of benthic communities on a high-latitude South Florida (Broward County, USA) reef system. *Coral Reefs,* 22(4): 447-464.

Muscatine L, McCloskey L, Marian R. 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respriration. *Limnology and Oceanography*, 26(4): 601-611.

Muscatine L, Porter J. 1977. Reef corals - mutualistic symbioses adapted to nutrient-poor environments. *Bioscience*, 27(7): 454-460.

Muscatine L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals. *Ecosystems of the World*, 25: 75-87.

Neale P, Banaszak A, Jarriel C. 1998. Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *Journal of Phycology,* 34(6): 928-938.

NOAA National Centers for Environmental information, Climate at a Glance: Global Time Series, published June 2019.

Odum H, Odum E. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. *Ecological Monographs*, 25(3): 291-320.

Pochon X, Montoya-Burgos J, Stadelmann B, Pawlowski J. 2006. Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium. Molecular Phylogenetics and Evolution,* 38(1): 20-30.

Pochon X, Putnam H, Gates R. 2014. Multi-gene analysis of *Symbiodinium* dinoflagellates: a perspective on rarity, symbiosis, and evolution. *PeerJ,* 2: e394.

Putnam H, Davidson J, Gates R. 2016. Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evolutionary Applications*, 9: 1165- 1178.

Reich H, Robertson D, Goodbody-Gringley G. 2017. Do the shuffle: changes in *Symbiodinium* consortia throughout juvenile coral development. *PLoS ONE,* 12 (2): e0171768.

Rodriguez-Lanetty M, Krupp D, Weis V. 2004. Distinct ITS types of *Symbiodinium* in clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Marine Ecology Progress Series*, 275: 97-102.

Rowan R, Knowlton N, Baker S, Jara J. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, 388(6639): 265-269.

Rowan R, Knowlton N. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. P*roceedings of the National Academy of Sciences*, 92(7): 2850-2853.

Rowan R, Powers D. 1991. Molecular genetic identification of symbiotitc dinoflagellates (zooxanthellae). *Marine Ecology Progress Series,* 71: 65-73.

Rowan R. 2004. Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* , 430(7001): 742-742.

St. Gelais A, Chaves-Fonnegra A, Brownless A, Kosmynin V, Moulding A, Gilliam D. 2016. Fecundity and sexual maturity of the coral *Siderastrea siderea* at high latitude along the Florida Reef Tract, USA. *Invertebrate Biology*, 135(1): 46-57.

St. Gelais A. 2010. Reproductive ecology of *Siderastrea siderea*: histological analysis of gametogenesis, spawning, and latitudinal fecundity variation. Master's thesis, Nova Southeastern University (FL), 80p.

Stat M, Morris E, Gates R. Functional diveristy in coral-dinoflagellate symbiosis. *Proceedings of the National Academy of Science,* 105: 9256 – 9261.

Stoddart D. 1969. Ecology and morphology of recent coral reefs. *Biological Reviews of the Cambridge Philosophical Society*, 44(4): 433-435.

Swain T, Chandler J, Backman V, Marcelino L. 2017. Consensus thermotolerance ranking for 110 *Symbiodinium* phylotypes: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential. *Functional Ecology*, 31: 172-183.

Talbot F, Wilkinson C. 2001. Coral reefs, mangroves and seagrasses: a sourcebook for managers. *Australian Institute for Marine Science:* 1-193.

Thornhill D, Lewis A, Wham D, LaJeunesse T. 2014. Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*, 68: 352-367.

Thronhill D, LaJeunesse T, Kemp D, Fitt W, Schmidt G. 2006. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, 148: 711- 722.

Trench R. 1979. The cell biology of plant-animal symbiosis. *Annual Review of Plant Physiology*, 30(1): 485-531.

Ulstrup K, van Oppen M. 2003. Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology,* 12(12): 3477-3484.

van Oppen M, Mahiny A, Done T. 2005. Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs* 24(3): 482-487.

van Oppen M, Palstra F, Piquet A, Miller D. 2001.Patterns of coral–dinoflagellate associations in Acropora: significance of local availability and physiology of Symbiodinium strains and host–symbiont selectivity. *The Royal Society of London B: Biological Sciences*, 268(1478): 1759-1767.

van Oppen M. 2001. In vitro establishment of symbiosis in *Acropora millepora* planulae. *Coral Reefs*, 20(3): 200.

Walker B, Gilliam D. 2013. Determining the extent and characterizing coral reef habitats of the northern latitudes of the Florida Reef Tract (Martin County). *PloS ONE* 8(11): e80439.

Weis V, Reynolds W, Deboer M, Krupp D. 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs*, 20(3): 301-308.