


1-26-2018

# Rare Parthenogenic Reproduction in a Common Reef Coral, *Porites astreoides*

Alicia A. Vollmer  
aliciavollmer@gmail.com

Follow this and additional works at: [https://nsuworks.nova.edu/occ\\_stuetd](https://nsuworks.nova.edu/occ_stuetd)

 Part of the [Marine Biology Commons](#), and the [Oceanography and Atmospheric Sciences and Meteorology Commons](#)

## Share Feedback About This Item

---

### NSUWorks Citation

Alicia A. Vollmer. 2018. *Rare Parthenogenic Reproduction in a Common Reef Coral, Porites astreoides*. Master's thesis. Nova Southeastern University. Retrieved from NSUWorks, . (464)  
[https://nsuworks.nova.edu/occ\\_stuetd/464](https://nsuworks.nova.edu/occ_stuetd/464).

This Thesis is brought to you by the HCNSO Student Work at NSUWorks. It has been accepted for inclusion in HCNSO Student Theses and Dissertations by an authorized administrator of NSUWorks. For more information, please contact [nsuworks@nova.edu](mailto:nsuworks@nova.edu).

---

Thesis of  
Alicia A. Vollmer

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science  
M.S. Marine Biology  
M.S. Coastal Zone Management

Nova Southeastern University  
Halmos College of Natural Sciences and Oceanography

January 2018

Approved:  
Thesis Committee

Major Professor: Nicole Fogarty

Committee Member: Joana Figueiredo

Committee Member: Xaymara Serrano

HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

RARE PARTHENOGENIC REPRODUCTION IN A COMMON REEF  
CORAL, *PORITES ASTREOIDES*

By

Alicia A. Vollmer

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  
and  
Coastal Zone Management

Nova Southeastern University

January 26, 2018

# **Thesis of Alicia A. Vollmer**

Submitted in Partial Fulfillment of the Requirements for the Degree of

## **Masters of Science: Marine Biology and Coastal Zone Management**

Nova Southeastern University  
Halmos College of Natural Sciences and Oceanography

January 2018

Approved:

Thesis Committee

Major Professor : \_\_\_\_\_  
Nicole Fogarty, Ph.D.

Committee Member : \_\_\_\_\_  
Joana Figueiredo, Ph.D.

Committee Member : \_\_\_\_\_  
Xaymara Serrano, Ph.D

## **ACKNOWLEDGEMENTS**

Many thanks to my major advisor Dr. Nicole Fogarty for being an excellent mentor and teacher throughout my graduate school experience. Being one of her graduate students has allowed me to gain numerous skills and experiences that have shaped me to become a better scientist. As one of her students, I have had the opportunity to work on many research projects, travel, and make connections with fellow marine scientists that have enhanced my experiences in graduate school. Through Dr. Fogarty's guidance and encouragement I was able to conduct my research both in the field and in the laboratory creating a successful research project. Additionally I would like to thank my committee members for their help throughout the entire process of my thesis work. Thank you to my committee member Dr. Xaymara Serrano for sharing your microsatellite primers and knowledge of genetics with me. Thank you Dr. Joana Figueiredo for your excellent feedback and knowledge of coral larvae research and literature that allowed me to enhance my writing skills.

I would like to thank Angel Rovira from Broward County for your help captaining the boat and deploying the experimental structures as well as Dr. Brian Walker from NSU for your help plotting my experimental structures in GIS. I would also like to thank both the former and current students in my lab for their help with my project. I enjoyed getting to know you and work with you all. A special thank you to Leah Harper for your help with this project from diving to our endless talks about our projects. Thank you to Hunter Noren for teaching me genetic skills and Hannah Nylander-Asplin for your help with DNA extraction.

Finally, I would like to thank my family and friends for all of their support throughout my life. You have shaped me into the woman I am today and I am forever grateful for all of your love and support.

This project was funded by the Nova Southeastern University President's Faculty Research and Development Grant. This work was conducted under permit no. SAL-15-1424-SRP issued by the Florida Fish and Wildlife Conservation Commission Division of Marine Fisheries Management.

## TABLE OF CONTENTS

LIST OF FIGURES .....	ii
LIST OF TABLES .....	ii
ABSTRACT.....	iii
<b>Chapter 1 – Introduction .....</b>	<b>1</b>
<i>Decline of Coral Reefs</i> .....	1
<i>Scleractinian Reproduction</i> .....	2
<i>Sexual Reproduction</i> .....	2
<i>Asexual Reproduction</i> .....	5
<i>Paternity in Corals</i> .....	8
<i>Study Species</i> .....	10
<i>Objectives</i> .....	11
<b>Chapter 2 – Publication .....</b>	<b>12</b>
<b>Introduction</b> .....	<b>12</b>
<b>Materials and Methods</b> .....	<b>14</b>
<i>Experimental Setup</i> .....	14
<i>Coral Collection/Planulation</i> .....	15
<i>Genetic Analysis</i> .....	16
<i>Histological Analysis</i> .....	17
<b>Results</b> .....	<b>20</b>
<i>Planulation</i> .....	20
<i>Genetic Analysis</i> .....	22
<i>Histological Analysis</i> .....	25
<b>Discussion</b> .....	<b>25</b>
<b>Chapter 3 – Discussion .....</b>	<b>31</b>
<b>References</b> .....	<b>39</b>

## LIST OF FIGURES

Figure 1. Modes of coral reproduction .....	3
Figure 2. Types of coral reproduction.....	4
Figure 3. Pathways to parthenogenesis .....	6
Figure 4. Cinderblock structures .....	15
Figure 5. Paternity array schematic .....	15
Figure 6. Planulation tank setup.....	16
Figure 7. Number of released larvae per day per array type.....	20
Figure 8. Number of released larvae per day per colony .....	21
Figure 9. Density array schematic with planulated colonies highlighted .....	21
Figure 10. Effect of adult size on number of planula/cm <sup>2</sup> .....	22
Figure 11. Percentage of parthenogenesis in Broward County, FL .....	24
Figure 12. Percentage of parthenogenesis in Summerland Key, FL.....	25

## LIST OF TABLES

Table 1. Microsatellite loci for <i>P. asteroides</i> .....	19
Table 2. Number of larvae genotyped by colony.....	23
Table 3. Characteristics of alleles .....	23
Table 4. Example of genetic results from a maternal colony and resultant larvae .....	24
Table 5. Self-fertilization genotype frequency changes over time .....	27

## ABSTRACT

Multiple stressors have caused a decline in coral populations. Broadcast spawning corals once dominated the Florida Reef Tract (FRT), but since their decline, smaller brooding corals, soft corals, and macroalgae are replacing them. Brooding corals are more resilient to current threats in part because they are reproductive throughout much of the year and their larvae are competent to settle after release. Despite the ubiquity of brooders on Florida reefs, much of their reproductive strategy remains unknown. This study aimed to examine paternity as a function of colony size and density in *Porites astreoides*, a common brooding coral in the FRT. *Porites astreoides* colonies were configured in arrays at three densities that were replicated three times. A focal colony was surrounded by six other colonies, separated from the focal colony at different distances (1m, 7m, and 15m) representing high, moderate, and low population densities, respectively. All arrays were placed in the field but were separated from the reef and naturally occurring *P. astreoides* colonies by at least 50 m. Four days before the new moon, colonies were transported to the laboratory for larval collection. Over a four day period, a total of 3,184 larvae were collected from 24 colonies, 13 of which released larvae over consecutive days. The resulting larvae were genotyped using seven microsatellite markers. All larvae had the exact genotypes of the colony from which the larvae were collected, i.e. maternal- egg donor. This suggested the larvae were parthenogenically produced and no sperm was used to fertilize the eggs. This is the first study to suggest that parthenogenesis is occurring in *P. astreoides*. In today's oceans that have been depleted of corals, parthenogenesis may be an advantageous reproductive strategy used to boost populations. However, parthenogenesis reduces the genetic diversity which could hinder successful sexual reproduction in the future causing fragmented populations.

**Keywords:** *Porites astreoides*, parthenogenesis, planulation, density dependence, asexual reproduction



## **CHAPTER 1- INTRODUCTION**

### ***Decline of Coral Reefs***

Coral reefs are one of the most diverse ecosystems with more than 25% of marine species relying on them for survival (Knowlton 2001; Spalding et al. 2001). However, coral reefs only make up only 0.1% of the ocean's area. Corals provide many benefits to humans; the goods and services corals provide have been valued at \$375 billion per year including food, tourism, shoreline protection, recreation, and pharmaceuticals (Costanza et al. 1997). Yet, coral reefs have been heavily exploited and have experienced drastic declines in diversity and biomass (Côté et al. 2005).

In the last four decades, Caribbean coral cover has declined from 50% to approximately 10% (Gardner et al. 2003; Sotka and Hay 2009; de Bakker et al. 2017). This decline is a result of global and local stressors. Globally, climate change and ocean acidification are shown to have negative impacts on reefs worldwide (Hoegh-Guldberg et al. 2007; Carpenter et al. 2008; Hughes et al. 2018). Carbon dioxide emissions and sea temperatures are projected to increase over the next 50 years to conditions that reefs have not experienced in the past 500,000 years (IPCC 2014). This phenomenon could cause further declines in coral populations because their ability to calcify will be compromised limiting coral growth and dissolving existing calcified structures, due to the availability of carbon in the water. Increased oceanic temperatures results in mass bleaching events around the globe leading to a loss of 19% of the reefs in the last 50 years (Wilkinson et al. 2008; Frieler et al. 2013; Hughes et al. 2018). Some scientists argue that the rate of change in the oceanic atmosphere is predicted to be beyond the adaptive capacity for scleractinian corals (Hoegh-Guldberg et al. 2007). The long generation times (5-100 years) and the variability of larval production means most coral species may fail to adapt to changes in their environment (Frieler et al. 2013). Multiple stressors in addition to changing oceanic conditions may have an additive or synergistic effect on coral health. Local anthropogenic stressors, such as overfishing, disease outbreaks and pollution, have contributed to the decline of coral reefs (Hoegh-Guldberg 2011).

Southeast Florida is home to the only living coral reef in the continental United States (FDEP 2011). The Florida Reef Tract (FRT) extends from Martin County through

Southeast Florida and the Florida Keys to the Dry Tortugas, making it the third largest barrier reef in the world. The Southeast Florida Reef Tract, near Broward and Miami-Dade counties, is composed of three reef tracts that run parallel to the coastline (Goldberg 1973; Moyer et al. 2003). Their proximity to high population centers has likely contributed to the rapid declines in coral cover in the past decades, with an average scleractinian coral cover of 13% in 1996 to less than 3% in 2016 (Chiappone and Sullivan 1996; Sathe et al. 2008; Gilliam et al. 2013; Gilliam et al. 2016). These reefs are exposed to high rates of shipping traffic (Port Everglades and the Port of Miami) and six sewer outfalls that negatively affect the reefs through sedimentation, nutrient loading, and the input of sex steroids onto the reefs (Lirman and Fong 2007). Additionally, reefs have been overfished both recreationally and commercially, leading to their overexploitation (McClenachan 2013). The southeast FRT is also at the northerly most limit of coral reef distribution, which can result in extreme temperature fluctuations. The variance in temperature may cause mass bleaching events that could result in further declines in coral populations (Wagner et al. 2010; Lirman et. al 2014). For corals to persist through these stressors, populations need to maintain high levels of recruitment and genetic diversity, which only occurs through successful sexual reproduction.

### ***Scleractinian reproduction***

#### *- Sexual reproduction*

Sexual reproduction is important to maintain genetic diversity within species (Johnson and Black 1984; Hedgecock 1986; Ayre et. al 1997; Ayre and Hughes 2000). Despite being simple organisms, scleractinian corals have adapted several mechanisms to reproduce sexually. Scleractinian sexual reproduction is categorized into four main groups: hermaphroditic broadcast spawners, hermaphroditic brooders, gonochoristic broadcast spawners, and gonochoristic brooders (Fig. 1) (Harrison and Wallace 1990; Richmond and Hunter 1990). Gonochoric refers to separate sexes per coral colony whereas hermaphroditic corals have both sexes in a single colony (Harrison 2011). When the probability of locating a mate is limited and self-fertilization is favored, hermaphroditism is advantageous over gonochorism. This is reflected in nature with 68% of reef building corals being hermaphroditic worldwide (Harrison and Wallace 1990; Baird et al. 2009). Coral sexual reproduction can be further divided into broadcast

spawning and brooding (Fig. 2) with 82.7 % broadcast spawning corals, 14.3% brooding, and 3% exhibit both modes worldwide (Baird et al. 2009; Harrison 2011).

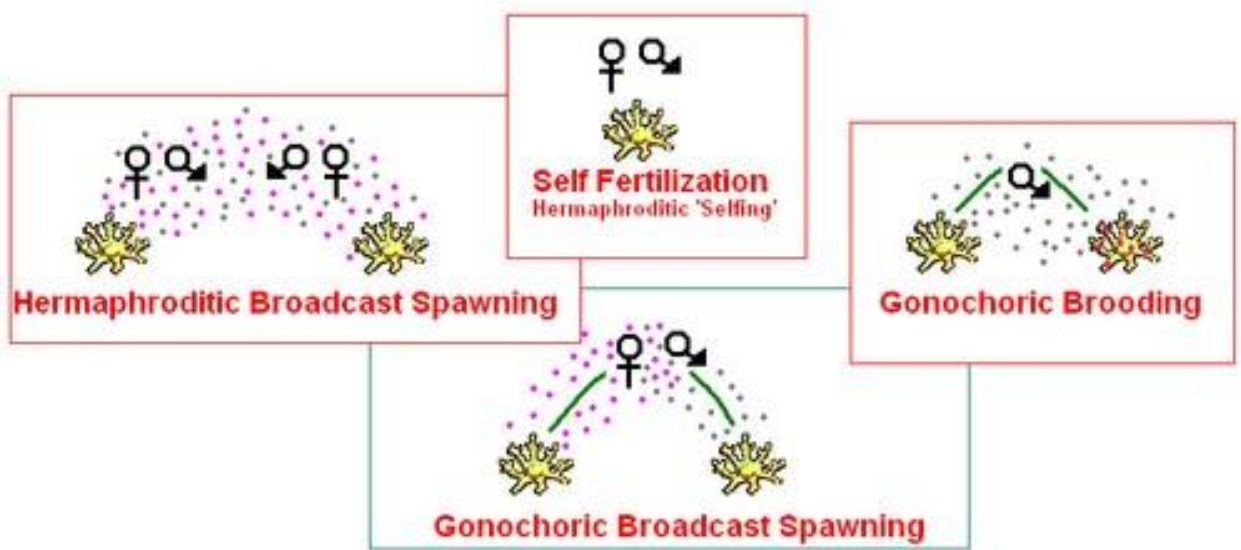


Figure 1. Modes of coral reproduction (Riddle 2008).

Broadcast spawners are typically larger reef building corals that have higher fecundity due to size (Szmant 1986). Gonochoric broadcast spawning corals release sperm and buoyant eggs, while hermaphroditic broadcast spawning corals release positively buoyant gamete bundles (eggs and sperm packed together) into the water column and external fertilization occurs when eggs and sperm from separate colonies mix at the surface. To optimize fertilization, conspecifics in the same geographic area spawn synchronously (Levitan et al. 2004). After fertilization, the embryo develops near the surface into a competent larva that is ready to settle and metamorphose (Edmunds 2011; Green and Edmunds 2011). Larvae from broadcast spawners can survive as plankton in the water column for weeks or even multiple months (Graham et al. 2008).

Alternatively, brooding corals release sperm and nearby colonies capture the sperm fertilizing their eggs internally (Harrison and Wallace 1990; Pemberton et al. 2003). Once fertilization occurs internally, fully competent larvae are released and settle and metamorphose almost immediately, typically near their parental colonies (Green and Edmunds 2011). Larvae of most brooding corals contain algal endosymbionts of the genus *Symbiodinium* from the parental colony, contrary to most broadcast spawners whose larvae are aposymbiotic (Baird et al. 2009) and thus have to obtain *Symbiodinium* from the environment (Edmunds et al. 2001; Cumbo et al. 2012). Because fertilization is

internal, little is known about the timing of fertilization or the paternity potential, specifically sperm dispersal distance, in most brooding coral species (Warner et al. 2016).

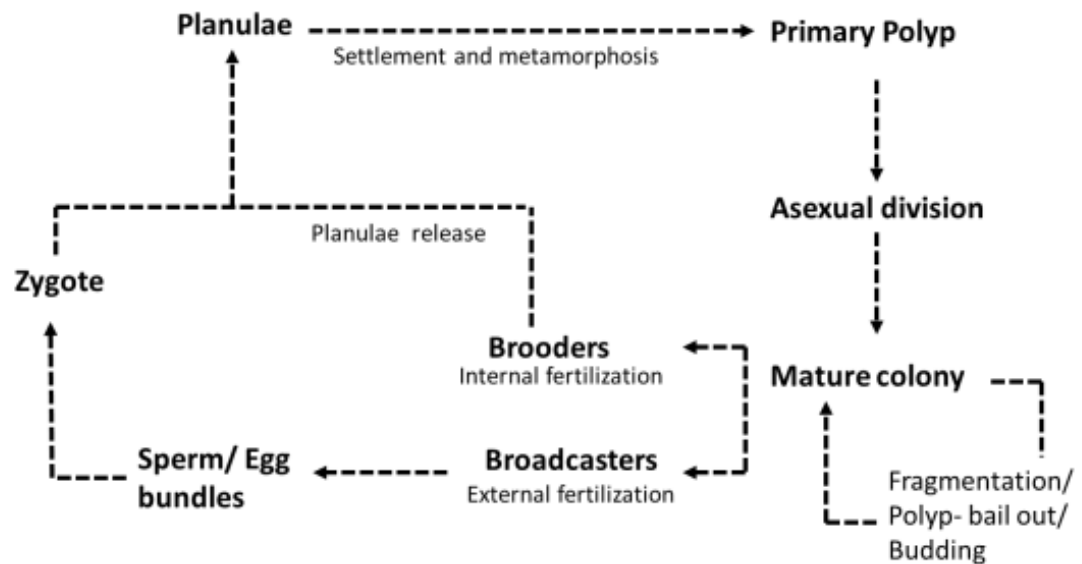


Figure 2. Coral reproduction cycle in both brooding and broadcasting corals. (Adapted from Vermeij et al. 2009)

Not all corals can have reproductive strategies that can be classified into the four typical sexual reproduction methods. At least 13 species have exhibited mixed patterns of reproduction (Harrison 2011). Species in the *Galaxea* genus were first observed as simultaneous hermaphrodites but through further observations, it was revealed that some colonies within species are female only while others are hermaphroditic (Harrison 1988). This trend also holds true in *Porites astreoides* where half of the colonies on the reef in Jamaica were females, while the other half were hermaphrodites (Chornesky and Peters 1987). Furthermore, some species of corals, such as *Stylophora pistillata*, change sex throughout their life cycle based on stress conditions and energy limitations (Rinkevich and Loya 1987).

Sexual reproduction does not always involve the genetic contribution from two unique individuals. Self-fertilization is when a hermaphroditic colony reproduces with itself or when ramets of the same genotype reproduce. A male and female chromosome are required to recombine and reproduce a larva but genes from that larva come from a single individual during self-fertilization (Darwin 1876; Dobzhansky and Dobzhansky 1970). One advantage of self-fertilization is that it lessens the need to find a mate. This is

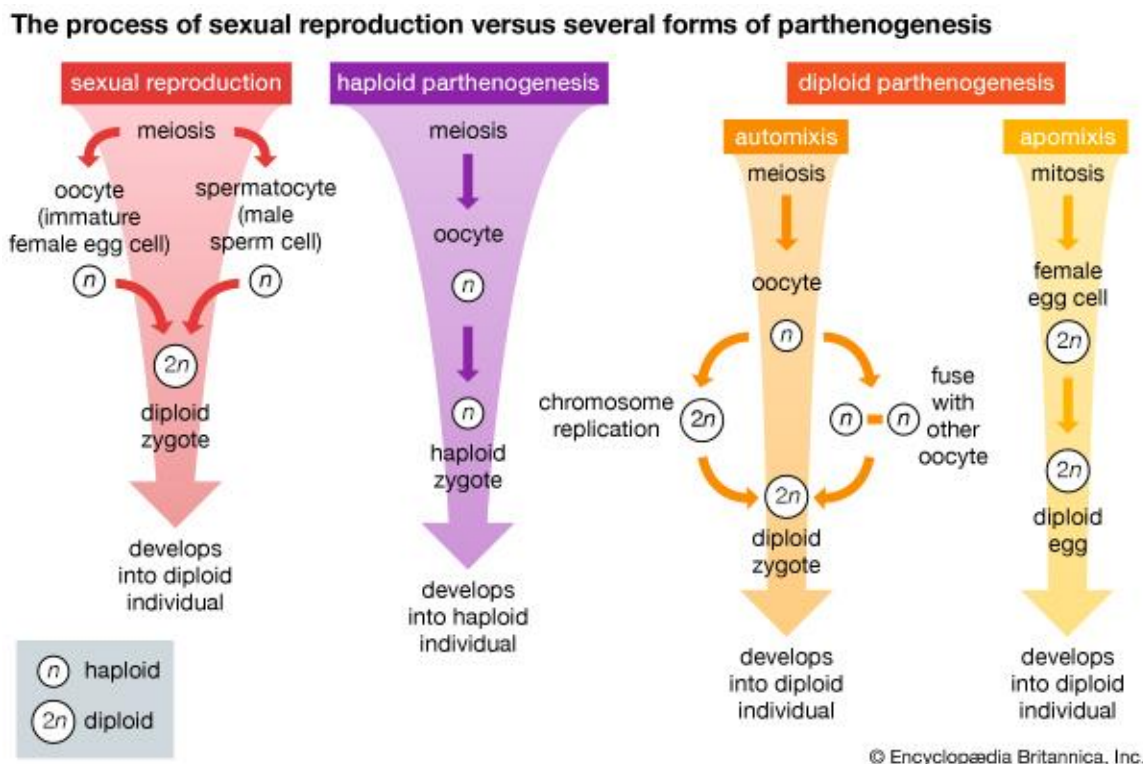
beneficial when the Allee Effect, a decline in individual fitness because of reproductive failure at low population levels that can result in extinctions, is evident (Courchamp et al. 2008). However, self-fertilization has the potential to cause inbreeding and diminishes the population's genetic diversity, reducing heterozygosity (Heyward and Babcock 1986; Brazeau et al. 1998; Stratton 2011). *Favia fragum* and *Porites astreoides* have been described as having high frequencies of self-fertilization under natural conditions potentially due to the high turbidity environments they occupy, which could hinder cross-fertilization (Brazeau et al. 1998).

- *Asexual reproduction*

Scleractinian corals also have the ability to reproduce asexually (Stoddart 1983). Genetically identical individuals are produced during asexual reproduction via fragmentation and fission, partial colony mortality, polyp bailout, and parthenogenesis. Fragmentation occurs when there is physical breakage of a colony into smaller parts that are dispersed by storms, wave action, or direct placement by humans (Van Veghel and Bak 1994). This form of asexual reproduction avoids high mortality of the larval and juvenile stages (Highsmith 1982). Partial colony mortality can be a result of predation, disease, or sedimentation causing parts of the colony to die and leaving isolates of the original colony (Aronson and Precht 2001). Polyp bailout is the process in which a single polyp ejects itself from their coral skeleton and drifts to a new location to settle. This is typically a last ditch effort to survive after intense stress (Sammarco 1982). Fragmentation, partial mortality, and polyp bailout result in smaller sexually immature colonies of genetically identical corals that may form monoclonal thickets, reducing sexual reproduction and genetic diversity (Lirman 2000). As the effects of climate change are predicted to rise and the severity of storms are predicted to increase, fragmentation is expected to become more likely and may impede the success of sexual reproduction (Wilkinson 2008).

The production of larvae via either self-fertilization or parthenogenesis is a rare occurrence that has been hypothesized to be a result of harsh environments that reduce successful sexual reproduction among individuals (Brazeau et al. 1998). Parthenogenesis is the reproduction of a female gamete or oocyte without the fertilization from a male gamete. Offspring from parthenogenesis can be a result of a haploid or diploid cell (Fig.

3). Haploid parthenogenesis occurs when a haploid egg produces a haploid adult. This rare form of parthenogenesis occurs in bees and nematodes (Stone et al. 2002). Diploid parthenogenesis, the more common form of parthenogenesis, can occur from two distinct pathways: automixis and apomixis. In automictic parthenogenesis the gametes are produced through meiosis forming a haploid oocyte. After meiosis, this oocyte may either duplicate its chromosomes or fuse with another oocyte formed in meiosis to produce a diploid zygote. Automictic organisms are considered half clones of the mother because during meiosis genetic material is separated and then recombines. Automixis has been described in lizards and sharks (Chapman et al. 2007). During apomictic parthenogenesis meiosis is forgone and mitosis creates two genetically identical diploid eggs. These diploid eggs then develop into offspring that are true clones of the mother. Common organisms that are apomictic include Bdelloid rotifers and velvet worms (Birky and Glibert 1971).



**Figure 3. The different pathways in which parthenogenesis can occur. (Encyclopedia Britannica 2016)**

Parthenogenesis is seldom observed in scleractinian coral species. The ability of corals to reproduce parthenogenically allows for reproductive insurance even when colonies are very sparsely distributed (Combosch and Vollmer 2013). Parthenogenesis in

corals was first documented in *Pocillopora damicornis* in populations from Western Australia (Stoddart 1983). *Pocillopora damicornis* was considered both a hermaphroditic brooding and broadcast spawner depending on geographic location. In high latitude marginal environments, it has been a documented broadcast spawning species, while in its tropical environments it is a brooding species (Richmond 1987; Sier et al. 1994). This form of reproductive plasticity may have resulted due to varying environmental stressors (Masse 2009). Other studies have since confirmed parthenogenesis in *P. damicornis* in Western Australia (Stoddart 1983; Ayre and Miller 2004; Sherman et al. 2006), and have suggested that most of larvae in this species are produced parthenogenically (94%), while a small portion are a result of sexual reproduction (6%) in regions where this species of coral is a brooder (Combosch and Vollmer 2013). It has been hypothesized that *P. damicornis* may interchange between sexual and asexual reproduction throughout their adult life stage (Barnes and Hughes 1999). Recently, Schmidt-Roach et al. (2014) split corals previously lumped as *P. damicornis* into five distinct species resulting in *P. damicornis* and *P. acuta* to be documented as known parthenogenic reproducers. Other species of corals produce parthenogenic larvae including *Tubastrea coccinea*, *T. diaphana*, and *Oulastrea crispata* (Ayre and Resing 1986; Nakano and Yamazato 1992; Lam 2000; Glynn et al. 2008). *Tubastrea spp.* are hermaphroditic brooding species which were determined to produce parthenogenic larvae through gel electrophoresis (Ayre and Resing 1986), while *O. crispata* are hermaphrodites and the only coral species known to have both brooding or broadcast spawning modes of reproduction (Ward 1992; Lam 2000). Additionally, *Favia fragum*, *Porites astreoides*, and *Agaricia agaricites*, all brooding species, have been previously identified as corals able to reproduce using self-fertilization determined using RAPD gel electrophoresis with 4 primers (Brazeau et al. 1998; Gleason et al. 2001). These studies may have incorrectly concluded self-fertilization when parthenogenesis may have been occurring because the authors defined selfing as larvae with the same genotype as the maternal colony.

Production of genetically identical larvae combined with natal philopatry may lead to higher local retention and result in more locally adapted larvae (Gleason et al. 2001); however, genetic diversity would decrease making the population vulnerable to disturbances (Szmant and Meadows 2006). Evidence of sexual and asexual reproductive

changes in corals implies that reproduction has a high level of plasticity (Harrison 1985). Corals may change their reproductive mode in order to adapt to environmental changes. It is unclear if this occurs, but if current reproductive modes in corals are not well understood, the adaptations of corals to their changing environment will not be detected.

### ***Paternity Studies***

Population connectivity, or the exchange of individuals among geographically separated subpopulations, begins with gametic fusion from different individuals (Underwood et al. 2006; Warner et al. 2016). Sessile organisms, such as plants and corals, as well as broadcast spawning individuals rely on the dispersal of gametes for reproductive success and population connectivity (Lasker et al. 2008). The dispersal of gametes allows for a higher rate of genetic diversity in the population. High genetic diversity may help a population recover following disturbances or adapt to environmental changes, such as thermal stress or acidic oceanic conditions associated with climate change (Ayre and Hughes 2004).

Genetic parentage analysis has previously been used to examine pollen dispersal in plant populations (Streiff et al. 1999; Bittencourt and Sebbenn 2008; Pluess et al. 2009; Gleiser et al. 2014; Sanchez-Robles et al. 2014; Saro et al. 2014) and marine fish connectivity (Jones et al. 2005; Planes et al. 2009; Harrison et al. 2012). Similar to results found in corals, multiple sires fertilize eggs through pollen transfer in terrestrial systems, as well as in mass fish spawning in aquatic systems. These types of studies have been used when assessing tree removal for the preservation of local clusters and for connectivity in marine protected areas and non-protected areas among fish populations. Sea urchin paternity has been widely examined to understand density dependent reproduction, sperm competition, sperm limitation, and sexual selection (Levitan et al. 1992; Chenuil et al. 2004; Levitan 2004; Levitan 2005; Maturana et al. 2017). When population densities are high, polyspermy (an egg that is fertilized by more than one sperm leading to an inviable zygote) is likely to occur but when population densities are low, sperm is a limiting factor (Levitan 1995). Sperm limitation is of evolutionary importance when examining egg size. If it is believed that a population is sperm limited, then a species that has larger eggs may have higher fertilization rates because the eggs create a larger target for the sperm (Levitan 1993). However, eggs are energetically



costly, therefore a later life stage may be negatively affected by this resource allocation. Sperm limitation leads to low paternity levels, effectively lowering the genetic diversity. In a changing environment, a loss of genetic diversity can ultimately lead to population declines because the organisms cannot adapt (Baums et al. 2006).

In brooding corals, the dispersal of sperm, allows colonies to remain genetically connected (Underwood et al. 2006; Warner et al. 2016). Yet, very little is known about sperm dispersal in these internally fertilizing corals. Warner et al. (2016) conducted the first parentage analysis in a brooding coral, *Seriatopora hystrix*, to determine the average distance sperm disperse and genetic diversity within a natural population. *Seriatopora hystrix* is native to the Indo-Pacific region and forms bushy clumps of thin branches that can grow to a meter across (Veron 2000). This study determined mating occurred between colonies within 10 m of each other with mean sperm dispersal of 5.5 m (Warner et al. 2016). The most larvae assigned to one genetically unique sire was 18 larvae (out of 495 tested), with no determined relationship between paternal colony size or paternity share (Warner et al. 2016). Self-fertilization rates were also noted in four colonies ranging from 2% to 23% (Warner et al. 2016).

Self-fertilization is thought to be a moderately important reproductive strategy for brooding coral species (Carlon 1999; Carlon et al. 2011; Warner et al. 2016). Most brooding coral species express both sexes simultaneously leading to a higher potential for self-fertilization causing inbreeding (Carlon 1999). In *Porites astreoides*, selfing rates ranged from 0 to 0.79 (Brazeau et al. 1998) while another study found self-fertilization was <1% in all colonies except for one which was < 3% (Miller and Babcock 1997). Natural self-fertilization rates were highest at 34% in *Porites astreoides* in the Florida Keys indicating that this form of sexual reproduction is an important reproductive strategy (Brazeau et al. 1998; Gleason et al. 2001). However, these studies may have incorrectly identified self-fertilization when parthenogenesis was actually occurring.

Prior to the study by Warner et al. (2016), parentage analysis through microsatellite markers had only been examined in broadcast spawning corals typically when testing gametic compatibility between larval crosses during laboratory experiments (Willis et al. 1997; Levitan et al. 2004; Fogarty et al. 2011; Fogarty et al. 2012; Baums et

al. 2013). These studies examined distributions of parental and hybrid corals, tested reproductive isolating mechanisms, and examined egg choice when in the presence of conspecific or heterospecific sperm. Miller and Ayre (2006) found the brooding coral *Acropora palifera* paternity estimates were low based on adult genotype frequencies, implying that multiple sires fertilized eggs from a single colony. This trend is common in broadcast spawning corals in which sires from multiple colonies have the ability to fertilize eggs due to synchronous mass spawning events. A mating system study on *Favia fragum* demonstrated that colonies appear as either tall or short ecomorphs depending on what habitat they inhabit, tall ecomorphs are found in seagrass beds whereas short ecomorphs can be found on reefs. This study used microsatellite markers to determine if the larvae from each ecomorph will have the same ecomorph as the parental colony. All larvae were assigned to the same population ecomorph as the parent demonstrating that there was no outcrossing between each ecomorph (Carlson and Lippe 2011). In a changing environment, a loss of genetic diversity can ultimately lead to population declines because the organisms may struggle to adapt (Baums et al. 2006).

Paternity studies aim to address unknown questions about sperm dispersal that are necessary when trying to maintain genetic diversity in a population. This study aims to understand how breeding systems are affected by size and density of colonies, as well as how paternity shares change during the planulation period. These gaps in knowledge are needed to understand how corals currently reproduce and how reproduction may be altered in the changing marine environment.

### ***Study Species***

*Porites astreoides*, commonly known as the mustard hill coral, is a brooding coral that inhabits the waters of the Caribbean, Gulf of Mexico, and the Eastern Atlantic at depths between 1-15 meters (Colin 1978). Colonies exhibit diverse growth patterns, such as mounding, plating, and encrusting morphologies, in relation to water depth and light availability (Chornesky and Peters 1987; Aronson et al. 2008). Additionally, *P. astreoides* is known to have two distinct color morphs, a green morph and a brown morph, that typically inhabit distinct environments. The green morph is found in shallow reefs environments (3 m) whereas the brown morph is more common on deeper reefs (5-

15m) (Thornton 1999). However, both color morphs are genetically connected (Serrano et al. 2016). Colonies are relatively small compared to larger reef building corals and individual polyps can range in size from 0.5 to 1.5 mm in diameter (Humann 1996). *Porites astreoides* was chosen for this study because of its relatively high abundance in South Florida, high fecundity, ease of larvae collection, and availability of DNA microsatellite markers (Kenkel et al. 2013; Serrano et al. 2016), and ease of larvae collection. This species exhibits high settlement rates and a short life span (Bak and Engel 1979; McGuire 1998). Like many other brooding corals, most *P. astreoides* are hermaphroditic although a few are solely female (determined from a reef in Jamaica Bay using n=100) (Chornesky and Peters 1987). Brazeau et al. (1998) conducted genetic analyses to parent and offspring collected in the field in the Florida Keys, and interpreted the results as being 34 % of self-fertilization. However, the results of thier study are actually consistent with parthenogenesis, not self-fertilization.

*Porites astreoides*' reproductive development occurs monthly throughout much of the year (Richmond and Hunter 1990; McGuire 1998). Sperm are assumed to be released from colonies around the full moon and polyps from conspecific colonies capture the sperm to undergo internal fertilization (Szmant 1986; Chornesky and Peters 1987; Brazeau et al. 1998; McGuire 1998). Colonies typically release competent planula larvae from April-August over 3-5 consecutive days around the new moon with peak planulation from May-June (Szmant 1986; Chornesky and Peters 1987; Brazeau et al. 1998; McGuire 1998).

### ***Objectives***

Caribbean coral populations have shifted from large broadcast spawners to small brooding corals (Gilliam et al. 2013). Fertilization successes have yet to be studied in the brooding coral *Porites astreoides*. In this study, I will examine how reproductive mode and paternity are influenced by colony size and density. The questions I will address are:

- 1. What percentage of colonies self-fertilize (mating between gametes of the same colony) or produce parthenogenic larvae?**
- 2. How many sires fertilize eggs of one colony?**
- 3. Does colony size and density influence paternity patterns?**
- 4. How does paternity share change over multiple days?**

## CHAPTER 2

### INTRODUCTION

Coral reefs worldwide have been experiencing mass declines in biomass and diversity (Côté et al. 2005). Caribbean coral reefs are among those that have seen the greatest losses, decreasing from 50% to 10% coral cover in the last four decades (Gardner et al. 2003; Sotka and Hay 2009). The main factors driving this decline are climate change, pollution, and overfishing which has led to a shift from large broadcast spawning coral domination to smaller brooding corals, soft corals, and macroalgae dominated reefs (Hoegh-Guldberg et al. 2011). To counteract these stressors, coral must maintain high genetic diversity allowing natural selection to act on the population and select the most fit individuals. Genetic diversity occurs through successful sexual reproduction.

Scleractinian corals reproduce both sexually and asexually (Szmant 1986). Sexual reproduction in corals is either through broadcast spawning or brooding. Broadcast spawning corals release positively buoyant gamete bundles (eggs and sperm packed together) into the water column and external fertilization occurs when eggs and sperm from separate colonies mix at the surface. Alternatively, brooding corals release sperm and nearby colonies capture the sperm and fertilize their eggs internally (Harrison and Wallace 1990; Pemberton et al. 2003). Once fertilization occurs internally, fully competent larvae are released and settle almost immediately (Green and Edmunds 2011). Genetically identical individuals are produced during asexual reproduction via fragmentation, partial colony mortality, polyp bailout, and parthenogenesis. The majority of asexual reproduction in coral populations occurs when part of a colony breaks off and forms a new genetically identical colony (Lirman 2000). The production of larvae via asexual reproduction is a rare occurrence that has been attributed to harsh environments that reduce successful sexual reproduction (Brazeau et al. 1998). The few coral species that have been shown to produce larvae asexually through parthenogenesis also benefit from genetic recombination (sexual reproduction) to maintain genetic diversity in a population (Combosch and Vollmer 2013).

Because fertilization is internal, little is known about the dynamics of fertilization or the paternity potential in most brooding coral species. Sessile organisms, such as

plants and corals, rely on the dispersal of gametes for reproductive success and population connectivity (Lasker et al. 2008). The dispersal of gametes allows for a higher rate of genetic diversity in the population. High genetic diversity may help a population recover following disturbances or adapt to environmental changes, such as thermal stress or acidic ocean conditions associated with climate change (Ayre and Hughes 2004).

Some brooding coral species have escaped declines and may actually be increasing in relative abundance. *Porites astreoides*, for instance, has increased in density from 0.18 colonies/m<sup>2</sup> in 2012 to 0.30 colonies/m<sup>2</sup> in 2015 on the reefs of Southeast Florida (Gilliam et al. 2016), the Florida Keys (Ruzicka et al. 2016), and throughout the Caribbean (Green et al. 2008) have seen increases as well. Little is known about the driving forces of *P. astreoides* reproductive success and genetic diversity, (see Serrano et al. 2016 for exception), therefore it is important to understand its reproductive strategy. *Porites astreoides* is a brooding coral meaning sperm released from nearby colonies is captured by other polyps and used to fertilize the eggs internally (Harrison and Wallace 1990; Pemberton et al. 2003). Planula larvae are released and are competent to settle almost immediately. In the Caribbean, brooders generally exhibit high recruitment rates and high fecundity (Soong 1991; Harper et al. unpublished data). Although brooded larvae typically settle near their maternal colonies, it has been documented that there is ~10% clonality among the species and high levels of gene flow both horizontally and vertically along the Florida reef tract (Serrano et al. 2016). However, Serrano et al. (2016) sampled colonies every 5m which may not have captured fine-scale population dynamics therefore, clonality may be higher. In previous studies, *P. astreoides* was thought to sexually reproduce larvae as well as reproduce through self-fertilization (Brazeau et al. 1998; Gleason et al. 2001). Reproductive plasticity has been documented in other hermaphroditic brooding coral species. In marginal habitats these species may switch reproductive modes from brooding to broadcast spawning or switch reproductive strategies from sexual reproduction to primarily asexual reproduction through parthenogenesis (Ward 1992; Lam 2000; Combosch and Vollmer 2013). In a quickly changing marine environment, it is necessary to understand the reproductive strategies of corals in order anticipate how they will adapt to their changing environments.

In this study, we used density arrays and microsatellite markers to investigate paternity patterns and reproductive strategies of *Porites astreoides*. Comparisons between maternal colonies and the larvae they released were genotyped to determine paternity patterns as it relates to colony size and density.

## **METHODS**

### *Experimental Setup*

Sixty-three adult *P. astreoides* colonies of the brown color morph were collected from the Florida Reef Tract in Broward County, Florida following the methods from Kuffner et al. (2006) in March 2016. Colonies were collected from 2 reefs, Barracuda reef (N 26.08317°, W 80.09533°) and BCA (N 26.14938°, W 80.08940°). Colonies collected were at least 5m apart from other collected colonies. Colonies approximately 12 cm in diameter (32.9 cm<sup>2</sup> to 247.3 cm<sup>2</sup> in total surface area of live tissue) were collected using a hammer and chisel, to break the colony from the reef at the base, and placed into a cooler filled with seawater. The colonies were transported back to NSU on-shore nursery so colonies could have a plastic screw epoxied on the bottom, be tagged with a unique identifier, and have total surface area of live tissue measured. Colony size was measured by calculating the surface area of live tissue using the area of an ellipse defined by the two largest perpendicular diameters (Chronesky and Peters 1987).

The following day, colonies were secured to concrete cinderblock structures (Fig. 4), and transported off-shore to a sandy habitat (26° 8.119' N, 80° 5.806' W). The off-shore sandy habitat was chosen as the location for this experiment because it is more than 50 m from reefs in all directions outside of the experimental area (210 m x 210 m) and there are no *P. astreoides* colonies located in the area. We installed structures in three different density arrays [1m, 7m, and 15m (Fig. 4 & 5)]. A focal colony was placed in the center of each array surrounded by six other colonies. Differing distances represent high, moderate, and low population densities, respectively. Each array was replicated three times.



Figure 4. Cinderblock structure that the *Porites astreoides* coral sat affixed to during the experiment.

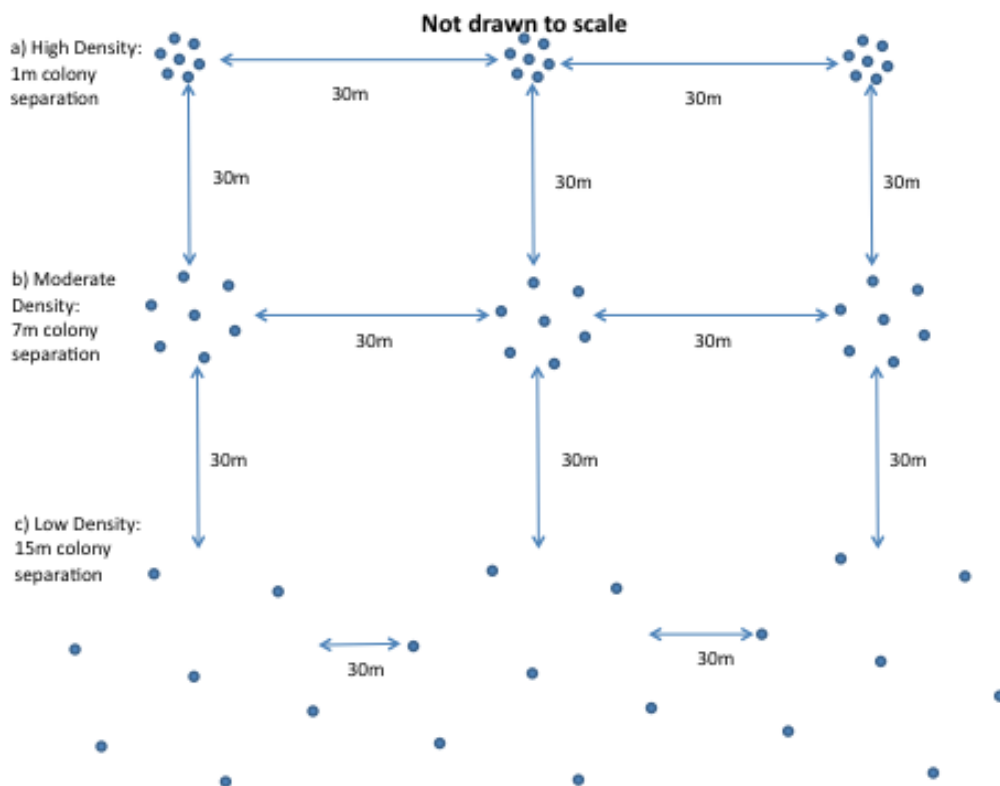


Figure 5. Schematic drawing of coral density arrays, not drawn to scale.

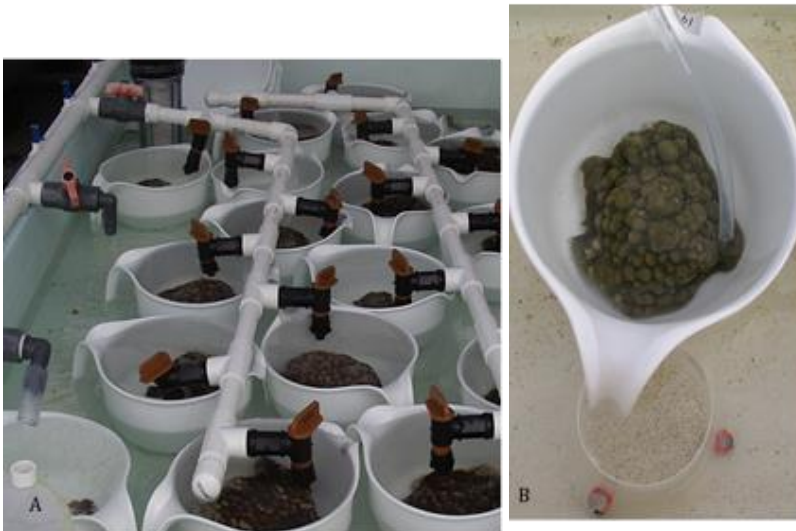
#### *Coral Planulae Collection*

On May 2, 2016, four days before the new moon, 58 of the original 63 colonies (the remaining 5 were found dead) were collected from the experimental arrays, and brought back to Nova Southeastern University for planulation. The colonies were placed into shallow tanks in individual plastic pitchers, each with tubing that supplied seawater.

Each pitcher handle was placed inside a plastic tri-pour beaker with a nitex bottom for larval collection; when larvae were released, they slide down the handle, and into a tri-

pour (Fig. 6). Larvae were collected from colonies every morning for a four-day period (May 3- May 6, 2016) and placed into individual 0.2ul microcentrifuge tubes with 25  $\mu$ l of Chaos (4 M guanidine thiocyanate, 0.1% sodium N-lauroyl sarcosine, 10 mM Tris-HCl pH8, 0.1 M 2- mercaptoethanol) (Fukami et al. 2004) for DNA digestion and preservation.

Additionally, planulated larvae and maternal colony genetic clippings were collected from a natural reef off of Summerland Key, FL in April 2015 from a prior planulation study. Fourteen maternal colonies and 50 of their planulae were collected and preserved as above. Four of the maternal colonies and eight larvae from each colony were tested for genetic analysis using the four most polymorphic primers to compare larval genetics among years and locations.



**Figure 6. Images of similar planulation tank setup. (A) coral colonies were kept in individual buckets with water circulation system to each colony. (B) Tripours were added under handle to collect larvae. (Raphael Ritson- Williams)**

### *Genetic Analysis*

Tissue samples were collected from all adult colonies used during the experiment. All tissue samples and larvae were preserved in Chaos and frozen until genetic analysis was conducted. DNA was extracted from twenty-four larvae collected from every planulating colony on each day of planulation. For the initial analysis, all adults and 6 sets of larvae were genotyped at all seven loci. Seven polymorphic microsatellite primers were used to genotype samples (Kenkel et al. 2013; Serrano et al. 2016). Clear, consistent



results were obtained for the initial six colonies. Therefore, in effort to maintain cost efficiency, the four most polymorphic primers were used to genotype eight larvae from each maternal colony. Forward primers were fluorescently labeled with 6FAM, NED, or VIC (Applied Biosystems, CA). These primers (Past\_3 (CAT) <sub>x</sub>, Past\_17 (ATTG) <sub>x</sub>, Past\_21 (ATG<sub>x</sub>) <sub>x</sub>, PA3 (TTCTT) imperfect, PA7 (CGTC)<sub>2</sub> CATC (CGTC)<sub>6</sub>, PA13 (ATT)<sub>2</sub> ATG (ATT)<sub>9</sub>, PA69 (ATT) imperfect) were performed in multiplex reactions (11 µl total volume, consisting of 2 primer pairs each) using 1 µl of DNA. Reactions were run on a Promega PCR cocktail (Promega, Madison, WI). The PCR recipe consisted of primer concentrations specific to each locus (Table 1), 5X PCR buffer, 2.75 mM of MgCl<sub>2</sub>, 0.8 mM of dNTPs, and 0.5 U of Taq polymerase.

Thermal cycling for all reactions was performed with an initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 1 min; 57°C (annealing temperature) for 1 min; 74°C for 1 min; and a final extension of 7 min at 74°C. PCR products were multiplexed with 8.8µl HiDI Foramide (1:12) and 0.2µl of an internal size standard, Genescan LIZ-500 (Applied Biosystems, Foster City, CA) and sent for analysis at Cornell University's Biotechnology Department using their ABI 3730xl DNA Analyzer. Samples that failed to amplify were re-run a maximum of two additional times.

All samples that successfully amplified were binned using GeneMapper5 and then placed into MicroChecker 2.3.3 to identify stutter peaks, large allele dropout, and potential null alleles.

### *Histological Analysis*

Tissue samples (3 cm) from five *P. astreoides* colonies were collected from one of the collection sites (Barracuda Reef) for histological analysis of oocytes and spermaries in early April 2016. Samples were placed in labeled 50-mL plastic falcon tubes with Z-Fix Concentration (Anatech, Ltd., 1:4 dilution in seawater). Samples were decalcified using a 5% EDTA solution that was changed every 24 h for a 3 day period. After decalcification, samples were cut and were placed in cassettes in 70% ethanol. Cassettes were processed through a series of ethanol, cleared with xylene, and infiltrated with molten paraffin wax. Samples were embedded into blocks with paraffin wax and were then sectioned at a 4 µm thickness. Sections were mounted onto glass microscope

slides and were stained with Harris' hematoxylin and eosin before adding a coverslip. The slides were examined for the presence of spermaries and oocytes using an Olympus BX 43 light microscope and computer imaging using 10x magnification. These slides were compared to slides collected in 2006 which were prepared using Heidenhain's stain (Renegar, unpublished data).

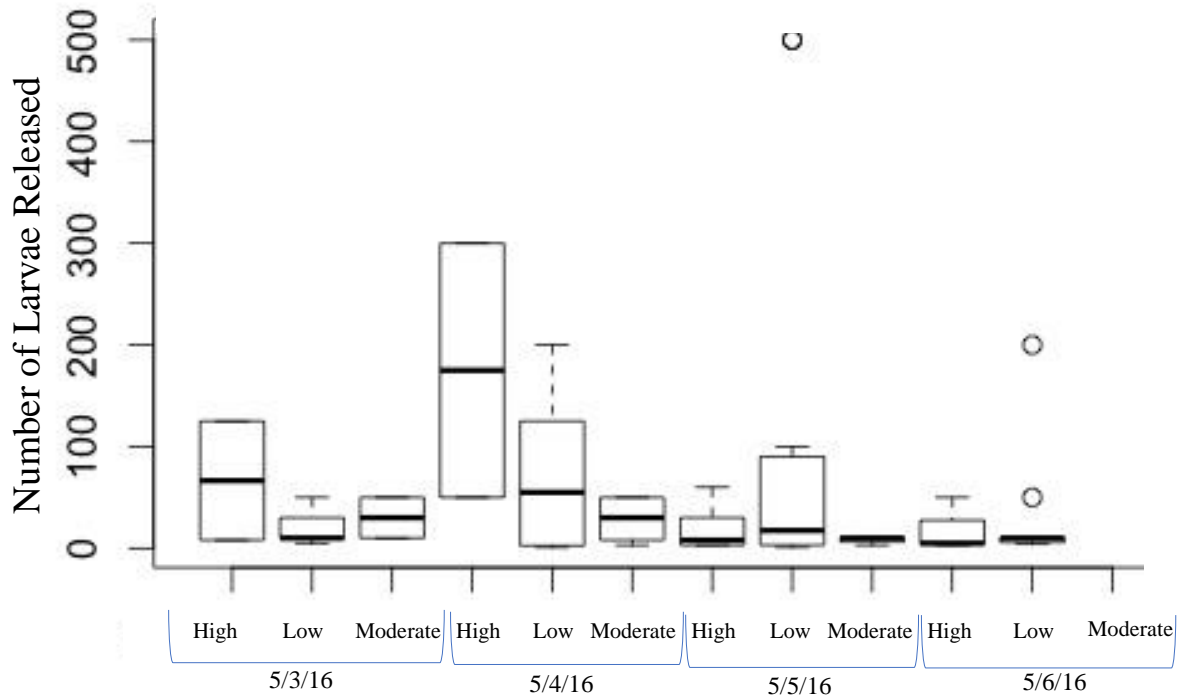
**Table 1. Microsatellite loci for *Porites astreoides*.**

<b>Locus</b>	<b>Primer Sequence(5'-3')</b>	<b>Motif type</b>	<b>Allele Size Range (bp)</b>	<b>Multiplex</b>	<b>Reference</b>
<b>Past_3</b>	F: FAM-CAGTTGTTCTAAGCTCGCCC R: GGGTTTTGAAGTGCCAGAAA	(CAT) <sub>x</sub>	406-439	C	Kenkel et al. 2013
<b>Past_17</b>	F: FAM-ACCAAAATGCTTCCTCGTTG R: AGCGGCCACTTTCTTCTGTA	(ATTG) <sub>x</sub>	249-293	D	Kenkel et al. 2013
<b>Past_21</b>	F: FAM-TTGGAGATCAGTCGCACAAA R: TCTCTCACTTGCGGGTTCTT	(ATG) <sub>x</sub>	142-194	D	Kenkel et al. 2013
<b>PA3</b>	F: VIC-CATTAACCGACTACAGTCCGT R: ACGTAAATCGCAGGACCTC	(TTCTT)imperfect	338-368	A	Serrano et al. 2016
<b>PA7</b>	F:6FAM-TTACAGTGGTCAAGCCTGG R: TTACAGGCTCCCACATAGC	(CGTC) <sub>2</sub> CATC (CGTC) <sub>6</sub>	256-268	A	Serrano et al. 2016
<b>PA13</b>	F: NED-AGATCCGCCAAGGCGAGTT R: GAGCGACGTAGGCGCAAAGAT	(ATT) <sub>2</sub> ATG (ATT) <sub>9</sub>	163-175	B	Serrano et al. 2016
<b>PA69</b>	F: 6FAM-GCCTACCATGTAAATCCTTG R: TGGTGTAAGTGAAGGTCACA	(ATT) imperfect	169-193	B	Serrano et al. 2016

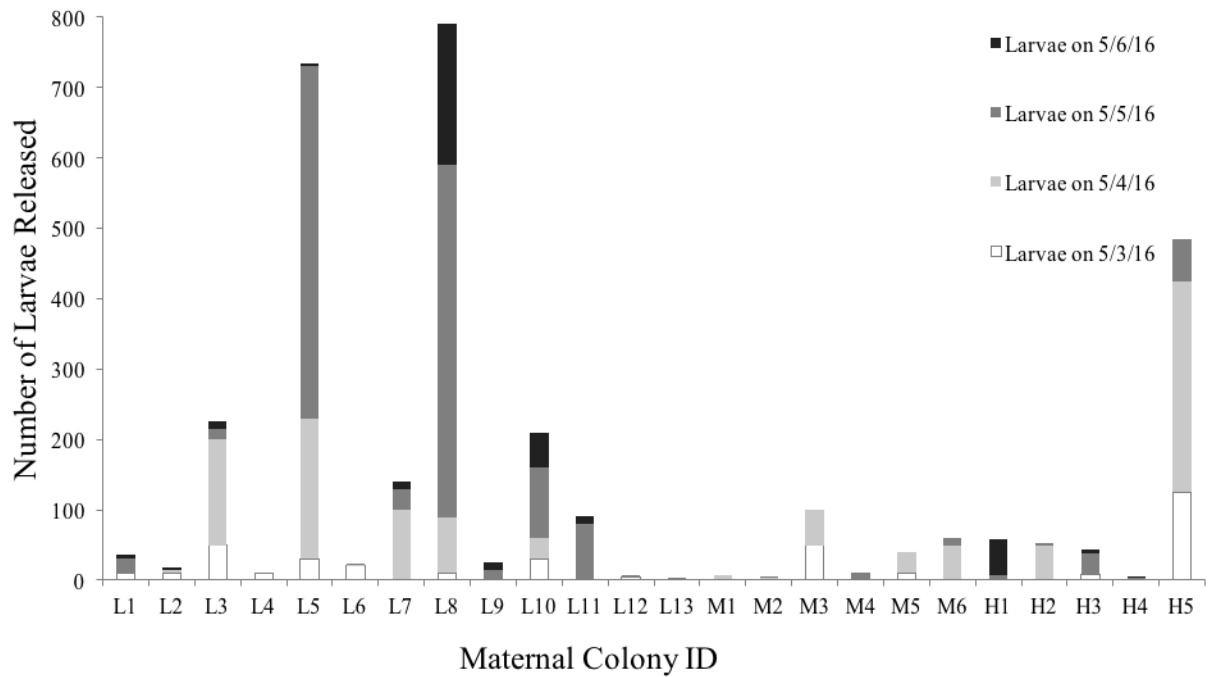
## RESULTS

### Planulation

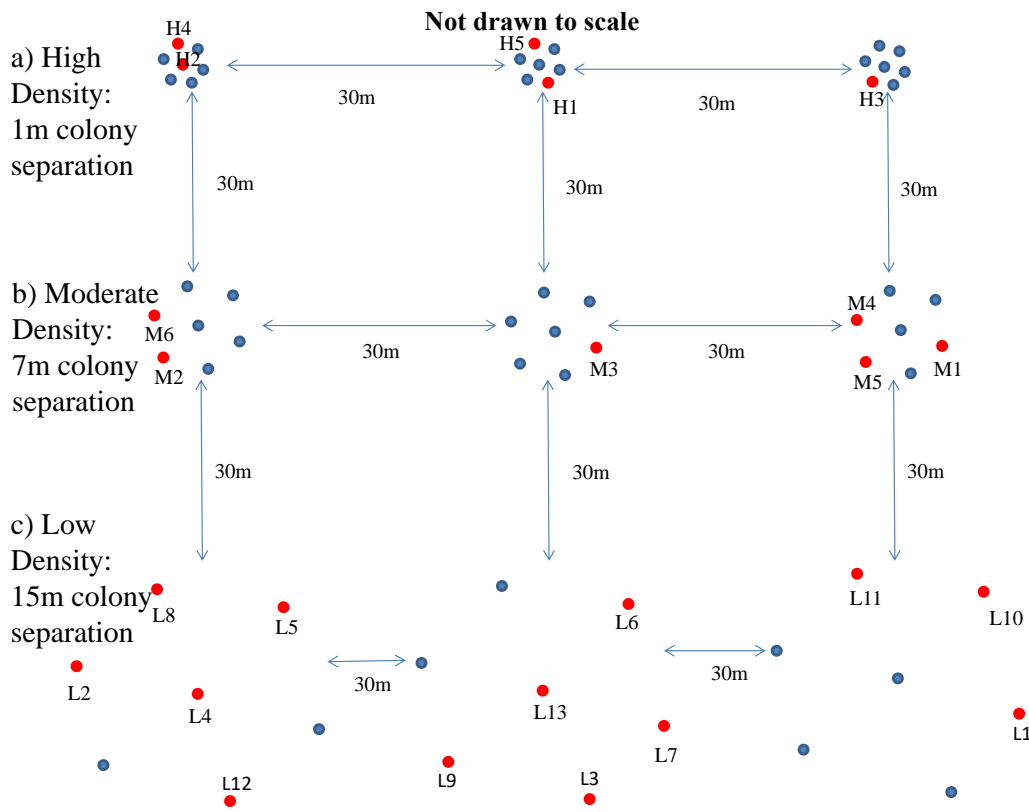
Throughout the duration of this experiment, 5 *P. astreoides* adult colonies became dislodged from the cinderblock structure and died, resulting in 58 adult colonies of which 24 colonies released larvae during the four-day collection period in May 2016 (Fig. 7 & 8). The percentage of colonies that planulated was 62 (13 of 21), 29 (6 of 21), and 16 (5 of 19) in the low, moderate, and high densities, respectively (Fig.9). Peak planulation (19 colonies) occurred on May 5, 2016, one day before the new moon. The number of planulae released was not significantly different among the four dates or three densities [Kruskal-Wallis,  $\chi^2=6.1978$ ,  $p=0.798$ ]. Maternal colonies #340 and #478 produced the most larvae and were both in low density arrays (separated from other colonies by at least 15m). These two colonies each released more than 500 larvae each on May 5, 2016.



**Figure 7. Average number of larvae released per day from each density array over the four-day monitoring period.**

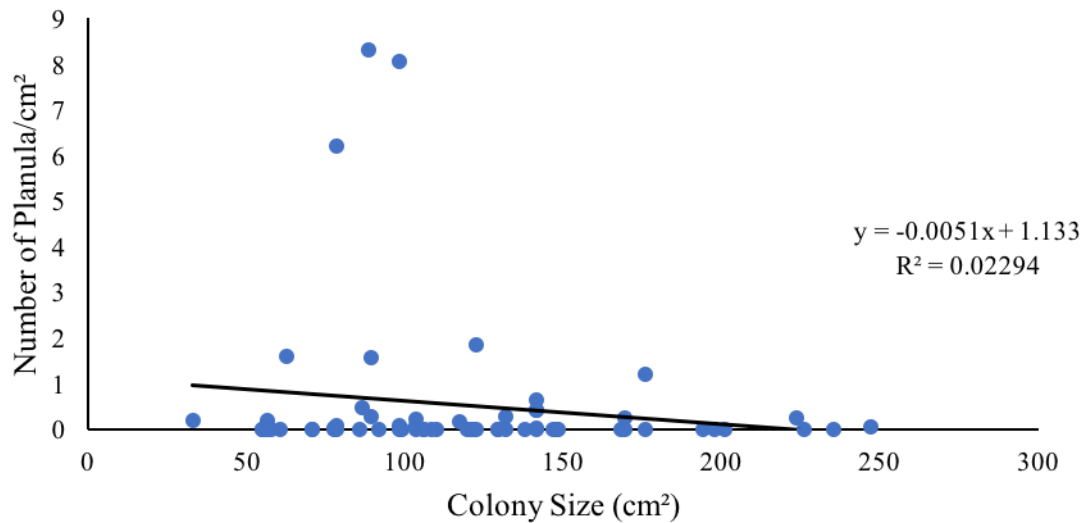


**Figure 8.** Number of larvae released per day from each maternal colony over the four-day monitoring period. Arbitrary numbers were assigned to planulated colonies L=low density, M=moderate density, H=high density.



**Figure 9.** Density array schematic with colonies that planulated in red.

Colony size, total surface area of live tissue, was measured for each maternal colony to determine the effects of size on number of planula released per cm<sup>2</sup> (Fig. 10). A linear regression suggests that colony size has no significant effect on the number of planula released/cm<sup>2</sup> [ $R^2=0.02$ ,  $p=0.0644$ ].



**Figure 10.** Relationship between colony size (total surface area of live tissue cm<sup>2</sup>) and number of planula released per cm<sup>2</sup>.

#### *Genetics*

Twenty-four *P. astreoides* colonies released a total of 3000 larvae. Of which, 998 larvae were collected and preserved in Chaos for microsatellite genotyping, and 280 larvae were successfully genotyped at six or seven microsatellite loci (Table 2). Allelic diversity ranged from 2 to 11 in the adult colonies among the seven microsatellite loci (Table 3). Of the 58 maternal colonies, 8 colonies had non-unique genotypes leading to 13% clonality found among the corals used in this study.

**Table 2. Number of larvae released per maternal colony in Broward County, Florida. Number of larvae that were genotyped per day are in parentheses.**

Maternal Colony ID	Density Array Type	Total Larvae Planulated	# Larvae Released (# of Larvae Genotyped)			
			Larvae Planulated on 5/3/16	Larvae Planulated on 5/4/16	Larvae Planulated on 5/5/16	Larvae Planulated on 5/6/16
L1	Low	36	10 (8)	0	20	6
L2	Low	19	10 (8)	4	0	5
L3	Low	225	50 (24)	150 (8)	15	10
L4	Low	10	10 (8)	0	0	0
L5	Low	734	30 (24)	200 (8)	500	4
L6	Low	23	20 (6)	2 (2)	1	0
L7	Low	140	0	100 (24)	30	10
L8	Low	790	10 (8)	80 (24)	500 (8)	200 (8)
L9	Low	25	0	0	15 (8)	10
L10	Low	210	30 (24)	30 (8)	100	50
L11	Low	91	0	1	80 (8)	10
L12	Low	8	5 (2)	0	3 (3)	0
L13	Low	3	0	0	3 (3)	0
M1	Moderate	8	0	8 (8)	0	0
M2	Moderate	8	0	5 (5)	3	0
M3	Moderate	100	50 (8)	50	0	0
M4	Moderate	10	0	0	10 (5)	0
M5	Moderate	40	10 (8)	30	0	0
M6	Moderate	60	0	50 (6)	10 (8)	0
H1	High	58	0	0	8 (8)	50
H2	High	53	0	50 (8)	3	0
H3	High	43	8 (8)	0	30	5
H4	High	6	0	0	3 (1)	3
H5	High	485	125 (24)	300	60	0

**Table 3. Number of alleles in each microsatellite primer for the adult colonies.**

Primer Name	Number of Allele in Adults	Proportion of Heterozygous Colonies
PA3	4	0.48
PA7	2	0.19
PA13	4	0.29
PA69	6	0.28
Past_3	8	0.83
Past_17	9	0.62
Past_21	11	0.88

Of the 280 genotyped *P. astreoides* larvae from colonies collected from Southeast Florida, all had identical genotypes to their maternal colony and were thus determined to be parthenogenically produced (Fig. 11 & Table 4). The percentage of parthenogenic larvae produced did not change throughout the duration of the planulation event.

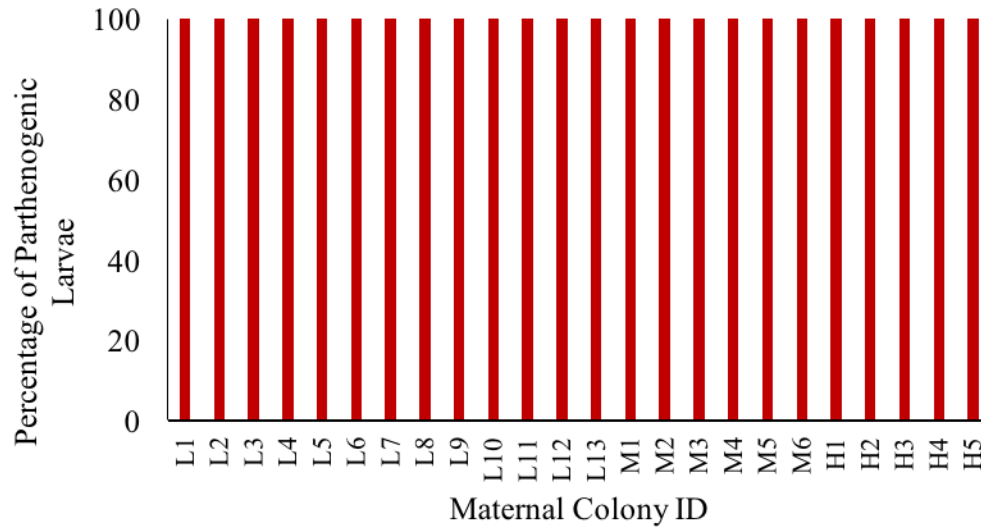


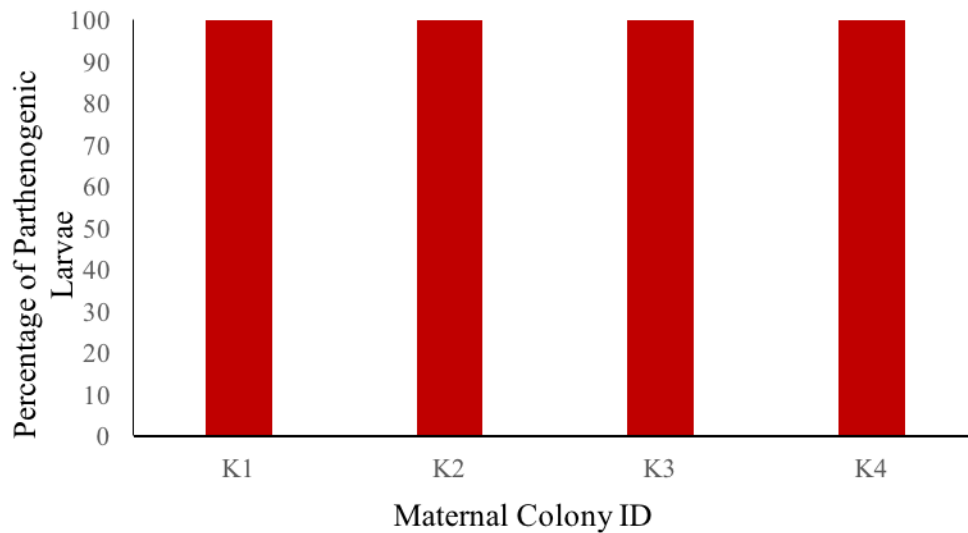
Figure 11. Percentage of parthenogenic larvae per maternal colony.

Table 4. Example of alleles from maternal colony H5 and eight of the planulated larvae using seven microsatellite primers.

Maternal/ Larvae ID	PA3		PA69		PA13		PA7		Past3		Past17		Past21	
H5	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-1	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-2	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-3	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-4	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-5	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-6	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-7	343	363	190	190	163	175	256	256	406	415	265	285	162	194
H5-8	343	363	190	190	163	175	256	256	406	415	265	285	162	194

Additionally, all larvae that planulated from the four *P. astreoides* colonies collected from the reefs off the lower Florida Keys also demonstrated the same pattern of the identical genotypes as their maternal colony and thus were derived from parthenogenesis (Fig. 12).





**Figure 12. Percentage of parthenogenic larvae per maternal colony from Summerland Key, FL collected in April 2015. All of the larvae that were genotyped were genetically identical to the maternal colony.**

### *Histology*

Five tissue samples from colonies on a natural reef in Broward County were collected in April 2016 and analyzed for the presence of oocytes and spermaries. Spermaries were not present in histologically slides from the five colonies examined. Without sperm present in colonies, colonies examined were either all female or had suppressed spermaries during the reproductive period and thus would only produced larvae without sperm, i.e., parthenogenesis. The five histology samples collected during this study were compared to ten samples collected from the same area in April 2006. The 2006 slides all had spermaries and oocytes present.

## **DISCUSSION**

All colonies of *P. astreoides* used in this study released larvae exclusively produced through parthenogenesis. Microsatellite data from 280 brooded larvae revealed they all share the same genotype as their maternal colony. There were no allelic mismatches observed between larvae and their respective maternal colonies, despite some loci demonstrating high allelic diversity. The microsatellite genotypes of the larvae strongly support the absence of paternal genetic contribution because the larvae had no unique alleles at any of the seven loci tested. Additionally, histology slides from five

corals collected on Barracuda reef (a sample site) in April 2016 showed spermaries were not present in any of the five slides. However, when compared to histology slides of *P. astreoides* from 2006 in the same regions, oocytes and spermaries were present in all ten slides. This further supports a shift to parthenogenic reproduction in the region.

While colonies used in this experiment were manipulated, this is not likely to have altered the results. Coral manipulation can stress corals and thus prompt them to resorb the sperm or disrupt sperm release (Jokiel and Guinther 1978; Loya and Rinkevich 1979; Kojis and Quinn 1984; Michalek-Wanger and Willis 2001). This seems unlikely because if gametes were resorbed due to stress, it would have included both sperm and eggs, yet nearly half of the colonies released larvae during May 3-6, 2016. Based on previous research, *P. astreoides* sperm are released into the water column around the full moon (Chornesky and Peters 1987), which was March 23<sup>rd</sup> and April 22<sup>nd</sup>, 2016 when the colonies were already placed in their experimental arrays. Furthermore, four corals collected from a reef in the Florida Keys in April 2015 were immediately brought into the laboratory for planulation and still produced parthenogenic larvae.

It remains unclear if the observed 100% parthenogenesis is a characteristic of the Broward County population, or if sexual reproduction only occurs during some months or under certain conditions, known as facultative parthenogenesis. Facultative parthenogenesis has been seen in other scleractinian coral species (Combosch and Vollmer 2013). Larvae produced by either self-fertilization or parthenogenesis are most observed in brooding species of corals that reproduce multiple times throughout the year (Lively and Johnson 1994). It is possible that based on environmental conditions or colony health, *P. astreoides* changes its reproductive strategy throughout its reproductive cycle. Previous studies document self-fertilization in *Porites astreoides* at a frequency of 34% in the upper Florida Keys likely misinterpreted their results. Brazeau et al. (1998) and Gleason et al. (2001) used electrophoresis and 4 RAPD PCR primers to determine if outcrossing or self-fertilization was occurring among the larvae and their maternal colonies. Outcrossing was scored as having one or more bands for any primer that was not present in the maternal colony leaving self-fertilization to be categorized as larvae that had the same bands for each primer as the maternal colony (Brazeau et al. 1998; Gleason et al. 2001). If self-fertilization were observed, there would be recombination of

the genes during DNA replication and crossing over. As self-fertilization continued to occur, there would be an increase in the frequency of homozygous genotypes and a decrease in heterozygosity (Table 5). However, this trend is not observed in *P. astreoides* due to the high proportion of heterozygous colonies and larvae. This pattern in matching bands the authors were observing is actually parthenogenesis, in which the larvae and the maternal colony have the same genotype. In the present study parthenogenesis was demonstrated, both in SE Florida and in the Florida Keys, using microsatellite markers determining genetic similarity through fragment analysis. Therefore, in Florida, *P. astreoides* has relied on parthenogenesis to some extent for at least two decades. It remains unclear why *P. astreoides* has shifted from relying on parthenogenesis only about a third of the time to 100% of the time.

**Table 5. Genotype frequency changes over time as a result of self-fertilization. (Stratton 2011)**

Genotype	Initial Frequency	Frequency after selfing
AA	$P$	$P + 1/4 H$
Aa	$H$	$1/2 H$
aa	$Q$	$Q + 1/4 H$

A few hypotheses for this shift to parthenogenesis include increased temperature stress, disease outbreaks, and endocrine disrupting compounds causing changes in reproduction or a bet-hedging strategy. Globally, temperatures have been increasing as a result of climate change and in 2014 temperatures on Florida reefs were higher than the coral bleaching threshold of 30.4 °C for more than 2 weeks (NOAA). In 2014–2015 following a thermal stress bleaching event, surveys throughout South Florida recorded 163 colonies of *P. astreoides* with a disease resembling rapid tissue loss and declines in mean live tissue area of *P. astreoides* at monitored sites (Gilliam et al. 2016). These stressors may be causing a shift in the sex ratio of these corals or *P. astreoides* may be producing parthenogenic larvae that are locally adapted to these environments. Southeast Florida’s reefs are close to large population centers which pump secondarily treated sewage out into the ocean. Additionally, the Florida Keys had relied on septic tanks for decades and only recently gone to a sewer system which could have caused inputs of sewage onto the reef in the past. Without the complete removal of sex steroids, endocrine-disrupting compounds may affect the natural hormone processes of corals

(Auriol et al. 2006). Endocrine-disrupting compounds have caused changes in reproductive development and in some cases complete sex reversal to all female populations (Langston et al. 2005; Andrew et al. 2010). These changes in population structure due to sex steroids may be what is driving populations of *P. astreoides* to become parthenogenic.

Parthenogenesis has been documented in a few species of scleractinian corals, but this is the first documented case in the Caribbean. It has been demonstrated that the majority of the larvae produced by *P. damicornis*, another common hermaphroditic brooding coral, are parthenogenic and only a few of the larvae are sexually produced (Combosch and Vollmer 2013). This form of mixed reproduction seen in *P. damicornis* and *O. crispata* allows for some genetic diversity within the population through sexual reproduction, but also permits the spread of successful genotypes in the populations through asexually derived larvae (Nakano and Yamazato 1992; Combosch and Vollmer 2013). Therefore, it may be a bet-hedging strategy for corals to reproduce parthenogenically to spread successful genotypes in this species northern most limit of distribution. To maintain some genetic diversity, it is still beneficial to have a small number of corals in the population or a small percentage of total larvae produced through sexual reproduction. *Tubastraea diaphana* and *T. coccinea* are hermaphroditic brooding corals whose larvae are all parthenogenic (Ayre and Resing 1986). *Tubastraea coccinea* is an invasive coral throughout the Caribbean and Gulf of Mexico and outcompetes other corals for space (Creed 2006). The success of this invasive species may be a result of its parthenogenic larvae having an advantage over other corals because they are already adapted to the environment in which they settle, assuming settlement is localized. Additionally, parthenogenesis can make invasive species highly successful because they colonize new areas, they don't have to rely on male colonies or successful fertilization.

Parthenogenesis is known to occur in marine and freshwater invertebrates and vertebrates (Lively and Johnson 1994). One of the most common examples of parthenogenesis occurs in Bdelloid rotifers, which reproduce exclusively through parthenogenesis (Birky and Glibert 1971). They are apomictic females that produce mature eggs cells by mitotic divisions and those resultant cells develop into embryos. These organisms have rapid rates of reproduction and during periods of exponential

reproduction, recessive mutations are known to occur (Birky and Gilbert 1971). Recessive mutations can accumulate in the genome and provide sufficient heterozygosity during period of amictic reproduction in rotifers (Birky and Gilbert 1971). The evolutionary loss of sexual reproduction would likely not be detrimental to the population because of these mutation events (Birky and Gilbert 1971). Sharks are also known to reproduce through parthenogenesis often called “virgin birth” (Chapman et al. 2007). Hammerhead sharks (*Sphyrna tiburo*) are shown to reproduce through automictic parthenogenesis, parthenogenesis involving meiosis in which the offspring may differ from one another and their mother, also known as half clones due to crossing over during meiosis (Chapman et al. 2007). Other studies have shown parthenogenesis may be facultative in the absences of males either in low population densities or in captive environments, such as aquaria (Castro et al. 1988). Sea stars also show evidence of parthenogenesis: mature eggs spawned by females formed bipinnaria larvae without fertilization from sperm due to female isolation, suggesting that isolated adults can produce successful larvae without outcrossing (Sunday et al. 2009).

Although parthenogenesis is a necessary form of reproduction in populations where mates are scarce, in the population of *P. astreoides* studied here, there is no shortage of possible mates leading to the hypothesis that facultative parthenogenesis is occurring due to a stressful environment or corals are employing different reproductive strategies throughout their life history (Brazaeu et al. 1998; Barnes and Hughes 1999). Genetic diversity among colonies of *P. astreoides* is high, noting that there is only approximately 10% clonality (Serrano et al. 2016), thus facultative parthenogenesis is hypothesized. In order to confirm that *P. astreoides* is reproducing using facultative parthenogenesis, genetics from both maternal colonies and resulting larvae would need to be collected and analyzed using similar methods to this study during multiple months of planulation, multiple years, and various locations throughout the Caribbean to determine if parthenogenesis is location specific due to relatively high latitudes or exposure to endocrine disrupting compounds. If all of the larvae throughout multiple, months, years and locations have the same genotypes as the maternal colonies, then results would suggest obligate parthenogenesis but if a portion of the larvae are genetically unique, then results would suggest facultative parthenogenesis. If *P. astreoides* is an obligate

parthenogenic reproducer, populations would become highly fragmented and not be able to adapt to a changing environment. If any disturbance were to occur (i.e. disease affecting specific *P. astreoides* genotypes) then genetic diversity could drastically decline and prevent the population from ever rebuilding the genetic diversity. However, if *P. astreoides* is a facultative parthenogenic reproducer, the population would be fragmented due to parthenogenesis but could possibly rebuild some genetic diversity through periodic sexual reproduction. Colony density would be a key factor in determining the potential for genetic diversity because if populations are too fragmented, sperm may have a short dispersal distance and cannot sexually reproduce with another colony, reducing genetic diversity.

### *Conclusion*

It is hypothesized that parthenogenic larvae are not exclusively adapted to the environmental condition the parental colonies inhabit but would benefit the species by dispersing to distant locations to maintain genetic diversity across habitats (Stoddart 1983). However, the planula larvae produced by *P. astreoides* are competent to settle almost immediately after release (Duerden 1902) and remain competent for a reduced time period. This short competency period means that *P. astreoides* larvae may not disperse far from their maternal colonies, and thus large populations of genetically identical individuals may form. One study suggests that there is approximately 10% clonality in Florida populations of *P. astreoides* (Serrano et al. 2016); however, sampling was conducted at least every 5 m and may not reveal fine-scale monoclonal clusters. Although this pattern of clonality may be interpreted as asexual fragmentation, findings from this study suggests this may be due to parthenogenesis. *Porites astreoides* currently comprises 30% of the adult stony coral density on reefs in SE Florida and in the Florida Keys (Gilliam et al. 2016). If parthenogenesis dominates the reproductive strategy of *P. astreoides* in SE Florida, then genetic diversity and thus adaptation to new environmental conditions in the future may become limited, posing a threat to the persistence of the species through climate change.

## CHAPTER 3- DISCUSSION

### *Parthenogenesis in Other Organisms*

Parthenogenesis is known to occur in other aquatic and terrestrial organisms, invertebrates and vertebrates alike. It can be a species only mode of reproduction known as obligate parthenogenesis, or an organism can switch from sexual reproduction to parthenogenesis throughout its reproductive cycle, called facultative parthenogenesis (Birky and Glibert 1971). Facultative parthenogenesis can be triggered by a lack of males or environmental conditions favoring population growth (seen in rotifers and daphnia), or it can be triggered by seasonal changes (seen in aphids).

Parthenogenesis can produce progeny that are female only (thelytoky), males only (arrhenotoky), or males and females (deuterotoky) (Gavrilov and Kuznetsova 2007). The velvet worm, *Epiperipatus imthurni*, and Bdelloid rotifers are two common examples of thelytoky parthenogenesis. No male specimens of *E. imthurni* or Bdelloid rotifers have ever been documented (Read 1988; Stelzer et al. 2010). In a study about the origin of obligate parthenogenic reproduction, it was discovered that these rotifers had lost their ability to respond to sex-inducing signals due to the inheritance of the *op* gene (Stelzer et al. 2010). Some reptiles use a ZW chromosome system in which a male is homozygous for the Z allele and a female is heterozygous for the W allele. Homozygous W alleles were thought to be unviable until 2010 when a female boa constrictor produced parthenogenic female offspring with a chromosomal makeup of WW, meaning the offspring are half clones of the mother (Walker 2010).

Arrhenotoky, the production of only male offspring through parthenogenesis, has been extensively described in honey bees. In bee populations, the queen bee is the only fertile female in the colony. However, if the queen bee dies, worker bees can produce male offspring through parthenogenesis. Eventually, the worker bees in the relict population die if they do not find a queen from another colony to mate with. This parthenogenic reproduction allows the colony's genetic diversity to be maintained in a new population. Deuterotoky, the parthenogenic reproduction of both male and female offspring, is common in gall wasps (Stone et al. 2002). *Biorhiza pallida* parthenogenically produces offspring at a 1:1 female to male ratio while other species of

gall wasps favor one sex (Atkinson 2000). Parthenogenesis has evolved to be a viable reproductive strategy in many species in both the plant and animal kingdoms.

### ***Genetic Interpretations***

Self-fertilization has been previously documented in *Porites astreoides* at a frequency of 34% in the upper Florida Keys (Brazeau et al. 1998; Gleason et al. 2001). Brazeau et al. (1998) used electrophoresis and 4 RAPD PCR primers to determine if outcrossing or self-fertilization was occurring among the larvae and their maternal colonies. Outcrossing was scored as having one or more bands for any primer that was not present in the maternal colony, leaving self-fertilization to be categorized as larvae that had the same bands for each primer as the maternal colony (Brazeau et al. 1998; Gleason et al. 2001). If self-fertilization were observed, there would be recombination of the genes during DNA replication and crossing over. This pattern in matching bands the authors were observing is actually indicative of parthenogenesis, because the larvae and their maternal colonies had identical genotypes. The same reproductive strategy was confirmed in this study with 100% of the resultant larvae having the same genotypes as the maternal colonies.

Other cases in which exact allele matches may occur include low allelic diversity or low heterozygosity among individuals as well as a lack of Hardy-Weinberg equilibrium. Additionally, there could have been a lack of resolution in the microsatellite markers to distinguish among potential siblings; however, these markers were used in a prior study on the genetic diversity of this species and demonstrated a high resolution (Serrano et al. 2016). If a population has low allelic diversity or low heterozygosity, individuals may appear to be exact allelic matches. However, in this study, allelic diversity ranges from 2 to 11 alleles per microsatellite marker and the proportion of heterozygosity among adults ranges from 19% to 88% per marker. Therefore, it is highly unlikely that parthenogenesis has been misinterpreted.

### ***Parthenogenesis in Porites astreoides***

This study is the first to correctly identify parthenogenesis in *Porites astreoides*. Of the 280 larvae tested for paternity in this study, all of the larvae were



produced through parthenogenesis. The pathway of evolution to parthenogenesis in *P. astreoides* is unknown, but we have demonstrated that adult colonies have different genotypes while larvae are clones of their maternal colony. Serrano et al. (2016) found approximately 10% clonality in populations of *P. astreoides* from the Florida Keys, which may be a result of asexual reproduction from parthenogenesis or fragmentation but based on *P. astreoides* morphology, and evidence here, parthenogenesis is more likely. Parthenogenesis was observed as the only method of reproduction in this study using coral colonies collected at two reefs off Broward County and one reef off Summerland Key, Florida. Parthenogenic larvae could be dispersing to other reefs, and thus there is still genetic diversity. This hypothesis is supported by the high amount of gene flow both vertically and horizontally within Florida populations and within the USVI populations of *P. astreoides* that was reported in a previous study, which used the same genetic markers as the current study (Serrano et al. 2016). However, this challenges the notion that asexual reproduction (*i.e.*, parthenogenesis) is used to maintain locally adapted clones. Additionally, maternal colony genetic diversity could have resulted from previous facultative parthenogenesis, evidenced in Brazeau et al. (1998), and these genetically diverse colonies are now reproducing solely through parthenogenesis. However, until a disturbance occurs that reduces the genetic diversity, the shift to parthenogenesis would likely not be observed in the population genetic signature.

Parthenogenesis in *P. damicornis* was observed in localized populations on Hawaiian reefs, but was rare on Australia's Great Barrier Reef (Ayre and Miller 2004). The current study on *P. astreoides* found parthenogenesis in two regions of the Florida Reef Tract, Broward County and in the Florida Keys which are relatively high latitude reefs. It will be interesting to determine if *P. astreoides* in other regions of the Western Atlantic also produce parthenogenic larvae, or if it is highly localized to higher latitudes. If *P. astreoides* is only parthenogenic on high latitude reefs, currents may be dispersing larvae from other locations to these high latitude reefs which is depicted in the genetic diversity of maternal colonies. The high latitude reefs may be a sink for sexual produced *P. astreoides* larvae.

### ***Environmental Changes***

With the decline of coral reefs around the world due to a combination of local and global stressors, high genetic diversity is crucial for surviving populations (Knowlton and Jackson 1993). Coral cover in the Caribbean has plummeted from 50% in the 1970's to less than 10% today (Gardner et al. 2006; Gilliam et al. 2016; de Bakker et al. 2017). Stress on corals from anthropogenic changes may have led to the changes in parthenogenesis observed along the Florida Reef Tract from 34% in May and June of 1995 (Brazeau et al. 1998) to 100% in May 2016, as seen in the current study.

Reefs in Florida have been severely impacted by anthropogenic stressors due to their proximity to major population centers. Southeast Florida reefs are impacted by poor water quality due to six ocean outfalls in which treated sewage is pumped into the ocean for disposal. Cumulatively, the outfalls release 400 million gallons of effluent per day, which receives secondary treatment (Koopman et al. 2006). Secondary treatment of waste is estimated to remove 70-80% of sex steroids from the water (Auriol et al. 2006). Without the complete removal of sex steroids, endocrine-disrupting compounds may affect the natural hormone processes of corals (Auriol et al. 2006). These endocrine-disrupting compounds can cause changes in reproductive development and in some cases complete sex reversal to all female populations (Langston et al. 2005; Andrew et al. 2010). These changes in population structure due to sex steroids may be what is driving populations of *P. astreoides* to become parthenogenic. To test this hypothesis, one could test the concentration of sex hormones in the water near coral colonies to determine if levels are higher than predicted and histological analyses could be used to determine if spermaries are present in colonies.

Additional stressors to corals along the Florida Reef Tract include land-based pollution, overfishing, habitat destruction, and disease outbreaks. Globally, temperatures have been increasing due to climate change and in 2014 temperatures on Florida reefs were higher than the coral bleaching threshold of 30.4 °C for more than 2 weeks (NOAA). Recently, in 2014–2015 following a thermal stress bleaching event, surveys throughout South Florida recorded 163 colonies of *P. astreoides* with a disease resembling rapid tissue loss and declines in mean live tissue area of *P. astreoides* at monitored sites (Gilliam et al. 2016). Additionally, temperature dependent sex

determination is known to occur in some marine organisms including reptiles and teleost fishes (Ospina-Alvarez and Piferrer 2008; Warner et al. 2008; Bachtrog et al. 2014). Although the molecular mechanisms are not understood for temperature dependent sex determination increases in global temperatures altering sex ratios of organisms may become more common to the detriment of a species (Shen and Wang 2014). Recent stress on Southeast Florida's *P. astreoides* population due to disease outbreaks and climate change may be causing these corals to transition to parthenogenic reproduction.

### ***Ecological Implications***

*Porites astreoides* comprises 30% of the adult stony coral density of Florida reefs (Gilliam et al. 2016) and appears to be thriving in the region, though they contribute little to reef structure. This species does not appear to be severely affected by disease outbreaks (Gilliam et al. 2016) and although colonies exhibit mortality in periods of cold temperatures, it has been documented to have high recruitment post-disturbances (Kemp et al. 2016). A concurrent study in Broward County demonstrated that *P. astreoides* had the highest recruitment rates of all scleractinian coral species in 2016 and 2017 (Harper et al., unpublished data). This recruitment data suggests that although *P. astreoides* may be reproducing exclusively through parthenogenesis, the larvae are viable through the earliest life history stages possibly due to high maternal reserves from brooding. The recruitment success of *P. astreoides*, coupled with the species' ability to survive in unstable conditions (Aronson et al. 2008), provides *P. astreoides* with a competitive advantage over other stony coral species and may explain this species' recent population increase.

In populations where the Allee Effect is present, parthenogenesis removes the need to find a mate therefore isolated individuals may still reproduce. Thelytoky, the reproduction of female only offspring, is the most common form of parthenogenesis (Stouthamer 1989). Females directly contribute to the population of a species because they can produce the next generation of offspring. Obligate parthenogenic reproduction may increase *P. astreoides* survivorship due to rapid population growth, but at the risk of genetically diverse populations. Without genetic variation, the population may not adapt

in response to changing environments, such as climate change, and may face an increased risk of extinction (Frankham et al. 2010).

### ***Future studies***

Because paternity was not apparent in this study, the main questions that this study originally sought to answer could not be addressed: colony size and density did not influence paternity, paternity share did not change over multiple days of planulation, and the number of sires fertilizing eggs from one colony was zero. These questions are still important and need to be answered in *P. astreoides* to understand if the trend seen in this study is facultative parthenogenesis. Future studies to understand these objectives of paternity patterns could use other common brooding corals, such as those in the genus *Agaricia*.

Of the 280 larvae tested for paternity in the present study, all were produced through parthenogenesis. More studies need to be conducted to determine if this is a result of facultative parthenogenesis that can be seen during different months or years of the reproductive cycle or a rare occurrence due to stress. This can be completed using both genetic and histological analysis from colonies and larvae collected throughout the planulation cycle, over multiple years and across locations. Genetic samples would explain if larvae are a result of outcrossing or parthenogenesis while the histology samples could be used to determine if spermaries are present in colonies.

*Porites astreoides* is known to have two distinct color morphs, a green and a brown morph, that live in distinct environments, however, they are genetically connected (Serrano et al. 2016). The green morph is found in shallow reefs environments (3 m) whereas the brown morph is more common on deeper reefs (Thornton 1999). The two morphs do not have different growth rates, but they do differ in their capacity for UV light protection and sediment removal (Gleason 1993, 1998). A study conducted on the reproductive strategies of these two color morphs might help to explain genetic diversity in the maternal colonies. In the present study, only the brown color morph of *P. astreoides* was tested so it would be interesting to know if similar parthenogenetic patterns occur in the green morph. Much like the research conducted on *P. damicornis*

larval movement, the dispersal of parthenogenic larvae could be investigated to determine where they settle in relation to their maternal colony.

Additionally, determining the presence of location specific patterns of parthenogenesis may explain if parthenogenesis is occurring due to stress (high anthropogenic influence on reefs) or latitudinal limits for *P. astreoides*. *Porites astreoides* is a species found throughout the Caribbean and Bermuda therefore determining in the frequency of parthenogenesis in other populations of this species could have important implications for the genetic diversity of this abundant species. If *P. astreoides* is an obligate parthenogenic reproducer, populations would become highly fragmented and not be able to adapt to a changing environment. If any disturbance were to occur (*i.e.*, disease affecting *P. astreoides*) then genetic diversity could drastically decline and prevent the population from ever rebuilding the genetic diversity. However, if *P. astreoides* is a facultative parthenogenic reproducer, the population would be fragmented due to parthenogenesis but could possibly rebuild some genetic diversity through times of sexual reproduction. Colony density would be a key factor in determining the potential for genetic diversity because if populations are too fragmented, sperm may have a short dispersal distance and cannot sexually reproduce with another colony, reducing genetic diversity.

Future efforts should examine if parthenogenesis is a common reproductive strategy in other brooding corals such as those in the genera *Agaricia*, *Favia*, and *Mycetophyllia* (Szmant 1986). Agaricids are known to be the dominant brooding coral on reefs elsewhere in the Caribbean (Bak and Engel 1979); therefore, it would be important to understand if this reproductive strategy is the reason for their success.

### ***Conclusion***

This study is the first to correctly identify parthenogenesis as a reproductive strategy in a Caribbean coral. There were no allelic mismatches observed between larvae and their respective maternal colonies demonstrating parthenogenesis as the only form of reproduction noted throughout the study. With the worldwide decline in coral populations, facultative parthenogenesis may be an advantageous reproductive strategy used to boost populations. However, more research is needed to fully understand the

evolution of parthenogenesis in *P. astreoides* and the ecological impacts it may ultimately have on coral reefs.

## REFERENCES

- Andrew MN, O'Connor WA, Dunstan RH, MacFarlane GR (2010) Exposure to 17 $\alpha$ -ethynylestradiol causes dose and temporally dependent changes in intersex, females and vitellogenin production in the Sydney rock oyster. *Ecotoxicology*, 19(8): 1440-1451
- Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. *The ecology and etiology of newly emerging marine diseases*, pp25-38
- Atkinson RJ (2000) *The genetic analysis of natural history, reproductive strategy and population structure in European oak gallwasps (Hymenoptera: Cynipidae)*. University of Oxford
- Auriol M, Filali-Meknassi Y, Tyagi RD, Adams CD, Surampalli RY (2006) Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochemistry* 41:525-539
- Ayre D, Resing J (1986) Sexual and asexual production of planulae in reef corals. *Marine Biology* 90:187-190
- Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* 54:1590-1605
- Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters* 7:273-278
- Ayre DJ, Miller KJ (2004) Where do clonal coral larvae go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. *Marine Ecology Progress Series* 277:95-105
- Ayre DJ, Miller K (2006) Random mating in the brooding coral *Acropora palifera*. *Marine Ecology Progress Series* 307:155-160
- Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. *Marine Ecology Progress Series*:175-187
- Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman T-L, Hahn MW, Kitano J, Mayrose I, Ming R (2014) Sex determination: why so many ways of doing it? *PLoS Biology* 12:e1001899
- Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology, Evolution, and Systematics* 40:551-571

- Bak R, Engel M (1979) Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. *Marine Biology* 54:341-352
- Barnes RSK, Hughes RN (1999) An introduction to marine ecology. John Wiley & Sons
- Baums I, Devlin-Durante M, Polato N, Xu D, Giri S, Altman N, Ruiz D, Parkinson J, Boulay J (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral, *Acropora palmata*. *Coral Reefs* 32:703-717
- Baums IB, Miller MW, Hellberg ME (2006) Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. *Ecological Monographs* 76:503-519
- Birky Jr CW, Gilbert JJ (1971) Parthenogenesis in rotifers: the control of sexual and asexual reproduction. *American Zoologist* 11:245-266
- Bittencourt JVM, Sebbenn AM (2008) Pollen movement within a continuous forest of wind-pollinated *Araucaria angustifolia*, inferred from paternity and TwoGener analysis. *Conservation Genetics* 9:855-868
- Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. *Journal of Experimental Marine Biology and Ecology* 231:225-238
- Carlson DB (1999) The evolution of mating systems in tropical reef corals. *Trends in Ecology & Evolution* 14:491-495
- Carlson DB, Lippe C (2011) Estimation of mating systems in Short and Tall ecomorphs of the coral *Favia fragum*. *Molecular Ecology* 20:812-828
- Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, DeVantier L (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 321:560-563
- Castro JI, Bubucis PM, Overstrom NA (1988) The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. *Copeia*:740-746
- Chapman DD, Shivji MS, Louis E, Sommer J, Fletcher H, Prodöhl PA (2007) Virgin birth in a hammerhead shark. *Biology Letters* 3:425-427
- Chenuil A, Gault A, Feral J-P (2004) Paternity analysis in the Antarctic brooding sea urchin *Abatus nimrodi*. A pilot study. *Polar Biology* 27:177-182



- Chiappone M, Sullivan K (1996) Distribution, abundance and species composition of juvenile scleractinian corals in the Florida reef tract. *Bulletin of Marine Science* 58:555-569
- Chornesky EA, Peters EC (1987) Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. *The Biological Bulletin* 172:161-177
- Colin PL (1978) Caribbean Reef Invertebrates and Plants: A field guide to the invertebrates and plants occurring on coral reefs of the Caribbean, the Bahamas and Florida. TFH Publications
- Combosch DJ, Vollmer SV (2013) Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. *Ecology and Evolution* 3:3379-3387
- Costanza R, d'Arge R, De Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253-260
- Côté I, Gill J, Gardner T, Watkinson A (2005) Measuring coral reef decline through meta-analyses. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 360:385-395
- Courchamp F, Berec L, Gascoigne J (2008) Allee effects in ecology and conservation. Oxford University Press
- Creed JC (2006) Two invasive alien azooxanthellate corals, *Tubastraea coccinea* and *Tubastraea tagusensis*, dominate the native zooxanthellate *Mussismilia hispida* in Brazil. *Coral Reefs* 25:350-350
- Darwin C (1876) The effects of cross and self fertilisation in the vegetable kingdom. J. Murray
- De Bakker DM, Van Duyl FC, Bak RP, Nugues MM, Nieuwland G, Meesters EH (2017) 40 years of benthic community change on the Caribbean reefs of Curaçao and Bonaire: the rise of slimy cyanobacterial mats. *Coral Reefs* 36:355-367
- Dobzhansky T, Dobzhansky TG (1970) Genetics of the evolutionary process. Columbia University Press
- Duerden J (1902) Aggregated colonies in madreporarian corals. *The American Naturalist* 36:461-471
- Edmunds P, Gates R, Gleason D (2001) The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances. *Marine Biology* 139:981-989

- Edmunds PJ (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites spp.* *Limnology and Oceanography* 56:2402-2410
- Florida Department of Environmental Protection (2011) Florida's Coral Reefs
- Fogarty ND (2010) Reproductive isolation and hybridization dynamics in threatened Caribbean acroporid corals. The Florida State University
- Fogarty ND (2012) Caribbean acroporid coral hybrids are viable across life history stages. *Marine Ecology Progress Series* 446:145-159
- Fogarty ND, Vollmer SV, Levitan DR (2011) Weak prezygotic isolating mechanisms in threatened Caribbean Acropora corals. *PLoS One* 7:e30486
- Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics. Cambridge University Press
- Frieler K, Meinshausen M, Golly A, Mengel M, Lebek K, Donner S, Hoegh-Guldberg O (2013) Limiting global warming to 2° C is unlikely to save most coral reefs. *Nature Climate Change* 3:165-170
- Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004) Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. *Evolution* 58:324-337
- Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. *Science* 301:958-960
- Gavrilov I, Kuznetsova V (2007) On some terms used in the cytogenetics and reproductive biology of scale insects (Homoptera: Coccinea). *Comparative Cytogenetics* 1:169-174
- Gilliam DS (2013) Southeast Florida Coral Reef Evaluation and Monitoring Project 2012 Year 10 Final Report 49
- Gilliam DS, Walton, C.J., Brinkhuis, V., Ruzicka, R., and Hayes, N.K. (2016) Southeast Florida Coral Reef Evaluation and Monitoring Project 2015, Year 13 Executive Summary. Florida DEP Report #RM143- 18
- Gleason DF (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. *Limnology and Oceanography* 38:1452-1463
- Gleason DF, Brazeau DA, Munfus D (2001) Can self-fertilizing coral species be used to enhance restoration of Caribbean reefs? *Bulletin of Marine Science* 69:933-943

- Gleiser G, Internicola AI, Austerlitz F, Bernasconi G (2014) Stabilizing selection on nectar concentration in wild *Petunia axillaris*, as revealed by genetic analysis of pollen dispersal. *Evolutionary Ecology* 28:869-884
- Glynn PW, Colley SB, Maté J, Cortés J, Guzman HM, Bailey R, Feingold JS, Enochs I (2008) Reproductive ecology of the azooxanthellate coral *Tubastraea coccinea* in the Equatorial Eastern Pacific: Part V. Dendrophylliidae. *Marine Biology* 153:529
- Goldberg WM (1973) The ecology of the coral-octocoral communities off the southeast Florida coast: geomorphology, species composition, and zonation. *Bulletin of Marine Science* 23:465-488
- Graham E, Baird A, Connolly S (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. *Coral Reefs* 27:529-539
- Green DH, Edmunds PJ (2011) Spatio-temporal variability of coral recruitment on shallow reefs in St. John, US Virgin Islands. *Journal of Experimental Marine Biology and Ecology* 397:220-229
- Green DH, Edmunds PJ, Carpenter RC (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series* 359:1-10
- Harper L (2018) *Variability in Coral Recruitment in Southeast Florida*. Nova Southeastern University
- Harrison HB, Williamson DH, Evans RD, Almany GR, Thorrold SR, Russ GR, Feldheim KA, Van Herwerden L, Planes S, Srinivasan M (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current biology* 22:1023-1028
- Harrison P, Wallace C, Dubinsky Z (1990) Ecosystems of the world: coral reefs. *Coral Reproduction*:133-208
- Harrison PL (1985) Sexual characteristics of scleractinian corals: systematic and evolutionary implications
- Harrison PL (1988) Pseudo-gynodioecy: an unusual breeding system in the scleractinian coral *Galaxea fascicularia*
- Harrison PL (2011) Sexual reproduction of scleractinian corals *Coral reefs: an ecosystem in transition*. Springer, pp59-85
- Hedgecock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of marine science* 39:550-564

- Heyward A, Babcock R (1986) Self-and cross-fertilization in scleractinian corals. *Marine Biology* 90:191-195
- Highsmith RC (1982) Reproduction by fragmentation in corals. *Marine Ecology Progress Series*:207-226
- Hoegh-Guldberg O (2011) Coral reef ecosystems and anthropogenic climate change. *Regional Environmental Change* 11:215-227
- Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737-1742
- Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JB, Kleypas J (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* 301:929-933
- Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC (2018) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* 359:80-83
- Humann PD (1996) Reef coral identification Florida, Caribbean, Bahamas: including marine plants
- IPCC (2014) Climate Change 2104: Synthesis Report 151
- Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* 38:1371-1383
- Jokiel PL, Guinther EB (1978) Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*. *Bulliten of Marine Science* 28:786-789
- Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. *Current Biology* 15:1314-1318
- Kemp DW, Colella MA, Bartlett LA, Ruzicka RR, Porter JW, Fitt WK (2016) Life after cold death: reef coral and coral reef responses to the 2010 cold water anomaly in the Florida Keys. *Ecosphere* 7
- Kenkel C, Meyer E, Matz M (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. *Molecular Ecology* 22:4322-4334
- Knowlton N (2001) The Future of Coral Reefs. *Proceedings of the National Academy of Sciences of the United States of America* 98:5419-5425

- Knowlton N, Jackson JB (1993) Inbreeding and outbreeding in marine invertebrates. The natural history of inbreeding and outbreeding University of Chicago Press, Chicago:200-249
- Kojis BL, Quinn NJ (1984) Seasonal and depth variation in fecundity of *Acropora palifera* at two reefs in Papua New Guinea. *Coral reefs* 3:165-172
- Koopman B, Heaney JP, Cakir FY, Rembold M, Indeglia P, Kini G (2006) Ocean outfall study. Final Report, Florida Department of Environmental Protection, Tallahassee, FL (<http://www/dep.state.fl.us/water/reuse/docs/OceanOutfallStudy.pdf>)
- Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. *Marine Ecology Progress Series* 323:107-117
- Lam KK (2000) Sexual reproduction of a low-temperature tolerant coral *Oulastrea crispata* (Scleractinia, Faviidae) in Hong Kong, China. *Marine Ecology Progress Series* 205:101-111
- Langston W, Burt G, Chesman B, Vane C (2005) Partitioning, bioavailability and effects of oestrogens and xeno-oestrogens in the aquatic environment. *Journal of the Marine Biological Association of the United Kingdom* 85:1-31
- Lasker HR, Gutiérrez-Rodríguez C, Bala K, Hannes A, Bilewitch JP (2008) Male reproductive success during spawning events of the octocoral *Pseudopterogorgia elisabethae*. *Marine Ecology Progress Series* 367:153-161
- Levitan DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. *The American Naturalist* 141:517-536
- Levitan DR (2004) Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. *The American Naturalist* 164:298-309
- Levitan DR (2005) Sex-specific spawning behavior and its consequences in an external fertilizer. *The American Naturalist* 165:682-694
- Levitan DR, Sewell MA, Chia F-S (1992) How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* 73:248-254
- Levitan DR, Fukami H, Jara J, Kline D, McGovern TM, McGhee KE, Swanson CA, Knowlton N (2004) Mechanisms of reproductive isolation among sympatric

- broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution* 58:308-323
- Lirman D (2000) Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. *Journal of Experimental Marine Biology and Ecology* 251:41-57
- Lirman D, Fong P (2007) Is proximity to land-based sources of coral stressors an appropriate measure of risk to coral reefs? An example from the Florida Reef Tract. *Marine Pollution Bulletin* 54:779-791
- Lirman D, Formel N, Schopmeyer S, Ault J, Smith S, Gilliam D, Riegl B (2014) Percent recent mortality (PRM) of stony corals as an ecological indicator of coral reef condition. *Ecological Indicators* 44:120-127
- Lively CM, Johnson SG (1994) Brooding and the evolution of parthenogenesis: strategy models and evidence from aquatic invertebrates. *Proc R Soc Lond B* 256:89-95
- Loya Y, Rinkevich B (1979) Abortion effect in corals induced by oil pollution. *Marine Ecology Progress Series*:77-80
- Masse LM (2009) Sexual Reproduction of *Pocillopora damicornis* (Cnidaria: Hexacorallia) at High Latitude Off Durban, South Africa. *Citeseer*
- Maturana CS, Gérard K, Díaz A, David B, Féral J-P, Poulin E (2017) Mating system and evidence of multiple paternity in the Antarctic brooding sea urchin *Abatus agassizii*. *Polar Biology* 40:787-797
- McClenachan L (2012) Recreation and the “right to fish” movement: anglers and ecological degradation in the Florida Keys. *Environmental History* 18:76-87
- McGuire M (1998) Timing of larval release by *Porites astreoides* in the northern Florida Keys. *Coral Reefs* 17:369-375
- Michalek-Wagner K, Willis B (2001) Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. *Coral Reefs* 19:231-239
- Miller K, Babcock R (1997) Conflicting morphological and reproductive species boundaries in the coral genus *Platygyra*. *The Biological Bulletin* 192:98-110
- Moyer RP, Riegl B, Banks K, Dodge RE (2003) Spatial patterns and ecology of benthic communities on a high-latitude South Florida (Broward County, USA) reef system. *Coral Reefs* 22:447-464

- Nakano Y, Yamazato K (1992) Ecological study of reproduction of *Oulastrea cispata* in Okinawa.(Behavior Biology and Ecology) Proceedings of the Sixty-Third Annual Meeting of the Zoological Society of Japan. Zoological Science 9:1292
- National Oceanic and Atmospheric Administration (2018) National Data Buoy Center
- Ospina-Alvarez N, Piferrer F (2008) Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. PLoS One 3:e2837
- Pemberton AJ, Hughes RN, Manríquez PH, Bishop JD (2003) Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained. Proceedings of the Royal Society of London B: Biological Sciences 270:S223-S226
- Pluess AR, Sork VL, Dolan B, Davis FW, Grivet D, Merg K, Papp J, Smouse PE (2009) Short distance pollen movement in a wind-pollinated tree, *Quercus lobata* (Fagaceae). Forest Ecology and Management 258:735-744
- Read VSJ (1988) The Onychophora of Trinidad, Tobago and the Lesser Antilles. Zoological Journal of the Linnean Society 93:225-257
- Richmond RH (1987) Energetic relationships and biogeographical differences among fecundity, growth and reproduction in the reef coral *Pocillopora damicornis*. Bulletin of Marine Science 41:594-604
- Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Marine Ecology Progress Series:185-203
- Ruzicka R CM, Brinkhuis V (2016) Coral Reef Evaluation and Monitoring Project 2016 Executive Summary
- Sammarco PW (1982) Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. Marine Ecology Progress Series:57-65
- Sánchez-Robles JM, García-Castaño JL, Balao F, Terrab A, Navarro-Sampedro L, Tremetsberger K, Talavera S (2014) Effects of tree architecture on pollen dispersal and mating patterns in *Abies pinsapo* Boiss.(Pinaceae). Molecular Ecology 23:6165-6178
- Saro I, Robledo-Arnuncio J, González-Pérez M, Sosa P (2014) Patterns of pollen dispersal in a small population of the Canarian endemic palm (*Phoenix canariensis*). Heredity 113:215-223
- Sathe M, Gilliam DS, Dodge RE, Fisher L (2008) Patterns in southeast Florida coral reef community composition. NSU Works

- Schmidt-Roach S, Miller KJ, Lundgren P, Andreakis N (2014) With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society* 170:1-33
- Serrano XM, Baums IB, Smith TB, Jones RJ, Shearer TL, Baker AC (2016) Long distance dispersal and vertical gene flow in the Caribbean brooding coral *Porites astreoides*. *Scientific Reports* 6: 21619
- Shen Z-G, Wang H-P (2014) Molecular players involved in temperature-dependent sex determination and sex differentiation in Teleost fish. *Genetics Selection Evolution* 46:26
- Sherman C, Ayre DJ, Miller K (2006) Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. *Coral Reefs* 25:7-18
- Sier C, Olive P (1994) Reproduction and reproductive variability in the coral *Pocillopora verrucosa* from the Republic of Maldives. *Marine Biology* 118:713-722
- Soong K (1991) Sexual reproductive patterns of shallow-water reef corals in Panama. *Bulletin of Marine Science* 49:832-846
- Sotka E, Hay M (2009) Effects of herbivores, nutrient enrichment, and their interactions on macroalgal proliferation and coral growth. *Coral Reefs* 28:555-568
- Spalding M, Ravilious C, Green EP (2001) *World atlas of coral reefs*. Univ of California Press
- Stelzer C-P, Schmidt J, Wiedlroither A, Riss S (2010) Loss of sexual reproduction and dwarfing in a small metazoan. *PLoS One* 5:e12854
- Stoddart J (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine Biology* 76:279-284
- Stone GN, Schönrogge K, Atkinson RJ, Bellido D, Pujade-Villar J (2002) The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology* 47:633-668
- Stouthamer R (1990) Causes of thelytoky and crossing incompatibility in several *Trichogramma* species (Hymenoptera: Trichogrammatidae)
- Streiff R, Ducouso A, Lexer C, Steinkellner H, Gloessl J, Kremer A (1999) Pollen dispersal inferred from paternity analysis in a mixed oak stand of *Quercus robur* L. and *Q. petraea*. *Molecular Ecology* 8:831-841



- Sunday J, Raeburn L, Stewart H, Hart MW (2009) Allelic inheritance in naturally occurring parthenogenetic offspring of the gonochoric sea star *Patiria miniata*. *Invertebrate Biology* 128:276-282
- Szmant AM (1986) Reproductive ecology of Caribbean reef corals. *Coral Reefs* 5:43-53
- Szmant AM, Meadows MG (2006) Developmental changes in coral larval buoyancy and vertical swimming behavior: implications for dispersal and connectivity. *Proceedings of the 10th International Coral Reef Symposium* 1:431-437
- Thornton SL (1999) Depth Distribution, Growth Rates, and Photoacclimation of the Green and Brown Color Morphotypes of the Scleractinian Coral *Porites astreoides*. NSU Works
- Underwood JN, Smith LD, van Oppen MJ, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology* 16:771-784
- Van Veghel ML and Bak RP (1994) Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis* : Reproduction in damaged and regenerating colonies. *Marine Ecology Progress Series* 229-233
- Veron JE (2000) *Corals of the World*, vol. 1–3. Australian Institute of Marine Science, Townsville:404-405
- Wagner DE, Kramer P, Van Woesik R (2010) Species composition, habitat, and water quality influence coral bleaching in southern Florida. *Marine Ecology Progress Series* 408:65-78
- Walker M (2010) Snake gives ‘virgin birth’ to extraordinary babies. *BBC Earth News*
- Ward S (1992) Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. *Marine Biology* 112:641-646
- Warner D, Shine R (2008) The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* 451:566-568
- Warner PA, Willis BL, Oppen MJ (2016) Sperm dispersal distances estimated by parentage analysis in a brooding scleractinian coral. *Molecular Ecology* 25:1398-1415
- Wilkinson C (2008) *Status of coral reefs of the world*. Australia: Australian Institute of Marine Science

Willis B, Babcock R, Harrison PL, Wallace C (1997) Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. *Coral Reefs* 16:S53-S65