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Population Dynamics and Genotypic Richness of the Threatened Acropora spp. and their Hybrid in the U.S. Virgin Islands

Hannah F. Nylander-Asplin
Nova Southeastern University, hnylander16@gmail.com

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Hannah F. Nylander-Asplin

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Approved:
Thesis Committee

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

Committee Member: Ronald Hill, Ph.D.

Committee Member: Lisa Greer, Ph.D.

Committee Member: Bernhard Riegl, Ph.D.

HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

POPULATION DYNAMICS AND GENOTYPIC RICHNESS OF THE THREATENED *ACROPORA* SPP. AND THEIR HYBRID IN THE U.S. VIRGIN ISLANDS

By

Hannah F. Nylander-Asplin

Submitted to the Faculty of

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in partial fulfillment of the requirements for the degree of Master of Science with a specialty in:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Since the 1980's, there has been an unprecedented decline in the reef-building Caribbean corals, *Acropora cervicornis* and *A. palmata,* which has led to their listing as "threatened" under the U.S Endangered Species Act. Despite this protective status, these *Acropora* species continue to experience declines primarily attributed to disease, global climate change, and storm damage. Recent evidence suggests the hybrid of these threatened species (*A. prolifera*) is found at abundances similar to or higher than the parental species at many sites throughout the Caribbean. However, there is still much that is unknown as to how and why hybrids may be increasing in abundance at select sites.

In 2007, scientists from NOAA NMFS established 9 permanent transects at three sites in the USVI to quantify fish diversity and coral tissue condition in *A. cervicornis* thickets. Over the years, they observed that *A. prolifera* seemed to be increasing in abundance on transects that were once dominated by *A. cervicornis.* This dataset provided a unique opportunity to investigate whether a shift from a threatened parental species to its hybrid may have occurred. This study has two objectives, (1) to quantify the change in *A. cervicornis* and *A. prolifera* percent cover and colony health over a 9-year period, and (2) to compare the genotypic diversity among the three Caribbean acroporids on and near the transects to determine the primary method of propagation, i.e., sexual versus asexual. For this study, I used transect photographs taken in March, July and November 2009, April 2012, and August 2017 to compare intra- and interannual variation in acroporid cover and colony health.

Striking losses were observed in *A. cervicornis* cover between March 2009 and August 2017. At Thatch Cay, *A. cervicornis* declined from 25.7% to 8.9% between March 2009 and November 2009, but remained stable (10.2%) up to August 2017. *Acropora cervicornis* cover declined from 13.2% to 0% at Lovango Cay, and from 8.2% to 0% at No-Name Bay. At the one site (No-Name Bay) that *A. prolifera* was present during the original surveys of the transects, the percent cover remained relatively high and stable over the sample period. At No-Name Bay, *A. prolifera* percent cover (18.2%) was significantly higher than *A. cervicornis* (5.4%) by November 2009. It appears that *A. prolifera* expanded in the habitat left void by the decline in *A. cervicornis*. The general health of *A. cervicornis* based on the amount of healthy versus white and pale tissue appeared to decline at all sites between March 2009 and November 2009. To determine if the high percent cover on some transects was derived from asexual propagation or sexual recruitment, 139 tissue samples were collected in 2017 and genotyped using five microsatellite markers. No significant difference in genotypic richness (number of unique genotypes divided by the sample size) was observed among *A. cervicornis* (0.62), *A. prolifera* (0.64), and *A. palmata* (0.68). This suggests that the hybrid colonization is from multiple sexually derived individuals, not just asexual propagation from a rare hybridization event. High genotypic diversity, stable population abundance, and healthier colonies, suggest acroporid hybrids may become the primary habitat building coral of shallow reefs in the U.S. Virgin Islands. Due to considerable differences in morphologies between *A. cervicornis* and *A. prolifera*, it is unclear how a shift to the hybrid may affect the organisms that occupy acroporid structure and if the same ecological functions can be fulfilled.

Keywords: *Acropora cervicornis*, *Acropora palmata*, *Acropora prolifera*, hybridization, population structure, coral reefs

CHAPTER 1- INTRODUCTION

Hybridizing Systems in Nature

Hybridization occurs in every major phyla and has been observed in marine, freshwater and terrestrial environments (Stebbins 1959, Abbott 1992, Arnold et al. 1999, Arnold and Fogarty 2009). The impacts of hybridization remain unclear for most species complexes. Specifically, there are two contradictory outcomes of introgressive hybridization (i.e., gene flow between species via hybrids mating with one or both parental species): (1) increased genetic diversity via the introduction of unique genes or (2) reduced biodiversity through gene swamping and extinction (Rhymer and Simberloff 1996; Martinsen et al. 2001, Arnold 2006). The likelihood of these outcomes depends upon the direction and strength of selection, but changes in the environment or parental species abundances can greatly influence the extent of introgression. This makes threatened marine species that are subjected to changing ocean condition the most vulnerable. However, low levels of gene exchanges facilitated by introgression could benefit threatened species that are prone to inbreeding depression, or need to rapidly adapt to a new environment. The exchange of novel genes via introgressive hybridization has the potential to facilitate adaptation to climate change due to altered selection regimes (Anderson 1948, Traill et al. 2010, Chunco 2014). Climate change is expected to increase introgressive hybridization by breaking down spatial, temporal, and behavioral isolating barriers. Spatial isolation refers to a physical barrier that prevents two species from breeding. Hybrid zones can form if environmental conditions are conducive to the removal of specific habitat barriers (Palumbi 1994, Thomas et al. 2004). In marine systems, currents act as a major spatial barrier that dictates the movement of planktonic life stages and determines if gamete bundles from different populations mix (Veron 1995). Spatial barriers in marine systems are less rigid than terrestrial environments especially in benthic organisms that can inhabit a range of depths (Palumbi 1994, Hubbard 1988). Depth may not be a sufficient spatial barrier as gamete bundles released during spawning will float to the surface irrespective of depth. Therefore, isolation by distance may be weakest where populations are connected

through relatively small vertical and horizontal gradients (Palumbi 1994). Climate change, particularly an increase in temperature, can change surface currents, increase the severity of storms, and alter other spatial barriers such as latitudinal range boundaries (Hughes 1994, Hoegh-Guldberg and Bruno 2010). Temporal isolation can be an effective barrier to reproduction in both marine and terrestrial environments (Coyne and Orr 2004). Reproductive events, specifically in broadcast spawning species, can be widely influenced by temperature among other factors used as cues for gamete release and, if synchrony is disrupted, may subsequently increase the chances of hybridization (Van Oppen et al. 2002, Fukami et al. 2003, Levitan et al. 2011). Finally, behavioral isolation is known to impact hybridization in some species (Chunco 2014). Although behavioral isolation is less likely in adult benthic organisms, it has been well documented as an additional barrier to species where individuals can control their movements and reproductive partners (Burton and Feldman 1982, Avise et al. 1986).

If reproductive isolating barriers are removed or weakened, the likelihood for extensive introgressive hybridization and eventual reticulation, where species undergo repeated separation and fusing over evolutionary time, becomes more probable. In terrestrial environments, many plants species have been documented to undergo reticulate evolution, but it is less observed in animals and aquatic habitats (Arnold 1992, Arnold 2006, for exceptions see Arnold and Fogarty, 2009). However, introgressive hybridization and reticulate evolution have been documented in corals (Veron 1995, Hatta et al. 1999, Willis et al. 2006). *Acropora spp*. in the Indo-Pacific have the highest diversity of coral species in the world, with over 150 identified species (Wallace and Willis 1994), and the greatest evidence for hybridization and reticulate evolution (Veron 2000, Wallace 1999). Caribbean acroporids, on the other hand, are only composed of two parental species, *Acropora palmata* and *A. cervicornis* and their hybrid, *A. prolifera* (Lamarck 1816). The likelihood of reticulation in Caribbean acroporids remains controversial (Van Oppen et al. 2000, Vollmer and Palumbi 2002). In general, the evolutionary and ecological consequences of hybridization are broad, and the effect *A. prolifera* may have on their parental species and on Caribbean coral reefs has yet to be determined.

Caribbean Acroporids

Acroporid corals are primary reef-building species that provide a solid foundation for invertebrates, and provide habitat for numerous fish species (Gilmore and Hall 1976). The parental species occupy distinct habitat ranges, with *A. cervicornis* typically inhabiting the fore-reefs along intermediate depths up to 25 m, and *A. palmata* occupying reef crests and shallow habitats (Hubbard 1988). Both species have a branching morphology and are fast growing. *Acropora cervicornis* form characteristic thickets of cylindrical branches ranging from 2-7 cm across, up to 2 m long, with an apical polyp on each tip (Plater 2004). *Acropora palmata* are characterized by flattened branches that broaden toward their tips, and allow them to withstand intense wave action along the reef crest (Plater 2004). Annual linear extension can exceed 71 mm/yr in *A. cervicornis* and 47-99 mm/yr in *A. palmata* (Gladfelter et al. 1978, Bak et al. 2009). The quick expansion and unique branching structures of *A. cervicornis* and *A. palmata* contribute to high rugosity and structural complexity in shallow reefs, and thus are considered an irreplaceable taxa (Friedlander and Parrish 1998, Bruckner 2002).

Unlike the *A. cervicornis* and *A. palmata*, which are found in the fossil records between 6-7 million years ago, *A. prolifera* is not found consistently in the fossil record and appeared recently in the Holocene, 11,500 years ago to present (Budd and Johnson 1999, McNeill et al. 1997). The hybrid displays intermediate morphology between *A. cervicornis* and *A. palmata* (Fig. 1C, D) and is found in marginal or intermediate habitats to that of the parental species (Plater 2004, Van Oppen et al. 2000, Fogarty 2010). Morphological difference between populations of *A. prolifera* can vary, displaying characteristics more similar to *A. cervicornis* or *A. palmata*. Most noted among these variations are fused branches at the apical tips of each arm (Fig 1D). *Acropora prolifera* was confirmed to be an F1 hybrid after molecular analysis revealed that all sampled individuals were heterozygous at three nuclear loci, indicative of a first generation hybrid (Van Oppen et al. 2000, Vollmer and Palumbi 2002).

Although *A. cervicornis* and *A. palmata* are considered primary reef building species in the Caribbean, they have undergone recent and extensive declines. In 1981, the parental species accounted for approximately 97% of coral cover on shallow Caribbean

reefs (Wells and Hanna 1992). An outbreak of white band disease (WBD) reduced these populations to just 3% cover in some areas of the Caribbean (Aronson and Precht 2001). Rapid tissue loss (RTL) is a similar affliction that has symptoms analogous to WBD (Williams and Miller 2005), but is uniquely characterized by rapid expansion (up to 4 cm per day) and irregular tissue margins followed immediately by skeleton denuded of tissue (Miller et al. 2014). In addition to changes in ocean-wide conditions such as increased sea surface temperature and reduced pH, local anthropogenic stressors including pollution, overfishing, high sediment runoff, and algal growth due to excess nutrients have also contributed to a reduction in overall coral cover. (Carpenter et al. 2008, Jackson and Sala 2001, Smith and Buddemeier 1992). Due to these unprecedented losses and continued threats, the Caribbean acroporids were listed as "threatened" under the Endangered Species Act in 2006, (Williams and Miller 2005, Hogarth 2006) and as "critically endangered" on the International Union for Conservation of Nature (IUCN) Red List in 2008 (Aronson et al. 2008). The loss of the reef architecture that *A. palmata* and *A. cervicornis* historically provided has had devastating effects on the organisms that once inhabit their abundant thickets. It remains unclear if their hybrid, which appears to have recently increased at some sites in the Caribbean (Fogarty 2010), call fill the same ecological function of the parental species.

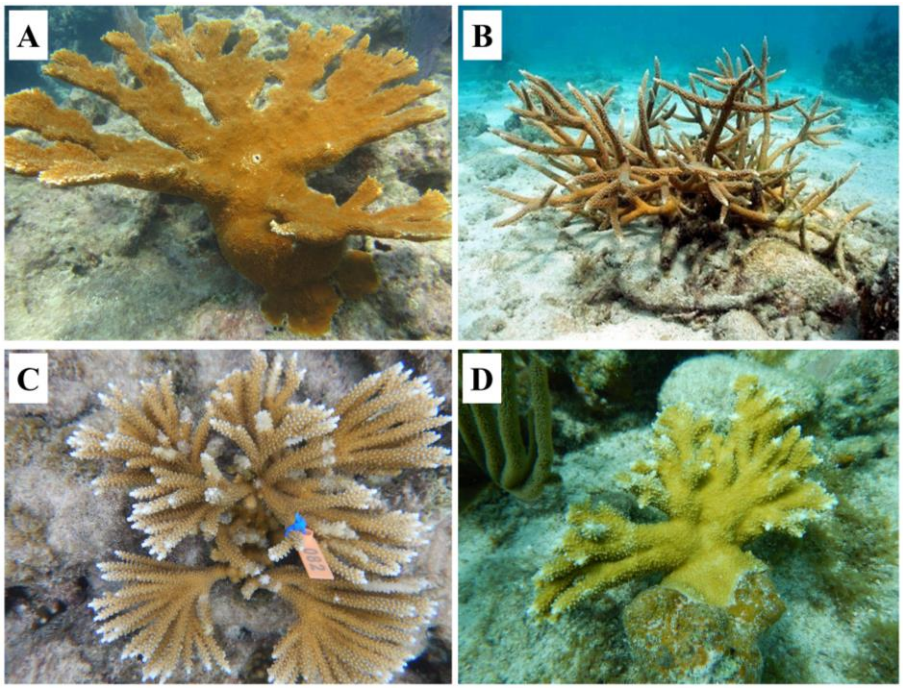

Figure 1: Morphological differences between *Acropora palmata* (A), *A. cervicornis* (B) and *A. prolifera* (C, D). The hybrid shows intermediate morphology between the parental species. (photo credit A: http://coralpedia.bio.warwick.ac.uk B: FWC.)

Acropora cervicornis and *A. palmata* can reproduce both sexually and asexually (Bothwell 1981, Wallace 1985). During asexual reproduction, broken branches or individual polyps can reattach to a suitable substrate and grow. These fragmented individuals are genetic clones of the original colony. Alternatively, sexual reproduction is achieved via broadcast spawning (Szmant 1986), in which large quantities of egg and sperm bundles are released into the water column for external fertilization, which creates a unique opportunity for hybridization. While many broadcast spawning species have pre-zygotic mechanisms to maintain reproductive isolation, Caribbean acroporids have been shown to have weak pre-zygotic barriers and are therefore uniquely susceptible to hybridization (Fogarty et al. 2012). For example, Caribbean acroporids synchronously release gametes 2 to 6 days after the full moon in July, August, and September, so the prezygotic barrier of asynchronous gamete release is unlikely (Szmant 1986, Fogarty et al. 2012, Jordan 2018). Additionally, choice (where both species of sperm compete) and no-choice (where each species is crossed in the absence of sperm competition) fertilization crosses concluded that *A. cervicornis* and *A. palmata* eggs are compatible with conspecific

and heterospecific sperm, further supporting weak pre-zygotic barriers in Caribbean acroporids (Fogarty et al. 2012).

Along with biological and environmental factors that affect fertilization success, the density of parental species is crucial for successful reproduction (Levitan and McGovern 2005). Due to the drastic and consistent losses in *A. cervicornis* and *A. palmata*, density dependent pre-zygotic barriers may explain the recent increases in hybrid cover at some locations (Fogarty et al. 2012). When populations of the parental species were abundant, it was highly likely that eggs were fertilized by sperm from nearby conspecifics, thus reducing the likelihood of hybridization (Fogarty et al. 2012). As populations of *A. cervicornis* and *A. palmata* declined due to WBD, the prevalence of nearby conspecifics decreased, and thus the likelihood of a hybrid embryo formation may have increased (Fogarty et al. 2012). This mechanism may explain increased hybridization at some sites particularly where hybrids have been recently observed (Fogarty et al. 2012). However, a recent study using somatic mutation to age genets suggests that some *A. prolifera* clones range from 156–281 years old (Irwin et al. 2017), suggesting that the hybrid expansion may also be linked to asexual propagation (Fogarty 2010), and possible hybrid vigor (Fogarty et al. 2012).

Potential Impacts of Hybridization

According to mitochondrial sequence data from Vollmer and Palumbi (2002), the hybrid can be produced from both *A. cervicornis* and *A. palmata* eggs. Unidirectional introgression also occurs with genes flowing from *A. palmata* into *A. cervicornis* (Van Oppen et al. 2000, Vollmer and Palumbi 2002). This one-way introgression suggests that the hybrid is only capable of backcrossing with *A. cervicornis*. However, recent data (Baums et al. in prep) demonstrate conflicting results with introgression occurring from *A. cervicornis* into *A. palmata*. Further studies are needed to reconcile these findings and determine the potential direction of gene flow between the two parental species and their hybrid.

Introgressive hybridization can have contradictory impacts on the parental species (i.e., facilitate advantageous adaptations through limited gene flow or reduce fitness

through outbreed depression and genetic swamping). Introgression can generate novel genotypes that may promote the colonization of new or previously unoccupied habitats (Lewontin and Birch 1966, Willis et al. 2006, Van Oppen and Gates 2006) or facilitate rapid adaptations to climate change and other environmental stressors, such as disease (Baums 2008, Willis et al. 2006). Alternatively, hybridization has the potential to threaten the long-term survival of the parental species through outbreeding depression or genetic swamping, which may contribute to extinction of the parental species (Rhymer and Simberloff 1996, Frankham et al. 2002, Levin 2002). Hybridization, as a means of genetic rescue, is an important concept to investigate as climate change continues to pressure these fragile ecosystems (Willis et al. 2006). In general, the evolutionary consequences of hybridization are broad, and it has yet to be determined what effect *A. prolifera* may have on the evolutionary and ecological trajectory of Caribbean coral reefs.

Recent evidence suggests that the hybrid is increasing in abundance and expanding into parental zones at various sites throughout the Caribbean (Fogarty 2010). Although observations of hybrid range expansion exist, there is no quantitative documentation of the hybrid replacing parental species in habitats left void after recent and unprecedented losses in acroporid abundance. The lack of recovery of *A. cervicornis* could be due to its asexual reproductive habits and lack of sexual recruitment (Tunnicliffe 1981, Highsmith 1982, Bak and Engel 1979). In order to successfully repopulate an area, larvae must recruit to increase the genetic diversity (Vollmer and Palumbi 2007). Additionally, it would be expected that if the hybrid is propagating asexually via fragmentation, the genetic diversity would be low. Results from Fogarty (2010), indicate that hybrid genetic diversity varies among locations, but in general, is comparable to the parental species. It is crucial to distinguish how hybrids, such as *A. prolifera*, can recruit, compete, and persist to enhance our understanding of coral resistance and adaptations to environmental stressors. It is imperative to further investigate the ecological potential of *A. prolifera* to understand if the hybrid can fill the same crucial ecological role of the parent and determine the fate of shallow coral reefs in the Caribbean.

Objectives

Acroporid hybridization may have key ecological and evolutionary consequences for Caribbean coral reefs, yet if the hybrid can provide an ecological replacement for one or both parental species is unclear. This study aims to document the transition from *A. cervicornis* to the hybrid by quantifying long-term photographic data. Additionally, I will determine whether this transition is the result of a rare hybrid recruitment event that asexually propagated or of multiple-hybrid colonization events. The objectives for this research are:

1. To quantify the abundance and assess the tissue condition of *A. cervicornis* and *A. prolifera* using photographs and mosaic methods on nine NOAA transects from 2009-2017.

2. To compare the genotypic richness of *A. prolifera*, *A. cervicornis*, and *A. palmata* at these sites to determine whether hybrid populations are derived primarily from sexual or asexual propagation.

CHAPTER 2

INTRODUCTION

Coral reefs have experienced unprecedented declines in diversity and total cover worldwide as a result of a multitude of biological and anthropogenic stressors (Hughes and Tanner 2000, Gardner et al. 2003, Côté et al. 2005). Globally, reefs have experienced declines or degradation, with over one-third of scleractinian corals are at risk of extinction from climate change and local stressors (Carpenter et al. 2008, Jackson et al. 2014). In just eight months in 2016, upwards of 50.3% of coral cover was lost along a 700 km-long section of the Great Barrier Reef (Hughes et al. 2018). The recent declines in coral reefs have global implications, as reefs provide sustenance for hundreds of millions of people, protect shorelines from storms, and support over $30 billion via ecotourism and other goods and services (Moberg and Folke 1999, Cesar et al. 2003). These declines can be attributed to global (e.g., ocean acidification, increased temperature) and local (e.g., pollution, storm damage, over-fishing, disease outbreaks) factors (Hoegh-Guldberg et al. 2007, Hoegh-Guldberg and Bruno 2010). Caribbean coral reefs have experienced some of the greatest declines, with total coral cover being reduced from 35% to 10% in only four decades (Gardner et al. 2003, Jackson et al. 2014).

Among the Caribbean corals with the greatest decline are the *Acropora* spp. In the 1980's, white band disease (WBD) led to drastic declines in Caribbean *Acropora cervicornis* (staghorn) and *A. palmata* (elkhorn) corals, reducing percent cover by up to 97% in some locations (Gladfelter 1982, Wells and Hanna 1992, Aronson and Precht 2001, Gignoux-Wolfsohn et al. 2012, Randall and Van Woesik 2015). These losses are devastating to shallow water coral reefs because Caribbean acroporids are considered irreplaceable due to their rapid growth rates and ability to create unique 3-D structures that contribute to reef rugosity (Gladfelter et al. 1978, Brock et al. 2004).

Despite these declines, the naturally occurring hybrid, *Acropora prolifera*, appears to be increasing in abundance and is found at equal or higher abundances than the parental species at some sites throughout the Caribbean. (Fogarty 2010, 2012, Japaud et al. 2014).

The parental species can be found reliably in the fossil record throughout the Holocene, Pleistocene, and even the Pliocene (up to 6 mya) (Budd and Johnson 1999), but *A. prolifera* has no reliable fossil record (Budd et al. 1999). It has been hypothesized that the declines in parental species and weak pre-and post-zygotic barriers have contributed to the recent increase in hybrid abundance at some sites (Fogarty et al. 2012, Fogarty 2010).

Acropora cervicornis and *A. palmata* are broadcast spawning corals with overlapping spawning times and compatible gametes, albeit *A. cervicornis* eggs are more likely to hybridize than *A. palmata* eggs (Fogarty et al. 2012). As a result of the declines in the parental population densities, it is likely that eggs float unfertilized for extended periods of time. Because of a lack of prezygotic isolating barriers especially in *A. cervicornis*, it is likely that whichever species' sperm the egg encounters will fertilize it, therefore increasing the probability of hybrid embryo formation. Historically when adult densities were high, eggs were likely immediately swamped by conspecific sperm, decreasing the probability of hybrid embryo formation(Fogarty et al. 2012). This density-dependent reproductive isolation may be the reason for an increase in hybridization in recent years at some sites (Fogarty et al. 2012, Japaud et al. 2014).

Populations with asymmetric loss where *A. cervicornis* densities are much lower relative to *A. palmata* are perhaps the most vulnerable to hybridization (Fogarty 2010). Asymmetric losses of parental species have led to increased hybridization in other systems. The process of asymmetric parental populations leading to hybridization through gene flow, known as the desperate hypothesis (Hubbs 1955) was first described in several Centrarchidae fish species and has been extensively documented in waterfowl [Anatidae (McCracken and Wilson 2011)]. Hybridization and the expansion of hybrids into parental zones in particular, has also been documented in several other systems including cord grass [*Spartina* spp. (Ayres et al. 2004)], rusty crayfish [*Orconectes rusticus* (Perry et al. 2001)], western sunflowers [*Helianthus anomalus* (Heiser et al. 1969)], and pupfish [*Cyprinodon pecosensis, C. varigatus* (Rosenfield et al. 2004)]. Therefore, it is plausible that a similar scenario may be occurring in Caribbean acroporids as well (Allendorf et al. 2001, Rosenfield et al. 2004). Not only does *A. prolifera* appear to be increasing at select Caribbean sites (Aguilar-Perera and Hernández-Landa 2017, Japaud et al. 2014, Lucas and Weil 2016), there is evidence of the hybrid co-occurring (Figure 1) with the parental

species [Figure 1 (Fogarty 2010, 2012)] . However, no quantitative documentation of this co-occurrence, hybrid expansion or parental species replacement on a long-term basis currently exists.

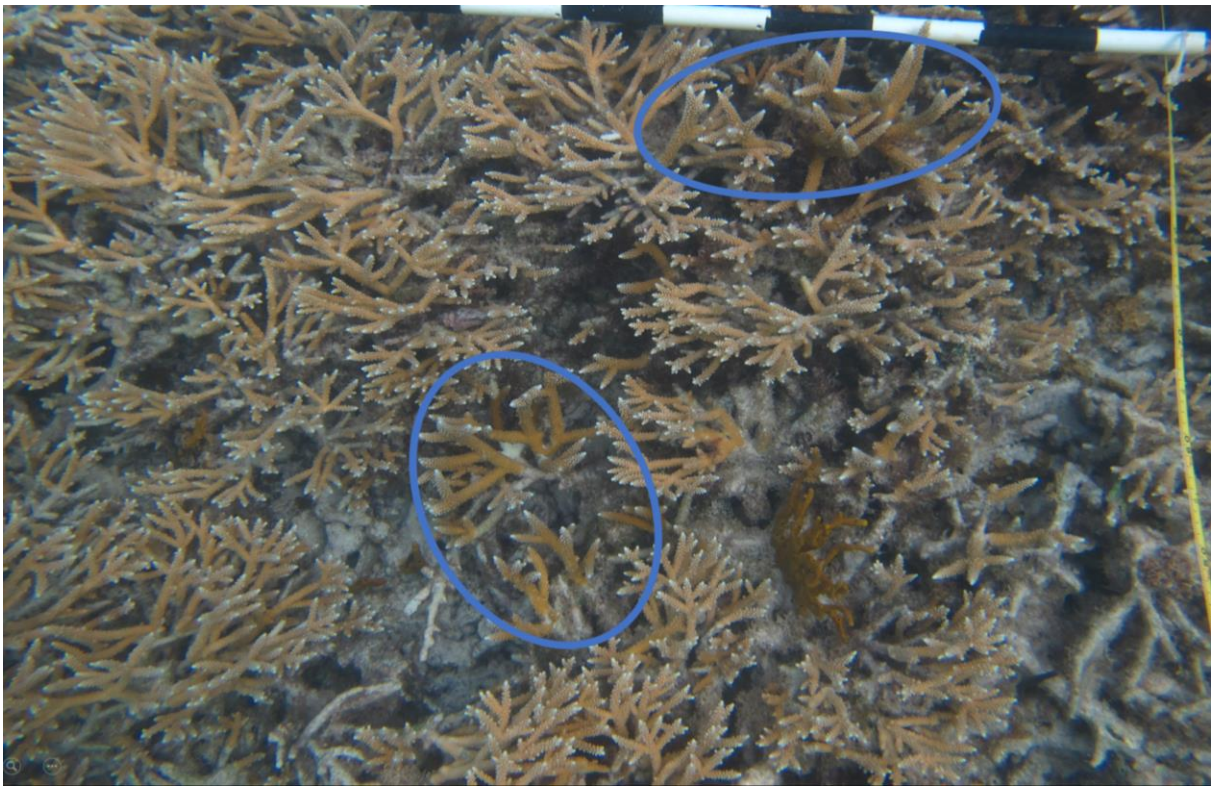

Figure 1. *Acropora cervicornis* (blue) co-occurring with the hybrid (*A. prolifera*) at No-Name Bay in the U.S. Virgin Islands in April 2012.

Hybridization can have critical evolutionary and ecological impacts on the parental species. Paradoxically, hybridization can cause the extinction of the parents through outbreed depression and genetic swamping or provide novel genetic variation into the population that provides a rapid avenue for adaptation, saving the species from extinction (Rhymer and Simberloff 1996, Allendorf et al. 2001, Chunco 2014). If selection pressures are high, beneficial mutations can spread across a population, thus allowing the population to persist (Rieseberg and Burke 2001). In the Caribbean, unidirectional gene flow from *A. palmata* into *A. cervicornis* has been documented (Vollmer and Palumbi 2002), although conflicting evidence, showing gene flow primarily into *A. palmata*, now also exists (Baum unpubl. data). The ecological effect of acroporid hybridization is still unknown,

particularly if the hybrid could potentially replace the ecological service of one or both parental species. Vigorous hybrids have the potential to serve as ecosystem engineers and effectively outcompete the parental species for habitat and resources. Additional anthropogenic stressors and habitat degradation that leads to the decline of the parental species can support further colonization by the hybrid if it exhibits a higher fitness relative to the parental species (Allendorf et al. 2001). In Caribbean acroporids, the ability of the hybrid to fill similar ecological functions of the parental species could hinge partly on the differences in the branching structure of the colonies. The hybrid appears to show extensive phenotypic plasticity, where the density of the skeleton and the shape of the colony itself is related to the environment (Vollmer and Palumbi 2002, Japaud et al. 2014, Aguilar-Perera and Hernández-Landa 2017). *Acropora palmata* has been observed hosting significantly higher fish populations, including grunts, snappers and damselfish, compared to an area with other coral species, including *A. cervicornis* (Lirman 1999). Although the hybrid has been observed inhabiting similar geographical ranges, the likelihood of it providing the same spatial niche as the parental species remains unclear.

Since 2007, scientists from the National Oceanic and Atmospheric Administration National Marine Fisheries Service (NOAA NMFS) have conducted annual surveys at three sites in the U.S. Virgin Islands where both parental species and the hybrid are present. Using these long-term monitoring sites, the main goals of this research are to 1) quantify the abundance and assess the general tissue condition of *A. cervicornis* and *A. prolifera* at Thatch Cay, Lovango Cay, and No-Name Bay in the US Virgin Islands using long-term monitored photo-transects between 2009-2017, and 2) compare the genotypic richness of *A. prolifera*, *A. cervicornis*, and *A. palmata* to determine whether hybrid populations are derived from sexual recruitment or asexual propagation at these sites.

MATERIALS AND METHODS

NOAA Permanent Transects in the U.S Virgin Islands

Long-term transects established by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA NMFS) have been used to document *A. cervicornis* rich habitats 1-3 times annually at Thatch Cay, St. Thomas and Lovango Cay, and No-Name Bay in St. John, U.S. Virgin Islands (Fig. 2). A total of nine permanent transects (10 x 2m) were established near *A. cervicornis* thickets using permanent steel posts. During each survey, photographs were taken every 1m along both sides of the transect. Therefore, each transect was comprised of 20 photographs. A 1-m PVC stick placed perpendicularly to the transect tape was used to provide a known length to aid in photo analysis. Transect surveys included a visual fish census, a traditional point intercept survey of benthic cover, and an estimation of colony dimension (e.g., to estimate volume). Additionally, environmental parameters including temperature, were recorded during the survey (Hill and Doerr 2009). Long-term temperature data in 2009 was analyzed to determine interannual variations. Only temperature data with multiple sampling efforts per year was included. For the purposes of this study, only the photographs are used to quantify percent live coral cover and tissue condition.

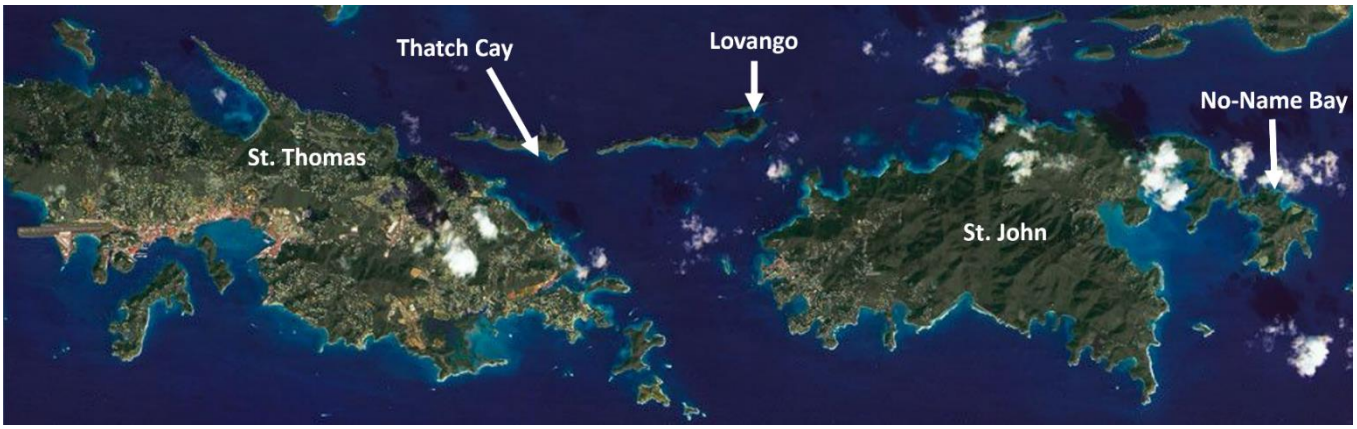

Figure 2: Study location in the U.S. Virgin Islands. Study sites include Thatch Cay (4 transects), Lovango Cay (2 transects), and No-Name Bay (3 transects).

Percent Live Coral Cover Analysis

Photographs (n=900) were analyzed from surveys conducted in 2009 (March, July, November), 2012 (April), 2017 (August), and 2018 (July). Analysis methods using Matlab and Adobe Illustrator (developed by L. Greer and colleagues) were used to quantify the percent cover of each square meter along either side of each transect (Yang et al. 2009). Each transect photo was scaled and rectified using MatLab R2017b. Live tissue (healthy, pale, or white in color) was traced using a brush tool (size 3, black, transparency 0%) using Adobe Illustrator 2017 software. The traced image was then overlaid on a white background to isolate live tissue from skeleton, algae, and other benthic cover. The composite black and white image was analyzed using MatLab R2017b to quantify the percentage of total coral tissue cover (Fig. 3). The data were analyzed with a particular concentration on variations within and between sites to determine if similar changes in coral cover was occurring. Similarly, the differences in live coral cover were compared between the sampling periods. In 2009, transect surveys were conducted three times, thus providing interannual variation data as well.

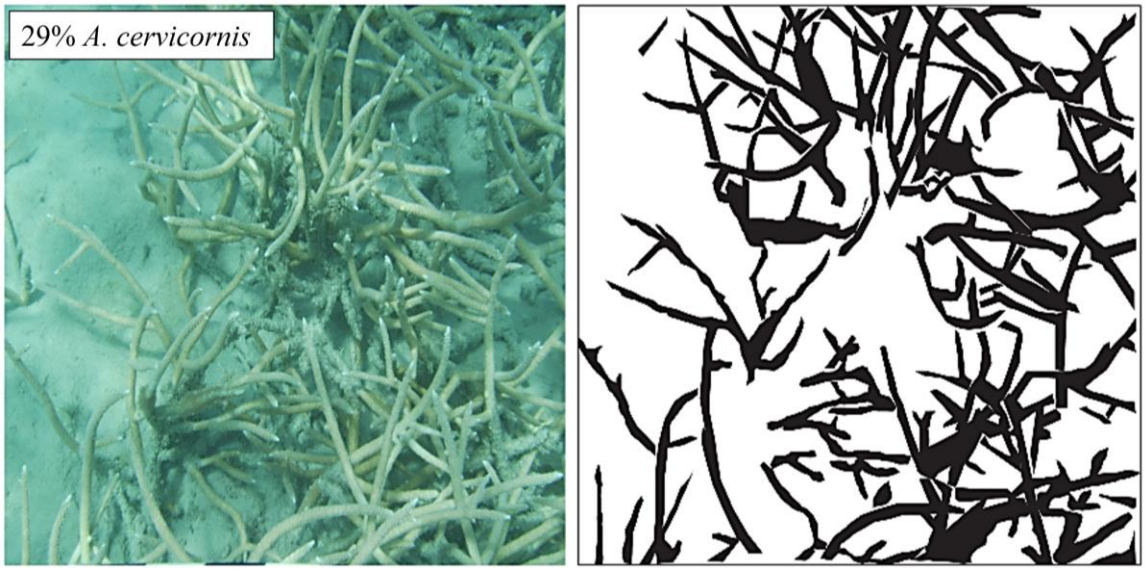

Figure 3: Example of a standardized transect photograph (left) and the completed outline (right). This image of *A. cervicornis* colonies from Thatch Cay represents 29% coral cover including healthy and pale tissue.

Tissue Condition Analysis

The condition of the tissue on each colony was analyzed similar to percent cover. Using post-standardized photos from the initial analysis, areas of coral colonies that were pale or white were isolated and quantified separately. Tissue that was not dark relative to other colonies was identified as pale. To avoid bias in identifying pale or healthy tissue, the same individual quantified all photographs for the tissue analysis. It was impossible to determine the cause of the white areas (i.e., bleached or denuded skeleton) from the photographs, as they could have been caused by disease, bleaching, or predation events. Therefore, any tissue identified as 'white' was excluded from the total percent cover analysis. Once the tissue condition was isolated, it was quantified using a MatLab script to determine the percent cover of each tissue type (i.e., healthy, pale, white).

Tissue Sampling

During August 3-6, 2017, 1 cm tissue samples were collected from the apical tips of *A. cervicornis* (n=50), *A. palmata* (n=40), and *A. prolifera* (n=39) at all three locations along the NOAA NMFS transects (Table 1). Once samples on the transects were collected, additional colonies from adjacent areas were selected haphazardly at each site to standardize sample size and distribution as much as possible (Fig. 4). Due to high abundances of *A. prolifera* at No-Name Bay, the majority of hybrid samples were collected at that site. *Acropora palmata* colony were sampled haphazardly at all sites due to variations in population size. Tissue samples were preserved in 96% molecular grade ethanol and stored at -20°C until extraction.

Table 1: Sampling distribution across sites.

	Thatch Cay	Lovango Cay	No-Name Bay	**Total**
A. cervicornis	22	12	16	50
A. prolifera	8	6	25	39
A. palmata	17	12	11	40
Total	**47**	**30**	**52**	**129**

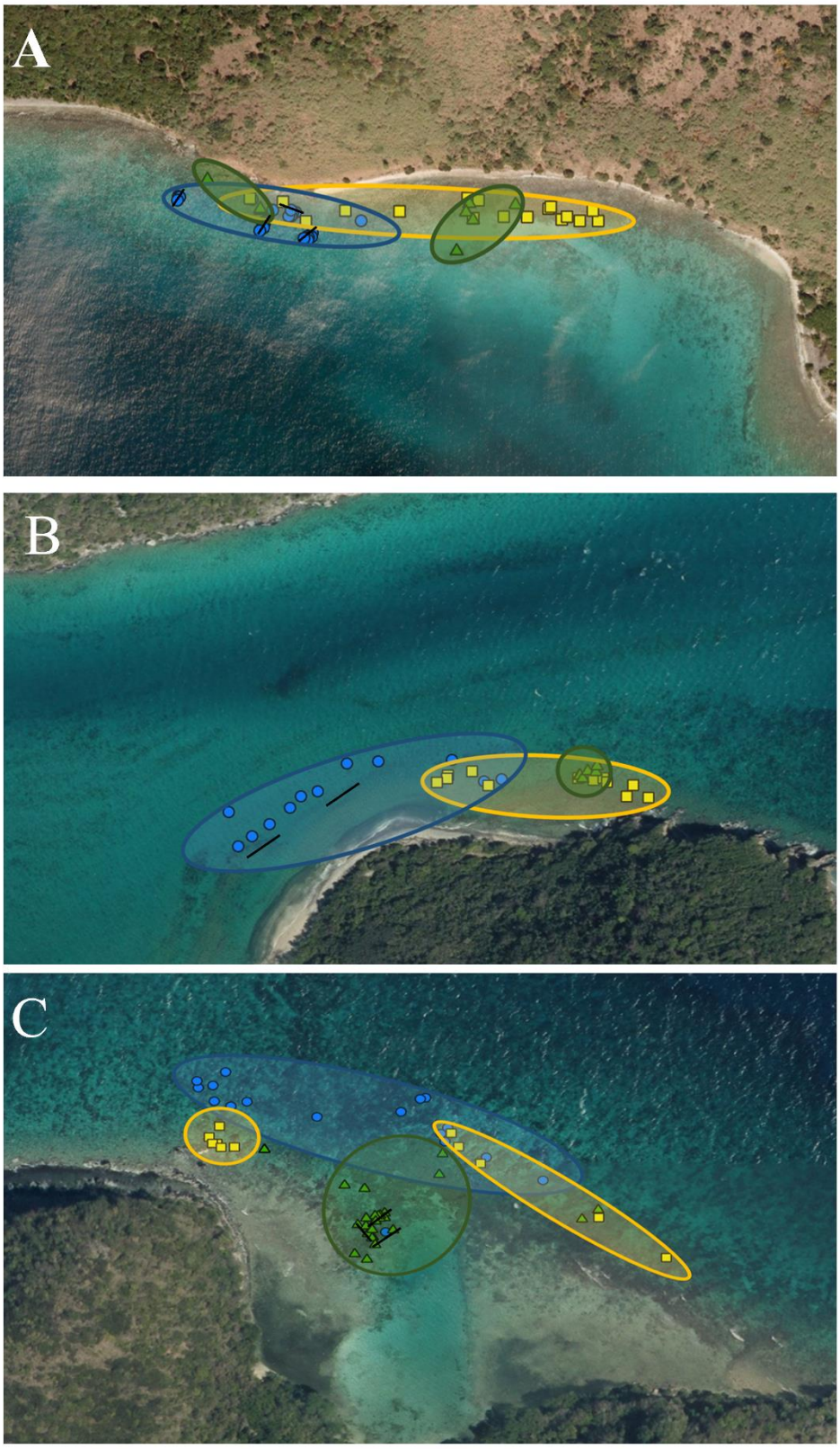

Figure 4: Sampling locations at Thatch Cay (A), Lovango Cay (B) and No-Name Bay (C) in the U.S. Virgin Islands in August 2017. Shaded areas represent locations where *A. cervicornis* (blue), *A. palmata (yellow),* and the hybrid, *A. prolifera* (green), were most prevalent. Black lines delineate permanent transects.

Genetic Analysis

Samples were genotyped using microsatellites developed by Baums et al. (2009), and protocols slightly modified by Fogarty et al. (2012). Tissue samples were transferred to CHAOS (4M guanidine thicyanate 0.1% N-lauroyl sarcosine sodium, 23 mM Tris pH 8, 0.1M 2-mercaptoethanol, ultra-pure water) for tissue digestion for 3-5 days prior to extraction. DNA was then extracted using a SprintPrep DNA Purification kit, magnetic bead-based protocol (Beckman Coulter Genomics/Agencourt Bioscience Corporation). For each sample, 50 µl of tissue was mixed with10 µl of Agencourt AMPure XP (magnetic beads), and 80 µl of 100% isopropyl. After mixing, the deep well plate was affixed to a magnetic plate for 10 minutes, and drained by inverting. Once drained, a sequence of 5 rinses were performed using 200 µl of cold 70% ethanol and dried for 1 hour. When the beads were observed to be dried and cracked, 50 µl of 1X TE buffer was added to each sample and placed on a shaker plate for 60 minutes, rotating 90 degrees each 15 minutes. Finally, the supernatant was pipetted from each well after an additional 15 minutes on the magnetic plate. DNA was quantified using a microplate spectrophotometer (ThermoFischer Scientific).

The extracted DNA was PCR amplified using 5 microsatellite primers [loci 166, 181, 187, 182, 207 (Baums et al. 2009)]. Per modified protocols in Fogarty (2010) and Fogarty (2012), each microsatellite primer was PCR separately using 5X PCR buffer, 2.75 mM of $MgCl_2$, 0.8 mM of dNTPs and 0.5 µl of Taq polymerase. The annealing temperature was loci-specific, with an initial denaturation step of 94°C for 3 minutes, followed by 35 cycles of 94°C for 20 sec, either 55°C (for primer 207), 56°C (for primer 182) or 59°C (for primer 166, 181 and 187) for 20 seconds, and 72°C for 30 seconds, followed by a final extension of 72°C for 30 minutes.

PCR products were then multiplexed in two combinations with primers 166,181, and 187 in a single multiplex, and primers 182 and 207 in another. The multiplex was completed using 12.5µl HiDI Foramide (1:12) and 0.5µl of an internal size standard, Rox 400x (Applied Biosystems, Foster City, CA). Samples were sent to Florida State University Sequencing Facility for fragment analysis. Any samples that were not successfully amplified were re-run individually. Samples were then binned and analyzed using GeneMapper5 software. Finally, Microchecker 2.3.3 was used to isolate stutter peaks,

allele dropout and null alleles, if present. Genotypic richness (the total number of unique genotypes divided by the total number of samples) was calculated for each site.

Statistical Analysis

Statistical analysis was conducted using R 3.5.1 Statistical Software. Coral cover, general health assessments and genotypic diversity was tested for normality (Shapiro-Wilks test) and homogeneity of variances (Bartlett's test). Log transformations were used to normalize genotypic richness. Once parametric assumptions were met, significance was tested using a t-test or an analysis of variance (ANOVA, one-way). A simple linear regression was used to determine if time was a reliable predictor of coral cover, such that as time continues, the amount of coral tissue increased or decreased.

RESULTS

Acroporid Distribution

Using long term photographs, the change in percent coral cover was analyzed at all sites. The initial site selection and transect setup was specific for *A. cervicornis*, therefore *A. palmata* was not included in the coral cover analysis. Thatch Cay (transects 1-4) and Lovango Cay (transects 5-6) contained solely *A. cervicornis* on the permanent transect. No-Name Bay had both *A. cervicornis* and *A. prolifera* within the permanent transects in 2009. It remains unclear if *A. prolifera* colonies inhabited the transects at No-Name Bay prior to 2009, or if *A. prolifera* colonies at Thatch Cay and Lovango Cay were present prior to sampling in 2017.

Intra-site Variation

At Thatch Cay, the amount of paling tissue increased between July 2009 (2.1%) and November 2009 (7.3%) [Fig. 5 (t-test, p=0.002, t-stat=4.2)]. The relative about of healthy tissue also declined as the total percent cover decreased. Specifically, transect 3 was observed to have a reduction from 13.6% healthy tissue in July to 7.5% in November

2009 (t-test, p=0.02, t-stat=3.18). These results seem to suggest that an increase of pale and white tissue may be an indication of the losses in total cover seen during future surveys.

From November 2009 to August 2017 at Thatch Cay (the only site that was not surveyed in 2012), *A. cervicornis* showed significant losses along transects 1-3 from 25.7% to 10.1% (ANOVA, p=0.01), while transect 4 showed an increase in coral cover from 6.3% to 16.3% (ANOVA, p=0.002; Fig. 4). The intra-site variation in coral cover at Thatch may be due to the location and depth of the transects. Transect 4 was slightly deeper (3.9-4.6m) than transect 1-3 (2-2.5m). It is possible that storms moved *A. cervicornis* colonies towards Transect 4 or the deeper colonies were protected from UV radiation and thermal stress, thus increasing their survival and/or growth.

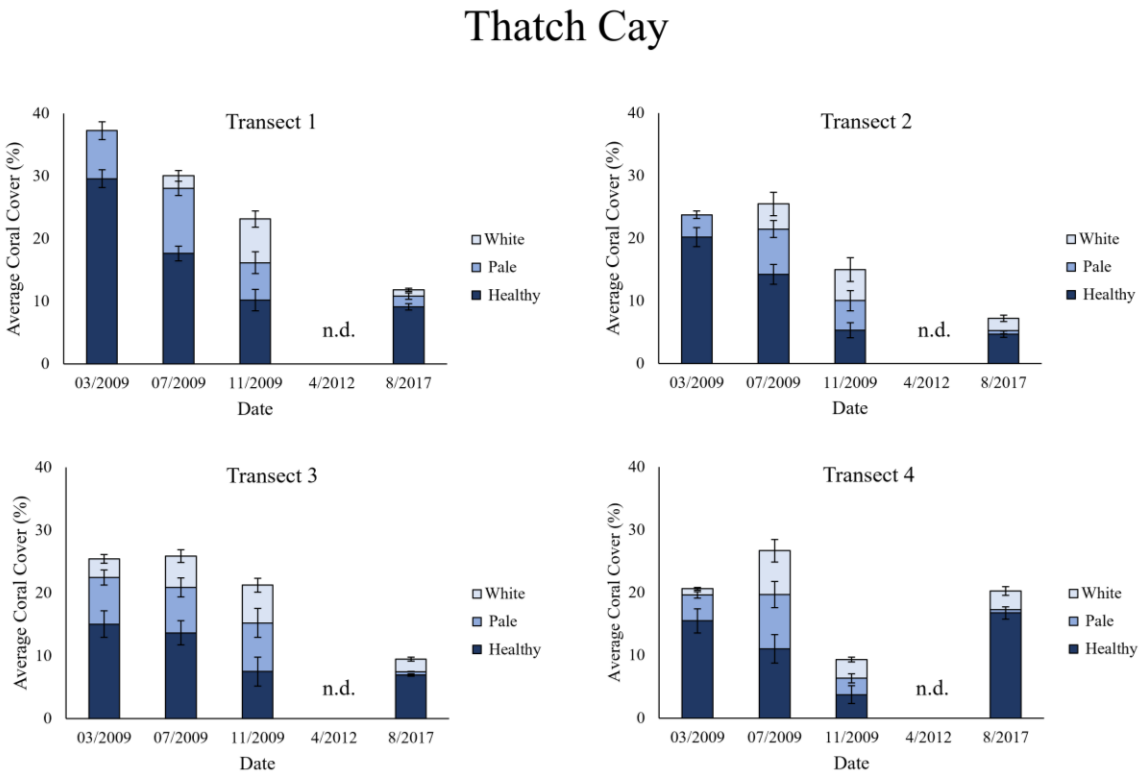

Figure 5: Average percent coral cover and general health of *A. cervicornis* colonies at Thatch Cay between 2009 and 2017 ±SE. Dark blue represents visually healthy tissue, intermediate blue characterizes pale tissue, and light blue represents white tissue or skeleton. Surveys were not conducted at this site in 2012.

Lovango Cay experienced similar declines in *A. cervicornis* to Thatch Cay. Transects 5 and 6 both experienced significant loses between 2009 and 2017, with no live coral cover observed in 2017 (Linear regression, p-value=0.001; Figure 6). There was a significant loss of *A. cervicornis* cover on Transect 5 between November 2009 and April 2012 (t-test, p=0.01). Transect 6 was observed to have a significant loss of *A. cervicornis* tissue (healthy and pale) between March and July 2009 (ANOVA, p=0.02), but retained a similar amount of total tissue between July 2009 and April 2012. During sampling in 2017, no *A. cervicornis* colonies were observed on either transect at Lovango Cay, although all three taxa were observed at locations down-current of the transects (Figure 4).

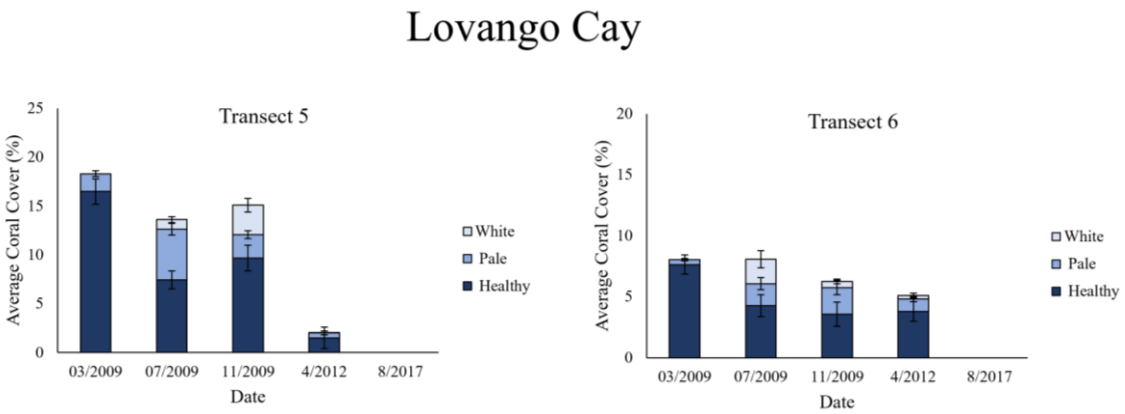

Figure 6: Average coral cover ±SE of *A. cervicornis* at Lovango Cay between 2009 and 2017. No live colonies were present in 2012 and 2017 Dark colors denote healthy tissue, intermediate color characterized pale tissue and light colors designate white tissue or skeleton recently denuded of tissue. There was no live *A. cervicornis* on either transect in 2017.

Through 2009, the percent cover and general tissue condition of *A. cervicornis* at Lovango Cay was observed to decline steadily, with a complete loss of tissue by 2017. The amount of pale tissue on transect 5 increased significantly between March 2009 and November 2009 (ANOVA, p=0.001). Transect 5 experienced an increase in white tissue or denuded skeleton from 1.8% in March 2009 to 3.1% in July 2009 (t-test, p=0.03).

Acropora cervicornis steadily decreased on all three transects within No-Name Bay, but the hybrid remained fairly stable (Fig. 7). The hybrid was significantly more

abundant than *A. cervicornis* in transect 8 for all sampling timepoints and maintainted a relative abundance around 20% cover (paired t-test, p = <0.01, t-stat=6.19). By 2017, transect 7 was void of *A. cervicornis*, but *A. prolifera* persisted. Transect 7 experienced a significant increase in *A. prolifera* tissue cover between March (7.9%) and July 2009 [15.1% (t-test, p=<0.01, t-stat=4.49)] but decreased significantly by August 2017 [6.8% (t-test, p=0.01, t-stat=4.33)]. From July 2009 to 2017, *A. prolifera* remained relatively stable on transects 8 and 9 (ANOVA, p=0.96).

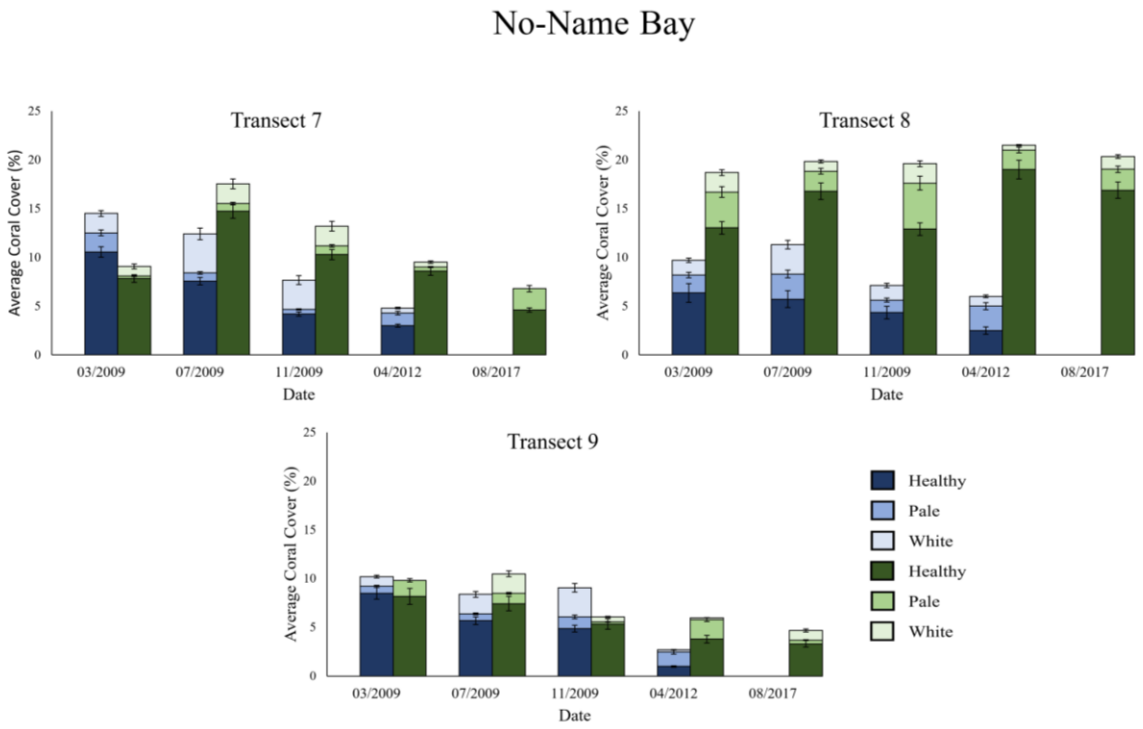

Figure 7: Average coral cover of *A. cervicornis* (blue) and *A. prolifera* (green) at No-Name Bay between 2009 and 2017 ±SE. The dark shade denotes healthy tissue, intermediate colors characterize pale tissue, and light colors designate white tissue.

Inter-site Variation

Among the three sites, the highest total live coral cover was at Thatch Cay, with an average *A. cervicornis* percent cover (healthy and pale tissue) of 27.6% in March 2009. Generally, *A. cervicornis* was observed to decline at all sites between 2009 and 2017, with a complete loss at Lovango Cay and No-Name Bay by 2017 (Fig. 8). Hybrid

populations remained relatively stable throughout the sampling period (ANOVA, p=0.96).

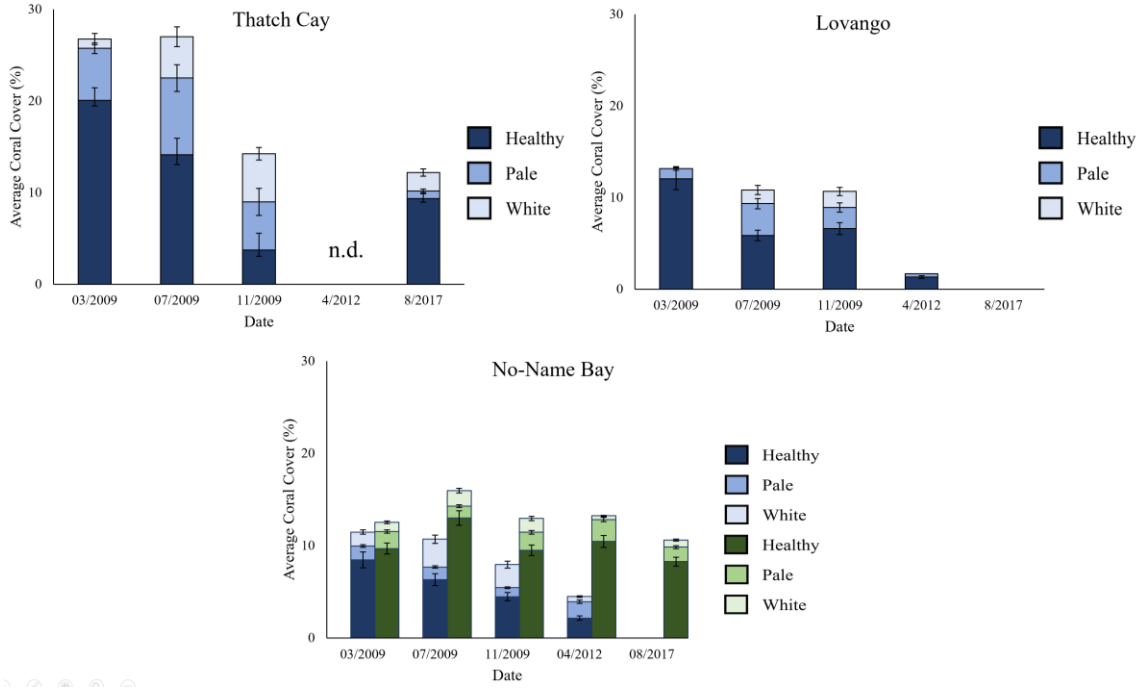

Figure 8: Average percent cover for each site to compare inter-sites variation. This data represents the average percent cover for *Acropora cervicornis* (blue) at Thatch Cay, Lovango Cay, No-Name Bay, and *A. prolifera* (green) at No-Name Bay between 2009 and 2017 ±SE. Dark colors represent healthy tissue, intermediate colors represent pale tissue, and light colors represent white tissue.

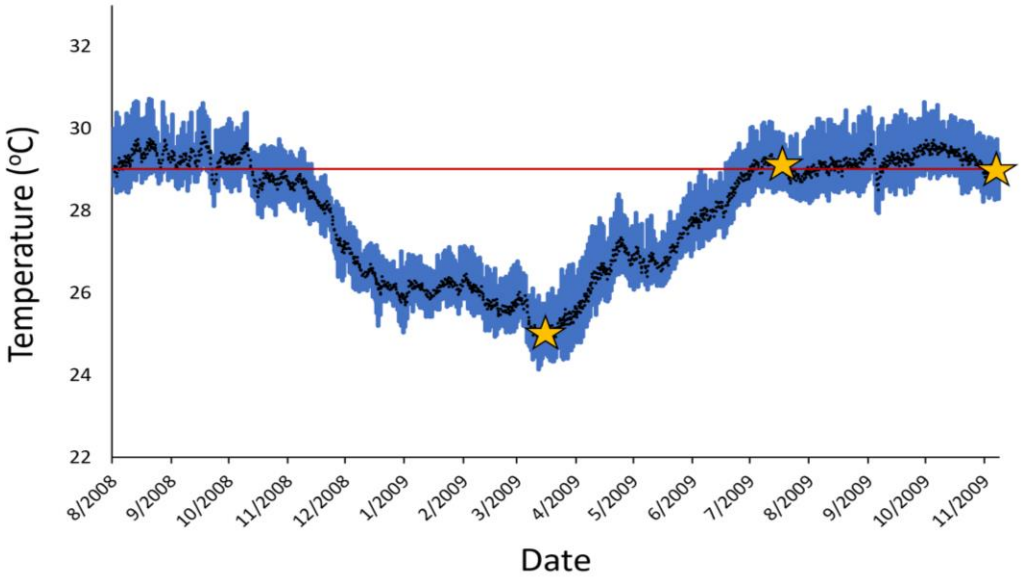

Figure 9. Sea surface temperatures at No-Name Bay (Transect 7) from August 2008 to November 2009. The blue area represents the high and low temperature for each day, while black dots represent daily averages. The red line indicates the bleaching threshold of 29.4°C. Yellow stars indicated dates when long-term transect photographs were collected.

Genotypic Analysis

The multiplexes used to analyze the genetic diversity were dependent on the primer and color of fluorescence (Table 2). The product length varied between 15 to 59 base pairs, with 6-12 alleles being detected. Micro-Checker analysis was used to verify the absence of stutter peaks, large allele drops, and null alleles. A low frequency (ρ= 0.11) of null alleles were only found in loci 207. Overall, this suggests there were limited issues with using these microsatellite markers in this population, increasing the overall confidence in these data.

The results of this study suggest that there is similar genetic richness of both parental species and the hybrid (Fig. 10). When considering genotypes within the total sample size, *A. prolifera* exhibited an intermediate genotypic richness relative to the parental species between all sites. The highest observed genotypic richness (N_g/N) was *A.*

palmata sampled at Lovango Cay, with a genotypic richness of 0.83 (Table 3). Interestingly, the lowest genotypic richness was also sampled at Lovango of *A. cervicornis* (0.42) with the hybrid exhibiting an intermediate richness (0.66). The total genotypic richness was not significantly different between either parental species or the hybrid [ANOVA, p=0.65].

Table 2. Characteristics of the microsatellite loci used to analyze genotypic richness.

Multiplex	Primer Name	Product Length (bp)	Number of Alleles	Number of Triploids
1	166	116-176	7	1
	181	141-186	7	0
	187	103-118	6	3
2	182	138-190	10	10
	207	146-197	12	4

Table 3. Genotypic Richness among sites. N is the number of sampled colonies, N_g number of unique genets, N_g/N represents the genotypic richness.

	Thatch Cay			Lovango			No-Name Bay			Total
	N	N_g	N_g/N	N	N_g	Ng/N	N	N_g	N_g/N	N_g/N
A. cervicornis	22	15	0.68	12	5	0.42	16	11	0.69	0.62
A. palmata	17	9	0.53	12	10	0.83	11	8	0.73	0.67
A. prolifera	8	6	0.75	6	4	0.66	25	25	0.6	0.64

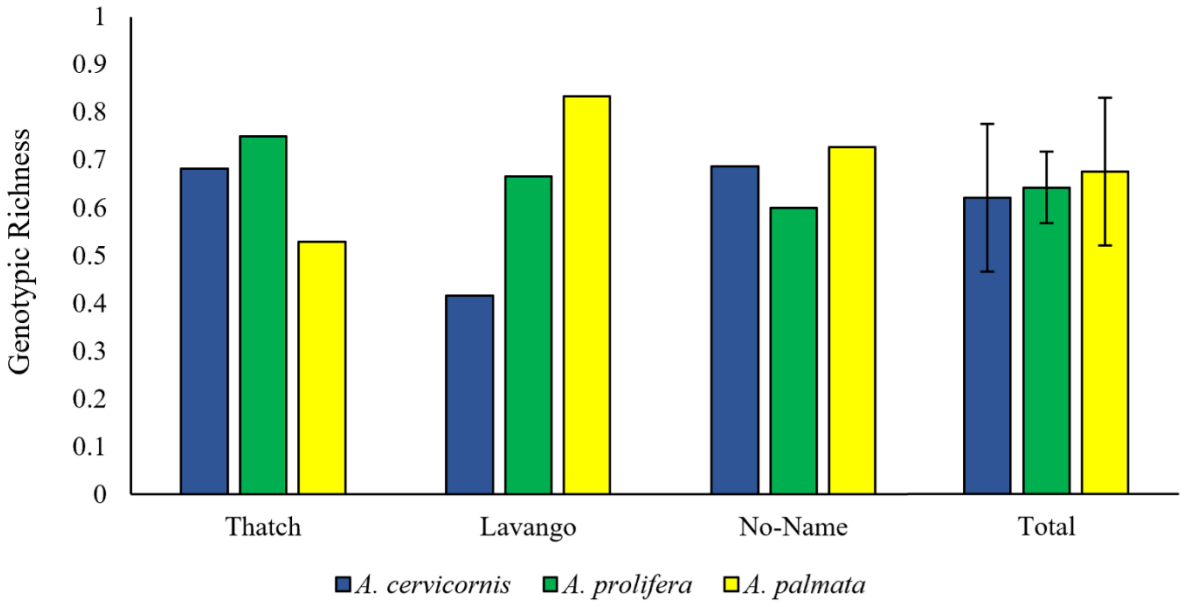

Figure 10. Relative genotypic richness of Caribbean acroporids at three distinct study locations in the U.S. Virgin Islands. Error bars represent ±SE.

DISCUSSION

The hybridization of acroporids in the Caribbean is thought to be a relatively new event in the evolutionary history of this taxa, but the impact of the hybrid on an ecological level is unknown. Hybrids were samples at all sites used in this study, and were found to dominate the shallow benthic habitat at No-Name Bay. Unlike *A. cervicornis*, which was observed to decline significantly between 2009 and 2017 at all sites, the hybrid maintained relatively high abundances (~20%) at No-Name Bay during the same time period. *Acropora cervicornis* still remained at No-Name Bay and was sampled in 2017, but consisted of sparse colonies that inhabited deeper water relative to the hybrid colonies. The average genotypic diversity among all three acroporids was similar across at all sites. Thus, acroporid colonies sampled in this study were likely derived from sexual reproduction from colonies that occasionally propagated asexually.

Trends in Long term abundance and Tissue Condition

During this study, several trends in the abundance and condition of the tissue conditions were observed. The most notable change was the significant decline of *A. cervicornis* at all sites between 2009 and 2017. This decline was most dramatic in 2009, with sustained losses between March and November. On October 16, 2008, the Category 4 Hurricane Omar, impacted the U.S. and British Virgin Islands (Brown et al. 2010). In addition to Hurricane Omar, several large hurricanes affected the U.S. Virgin Islands directly during the yearly sampling between 2007 and 2018 including Hurricane Earl and Otto (2010), Hurricane Irene (2011), Hurricane Bertha (2014), Hurricane Danny (2015) and Hurricane Irma and Maria (2017). It is possible that large hurricanes increase rates of disease and loss of coral through scouring, being smothered by sedimentation, and colony breakage (Scoffin 1993, Rogers 1993). New recruits and colonies less than 10 cm produced from a storm, such as Omar, usually die (Tunnicliffe 1981). In addition to potential damage from large storm events, the sea surface temperature in 2009 was observed to be the fifth warmest September on record, 0.50°C above the 20th century average, and has increased in subsequent years (NOAA, 2009). Temperature data

collected hourly at Transect 9 at No-Name Bay was recorded between August 2008 and November 2009. The hottest temperature was recorded to be 30.7° C, where the summertime average was 28.4°C, and the lowest temperature was 24.2°C (Figure 9).

An increase of 1°C above the mean summer temperature is often used to determine the bleaching threshold. During sampling in April 2009 through November 2009, temperatures often remained near or above this bleaching threshold of 29.4°C. A prolonged increase in sea surface temperatures is known to induce bleaching, which can reduce the ability of a coral to grow, reproduce and defense itself from a variety of biological stressors. It is likely that increased sea surface temperature exacerbated paling and subsequent white tissue observed in 2009. Although the cause of white tissue cannot be distinguished between bleaching, disease, or skeleton recently denuded of tissue, increased sea surface temperatures are strongly correlated with increased rates of disease (Muller et al. 2008, Miller et al. 2009). Previous declines in *A. cervicornis* cover appeared to partially recover by 2017 at Thatch Cay, with the amount of healthy tissue at a higher percent and the amount of pale and white areas at lower percentages than in November 2009. At Lovango Cay and No-Name Bay, the transects were devoid of any *A. cervicornis* cover by 2017. However, there were colonies present off the transects at these sites in 2017, allowing genotypic analysis. These colonies, however, were small and appeared to show signs of disease or paling tissue. The *A. cervicornis* colonies found outside the permanent transect could be attributed to a lack of potential habitat as *A. prolifera* increased in abundance, or that deeper water provided a reprieve from storms and warmer water that could contribute to bleaching and disease.

The healthiest and most abundant corals in 2017 were observed along transect 8 at No-Name Bay. This transect also had significantly higher hybrid coral cover relative to *A. cervicornis*, and did not change significantly at any time between 2009 and 2017. Perhaps, these hybrid genotypes are not as susceptible to bleaching and disease as the *A. cervicornis* colonies, which decreased steadily throughout the study period. My findings of healthier hybrids than parental species is consistent with other research that found fewer afflictions (i.e., disease, predation, bleaching, parasitism) in the hybrids at all sites across the Caribbean (Fogarty 2012). Likewise, Fogarty et al. (2012) found that *A. prolifera* were co-existing at sites with either *A. cervicornis* or *A. palmata*. Similar to No-

Name Bay, hybrid densities were found to be equivalent or greater than the parental species at several sites (East Rock, Antigua; Flat Key, St. Thomas; Sea Aquarium, Curaçao; and Caye Caulker, Belize) (Fogarty 2012). Genetic data was used to determine if acroporids at these sites were derived sexually or from asexual propagation. Although the genotypic richness varied, it is clear that most sites were not composed of only one rare hybrid that asexually propagated (Fogarty 2010).

The analysis methods used to quantify the general health of the coral tissue at these sites could potentially impact the results of the study. The amount of coral in each image varied based on angle, cloud cover, image quality, and position of the camera. Although the size of the image was standardized, the amount of easy distinguishable tissue varied among transects and years. The amount of paling on the tissue could also be subjective. This potential bias was controlled by having the same individual quantify all photographs used in this analysis. Additionally, tissue that was labeled as 'white' was used as a relative term for any tissue that displayed a white tissue due to bleaching, disease, predation, or skeletal tissue that was recently denuded. However, the cause of the tissue loss could not be differentiated. Regardless of whether the white area was pale, bleached, diseased, or recently predated upon, it was not healthy.

Genotypic Richness

When comparing genotypes within a population, there are two distinct methodologies that are used; genotypic diversity and genotypic richness. Genotypic diversity is the number of unique multilocus genotypes in the sample population (Baums et al. 2006). Comparisons of genotypic richness are influenced by both the number of unique genotypes observed in a sample and the evenness of the distribution (sample size) (Stoddart and Taylor 1988, Baums et al. 2006). Genotypic richness was used here because genotypic diversity has inherent problems in low diversity or unevenly distributed sample sizes. Due to the nature of sample collection, it was difficult to ensure completely comparable sample sizes. For example, samples from all hybrids colonies at Thatch Cay were collected, yet the sample size was still significantly lower than at No-

Name Bay. The numbers of hybrid samples collected at No-Name Bay could overshadow the number of unique genets compared to the other sites. By using genotypic richness, the impact of uneven sample sizes could be eliminated or reduced.

The genotypic richness of acroporids at Thatch Cay, Lovango Cay and No-Name Bay provide important information regarding the dominant method of dispersal. Of the hybrid colonies sampled for this study, the average genotypic richness (0.64) of all sites was not significantly different from that of the parents, *A. cervicornis* (0.62) and *A. palmata* (0.68). Genotypic richness values at or above 0.6 suggest that the population is derived primarily through sexual reproduction (Baums et al. 2006). The high genotypic richness found within these sites suggests that this propagation occurred from multiple sexually-derived hybridization events. The alternative hypothesis that the general hybrid populations only propagate asexually following a rare hybridization event (Vollmer and Palumbi 2002) is not supported here.

There were some significant differences between the genotypic richness of the parental species, and the hybrid when compared among sites. *Acropora palmata* was observed to have the highest genotypic richness of any site at Lovango Cay, where *A. cervicornis* was observed to have the lowest. This stark differentiation in genotypic richness between the parental species could be attributed to the site and the geomorphology of the reef itself. Lovango Cay is a narrow E-W running ledge found between two small islands. It is well known that the current within this inlet can be quite rapid, as large volumes of water funnel through this location upon changes in tidal state. It is hypothesized that the *A. palmata*, which are found in shallower and on more protected ledges along this site, may reduce the amount of scouring or fragmentation due to irregular sea conditions. The size and vertical expansion of *A. palmata* colonies may also contribute to their ability to withstand these abiotic stressors. *Acropora cervicornis* is well documented to fragment and break more easily than *A. palmata*, propagating genotypically identical clones. This is particularly true for areas that undergo constant wave action or frequent storms. The low genotypic diversity of *A. cervicornis* at Lovango Cay may be explained by the asexual fragmentation of several colonies near the colonies that were sampled for genotypic analysis.

Other studies that have analyzed the genotypic richness of the hybrid have also found relatively high genotypic richness values between the parental species and the hybrid. Fogarty (2010) found an average genotypic richness at nine sites throughout the Caribbean of 0.41 (genets/sample size) that were haphazardly sampled in a manner similar to this study. In this study, the genotypic richness of *A. cervicornis* and *A. palmata* were higher at these sites relative to other studies throughout the Caribbean. Irwin et al. (2017) reported the highest genotypic richness of 0.53 for *A. palmata* at Manatee Channel, Belize. Similarly, the highest genotypic richness in this study was also determined to be from *A. palmata* (0.83). Similar genotypic richness was also reported throughout the Caribbean, where richness values of *A. palmata* in in the U.S Virgin Island varied between 0.36 to 0.70 (Fogarty 2010). Other studies throughout the Caribbean found genotypic richness between 0.05 and 1, with an average of 0.52 (Baums et al. 2006). The genotypic richness of *A. cervicornis* was relatively lower in the western Caribbean compared to the eastern region [0.59 vs. 0.62 (Vollmer and Palumbi 2006)].

Prior to genetic sampling in 2017, hybrids were only observed on No-Name Bay transects. Several hybrid colonies were observed near *A. palmata* colonies at Thatch Cay and Lovango Cay, but not on the permanent transects. There are two potential sources of hybridization: sexually derived hybrids that recruited to this area, or fragments that lived in the periphery and were transported into the area during storms. Genotypic analysis of these isolated colonies suggests the former, with high genotypic richness at these sites. Based in the genotypic data and size of the hybrids, it is likely that the hybrids had recruited to the reef prior to 2017, but were not detected due to their location away from the permanent transects. It is unclear whether these data support the density dependent isolation hypothesis where sexual reproduction due to low parental population densities is a primary method of hybrid propagation at select Caribbean sites (Fogarty 2010).

It is possible that these genotypes recruited into the study area after the decline of the parental species. It is also possible that these genotypes are old and have only fragmented occasionally. At other sites somatic mutations in hybrid colonies have been used to determine their age. In Belize, hybrid genets were between 156-281 years old (Irwin et al. 2017). In order to determine if the U.S. Virgin Islands hybrid population was

derived by density dependent reproductive processes, somatic mutations would need to be used to age the genets.

Impacts of Hybridization

Hybrids at No-Name Bay were observed and quantified dominating habitat that was previously shared with *A. cervicornis*, and could have contributed to the complete loss of the parental species on the long-term monitoring transects. The persistence of relatively high percent cover and healthier tissue of *A. prolifera* suggests that the hybrids are outcompeting *A. cervicornis* spatially at No-Name Bay. The ability of the hybrid to quickly reattach after fragmentation and their apparent resistance to disease and bleaching could be driving this shift in the hybrid zone at No-Name Bay.

The formation of some hybrid zones, and the potential increase of hybrid abundances could be due to human-mediated impacts such as increased disturbances, fragmentation and shifts in the habitat range itself (Hulme 2008, Brennan et al. 2015). For Caribbean acroporids, the impacts of introgressive hybridization are paradoxical, such that the parental species could be saved (i.e., genetic rescue through the sharing of beneficial alleles) or become homogenized [(i.e., genetic swamping)(Rieseberg et al. 1993, Ellstrand and Schierenbeck 2000, Rieseberg and Burke 2001)]. What is less understood though, are the impacts of the Caribbean hybrid ecologically. In several other biological systems, successful hybrids can act as an invasive species through the removal, displacement, and reduction of native taxa including the parental species (Lee 2002). This shift from native or parental dominated habitat to that of the hybrid could promote further hybridization in the organisms that occupy the newly formed habitat (Schwarz et al. 2005). Changes in available habitat can impact the array and diversity of organisms in that area, and could be occurring at No-Name Bay where the hybrid is thriving.

CONCLUSION

No study has attempted to quantify habitat exchanges between *A. cervicornis* and *A. prolifera*. Understanding habitat boundaries is a crucial component in solving the unknown impacts of the hybrid on the parental species. The shift from *A. cervicornis* to *A. prolifera* at No-Name Bay is a novel finding that has important ecological implications. It is possible that the shift from parental species to hybrid would support different fish and invertebrate species, and therefore restructure shallow coral reefs. Additionally, the growth patterns of the hybrid can be vastly different than the large, branching structures created by *A. palmata* and the tumbleweed-like thickets of *A. cervicornis*. Hybridization can lead to the formation of novel alleles that can be shared with the parental species through backcrossing, effectively facilitating rapid evolution against deleterious environmental conditions (Grant 1981, Arnold 1992, Mallet 2007). Alternatively, if introgression rates increase because of more prevalent hybridization, it could lead to a decline in *A. cervicornis* or *A. palmata* via genetic swamping. Hybrid vigor may cause further declines in *A. cervicornis* at No-Name Bay, where the hybrid appears to be to be less susceptible to environmental stressors and could outcompete the parental species spatially. Furthermore, the unique morphologies of the hybrid have the potential to fill or create unique niches without speciation (Vollmer and Palumbi 2002). Although hybridization is well documented in the Pacific (Veron 2000), it is a novel occurrence in the Caribbean and could have a sustained impact on the success of the parental species and the organisms that utilize them, now and in the future.

CHAPTER 3

DISCUSSION

As climate change continues to alter natural habitats, the opportunity for hybridization increases through the breakdown of biological and environmental barriers (Vallejo-Marín and Hiscock 2016). Specifically, global climate change can reduce the efficacy of these barriers through changes in temperature, ecological patterns and geographic limitations of a population (Chunco 2014, Brennan et al. 2015). Understanding the population dynamics of acroporids in the Caribbean is a crucial step to determine the impacts that more abundant hybrids have on the reduced parental species, and what this could mean for the future of these important, fast-growing and keystone species. The sites investigated in this study have distinct geomorphologies and population abundances of *A. cervicornis*, *A. palmata*, and *A. prolifera*. It is important to determine how hybrid populations may influence the ecological success and evolutionary trajectory of the parental species.

In other hybridizing systems, asymmetries in parental abundances have led to increases in hybridization, including the rusty crayfish [*Orconectes rusticus* (Perry et al. 2001)], western sunflowers [*Helianthus anomalus* (Heiser et al. 1969)], and pupfish [*Cyprinodon pecosensis, C. varigatus* (Rosenfield et al. 2004)]. It is possible that the asymmetries in Caribbean acroporids (where *A. palmata* is higher than *A. cervicornis*) has led to increased rates of hybrid formation, which then propagated asexually. Increased hybrid formation could have contributed to the apparent increase in hybrid coral cover at No-Name Bay (Fogarty 2010, Lang et al. 1998). *Acropora palmata* was more abundant at No-Name Bay and Thatch Cay, which mirrors the asymmetries observed at other sites in the Caribbean (Fogarty 2010). Although both parental species abundances are important to consider, *A. palmata* was not included in the long-term monitoring analysis as no colonies were observed on the permanent transect at any point during this study. This was primarily because the initial objective of the NOAA project was to follow *A. cervicornis* and the associated fish populations. However, I was able to

sample *A. palmata* in areas immediately adjacent to the transects for comparison of genetic make-up.

During the time period investigated in this study, Lovango Cay experienced dramatic and significant losses in *A. cervicornis*. These declines could be attributed to reported bleaching events in 2005 and 2009 (Muller et al. 2008, Rogers and Muller 2012). Long-term monitoring sites at Haulover Bay, near No-Name Bay reported that 89.9% of *A. palmata* colonies exhibiting disease between 2003 and 2009 (Rogers and Muller 2012). Additionally, the highest rates of disease were recorded in November 2009 (57% of sampled colonies), which support similar results found in this study with increased rates of paling and white tissue in *A. cervicornis* (Rogers and Muller 2012). The complete loss of *A. cervicornis* on permanent transects at Lovango Cay and No-Name Bay by 2017 could be attributed to these increased rates of disease and bleaching. The samples of *A. cervicornis* collected at Lovango Cay likely propagated primarily through asexual fragmentation, primarily due to the rapid currents experienced in this area, and could explain the low genotypic richness (0.42) found there.

Although Thatch Cay and Lovango Cay both experienced declines in *A. cervicornis*, the genotypic richness of the sampled colonies were much different. *Acropora cervicornis* was observed to have the highest genotypic richness (0.68) at Thatch Cay. Similarly, the geomorphology of this location could impact these findings. The Thatch Cay site is off the southern portion of the island, with a steady depth gradient from rocky shore to sandy bottom. A majority of the individuals sampled here were found between 0 and 3 meters, with *A. palmata* and *A. prolifera* inhabiting shallower zones relative to *A. cervicornis*. Within the individual *A. prolifera* colonies, there were two distinct morphologies detected. Individual hybrid colonies sampled for this study that were collected in the shallowest habitats, near several *A. palmata* colonies were observed to be 12.5 cm in width, with a majority of the apical polys being completely fused. Hybrid colonies that were sampled in deeper locations were observed to have less fusion along the apical polyps and were larger than 12.5 cm in width. These distinct morphologies are likely from phenotypic plasticity where the environment influences hybrid morphology. However, morphological variations could be explained by genetic

differences, specifically if genets are made of up a mix of F1 and backcrossed individuals.

Of the locations included in this study, No-Name Bay was the only site to have large thickets of hybrid colonies that dominated areas that were previously shared with *A. cervicornis*. The high percentage of hybrid colonies, and thus the number of colonies sampled, could have contributed to the low genotypic richness at No-Name Bay. Although genotypic richness standardizes for a skewed sample size, it is possible that the low richness here was an artifact of the large sample size. The hybrids at this location formed large, dense thickets with skeletal fragments below live tissue. In several areas, a single thicket was larger than 10m across, covering the entire length of the transect. The range of this hybrid zone extended from less than 1 meters to 3-4 meters in depth and was located near the mouth of a shallow bay. The parental species were sampled intermediately between and within the mouth of a shallow inlet. *Acropora palmata* was found in the upper reef, in water less than 1m, while *A. cervicornis* was found in deeper habitats up to 5 m deep. Because of the large thickets of hybrid, and low genotypic diversity at No-Name Bay, it could be hypothesized that these corals in particular, propagate asexually more than at other sites.

Caribbean acroporids, especially *A. cervicornis* and *A. prolifera* colonies that have been shown to directly compete for the same spatial habitat, may be experiencing competitive exclusion(Hardin 1960, Bruno et al. 2003). This principle, also known as Gause's Law, is used to explain why species that compete for the same resources cannot coexist in the same ecological niche (Hardin 1960). This principle has potential implications for the acroporid hybrid system

Once established, the outcomes of hybridization can be profound. Globally, inter-specific hybridization occurs in as many as 25% of plant species and 10% of animals species (Mallet 2007). The formation of some hybrid zones can be attributed to human-mediated impacts on the ecosystem itself, including increased disturbance and fragmentation (Hulme 2008). Additionally, hybridization may be mediated through shifts in habitat range (Brennan et al. 2015). Specifically, global changes may increase the latitudinal ranges of corals, thus promoting hybridization. (Stebbins 1959, Buggs 2007).

The impacts of altered habitats and ranges are particularly evident in plant systems, where habitat modification may facilitate hybridization [*Silene dioica, Silene latifolia* (Marren 1999)] and determine if the hybrids are viable and able to persist [Asteraceae(Abbott et al. 2009)]. The impacts of introgressive hybridization can vary. In one scenario, repeated backcrossing can result in genetic swamping, where genes are rapidly and indiscriminately exchanged between the taxa until the lines of speciation become blurred. This results in the homogenization of the parental species (Rieseberg and Burke 2001, Rieseberg et al. 1993). Alternatively, hybridization can facilitate genetic rescue by transferring beneficial mutations and allelic variations between and within species (Rieseberg et al. 1993, Ellstrand and Schierenbeck 2000).

The persistence of hybridization can be achieved by the stabilization of the hybrid zone, the expansion of the hybrids into new niches, complete speciation, or the spatial displacement of the parental species (Rieseberg and Burke 2001, Chunco 2014). Here, it appears the later may be occurring at No-Name Bay through the formation and propagation of sexually derived hybrid recruits. Hybrid vigor has been observed in vertebrates [*Cyprinodon pecosensis, C. variegatus*(Rosenfield et al. 2004)], invertebrates [*Melanoides turerculata* (Facon et al. 2005)], and plants (Barbour et al. 2003, Marren 1999). On an ecological level, successful hybrids may act similar to invasive species due to their tendency to displace not only the parental species, but potentially compete with other native taxa (Lee 2002, Muhlfeld et al. 2014). The displacement of parental species can lead to shifts in species distributions, such that previously common habitats become rare while hybrid colonies support different species arrangements. This shift from parental dominated habitat to the hybrid (or invader) can facilitate rapid hybridization in the animals that occupy these habitats (Schwarz et al. 2005). Additionally, hybrids themselves may be formed through introduced species that then outcompete the original parental species (Ayres et al. 2004, Huxel 1999). This ecological replacement of parental species by the hybrid may be occurring in Caribbean acroporids, and at No-Name Bay in particular.

CONCLUSION

The results of this study highlight a complex and dynamic relationship between the parental species *A. cervicornis* and *A. palmata* and their hybrid, *A. prolifera*. The ecological function of *A. cervicornis* and *A. palmata* is a crucial one. By increasing the complexity of the reef and contributing to rugosity, these reef building species offer habitat for innumerable fish and invertebrate species. Based on this research, it can be concluded that the hybrid may be replacing *A. cervicornis* at one of the study locations. It is still unknown if the hybrid can fully replace the parental species ecologically.

The reduced percentage of paling and disease in 2017 at No-Name Bay suggest that an increased resistance to environmental stressors may have been achieved by the hybrid. It is important to consider the impact of future environmental and ecological stress to the coral ecosystems. As global temperatures continue to rise, bleaching and disease will continue to plague the acroporid corals. Additionally, increased severity of hurricanes and reduced ocean pH could have negative long-term impacts on the entire coral ecosystem.

In the future, it would be advantageous to compare the fish populations at each site to determine if the ecological services provided by *A. cervicornis* are also supported by the hybrid. More transects could be added that include *A. palmata* to compare the percent cover relative to *A. cervicornis* and *A. prolifera*. Finally, the number of sites throughout the U.S. Virgin Islands can be expanded, while adding genotypic samples at these additional sites to determine how site location influences hybrid population dynamics.

In all, coral reefs are irreplaceable ecosystems and understanding them on fundamental genetic and function related scales are imperative to prevent future declines. Hybridization is a novel occurrence in Caribbean acroporids, but has historically occurred in the Pacific (Veron 2000, Richards and Hobbs 2015). The success of Caribbean acroporids may depend on hybridization for the successful propagation of the species or could be the cause of their demise.

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