AN ABSTRACT OF THE THESIS OF Nicole M. Burns for the Master of Science in Biology presented March 6, 2018.

Title: Using Survivor Populations to Mitigate Bleaching Mortality of Staghorn *Acropora* Corals

Approved: _____
Laurie J. Raymundo, Chair, Thesis Committee

As the effects of climate change on marine ecosystems intensify, efforts are being channeled to monitor and actively mitigate these impacts. Coral reefs, in particular, are rapidly declining due to coral bleaching, a direct result of climate change-induced ocean warming. Staghorn *Acropora* spp. are especially vulnerable to coral bleaching. Guam's coral reefs were impacted by coral bleaching in 2013, 2014, 2016, and 2017. A novel approach to rehabilitate dying reefs is to grow corals in a coral nursery, then plant the corals onto degraded reefs. For this study, which was conducted on Guam, fragments of four species of staghorn *Acropora* (*A.* cf. *intermedia, A. aspera, A. muricata,* and *A.* cf. *pulchra*) from five surviving source populations were collected and propagated in an *in situ* coral nursery. To test the methods of culturing corals in a nursery, we made comparisons between fragments fixed in place vs. hanging freely, between species, and between source populations (only comparing *A.* cf. *pulchra* from difference sources). After a 10-month nursery phase, fragments of *A.* cf. *pulchra* from West Agaña were pruned from the nursery and simultaneously outplanted along with fragments collected from the same wild source population. To test the effectiveness of the nursery phase on subsequent performance of outplanted corals, we compared the relative growth and

survival of attached nursery-sourced corals, attached wild-source fragments, and unattached wild-sourced fragments. All populations of hanging fragments had significantly higher growth rates than their respective fixed fragments ($p<0.01$). Fragments of *A.* cf. *pulchra* from Togcha had significantly higher growth rates than those from West Agaña ($p=0.002$), but not those from Agat. Fragments of *A.* cf. *pulchra* from Togcha had significantly higher growth rates that *A. muricata* ($p<0.001$) and *A. aspera* ($p=0.009$). All of the loose fragments either died or were lost, and the nursery-sourced corals had significantly higher growth rates that the wild-sourced fragments ($p<0.001$). This study offers valuable, specific methods for future restoration activities on Guam, and recommendations that can be applied elsewhere.

TO THE OFFICE OF GRADUATE STUDIES

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USING SURVIVOR POPULATIONS TO MITIGATE BLEACHING MORTALITY OF STAGHORN *ACROPORA* CORALS

BY

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INTRODUCTION

Coral reefs worldwide are facing drastic declines in health and diversity as the oceans continue to rapidly change (Wilkinson 2002; Hughes et al. 2003; Bellwood et al. 2004). Bruno & Selig (2007) found that globally, coral reefs experienced an annual average decline of 1% in total coral cover over the previous two decades, and a 2% decline annually from 1997 to 2003. In more recent years, however, reef decline has been increasing at much higher rate. Hughes et al. (2018) found that the Great Barrier Reef lost 30% of its coral cover in only nine months in 2016. When reefs provide roughly $30 billion a year in goods and services, their decline will have significant impacts on the global economy (Cesar et al. 2003).

In 2007, Guam had 108 km^2 of coral reef habitat consisting of submerged reefs, fringing reefs, offshore banks, patch reefs, and barrier reefs. Guam's reefs have suffered much higher losses in recent decades than the 1% coral cover decline globally. Estimates from coral community surveys in the 1960s found ~50% average coral cover around the island (Randall 1971). However, 2005 surveys estimated that the average coral cover was only 26.1%, with multiple survey sites as low as 11.8% (Burdick et al. 2008). Furthermore, from 2003 to 2014, coral cover on the east side of Guam declined from 17% to 7% (NOAA PIFSC-CRED, unpublished data). While different methods were used amongst these surveys, the island's reefs indisputably lost a significant amount of coral cover in only 40 years. With much of Guam's economy and culture reliant on its reefs, such significant losses can have devastating effects (Burdick et al. 2008).

A 2007 Total Economic Value (TEV) study estimated Guam's reefs to be worth $127.28 million per year (van Beukering et al. 2007), or $150.47 million today (US

Inflation Calculator). The TEV takes into account numerous goods and services that coral reefs provide, including tourism and recreation, food fish habitat, and coastal protection. Culturally, Guam's coral reefs are important for fishing and providing supplemental food (Amesbury & Hunter-Anderson 2003). While subsistence fishing is not as prevalent as it once was, traditional and modern fishing practices are still an important part of the cultural, economic, and social life on the island (van Beukering et al. 2007). Therefore, the continuing decline in coral cover is likely to have a strong negative effect on the island's people and economy.

The coral reefs around Guam face natural stressors such as crown-of-thorns starfish predation (Chesher 1969; Gawel 1999), typhoon damage, and occasional extreme low tides (Raymundo et al. 2017), as well as many local anthropogenic stressors such as sewage runoff (Redding et al. 2013), stormwater runoff and sedimentation (Wolanski et al. 2003), physical damage due to recreational misuse (Sturm 2017), over-harvesting (Houk et al. 2012), and global climate change (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; van Beukering et al. 2007; Burdick et al. 2008). The southern coast of Guam, for example, sees large amounts of stormwater runoff and river flooding, which carries eroded soil into the nearshore reef flats (Wolanski et al. 2003). This increased sedimentation can smother existing coral colonies, and hinder the growth and survival of juvenile corals (Humanes et al. 2017). In popular tourist areas, such as Tumon Bay, corals on shallow reefs flats are particularly susceptible to physical damage from recreational activities—such as snorkeling or SCUBA diving (Sturm 2017).

The genus *Acropora* is the most speciose of all extant genera of scleractinian corals, with approximately 180 species distributed globally, but are predominantly found

in the Indo-Pacific (Veron 2000). Members of the genus are generally strong competitors; these corals have rapid growth rates, can fragment as a means of asexually reproducing, and display high rates of monopolization of space (Bak & Engel 1979; Kojis & Quinn 1994; Obura 2001)—traits that allow them to quickly dominate a reef. They also provide critical habitat to a variety of juvenile reef fishes (Floros & Schleyer 2016). However, *Acropora* corals are highly vulnerable to climate change impacts, and of particular concern are warming ocean temperatures. Acroporids generally have a low stress resistance to elevated temperatures, which is thought to be a result of their high metabolic rate (Kinsman 1964; Yap & Gomez 1981). When exposed to prolonged stress, including periods of elevated temperatures, the corals may undergo coral bleaching, which can result in mortality (Salvat 1991; Obura 2001). Bleaching occurs when symbiotic zooxanthellae that live in the coral tissue are expelled, leaving the coral tissue not only colorless, but also deprived of food and susceptible to starvation (Buddemeier & Fautin 1993).

Arborescent, branching *Acropora*, referred to hereafter as staghorn *Acropora*, often form large thickets or stands. On Guam, these species are generally found in shallow reef flats, Cocos Lagoon, and the shoals of Apra Harbor (Raymundo et al. 2017; Lapacek 2017). The most common species of staghorn found around the island is *A.* cf. *pulchra*, while other less common species include *A.* cf. *intermedia*, *A. muricata*, and *A. aspera*. Uncommon or rare species of staghorn include *A. teres, A. austera, A. virgata,* and *A. vaughani* (Raymundo et al. 2017).

Small-scale bleaching events were reported throughout the Mariana Islands in 1994, 1996, 2006, and 2007 (Paulay & Benayahu 1999; Burdick et al. 2008). The first

widespread, severe bleaching event was recorded in 2013, during which 85% of corals bleached across Guam, Rota, and Saipan (Reynolds et al. 2014). After the successive 2013 and 2014 bleaching events, Guam's reefs lost 53% of total cover of staghorn *Acropora*; eight of the 21 survey sites experienced ≥75% mortality (Raymundo et al. 2017). Guam's staghorn beds are a critical habitat for many reef fish species throughout their lives, so their loss will likely negatively affect reef fish populations around the island (Holbrook et al. 2015; Raymundo et al. 2017). Although species of corals vary in their ability to acclimatize to changing environments (Baker et al. 2004; Palumbi et al. 2014; Bay et al. 2014; Heron et al. 2016; Bay et al. 2017), it is unknown if they will be able to survive more severe and frequent mass bleaching episodes predicted for the near future (McWilliams et al. 2005; Maynard et al. 2008; van Hooidonk et al. 2013a). Furthermore, while bleaching projections have shown variation at a regional level (van Hooidonk et al. 2016), annual bleaching events are predicted as early as 2040, with the last reef refugia experiencing annual bleaching by 2055 (van Hooidonk et al. 2013b).

To ensure the recovery and survival of these ecologically-important corals, urgent conservation measures are needed. Traditionally, these measures took the form of reestablishing small reef communities by directly transplanting certain species of coral fragments or colonies (Rinkevich 1995). In some instances, this method has been effective in rehabilitating dead or damaged reefs. In Costa Rica, Guzman (1991) transplanted fragments of *Pocillopora* spp. and reported low mortality rates (21% and 17% on the two study sites) and found that natural fragmentation from the transplanted colonies led to an additional 41% and 115% increase in new colonies over the original transplanted colonies. Bowden-Kerby (1997) transplanted fragments of *A. cervicornis*

and *A. prolifera* onto sites with reef flat rubble and found up to 100% and 88% survival rates (respectively) for large-sized fragments, as well as 95% survival of large, nursery-reared coral colonies that were transplanted into lagoonal areas. Rinkevich (2000) found that up to 90% survivorship can be achieved using even small branches of *Stylophora pistillata* (<4 cm length), leading to successful reef rehabilitation. Furthermore, dela Cruz et al. (2014) experimented with two species of *Acropora*—*A. pulchra* and *A. intermedia*—in the Philippines, and found high levels of survivorship and significant increases in growth (15-fold increase in ecological volumes). The researchers also found significant increases in fish and macroinvertebrate biomass in their experimental plots, concluding that these areas of the reef had successfully been restored due to their transplants.

However, there can be negative consequences from transplantation that can actually stunt the growth of transplanted fragments. Several studies have found that the act of transplanting causes an initial "transplantation stress" on the coral, when the initial growth of the transplant is inhibited, sometimes resulting in negative growth, until the period of stress is surpassed (Yap & Gomez 1985; Yap et al. 1992; Raymundo 2001). Another concern of transplantation is the risk of introducing and spreading diseases from infected coral transplants. Raymundo (2001) found that only fragments transplanted to a poor-quality recipient site—heavy siltation and agricultural runoff—became infected with *Porites* ulcerative white spot disease (PUWS), which subsequently spread to 45% of *Porites* spp. colonies elsewhere on the reef by the following year. Furthermore, a common criticism of transplanting corals for rehabilitation involves the logistics of doing it on a large-scale, while not negatively impacting the donor site. Edwards & Clark

(1999) discuss the potential for donor reefs to lose too much coral cover and experience little to no recovery, resulting in negative consequences possibly outweighing the benefits of the practice.

To resolve this problem, a "coral gardening" technique was developed by Rinkevich (1995) that adds an *in situ* ocean nursery phase prior to transplanting the corals to their final home. Nursery structures range in design, but a popular example is the mid-water suspended nursery which offers an ideal period of growth by elevating corals off the sea floor and protecting them from negative impacts such as sedimentation and benthic corallivores (Bongiorni et al. 2003; Shafir et al. 2006). This method allows corals to be fragmented at a small size, reducing the impact on donor colonies, and allowing the fragments to acclimate and recover from harvesting while growing to larger sizes for transplantation. This technique has repeatedly shown positive results, producing higher survival rates post-transplantation, and overall physiologically more fit fragments—or more likely to survive—compared to those directly transplanted (Epstein et al. 2003; Rinkevich 2014).

Many studies have used the asexual reproductive strategy of corals (i.e. fragmentation) in different methods to grow fragments of varying species and sizes in the coral nursery, with high survival and growth rates. Dela Cruz et al. (2015) found that nubbins (fragments 3-4 cm in length) of *Echinopora lamellosa* grown in a nursery had significantly higher survival and greater skeletal weight than those that were directly transplanted onto a denuded reef. In Singapore, Bongiorni et al. (2011) cultured nubbins of 13 species of corals of varying morphologies in both a degraded reef site and a healthy

site. They found significant increases in growth for multiple species at both locations, concluding that nurseries can even be successful in sites with poor environmental quality.

The manner in which the corals are grown in the nursery and the species used has also been experimented with. Kuffner et al. (2017) found that fragments of *A. cervicornis* grown on a substrate had lower rates of linear extension and higher skeletal density, whereas fragments hung on strings produced the opposite results. Putchim et al. (2008) cultured two species of *Acropora* in Thailand, *A. grandis* and *A. muricata*, and found both high survival and comparable rates of linear extension (0.41 and 0.23 cm/month, respectively) of nursery-grown corals and corals post-transplantation as found in other studies. Soong and Chen (2003) grew fragments of *A. pulchra* and found that fragments grew ~1cm/month.

Other studies have experimented with manipulating sexual reproduction of corals for use in coral gardening for reef rehabilitation. Bongiorni et al. (2003) found that corals reared in a nursery showed improved reproductive output compared to fragments transplanted directly from wild stock. If these fragments can produce more larvae, then it could be yet another step in aiding in reef recruitment and recovery. Amar & Rinkevich (2007) discuss how larvae from nursery-sourced corals were larger, with more symbionts, and had faster growth than those from *in situ* corals on the reef. With 10,000 colonies in the researchers' nursery, they refer to their site as a "larval dispersion hub," that has capabilities to aid in generation of new corals for recovery of a reef. Furthermore, Guest et al. (2013) found that sexually propagated transplants of *Acropora millepora* that had a longer ocean nursery phase (19 months) showed 46.7% survival rate at the end of the surveying period, compared to transplants that only had a seven month (8.3%) or 14-

month (11.7%) ocean nursery phase. They concluded that there was a positive correlation between rate of survival post-transplantation and time spent in the *in situ* nursery.

Using a nursery phase in which small fragments can be grown out to larger sizes for outplanting is a way to eliminate significant damage to donor reefs. This can be accomplished for large-scale reef restoration without causing significant damage to donor reefs. Muñoz-Chagín (1997) transplanted all of the coral reef fauna from one location to another in order to prevent harm to the organisms from a construction project. The researchers were able to move 3,106 marine animals from one location to another—the significant majority being Scleractinia, sponges, and octocorals—with a total mortality of only 3% one month after transplantation. Montoya-Maya et al. (2016) outplanted 24,431 nursery-grown corals to a degraded reef site spanning 5,225 m^2. They considered the experiment successful in restoring the degraded reef area, citing the significant increase in both coral spat recruitment and coral juvenile recruitment to the transplanted site.

Van Oppen et al. (2015) encourages the use of assisted evolution—by speeding up and/or facilitating natural selection processes in corals in order to improve their ability to tolerate stressors—to enhance resilience in corals. An example of this includes the acclimatization of coral stocks to natural stressors, such as increased temperatures. The bleaching episodes in Guam may have acted as this stressor, which may have acclimatized surviving populations of *Acropora* that are more resilient to thermal stress. Fragments from these populations (Fig. 1; Table 1), were collected, grown in an *in situ* nursery, and a subset was outplanted in a pilot study. Their performance was documented over the course of two years, in order to determine if certain species from specific populations perform better than others, and whether or not position in the nursery affects

that growth, as well as if nursery-sourced fragments grow significantly better than fragments directly outplanted from *in situ* populations.

HYPOTHESES

$H1_0$: Fragments of *A.* cf. *intermedia, A. aspera, A. muricata,* and *A.* cf. *pulchra* that are fixed upright will not grow at significantly different rates than those that are hanging freely.

> $H1_a$: Fragments of *A.* cf. *intermedia, A. aspera, A. muricata, & A.* cf. *pulchra* that are fixed in place will grow at a significantly different rate than those that are hanging freely.

$H2_0$: The growth rates of *A.* cf. *intermedia, A. aspera, A. muricata, & A.* cf. *pulchra* will not differ significantly.

> $H2_a$: The growth rates of *A.* cf. *intermedia, A. aspera, A. muricata, & A.* cf. *pulchra* will be significantly different.

$H3_0$: Fragments of *A.* cf. *pulchra* from different source populations will not grow at significantly different rates.

> $H3_a$: Fragments of *A.* cf. *pulchra* from different source populations will grow at significantly different rates.

$H4_0$: There will be no difference in the survival and growth of corals outplanted from an *in situ* nursery phase versus coral fragments outplanted directly from wild populations.

> $H4_a$: Corals grown with an *in situ* nursery phase will survive and grow significantly more than coral fragments transplanted directly from wild populations.

MATERIALS & METHODS

Sites

Guam is a tropical island in the Mariana Archipelago in the Pacific Ocean, located at ~13.44° N and 144.79° E. Due to its tropical location, the temperature is relatively consistent year-round. The source populations were stands of staghorn *Acropora* from the reef flats around the island (~ 0.3 to 1.5 m deep) that survived both the 2013 and 2014 bleaching events. The four species of *Acropora* and their respective source population locations around the island are described in Table 1.

The sampled sites were chosen for the size and health of their remaining stands of staghorns and ease of access (Fig. 1). The Tumon site is located in the Tumon Bay Marine Preserve (MP), directly in front of the Outrigger Guam Beach Resort, where there are several patchy stands of both *A.* cf. *pulchra* and *A.* cf. *intermedia*. Because the area is in the hub of the tourism industry, these populations are often subjected to nutrient pollution from the nearby hotels and recreational damage. The West Agaña site has one of the largest remaining stands of staghorns (28,967 m^2, Raymundo et al. 2017), consisting mostly of large, contiguous stands of *A.* cf. *pulchra*. The Agat stand of staghorns is also mostly contiguous, and consists of *A.* cf. *pulchra*, *A.* cf. *intermedia*, and *A. muricata*. Both of those sites are situated next to wastewater treatment facilities and have been subjected to wastewater effluent. The Achang site is immediately to the left boundary of the Achang Reef Flat Marine Preserve, is the southernmost site, and is only accessible by boat. The site contains patchy stands of both *A.* cf. *pulchra* and the only known remaining stand of *A. aspera*. The Togcha site only contains *A.* cf. *pulchra* and

contains the only known remaining stand of staghorns on that side of the island. The site can be difficult to access due to its position on the windward side of Guam.

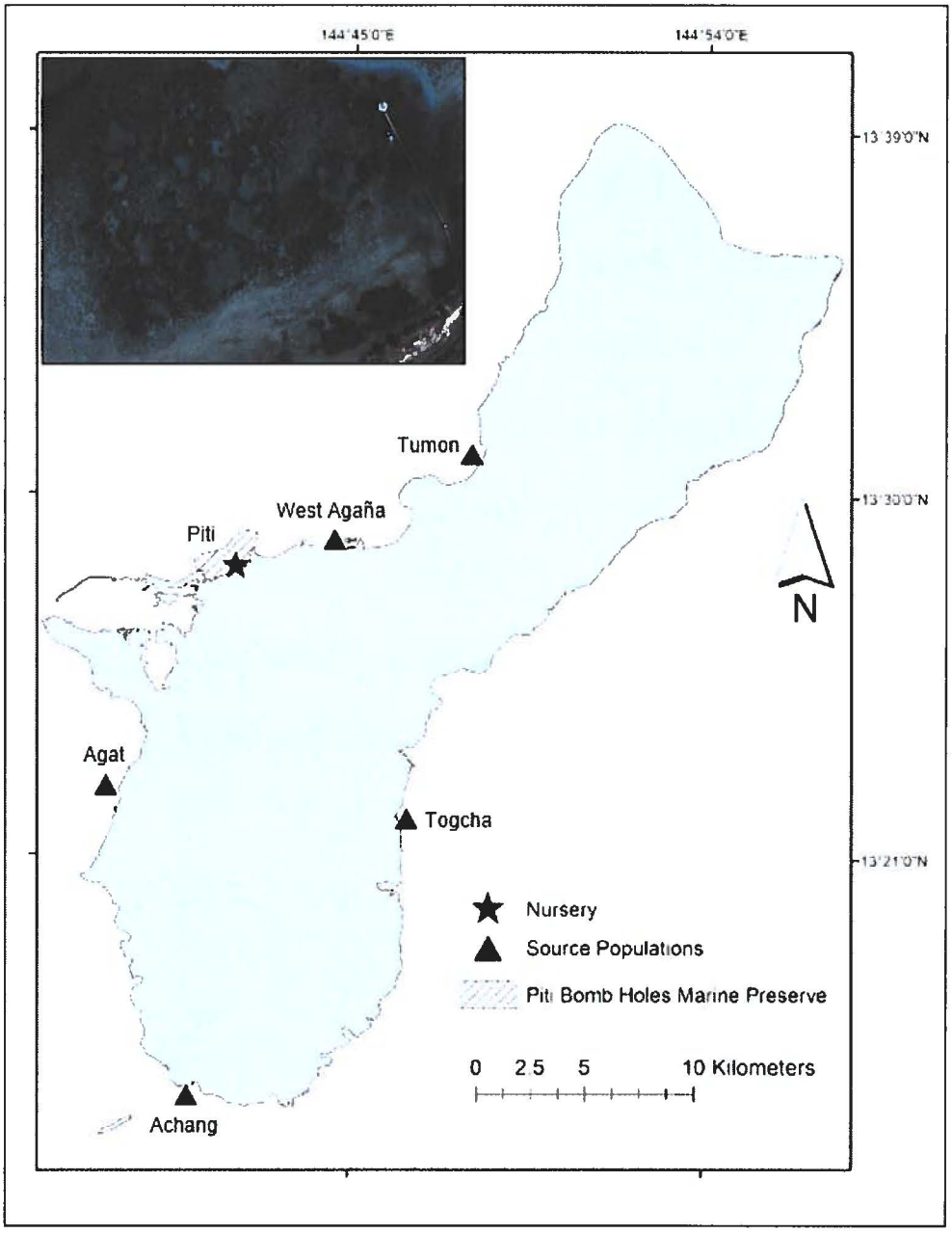

Fig. 1. Map of Guam: the hashed area indicates the boundaries of the Piti Bomb Holes Marine Preserve; the star indicates the site of the nursery with in the preserve; the triangles indicate source populations from which we collected corals. The light blue patches in the inset photo show the natural indentations in the sea floor, or the "bomb holes" for which the preserve is named.

Table 1. List of source population location and the species of staghorn *Acropora* for the *in situ* nursery in Piti Bomb Holes Marine Reserve.

Species	Source Population	Species-Population Code	Initial Fragments	Date Collected
A. cf. *intermedia*	Tumon	TUMACU	58	10/30/2015
A. aspera	Achang	ACHASP	156	10/5/2015
A. muricata	Agat	AGTMUR	84	7/29/2015
A. cf. *pulchra*:	Achang	ACHPUL	48	10/5/2015
	Agat	AGTPUL	96	7/29/2015
	Togcha	TOGPUL	44	9/4/2015
	Tumon	TUMPUL	106	10/30/2015
	W. Agaña	WAGPUL	106	6/29/2015

This study used an existing mid-water suspended nursery in the Piti Bomb Holes Marine Preserve—an area that has natural, large indentations in the sea floor that resemble bomb craters but are actually karst sinkholes (Fig. 1 inset photo). These 'holes' were a desirable site for the nursery for their ability to provide depth and protection from strong currents, waves, and storm activity. The nursery structure was originally deployed in 2013 as part of a SECORE (Sexual Coral Reproduction) International spawning workshop. Three 1.8 m long by 1.3 m wide PVC frames (3.81 cm pipe; schedule 80) were installed at 4.5 m deep, and were labeled A, B, & C. The frames are suspended approximately 1.5 m off of the sea floor, and are chained to cement blocks and attached to buoys to keep them suspended in the water column (Fig. 2).

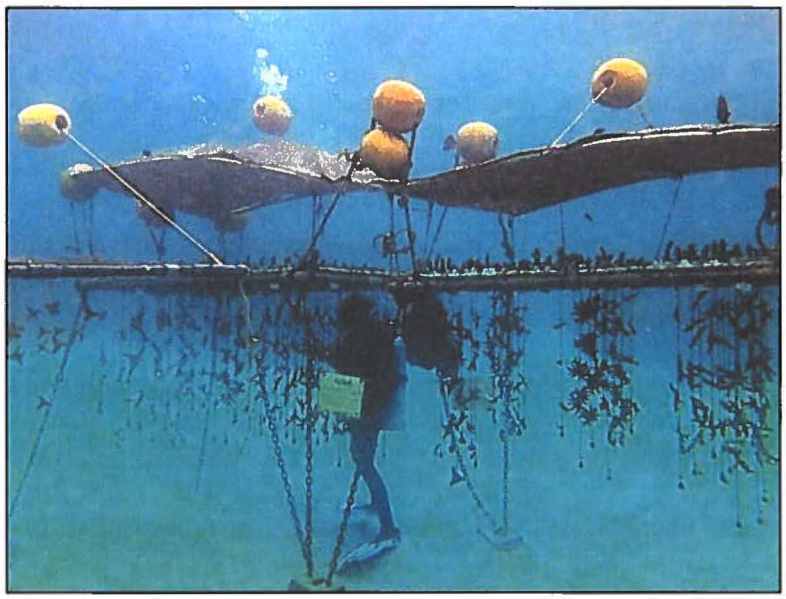

Fig. 2. Picture demonstrating the general set-up and organization of the frames with shade cloths (see p. 16) deployed in the ocean coral nursery in Piti Bomb Holes Marine Preserve. (Photo credit: Laurie Raymundo.)

Collection of Fragments

Coral fragments were collected between of June and October 2015 (Table 1). Unbranched coral fragments ~ 6-7 cm in length were cut from source colonies with wire cutters (no more than 4 branches per colony). To minimize damage and stress to donor colonies, fragment collection did not exceed 10% of total colony size (after Epstein et al. 2001). They were placed into plastic Ziploc baggies filled with fresh seawater. Samples were transported immediately to the nursery site in coolers filled with fresh seawater and SCUBA was used to complete fragment attachment to nursery frames. The scars where donor colonies were fragmented were monitored twice (once a month for two months) to ensure the colonies were not negatively impacted by sampling.

Positioning in Nursery

Initial setup of the nursery in June 2015 mimicked Shafir et al. (2006), with plastic PVC mesh stretched taut across the existing frames to secure coral. Pieces of latex tubing were pulled through the holes of the mesh so that each end came through the top of the mesh, and then one fragment was placed in each end of the tubing (see Fig. 3b). These fragments are referred to as "fixed" fragments. Fragments and tubing were secured into the mesh with ultraviolet-resistant cable ties, organized into rows of 16 across (Fig. 3a & 3b). Fragments were also hung on mildew-resistant string, four down, from the underside of the mesh. These fragments are referred to as "hanging" fragments. Fragments were both fixed and hung to determine of the position in the nursery affects growth. Fragments from each of the six source populations were placed into both fixed and hanging positions on all three frames in the nursery. Fragments of *A.* cf. *pulchra* from Achang were only placed in the fixed position because very few were collected at the time, so they were aggregated in one position. Fragments of *A.* cf. *pulchra* and *A.* cf. *intermedia* from Tumon were all initially hung in the nursery, but none were transferred to the fixed position due to heavy predation (see results).

Fig. 3a. One of the frames in the nursery with the initial design of plastic mesh and latex tubing.

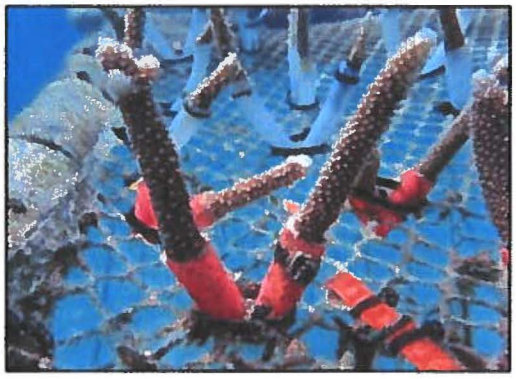

Fig. 3b. Close-up of *A.* cf. *pulchra* fragments from West Agaña in the initial setup of placing fragments in each end of latex tubing and looping it through a hole in the plastic mesh.

After approximately eight months, the plastic mesh began to degrade. Ten months after the start of the experiment the mesh and tubing were removed and replaced with three PVC grids per frame (1.27 cm pipe; schedule 40). Each grid had ten rows with PVC "T" fittings in which the fragments were affixed. The two edge grids held ten rows with five fragments in each row, while the middle grid held ten rows with four fragments in each row (Fig. 4a). Each of the three grids was divided into two sections (five rows each), with each section holding a specific sample population. Each fragment was secured into place with modeling clay and marine epoxy (Fig. 4b). There were occasions where the fragment would detach from the fitting and fall below, making it susceptible to sand abrasion and burial. If the fragment still had at least 10% live tissue, it was placed back in its fitting. If the fragment had less than 10% living tissue, it was considered dead and was removed from the nursery.

Fig. 4a. One of the frames in the nursery after it was rearranged with the three PVC grids.

Fig. 4b. Close-up of the newly arranged *A* cf. *pulchra* fragments from West Agaña secured with the modeling clay and epoxy.

Maintenance, Monitoring, & Environmental Measurements

Nursery fragments were monitored weekly for health (e.g. alive, dead, missing, diseased, detached, predated). Fragments that were dead, diseased, or completely overgrown by algae were removed from the nursery. During these surveys, the frames were also cleaned of algal overgrowth with steel brushes. Bi-weekly cleaning efforts were necessary in the first year of monitoring, but after approximately one year the herbivorous fish population controlled algal fouling sufficiently, so cleaning efforts significantly diminished to approximately once per month.

Shade cloths were deployed in order to prevent intense UV radiation from damaging the corals during the bleaching season, from June to October 2016 (Fig.2). Frames were constructed of PVC (1.90 cm pipe; schedule 40) to match the length and width of the nursery frames. Black shade cloth was stretched across the new frames and attached with cable ties. Buoys were attached to the shade cloth frames to keep them suspended in the water and off of the corals and the shade cloth frames were attached to the nursery frames with rope. Maintenance increased during the period shade cloths were

attached because algae and fouling accrued more quickly. Therefore, scrubbing of the shades and frames was done bi-weekly. Due to the success of the shade cloths in preventing bleaching, they have been deployed annually since that time.

Both temperature and irradiance were compared to growth data to determine if growth rate varied with temperature or correlated with change in light. Water temperature and irradiance in the nursery were recorded with the use of Onset HOBO TidbiT® v2 Water Temperature data loggers and Onset HOBO Pendant® Temperature/Light data loggers, respectively. One HOBO Tidbit® was deployed on each frame for three months at a time, and temperature was recorded every hour in °C. Four HOBO Pendants® were tied to a string, matching the design of the hanging fragments, and irradiance was recorded every hour in lum/ft^2. The pendants were hung in that way to measure the amount of light each level of fragments received on the string. These pendants were only hung on frame A and only recorded for one month.

Growth Measurements

Ten fragments were randomly selected for measuring from each species-population, in each position (fixed and hanging), on each nursery frame (after Levy et al. 2010). Initially, growth was assessed by measuring linear extension (cm) of the central axis of each fragment. However, once data analysis began, this proved to be insufficient. The shape of the staghorn coral fragment changes as it branches and grows; therefore, the central axis of the fragment was no longer the longest axis and could not accurately represent the growth of the fragment. Only considering the linear extension of the original branch as it grows does not take into consideration the morphological change and growth of the coral. Therefore, a new method was tested which involved deriving an

ellipsoid volume representing monthly growth rate (cm^3/month). Kiel et al. (2012) compared total linear extension (TLE) of colonies of *A. cervicornis* and their ellipsoid volume (EV) and found a strong correlation between the two ($R^2 = 0.94$, $p < 0.001$). This relationship was consistently significant regardless of location on the reef (shallow forereef, patch reef, and deep forereef), or origin (nursery-sourced and transplanted, wild-sourced and transplanted, and unmanipulated wild). Thus, ellipsoid volume can be used as a proxy for total colony size. The equation I used for my study follows Kiel et al. (2012):

$$EV = (4/3) \times \pi \times H/2 \times L/2 \times W/2$$

where height (H) is the maximum colony height, length (L) is the maximum colony diameter, and width (W) is perpendicular to maximum colony diameter. To find those dimensions, two photos (using an Olympus Tough TG-4) of each coral were taken with a ruler attached to a clipboard in the background for scale. One photo was taken to acquire height and length (Fig. 5a), then the coral was rotated 90° to the right and a second photo was taken to acquire the width (Fig. 5b). Each photo was analyzed using Digimizer software.

The same process was used to find a mean initial EV (IEV) for each nursery source population (Table A1). The IEV was used because initial photographs with a scale were not taken for every measured fragment, so not every measured fragment would have its own initial EV. To obtain a mean IEV against which to compare individual fragment growth, 30 branches ~6-7 cm in length were randomly selected from each *in situ* source population and species; photographs were taken of each branch and analyzed using the above method. The IEV was subtracted from the final EV (FEV) of each coral analyzed

in the nursery, and then divided by the number of months (t) of growth in nursery. The amount of time that each population was in the nursery varied, so (t) was standardized into the number of months spent in the nursery. The final result was a mean monthly EV growth rate (MEV) for each measured fragment, which was the metric that was used for all growth comparisons, and can be summarized as follows:

$$MEV = [(FEV - IEV) / t]$$

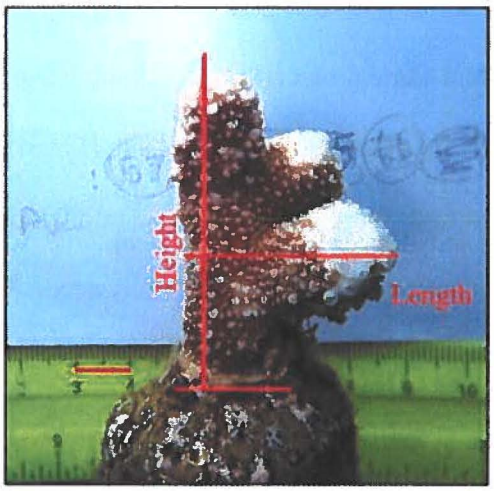

Fig. 5a. Example of the first photo taken of the coral to acquire height and length.

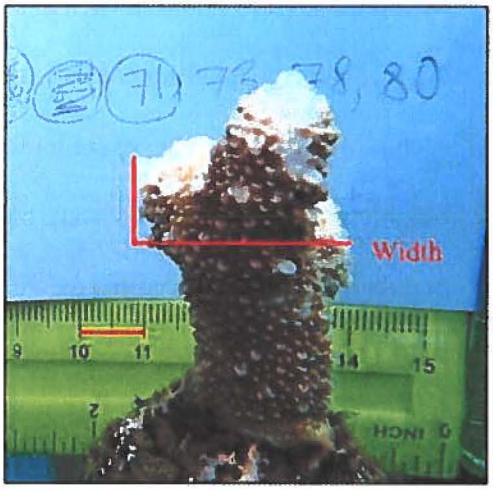

Fig. 5b. Example of the second photo taken of the coral to acquire width.

Control measurements were taken on one branch from five haphazardly selected colonies from each species from each source population (40 branches total). This was to determine how nursery growth rates compared to those observed in nature. Control branches were tagged with a cable tie ~6-7 cm from the tip to be able to find easily, and to standardize where each branch was measured each time. Measurements were taken with hand-held calipers from the base of the cable tie to the tip of each branch (total length in cm). Total length measurements and number of branches (if present) were recorded once a month for three consecutive months between March and May 2016.

Outplanting Experiment

In order to test the effectiveness of the nursery phase on coral performance (i.e. differences in survival, growth, and establishment), an experiment was designed to compare the growth of nursery-sourced fragments to the growth of fragments taken directly from the wild population. Three replicate plots were established on the reef flats of Piti Bomb Holes MP. This site was chosen for its close proximity to the nursery, and each plot (labeled A, B, & C) was chosen for its substrate (a mix of rubble and sand) and coverage by surrounding corals which offers some protection from strong wave action. In each plot, 27 rebar stakes were hammered into the seafloor at 2 m depth (Fig. 6). Fragments of *A.* cf. *pulchra* were collected from the West Agaña wild population. This population is one of the most extensive remaining stands and it is easily accessible. Furthermore, the nursery housed fragments from the same West Agaña source population that would be used in this experiment, so comparisons of performance can account for source site factors.

Each plot housed three experimental treatments: 1) wild-sourced fragments directly laid haphazardly on the substrate, mimicking this species' mode of asexual reproduction via fragmentation; 2) wild-sourced fragments, attached upright with cable ties to rebar stakes, to provide stability and avoid tissue loss from sand abrasion; and 3) 10-month-old nursery-sourced corals originally from West Agaña, also attached upright to rebar stakes. Thus, the nursery-sourced fragments were larger (and some had branches) than the wild-sourced fragments at the start of the outplant experiment. A fourth treatment placed wild-sourced fragments into the nursery to simultaneously evaluate the effectiveness of a nursery grow-out phase on growth performance (Fig. 6).

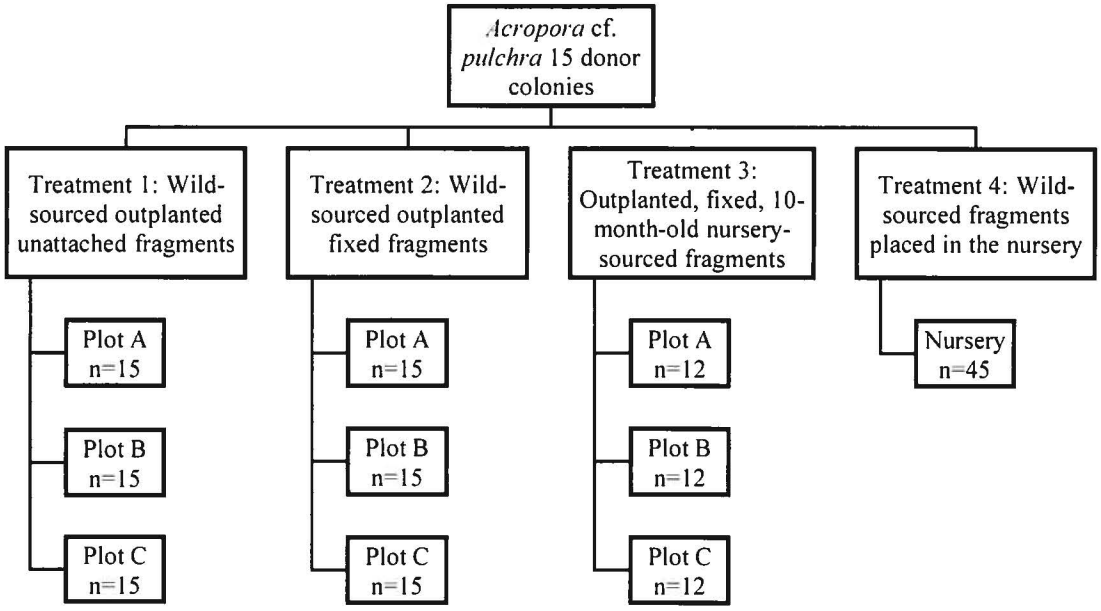

Fig. 6. Experimental design: n= 9 fragments from each wild-source donor colony, 15 donor colonies; N_{Total}= 9 x 15 = 135 total wild-sourced fragments outplanted to plots or placed in the nursery. Each plot holds treatments 1, 2, & 3. Wild-sourced outplanted fragments that go into the plots will either undergo treatment 1 or 2, resulting in 30 wild-sourced outplanted fragments in each plot. N_{Plot}= 30 + 12 = 42 total fragments in each plot. The remaining 45 wild-sourced fragments were randomly placed into the nursery in both the fixed and hanging positions.

A clonal design was used in the setup of the plots: by placing clonal fragments in each of the plots, genetically-based differences in growth or survivorship are accounted for (Raymundo 2001). Clonal fragments (fragments from the same colony) were haphazardly assigned to treatment and position in each plot. Collection and transportation of freshly obtained fragments followed the same protocol as that of the fragments placed in the nursery.

Initial photographs with scale were not taken for every measured fragment, so not every measured fragment would have its own initial EV. Therefore, for treatments 1, 2, and 4 which involved wild-sourced fragments, I used the mean IEV from the West Agaña *A.* cf. *pulchra* population that I found earlier (3.1542 ± 0.9549 cm^3), subtracted it from

the FEV of fragments in the plots, then divided that by the number of months of growth in the plots. Nursery-sourced corals in treatment 3 started out larger than those fragmented from the wild population, so I derived estimated IEV values for those corals at the time they were pruned from the nursery and put into the plots. I used estimated IEV values because as with the nursery fragments, I did not have photographs of the corals when they were initially placed into the plots that were necessary to find true IEV values. I did this by first running a regression between the final height and the FEV of the nursery-sourced corals, to test the strength of the correlation between those two variables. If there was a strong correlation, I could use the initial height measurements that I did have for each coral to derive estimated IEV values. The FEV values were non-normal, so both the final height and the FEV values were log-transformed. With the log-transformed values I found that there was a significant and fairly strong correlation ($r^2 = 0.777$; $p<0.001$), so I performed this process to calculate the estimated IEV, which served as a proxy for initial fragment size. Because those values were found with log-transformed variables, I back-transformed them to get the estimated IEV values. Then I subtracted them from the FEV values, and divided by the number of months of growth in the plots to get the MEV values. Fragment health was recorded monthly, which included monitoring for attachment to rebar or ocean floor, bleaching, algal overgrowth, diseases, predation, and other sources of mortality.

Data Analysis

All data were tested for normality with Shapiro-Wilks test and homoscedasticity with Levene's test, and were transformed to meet those assumptions. For comparisons between position, species-population, and source populations of *A.* cf. *pulchra*

(hypotheses 1, 2, and 3), calculations were performed with square-root-transformed data. Mixed effects one-way ANOVAs were performed on each nursery populations' growth rates to test for significant differences between fixed and hanging fragments. This test was chosen because there between the same position/species-population groups that were on different frames. No analysis was run on *A.* cf. *pulchra* from Achang because there were no fragments in the hanging position, so no comparison could be made for this treatment. Fixed fragments were excluded from analyses for hypotheses 2 and 3 because hanging fragments had such significantly higher growth rates. A two-way ANOVA was performed on all nursery populations' growth rates, with frame and the species-population code (Table 1) as factors. A two-way ANOVA was also performed on growth rates of *A.* cf. *pulchra* from different source populations; the source population and the frame were the factors. Bonferroni t-tests were used in all pairwise comparisons. Measurements for outplant comparisons (hypothesis 4) were non-normal, so statistical analyses are non-parametric. A Kruskal-Wallis One-Way ANOVA on ranks was performed on log-transformed growth rates of all outplanted attached fragments, nursery-sourced corals, and wild-sourced fragments put into the nursery. All data were graphically presented with non-transformed data. Finally, tests were run in the programs R and SigmaPlot.

RESULTS

Fragment Survival, Mortality Rates, & Causes

In total, 698 fragments of four *Acropora* species from five source populations were placed into the nursery over a period of four months. Monitoring of nursery fragments began on 6/29/2015 and ended when the final photos were taken on 7/22/2017,

approximately 25 months later. During this period, overall survival of nursery-grown fragments was 90.5%. The source population with the highest percent survival was *A.* cf. *intermedia* from Tumon (100%), and the population with the lowest was *A.* cf. *pulchra* from Achang (68.7%) (Table 2).

Overall, 66 fragments died or were lost. Of the total, 16 died due to disease and three died due to fish predation. The largest source of morality was due to detachment and subsequent loss or sand abrasion (47) (Table 2). The second largest source of mortality was due to white syndrome, a coral disease (16) (Raymundo et al. 2008). In all instances, the disease first manifested as a small white spot with a distinct barrier between live tissue and dead tissue. The disease rapidly spread and fragments were completely dead within one week. Affected fragments were often clustered and from the same population. The skeletons of dead fragments were removed from the nursery. Though very few fragments died due to predation, the fragments from Tumon experienced severe damage caused by intense predation immediately after attachment in the nursery. The remaining populations in the nursery survived, however, the mortality resulting from predation would have created an effect on their results and future comparisons, so they were excluded from further analyses.

Table 2. Fragment (Frag) survival and causes of mortality broken down by populations. WS = white syndrome, which is what I hypothesize was the observed disease.

Species	Source Pop.	Initial Frags. No.	Total Frags. Died/ Lost	Percent survival	Causes of Mortality		
					WS	Detachment	Predation
A. cf. *intermedia*	Tumon	58	0	100	0	0	0
A. aspera	Achang	156	11	92.9	4	7	0
A. muricata	Agat	84	10	88.1	3	7	0
A. cf. *pulchra*:	Achang	48	15	68.7	8	7	0
	Agat	96	10	89.6	0	10	0
	Togcha	44	10	77.3	0	10	0
	Tumon	106	3	97.1	0	0	3
	W. Agaña	106	7	93.4	1	6	0

Factors Influencing Growth Rates

Position in the nursery had a significant effect on the growth rates of all five applicable populations. For all populations, the hanging fragments had significantly higher growth rates than the fixed fragments (Mixed Effects One-Way ANOVA, $p<0.01$) (Fig. 7).

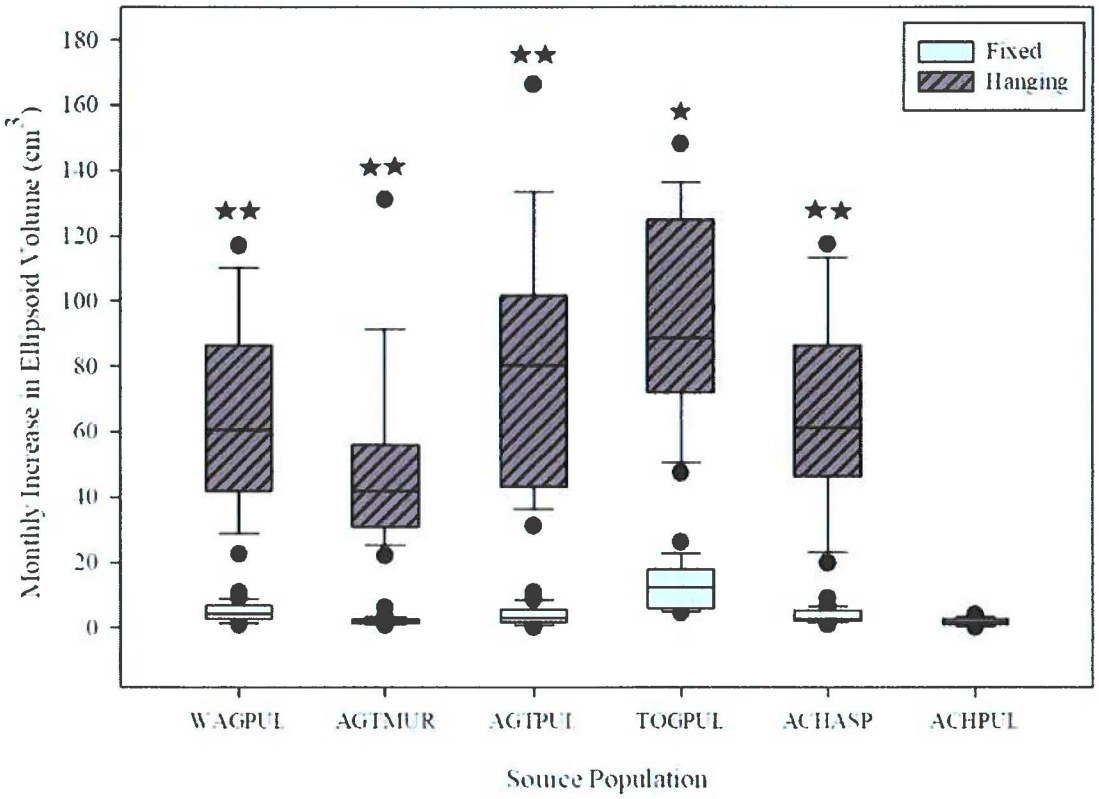

Fig. 7. Mean monthly ellipsoid volume (EV) growth rate comparing fixed coral fragments and hanging coral fragments, separated by source population. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median. Whiskers above and below represent the 90th and the 10th percentile, respectively. Circles above and below represent the 95th and 5th percentile outliers, respectively. Asterisks represent levels of significance ★ = <0.01; ★★ = <0.001; all assessed at $\alpha = 0.05$.

Fixed fragments were excluded from all further analyses—values are representative of hanging fragments only. Species-populations had significantly different growth rates ($p<0.001$) (Fig. 8), but frame did not have a significant effect on growth rate ($p=0.070$), nor did the interaction between the two ($p=0.359$). All pairwise comparisons between species-populations can be found in Table 3.

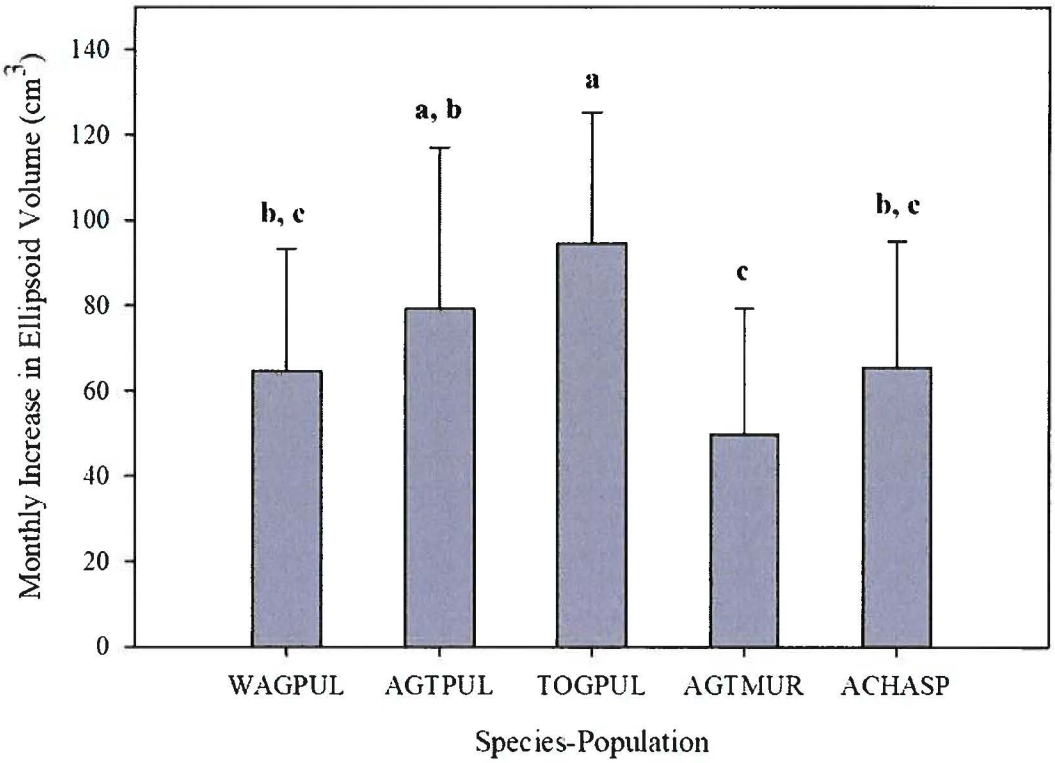

Fig. 8. Mean (± SD) monthly increase in ellipsoid volume of coral fragments analyzed by species-populations. Groups labeled with the same letters are not significantly different from one another at α=0.05.

Table 3. All pairwise multiple comparison procedures for the species-population factor (Bonferroni t-test).

Source Populations	P-value
TOGPUL vs. AGTMUR	**<0.001**
AGTPUL vs. AGTMUR	**0.001**
TOGPUL vs. WAGPUL	**0.008**
TOGPUL vs. ACHASP	**0.009**
ACHASP vs. AGTMUR	0.194
WAGPUL vs. AGTMUR	0.205
TOGPUL vs. AGTPUL	0.515
AGTPUL vs. WAGPUL	1.000
AGTPUL vs. ACHASP	1.000
ACHASP vs. WAGPUL	1.000

Fragments of *A.* cf. *pulchra* from some different source populations grew significantly differently ($p=0.003$). Fragments from Togcha had significantly higher growth rates than those from W. Agaña (Bonferroni t-test, $p=0.002$), while fragments from Agat were not significantly different from either W. Agaña or Togcha. (Fig. 9; Table 4).

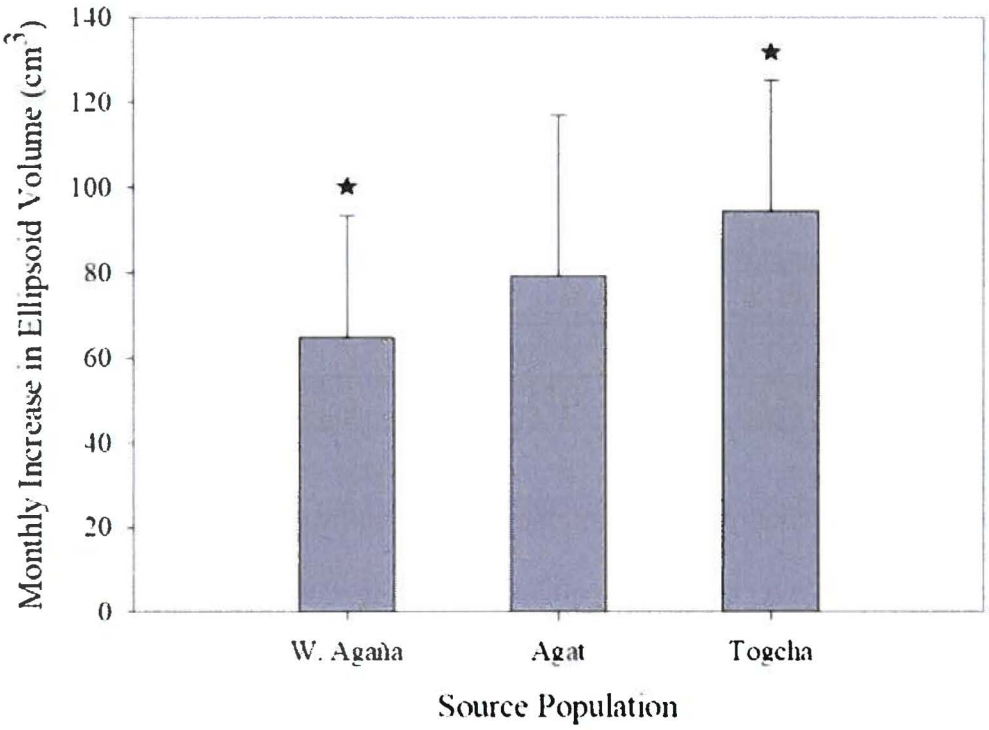

Fig. 9. Mean (± SD) monthly ellipsoid volume growth rate of hanging *A.* cf. *pulchra* fragments separated by source population. ★ = significant difference between the two populations at $\alpha = 0.05$.

Table 4. All pairwise multiple comparisons between fragments of *A.* cf. *pulchra* from different source populations and their respective P-values (Bonferroni t-test).

Position	Source Populations	P-value
Hanging	Togcha vs. W. Agaña	0.002
	Togcha vs. Agat	0.154
	W. Agaña vs. Agat	0.275

Fragments grown on different frames also had significantly different growth rates (Two-Way ANOVA, p=0.016). Fragment growth rates from both frame B and C were significantly greater than those from A (Bonferroni t-tests, p=0.037 and p=0.034, respectively) but there was no difference between B and C (Table 5). The interaction

between source population and frame did not have a significant effect on the growth rate of any of the populations.

Table 5. All pairwise comparison between hanging fragments of *A.* cf. *pulchra* on different frames and their respective P-values (Bonferroni t-test).

Frames	P-value
A < B	0.034
A < C	0.037
B = C	1.000

In *in situ* control populations, there was a significant difference in mean branch length between source populations after one month (p=0.043) and also a significant difference in mean branch length after two months (p=0.013). WAGPUL grew significantly longer than branches of TOGPUL, AGTPUL, and AGTMUR, but not ACHASP or ACHPUL. There was no significant difference in mean branch length between source populations on initial survey in 03/2016. After one month in 4/2016, branches of WAGPUL were significantly longer than branches of TOGPUL, AGTPUL, and AGTMUR (One-Way ANOVA, p=0.007, p=0.041, and p=0.005, respectively) (Fig. 10). After another month in 5/2016, branches of WAGUL were again significantly longer than branches of TOGPUL (p=0.006), AGTPUL (p=0.018), and AGTMUR (p=0.001). Also, branches of *A. aspera* were significantly longer than branches of both *A.* cf. *pulchra* from Togcha (p=0.045) and *A. muricata* (p=0.014) (Fig. 10).

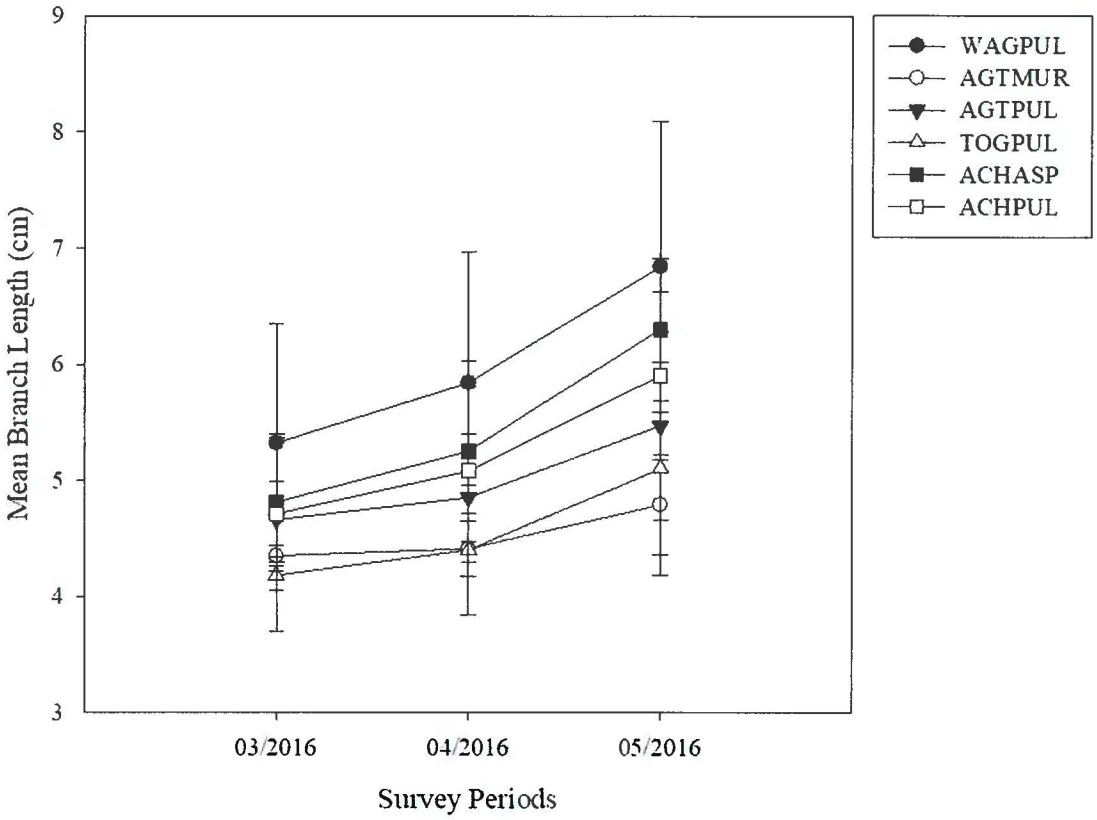

Fig. 10. Mean (± SD) length of branches from control populations, measured *in situ*. Lengths represent the mean of five branches from each *in situ* source population (n=5).

Outplanting Experiment

To test the effectiveness of the nursery phase on coral performance (i.e. survival, growth, and establishment), I designed an experiment to compare the growth of nursery-sourced fragments to the growth of wild-sourced fragments taken directly from the wild population. We also compared the growth of wild-sourced fragments growing in the nursery to fragments transplanted directly from the wild population to the outplanting site, without a nursery phase. Percent survival of plot treatment in order of highest to lowest is as follows: attached wild-sourced 100%; attached nursery-sourced 97.2%; wild-sourced in nursery 48.9%; loose wild-sourced 0%. Both nursery-sourced corals and wild-

sourced fragments growing in the nursery had significantly greater growth rates than the wild-sourced attached fragments (t-test, $p<0.001$), and wild-sourced fragments in the nursery also had significantly higher growth rates than nursery-sourced corals (Mann-Whitney Rank Sum test, $p<0.001$) (Fig. 11). By the end of the monitoring period, all loose fragments died due to sand abrasion and/or smothering (Table 6). For this reason, they were not included in statistical analyses.

Table 6. Percent survival of each treatment pooled across plots.

Treatment	Initial Frags. No.	Total Frags. Died/Lost	Percent Survival
Loose: Wild-Sourced	45	45	0
Attached: Wild-Sourced	45	0	100
Attached: Nursery-Sourced	36	1	97.2
Wild-Sourced in Nursery	45	23	48.9

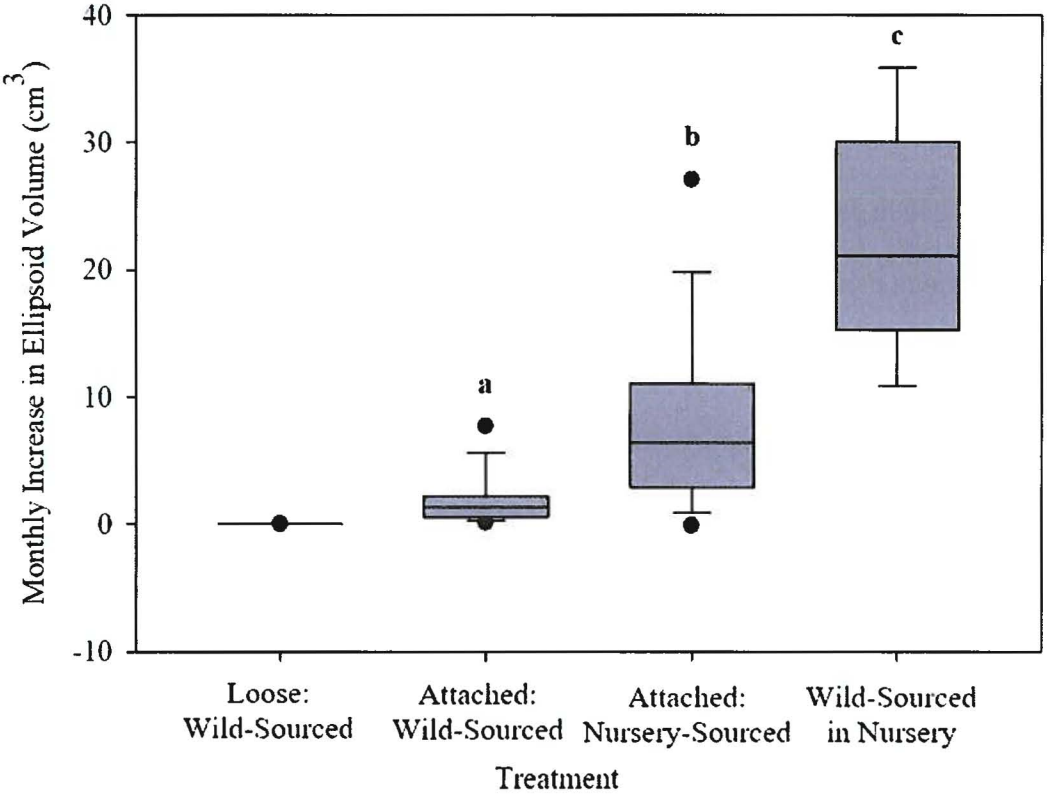

Fig 11. Mean monthly change in ellipsoid volume of wild-sourced outplants that were loose, attached, and in the nursery, and nursery-sourced outplants that were attached. Boxes represent the 25th and 75th quartile range, horizontal cross lines represent the median. Whiskers above and below represent the 90th and the 10th percentile, respectively. Circles above and below represent the 95th and 5th percentile outliers, respectively. Groups labeled with different letters show a significant difference ≤ 0.001 at $\alpha=0.05$.

Maintenance, Monitoring, & Environmental Measurements

In the beginning of the experiment, we cleaned the nursery frames and strings extensively. However, one year after placing the first fragments into the nursery, several species of herbivorous fishes (Table A13) had recruited to the nursery. With an increase in fish herbivory keeping the nursery clean from various types of biofouling, human cleaning efforts significantly decreased thereafter.

Monthly mean temperature either met or exceeded the bleaching threshold temperature of 30°C seven times during the 25-month-long monitoring period (Fig. A1), and either met or surpassed the maximum monthly mean sea surface temperature (SST) recorded in all but nine months (NOAA Coral Reef Watch 2015) (Fig. A1). The minimum mean monthly temperature was in Feb. 2016, at 27.28 °C, and the maximum was in Oct. 2015, at 31.1 °C.

There was a significant difference in light attenuation/availability at different hanging positions on the strings (Kruskal-Wallis One-Way ANOVA on ranks, $p<0.001$) (Fig. 12). Position 3 (third down the string from top to bottom) received significantly less light than positions 1, 2, and 4, but there was no significant difference between positions 1 and 2, 1 and 4, or 2 and 4. A two-way ANOVA was performed on the MEV of hanging fragments from frame A (where the light loggers were placed), and no significant difference was detected in either of the factors with species-population ($p=0.192$) and position on the string ($p=0.180$), nor in the interaction between the two ($p=0.167$).

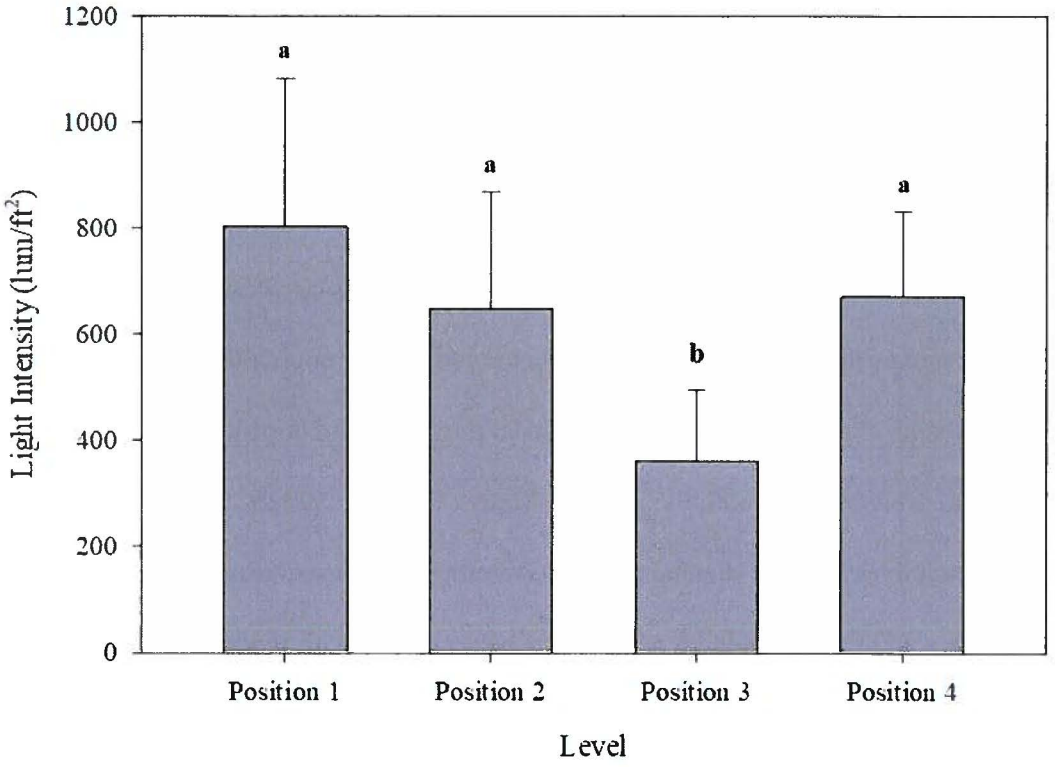

Fig. 12. Mean (± SD) light intensity of each level of hanging fragments after one month of monitoring. Light loggers were placed on the outside of the frame, and only on frame A. Columns represent means for each level. Groups labeled with the same letter are not significantly different from one another at α=0.05.

DISCUSSION

Through this study, I found several results that are important to consider when culturing staghorns, regarding how to grow them, which corals to sample, and whether or not the nursery grow-out phase is an efficient method for rehabilitating Guam's reefs. The first result is that fixed fragments grow significantly less than hanging fragments, independent of the species or source population. I found that different species-populations of staghorns grow at different rates, and for Guam's staghorns, *A.* cf. *pulchra* from Togcha had the highest growth rate. I also found that nursery-grown corals grew significantly more than wild-sourced fragments from the same source population, proving

the effectiveness of the nursery phase on coral growth and survival. Together, these results demonstrate that the nursery grow-out phase is a viable tool for reef rehabilitation.

Fragment Survival

The leading cause of mortality was detachment from the nursery structure and subsequent abrasion or burial in the sand. Other studies cite detachment from the nursery structure as the main cause of mortality as well (Shafir et al. 2006; Putchim et al. 2008). Fragments that lost live tissue from partial burial or sand abrasion, but still had some live tissue, were put back into the nursery. Many of those fragments recovered and tissue re-sheeted over the bare skeletal patches. But after the nursery was redesigned with the PVC fittings and fragments were meant to be held in place with modelling clay, the fragments did not hold in place as well as anticipated. After experiencing these loses, Z-SPAR A-788 Splash Zone two-part epoxy was used to better secure the fragments.

The second most common cause of mortality was due to disease, and all cases appeared to be white syndrome (Raymundo et al. 2008). The affected corals showed white, bare skeleton, without a distinct band separating the skeleton from living tissue, followed by rapid total mortality (1-2 weeks). All cases were separate populations, but diseased fragments were contained in one spot within that population, where only 1-3 fragments were affected in each instance/episode/event. Disease is known to affect damaged corals more easily than healthy corals (Brandt et al. 2013; Bright et al. 2016), so it is not surprising that the open wounds on the coral fragments caused by transplantation made them more susceptible to disease, even when the source colony appeared healthy.

Sweet & Brown (2016) point out that many coral pathogens actually reside within the coral, and only become harmful when the coral surpasses a stress threshold. This

could happen when stress due to physical damage is compounded with thermal stress, which is also known to hinder the coral's immune responses and make them more susceptible to disease (Palmer et al. 2010; van Woesik & Randall 2017). This may be the case with *A. muricata* which was placed into the nursery in July 2015. That month, and the two following it, had temperatures above the max monthly mean SST, and even surpassed the bleaching threshold (Fig. A1). Local bleaching thresholds can vary (Jokiel & Coles 1990; Buddemeier & Fautin 1993), and any exceedance as small as 0.1°C beyond the local mean SST can cause corals to become more susceptible to bleaching (McWilliams et al. 2005). So, it stands to reason why that population had fragments that became diseased. Furthermore, in some instances, disease has been known to take some time to affect the coral after it experiences stressors (Brandt & McManus 2009). This could also explain why *A. aspera* fragments, although placed in the nursery in the cooler month of October 2015, did not become diseased until nearly three weeks after placement in the nursery.

As for the vector of disease, water-borne transmission, albeit a possibility, seems unlikely because more fragments would have been affected on a larger spatial scale, instead of the clustered instances that occurred in this experiment. The clustered nature may also suggest the host corals were stressed and possibly already diseased. Another possible source of transmission would have been by a corallivorous fish, and it is well documented that these organisms are often considered to be vectors of disease (Sussman et al. 2003; Abey 2007; Raymundo et al. 2009; Bright et al. 2016).

While predation was the rarest source of mortality, it was still a serious impact on the nursery populations. Nearly 100% of fragments of *A.* cf. *pulchra* and *A.* cf.

intermedia from Tumon experienced predation after being placed in the nursery (as evident by the healing of the wounds I observed two weeks later). Damage and mortality due to predation could be expected as corallivorous marine life—such as snails, butterflyfish, and parrotfish—are known to be attracted to stressed or damaged corals (Knowlton et al. 1990; Morton et al. 2002; Bright et al. 2015; Bright et al. 2016). Throughout the process of coral collection, the intention was to transplant the corals into the nursery within two hours of harvesting to avoid the water in their container getting fouled and possibly stressing the corals further. However, I hypothesize that because the corals from Tumon were bagged for longer than two hours, the transportation process could have caused severe stress, and therefore caused the coral fragments to secrete a mucus or chemical cues that attracted the corallivorous fish predators.

When corals are physically damaged, such as when they are cut from the donor colonies, they release mucus containing chemical cues (Daumas & Thomassin 1977). Many studies suggest that those secretions are what attract the corallivorous predators. The invertebrates, *Drupella rugosa* and *Coralliophilia abbreviata*, were both found to be more attracted to damaged pieces of *Acropora* spp. over undamaged pieces (Morton et al. 2002; Bright et al. 2015). Butterflyfish preferentially feed on open, diseased lesions on species of *Acropora* (Chong-Seng et al. 2011), and correlations were found between higher levels of damselfish predation and *A. cervicornis* damaged from a natural disturbance (Knowlton et al. 1990). However, based on the amount of tissue and skeleton taken, it is unlikely that corallivorous butterflyfish were the predators, and the fact that predation was on hanging fragments where benthic predators could not access eliminates snails. Damselfish are more capable of removing chunks of skeleton when scraping the

coral tissue (Rotjan & Lewis 2008), but they rarely predate upon corals unless in the process of creating farming territories (Kaufman 1977). Parrotfish, however, are known for the way they intensely scrape and excavate corals, often removing much of the live tissue and skeleton (Bellwood & Choat 1990). Furthermore, McIlwain & Jones (1997) found that corallivorous wrasses, *Labrichthys unilineatus*, were more attracted to damaged corals than undamaged corals, again citing the corals' secretions as the reason for the increased attraction. Therefore, based on the extent of the damage and the way in which much of the skeleton was gone, I hypothesize that parrotfish were the predators (Table A13).

Growth Measurements

Position: Fixed vs. Hanging

When comparing the position of fragments in the nursery, the hanging fragments had significantly higher growth rates across all of the source populations (Fig. 7). Other studies have seen this same result when comparing these two methods. Lirman et al. (2014) found that *A. cervicornis* suspended on ropes had significantly higher annual productivity (6.6 cm) than corals fixed on a frame (4.8 cm). Hernández-Delgado et al. (2014) found that hanging fragments of *A. cervicornis* resulted in significantly higher total lengths (40.0 cm/yr) compared to fragments fixed on a frame (27.0 cm/yr). What is also interesting is the major difference observed in fragment morphology. While I did not record fragment diameter, I observed that fixed fragments were wider than their hanging counterparts (Fig. 13a & 13b). These results were also seen by Kuffner et al. (2017), who found that fixed corals had significantly lower linear extension rates but also greater skeletal densities than hanging fragments, suggesting plasticity in those traits.

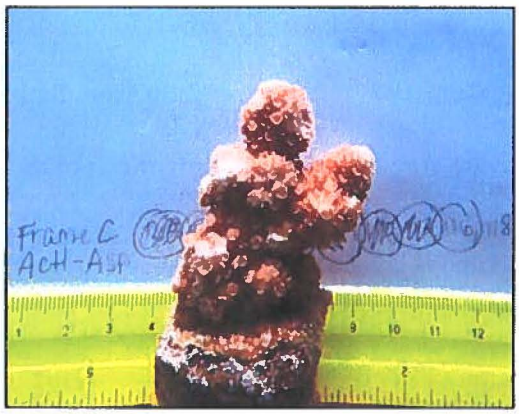

Fig. 13a. A fragment from *A. aspera* population in the fixed position, demonstrating shorter length but a thicker diameter.

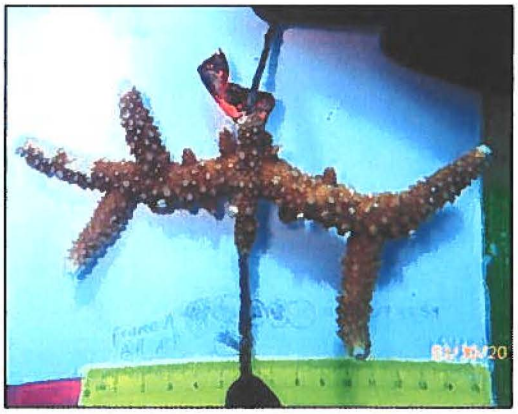

Fig. 13b. A fragment from *A. aspera* population in the hanging position, demonstrating longer lengths but a thinner diameter.

I hypothesize that fixed fragments put more energy into stabilizing themselves onto their substrate and strengthening their skeletons, because they likely experience greater force from water currents. Hanging fragments, however, are able to move with the water and do not need to fortify themselves as much, and so they can put their energy into growing in multiple directions instead of in one direction (Bottjer 1980; O'Donnell et al. 2014). Relating this to outplanting, these different growth strategies may have varying levels of success once outplanted. Shorter, denser outplants may be successful because the outplanting technique is similar to their nursery environment, being fixed in place and receiving greater force from water currents. However, shorter outplants are closer to the ocean floor and thus at greater risk of being smothered by sand or sediment, whereas taller outplants would be higher up and away from sand and sediments, but may be at higher risk of breakage due to having a thinner skeleton.

Other possible explanations include the hanging fragments' ability to have more all-over-contact with water in the water column, and therefore be open to more heterotrophic feeding, resulting in higher growth rates. Jokiel (1978) suggests that

increased water motion delivers more planktonic food, and could explain the increased growth in his experiment of water motion on growth of corals. Becker & Mueller (2001) found that the corals in their experiment that received more water flow grew faster than those that received less water flow, and they suggest a correlation between greater water flow and greater plankton availability to explain the faster growth. Also, Wijgerde et al. (2012) found that both single polyps and colonies of *Galaxea fascicularis* captured and retained more zooplankton when in higher flow rates, concluding that water flow rate has a significant effect on amount of prey corals are able to capture.

Finally, consideration should be made regarding the fixed fragments receiving more direct photosynthetically available radiation (PAR), which has been correlated with decreased growth (Mercado-Molina et al. 2016). I only deployed light loggers along the four hanging levels on the string—none were deployed in the fixed position. However, the light received on the first hanging level was significantly greater than that on the third hanging level (Kruskal-Wallis One-Way ANOVA on ranks, $p<0.001$) (Fig. 13), which suggests that fragments higher up receive more light, and likely more PAR. I hypothesize then that fixed fragments received more PAR than some of the hanging fragments, which could explain why the fixed fragments had lower growth rates than the hanging fragments. However, because there was no significant difference detected between the MEV's of frame A's hanging fragments in the different positions on the string, I conclude then that light received by the hanging fragments did not have an effect on their growth.

Between-Species Comparison

The genus *Acropora* is generally fast-growing (Alcala et al. 1979; Alcala et al. 1981; Yap & Gomez 1981), and many Acroporids are used in restoration activities around the world (Yap et al. 1992; Soong & Chen 2003; dela Cruz et al. 2014; Xin et al. 2016; Coelho et al. 2017). However, when considering localized restoration efforts, it is useful to know which species of *Acropora* grow the most, and therefore which species would grow well in large-scale restoration efforts. This study found that *A. muricata* had the lowest growth rates (49.55 ± 29.72 cm^3/month) of the five species-population groups that were tested, almost half the growth rate of *A.* cf. *pulchra* from Togcha which had the highest growth rates (94.51 ± 30.73 cm^3/month) (Table A8). Putchim et al. (2008) found comparable results in that *A. muricata* had a significantly slower linear extension growth rate compared to *A. grandis* in a nursery (0.23 cm/month vs. 0.41 cm/month, respectively). Mbije et al. (2013) also found that *A. muricata* grew significantly slower than *A. nasuta*, the ecological volume of *A. nasuta* increased 12 and 17 times in two experimental plots, while *A. muricata* only increased 6 and 7 times.

My findings are supported by the literature in that *A.* cf. *pulchra* has some of the highest growth rates among the commonly propagated species of *Acropora* (Yap & Gomez 1981: 1.5 cm/month; Soong & Chen 2003: 1 cm/month). Dela Cruz et al. (2014) looked at the ecological volumes of two transplanted species of *Acropora* and found that transplanted *A. pulchra* had significantly higher growth rates compared to *A. intermedia* (1333.2 vs. 934.20 cm^3/month, respectively). In my nursery study, I found that the growth rates of *A.* cf. *pulchra* from all three source populations were also either similar or higher than the other two species-populations of staghorns used. Yucharoen et al. (2013),

however, found that *A. aspera* had higher growth rates than both *A. pulchra* and *A. formosa* (9.8 vs. 9.2 vs. 7 cm/year, respectively). Those results are also comparable to my study though in that the growth rates of *A. aspera* were very close to those of one population of *A.* cf. *pulchra* from West Agaña.

There are a number of ways in which differences in growth rates could be further assessed, and the first suggestion would be to focus on the method of assessing growth. The best way to measure growth would be to establish growth intervals by measuring the fragments every two to three months. This was the original intention of this study, however because the original method of collecting growth data was insufficient, the new method could only be based on an initial and final measurement. With interval measurements, it is possible to get more precise growth rates. Also, with interval growth rates, it would then be possible to detect post-transplant stress and seasonal effects which can help to explain growth variation.

Furthermore, it would be helpful to have genetic samples of populations of each species from more than one source population to determine if growth rate differences can be explained by genotype. Because *A.* cf. *pulchra* is the most common and abundant staghorn on Guam, it is easy to sample from more than one source. Species such as *A. muricata* are far less abundant, and *A. aspera* has been reduced to only one population on Guam. Other staghorn populations found on Guam, such as *Acropora austera* and *Acropora teres*, were also severely depleted after the 2013-2014 bleaching events, and only rare, scattered, small populations remain, making it difficult to sample from multiple source populations for culturing. In conclusion, this suggestion may be limited to only *A.* cf. *pulchra, A. muricata*, and *A. intermedia* but those would still be strong comparisons to

make in order to more reliably say one species grows more than another, or if differences could be linked to environmental conditions instead.

Source Population: Comparing fragments of *A.* cf. *pulchra*

Comparing the fragments of *A.* cf. *pulchra* populations produced two results: fragments from Togcha had significantly higher growth rates than the fragments from West Agaña, but not Agat; and secondly, there was an effect of the frame on growth.

Branches from West Agaña *in situ* populations had the highest rates of linear extension, and branches from Togcha had the second lowest rates of linear extension. Branches from Agat were lower than those from West Agaña, but higher than those from Togcha. I expected to see similar results in the nursery, but it was the exact opposite: fragments from Togcha had significantly higher growth rates than those from West Agaña, and were also higher than those from Agat. Variation could be explained by intraspecies plasticity in growth rates. Kuffner et al. (2017) experimented with *A. cervicornis* in a nursery grow-out phase and concluded that extension rates are plastic traits that varied on environmental factors. Mass & Genin (2008) also found that morphological differences in *Pocillopora verrucosa* were highly plastic and were strongly influenced by environmental conditions, in their case levels of water flow intensity. Therefore, I hypothesize that the intraspecific variation is due to environmental differences. The three source populations are exposed to very different habitats: Togcha is windward, experiencing high wave energy for much of the year, whereas West Agaña and Agat are leeward and are relatively sheltered. Bottjer (1980) found that the direction that branches of *A. cervicornis* grew was correlated with the amount of wave energy the colonies received, meaning that low wave energy colonies grew vertically, whereas high

wave energy colonies grew more horizontally and with the direction of the current. This would explain then why the *in situ* branches from Togcha grew less than those from West Agaña and Agat.

Once the Togcha fragments were put into the calmer waters of the nursery, they had the chance to grow much more than they would otherwise in the wild populations, whereas the West Agaña and Agat fragments were experiencing similar conditions as they did in their source populations. Studies have shown that coral colonies can undergo morphological changes or changes in growth patterns when moved to a site with different environmental conditions (Foster 1979; Bruno & Edmunds 1997; Rocker & Brandl 2014; Drury et al. 2017). This suggests that environmental conditions are a significant driver of corals phenotypic responses, and that often corals will change their growth based on the environmental conditions. Therefore, only comparing growth of the wild populations may not be sufficient when deciding which source populations to sample from for restoration purposes. Based on control data, the West Agaña population would be the best to supply the nursery, followed by Agat, and Togcha would not be a strong source to consider. However, after looking at the nursery results, the Togcha population should definitely be a priority.

Two problems arise when considering sampling from the Togcha wild population: it is one of the more difficult sites to access of the *A.* cf. *pulchra* populations from which we sampled; also, it suffered significant declines after recent bleaching events. Raymundo et al. (2017) estimates that after the 2014 bleaching event, the staghorn population at Togcha experienced a mean 65% mortality rate. The staghorn *Acropora* population in West Agaña experienced a mean 55% mortality rate, however that

population was significantly larger before the bleaching event. Even a slightly greater mortality rate at Togcha would have a larger effect on its population than a slightly smaller mortality rate on the West Agaña population. So, while sampling from the Togcha population may still be viable, sampling effort would have to be less than it could be on the other populations of *A.* cf. *pulchra*, like at Agat which only saw a mean 25% mortality rate, or Achang which saw a mean 30% mortality rate, both of which ended with an ~8-10 times larger area post-bleaching than Togcha. With all of this in mind, Togcha should remain a valid donor site, but caution should be made on how much is sampled. Agat is also a good source to consider, because fragments from there had higher growth rates than West Agaña, and it still has a large remaining stand of staghorns from which more can be sampled.

The second result to consider is how or why the frame position influenced the growth rates. At present, frames are lined up in a row, with frame A closest to the reef break, and frame C, the furthest inside the bomb hole. However, because the frames are the same in all other regards, it is unlikely that any other environmental conditions would explain the frame effect. Furthermore, the frame effect was only seen in the test between fragments of one species, *A.* cf. *pulchra*, and not between all species, which points towards intraspecific stochastic variation in growth.

Outplanting Experiment

The final part of this study was to determine if nursery-sourced fragments would perform better than fragments that were directly transplanted from wild-sourced populations. When considering restoration approaches, a cost-benefit approach is a productive way to compare strategies. Studies have shown that larger transplants survive

better (Harriot & Fisk 1988a; Harriot & Fisk 1988b; Bowden-Kirby 1997; Smith & Hughes 1999). It is logical then that transplanted corals that have had a nursery grow-out phase, in which they can develop to a larger size, survive better and grow more than smaller fragments directly transplanted from wild populations (Sujirachato et al. 2013). Okubo et al. (2005) confirms this hypothesis, when they found survival rate of transplanted fragments to be positively correlated with fragment size—larger fragments had higher survival rates. Yucharoen et al. (2013) reported low survival of smaller *A. formosa* when transplanted onto the reef, while their larger, nursery-grown fragments had higher survival rates. If taking small fragments from the wild and growing them out to a larger size shows positive results, then it is a viable alternative to taking larger fragments from wild populations, which puts more stress on donor colonies. My results agree with the current literature on this topic—fragments given a nursery grow-out phase will grow more than fragments directly transplanted from the wild population.

Furthermore, Lirman et al. (2014) found that wild-sourced fragments of *A. cervicornis* grown in a nursery had significantly higher growth than nursery-grown corals transplanted onto the reef. My results also agree with theirs, as I found that wild-sourced fragments simultaneously grown in a nursery grew more than nursery-grown corals from the same source population once transplanted to the reef. This result is of particular interest to me, because the wild-sourced fragments in the nursery experienced heavy predation upon nursery placement, and yet they still had significantly higher growth rates than the nursery-reared corals that were outplanted. Lirman et al. (2014) compared fixed wild-sourced fragments to the nursery-grown outplanted fragments and still found that the wild-sourced grew significantly more. These results confirm the effectiveness of the

nursery grow-out phase when considering using the coral gardening concept in reef rehabilitation.

To further test the idea that bigger size equates to higher survival and growth rates, experiments may compare nursery-sourced corals to larger, directly-transplanted corals of the same size. Caution, should be taken, however, as a main focus of coral gardening with a nursery is to reduce dependence on collecting from wild population donor colonies as a means of active restoration. A main reason that a nursery is a valuable tool is because it can be used as a source population for repeated propagation, as long as large enough nubbins are left in the nursery post-pruning so that they can regenerate. This reduces dependence on wild populations to seed restoration activities. So, while a small-scale experiment would be valuable to test the question of bigger size means more growth, it should be done with caution and with respect to the size of the donor colonies and the size of the wild source population.

Furthermore, all 15 wild-sourced, loosely laid fragments in each plot (45 total) were either lost or had died by the end of the monitoring period. This treatment was designed to mimic staghorns' natural mode of asexual reproduction, by which pieces break off and have the potential to form new colonies elsewhere. While fragmentation is considered an effective form of asexual reproduction for wild populations, the results of this study suggest that it is not an effective method for active restoration efforts.

RECOMMENDATIONS

Because the leading cause of mortality to fragments was detachment from the fixed position, recommendations for securing the fragments in the future include using a common method of attaching the coral fragment with a strong glue or epoxy to a small

disk or ramet (Rinkevich 1995; Shafir et al. 2001; Shafir et al. 2006; Shaish et al. 2010; Kuffner et al. 2017; O'Donnell et al. 2017). While this may cause minor death on the glued end of the fragment, or possibly initial negative growth, it would mean the fragment is more secured for the long-term and would result in fewer corals dying from detachment. This may also help to avoid the spread of disease, the second leading cause of fragment mortality. After cleaning the coral of any debris in fresh seawater, gluing the end of the fragments acts like a cauterizing process to seal off the open wound, which would prohibit potential water-borne diseases from infecting the fragments. Furthermore, sealing the wound would prohibit the release of stress hormones that may attract corallivorous predators. This would also make the corals easier to move for transplantation, etc., as they would already be settled on a substrate.

Further recommendations to tease apart variability in growth rates should focus on differences in genetics and environmental conditions—both within source populations and in the nursery. Future studies should collect samples from multiple populations of the same species (where possible) in order to determine the genetic relatedness between the populations. If there is high genetic structure (or low genetic relatedness), that may help explain why one population performed better or worse than others (Goergen et al. 2017). This may also help to resolve the taxonomic questions around *A.*cf. *pulchra* and *A.* cf. *intermedia*.

Measuring environmental parameters would also be a useful tool to explain differences in growth. Loggers should be installed to quantify water flow and light received in the nursery in different places within the hanging fragments, and also throughout the fixed fragments. With these measurements, correlations could be made

between the amount of water flow or light that different fragments receive in different positions, and differences in growth. These data may also help to explain the frame effect that was detected in the comparison of fragments of *A.* cf. *pulchra*. Furthermore, in conjunction with this environmental data, interval growth rates would be useful to determine if there is any seasonal effect on the growth rates (e.g., if more light or higher temperature received during the summer months resulted in slower growth). Finally, tide loggers and water flow loggers should be installed in the source populations in order to determine if significant differences exist which could further explain variation in their growth rates.

CONCLUSIONS

Coral reefs are critical ecosystems for innumerable marine organisms and play a vital role in sustaining biodiversity on Earth. As reefs continuing to decline worldwide, considerations on how to mitigate losses are crucial. While the possibility for corals to adapt to the warming oceans exists, the feasibility of this remains unclear. On the other hand, many studies have shown positive results when using methods of active reef restoration to explore the possibilities of restoring dead or dying reefs, . These data provide clear evidence that the best species of staghorn *Acropora* to grow in an *in situ* coral nursery is *A.* cf. *pulchra*, and that they should be allowed to hang freely on strings in order to optimize growth rates. Furthermore, this study demonstrates that using a coral nursery in the coral gardening concept of reef restoration is an effective means to grow corals that will survive better once outplanted, compared to direct transplantation from wild populations. Finally, this study is particularly beneficial as a launchpad from which

to develop further useful techniques that can continue to be used to restore Guam's coral reefs, and reefs around the world.

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APPENDIX

Table A1. Mean and SD used for IEV of each species-population.

Population Code	Mean	SD
WAGPUL	3.1542	0.9549
AGTMUR	6.3825	2.5505
AGTPUL	3.5294	1.7905
TOGPUL	6.9353	2.6532
ACHPUL	3.2638	0.9330
ACHASP	5.5723	1.2620

Table A2. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of hanging fragments of *A.* cf. *pulchra* from West Agaña. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
PL27WAG	1325.626	1322.472	61.91347
PL28WAG	475.7081	472.5539	22.12331
PL31WAG	922.6655	919.5113	43.16954
PL33WAG	1302.762	1299.608	61.01445
PL36WAG	779.6614	776.5072	36.45574
PL38WAG	988.5651	985.411	46.26343
PL39WAG	654.1846	651.0304	30.56481
PL40WAG	2348.249	2345.095	110.0983
PL41WAG	2091.248	2088.093	86.28485
PL42WAG	2476.577	2473.423	102.2076
PL43WAG	1576.712	1573.558	73.66843
PL44WAG	2610.449	2607.295	122.0644
PL45WAG	1470.181	1467.027	68.68102
PL46WAG	1668.287	1665.133	77.95567
PL47WAG	754.147	750.9928	35.15884
PL48WAG	1095.715	1092.56	45.14712
PL49WAG	2412.906	2409.752	112.8161
PL50WAG	1946.989	1943.835	91.00352
PL51WAG	614.1741	611.0199	28.68638
PL52WAG	488.5419	485.3877	22.78815
PL91WAG	2121.806	2118.652	87.54761
PL92WAG	1585.814	1582.66	65.39918
PL95WAG	2071.99	2068.836	85.4891
PL96WAG	2655.873	2652.719	109.6165
PL97WAG	1269.603	1266.449	59.29069
PL100WAG	1203.295	1200.141	56.18638
PL101WAG	822.9658	819.8117	38.3807
PL102WAG	1182.446	1179.292	55.21031
PL104WAG	1462.17	1459.016	60.28992
PL106WAG	975.2175	972.0634	45.50858

Table A3. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of hanging fragments of *A. muricata* from Agat. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
MR19AGT	687.1999	680.8174	30.35298
MR20AGT	1082.441	1076.058	47.97406
MR22AGT	3904.19	3897.807	173.7765
MR24AGT	1443.078	1436.695	64.05239
MR26AGT	1258.529	1252.147	55.82464
MR27AGT	1537.897	1531.514	68.27972
MR28AGT	930.8733	924.4907	41.21671
MR29AGT	468.1168	461.7343	20.58557
MR30AGT	700.0916	693.7091	30.92774
MR32AGT	600.1996	593.8171	26.47423
MR33AGT	953.5729	947.1903	41.00391
MR34AGT	2176.466	2170.083	93.943
MR35AGT	1579.004	1572.622	68.07887
MR36AGT	1005.41	999.0273	43.24794
MR37AGT	898.0807	891.6981	38.60165
MR38AGT	1031.031	1024.649	44.35709
MR39AGT	1306.803	1300.42	56.29525
MR40AGT	912.1894	905.8069	39.21242
MR41AGT	1103.396	1097.014	47.48978
MR42AGT	721.5205	715.138	30.95836
MR73AGT	651.5971	645.2146	28.7657
MR74AGT	574.2114	567.8289	25.3156
MR76AGT	871.8285	865.446	38.58431
MR77AGT	942.0037	935.6211	41.71294
MR78AGT	526.7365	520.354	23.19902
MR79AGT	824.7733	818.3908	36.48644
MR80AGT	948.256	941.8735	41.99168
MR82AGT	2162.908	2156.525	96.14468
MR83AGT	1107.174	1100.791	49.07675
MR84AGT	964.0033	957.6208	42.69375

Table A4. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of hanging fragments of *A.* cf. *pulchra* from Agat. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
PL21AGT	1012.85	1009.32	43.26276
PL23AGT	4075.337	4071.808	174.531
PL24AGT	1354.236	1350.707	57.89572
PL26AGT	2129.421	2125.891	91.12265
PL27AGT	2123.007	2119.477	90.84772
PL29AGT	2172.654	2169.124	92.97575
PL30AGT	942.5762	939.0469	40.25062
PL33AGT	2917.905	2914.375	126.1634
PL34AGT	1936.046	1932.517	83.65875
PL36AGT	619.8854	616.356	26.68208
PL37AGT	2439.05	2435.52	105.4338
PL39AGT	808.3707	804.8414	34.84162
PL40AGT	911.7672	908.2378	39.31765
PL41AGT	907.2037	903.6743	38.73443
PL43AGT	3140.008	3136.479	134.4397
PL44AGT	1306.712	1303.182	55.85864
PL45AGT	1401.503	1397.974	60.51834
PL46AGT	980.859	977.3297	42.30864
PL49AGT	1230.317	1226.787	53.10767
PL51AGT	1787.349	1783.819	77.22162
PL81AGT	846.3418	842.8125	36.1257
PL82AGT	1976.633	1973.104	84.57367
PL85AGT	2250.422	2246.893	96.30916
PL87AGT	2196.036	2192.506	93.97799
PL88AGT	3727.339	3723.809	159.6146
PL89AGT	1652.88	1649.351	70.69656
PL91AGT	2361.422	2357.893	101.067
PL92AGT	2582.919	2579.39	110.5611
PL94AGT	1133.417	1129.887	48.43066
PL95AGT	2410.689	2407.159	103.1787

Table A5. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of hanging fragments of *A.* cf. *pulchra* from Togcha. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
PL23TOG	937.996	931.0606	47.02326
PL24TOG	1496.718	1489.783	71.72762
PL25TOG	1839.309	1832.374	92.54415
PL26TOG	1733.103	1726.168	83.10871
PL27TOG	1309.201	1302.266	62.69937
PL28TOG	978.4992	971.5639	49.06888
PL29TOG	1736.546	1729.61	83.27446
PL30TOG	1995.425	1988.49	95.73856
PL31TOG	1135.066	1128.13	54.31537
PL32TOG	1768.785	1761.849	78.54879
PL33TOG	2713.778	2706.843	130.3246
PL34TOG	2568.541	2561.605	123.332
PL35TOG	3125.698	3118.763	150.1571
PL36TOG	1504.691	1497.756	72.11151
PL37TOG	3082.277	3075.342	137.1084
PL38TOG	1678.202	1671.267	80.46542
PL39TOG	1946.887	1939.951	93.4016
PL40TOG	1771.297	1764.361	84.94759
PL41TOG	2561.251	2554.316	122.981
PL42TOG	2808.327	2801.392	134.8768
PL43TOG	2024.843	2017.908	97.15494
PL44TOG	2798.495	2791.559	134.4034

Table A6. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of fixed fragments of *A.* cf. *pulchra* from Achang. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
PL1ACH	63.89971	60.63587	3.308013
PL2ACH	43.15068	39.88684	2.176041
PL4ACH	50.09968	46.83584	2.555147
PL6ACH	11.87179	8.607948	0.46961
PL8ACH	59.32507	56.06123	3.058441
PL9ACH	58.78411	55.52027	3.028929
PL10ACH	16.45536	13.19152	0.719668
PL11ACH	14.17138	10.90754	0.595065
PL12ACH	46.9966	43.73276	2.385857
PL14ACH	7.086305	3.822463	0.208536
PL17ACH	29.89434	26.6305	1.437156
PL18ACH	26.65954	23.39569	1.262585
PL20ACH	24.12233	20.85849	1.12566
PL21ACH	29.8128	26.54896	1.432756
PL22ACH	53.23841	49.97457	2.696955
PL23ACH	23.51724	20.2534	1.093006
PL27ACH	22.3405	19.07665	1.029501
PL28ACH	10.31288	7.049038	0.380412
PL31ACH	66.92913	63.66529	3.435795
PL32ACH	37.92522	34.66138	1.870555
PL33ACH	29.44128	26.17743	1.412706
PL34ACH	57.4108	54.14696	2.922124
PL35ACH	33.67228	30.40843	1.641038
PL36ACH	31.49928	28.23544	1.523769
PL40ACH	43.76115	40.49731	2.1855
PL42ACH	5.47545	2.211608	0.119353
PL43ACH	63.03596	59.77211	3.225694
PL44ACH	77.40904	74.1452	4.00136
PL48ACH	40.45565	37.19181	2.007113

Table A7. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate of hanging fragments of (MEV) of *A. aspera* from Achang. Volumes are based on 25 months of growth in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
AS17ACH	753.2984	747.7261	43.04698
AS18ACH	2059.408	2053.836	97.01635
AS20ACH	609.6858	604.1134	34.77913
AS21ACH	833.9582	828.3859	47.69061
AS22ACH	2320.924	2315.352	109.3695
AS27ACH	1527.174	1521.601	71.87536
AS35ACH	964.3312	958.7589	55.19625
AS39ACH	938.0297	932.4574	53.68206
AS48ACH	719.5208	713.9485	41.10239
AS52ACH	1775.539	1769.966	83.60728
AS73ACH	514.0217	508.4493	29.00453
AS75ACH	847.6407	842.0684	48.03585
AS79ACH	827.6121	822.0398	46.89331
AS81ACH	1386.186	1380.614	78.75719
AS87ACH	2003.099	1997.526	94.35646
AS89ACH	841.5957	836.0234	47.69101
AS92ACH	2406.526	2400.954	113.413
AS97ACH	319.474	313.9017	17.90654
AS101ACH	847.8273	842.255	48.04649
AS102ACH	393.2895	387.7172	22.11735
AS125ACH	1413.925	1408.353	80.02004
AS134ACH	2134.29	2128.717	120.9499
AS137ACH	369.142	363.5697	20.65737
AS140ACH	1308.239	1302.666	74.01513
AS142ACH	900.9884	895.4161	50.87591
AS145ACH	1283.097	1277.524	72.5866
AS147ACH	2012.344	2006.772	114.0211
AS150ACH	1297.442	1291.869	73.40167
AS152ACH	1174.372	1168.8	66.40908
AS155ACH	1814.422	1808.85	102.7756

Table A8. Mean ± SD of MEV growth rates of hanging fragments of each species-population.

Population Code	Mean (cm^3)	SD (cm^3)
WAGPUL	64.6995	28.5615
AGTMUR	49.5541	29.7163
AGTPUL	79.1236	37.8718
TOGPUL	94.5143	30.7351
ACHASP	65.3100	29.5878

Table A9. Mean ± SD of MEV growth rates of wild-sourced loose, wild-sourced attached, nursery-sourced attached, and wild-sourced in nursery fragments (hanging only) at the end of the monitoring period.

Treatment	Mean (cm^3)	SD (cm^3)
Wild-sourced loose	0	0
Wild-sourced attached	2.0341	2.4334
Nursery-sourced	11.6815	8.4195
Wild-sourced in nursery	22.1163	8.3106

Table A10. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of wild-sourced attached fragments of *A.* cf. *pulchra* from West Agaña.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
A1A	10.12055396	6.966388607	0.52775671
A2A	9.591055935	6.436890581	0.48764323
A3A	26.12630534	22.97213998	1.74031364
A4A	110.8048442	107.6506788	8.15535445
A5A	36.45918078	33.30501542	2.52310723
A6A	15.33762915	12.1834638	0.92298968
A7A	39.99574012	36.84157477	2.79102839
A8A	19.93602636	16.78186101	1.27135311
A9A	82.52506603	79.37090068	6.01294702
A10A	10.52697645	7.3728111	0.5585463
A11A	6.545283568	3.391118214	0.2569029
A12A	18.39295732	15.23879197	1.15445394
A13A	33.01485284	29.86068749	2.26217329
A14A	31.31135173	28.15718638	2.13312018
A15A	17.29368692	14.13952157	1.07117588
B1A	9.7066415	6.552476164	0.49639971
B2A	3.6349728	0.480807466	0.03642481
B3A	19.177873	16.02370794	1.21391727
B4A	3.8719895	0.717824167	0.05438062
B5A	53.333351	50.17918613	3.80145349
B6A	28.060356	24.90619105	1.88683265
B7A	28.808173	25.65400768	1.94348543
B8A	13.788072	10.63390619	0.80559895
B9A	176.56006	173.405899	13.1368105
B10A	28.922377	25.76821123	1.95213721
B11A	26.941995	23.78783009	1.80210834
B12A	31.180106	28.02594063	2.12317732
B13A	8.4329645	5.278799169	0.39990903
B14A	89.291088	86.13692241	6.52552443
B15A	15.084081	11.92991601	0.90378152
C1A	11.310732	8.156567062	0.61792175
C2A	10.369296	7.215130677	0.54522398
C3A	11.018303	7.864137223	0.5942673
C4A	10.033355	6.879189684	0.51983801
C5A	6.3106579	3.156492564	0.23852589
C6A	41.063709	37.90954334	2.86470101
C7A	29.144081	25.98991547	1.96893299
C8A	16.720038	13.56587269	1.02512892
C9A	73.193324	70.03915878	5.29263165
C10A	6.3612913	3.20712593	0.24235209
C11A	14.046805	10.89263937	0.82312136
C12A	31.002879	27.84871371	2.10975104
C13A	25.993765	22.83959989	1.72591435
C14A	21.464123	18.30995783	1.38711802
C15A	37.950606	34.79644046	2.62945394

Table A11. Proxy initial ellipsoid volume (IEV), final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of nursery-sourced corals of *A.* cf. *pulchra* from West Agaña.

Frag. Code	IEV (cm^3)	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
A1N	27.5644	68.8835	41.3191	3.1302
A2N	51.0969	56.1057	5.0088	0.3795
A3N	51.0969	141.6034	90.5065	6.8566
A4N	82.8578	69.9489	-12.9089	-0.9779
A5N	80.1121	162.1636	82.0515	6.2160
A6N	41.0988	186.9983	145.8995	11.0530
A7N	29.0939	47.8723	18.7784	1.4226
A8N	43.0023	118.8657	75.8634	5.7472
A9N	59.9711	98.5107	38.5396	2.9197
A10N	29.0939	123.6944	94.6005	7.1667
A11N	57.6787	104.2842	46.6055	3.5307
A12N	37.4344	258.7580	221.3235	16.7669
B1N	126.7695	433.4926	306.7231	23.2366
B2N	35.6731	176.1208	140.4478	10.6400
B3N	43.0023	117.8626	74.8602	5.6712
B4N	41.0988	108.8807	67.7819	5.1350
B5N	82.8578	271.2927	188.4348	14.2754
B6N	77.4174	426.1987	348.7813	26.4228
B7N	49.0007	170.0149	121.0142	9.1677
B8N	97.3603	118.7456	21.3853	1.6201
B9N	39.2429	280.7844	241.5414	18.2986
B10N	21.8998	146.8262	124.9264	9.4641
B11N	29.0939	80.3595	51.2656	3.8838
B12N	35.6731	210.0579	174.3848	13.2110
C1N	27.5644	49.0022	21.4378	1.6200
C2N	55.4357	200.2480	144.8123	10.9430
C3N	72.1809	474.9532	402.7723	30.4362
C4N	29.0939	124.1256	95.0318	7.1994
C5N	59.9711	146.1648	86.1937	6.5298
C6N	19.3355	71.5163	52.1807	3.9531
C7N	59.9711	260.5629	200.5918	15.1963
C8N	29.0939	0.0000	-29.0939	0.0000
C9N	57.6787	71.5105	13.8318	1.0452
C10N	43.0023	81.1786	38.1762	2.8849
C11N	29.0939	161.9661	132.8722	10.0661
C12N	23.2485	44.0603	20.8118	1.5767

Table A12. Final ellipsoid volume (FEV), total growth, and monthly ellipsoid volume growth rate (MEV) of wild-sourced fragments of *A.* cf. *pulchra* from West Agaña in the nursery.

Frag. Code	FEV (cm^3)	FEV-IEV (Total Growth) (cm^3)	MEV (cm^3)
N11	632.1948518	629.04	32.0939126
N12	381.7392968	378.59	19.3155679
N13	202.2623054	199.11	10.1585786
N61	470.3559762	467.2	23.8368271
N62	249.3274221	246.17	12.55986
N63	373.9466781	370.79	18.9179853
N71	736.2311257	733.08	37.4018857
N72	450.4899007	447.34	22.8232518
N73	633.3416444	630.19	32.1524224
N101	397.2280541	394.07	20.1058106
N102	434.9652868	431.81	22.0311797
N103	277.5157199	274.36	13.9980385

Table A13. Fish species recorded in the nursery throughout the 25-month monitoring period.

Species
Acanthurus triostegus
Monotaxis grandoculis
Rhinecanthus aculeatus
Chrysiptera traceyi
Blennioidei sp.
Acanthurus sp.
Aulostomus chinensis
Canthigaster valentine
Chromis sp.
Ctenochaetus striatus
Epibulus insidiator
Lutjanus bohar
Meiacanthus atrodorsalis
Chaetodon ornatissimus
Bothus sp.
Arothron mappa
Oxymonacanthus longirostris

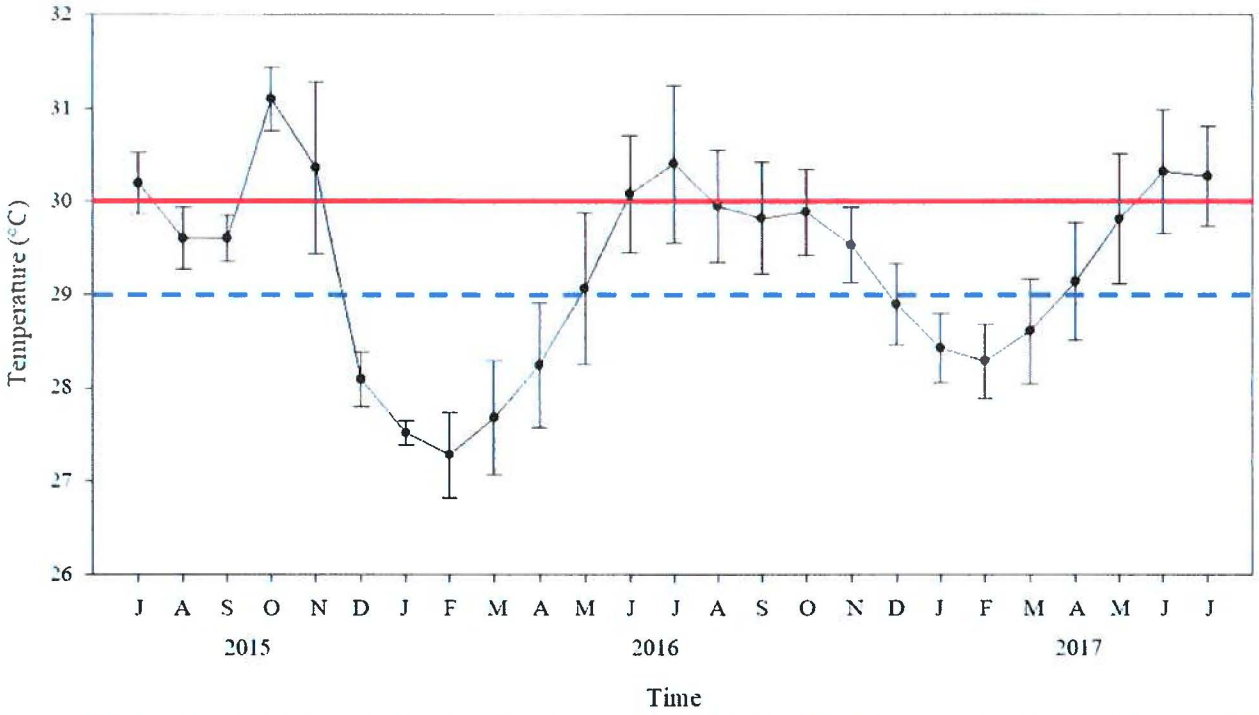

Fig. A1. Mean (± SD) monthly temperature recorded in the nursery in Piti Bomb Holes Marine Preserve over the duration of the monitoring period. The horizontal lines represent NOAA Coral Reef Watch maximum monthly mean SST (blue dashed), and the coral bleaching threshold SST (red solid).

Table A14. Mean daily light received (lum/ft^2) at each fragment position on the string.

Date	Position 1	Position 2	Position 3	Position 3
5/5/2016	636.6428571	785.8571429	628.2857143	566.5714286
5/6/2016	540.8076923	464.3461538	456.8461538	521.1538462
5/7/2016	892.0769231	504.2307692	641.1923077	740
5/8/2016	683.3461538	561.3461538	630.6538462	785.0384615
5/9/2016	578.4230769	759.6538462	500.4615385	780.3461538
5/10/2016	737.6538462	432.1538462	405.0384615	609.9230769
5/11/2016	900.4230769	532.0384615	513.0384615	866.0384615
5/12/2016	740.1538462	482.8076923	241.7692308	765.0769231
5/13/2016	945.9615385	654.5769231	277.0384615	780.0384615
5/14/2016	877.1923077	897.5769231	483.4615385	764.1153846
5/15/2016	1529.346154	1094.115385	375.4230769	866.1923077
5/16/2016	920.9615385	701.2307692	350	538.6538462
5/17/2016	851	880.7692308	315.6923077	525.6923077
5/18/2016	877.8076923	704.3846154	296.8076923	465.0384615
5/19/2016	624.2307692	638.5	294.7307692	579.2307692
5/20/2016	118.75	85.25	39	80.75
5/21/2016	838.1153846	821.1538462	273.1538462	616.9615385
5/22/2016	1228.423077	787.8846154	336.0384615	881.6923077
5/23/2016	965.4230769	961.3846154	384.1153846	882.8846154
5/24/2016	1142.807692	1038.653846	267.2692308	899.3461538
5/25/2016	1319.538462	701.5769231	268.6923077	414.5
5/26/2016	813.4615385	474.8846154	294.5769231	599.1923077
5/27/2016	661	758.9615385	434.6153846	714.0384615
5/28/2016	336.5	306.2307692	217.0384615	539.3461538
5/29/2016	707.3461538	558.9230769	321.2307692	788.3461538
5/30/2016	462.1538462	262.0384615	170.0384615	419.3076923
5/31/2016	473.8461538	338.6153846	227.1538462	808.1538462
Position Mean	**824.5435115**	**658.3358779**	**365.4610687**	**677.3389313**
Position SD	**293.2512333**	**238.0855184**	**141.3553**	**186.2757479**