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PHENOTYPIC PLASTICITY IN *ACROPORA ASPERA* AND ITS IMPLICATIONS FOR CORAL RESTORATION

BY

RENEE SCHNABEL CRISOSTOMO

A thesis defense submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

SUPERVISORY COMMITTEE

Dr. Laurie Raymundo, Chair

Dr. David Combosch

Mr. David Burdick

UNIVERSITY OF GUAM

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AN ABSTRACT OF THE THESIS of Renee Schnabel Crisostomo for the Master of Science in Biology, presented, August 22, 2023

Title: Phenotypic plasticity in *Acropora aspera* and its implications for coral restoration

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

Abstract

Staghorn *Acropora* species in Guam have substantially declined due to increased local anthropogenic stressors and climate change. This loss has prompted the establishment of *in situ* ocean nurseries with subsequent restoration of vulnerable populations. *Acropora aspera* is a highly vulnerable staghorn species in Guam currently reduced to a single wild population and is thus at high risk for extirpation. In our ocean nursery, *A. aspera* undergoes pronounced morphological changes from the wild population, likely a response to a different environmental regime. At present, the consequences of phenotypic changes in corals in response to nursery culture is poorly understood, though these changes could affect the outcome of restoration efforts. This study sought to quantify the morphological plasticity of *A. aspera* under two different restoration methods: direct transplantation vs. nursery-rearing with subsequent outplanting. Plasticity was quantified by following survival, linear extension rates, changes in corallite structure using 3D scanning, bleaching severity, predation, and disease susceptibility over the course of a year. I found that *A. aspera* exhibited an ability to adapt to differing restoration environments by expressing a suitable phenotype-environment match. Nursery-reared colonies experienced greater phenotypic change throughout the restoration process when compared to directly transplanted colonies, likely due to

the reduced environmental differences during nursery-rearing compared to direct transplantation. The increased phenotypic plasticity displayed in nursery-reared corals was likely a bet-hedging technique to increase their ability to survive in response to a shift in environment. Nursery-reared colonies also endured a longer post-outplant recovery period when compared to transplanted colonies, which could be a trade-off incurred from their plastic response. These results should inform future restoration efforts and advance species management interventions in Guam, regionally, and globally.

TO THE OFFICE OF GRADUATE STUDIES

The members of the committee approve the thesis of Renee Schnabel Crisostomo presented on August 22, 2023

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1 Introduction

Coral reefs are among the most biologically diverse and valuable ecosystems in the world. They support the economic and cultural stability of many coastal communities globally through fisheries, coastal protection, and tourism (Moberg & Folke, 1999; Hoegh-Guldberg et al., 2007). Over the past few decades, coral reef communities have seen major losses as a result of anthropogenic stressors. Local stressors, such as sedimentation, nutrient enrichment, and over-fishing practices, diminish the health of coral reef ecosystems (Hoegh-Guldberg et al., 2007; Williams et al., 2015). On a global scale, rapid increases in atmospheric CO_2 are driving ocean acidification and warmer sea surface temperatures (SSTs) which reduce calcification and growth rates of corals and trigger bleaching events and mass mortality (Hoegh-Guldberg et al., 2007, Hughes et al., 2018). Although corals have an innate capacity for natural recovery, the frequency and intensity of mass bleaching events has made it increasingly difficult for reef ecosystems to recover (Hughes et al., 2018). In recent decades, the active restoration of degraded coral reefs has grown considerably as a major tool for reef rehabilitation, conservation, and management (Rinkevich, 2005).

Ideally, coral reef restoration works to maintain or recover key ecosystem processes, functions, and services and promotes reef resilience in the face of climate change and increasing anthropogenic stressors (Hein et al., 2021). Active coral restoration efforts are a complementary management tool to passive conservation strategies such as the establishment of marine protected areas, fisheries management, land-based source pollution control, sustainable use for recreation and tourism, education, and climate change adaptation (Young et al., 2012; Guam Coral Reef Initiative, 2019; Boström-Einarsson et al., 2020). This multi-pronged management allows for active coral restoration to preserve reef biodiversity in the short-term as passive conservation

strategies work to take effect (Boström-Einarsson et al., 2020). The need for appropriately scaled restoration methods in response to large-scale ecological degradation has created a demand for low-cost, low-tech approaches that can be implemented world-wide (Lirman & Schopmeyer, 2016; Ceccarelli et al., 2020). A few examples of these approaches include direct transplantation, coral gardening, larval enhancement, and substrate provisioning (Boström-Einarsson et al., 2020; Suggett & van Oppen, 2022).

In 1995, Rinkevich pioneered the coral gardening method wherein an initial population of coral fragments are propagated within nurseries prior to outplanting to a suitable recipient site. The grow-out stage in *in situ* or *ex situ* nurseries helps to maximize coral survivorship and productivity while reducing impacts to source colonies by limiting harvesting to small fragments (Johnson et al., 2011; Lirman & Schopmeyer, 2016). The ability to repeatedly prune and allow for regrowth of fragments in nurseries helps to minimize negative impacts on existing wild populations but also multiplies the number of fragments available for outplanting (Rinkevich, 2005; dela Cruz et al., 2015; Boström-Einarsson et al., 2020). The coral gardening method has been largely successful and performed on over 100 coral species globally with outcomes of increased survival, growth, and reproductive outputs (dela Cruz et al., 2015; Boström-Einarsson et al., 2020; Rinkevich, 2021). However, due to cost and limited resources most coral gardening is confined to small-scale projects where success of efforts and survival of outplants are variable (Ross, 2014; Goergen & Gilliam, 2018; Hein et al., 2020; Calle-Triviño et al., 2021). The drivers of variable outplant survivorship are not well known but may be attributed to environmental factors of outplant sites, stress incurred from outplanting techniques, reduction of genetic diversity, or the selection of less stress tolerant genotypes (Lohr et al., 2017; Banister & van Woesik, 2021; Lock et al., 2022).

One important aspect of coral restoration is site selection. In theory, the distribution and status of remaining wild populations should guide site selection as this provides important information for determining suitable environments ideal for the target species' growth and survival. Factors to consider include, but are not limited to, depth, water motion, water quality, herbivory, predation, accessibility, size of restoration area, and substrate type (Johnson et al., 2011). However, there are many challenges involved in selecting and predicting a suitable restoration site. Not all physical or biological factors may be available or unexpected factors may exist that jeopardize long-term survivorship and growth of coral colonies at selected sites. Sites chosen for coral restoration may be less than optimum for a particular species but may constitute the best site available sometimes influenced by political, cultural, or economic reasons. Another important aspect of coral restoration is selecting the appropriate species to be restored. Coral restoration tends to focus efforts on damaged, depleted, or destroyed coral populations to assist in their recovery (Rinkevich, 2005; Young et al., 2012). Most coral restoration projects focus primarily on fast-growing branching corals, such as the staghorn coral genus *Acropora*, as they are important reef-building taxa but have also suffered significant population declines globally (Young et al., 2012; Raymundo et al., 2017; Boström-Einarsson et al., 2020). In Guam, staghorn *Acropora* populations have experienced a massive decline which has made them an important target genus for coral restoration (Raymundo et al., 2017; Raymundo et al., 2019).

The genus *Acropora* (Anthozoa: Acroporidae) is the most diverse and speciose coral genus, comprising over 15% of all reef-building species globally (Renema et al., 2016). In Guam, staghorn *Acropora* communities are generally found in shallow reef flats and lagoonal patch reefs (Raymundo et al., 2017). They provide essential habitats for fish and invertebrate species while serving as economically and culturally important resources vital to traditional use, tourism,

recreation, fisheries, and shoreline and infrastructure protection (Burdick et al., 2008; Raymundo et al., 2017). However, due to the combination of climate change and increased anthropogenic stressors, staghorn *Acropora* populations have suffered significant degradation globally and locally (Burdick et al., 2008; Young et al., 2012; Raymundo et al., 2019). Staghorn *Acropora* species have relatively low genetic diversity due to their dominant reproductive strategy of asexual fragmentation (Baums et al., 2006). Their thin tissue, high growth rates, and low metabolic rates also mean quick acclimation to changing environments is difficult (Loya et al., 2001; Hoegh-Guldberg et al., 2007). Due to observed losses globally, their ecological importance, and their ability to rapidly recover, staghorn *Acropora* have become a key target genus for coral conservation, restoration, and management efforts.

From 2013 through 2017, Guam's reefs were severely hit with consecutive island-wide bleaching events due to elevated SSTs, extreme low tides, and disease outbreaks which resulted in an estimated 36% live coral cover loss for staghorn *Acropora* populations (Raymundo et al., 2019). A set of surveys conducted in 2020 through 2021 found that live coral cover of staghorn *Acropora* populations have been greatly reduced relative to prior records (Raymundo et al., 2022). Currently, there are eight recorded putative staghorn species in Guam and four species, *A. aspera, A. vaughani, A. acuminata,* and *A. austera,* are now limited to single populations. *Acropora aspera* is a species of staghorn coral characterized by its labellate radial corallites and low, sprawling colonies which can appear either corymbose or arborescent in growth and generally occur in shallow protected habitats (Wallace, 1999). In Guam, the single remaining *A. aspera* wild population is found on the eastern edge of Cocos Lagoon in a shallow area exposed to moderate to high water flow throughout the year. This extremely vulnerable population has seen an estimated 30% coral cover loss due to low tide exposure, *Terpios hoshionota* sponge overgrowth, thermal

stress, and damselfish algal farming mainly observed in the center area of the extensive thicket (Raymundo et al., 2022). Local restoration efforts have concentrated heavily on *A. aspera,* as it is currently one of the most outplanted species to date (Raymundo & Andersen, unpublished data).

With subsequent coral mortality events that Guam has experienced, marine management entities prioritized the need to develop strategies to restore and maintain ecological functions of its reefs (Guam Coral Reef Initiative, 2019). The main goals of local restoration efforts focus on rescuing at-risk species of extirpation, replenishment of degraded populations, and preservation for genetic research (Raymundo et al., 2022). Currently, coral gardening is a popular restoration strategy in Guam with the establishment of two *in situ* coral nurseries located in the Piti Bomb Holes Marine Preserve and Cocos Lagoon. The coral nurseries target the restoration of some of Guam's most vulnerable species, such as *A. aspera*. Although both coral ocean nursery locations provide desirable space and substrate for different structures, their environmental characteristics can differ from species' original source populations. In Guam, finding a restoration site that is suitable across all desirable factors can prove to be difficult. The establishment of both *in situ* nurseries in Guam required an environment that was deep enough to provide protection during storms which limited the scope of suitable locations within the reef crest margin. The environmental differences between source population and restoration site may provoke genotypic and phenotypic responses within corals to adapt to varying environmental states (Rinkevich, 2021).

Within Cocos Lagoon, the Merizo Ocean Coral Nursery sits at a depth of 9 m (30 ft) and is in an area subject to high turbidity from river runoff and low fish herbivory due to heavy fishing pressure. This differs from the shallow reef localities in which staghorn *Acropora* populations in Guam are generally found, although records have observed healthy staghorn populations within

the lagoon that have now died out (Randall & Sherwood, 1982, Raymundo et al., 2017, Raymundo et al., 2022). From personal observations, staghorn *Acropora* species, specifically *A. aspera*, growing in the Merizo Ocean Coral Nursery tend to undergo morphological changes, such as rapid linear extension rates, a reduction in radial corallite length, decreased radial crowding, and changes in the length of their radial walls, hypothesized to represent phenotypic plasticity in response to the environmental differences that exist between source population and nursery location (Figure 1a-b). *Acropora aspera* characters currently used to identify colonies *in situ* are their corymbose appearance, the proximity of their radial corallites, the length and thickness of their radial walls, and their axial tip diameter (Figure 1a) (Wallace, 1999). However, after undergoing a growth phase within the nursery, species start to lose their taxonomic morphology characteristic of wild populations (Figure 1b).

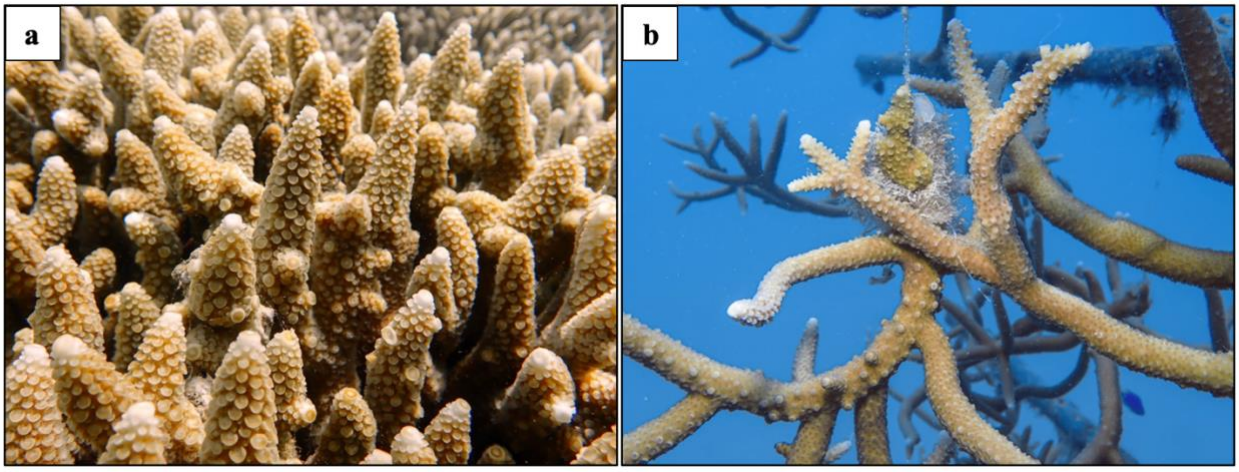

Figure 1. Contrasting morphology of *A. aspera* in two different environments **a.** Wild population of *A. aspera* **b.** *A. aspera* in the Merizo Ocean Coral Nursery after approximately 2 years of growth

Phenotypic plasticity is the ability of an organism to express phenotypic variation, such as changes in behavior, morphology, or physiology, in response to different environments (Schlichting, 1989; Todd, 2008; Kelly, 2019; Rinkevich, 2021). An organism's plasticity can determine its fitness and functionality under diverse selective pressures (Gibbin et al., 2016;

Rinkevich, 2021). Intraspecific morphological variation in corals can be explained by the coral's genetically based ability to express phenotypic plasticity (Todd, 2008; Million et al., 2022). Most scleractinian corals are able to take advantage of phenotypic plasticity throughout their adult life, making it central to their survival in sub-optimal or highly competitive conditions (Torda et al., 2017; Rinkevich, 2021). However, the role of the genotype and the impact of the environment on coral responses, such as morphology, can vary among species (Todd, 2008). For example, the influence of light on fragments of *Acropora* spp. induced linear extension towards the direction of maximum irradiance in a low light environment (Kawaguti, 1937; Kawaguti, 1943). Temperature, irradiance, and wave energy have also been shown to affect various skeletal growth parameters in *A. aspera*, such as skeletal extension, skeletal accretion, and skeletal density (Brown et al., 1985). Similar responses have also been observed in other coral species (Todd, 2008).

The potentially negative impacts of phenotypic plasticity on corals are poorly understood. There is a limited understanding of how rapid acclimation may affect other traits or what trade-offs may exist between length of time to respond and the magnitude of expression the coral may achieve (Todd, 2008). For example, when introduced to a different environment, corals may experience a period of reduced fitness as they engage their plastic response (Todd, 2008; Murren et al., 2015). Corals can also incur costs by acquiring information about the environment that may involve additional energy or reduced efficiency (DeWitt et al., 1998). Genetic costs may also exist through linkage of plastic genes to low fitness genes, pleiotropy, and epistasis (DeWitt et al., 1998). However, work conducted so far has shown that phenotypic plasticity is beneficial for corals and can allow for a broader resilience and more elastic tolerance to environmental fluctuations, increasing the likelihood of survivorship (Murren et al., 2015; Kelly, 2019; Rinkevich, 2021). Corals subjected to high heat stress were found to express both short-term acclimatory and long-

term adaptive abilities of climate resistance (Bellantuono et al., 2012; Palumbi et al., 2014; Carballo-Bolaños, 2019). A study performed by Middlebrook et al. (2008) also found that *Acropora* colonies subjected to short-term thermal stress were more thermally tolerant during simulated bleaching events compared to colonies that had not been subjected to short-term thermal stress. Although plastic trait changes typically occur within a generation, short-term acclimation and alteration of fitness-related traits may be key for resilience in the face of climate change and useful to further inform restoration management (Million et al., 2022).

Within the context of coral restoration, phenotypic plasticity could be a useful tool, as it allows coral species to acclimate to environmental changes (Kuffner et al., 2017). Generally, the plastic response expressed by corals should allow for a broader resilience and more flexible tolerance to environmental fluctuations, making it a favorable rapid-response mechanism in the face of harsher conditions (Rinkevich, 2021). However, switching the coral's environment drastically, from the wild to a nursery, and then to their final outplant location, could be potentially harmful. Phenotypic responses that could affect the functionality and fitness of coral outplants, or that increase resistance to stress and allow corals to grow to maturity are important to identify to improve restoration outcomes (Lohr & Patterson, 2017; Rinkevich, 2021).

As the effects of climate change and anthropogenic stressors increase at an unprecedented rate, phenotypic plasticity in corals may provide a first step towards improving their adaptive capacity (Rinkevich, 2021). Although corals' plasticity may facilitate a more desirable phenotype-environment match, there is a dearth of studies on a potential role of phenotypic plasticity in coral restoration. It is unclear whether this innate flexibility provides them with the resilience to acclimate to and survive the restoration process or whether it involves additional energetic and genetic costs that result in lower outplant success. Studies that quantify fitness costs or the benefits

of plasticity will be important for species conservation and restoration, where survival of these species may be reliant on plastic traits and our ability to leverage them (Million et al., 2022). My study sought to quantify the morphological plasticity of *A. aspera* under two different restoration methods to investigate the potential trade-offs incurred from this observed plastic response.

2 Hypotheses

$H1_0$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show no differences in linear extension rates relative to colonies directly transplanted and their original source colonies.

$H1_a$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show differences in linear extension rates relative to colonies directly transplanted and their original source colonies.

$H2_0$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show no differences of morphometric characters relative to colonies directly transplanted and their original source colonies.

$H2_a$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show differences of morphometric characters relative to colonies directly transplanted and their original source colonies.

$H3_0$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show no differences of health performance metrics relative to colonies directly transplanted and their original source colonies.

$H3_a$: Colonies of *A. aspera* subject to nursery culture with subsequent outplanting will show differences of health performance metrics relative to colonies directly transplanted and their original source colonies.

3 Methods

3.1 Study site

Fragments were collected from selected source colonies within the last remaining wild population of *A. aspera* located on the eastern edge of Cocos Lagoon, which sits at a depth of 1m (Figure 2).

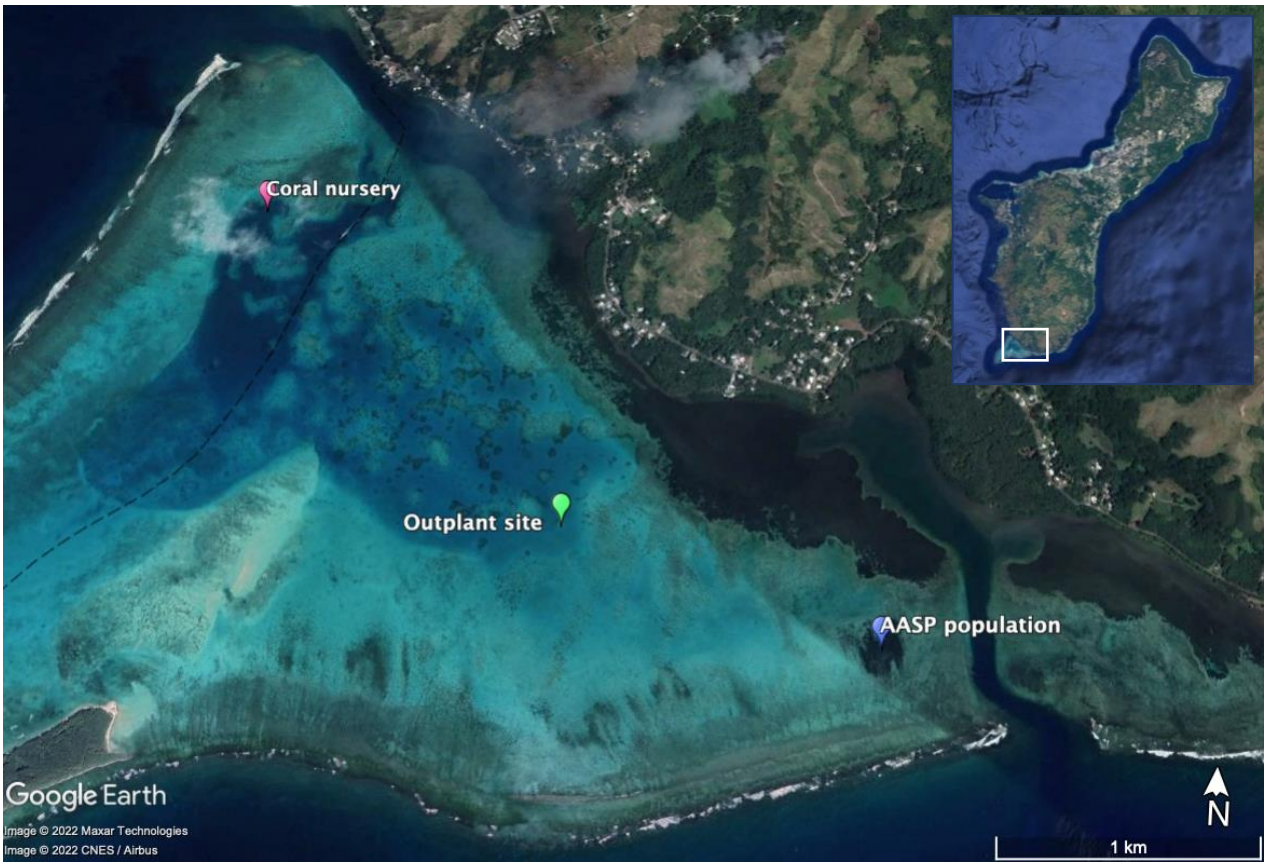

Figure 2. Study focus area of Cocos Lagoon which contains the last remaining wild *A. aspera* population, the Merizo Coral Nursery, and the outplant site chosen for this study. AASP represents the species *A. aspera*.

The Merizo Coral Nursery, also located within Cocos Lagoon (Figure 2), was selected for this study based on the observed morphological changes that certain staghorn species were experiencing in this nursery culture environment (Figure 1b). The Merizo Coral Nursery is in a deep, sandy bottom area (9 m) within the lagoon allowing for protection during storms. This area can experience a moderate current and is in proximity to a wild population of *Acropora virgata* and a healthy *Porites*-dominated community. The outplant site selected for this study was located

between the coral nursery and the *A. aspera* wild population. The site has a depth of 3.6 m with a sandy bottom and is also situated within an area of current active restoration and outplanting. GPS coordinates of all sites are described in Table A3.

3.2 Sampling source colonies

Ten source colonies within the *A. aspera* wild population were sampled and monitored throughout the duration of the study. Source colonies were selected approximately 60 m apart from each other to minimize replicate sampling of clonal fragments of the same genotype (Figure 3; Table A3). From each source colony, eight fragments were removed with wire cutters. Four fragments were used for direct transplantation and the other four fragments were used for nursery culture with subsequent outplanting. Direct transplantation is the restoration technique that

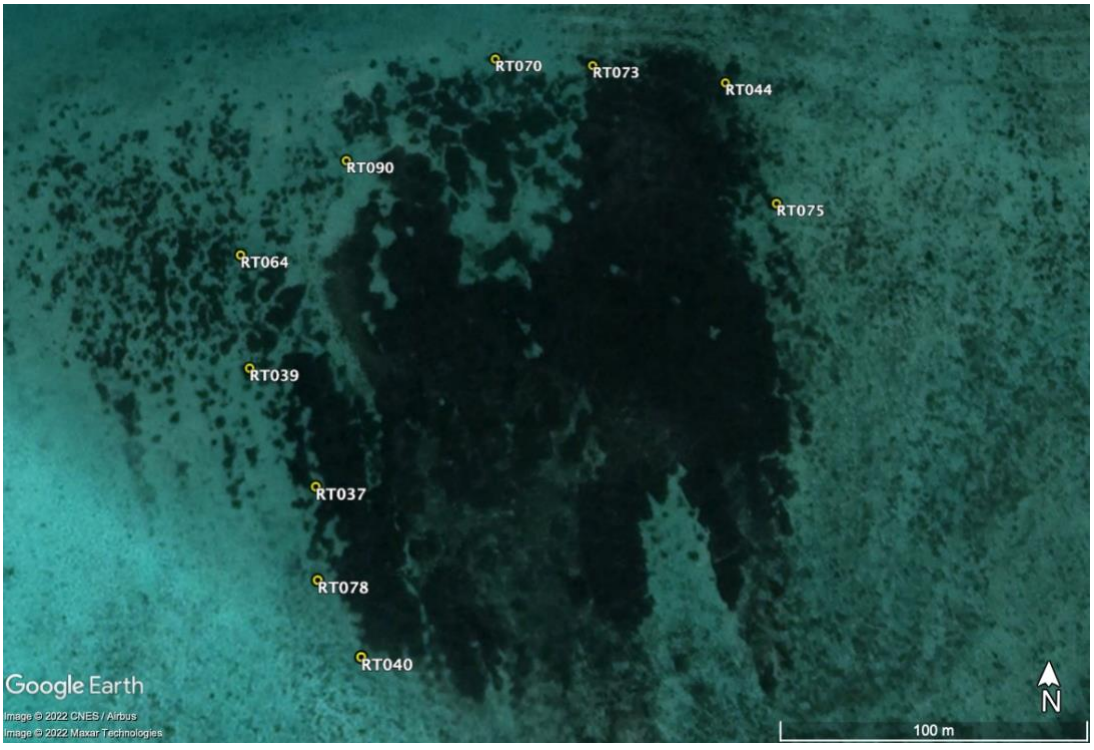

Figure 3. Location of *A. aspera* source colonies which were selected approximately 60 m apart along the population margins

transplants coral colonies or fragments without an intermediate nursery phase (Shaver et al., 2020). Nursery culture with subsequent outplanting refers to the restoration technique that involves

captive rearing and outplanting of coral colonies using an intermediate nursery phase, often referred to as coral gardening (Shaver et al., 2020).

Three branches on each source colony were tagged to track growth rate and health characteristics of *in situ* wild colonies. Tissue samples were collected for genotyping by the UOG Island Evolution Lab. Genetic analysis was not a part of this study but will contribute to the restoration efforts for this species.

3.3 Contrasting restoration methods and structures

This study used two different restoration methods: direct transplantation vs. nursery rearing with subsequent outplanting separated into two phases. In Phase 1, fragments of source colonies were sampled and separated by restoration method. Fragments used for direct transplantation were immediately transplanted to the outplant location. Fragments used for nursery culture were placed into the nursery where they underwent a 7-mo grow-out phase. In Phase 2, nursery-reared colonies were then outplanted to the outplant site. Both phases lasted for a period of 7 mos each.

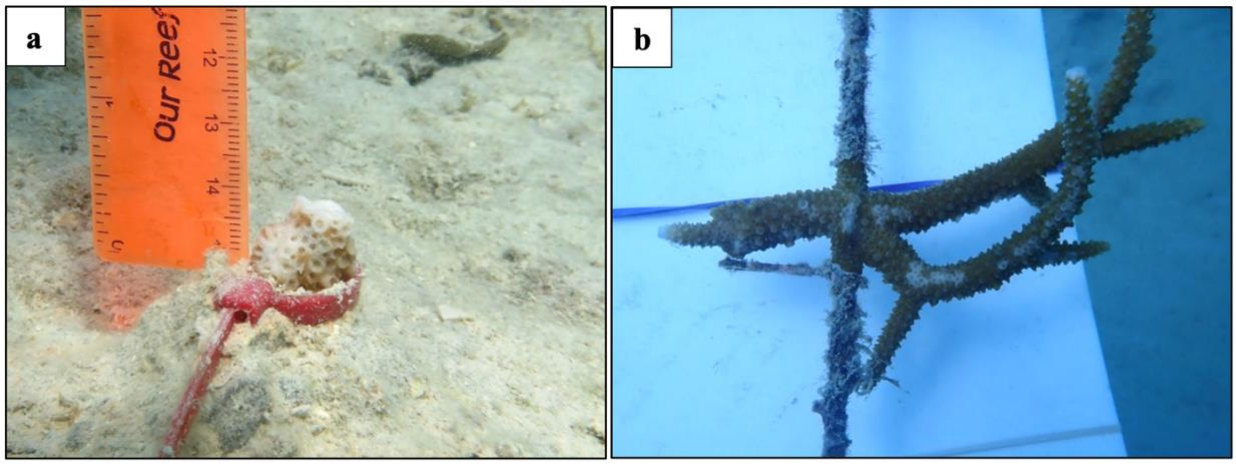

Figure 4. Predation on fragments during the study period **a.** Predation on initial direct transplant fragments **b.** Predation on rope nursery fragments

Directly transplanted fragments were initially cemented onto pavement substrate using a cement-plaster mix. However, because of severe and immediate fish predation of these fragments post-transplantation (Figure 4a), monitoring of these fragments were discontinued, source colonies were resampled, and C-frame structures were used as substrate platforms instead. C-frames are platforms constructed from chicken wire and plastic mesh that provide substrate in unstable, soft bottom areas. These structures are currently used in Guam's restoration strategy as substrate provision in sandy bottom areas to prevent the burial of small coral fragments. The C-frames used in this study were anchored into a sandy bottom using rebar. Fragments were then attached haphazardly to one of the four C-frames using zip ties (Figure 5a). C-frame fragments experienced no severe and immediate predation post-transplantation, likely because frames protected fragments from fish predators inhabiting nearby reef patches.

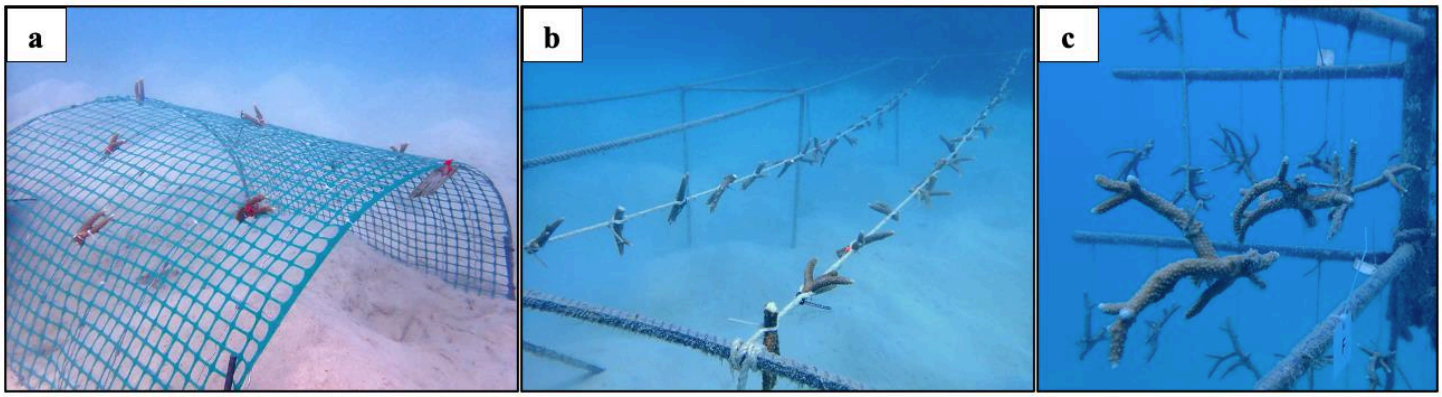

Figure 5. Structures used for restoration **a.** C-frames used for direct transplantation **b.** Rope nursery used for nursery culture for 5 mos **c.** Coral tree nursery used for the last 2 mos of nursery culture during the study

For nursery-rearing, fragments were initially placed in a rope nursery located in the Merizo Coral Nursery. The rope nursery is a structure, resembling a clothesline, that uses paired rebar anchors, where ropes are strung and suspended over substrate (Figure 5b). Coral fragments were arranged at regular intervals within the braids of the rope allowing for fragments to grow and attach themselves onto the rope. Fragments were cultured in the rope nursery for approximately 5 mos

until they experienced predation, after which they were promptly transferred to a coral tree within the Merizo Coral Nursery (Figure 4b). Fragments were placed on the bottom half branches of the coral tree to ensure consistent depth with the rope nursery (Figure 5c). No further predation occurred once fragments were relocated, and all predation wounds healed.

After the 7-mo nursery grow out phase, fragments in the nursery were outplanted onto the C-frames at the outplant site and arranged haphazardly. Within 3 mos post-outplanting, six nursery-reared colonies incurred disease lesions and were promptly moved to a quarantine C-frame. This C-frame was placed 10 m down current from the outplant location where fragments remained for the duration of the study. These diseased fragments were moved to avoid further spreading of disease on other healthy fragments. Diseased fragments remained a part of the study and were monitored regularly for growth and other performance metrics.

3.4 Monitoring performance metrics

Health metrics and growth data of colonies were collected for a period of 14 mos. Photographs of colonies were taken with a measurement scale and processed through ImageJ (Version 2.3.0 for Mac) to calculate total linear extension (TLE) in cm. TLE is defined as the sum of all branch lengths with live tissue of an entire colony (Kiel et al., 2012). Daily growth rates of all colonies were calculated using the equation $GR = \frac{(G_t - G_0)}{t}$, where G_t is TLE at t, G_0 is initial TLE, and t is time in days. Daily growth rates were multiplied by 28 to obtain standardized monthly average growth rates.

Health metrics were measured by conducting a rapid health assessment that is used to monitor bleaching severity, mortality of unknown cause, disease (after Myers & Raymundo, 2009), and predation of colonies (Appendix 11.3). Bleaching severity and changes in pigment concentration of colonies were measured using the CoralWatch Coral Health Chart. The chart is a

proxy indicator of symbiont density that uses a 6-point numerical scale and the four common colors typically expressed by corals (Siebeck et al., 2006). Each colored square corresponds to the concentration of symbiotic algae living in the coral tissue and is thus linked to coral health (Figure A17).

Source colonies were visited four times throughout the study period to collect growth and health data of tagged branches. Directly transplanted and nursery-reared fragments were visited every 5-6 weeks to collect growth and health data. Monthly maintenance of the restoration structures used in this study included removal of algae using various scrubbing instruments and replacing or tightening of zip ties to prevent movement and abrasion of fragments.

3.5 Morphometrics and skeletal density analyses

Morphometric and skeletal density analyses were conducted on fragment samples collected from source colonies, directly transplanted colonies, and nursery-reared colonies. Samples from source colonies were only collected at the beginning of the study to establish baseline morphometric data of wild *A. aspera* colonies (n=10). After Phase 1, one sample was collected from each source colony of directly transplanted colonies and nursery fragments before they were outplanted (n=20).

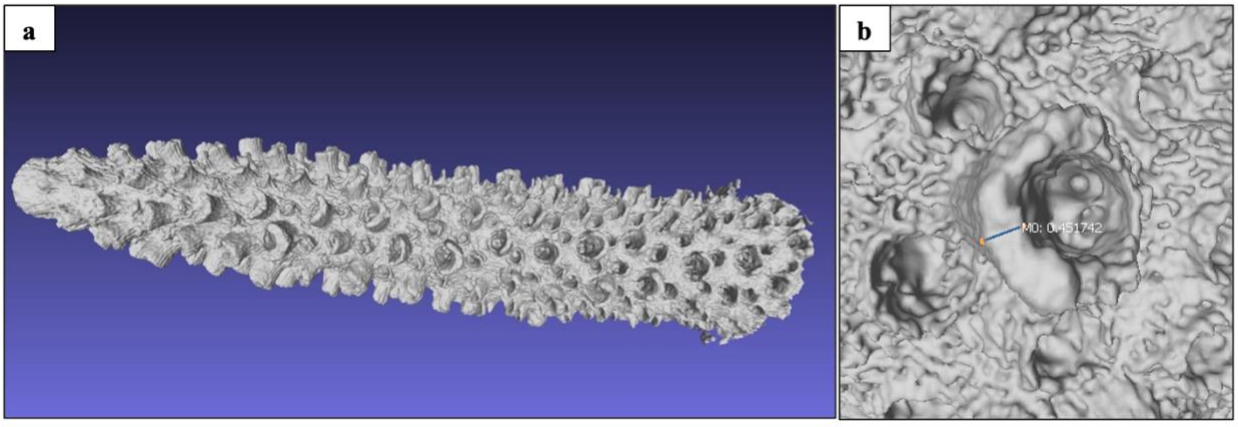

Figure 6. a. 3D model of a fragment sample. **b.** Measurement of radial wall thickness (Character 3, Table 1)

After Phase 2, one sample was again collected from each source colony of directly transplanted colonies and nursery outplant colonies (n=20). All fragment samples were bleached to remove live tissue and air dried. To prep fragments for scanning, each fragment was sprayed using a matte white finish to reduce surface light reflection and internal light scattering characteristic of the calcium carbonate skeletons of corals (Veal et al., 2010). Fragments were then scanned using the D3D-S 3D scanner and mesh files were then imported into MeshLab for post-processing. Mesh files of each fragment were aligned and underwent surface reconstruction to produce visual 3D models (Figure 6a).

Morphometric characters measured were chosen based on their use as traditional taxonomic characters of *A. aspera* (Wallace, 1999) (Table 1). Radial crowding was measured by counting the number of corallites intersecting a transect, created by placing a consistent selection around the fragment using selection tools within MeshLab. Counts of three different transects were collected and averaged to obtain average radial crowding for each fragment. Radial wall thickness, radial corallite profile length, radial corallite diameter, radial calice diameter, and axial tip diameter were measured by using the built-in measurement tool within MeshLab (Figure 6b). Radial wall thickness, radial corallite profile length, radial corallite diameter, and radial calice diameter measurements were collected on 30 corallites per fragment for each character. The first 2 cm of each fragment were not sampled to ensure that measurements of immature corallites did not skew measurements of matured corallites. Axial tip diameter only produced one measurement per fragment.

Table 1. Morphometric characters measured

No.	Character	Description
1	Radial crowding	Average number of emersed radial corallites / 3 transects
2	Radial wall thickness	Width of radial wall (Figure 7)

3	Radial corallite profile length	Maximum distance from base to outer edge of radial corallite (Figure 7)
4	Radial corallite diameter	Maximum diameter of radial corallite from inner to outer wall (Figure 7)
5	Radial calice diameter	Maximum diameter of radial calice from inner to outer wall (Figure 7)
6	Axial tip diameter	Maximum diameter of axial tip

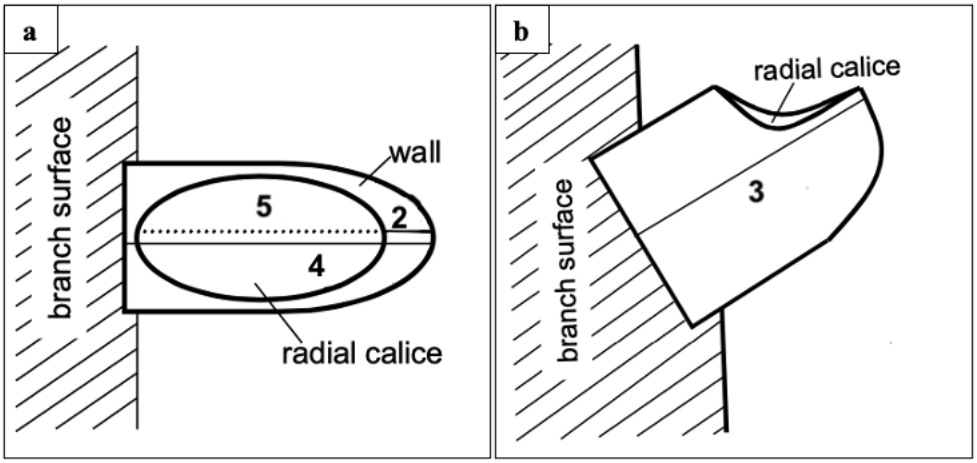

Figure 7. Diagram of morphometric characters 3-6 from **a.** Upper view of radial corallite **b.** Profile view of radial corallite. Numbers in red correspond to characters described in Table 1. (Source: Wolstenholme et al., 2003)

Skeletal density was estimated by obtaining volume measurements of each 3D scan in MeshLab and weighing each fragment for mass. Volume (mm^3) was calculated by using the Compute Geometric Measures function in MeshLab. Samples were weighed using an analytical balance to obtain mass (g). Skeletal density (g/mm^3) was then calculated using the following equation: $\rho = m/V$.

3.6 Environmental monitoring

Water temperature (°C) was monitored throughout the duration of the study by installing HOBO TidbiT v2 Water Temperature Data Loggers at each of the three sites. The outplant site was monitored for a total of 14 mos. The nursery was monitored only during the nursery grow out phase which lasted for a total of 7 mos. The wild population of *A. aspera* was monitored for

approximately 18 months, however, the logger installed at the site went missing during this monitoring period which created a 7-mo gap of temperature data at the site.

Light intensity (lux) was measured using a HOBO Pendant Temperature/Light Data Logger installed at each of the three sites. Light intensity was monitored at each site during the dry and rainy season to characterize seasonal changes that may have occurred during the study period. The dry season in Guam generally lasts from January to June, while the rainy season spans from July to December. During the dry season, light intensity was monitored from April 6, 2022 – April 21, 2022 at all three sites. During the rainy season, light intensity was measured at the outplant site and the wild population of *A. aspera* from October 25, 2022 – November 23, 2022. Light intensity data was not collected at the nursery during the rainy season due to availability of light loggers. Light intensity data that was collected in 2021 by the Raymundo Coral Lab at the Merizo Coral Nursery was used to characterize light intensity in the nursery during the rainy season for this study. Both temperature and light data were aggregated to daily averages at each site.

5.7 Data analysis

All data analyses were performed using R and R Studio (Version 1.3.1073). All data were tested for normality using Shapiro-Wilk's test and homogeneity of variance using Levene's test and transformed using a log10 scale accordingly to meet those assumptions. A non-parametric test was used when data did not meet normality assumptions. A principal components analysis (PCA) based on a correlation matrix was used to explore any explained variances of corallite morphometrics measured between methods. The morphometric characters analyzed for the PCA were radial corallite diameter, radial calice diameter, radial wall thickness, and radial corallite profile length. Graphs were produced using the R package ggplot2.

4 Results

4.1 Growth

Differences in average monthly growth rates, the rate at which colonies grew, between source colonies were not significant (fixed-effects ANOVA, p=0.069). Differences in average monthly TLE, the average cm grown monthly, between source colonies were also not significant (fixed-effects ANOVA, p=0.074). Because there were no significant growth differences of individual source colonies, data were pooled to examine differences in growth between methods: wild source colonies, direct transplants, and nursery-reared outplants. Average monthly growth rates and average monthly TLE between methods were significantly different (fixed-effects ANOVA, p<0.001) (Figure 8). Pairwise differences revealed significant differences of average monthly growth rates and monthly TLE between all methods except for fragments reared in the nursery and after they were outplanted (Table 2).

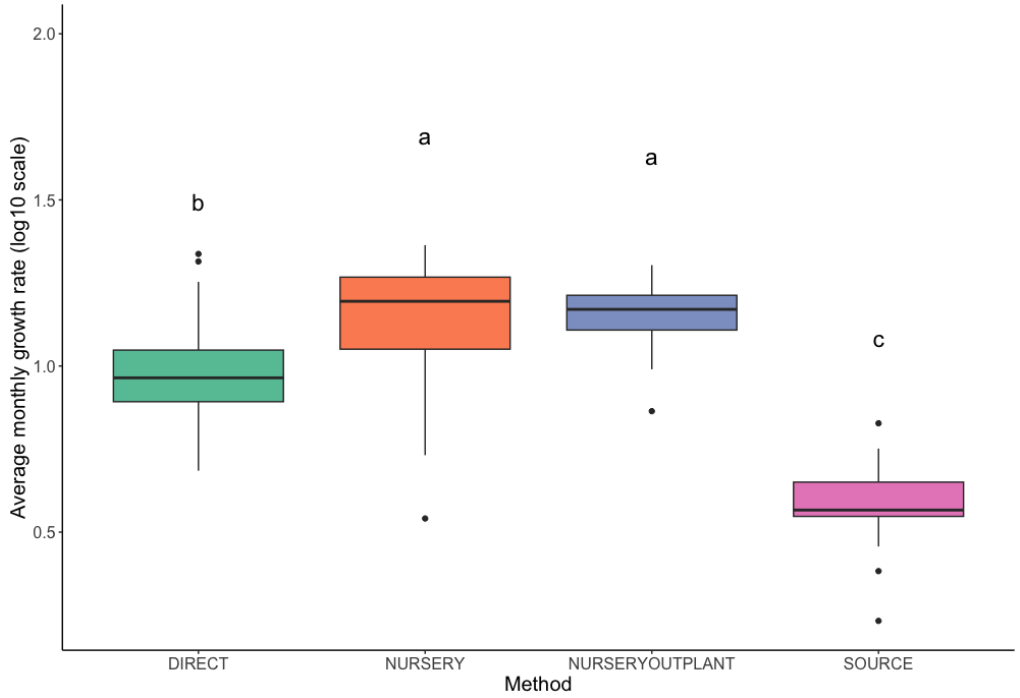

Figure 8. Boxplot of average monthly growth rates (log10 transformation) of colonies between methods. Groups labeled with different letters show a significant difference at α=0.05.

Table 2. Pairwise multiple differences of average monthly growth rates and average monthly TLE between methods and their respective p-values (Tukey's HSD test). * indicate significant difference between methods at α=0.05.

Method Comparison	P-value of average monthly growth rates (Tukey's HSD)	P-value of average monthly TLE (Tukey's HSD)
Nursery vs. Direct Transplant	<0.001*	<0.001*
Nursery Outplant vs. Direct Transplant	<0.001*	<0.001*
Source vs. Direct Transplant	<0.001*	<0.001*
Nursery Outplant vs. Nursery	0.997	0.894
Source vs. Nursery	<0.001*	<0.001*
Source vs. Nursery Outplant	<0.001*	<0.001*

Growth trends of average monthly TLE revealed that directly transplanted colonies underwent an initial recovery period post-transplantation where colonies experienced reduced growth (Figure 9). This recovery period lasted for about five weeks. Nursery-reared fragments underwent rapid linear extension until they experienced predation within the nursery that resulted in reduced TLE (Figure 9). Predation was no longer observed after fragments were moved out of the rope nursery (Figure 9). Nursery outplants underwent a similar post-outplant recovery period of reduced growth that lasted for 10 weeks (Figure 9). Source colony growth was difficult to consistently follow, due to lost markers and tags in the field, however monthly TLE was minimal (Figure 9).

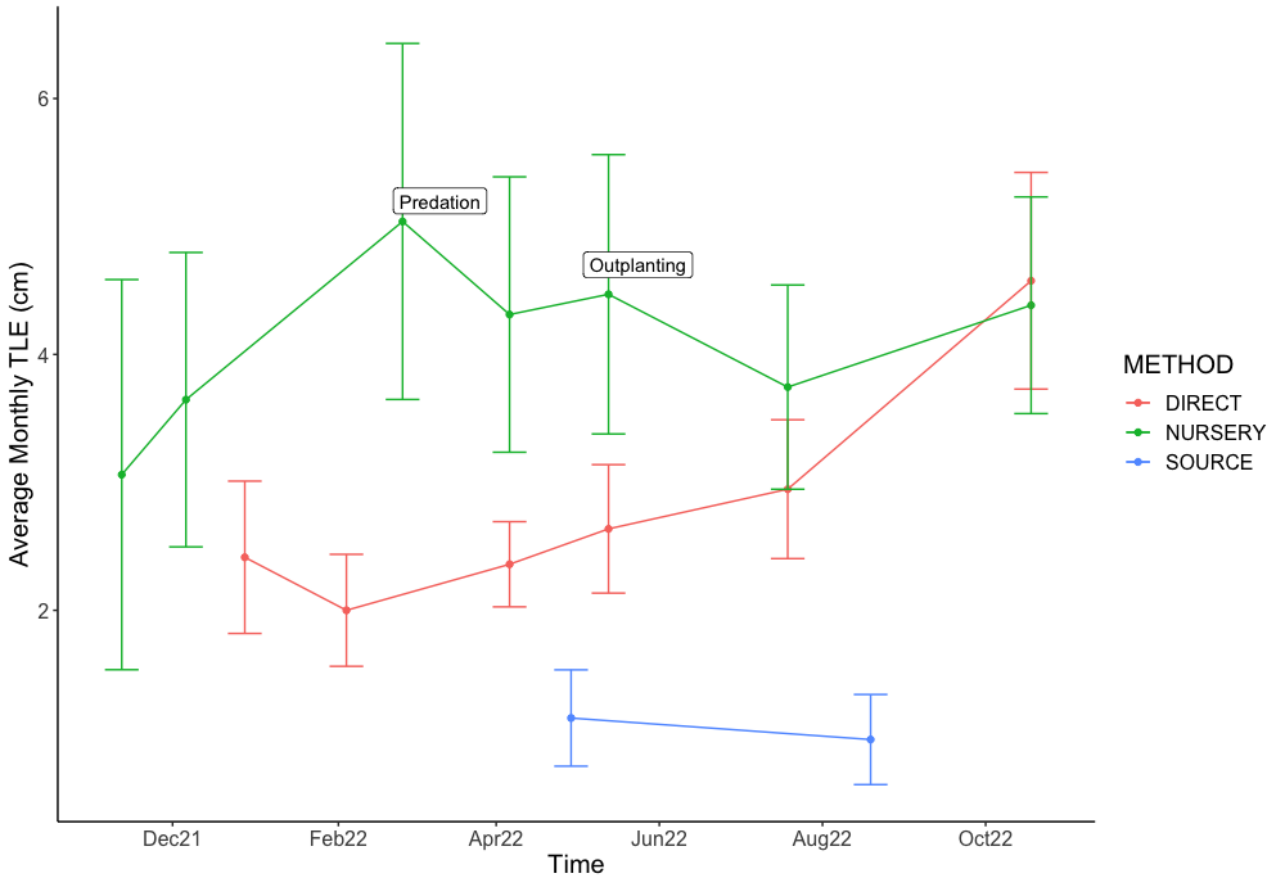

Figure 9. Average (mean±SE) monthly TLE time series trends of methods. Predation box marks when nursery colonies experienced predation. Outplanting box marks when nursery colonies were outplanted. Growth following outplanting of nursery colonies represents nursery outplant colonies.

4.2 Morphometrics and skeletal density

Results revealed that radial corallite profile length and radial corallite diameter had the most impact on variation among radial corallite morphometric characters (Figure 10, A19, A20). Nursery-reared fragment morphometrics showed the most variance from source colony morphometrics (Figure 10). Nursery outplant colonies showed the most similarity in morphometric characters with directly transplanted colonies after being in the same environment for a 7-mo period (Figure 10). Interestingly, directly transplanted colonies also displayed the highest within-group morphometric variation after 7 mos (Figure 10). Nursery fragments also showed the least

within-group morphometric variation compared to all methods (Figure 10). Average values of these morphometric characters are outlined in Table A10.

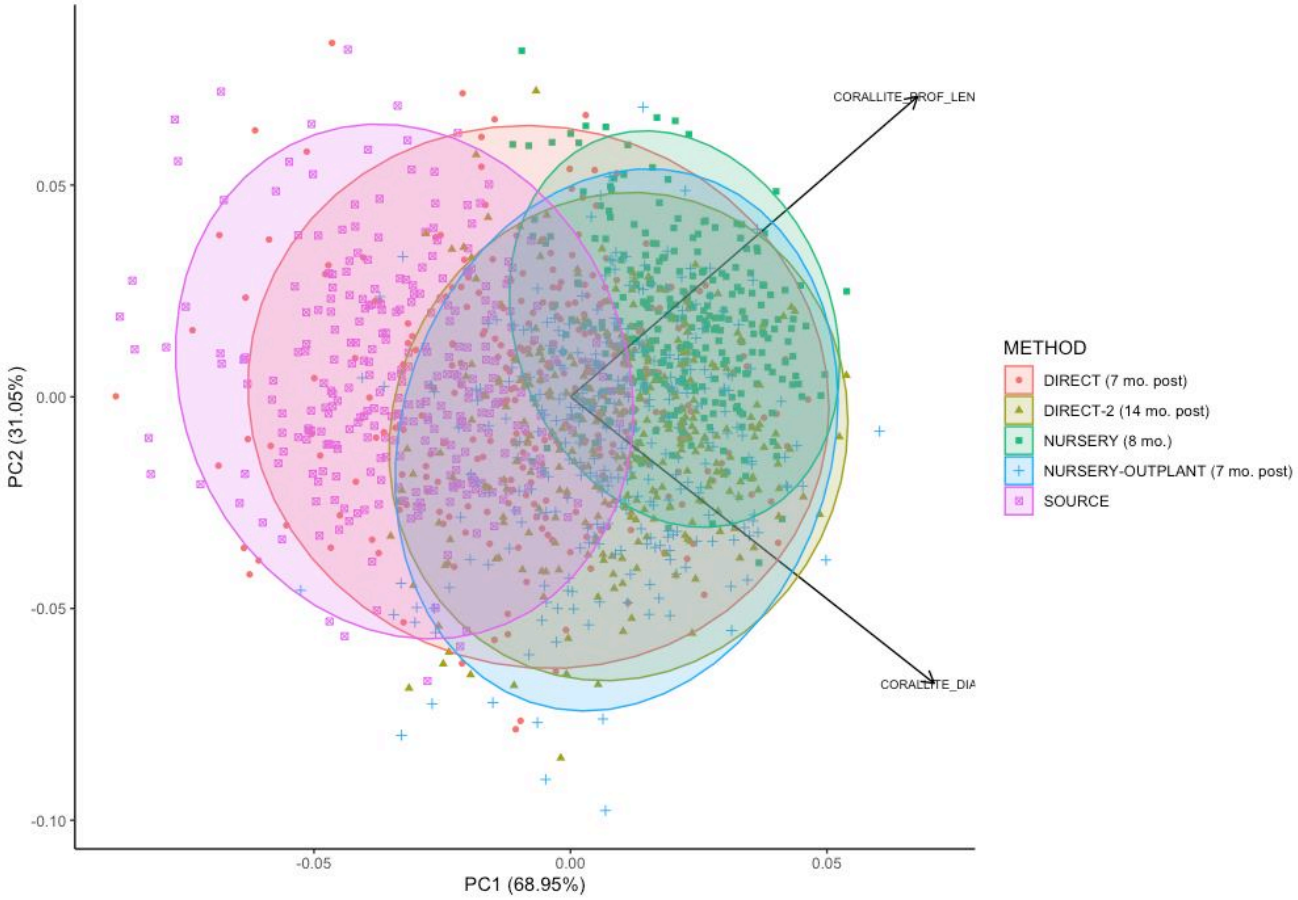

Figure 10. PCA of corallite morphometrics showing positions by method of the two most impactful morphometric characters measured: radial corallite diameter and radial corallite profile length. Each method is represented by a different color and shape. Each point represents a single corallite measurement.

Differences between methods in radial crowding were significantly different (fixed-effects ANOVA, $p<0.001$). Pairwise differences between methods showed that radial crowding in nursery-reared fragments were significantly different from direct and source colonies, while nursery outplant colonies were only significantly different from source colonies. (Tukey's HSD test, Table A4). Source colonies (16 count/transect ± 4) had the highest average radial crowding

while nursery (9 ± 2) and nursery outplant colonies (10 ± 2) had the lowest (Figure 11). Average radial crowding values of all methods are outlined in Table A5.

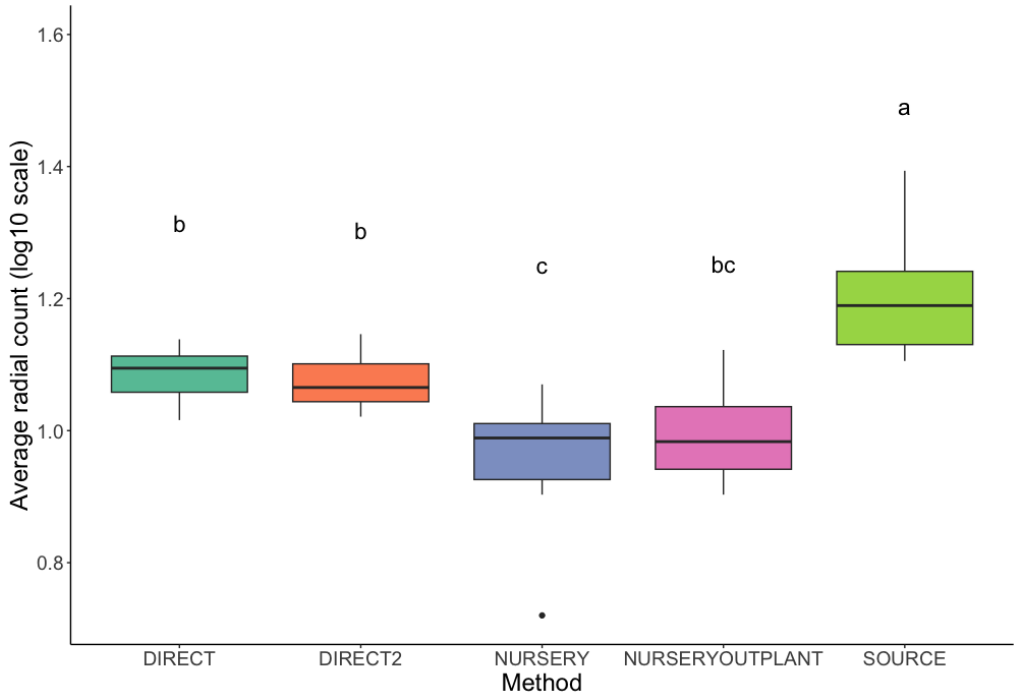

Figure 11. Boxplot of average radial crowding between methods. Groups labeled with different letters show a significant difference at α=0.05. Direct samples were taken 7 months post-transplant, and direct-2 samples were taken 14 months post-transplant.

Axial tip diameter between methods were also significantly different (Kruskal-Wallis, $p<0.001$). Source colony fragments had the largest observed axial tip diameter(3.74mm ± 0.2) while nursery-reared fragments had the smallest (2.75 ± 0.15) (Figure 12). Source colony axial tip diameter was significantly larger than directly transplanted colonies 7 mo post-transplant (3.19 ± 0.26), nursery fragments, and nursery outplant colonies (3.12 ± 0.18), however, they did not differ significantly from directly transplanted colonies 14 mo post-transplant (3.48 ± 0.48) (Figure 12; Dunn's test, Table A6). Nursery-reared fragment axial tip diameter was also significantly smaller than both directly transplanted time points (Figure 12; Dunn's test, Table A6). Average axial tip diameters are outlined in Table A7.

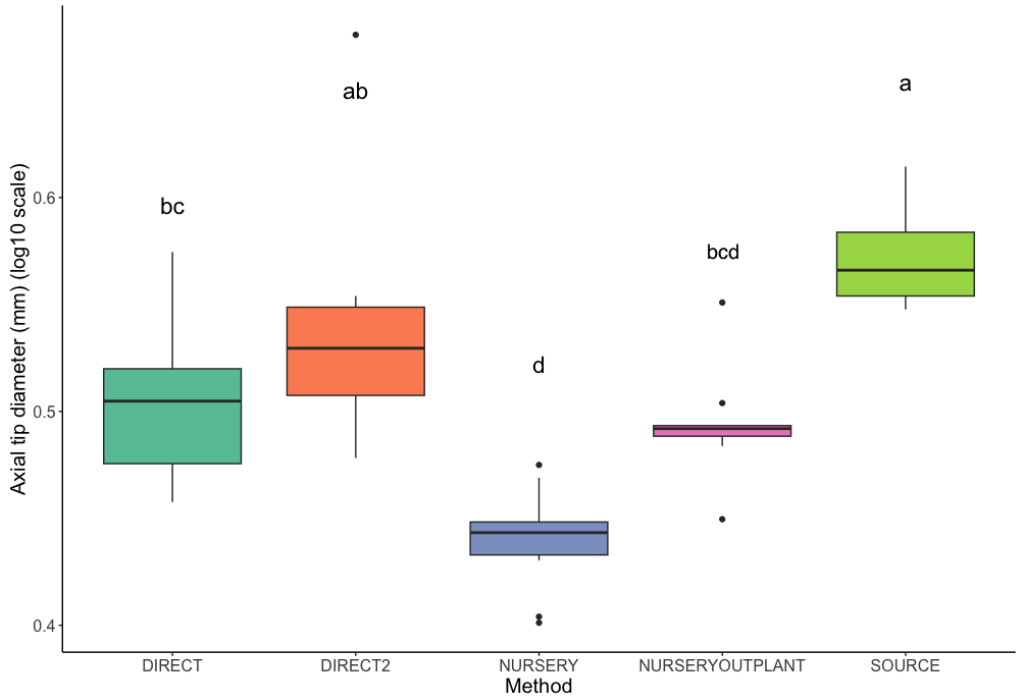

Figure 12. Boxplot of observed axial tip diameter between methods (log10 transformation). Groups labeled with different letters show a significant difference at α=0.05. Direct samples were taken 7 months post-transplant, and direct-2 samples were taken 14 months post-transplant.

Analysis of skeletal density measurements revealed significant results (Kruskal-Wallis, p=0.042). Differences in skeletal density were observed only between nursery outplant colonies and source colonies, where skeletal density of nursery fragments decreased after they were outplanted (Figure 13; Dunn's test, Table A8). Average skeletal density values are outlined in Table A9.

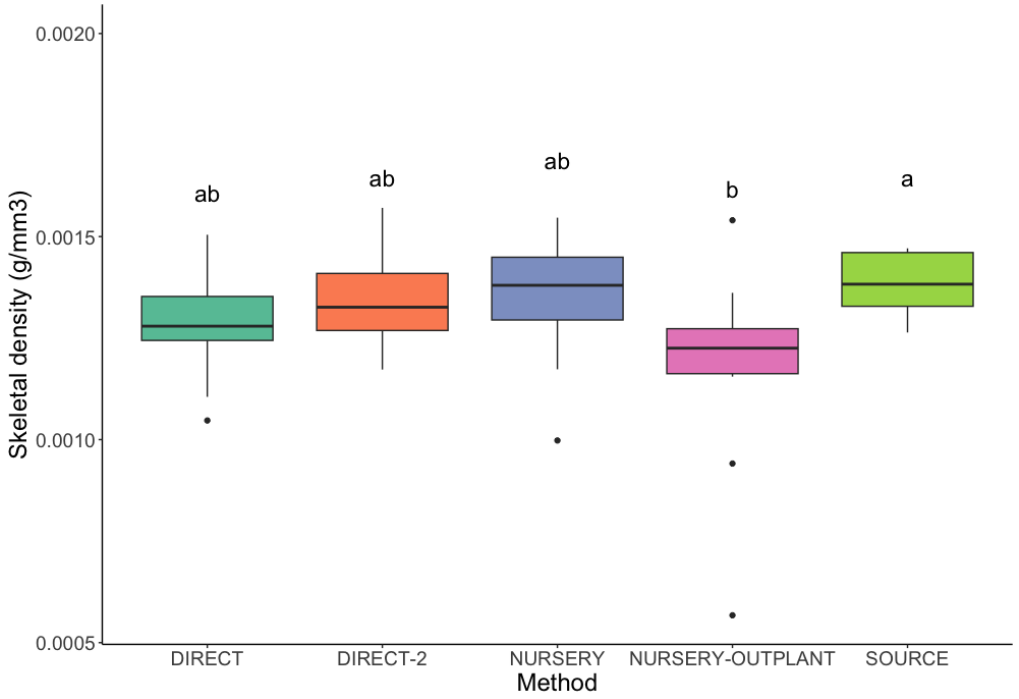

Figure 13. Boxplot of skeletal density measurements between methods. Groups labeled with different letters show a significant difference at α=0.05. Direct samples were taken 7 months post-transplant, and direct-2 samples were taken 14 months post-transplant.

4.3 Health metrics

All colonies followed throughout the duration of the study had a 100% survival rate. Predation impacts to colonies were measured using percent severity bins based on the percentage of the colony affected. Predation on initial direct transplants that experienced immediate and severe predation were not included in this study. There were no significant differences of predation between source colonies (Kruskal-Wallis, p=0.594) and no significant differences between methods (Kruskal-Wallis, p=0.196). Directly transplanted colonies and nursery-reared fragments had similar occurrences and severity of predation, whereas source colonies were observed to be

predated on the least (Figure 14). On a temporal scale, predation impacts to colonies followed no trends and appeared to be random.

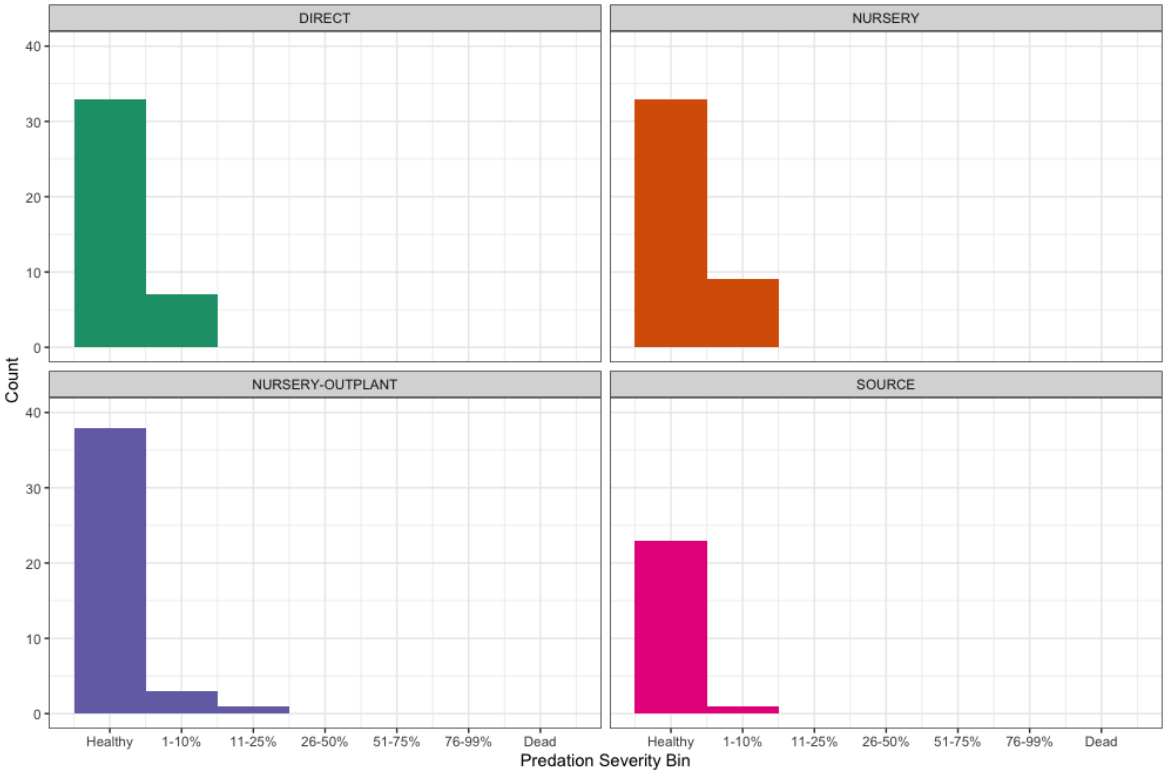

Figure 14. Predation impacts in observed colonies between methods.

Disease impacts to colonies were also measured using percent severity bins based on the percentage of the colony affected. Nursery outplant colonies were the only colonies observed to be infected with two main diseases: white syndrome and grey death (Figure 15). Infections occurred 12 weeks after outplanting. Once infected colonies were moved to their quarantine C-frame, spread of further disease was minimal and subsequent recovery of infected lesions was observed. Significant differences between source colonies infected with white syndrome (Kruskal-Wallis, p=0.3911) and grey death (Kruskal-Wallis, p=0.566) was not found. White syndrome infection was significant between methods (Kruskal-Wallis, p=0.003), where disease prevalence in nursery outplants were 16% (Figure 15A). However, grey death infection was not significant between methods (Kruskal-Wallis, p=0.166), where disease prevalence observed in nursery

outplant colonies was 4% (Figure 15B). It is important to note that infections of both diseases were observed in random colonies and did not follow a pattern suggesting spread between colonies in proximity.

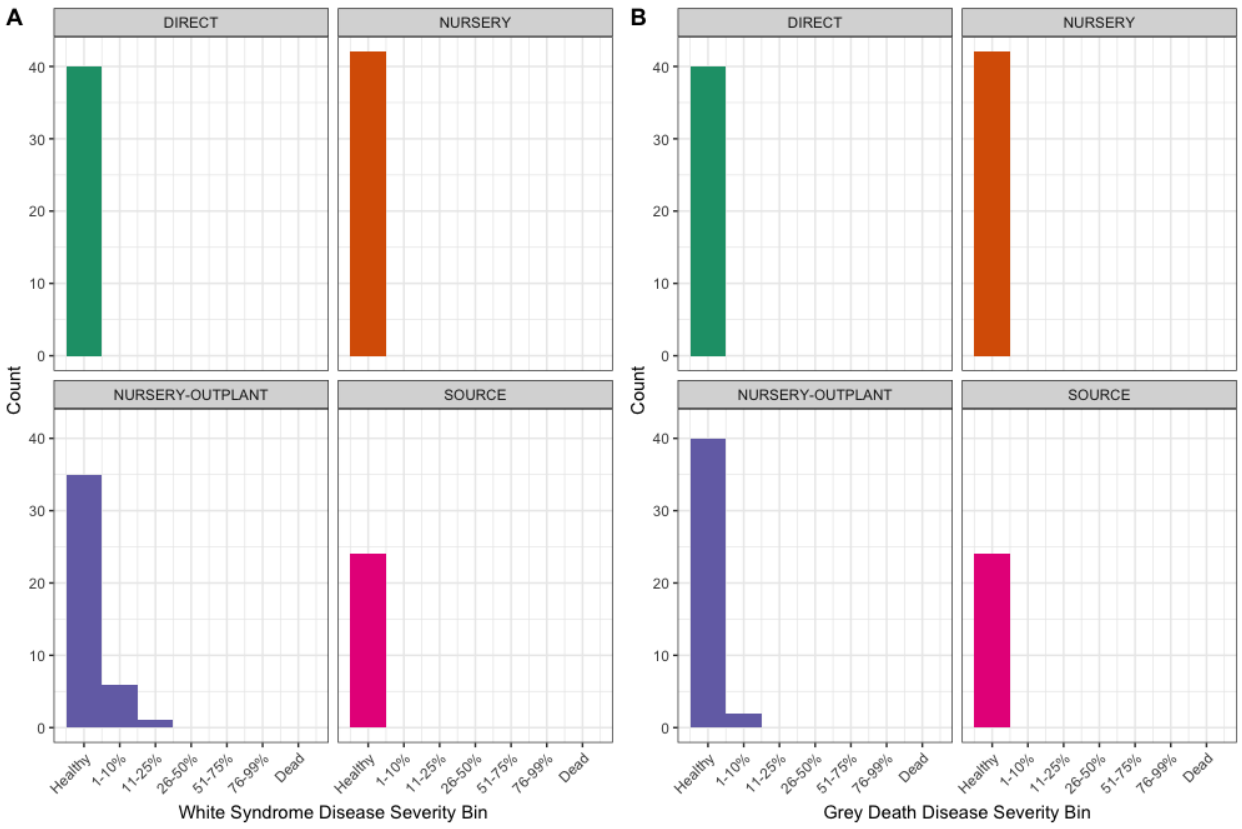

Figure 15. Disease impacts in observed colonies between methods. **A.** Impacts of colonies infected with white syndrome disease by method. **B.** Impacts of colonies infected with grey death disease by method.

Color of colonies fell within the "D" category of the CoralWatch Coral Health Chart index. Pigment concentrations corresponded with changes in environmental regimes. Nursery-reared fragments experienced an increase in concentration of symbiotic algae to D5 after being placed in the nursery, whereas directly transplanted colonies experienced a decrease to D3 after being transplanted (Figure 16). However, nursery outplants and directly transplanted colonies acclimated back to D4, which reflected the color of source colonies (Figure 16).

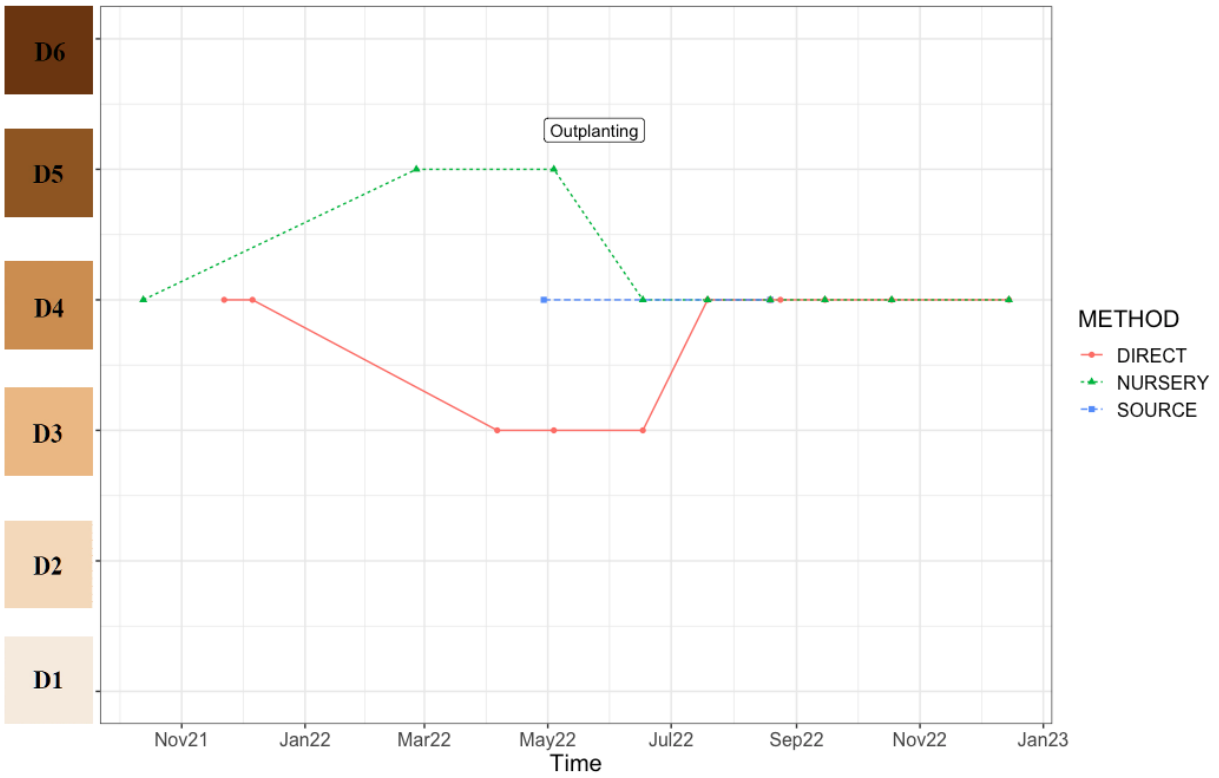

Figure 16. Temporal changes in color between methods. Color of observed colonies fall into the "D" category of the CoralWatch Coral Health Chart index. D1 represents the lowest concentration of symbiotic algae while D6 represents the highest.

4.4 Environmental monitoring

Average daily water temperature at each site followed general trends of Guam's waters, where SSTs are usually lower in the winter months and warmer during the summer (Figure 17). Although temperature was very similar at all three sites, the wild *A. aspera* patch had higher average daily temperatures than the outplant site and the nursery (Figure 17). During the summer months, average daily temperatures at all three sites exceeded both Guam's average max monthly mean and bleaching threshold (Figure 17). Although outplanted fragments did not experience bleaching, this rise in temperature coincided with the outplanting of nursery fragments in May 2022 in which colonies experienced paling (Figure 16).

Seasonally, daily average light intensity was lower during the rainy season than in the dry season at the outplant site and the wild *A. aspera* patch (Figure A22). Although light intensity was

not measured in the nursery during the 2022 rainy season, 2021 measurements showed that light intensity at the nursery was lower on average than light intensity at the outplant site and wild *A. aspera* patch during the 2022 rainy season. (Figure A22, A23).

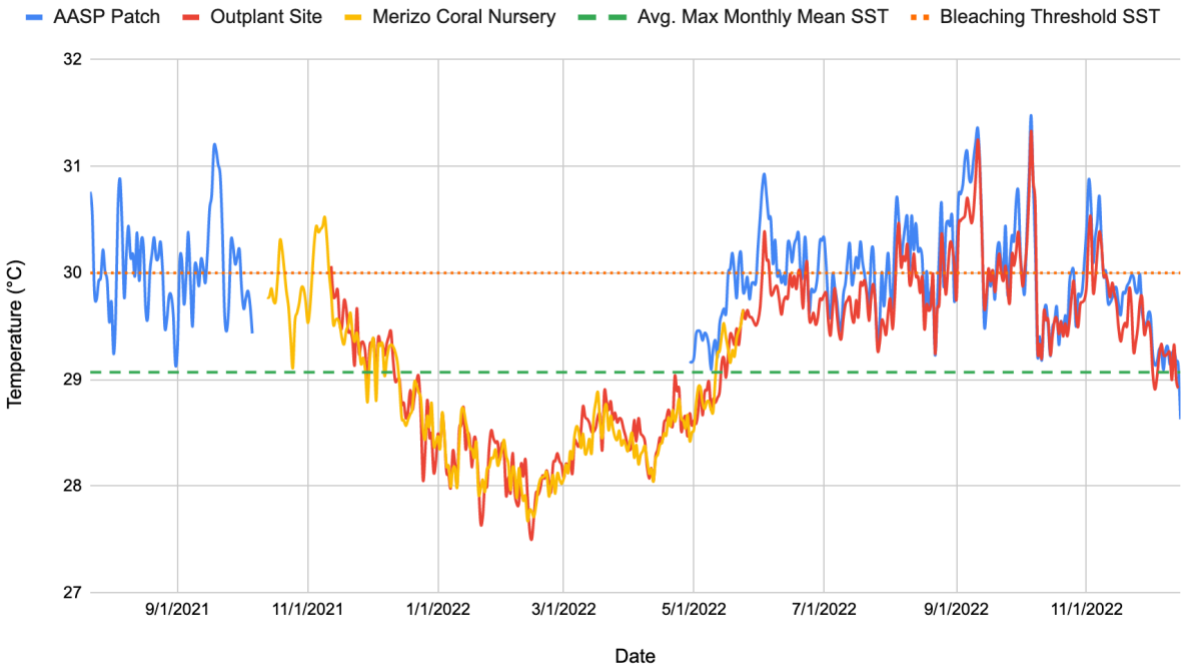

Figure 17. Average daily water temperature was measured at each site throughout the study period. Average max monthly mean SST and bleaching threshold SST data for Guam was obtained from the NOAA Satellite and Information Service Coral Reef Watch (https://coralreefwatch.noaa.gov/product/vs/gauges/guam.php). Gaps in AASP patch temperature data was due to a lost temperature logger in the field.

5 Discussion

Prior to this study, phenotypic plasticity observed throughout the restoration process and its implications on active coral restoration had not been explored. Understanding how species differentially respond to different restoration techniques is important for ensuring increased coral cover and restoration success. Here, I quantified the morphological plasticity in *A. aspera* under two different restoration methods: direct transplantation vs. nursery-rearing with subsequent outplanting, comparing them with extant wild colonies. Results demonstrate that *A. aspera*

displayed an ability to adapt to different restoration environments by expressing a suitable phenotype-environment match.

5.1 Growth trends

Analyses revealed that monthly growth rates and average TLE were significantly different between methods and their source population (Figure 8); fragments within the nursery experienced rapid growth, sometimes triple that of directly transplanted colonies (Figure 9). Although staghorn *Acropora* are inherently fast-growing species, studies have shown that fragments growing on a suspended nursery structure, such as the ones used during the nursery phase of this study, experience enhanced growth rates due to the availability of space in all directions (Griffin et al., 2012; Lirman et al., 2014; O'Donnell et al., 2017). Inversely, the slower growth rates of directly transplanted colonies can likely be explained by the energy investment in basal growth over branch growth to ensure stability onto available substrate (Lirman et al., 2014). Slow growth rates of source colonies reflect space limitations in the wild and the distribution of metabolic resources for other processes, such as reproduction (Lirman et al., 2014; Ware et al., 2020).

Nursery-reared fragments experienced a 10-week post-outplant recovery period where directly transplanted colonies experienced a shorter, 5-week recovery period (Figure 9). Studies have found that transplanted corals undergo shock periods post-transplantation, likely a response of acclimation to restoration site conditions or even from the transportation of coral fragments to the restoration site (Forrester et al., 2012; Stewart et al., 2021). The 10-week recovery period nursery outplants underwent is consistent with findings of a previous study comparing post-outplant stress responses of seven staghorn *Acropora* species reared in the Piti Bomb Holes Ocean Coral Nursery in Guam (Meade, 2022). Meade (2022) found that growth recovery of all seven species took approximately 10-weeks.

Because recovery patterns of nursery-reared corals were twice that of direct transplants, this could mean that the process of nursery-culture, potentially prolongs recovery periods of species. Nursery-reared fragments underwent rapid linear extension within the nursery environment and were also subjected to more environmental changes during the restoration process when compared to direct transplants. Exposure to multiple environmental changes coupled with energy investment into linear extension may potentially be prolonging recovery efforts once nursery-reared fragments are outplanted. Understanding possible trade-offs of nursery-culture of particularly vulnerable species is important to ensure the success of restoration efforts.

5.2 Morphometric plasticity and skeletal density

Radial corallite profile length and radial corallite diameter explained the most morphological differences observed between methods (Figure 10). Importantly, my analysis revealed that there were measurable phenotypic differences between source colonies and nursery-cultured fragments (Figure 10). Nursery-reared fragments and their outplants also displayed more space between radial corallites (Figure 11) and a smaller average axial tip diameter (Figure 12) than source colonies. Nursery outplants and directly transplanted colonies became more phenotypically similar and displayed less phenotypic variation overtime, displaying the ability of *A. aspera* to express phenotypic plasticity in a new environment (Figure 10). Therefore, it is important to note that different environmental regimes can change taxonomically important characteristics commonly used in field identification of *A. aspera*, which demonstrates a need for a multifaceted approach when defining species boundaries (Wallace, 1999; Todd, 2008; Stefani et al., 2011).

An increased plastic response has been associated with an increased probability of survival for corals in response to changing environments (Rinkevich, 2021; Million et al., 2022). By

examining morphological changes along with growth, I found that an increased phenotypic change was also associated with higher growth rates. Phenotypic plasticity in *A. aspera* was displayed as corals changed morphologically when placed in new environments during the restoration process, likely a response to increase their probability to survive.

Higher skeletal density was observed in source colonies and nursery-reared fragments, despite their higher growth rate (Figure 13). Interestingly, nursery-outplants experienced a decrease in skeletal density (Figure 13). Source colonies of *A. aspera* grow in a shallow reef flat area subject to high flow, which has been shown to be correlated with bulk skeletal density as well as decreased linear extension rates (Klein et al., 1993; Todd, 2008). Previous studies have shown that corals grown hanging in the water column tend to have lower skeletal density when compared to corals that are grown attached to surfaces (Kuffner et al., 2017; O'Donnell et al., 2017). In this study, corals grown on stable surfaces diverted more of their calcification effort into their basal growth for stability, whereas corals suspended in the water column invested more of their energy into linear extension (Lirman et al., 2014; Kuffner et al., 2017; O'Donnell et al., 2017). My results, however, showed that nursery-reared fragments grown on a hanging structure produced higher skeletal density than directly transplanted colonies. This counterintuitive response could be a result of the initial rope nursery method used for a majority of the nursery grow out phase, where fragments appeared more stable to water flow and weren't subjected to much movement in the water column. However, coral density and linear extension rates have been described as plastic growth traits that are more responsive to environmental forces, which could be at play here (Kuffner et al., 2017). My results suggest that creating denser skeleton may have been more beneficial to fragments within the nursery environment. Likewise, the decrease in skeletal density of nursery outplants could be a result of new growth being invested into basal growth and not

linear extension rates (Lirman et al., 2014). An increase in skeleton porosity toward the axial tips of *Acropora* branches has also been observed after transplantation efforts (Soong & Chen, 2003), which could also explain the decrease in skeletal density of nursery outplants.

The observed morphological changes in *A. aspera* shows that the environmental changes they experienced throughout the restoration process influenced how phenotypic plasticity was expressed. Understanding these changes in *A. aspera* will be important in informing species management efforts.

5.3 Survival and potential health trade-offs of nursery culture

Colonies between all methods had a survival rate of 100%. Million et al. (2022) observed that an increased plastic response of the staghorn *Acropora* species *A. cervicornis* was associated with an increased probability of survival. The ability of *A. aspera* to express a plastic morphological response at no cost to colony survival gives us a better insight into how phenotypic plasticity can be beneficial through the lens of coral restoration. Although the nursery phase exhibited higher growth and more dense skeletons, which are favorable characters for coral survival, the extended recovery period they experienced post-outplanting may be a cost of an intermediate nursery phase in an environment different than that of their wild population.

Predation pressure did not differ between methods, but all methods were affected at some point throughout the duration of the study (Figure 14) –observed in two main forms: breakage of axial tips and patches of tissue loss (Figure 4a-b). The sudden availability of *A. aspera* near reef patch populations could have presented itself as an opportunistic food source for corallivorous fishes within Cocos Lagoon. Corallivorous fishes, mainly Chaetodontidae, are obligate coral predators and have been observed to prefer staghorn *Acropora* in Guam (Reese, 1981). It is important to note that at the initial outplant site, directly transplanted corals were heavily predated

on, which led to total colony mortality of most colonies and the movement of plots to a location further from existing fish populations (Figure 4a). Meade (2022) observed similar predation patterns on seven different staghorn *Acropora* species, where predators did not appear to have a species preference. However, their study did observe a decline in predation of colonies after 10 weeks, which strongly suggests that the defense capability in staghorn *Acropora* species to ward of predators suffers during this post-outplanting recovery period (Meade, 2022). Corallivorous fish can threaten restoration success, which highlights the importance of understanding coral predator composition of restoration sites if possible (Cole et al., 2008; Meade, 2022).

Grey death disease, more commonly known as grey patch disease, is a poly-microbial coral disease found throughout Micronesia (Sweet et al., 2019). Although grey death was only observed in nursery outplants, its prevalence was low and, therefore, not significant between methods (Figure 15B). However, white syndrome prevalence was higher in nursery outplants (Figure 15A). White syndrome is an identification term used to describe several coral diseases that induce acute and rapid tissue loss (Ainsworth et al., 2006; Greene et al., 2020). It is one of the more widespread coral diseases found on reefs and its prevalence in Guam for certain species, including staghorn *Acropora*, increases during the warmer and wetter summer months (Greene et al., 2020). Nursery outplants were infected outside of the 10-week post-outplant recovery period (at week 12). Infection coincided with increasing temperatures at the restoration site (Figure 17), which are associated with an increase in coral susceptibility to disease (Rosenberg & Ben-Haim, 2002; Greene et al., 2020; Howells et al., 2020). Disease lesions on nursery outplant colonies also originated at the point of rope or monofilament contact, which were materials used during the nursery culture phase. Exposed coral skeleton or physically injured corals have been linked to an increased susceptibility to disease (Page & Willis, 2007; Katz et al., 2014). The prospect of these

materials as possible disease vectors should be explored further, and working to keep these points of contact clean and clear of debris in the nursery and ensure their removal when outplanting should also be prioritized. Although nursery outplant colonies were the only colonies infected, it is difficult to determine nursery culture and phenotypic plasticity as the main cause of disease infection when multiple abiotic and biotic factors exist at the restoration site. An increased plastic response may result in a prolonged period of susceptibility to disease, but the effects of phenotypic plasticity on the coral microbiome are understudied and should be explored further (Ziegler et al., 2019; Mohamed et al., 2022).

Changes in estimated symbiont density concentrations coincided with changes in restoration environments. Colonies that were directly transplanted displayed a shift to a lighter overall colony color post-transplantation (Figure 16). Bleaching or paling of colonies is a commonly observed stress response to transplantation or outplanting of colonies (Forrester et al., 2012; Pausch et al., 2018; Meade, 2020). Nursery culture fragments, however, displayed an opposite shift where overall colony color became darker in the nursery (Figure 16). Higher symbiont concentrations have been associated with deeper depths and decreased solar irradiance, which can explain the increased symbiont density concentrations observed in the nursery (Chen et al., 2005; Cohen & Dubinsky, 2015). Source colonies stayed consistent in symbiont density over time, with no signs of bleaching associated with the warmer, summer months during this study (Figure 16). Both directly transplanted and nursery outplant colonies eventually displayed symbiont concentrations that reflected their original state, however, directly transplanted colonies had a slower recovery period of symbiont density which lasted 14 weeks. Slow recovery of symbiont density could be a plastic response to the restoration process where maintaining lower

symbiont loads and associated lower growth rates minimize overall bleaching risk to colonies (Cornwell et al., 2021).

Acropora aspera colonies subjected to nursery-culture have shown to display an increase in phenotypic plasticity from the environmental changes that they experience throughout the restoration process. Understanding how this plastic response is associated with impacts of predation, disease, and symbiont density, among other health impacts, is important in order to guide future restoration monitoring and techniques for this species.

6 Conclusion

In this study I found that *A. aspera* displayed an ability to adapt to differing restoration environments by expressing phenotypic plasticity. This expression involved a process of acclimation and adjustment that occurred over a matter of weeks. Nursery-reared colonies expressed the most phenotypic plasticity throughout the restoration process when compared to directly transplanted colonies, likely a bet-hedging technique to increase their ability to survive in response to multiple changes in environment. Nursery-reared colonies also endured a longer post-outplant recovery period when compared to transplanted colonies, which could be a trade-off incurred from their plastic response. This post-outplant recovery period can leave colonies more susceptible to stressors present at the outplanting location. Understanding the potential trade-offs associated with nursery-culture is important when working with vulnerable species, such as *A. aspera*. Results from this study will work to fill gaps in understanding the role that phenotypic plasticity plays within the context of coral reef restoration and has important management implications that include the importance of understanding species level responses of nursery culture, the importance of prolonged monitoring efforts within outplanting recovery periods, and the importance of suitable site selection.

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8 Appendices

8.1 Tables

Table A3. GPS coordinates of site locations used during this project. Abandoned outplant locations were the initial sites where direct transplantation and outplanting were to occur but were abandoned and relocated due to severe predation on fragments.

Location	Latitude	Longitude
Outplant location	13.249362	144.673651
Abandoned outplant location (plot 1)	13.249722	144.672747
Abandoned outplant location (plot 2)	13.249896	144.672723
Cocos Coral Nursery	13.261117	144.66105
Source colony 40	13.244751	144.685022
Source colony 78	13.244971	144.684878
Source colony 37	13.245248	144.684857
Source colony 39	13.245609	144.684627
Source colony 64	13.24597	144.684574
Source colony 90	13.246287	144.684902
Source colony 70	13.246642	144.685385
Source colony 73	13.246624	144.685712
Source colony 44	13.24657	144.686156
Source colony 75	13.246157	144.686312

Table A4. Pairwise multiple differences of average radial crowding of morphometric samples between methods and their respective p-values (Tukey's HSD test). * indicate significant difference between methods at α=0.05.

Method Comparison	P-value (Tukey's HSD test)
Direct (7 mo.) vs. Direct (14 mo.)	0.996
Direct (7 mo.) vs. Nursery	0.004*
Nursery Outplant vs. Direct (7 mo.)	0.071
Source vs. Direct (7 mo.)	0.006*
Nursery vs. Direct (14 mo.)	0.013*
Nursery Outplant vs. Direct (14 mo.)	0.152
Source vs. Direct (14 mo.)	0.002*
Nursery Outplant vs. Nursery	0.847
Source vs. Nursery	<0.001*
Source vs. Nursery Outplant	<0.001*

Table A5. Average radial crowding count per transect and standard deviation values

Method	Average count
Direct (7 mo.)	12.55 ± 2.23
Nursery	9.38 ± 2.08
Source	16.45 ± 4.27
Nursery Outplant	10.03 ± 2.01
Direct (14 mo.)	11.9 ± 1.58

Table A6. Pairwise multiple differences of axial tip diameter of morphometric samples between methods and their respective p-values (Dunn test). * indicate significant difference between methods at α=0.05.

Method Comparison	P-value (Dunn test)
Direct (7 mo.) vs. Direct (14 mo.)	0.374
Direct (7 mo.) vs. Nursery	0.033*
Direct (14 mo.) vs. Nursery	<0.001*
Direct (7 mo.) vs. Nursery Outplant	0.602
Direct (14 mo.) vs. Nursery Outplant	0.263
Nursery vs. Nursery Outplant	0.121
Direct (7 mo.) vs. Source	0.026*
Direct (14 mo.) vs. Source	0.342
Nursery vs. Source	<0.001*
Nursery Outplant vs. Source	0.004*

Table A7. Average axial tip diameter and standard deviation values

Method	Average axial tip diameter (mm)
Direct (7 mo.)	3.2 ± 0.26
Nursery	2.76 ± 0.15
Source	3.74 ± 0.2
Nursery Outplant	3.12 ± 0.18
Direct (14 mo.)	3.48 ± 0.48

Table A8. Pairwise multiple differences of skeletal density measurements of morphometric samples between methods and their respective p-values (Dunn test). * indicate significant difference between methods at α=0.05.

Method Comparison	P-value (Dunn test)
Direct (7 mo.) vs. Direct (14 mo.)	1.000
Direct (7 mo.) vs. Nursery	1.000
Direct (14 mo.) vs. Nursery	1.000
Direct (7 mo.) vs. Nursery Outplant	1.000
Direct (14 mo.) vs. Nursery Outplant	0.490
Nursery vs. Nursery Outplant	0.170
Direct (7 mo.) vs. Source	0.581
Direct (14 mo.) vs. Source	1.000
Nursery vs. Source	0.634
Nursery Outplant vs. Source	0.048*

Table A9. Skeletal density estimates and standard deviation values

Method	Average skeletal density estimates (g/mm^3)
Direct (7 mo.)	0.00128289 ± 0.000139868
Nursery	0.001342568 ± 0.000161827
Source	0.001386657 ± 7.44972E-05
Nursery Outplant	0.001173246 ± 0.000261356
Direct (14 mo.)	0.001344989 ± 0.000116149

Table A10. Average values and standard deviation of morphometric characters: radial wall thickness, radial corallite profile length, radial corallite diameter, and radial calice diameter.

Method	Average radial wall thickness (mm)	Average radial corallite profile length (mm)	Average radial corallite diameter (mm)	Average radial calice diameter (mm)
Direct (7 mo.)	0.39 ± 0.09	1.4 ± 0.28	1.67 ± 0.29	1.11 ± 0.17
Direct (14 mo.)	0.3 ± 0.07	1.31 ± 0.24	1.45 ± 0.23	1.01 ± 0.12
Nursery	0.29 ± 0.06	1.03 ± 0.16	1.51 ± 0.2	0.97 ± 0.13
Nursery Outplant	0.28 ± 0.07	1.32 ± 0.26	1.45 ± 0.23	0.96 ± 0.12
Source	0.51 ± 0.13	1.62 ± 0.23	1.96 ± 0.26	1.24 ± 0.16

8.2 Figures

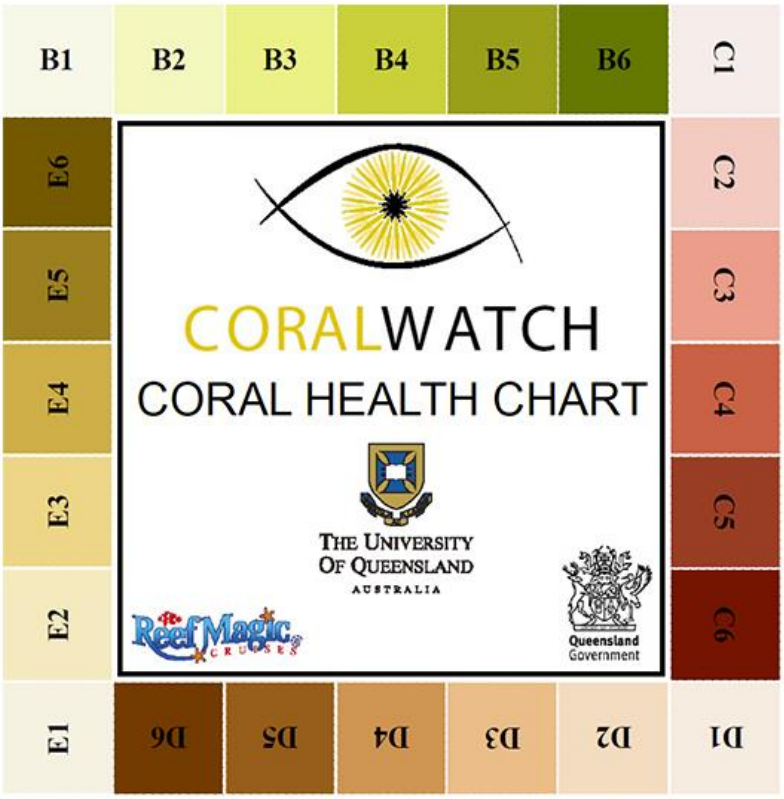

Figure A18. CoralWatch Color Health Chart used as a proxy indicator of symbiont density in corals using a 6-point numerical scale.

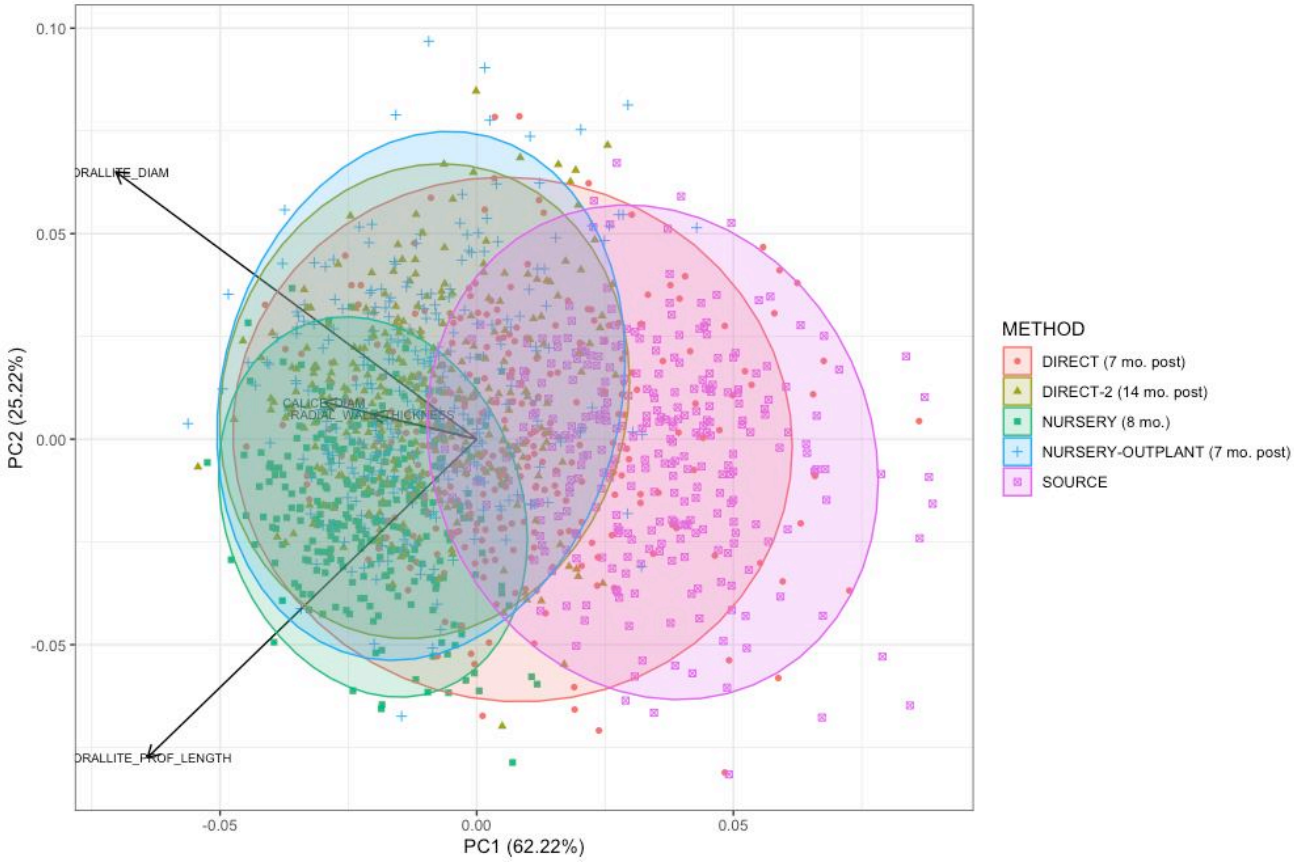

Figure A19. PCA of corallite morphometrics showing positions separated by method of four morphometric characters measured: radial corallite diameter, radial calice diameter, radial wall thickness, and radial corallite profile length. Each method is represented by a different color and color.

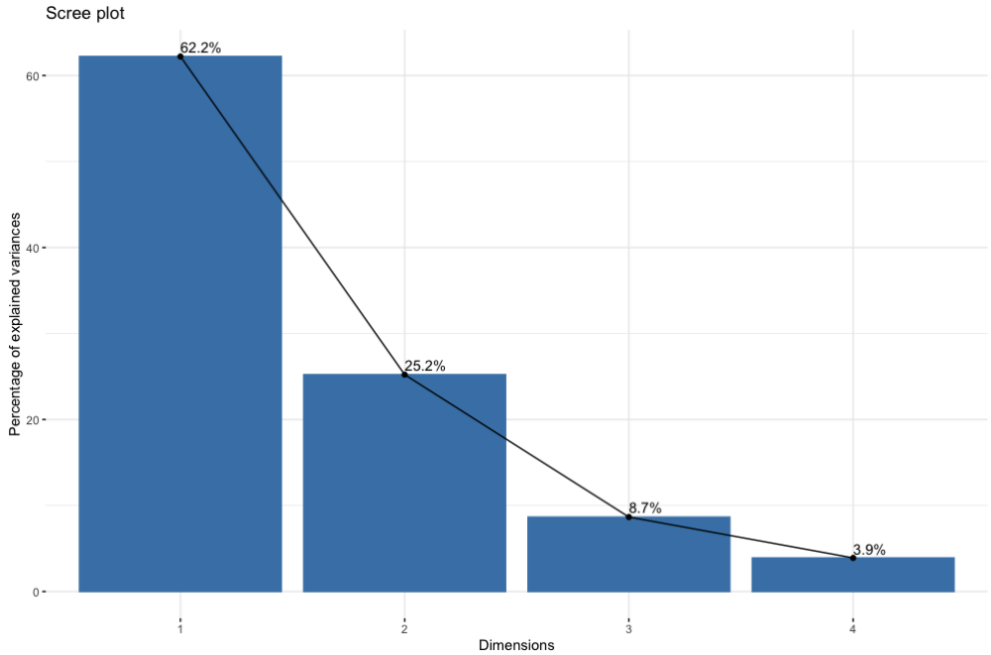

Figure A20. Scree plot for PCA analysis of four dimensions: radial wall thickness, radial corallite profile length, radial corallite diameter, radial calice diameter.

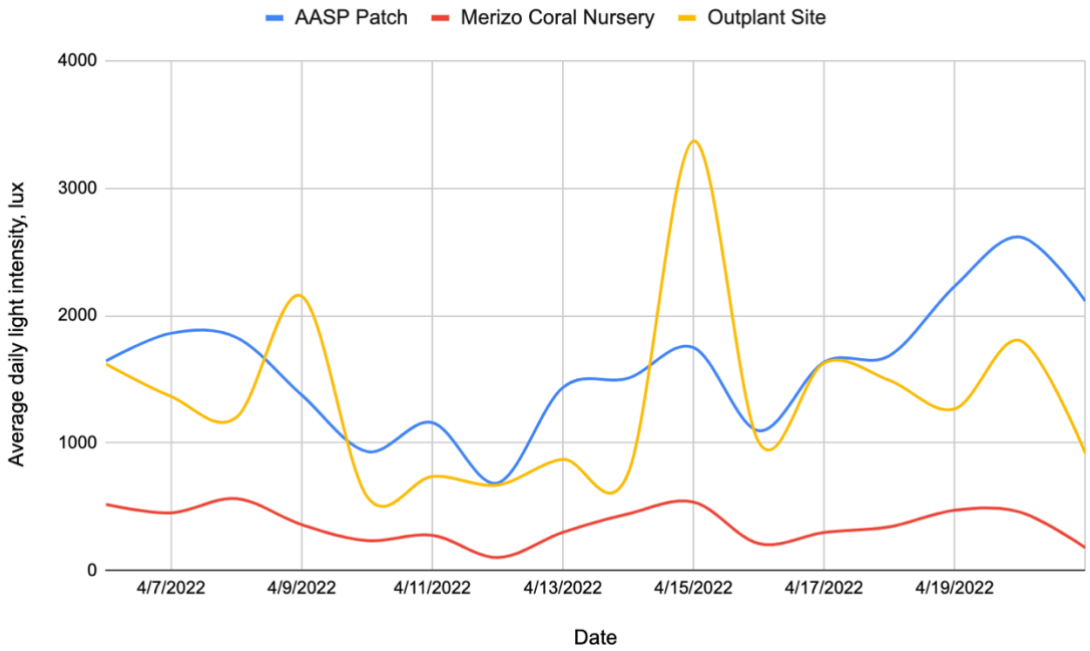

Figure A21. Dry season average daily light intensity (lux). The dry season typically lasts from January-June. Data was collected for a period of approximately two weeks.

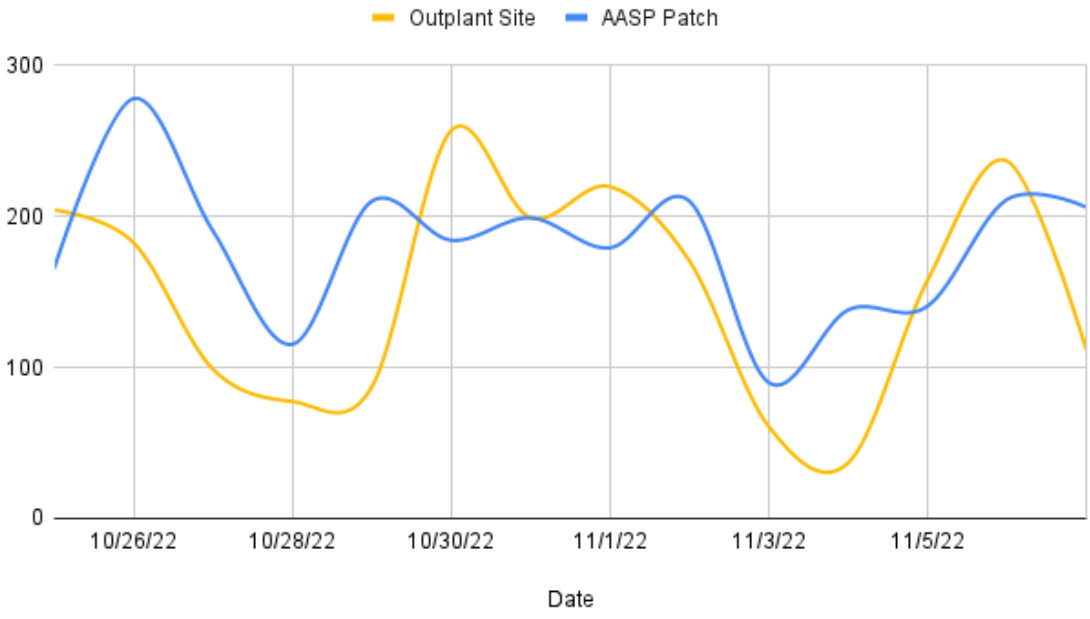

Figure A22. Rainy season average daily light intensity (lux). The rainy season typically lasts from July-December. Data was collected for a period of approximately four weeks.

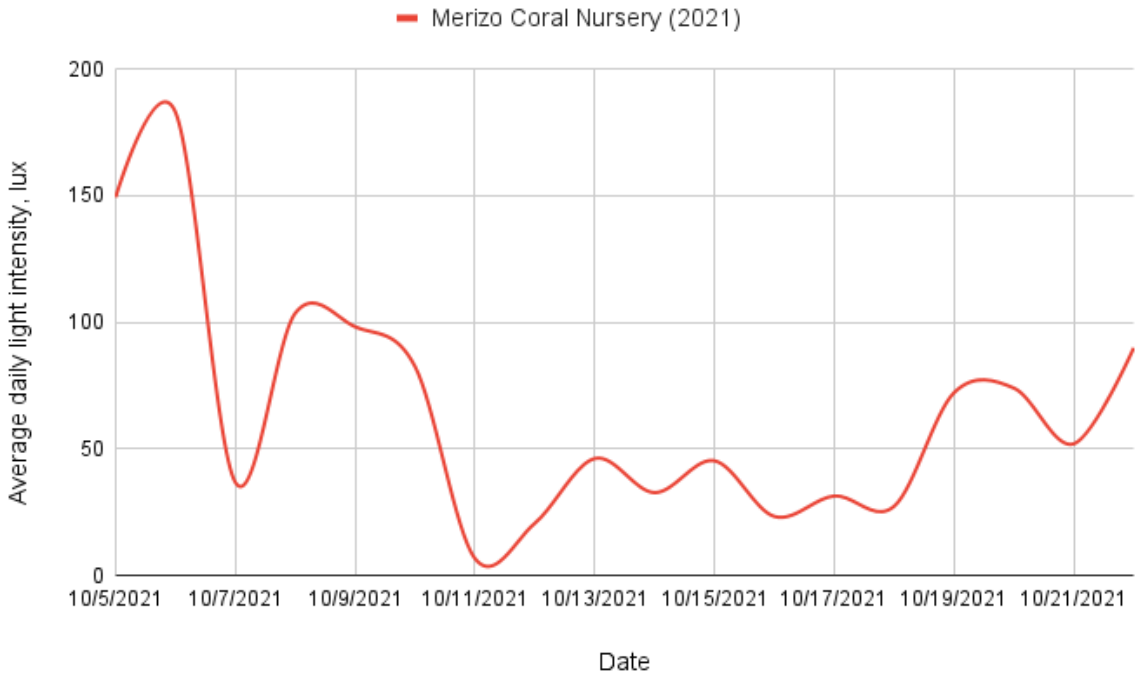

Figure A23. Rainy season average daily light intensity (lux). The rainy season typically lasts from July-December. Light data was not collected at the nursery during the rainy season for this study. Historical light intensity data measured at the Merizo Coral Nursery in 2021 was used.

8.3 Data sheets

NFWF Rapid Health Assessment

Site:	**Depth:**	**Date:**
Plot:		**Recorder:**

% PM bin: 0 = 100% alive; **1** = 1-24%; **2** = 25-49%; **3** = 50-74%; **4** = 75-99%; **5** = 100% dead

BleachSev: PPL=partial pale; **PL**=whole pale; **PBL**=partial bleach; **BL**=whole bleach; **BLM**= total bleach mortality

Impacts: WS; BBD; BrB; SEB; GA; CMD; PR; PRD; DMS; DRP; COTS; TP; ALOG; CYA; PR; PD; LTX; PM

% Severity Bin (active lesions from impact)**: 1**=1-10%; **2**=11-25%; **3**=26-50%; **4**=51-75%; **5**=76-99%; **6**=dead

Colony #	Coral Watch		Bleach Sev	% PM	Impacts and % Severity Bin	Notes
	Upper Br.	Lower Br.				