Scaling up production capacity for Hawaiian coral reef restoration: few large or many small?

NOAA grant #: NA18NOS4820110
Principal Investigator: Dr. Robert Toonen
Postdoctoral Researchers: Dr. Zac Forsman, Dr. Ingrid Knapp
Graduate Assistants: Erika Johnston, Austin Greene
Undergraduate Assistant: Clare Bardin
Reporting Period: 08/01/2018 - 01/31/2020

I. Abstract

As coral reefs decline worldwide, interest in active coral reef restoration has increased. The micro-fragmentation method is becoming widely used for massive and encrusting species such as those that dominate Hawaiian reefs. Micro-fragmentation takes advantage of the tendency of small (~1cm) coral fragments to rapidly spread tissue and fuse to cover a surface, resulting in increases in coral cover at rates that can be orders of magnitude higher than typical growth rates. Micro-fragmentation shows promise and flexibility for reef restoration; however, it is a relatively new method and there are many unanswered questions for optimal application at a particular restoration site. This project sought to explore relationships and tradeoffs between size, nursery residence time, and genotype for a variety of outplanting field sites. We developed coral micro-fragment growth assays to provide data on growth and survivorship for the predominant reef building corals in Hawai‘i. We deployed 126 assay modules consisting of 882 small, 378 medium, and 126 large fragments yielding 1,386 coral fragments in total across 3 distinct coral reef habitats (Kāne‘ohe Bay reef flat, Honolulu Harbor, and a South shore exposed reef). The assays were held in the nurseries and deployed over time in batches (direct transplantation (0 months), 4 months, and 8 months) to examine effects of nursery residence time. Health checks tracking mortality, partial mortality, and tissue discoloration were ranked, and coral tissue cover (cm^2) was measured from scaled digital 2D photographs and also estimated from 3D Structure from Motion (SfM) scaled photogrammetric models. The project coincided with a bleaching event in the summer, providing data on growth and survivorship before, during, and after the event. Survivorship was highly dependent on site, size, and genotype (i.e. donor colony). Colony genotype had a major impact on coral growth and survivorship, indicating that selection of genotypes is critical for restoration success. Several genotypes more than doubled in total coral cover and total colony surface area while one genotype was highly susceptible to bleaching and suffered total mortality. Growth data from 2D and 3D measurement methods showed highly similar results, although 3D measurements were less time consuming, while accounting for complex colony morphology. *In-situ* nursery residence time showed high temporal variability likely due to micro-spatial variation, temperature stress and micro-predator outbreaks. The *ex-situ* nursery in contrast had almost no mortality and the highest growth rates, but variable mortality when outplanted depending on size and genotype. Mortality was highest on the exposed reef and both harbor and reef sites had the highest mortality for smaller fragments, indicating that a ‘size refuge’ is highly site specific. Mortality also varied by colony genotype at both harbor and reef sites, with several colonies showing an interaction between genotype and environment. Final growth data at the AFRC sites could not yet be collected due to the COVID-19 shutdown; however, this project demonstrated that the assays successfully provided data on optimal colony size, genotype, and nursery residence time for a variety of reef locations.

II. Introduction and Background

Coral reef ecosystems are declining worldwide and novel management tools are needed to address emerging conservation challenges (Rinkevich 2005; Bruno and Selig 2007; Hoegh-Guldberg et al. 2007; Mumby et al. 2007). Corals form the structure and foundation of coral reefs, fulfilling an ecological role analogous to trees in terrestrial ecosystems. One of the most widely used conservation and management tools for terrestrial forests, is to incorporate a nursery phase where vulnerable seeds, saplings, or propagules are sheltered and provided conditions to greatly increase the probability of survivorship; the strategy has dramatically transformed forest ecosystems (Khurana and Singh 2001; Fox et al. 2004). Over the last two decades, coral nurseries have transitioned from small scale pilot projects, to large full scale operations dedicated to coral reef restoration (Epstein et al. 2003; Rinkevich 2008) (Rinkevich 2008), for the marine hobby industry (Delbeek 2001), and for the conservation of rare or endangered coral (Herlan and Lirman 2009; Griffin 2012).

The benefits of reef restoration activities vary from site to site, because natural recruitment and recovery rates are highly variable, both temporally and spatially (Connell et al. 1997; Kojis and Quinn 2001). Reefs surrounded by high coral cover might naturally recover from disturbance within a decade (Grigg and Maragos 1974; Connell et al. 1997), while some reef systems and entire ecological regions may take an order of magnitude longer if they ever recover at all (Smith 1992; Hughes and Tanner 2000; Salinas-de-León et al. 2012). Recruitment failure and high rates of post-settlement mortality can result in a downward spiral of ecosystem collapse and transition to alternative stable states (Hughes 1994; Hughes and Tanner 2000). Transitions in the other direction; from algal to coral dominated ecosystems are rare and challenging to document, but increased fish and coral recruitment have been documented to occur with some large scale reef restoration efforts in the Caribbean (Schopmeyer and Lirman 2015; Huntington et al. 2017; Opel et al. 2017) and the Pacific (Yap 2009; Cabaitan et al. 2015). In order for reef rehabilitation efforts to be effective at larger scales, knowledge of the optimal colony size and nursery residence time for each species while maintaining a high chance of survivorship in the field, is necessary to optimize efficiency (dela Cruz et al. 2015).

Larger colonies are thought to have a higher chance of survival. This so-called 'size refuge' is likely to vary both temporally and spatially, for each species. Previous studies on demography and size dependent survivorship for reef building corals, have found relationships between size and mortality to be dependent on nursery conditions (Forsman et al. 2006), habitat (Bruno, 1998), bleaching events (Depczynski et al. 2013), and competitive interactions (Ferrari et al. 2012). On the other hand, survivorship is highly stochastic in highly heterogeneous coral reef ecosystems (Babcock et al. 1991), and under the right conditions recovery from small fragments or patches of live tissue can occur surprisingly quickly (Roff et al. 2014). There are significant tradeoffs between strategies to outplant larger coral colonies, vs. approaches to outplant many smaller colonies, for example a new coral seeding approach that uses small concrete tetrapods with settled coral recruits found a 5 to 18 fold reduction in out-planting costs (Chamberland et al. 2017). This seeding approach dramatically reduces diver time in the water, which is the costliest aspect of reef restoration work. Survivorship in this initial study was low (9.6% over 1 year), however; if survivorship could be increased the approach has potential for enabling larger scale reef restoration. Determining the 'size refuge' for each species at a particular restoration site would enable a more targeted approach to mass producing and outplanting coral.

One of the main roles of a coral nursery is to improve survivorship of small fragments by housing them before outplanting. There are two main types of nursery either *in-situ* (in the water) or *ex-situ* (in tanks on land), each with tradeoffs and benefits. *In-situ* nurseries have minimal maintenance and equipment costs, but environmental conditions are more difficult to control (e.g. light, temperature, sedimentation, predation, disease). One key way to improve

efficiency for both types of nurseries is to reduce the amount of time that smaller colonies and micro-fragment covered modules need to be maintained in the nursery. Since the micro-fragmentation method can precisely control colony genotype and size growing over a given substrate, it has excellent potential as an assay to provide data on mortality and growth for replicated genotypes across a range of sizes at a particular restoration site. Knowledge of genotype and size specific mortality rates for a given site would allow for targeted mass production of resilient genotypes of an optimal size, improving the outcome of restoration and optimizing the efficiency and scale that can be restored.

Micro-fragmentation is primarily an *ex-situ* nursery based method which results in rapid two-dimensional spreading of tissue at rates that can be orders of magnitude higher than growth rates under typical field conditions (Forsman et al. 2015). The technique uses small (~1cm^2) fragments spaced approximately 2-3 cm apart over an artificial substrate, taking advantage of the tendency of these small fragments to spread thin layers of tissue, doubling or quadrupling in size in a few months. The use of the method was pioneered at the Ānuenue Fisheries Research Center (AFRC), the State of Hawai‘i Division of Aquatic Resources (DAR) Coral Restoration Nursery (CRN), where hundreds of 20cm to 1m diameter coral modules can be produced annually. The method is becoming widely used by NGOs including the Coral Vita in the Bahamas, and the Legacy Reef Foundation on Hawai‘i Island, and MOTE Marine Laboratory in the Florida Keys. MOTE Marine Labory uses micro-fragmentation to mass produce coral plugs that are attached to dead coral heads or ‘re-skinning’, which can result in bringing large endangered coral colonies back to life (Page et al. 2018). The Hawai‘i Institute of Marine Biology (HIMB) coral nursery consists of floating midwater platforms of coral that were rescued after naturally settling on decommissioned fish and dolphin pens. The primary function of the HIMB *in-situ* coral nursery is to conduct research on approaches including micro-fragmentation to increase the scale of restoration efforts. An advantage of the micro-fragmentation method is that genetic replicates of a wide variety of sizes can be produced, which is ideal for an assay to determine the size refuge for a variety of species in various habitats. Combining the micro-fragmentation method with a coral seeding approach has the potential to dramatically increase survivorship, while dramatically reducing costs, however: the optimal colony size and nursery residence time for such an approach are unknown. The broad goal of this project was to collect data on fragment size, nursery residence time and genotypic differences for both in water (the HIMB *in-situ* nursery) and land based (the DAR *ex-situ* nursery) nurseries to improve strategies for more cost-effective and time-efficient reef restoration at larger scales under a variety of outplanting conditions in the field (**Figure 1**).

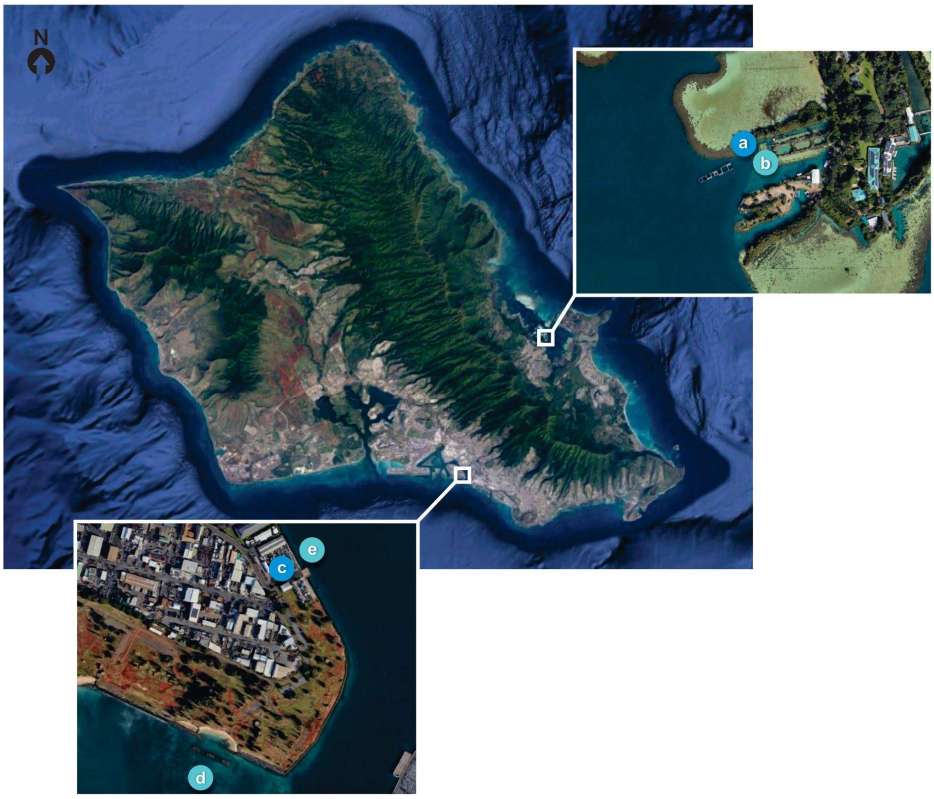


Figure 1. Map of the two experimental areas, with the darker blue indicating the nurseries and the lighter blue the outplanting sites where **(a)** HIMB nursery, **(b)** HIMB outplanting reef site, **(c)** AFRC nursery **(d)** AFRC outplanting reef site, and **(e)** AFRC outplanting harbor site (Honolulu harbor)

III. Specific goals and objectives

1. **Design and deploy coral growth assays** to allow comparisons across both *in-situ* and *ex-situ* nurseries and a range of reef sites.
2. Assess **coral health, change in coral cover, and three-dimensional growth** on the coral assays.
3. Determine if there is a 'size refuge' or **optimal colony size for a range of reef sites**.
4. Determine if there is an optimal *in-situ* and *ex-situ* **nursery residence time** to reduce costs and increase scale of restoration.
5. Examine **genotypic differences** in growth and survival between and within the dominant reef building corals in Hawai'i (*M. capitata*, *P. lobata*, and *P. compressa*).

IV. Methods

Assay design and deployment

Designing assays suitable across both types of nursery and outplanting sites was integral to the project. Our final design incorporated a central label and three sloped sides in a 'pyramid' shape (**Figure 2**) to reduce the horizontal surface and therefore mortality from sedimentation, while allowing space for corals to grow and fuse covering the substrate, blending into the reef substrate similar to the coral modules currently in use at AFRC. All pyramids were soaked in seawater for a month and allowed to dry in the sun for a further month before fragmentation.

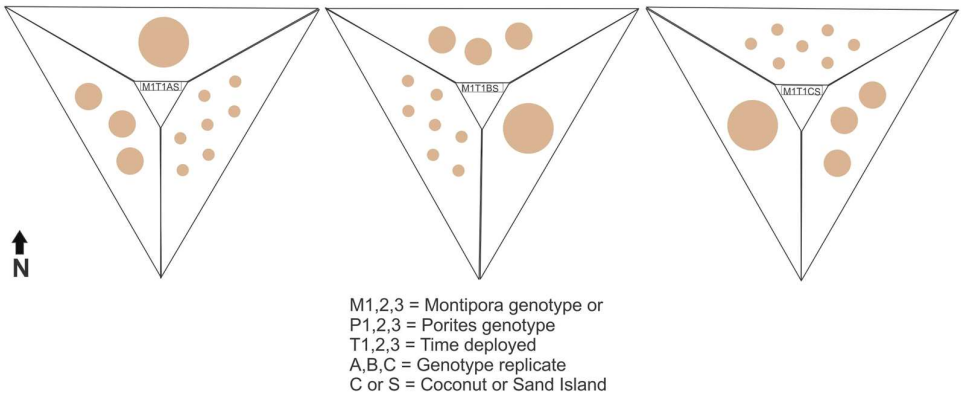


Figure 2. Rotational pyramid fragmentation protocol (above), where replicate assays rotated the location of each fragment size in order to expose all size fragments to all water flow and light conditions, assays were then outplanted facing the same direction, to provide an informative unique identifier for each pyramid assay. A label key is located below the diagram.

Three parent/donor colonies were collected for *M. capitata* and *P. lobata* at each site (**Figure 1**) resulting in six parent colonies per nursery. AFRC's standard quarantine period was also observed for the *ex-situ* nursery samples, whereby any parent colonies not already in the nursery had all other epifauna removed, they were then placed in a quarantine tank, and were required to remain clear of parasites for one month (**Appendix S1**). Parasites (*Phestella sp*.) on the *P. lobata* AFRC parent colonies resulted in delayed fragmentation and deployment by 2 months at this site.

Within each outplanting location, time and genotype there were three replicates and the location of each fragment size was alternated around the pyramid with a clearly visible label on the top (**Figure 2**). The pyramids were then outplanted with this label all facing the same direction, therefore each fragment size was exposed to all the potential variable light and water flow conditions. 126 assay modules were constructed and deployed across five environments, including the two nursery sites (HIMB *ex-situ* and AFRC *in-situ* nurseries), and the outplanting sites (HIMB reef flat, Sand Island exposed south shore reef, and Honolulu Harbor; **Figure 1**). The harbor site by AFRC was included as an additional site for comparison for the first phase of outplanting to provide data on variability due to contrasting habitats.

Three *M. capitata* and three *P. compressa* colonies, approximately 30 cm across, were selected as the donor colonies for each nursery. The corals were micro-fragmented to yield seven small (≈ 1cm^2 each, 7cm^2 total), three medium (≈ 3cm^2 each, 9cm^2 total), and one large (≈ 9cm^2 each and total) per pyramid (**Figure 2**) totaling 378 small, 162 medium and 54 large fragments for all the pyramids deployed at each reef outplanting site, with an additional 126 small, 54 medium, and 18 large at the harbor site at AFRC. All fragments were cut on a Gryphon XL Aquasaw and 42" diamond tipped stainless steel blade and fixed to the pyramid assays with cyanoacrylate (gel superglue). The top and all sides of the pyramid were photographed with a ruler before placing in the nursery or outplanting site.

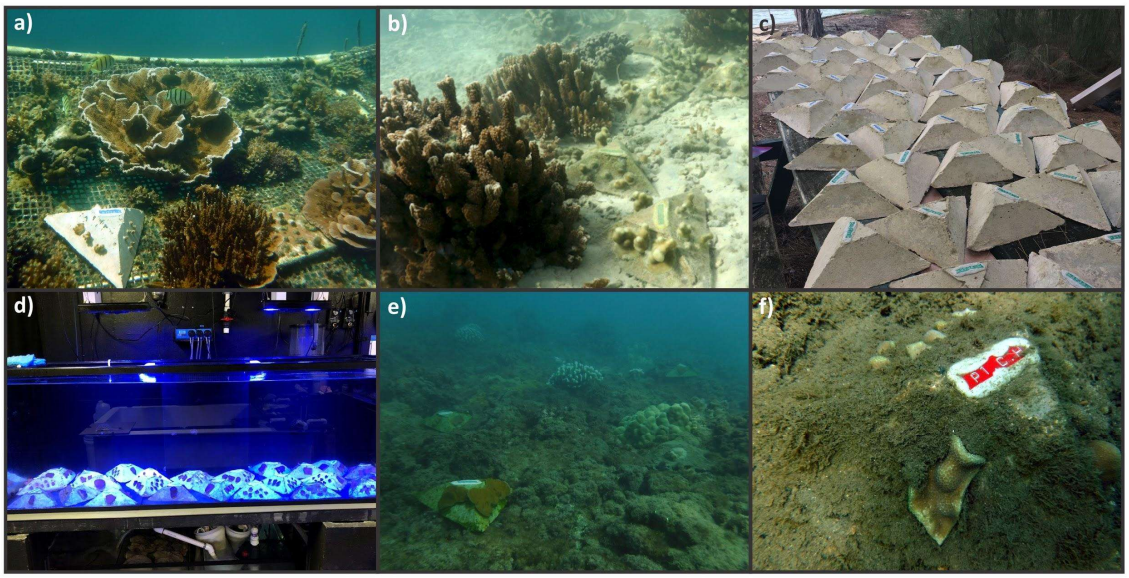


Figure 3. Photos of assays **(a)** in the HIMB coral nursery, and **(b)** HIMB outplanting reef, **(c)** prepared before fragmenting **(d)** in the AFRC coral restoration nursery, and AFRC outplanting sites **(e)** Sand Island beach park, and **(f)** Honolulu harbor.

The reef outplanting experiment was run as close to parallel as logistically feasible at the two nursery sites (**Figure 3a and d**). The assays were split into three identical groups consisting of 18 assays, giving a total of 54 pyramids (**Figure 3c**) per nursery and an additional set of 18 for deployment in Honolulu Harbor. After fragmentation the first set from both nurseries were immediately outplanted at their respective reef sites (**Figure 3b, e and f**), while the remaining two sets were kept in nursery conditions (**Figure 3a and d**). After 4 months the second set of 18 pyramids were deployed, leaving only one set in the nursery. After a further 4 months (8 months since the first set was deployed) the third set was outplanted.

Coral health checks were performed to provide data on temporal changes in mortality and tissue discoloration in the following categories (dead and/or partial mortality, paling/bleached, and healthy), approximately every 1-3 months at HIMB and 2-4 months at the AFRC locations. At the HIMB reef location, oysters were observed settling on bare substrate on the assays within a few months and the number of oysters per module were recorded to examine the potential for competition with coral tissue for space (**Appendix S2 and S3**). The initial and final fragment size of all 1,386 corals were manually measured from scaled digital photos using the program ImageJ (Rasband 2012). Change in area of live tissue (**Δ**cm^2) was measured from digital photographs of all assays taken at the point of fragmentation (0 months), outplanting of the final assay set on the reef (8 months) and a final growth measurement was then collected at HIMB 4 months after the final deployment (**Table 1**), giving a total growth time of no less than a year. Due to the COVID-19 closure, the collection of the final set of photographs at AFRC has been delayed but we expect to obtain these images and analyze the results as soon as possible for publication.

Table 1. Timeline of coral assay deployment. Phase 1 (0 months) set 1 was outplanted on the reef and set 2 and 3 were placed into the nursery. Phase 2 and 3 provided 4 months (for set 2) and 8 months (for set 3) of growth respectively in the nursery prior to transplantation on the reef. Phase 4 was the final growth measurement representing 12 months of growth since fragmentation.

Assay phase	HIMB (quantity)	AFRC (quantity)
Phase 1- Deployment (Set 1) (direct transplant)	12/10/2018 (n=54)	02/06/2019 (n=72)
Phase 2 - Deployment (Set 2) (4 month nursery growth)	04/04/2019 (n=18)	05/28/2019 (n=18)
Phase 3 - Deployment (Set 3) (8 month nursery growth)	08/16/2019 (n=18)	10/18/2019 (n=18)
Phase 4- Growth (Sets 1, 2 &3) (12 months growth)	12/12/2019	Pending

Estimating surface area from 3D photogrammetric models:

The coral fragments at both the HIMB nursery and field site grew with a higher degree of three dimensional structure than anticipated (**Figure 4**), therefore in addition to estimating growth from planer top down digital images, we investigated using photogrammetry to estimate surface area of living coral tissue at the HIMB locations.

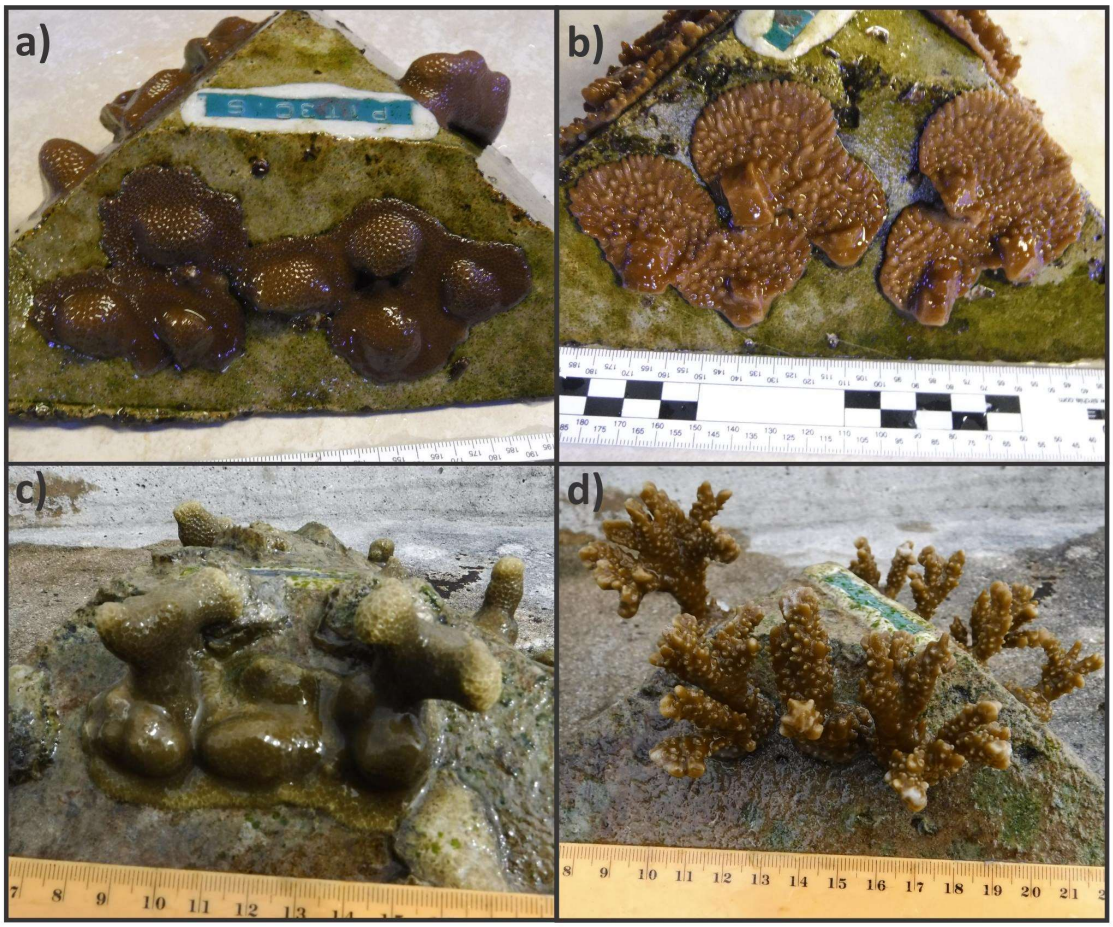


Figure 4. Examples of *Porites compressa* **(left)** and *Montipora capitata* **(right)** fragments spreading horizontally and fusing and/or growing vertically on assays at both AFRC (**a and b**), and the HIMB (**c and d**) coral nurseries.

A three dimensional structure from motion (3D SfM) photogrammetric model of coral assays at the HIMB site was constructed using Agisoft Metashape Pro v 1.5.5, from approximately 500 photos taken with a Canon Rebel EOS in an underwater housing on 12/19/2019 (**Figure 5**). Camera setting and assembly of the SfM model followed recommendations in Suka et al. (2020), briefly, manual camera settings were selected (auto ISO exposure, f-stop=F10, shutter speed=1/320, -⅓ exposure, broad point autofocus, repeat shutter, and large format photos). A batch script was followed in Agisoft Metashape Pro v 1.5.5 with the following settings (alignment = high accuracy and generic precision, 40k key point limit, 4k tie point limit, adaptive camera model fitting, Optimization = fit f and cx,cy, Build dense cloud = medium quality, mild depth filtering, build mesh = arbitrary surface type, medium depth map quality, build texture = generic mapping mode, texture from all cameras, and hole filling enabled, build tiled model = source data dense cloud with medium depth map quality). The resulting SfM model was scaled with a series of six printed targets, fixed in pairs 10cm apart. The accuracy of the three scale bars was 0.001 m (0.1 cm), with an overall estimated error of 0.000655 m (0.07 cm). The scaled 3D SfM model was exported into Cloudcompare v2.11 and areas with living coral tissue were segmented for inclusion with the segmentation tool. Corals on each side of the pyramid were grouped, labeled and colorized using an elevation model to highlight upward growth along the Z axis (**Figure 5**). Surface area

was estimated for each size category (*e.g.* fused or unfused corals were grouped together for estimation of total surface area for each size category). Finally, we compared two-dimensional area from top down measurements with three-dimensional surface area estimates from the SfM model by linear regression in R (R Core Team 2013).

Figure 5. Example of color-coded segmentation and computer labeling of living coral tissue on an HIMB coral assay module used for the estimation of surface area covering the complex geometry of coral colonies.

To examine *P. compressa* and *M. capitata* net growth (cm^2) obtained by either 2D top down photographs or 3D photogrammetry images and fragment size, nursery residence time and genotype we used generalized linear models (implemented in RStudio (2019)) along with pairwise comparisons using the "emmeans" package (Lenth, 2020). For size, time and genotypic comparisons, analysis of net growth was only conducted on fragments that survived to the end. Due to the skewness of the raw data prior to running the models the data was transformed with the Yeo Johnson transformation based on the "bestnormalize" package (Peterson, 2019) output, which contains a suite of transformation-estimating functions and suggests the most appropriate transformation method for a given dataset.

V. Results and discussion

Coral assay design and deployment

The concrete pyramid assay design was well suited to both *in-situ* and *ex-situ* nurseries and a variety of reef sites (patch reef flat, protected harbor, and exposed reef). They were also easy to handle and small enough to take up minimal space in each nursery. The concrete pyramid design was also ideally suited to outplanting as they were negatively buoyant, and allowed for placement in sandy rocky areas at HIMB and the AFRC harbor site and attachment

to the reef with epoxy at the AFRC reef site. The sloped sides reduced issues with sedimentation while still allowing for a clearly visible label, which was also used to orient the pyramids during outplanting. The design provided substrate for fusion of coral tissue and did not appear artificial, rapidly blending into the reef substrate.

Monitoring of coral health and mortality

At the HIMB *in-situ* nursery, the mean (± S.D) size of the micro-fragments was 0.74 (± 0.27), 2.86 (± 1.03), 8.19 (± 2.19), for the small, medium, and large size categories respectively. These micro-fragmented coral colonies had higher than 80% survivorship for the first months after fragmentation (**Figure 6 a-c**). With the onset of increasing summer temperatures leading up to the 2019 summer bleaching event, mortality began to increase, showing a clear size dependent trend in the *in-situ* nursery with 54, 71 and 72% overall survivorship of small, medium and large colonies respectively in August 2019. Assays on the reef flat also showed a size dependent trend with 45, 64, and 64% overall survivorship of small, medium and large colonies respectively. By the 8th month (8/16/2019), assays on the reef flat had higher mortality and partial mortality. After 12 months of monitoring at the close of the experiment, size dependent trends were no longer evident, although many of colonies in the medium and small categories had completely fused at this point, decoupling the relationship between the original health check categories and actual colony size (**Figure 4**). By the final monitoring period after 12 months, mean overall survivorship was 34%, with 37% and 31% of *M. capitata* and *P compressa* fragments surviving respectively (**Appendix S4 and S5)**. Overall survivorship of small, medium and large colonies for each species were 35, 35, and 52 % for *M. capitata* and 22, 44, and 52 % for *P compressa* respectively, suggesting a size dependent trend for both species with the 'large' size category having the highest survivorship for each species.

Mortality was highly dependent on the donor colony (i.e. genotype). The *M. capitata* colony M2 was particularly susceptible to bleaching, and began showing signs of paling and partial mortality at 2 (2/28/2019) and 5 (5/7/2019) months into the experiment (**Figure 7**). With the onset of increasing temperatures leading up to the bleaching event, partial mortality and mortality began to increase across all genotypes. By the 8th month (8/16/2019), only ~20% of colony M2 survived in the nursery and ~40% on the reef (**Figure 7d**). In contrast, colony M1, M3, and P3 showed higher resilience with ~80% survival in the nursery and ~60% survival on the reef flat (**Figure 7d**). By the end of the experiment at 12 months, M2 suffered 100% mortality, while M1 and M3 had 46 and 64% survivorship respectively, while P1, P2 and P3 had 20, 32, and 40% survivorship respectively (**Appendix S4 and S5**), indicating that mortality is highly dependent on the donor colony. Careful selection of colony genotypes for a restoration project is therefore likely to increase the chances of success at a given location and further study to understand interactions between coral genotypes and their environment will shed light on resilience to bleaching events and appropriate selection of outplanting sites.

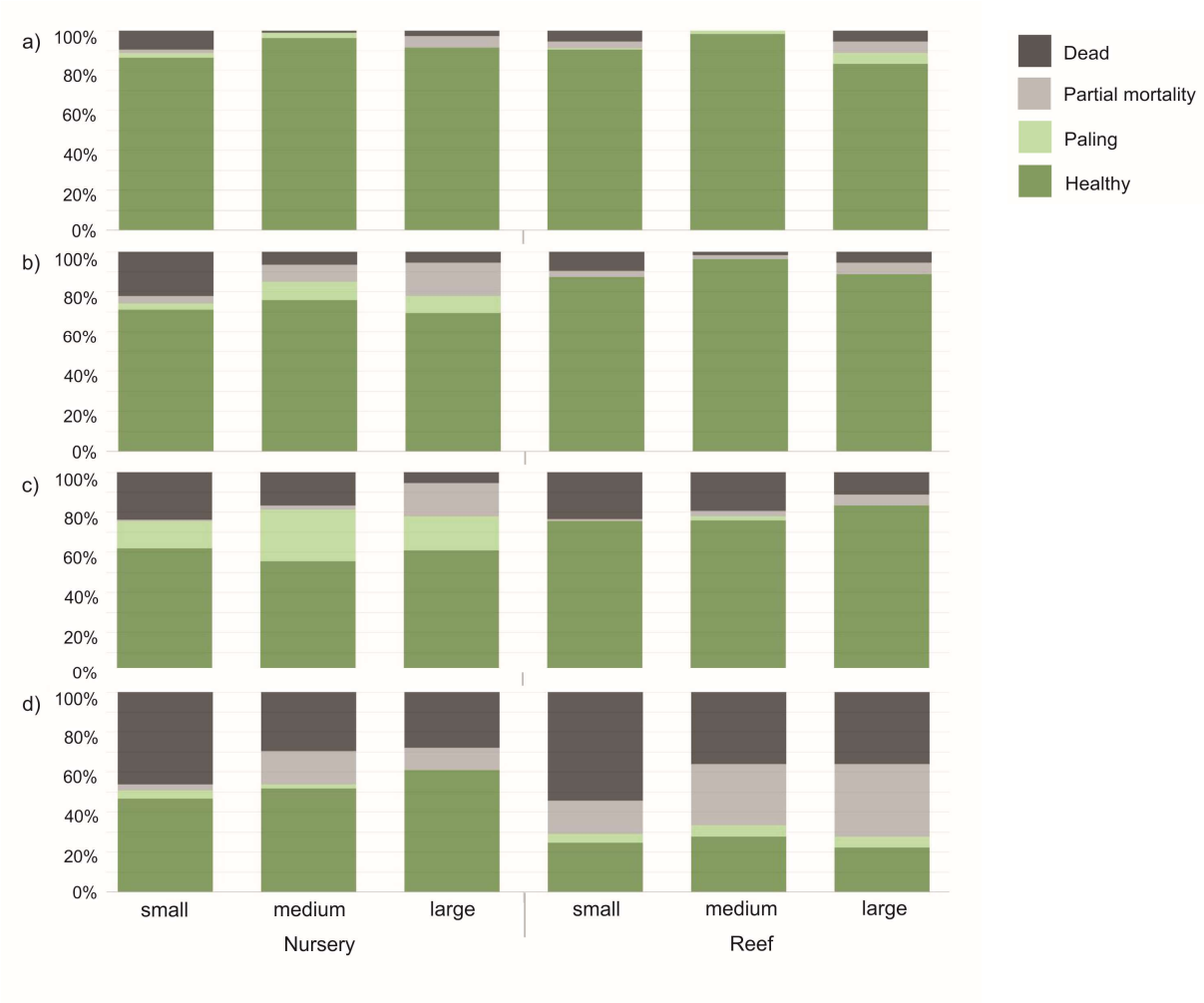


Figure 6. Survivorship, paling, partial mortality and mortality of coral fragments at HIMB for each size category in each environment over time **(a)** 1/15/19, **(b)** 2/28/19, **(c)** 5/7/19, **(d)** 8/16/19, 1, 2, 5 and 8 month/s after initial fragmentation respectively.

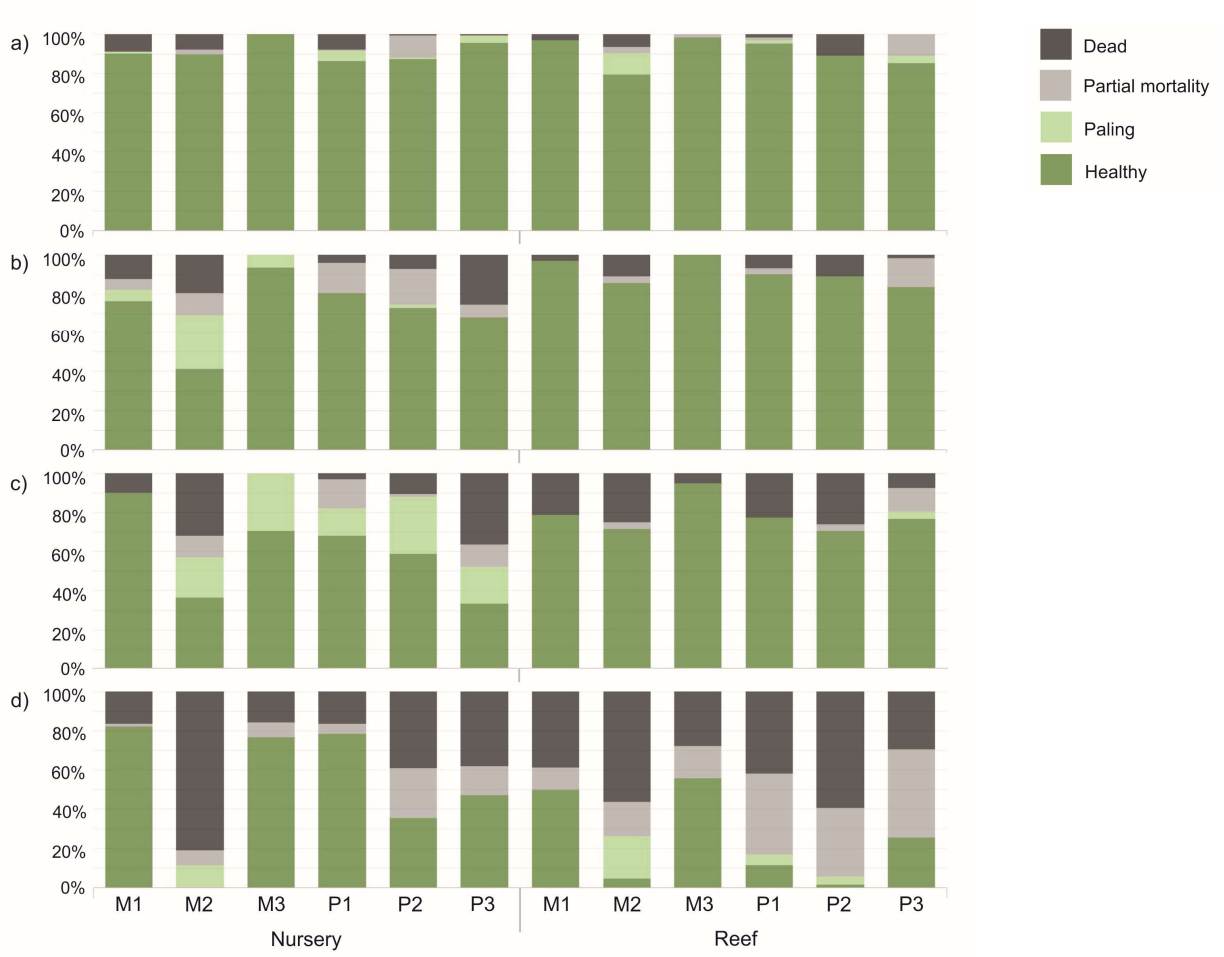


Figure 7. Survivorship, paling, partial mortality and mortality of coral fragments at HIMB for each genotype in each environment over time **(a)** 1/15/19, **(b)** 2/28/19, **(c)** 5/7/19, **(d)** 8/16/19, 1, 2, 5 and 8 month/s after initial fragmentation respectively.

At the AFRC *ex-situ* nursery the mean (± S.D) size of the micro-fragments was 1.16 (± 0.56), 4.95 (± 1.27), 12.64 (± 3.61) cm^2, or the small, medium, and large size categories respectively. Survival in the controlled conditions of the *ex-situ* nursery was nearly 100%, with only 2 small fragments lost. In contrast, the Sand Island reef location had a clear trend of sized dependent survival with 25, 80, and 90% after 1 month and 40, 75, and 85 % after 7 months survival for small, medium, and large colonies respectively (**Figure 8 a-b**). The Honolulu Harbor also had a trend of size dependent survival with 60, 80, and 95% and 50, 65, and 95% survivorship for small, medium and large colonies after 1 and 7 months respectively (**Figure 8 a-b**). The harbor had higher overall survivorship and tissue paling when compared to the Sand Island reef location (**Figure 8b**). Mortality was also highly dependent on the donor colony, although all colonies had almost 100% survivorship in the nursery (**Figure 8 a-d**). *M. capitata* colony M1 had only ~40% survivorship at the harbor site compared to ~75% at the Sand Island reef location, while colony M3 showed the opposite pattern with ~45% survival at the reef location compared to ~75% at the harbor location (**Figure 8 c-d)**. This may indicate that there are genotype by environment interactions, with certain genotypes more suited to a particular environment than others, further illustrating how selecting the appropriate genotype can influence the outcome of a restoration project.

Figure 8. Survivorship, paling, and mortality of coral fragments at the Sand Island (AFRC) locations at 1 month **(a and c)** and 7 months **(b and d)** after outplanting the first set of assays where **(a-b)** survivorship by size category at each location and **(b-c)** survivorship by colony genotypes, M = *Montipora capitata*, P = *Porites lobata*.

Effects of fragment size, nursery residence time and genotype on coral growth

Typically coral micro-fragments spread tissue in two dimensions, however; at the HIMB locations, the coral colonies rapidly grew into complex branching morphologies (see **Figure 4** for a contrast between AFRC and HIMB locations). To account for increased surface area due to three dimensionality, we estimated the surface area from 3D SfM models and compared these measurements to those obtained from planar area from scaled digital photographs. For both species, there was a strong relationship between surface area derived from these two methods (**Figure 9**), with a high proportion of the variance accounted for (r^2=0.82). We also used this method to estimate volume, although less of the variation was accounted for (r^2=0.72), which may be due to holes in the mesh model (**Appendix S6**). In addition to accounting for surface area due to 3D complexity, the SfM segmentation method was significantly faster and required less manual effort. Manual segmentation took approximately 5 minutes in the field and 3-5 hours of effort to build and segment the model. In contrast, planer measurements required approximately an hour of fieldwork (at least 4 digital photographs per module), and at least 20 hours of manually tracing coral tissue cover.

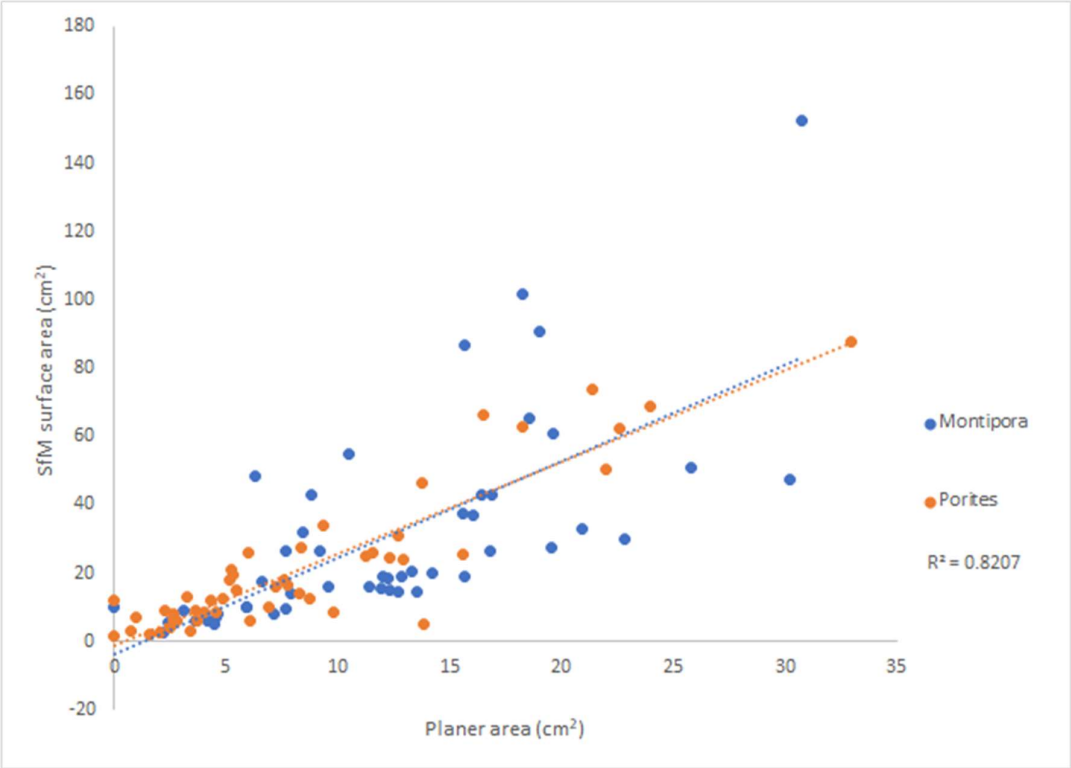


Figure 9. The relationship between two dimensional (planar) area in cm^2 to surface area derived from three dimensional

Both approaches of measuring the coral colonies resulted in highly similar trends (**Figure 10**), and yielded similar results according to the generalized linear model (GLM) and pairwise analyses (**Appendix S7 and S8**). Although there was a trend toward larger colonies showing higher growth rates for *M. capitata*, there were no significant differences in tissue growth found among sizes categories for both species. There were however, significant differences in net growth associated with genotype (i.e. colony of origin; **Figure 10a and Appendix S1**). According to the GLM, significant differences were found between *M. capitata*

genotypes M1 and M3 ($p = 0.0001$), while genotype M2 had a 100% mortality rate, and therefore was not able to be included in the model (**Appendix S7**). *P. compressa* genotype P3 was significantly different from P1 and P2 respectively ($p = 0.0380$, $p = 0.0015$) with the 2D photo measurements and between P3 and P2 ($p = 0.0194$) for the 3D analyses.

The most resilient genotypes (i.e. the colonies that had the lowest rates of mortality, see M1, M3, P3 in **Figure 7**) also had the highest rates of net tissue growth. Colony M1 had the highest total increase in two dimensional coral cover, a 153% increase over a year (from 120.5cm^2 to 305cm^2; **Appendix S9**). The total surface area of colony M1 estimated from the 3D SfM model, which more realistically accounts for the complex branching morphology, was 1,049cm^2 (a 770% increase over a year). Colony M2 in contrast completely died, while colony M3 grew in 2D area by 56% (201cm^2 to 313cm^2), or 126% (455cm^2) when 3D structure was taken into account (**Appendix S9**). Colony P1 and P2 decreased in two dimensional coral cover (-13.7cm^2 and -27.2cm^2 respectively), yet slightly increased in total area when 3D structure was taken into account (a 103% and 92% increase respectively). Colony P3 increased by only 41% (from 193.1cm^2 to 272.3cm^2) in 2D area and 239% (654.3cm^2) in surface area estimated from the SfM model (**Appendix S9**).

Hawai‘i experienced a bleaching event from August-November 2019, therefore this project provides information on growth for a range of sizes and genotypes, before, during and after a bleaching event. Although the total 2 dimensional live tissue area for all fragments declined by ~11% over the course of the year (from 1185cm^2 in December 2018 to 1060cm^2 in December 2019), when examining only those fragments which survived there was a 46% increase (from 725 to 1060cm^2), and accounting for 3D structure with the SfM model, total surface area for all fragments increased by 218% (2,584cm^2). In other words, even though the two dimensional area covered by coral tissue for all fragments decreased by ~11%, the surface area of tissue covering the complex branching morphology increased by 218%. In spite of mortality associated with the bleaching event, several genotypes had surprisingly large gains in both two dimensional coral cover, and surface area estimated from the SfM models, further illustrating that the selection of source genotypes is critically important for successful restoration outcomes.

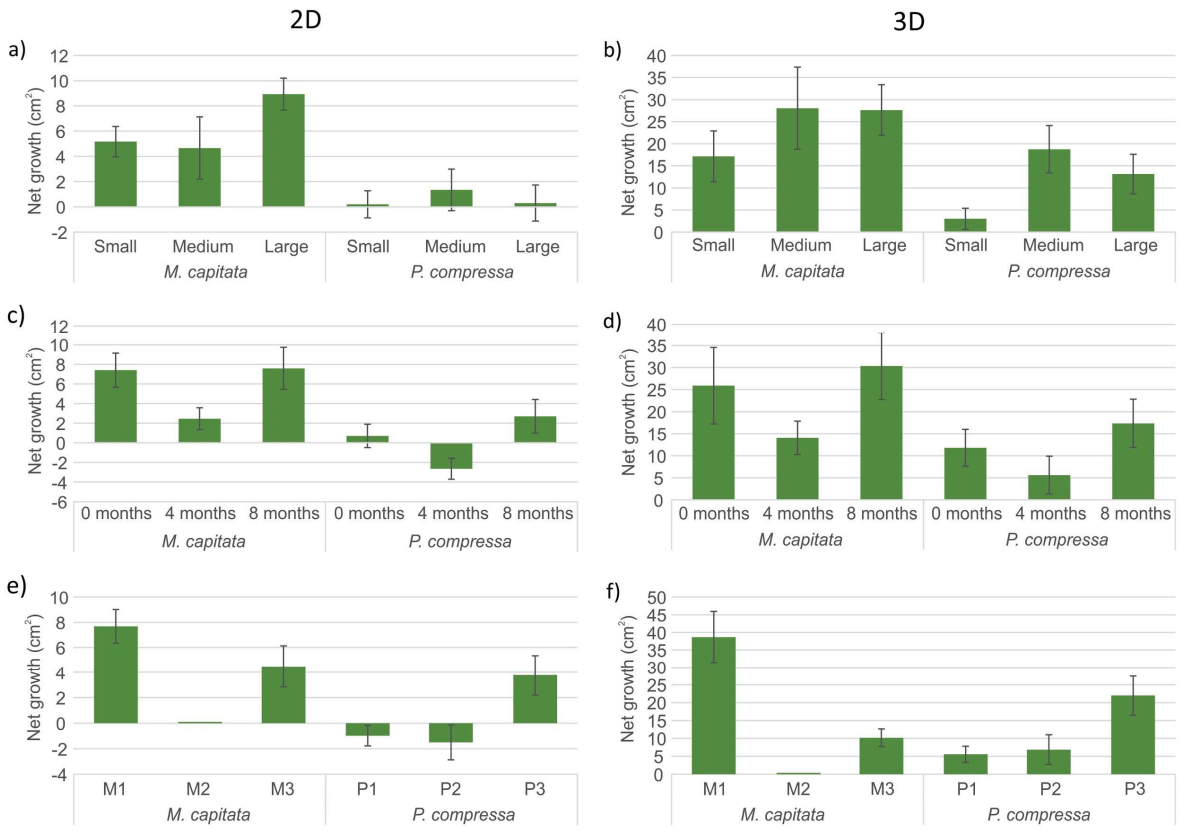


Figure 10. Mean (±SE) of net growth (cm^2) of *Porites compressa* and *Montipora capitata* area from 2D photographs **(a,c and e)** and 3D photogrammetry measurements **(b,d and f)** at HIMB of fragments that survived 12 months. Mean net growth by fragment sizes **(a-b)**, nursery residence time **(c-d)**, and genotype **(e-f)**.

The GLM model indicated that there were significant differences in growth rates associated with nursery residence time (i.e. between batches of assays deployed after 0, 4, or 8 months in the HIMB nursery; **Figure 10c**). Growth rates were similar between fragments placed directly on the reef with no *in-situ* nursery residence time (0 months), and fragments that spent 8 months in the nursery. It may not be surprising that growth rates are similar between an *in-situ* nursery and the nearby reef, however it is challenging to explain why fragments that spent 4 months in the nursery would have significantly less average net growth (2D area p=0.008, 3D surface area p=0.0134). Even though placement of the assays in the nursery was randomized, this batch effect could have been due to a number of factors, such as predation or parasite outbreaks (outbreaks of the corallivorous nudibranch *Phestilla* that feeds on *Porites* have been observed in the nursery), partial shading, plankton blooms, food availability, etc. Although net growth was lowest for the 4 month batch, the 8 month batch had the highest average net growth. Mortality, partial mortality, and tissue discoloration were less common in the HIMB nursery relative to the reef (**Figure 6d, 7d**). The modules that spent more time in the nursery had noticeably less biofouling (e.g. there were fewer oysters that settled next to the coral competing for space **Appendix S2, S3**) which may be due to juvenile herbivorous fish

that appear to be more abundant in the nursery compared to the reef. The temporal and microspatial factors that influence coral growth in an *in-situ* nursery environment are clearly in need of further study for optimization, since this method requires far less cost and effort than an *ex-situ* nursery. Although the collection of the final set of data from the AFRC field sites has been delayed, this project demonstrated that the coral assays were effective in providing key information for increasing the effectiveness and chances of success of a restoration project.

VII. Overall conclusions

1. We designed and deployed 126 assay modules consisting of 882 small, 378 medium, and 126 large fragments yielding 1,386 coral fragments in total across 3 distinct coral reef habitats (Kāneʻohe Bay reef flat, Honolulu Harbor, and an exposed South shore reef).
2. The assays successfully provided data on coral survivorship, partial mortality, bleaching, coral cover, and colony surface area, across a range of reef habitats. Growth data from 2D and 3D measurement methods showed highly similar results, although 3D measurements were less time consuming, while accounting for complex colony morphology.
3. The assays revealed size dependent mortality that was highly dependent on reef site, coral species, and colony genotype. In other words, the 'size refuge' or optimal colony size can be determined for a particular coral genotype at a particular site.
4. Nursery residence time showed high temporal variation for the *in-situ* nursery, which may be expected since many factors *in-situ* are challenging to control. Residence time data for the *in-situ* nursery has yet to be collected but we predict it will provide a head start and size refuge that is quantifiable using this methodology.
5. The assays revealed major differences in survivorship and growth by coral genotype (colony of origin). Several genotypes were highly resilient to bleaching and had major gains in coral cover and colony surface area, whereas other genotypes were highly susceptible to bleaching and suffered high mortality and tissue loss. The use of these methodologies to select genotypes suited to a particular environment will likely greatly increase the likelihood of success for a reef restoration project.

VII. Outcomes

A. *Presentations*

Knapp ISS, Forsman ZH, Toonen RJ (November, 2019) Planting coral:measuring the importance of size, genotype, site, and in-situ or ex-situ nursery residence time. Hawaiʻi coral restoration and bleaching symposium (~100 participants).

Forsman ZH, Knapp ISS, Toonen RJ (December 2019) Overview of reef restoration efforts in Hawaiʻi, Marine Biology (30 undergraduate students)

Forsman ZH, Knapp ISS, Toonen RJ (Jan 2020) Coral secrets: data from micro-fragmentation & DNA, Mote Marine Laboratory, Summerland Key & Sarasota FL (~70 participants)

B. *Student training*

Erika Johnston (PhD Candidate UHM, graduated 05/2019): Erika was responsible for deploying coral assays by snorkel and SCUBA, and she developed methods to score health data as well as initial measurements of coral tissue area. Erika was responsible for animal husbandry of the assay modules under the direction of the AFRC Coral Restoration Nursery Staff. Erika has assisted with statistical analysis in R and with report writing. Erika will assist with co-authorship of publications resulting from this project.

Austin Greene (PhD Candidate UHM): Austin assisted with field deployments and health checks of assay modules by snorkel and SCUBA. He continued the animal husbandry of the assay modules under the direction of the AFRC Coral Restoration Nursery Staff. He will assist with modeling, statistical analysis and co-authorship of publications resulting from this project.

Claire Bardin (undergraduate UHM): Claire is a work study undergraduate hired to assist with this project. Claire assisted with field deployment and health checks of assay modules by snorkel. She measured most of the corals from scaled digital models and assisted with all aspects of the project.

Volunteers: This project would not have been possible without additional assistance in the field and at the AFRC Coral Restoration Nursery. We would like to acknowledge the assistance of Dr. Eileen Nalley and the staff of the AFRC Coral Restoration Nursery, partlicularly Chelsea Wolke, Norton Chan, Dave Gulko, and nursery staff for assistance in the nursery and field.

VIII. References

Babcock RC, Monographs E, Sep N (1991) Comparative Demography of Three Species of Scleractinian Corals Using Age- and Size-Dependent Classifications. Ecological monographs 61:225–244

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS ONE 2:e711

Bruno JF (1998) Fragmentation in Madracis mirabilis (Duchassaing and Michelotti): how common is size-specific fragment survivorship in corals? Journal of Experimental Marine Biology and Ecology 230:169–181

Cabaitan PC, Yap HT, Gomez ED (2015) Performance of single versus mixed coral species for transplantation to restore degraded reefs. Restor. Ecol. 23:349–356

Chamberland VF, Petersen D, Guest JR, Petersen U, Brittsan M, Vermeij MJA (2017) New seeding approach reduces costs and time to outplant sexually propagated corals for reef restoration. Sci. Rep. 7:18076

Connell JH, Hughes TP, Wallace CC (1997) A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs 67:461–488

dela Cruz DW, Rinkevich B, Gomez ED, Yap HT (2015) Assessing an abridged nursery phase for slow growing corals used in coral restoration. Ecol. Eng. 84:408–415

Delbeek JC (2001) Coral farming: past, present and future trends. Aquarium Sciences and Conservation 995:171–181

Depczynski M, Gilmour JP, Ridgway T, Barnes H, Heyward AJ, Holmes TH, Moore JAY, Radford BT, Thomson DP, Tinkler P, Wilson SK (2013) Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef. Coral Reefs 32:233–238

Epstein N, Bak RPM, Rinkevich B (2003) Applying forest restoration principles to coral reef rehabilitation. Aquatic Conserv: Mar. Freshw. Ecosyst. 13:387–395

Ferrari R, Gonzalez-Rivero M, Mumby PJ (2012) Size matters in competition between corals and macroalgae. Mar. Ecol. Prog. Ser. 467:77–88

Forsman ZH, Page CA, Toonen RJ, Vaughan D (2015) Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ 3:e1313

Forsman ZH, Rinkevich B, Hunter CL (2006) Investigating fragment size for culturing reef-building corals (Porites lobata and P. compressa) in ex situ nurseries. Aquaculture 261:89–97

Fox TR, Jokela EJ, Allen HL (2004) The evolution of pine plantation silviculture in the southern United States. Southern forest science: past, present and future. Gen Tech Rept SRS-75. Asheville, NC: USDA Forest Service Southern Research Station

Griffin S (2012) Scaling up Acropora nurseries in the Caribbean and improving techniques. 9–13

Grigg RW, Maragos JE (1974) Recolonization of hermatypic corals on submerged lava flows in Hawai'i. Ecology 55:387–395

Herlan J, Lirman D (2009) Development of a coral nursery program for the threatened coral Acropora cervicornis in Florida. 1244–1249

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Khurana E, Singh JS (2001) Ecology of seed and seedling growth for conservation and restoration of tropical dry forest : a review. Environ. Conserv. 28:

Kojis BL, Quinn NJ (2001) The importance of regional differences in hard coral recruitment rates for determining the need for coral restoration. Bulletin of marine science 69:967–974

Lenth R (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.6.

https://CRAN.R-project.org/package=emmeans

Mumby PJ, Hastings A, Edwards HJ (2007) Thresholds and the resilience of Caribbean coral reefs. Nature 450:98–101

Page CA, Muller EM, Vaughan DE (2018) Microfragmenting for the successful restoration of slow growing massive corals. Ecol. Eng. 123:86–94

Peterson RA (2019). Ordered quantile normalization: a semiparametric transformation built for the cross-validation era. Journal of Applied

Statistics, 1-16.

Rasband WS (2012) ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA //imagej.nih.gov/ij/

Rinkevich B (2005) Conservation of coral reefs through active restoration measures: recent approaches and last decade progress. Environ. Sci. Technol. 39:4333–4342

Rinkevich B (2008) Management of coral reefs: we have gone wrong when neglecting active reef restoration. Mar. Pollut. Bull. 56:1821–1824

Roff G, Bejarano S, Bozec Y-M, Nugues M, Steneck RS, Mumby PJ (2014) Porites and the Phoenix effect: unprecedented recovery after a mass coral bleaching event at Rangiroa Atoll, French Polynesia. Mar. Biol. 161:1385–1393

RStudio Team (2019). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

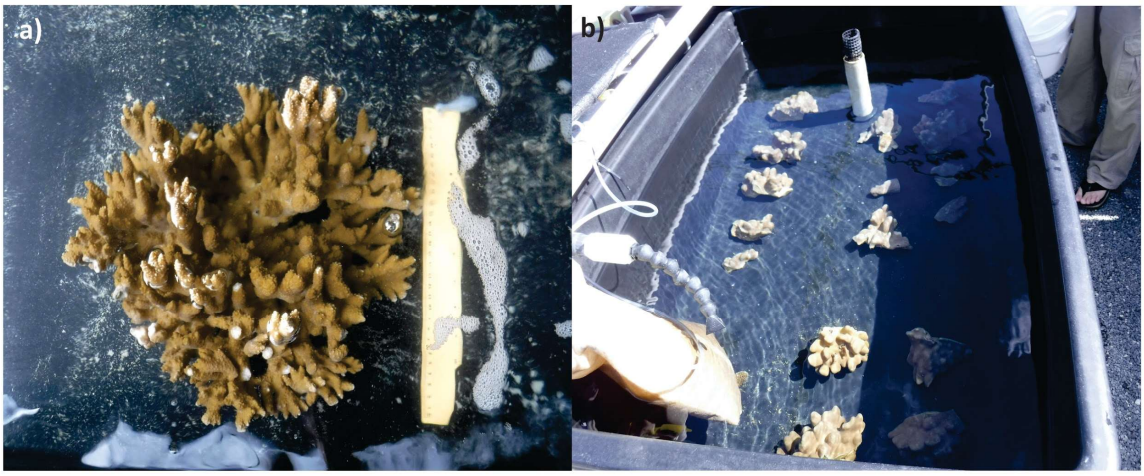
Suka R, Oliver T, Couch C, Asbury M, Gray A (2020) Standard operating procedures for Structure-from-motion surveys at fixed sites. NOAA Technical Memorandum NMFS-PIFSC

Yap HT (2009) Local changes in community diversity after coral transplantation. Mar. Ecol.

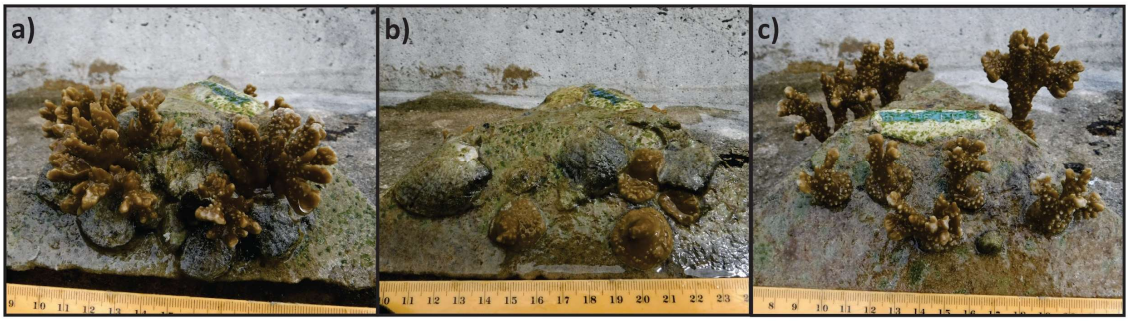
Prog. Ser. 374:33–41

X. Appendices

Appendix S1. **(a)** One of the three *Montipora capitata* parent colonies from HIMB and **(b)** *Porites lobata* genotypes 1, 2, and 3 in the quarantine tank at AFRC.



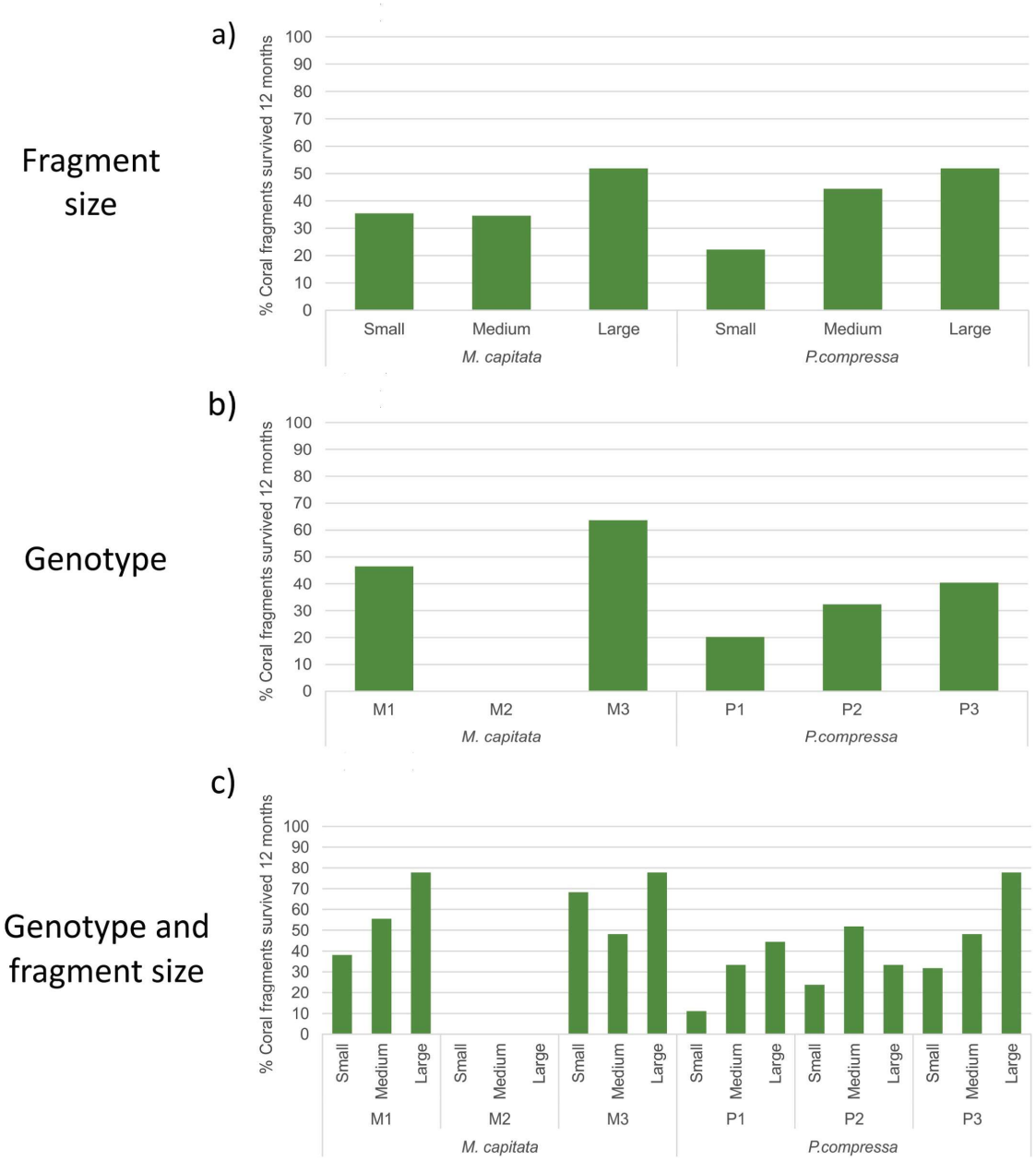
Appendix S2 Examples of HIMB pyramid assays with **(a)** 0 months in the nursery, **(b)** 4 months in the nursery before outplanting, and **(c)** 8 months in the nursery before outplanting with high to low numbers of oysters from a to b respectively.



Appendix S3 Oyster count on assays outplanted after 0, 4 and 8 months for *Montipora capitata* and *Porites compressa*.

	Number of oysters		
Residence time	*M. capitata*	*P. compressa*	Total
0 months	47	74	121
4 months	54	32	86
8 months	3	2	5
Total	104	108	212

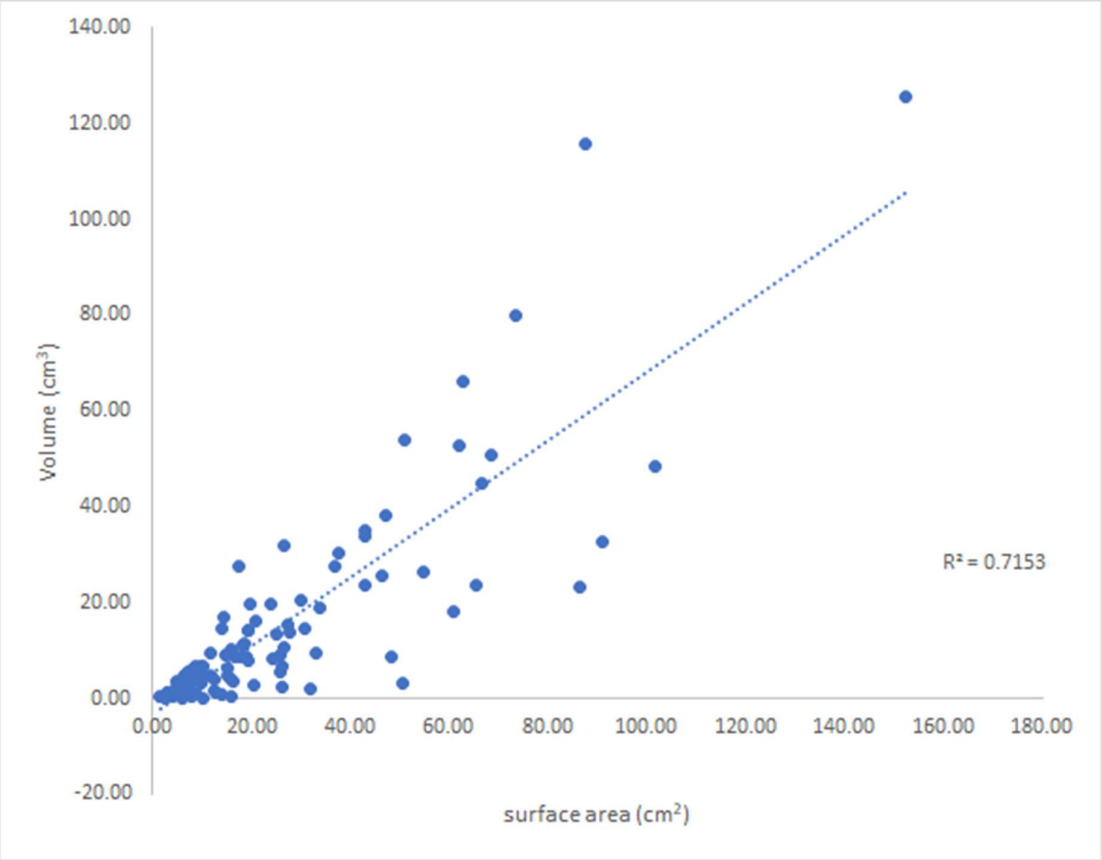
Appendix S4. Percentage of *Montipora capitata* and *Porites compressa* fragments with any live tissue which survived from December 2018-19 relative to **(a)** fragment size, **(b)** genotype, where M = *M. capitata* and P = *P. compressa*, and **(c)** genotype and fragment size at HIMB.



Appendix S5. Number of coral fragments with any live tissue over time, mortality rate from December 2018-2019, and percentage (%) of fragments which survived all 12 months at HIMB, by species, genotype (where M = *Montipora capitata* and P = *Porites compressa*), and fragment size.

	Number of coral fragments				
	Dec 2018	Aug 2019	Dec 2019	Mortality over 12 months	% survived
Montipora capitata	**297**	**171**	**109**	**188**	**37**
M1	**99**	**60**	**46**	**53**	**46**
Small	63	33	24	39	38
Medium	27	20	15	12	56
Large	9	7	7	2	78
M2	**99**	**37**	**0**	**99**	**0**
Small	63	24	0	63	0
Medium	27	10	0	27	0
Large	9	3	0	9	0
M3	**99**	**74**	**63**	**36**	**64**
Small	63	48	43	20	68
Medium	27	19	13	14	48
Large	9	7	7	2	78
Porites compressa	**297**	**170**	**92**	**205**	**31**
P1	**99**	**58**	**20**	**79**	**20**
Small	63	31	7	56	11
Medium	27	20	9	18	33
Large	9	7	4	5	44
P2	**99**	**52**	**32**	**67**	**32**
Small	63	31	15	48	24
Medium	27	16	14	13	52
Large	9	5	3	6	33
P3	**99**	**60**	**40**	**59**	**40**
Small	63	33	20	43	32
Medium	27	20	13	14	48
Large	9	7	7	2	78
Total	**594**	**341**	**201**	**393**	**34**

Appendix S6. Relationship comparing volume (cm^3) estimated from 3D Structure from Motion photogrammetry and 2D surface area (cm^2) estimated from top down scaled digital photographs taken in December 2019 of *Montipora capitata* and *Porites compressa* fragments at HIMB.



Appendix S7 Generalized linear model (GLM) output examining *Montipora capitata* net growth (cm^2) and fixed independent variables: genotype, fragment size and nursery residence over 12 months based from **(a)** 2D photographs and **(b)** 3D photogrammetry images and **(c)** accompanying pairwise analyses indicating a significant difference between *M. capitata* `intercepts and genotypes one and three with the 3D photogrammetry data, where * = significant (≤ 0.05).`

a)

Term_2D_glm_M.cap	Estimate	Std. error	F statistic	p value
(Intercept)	-0.7177	0.0451	-15.9072	**1.26081e-19***
Genotype_M.cap_3	-0.0497	0.0363	-1.3708	0.1775
Size_medium	-0.0646	0.0453	-1.4242	0.1615
Size_small	-0.0582	0.0458	-1.2720	0.2101
Time_4 months	-0.0769	0.0459	-1.6747	0.1012
Time_8 months	0.0027	0.0428	0.0641	0.9491

b)

Term_3D_glm_M.cap	Estimate	Std. error	F statistic	p value
(Intercept)	-0.8353	0.0311	-26.8266	**1.7540 e-28***
Genotype_M.cap_3	-0.0933	0.0250	-3.7269	**0.0005***
Size_medium	-0.0391	0.0313	-1.2490	0.2183
Size_small	-0.0358	0.0316	-1.1329	0.2634
Time_4 months	-0.0177	0.0317	-0.5597	0.5785
Time_8 months	-0.0056	0.0295	-0.1903	0.8498

c)

Level 1	Level 2	Estimate	Std. error	Z ratio	p value
M.cap_1	**M.cap_3**	0.0933	0.0250	3.7269	**0.0001***

Appendix S8 Generalized linear model (GLM) output **(a and c)** and pairwise analyses **(b and d)** examining *Porites compressa* net growth (cm^2) and fixed independent variables: genotype, fragment size and nursery residence time over 12 months based from **(a-b)** 2D photographs and **(c-d)** 3D photogrammetry images indicating significant differences between genotypes 3 when compared to 1 and 2, time 4 months and 8 months with 2D data and genotype 3 and 2, time 4 months and 8 months, and intercept with 3D data, where * = significant (≤ 0.05).

a)

Term_2D_glm_P.com	Estimate	Std. error	F statistic	p value
(Intercept)	0.0439	0.0614	0.7152	0.4780
Genotype_P.com_2	-0.0437	0.0592	-0.7385	0.4639
Genotype_P.com_3	0.1422	0.0580	2.4490	**0.0181***
Size_medium	0.0525	0.0571	0.9193	0.3627
Size_small	0.0329	0.0604	0.5446	0.5886
Time_4 months	-0.1302	0.0627	-2.0756	**0.0435***
Time_8 months	0.0619	0.0510	1.2142	0.2308

b)

Level 1	Level 2	Estimate	Std. error	Z ratio	p value
P.com_1	**P.com_2**	0.0437	0.0592	0.7385	0.7405
P.com_1	**P.com_3**	-0.1422	0.0580	-2.4490	**0.0380***
P.com_2	**P.com_3**	-0.1860	0.0536	-3.4666	**0.0015***
Time_0 months	**Time_4 months**	0.1302	0.0627	2.0756	0.0949
Time_0 months	**Time_8 months**	-0.0619	0.0510	-1.2142	0.4446
Time_4 months	**Time_8 months**	-0.1921	0.0644	-2.9829	**0.0080***

c)

Term_3D_glm_P.com	Estimate	Std. error	F statistic	p value
(Intercept)	-0.4283	0.0366	-11.7033	**2.1721 e-15***
Genotype P.com_2	-0.0151	0.0352	-0.4301	0.6691
Genotype P.com_3	0.0708	0.0345	2.0485	**0.0462***
Size_medium	0.0244	0.0340	0.7188	0.4758
Size_small	-0.0625	0.0359	-1.7375	0.0889
Time_4 months	-0.0800	0.0373	-2.1444	**0.0373***
Time_8 months	0.0278	0.0303	0.9188	0.3629

d)

Level 1	Level 2	Estimate	Std. error	Z ratio	p value
P.com_1	**P.com_2**	0.0151	0.0352	0.4301	0.9030
P.com_1	**P.com_3**	-0.0708	0.0345	-2.0485	0.1008
P.com_2	**P.com_3**	-0.0859	0.0319	-2.6924	**0.0194***
Time_0 months	**Time_4 months**	0.0800	0.0373	2.1444	0.0810
Time_0 months	**Time_8 months**	-0.0278	0.0303	-0.9188	0.6283
Time_4 months	**Time_8 months**	-0.1079	0.0383	-2.8160	**0.0134***

Appendix S9 Summary table of total area cover (cm^2) over 12 months of **all coral fragments** at the HIMB reef flat, **those fragments which survived till the end**, including the **change over time** made from 2D photograph measurements calculated from scaled images in ImageJ, along with the one set of **3D photogrammetry measurements** taken in December 2019.

	Total tissue area (cm^2) for all fragments				Total tissue area (cm^2) for fragments which survived 12 months				
	Dec 2018	Aug 2019	Dec 2019	Change over 12 months	Dec 2018	Aug 2019	Dec 2019	Change over 12 months	3D Dec 2019
Montipora capitata	**593.3**	**646.6**	**618.0**	**24.8**	**321.2**	**509.3**	**618.0**	**296.8**	**1504.0**
M1	**138.7**	**203.6**	**305.1**	**166.4**	**120.5**	**203.6**	**305.1**	**184.6**	**1048.8**
Small	27.9	44.5	64.4	36.5	25.2	44.5	64.4	39.1	299.9
Medium	53.8	83.9	133.2	79.4	53.8	83.9	133.2	79.4	492.6
Large	57.0	75.1	107.6	50.5	41.5	75.1	107.6	66.1	256.3
M2	**237.3**	**137.2**	**0.0**	**-237.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Small	47.2	53.6	0.0	-47.2	0.0	0.0	0.0	0.0	0.0
Medium	92.2	50.2	0.0	-92.2	0.0	0.0	0.0	0.0	0.0
Large	98.0	33.4	0.0	-98.0	0.0	0.0	0.0	0.0	0.0
M3	**217.2**	**305.7**	**312.9**	**95.8**	**200.7**	**305.7**	**312.9**	**112.3**	**455.2**
Small	48.9	76.3	97.8	48.8	48.9	76.3	97.8	48.8	181.2
Medium	98.9	126.3	103.4	4.5	98.9	126.3	103.4	4.5	164.9
Large	69.4	103.1	111.8	42.4	52.9	103.1	111.8	58.9	109.2
Porites compressa	**592.2**	**583.8**	**441.9**	**-150.3**	**403.6**	**527.5**	**441.9**	**38.3**	**1066.8**
P1	**145.2**	**179.8**	**62.7**	**-82.5**	**76.4**	**137.4**	**62.7**	**-13.7**	**154.7**
Small	41.0	43.5	12.1	-28.9	19.7	33.0	12.1	-7.6	46.6
Medium	49.0	80.1	30.3	-18.7	32.8	75.3	30.3	-2.5	84.5
Large	55.2	56.3	20.3	-34.9	23.9	29.1	20.3	-3.6	23.6
P2	**210.1**	**119.6**	**106.9**	**-103.1**	**134.1**	**105.8**	**106.9**	**-27.2**	**257.7**
Small	50.1	28.1	24.4	-25.6	32.5	19.1	24.4	-8.1	65.9
Medium	70.8	51.3	52.0	-18.8	70.8	51.3	52.0	-18.8	147.1
Large	89.2	40.2	30.5	-58.7	30.7	35.3	30.5	-0.3	44.8
P3	**237.0**	**284.4**	**272.3**	**35.3**	**193.1**	**284.4**	**272.3**	**79.2**	**654.3**
Small	65.1	72.4	63.0	-2.1	44.1	72.4	63.0	18.9	186.6
Medium	98.2	136.7	140.9	42.7	88.6	136.7	140.9	52.3	391.6
Large	73.6	75.2	68.4	-5.2	60.4	75.2	68.4	8.0	76.2
Total	**1185.5**	**1230.4**	**1060.0**	**-125.5**	**724.8**	**1036.8**	**1060.0**	**335.1**	**2570.8**
% diff. from Dec. 2018	**NA**	**3.8%**	**-10.6%**	**NA**	**NA**	**43.1%**	**46.3%**	**NA**	**254.7%**