Coral micro-fragmentation assays for optimizing active reef restoration efforts

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The global decline of coral reefs has driven considerable interest in active coral restoration. Despite their importance and dominance on mature reefs, relatively few coral restoration projects use slower growth forms like massive and encrusting coral species. Micro-fragmentation can increase coral cover by orders of magnitude faster than natural growth, which now allows cultivation of slow growing massive forms and shows promise and flexibility for active reef restoration. However, the major causes of variation in growth and survival of outplanted colonies remain poorly understood. Here, we report simple outplanting assays to aid in active reef restoration of slower growing species and increase the likelihood of restoration success. We used two different micro-fragmentation assays. Pyramid assays were used to examine variation associated with fragment size (ranging from ≈ 1-9cm^2), nursery residence time, and 2D vs 3D measurements of growth. Block assays were used to examine spatial variation among individual performance at outplanting sites in the field. We found 2D and 3D measurements correlated well, so we measured survivorship and growth using top-down planar images for two of the main Hawaiian reef building corals, the plating *Montipora capitata* and the massive *Porites compressa*. Pyramid assays housed and outplanted from the *in-situ* nursery showed no effect of residence time or size on overall survivorship or growth for either species. Results from the *ex-situ* nursery however varied by species, with *P. compressa* again showing no effect of time or size on survivorship or growth in the nursery. In contrast, nursery culture resulted in improved survivorship of small *M. capitata* fragments, but net growth showed a weak positive effect of nursery time for medium fragments. Small fragments still suffered higher mortality than either medium or large fragments. Due to their lower mortality, medium fragments (≈ 3cm^2) appear to be the best compromise between growth and

survivorship for outplanting. Likewise, given weak positive gains relative to the investment, our results suggest that it could be more cost-effective to simply outplant medium fragments as soon as possible, without intermediate culture in a nursery. Furthermore, the block assay revealed significant differences in survivorship and growth among sites for individuals of both species, emphasizing the importance of considering spatial variation in coral survival and growth following outplanting. These results highlight the value of short-term micro-fragmentation assays to provide key information on the sizes and location specific performance to optimize the efficiency of reef restoration activities and we advocate using them in advance to maximize the chances of success for active coral restoration projects.

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Abstract

The global decline of coral reefs has driven considerable interest in active coral restoration. Despite their importance and dominance on mature reefs, relatively few coral restoration projects use slower growth forms like massive and encrusting coral species. Micro-fragmentation can increase coral cover by orders of magnitude faster than natural growth, which now allows cultivation of slow growing massive forms and shows promise and flexibility for active reef restoration. However, the major causes of variation in growth and survival of outplanted colonies remain poorly understood. Here, we report simple outplanting assays to aid in active reef restoration of slower growing species and increase the likelihood of restoration success. We used two different micro-fragmentation assays. Pyramid assays were used to examine variation associated with fragment size (ranging from ≈ 1-9cm^2), nursery residence time (for both *in-situ* and *ex-situ* nurseries), and 2D vs 3D measurements of growth. Block assays were used to examine spatial variation among individual performance at outplanting sites in the field. We found 2D and 3D measurements correlated well, so measured survivorship and growth using top-down planar images for two of the main Hawaiian reef building corals, the plating *Montipora capitata* and the massive *Porites compressa*. Pyramid assays housed and outplanted from the *in-situ* nursery showed no effect of residence time or size on overall survivorship or growth for either species. Results from the *ex-situ* nursery however varied by species, with *P. compressa* again showing no effect of nursery residence time or size on survivorship or growth. In contrast, nursery culture resulted in improved survivorship of small *M. capitata* fragments, but net growth showed a weak positive effect of nursery time for medium fragments. Small fragments still suffered higher mortality than either medium or large fragments. Due to their lower mortality, medium fragments (≈ 3cm^2) appear to be the best compromise between growth and survivorship for outplanting. Likewise, given weak positive gains relative to the investment, our results suggest that it could be more cost-effective to simply outplant medium fragments as soon as possible, without intermediate culture in a nursery. Furthermore, the block assay revealed significant differences in survivorship and growth among sites for individuals of both species, emphasizing the importance of considering spatial variation in coral survival and growth following outplanting. These results highlight the value of using short-term micro-fragmentation assays prior to outplanting to assess size, and location specific performance, optimizing the efficiency of active reef restoration activities and maximizing the probability of success for active coral restoration projects.

Introduction

As coral reef ecosystems continue to decline worldwide, many have called for active intervention and innovative management tools to address conservation challenges and reverse the decline of coral reef habitats (Rinkevich, 2005; van Oppen et al., 2015, 2017; Anthony et al., 2017; Kleypas et al., 2021). Corals form the structure and foundation of coral reefs, fulfilling an ecosystem engineering role analogous to trees in terrestrial ecosystems. The ethics and scalability of active interventions to reverse coral reef decline remain a subject of debate (Williams et al., 2018;

Filbee-Dexter and Smajdor, 2019; Doropoulos et al., 2019; Anthony et. al., 2020; Caruso et al., 2021), but are common management strategies among terrestrial ecosystems. For example, one of the most widely used conservation and management tools for 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, a strategy that 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 scale operations dedicated to production for the marine hobby industry (Delbeek, 2001; Tlusty, 2002), the conservation of rare or endangered coral species (Herlan and Lirman, 2009; Griffin et al., 2012), or active coral reef restoration (Epstein et al., 2003; Rinkevich, 2008; Nedimyer, Gaines and Roach, 2011; Boström-Einarsson et al., 2020).

The potential 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). Some reefs surrounded by high coral cover might naturally recover from disturbance within a period of decades (Connell et al., 1997; Adjeroud et al., 2009; Jury and Toonen, 2019), whereas other reef systems may take an order of magnitude longer if they ever recover at all (Smith 1992; Hughes and Tanner 2000; Salinas-de-León et al. 2013). Recruitment failure and high rates of post-settlement mortality of corals can result in a downward spiral of ecosystem collapse and transition to a macroalgal dominated alternative state (Hughes and Tanner 2000; Dudgeon et al. 2010; Briggs et al. 2018). Once ecological systems transition to an alternative state, such as macroalgal dominance on coral reefs, it often requires much higher densities of herbivores to transition back than it did to maintain the previous state (Fung et al. 2011; Mumby et al. 2013; Schmitt et al. 2019). Thus, reverse transitions, from algal- to coral-dominated ecosystems are rarely observed, but increased fish and coral recruitment have been documented to occur with some large scale reef restoration efforts in both the Caribbean (Schopmeyer and Lirman 2015; Huntington et al. 2017; Opel et al. 2017) and the Pacific (Yap, 2009; Cabaitan et al., 2015).

Most reef restoration efforts seek to augment three-dimensional structure and live coral cover (Boström-Einarsson et al., 2020). To increase the productivity required to scale-up restoration, the success of such efforts is dependent on finding the optimal colony size and nursery residence time for outplants that maximizes effectiveness of the restoration (dela Cruz et al., 2015). Outplanting of larger coral fragments through rearing juveniles or small fragments in nurseries often translates to increased probability of survival for coral colonies (Raymundo and Maypa 2004; Page et al. 2018; van Woesik et al. 2021). Thus, most coral nurseries seek to provide safe environments in which corals are maintained under ideal conditions prior to outplanting until their risk of mortality is reduced (by reaching a size refuge). However, it takes time to grow large fragments even under nursery conditions, and the larger the starting size, the fewer total fragments can be taken from a parent colony. Prolonged nursery culture not only increases labor and maintenance costs but also requires substantially more space to maintain equivalent output, which impacts scaling during restoration efforts.

Nursery costs depend not only on duration of culture, but also the type of nursery: *in-situ* (in the water) and *ex-situ* (in tanks on land) culture each have a suite of costs and benefits to consider. *In-situ* nurseries have minimal maintenance and equipment costs, but environmental conditions are more difficult to control (e.g., light, temperature, sedimentation, competition, predation, disease), whereas *ex-situ* nurseries maintain perfect conditions at a premium in terms of labor, setup and operational (utilities, water quality, supplies, and infrastructure maintenance) costs. One key to improving efficiency and reducing cost for both types of nurseries is to reduce the amount of time that fragments need to be maintained prior to outplanting. Thus, identifying the ideal size for outplanting success is of high value and essential to optimizing efforts to scale restoration. However, the ideal size for trading off survivorship and costs is likely to vary both temporally and spatially, as well as among species, in the same way that individual growth rates vary in the same corals through time (Edmunds and Putnam 2020). For example, previous studies have found relationships between size and mortality vary with nursery conditions (Forsman et al., 2006), habitat (Bruno, 1998), bleaching events (Depczynski et al., 2013), and competitive interactions (Ferrari et al., 2012). Restoration efforts of slower growing species must optimize tradeoffs between a strategy to outplant larger coral colonies with higher survival but greater investment per individual against one of outplanting many smaller colonies with minimal investment in each. For example, artificial substrates seeded with new coral recruits showed a 5-18-fold reduction in out-planting costs by dramatically reducing diver labor, which is the costliest aspect of reef restoration work (Chamberland et al., 2017). Survivorship is often low among recruits and highly stochastic in coral reef ecosystems (Edmunds et al., 2004; Irizarry-Soto and Weil, 2009; Miller, 2014), so it is not surprising that only 9.6% of newly settled corals survived their first year, but this essentially offsets the initial cost savings. Because corals show variable sensitivity, both within and among species, to environmental parameters such as sedimentation, pollution, temperature, irradiance, salinity, and pH (Bahr et al. 2015; Fabricius 2005; Kleypas et al. 2021; Lough and Barnes 2000; Williams et. al. 2010) it is also important to determine whether there is variation in survival when attempting to scale-up restoration efforts. Therefore, prior information about genotype- and species-specific responses at a particular restoration site could maximize survival while minimizing cost and ensuring the most cost-effective approach to mass producing and outplanting corals for reef restoration.Coral micro-fragmentation (Forsman et al., 2015; Page et al., 2018) can precisely control colony size and genotype (donor colony) for outplanting, with the potential to develop highly flexible and cost-effective assays on site-specific data mortality and growth for replicated genotypes across a range of sizes. 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; Page et al., 2018). The technique typically uses small (~1cm^2) fragments all from the same donor colony (and therefore same genotype) spaced approximately 2-3 cm apart over an artificial substrate, taking advantage of the tendency of these small fragments to rapidly spread thin layers of tissue and fuse upon contact, doubling or quadrupling in size within a few months. When such fragments are attached

to dead coral heads they can quickly 're-skin' an entire colony, which can result in bringing large endangered corals back to life (Page et al., 2018). Knowledge of genotype- and size-specific mortality rates for corals at a given site would allow restoration efforts to target mass production of resilient genotypes of an optimal size, to maximize cost-effectiveness and scale while improving the outcome of restoration.

Here, we evaluate the impacts of fragment size and nursery residence time at both an *in-situ* (the Hawai'i Institute of Marine Biology (HIMB) mid-water coral farm) and an *ex-situ* (the Hawai'i Division of Aquatic Resources' Hawai'i Coral Restoration Nursery's (HCRN) land-based facility) coral nursery. We use that information to test spatial variation in outplanting performance across an environmental gradient then combine these approaches to propose a rapid assay approach to improve strategies to increase time- and cost-effectiveness of reef restoration efforts in the field.

Materials & Methods

1. Study species

We selected *Montipora capitata* (Family Acroporidae) and *Porites compressa* (Family Poritidae) for these assays. These are two of the dominant reef building coral species on O'ahu (Fletcher et al., 2008; Franklin et al. 2013), and represent two of the major life history categories of reef-building corals (Darling et al., 2012). *M. capitata* is a highly polymorphic encrusting species that forms plates and branches as it matures. *P. compressa* is a massive coral that forms large mounds with cylindrical branches that often fuse.

2. Micro-fragmentation

All experimental fragments for both assays were cut to size with a Gryphon XL Aquasaw and 42" diamond tipped stainless steel blade, and fixed to the substrate using cyanoacrylate (Bulk Reef Supply extra thick gel superglue). To standardize treatment all the undersides of fragments were freshly cut flat to ensure greater adhesion, even if the coral was flat before cutting. We aimed to cut the fragments leaving little to no skeleton exposed for algal growth. If any coral skeleton was exposed, we covered it in superglue to deter predators, particularly in the case of *P. compressa*, which is often heavily predated on by an aeolid nudibranch (*Phestilla* sp.). After gluing, but before moving the substrate, each fragment was checked to ensure it was firmly fixed to the assay to avoid any accidental loss of corals.

3. Experimental locations

In-situ nursery

The field-based nursery was located on Moku o Lo'e (Coconut Island) at the Hawai'i Institute of Marine Biology (HIMB) in Kāne'ohe Bay (**Figure 1A, B**) and was constructed to conduct research on improving the time and cost efficiency of reef restoration. The coral nursery consists of floating walkways surrounding and supporting suspended midwater platforms for coral

cultivation. It was constructed in 2017 from recycled materials salvaged from decommissioned aquaculture and marine mammal pens. The primary source of the over 1000 corals housed in the nursery are recovered from the retired pens and other 'corals of opportunity' relocated to the nursery from marine debris or other salvage which would otherwise be discarded.

Ex-situ nursery

The Division of Aquatic Resources' (DAR) Hawai'i Coral Restoration Nursery's (HCRN) *ex-situ* nursery is located at the Ānuenue Fisheries Research Center (AFRC) on Sand Island (**Figure 1C, D**). It was built for the purpose of improving methods of coral culture and outplanting, to restore and conserve Hawai'i's coral reefs, and consists of small to large indoor and outdoor tanks with varying levels of filtration and control of temperature, lighting, water chemistry, and biotic communities. A full-time staff of professional aquarists provide daily husbandry for the maintained corals. The facility primarily outplants a range of Hawaiian species ranging in size from 15cm to over 1m in length. The source material for this land-based nursery is predominantly coral that would otherwise have been destroyed from various state and federal projects such as harbor improvements or dredging.

Outplanting sites

Each nursery had an adjacent natural reef outplanting site. The site for the *in-situ* nursery outplanting (**Figure 1B**) was located in an enclosed bay with low water flow and composed of a sandy substrate situated next to existing mature *M. capitata* and *P. compressa* coral reef structure with roughly 70% coral cover (McGilly 2019). This site was selected so assays could be placed on the adjacent sand flat rather than affixed to the reef. The site for *ex-situ* nursery outplanting (**Figure 1D)** was located on the south shore of O'ahu with hard bottom pavement and due to high water flow conditions assays were fixed to the reef with Z-SPAR A-788 splash zone epoxy (Pettit Paint, NJ) in open patches among live coral colonies.

4. Pyramid assays

Pyramid assays were developed to study the effect of fragment size (small, medium or large) and nursery residence time (0, 4 and 8 months) on coral survivorship and net growth from both *in-situ* and *ex-situ* nurseries (**Figure 1**). The important role of genotypic variation (Baums et al. 2008; Grotolli et al. 2021) was incorporated in this experiment as a random effect with three separate colonies selected per species (*M. capitata,* and *P. compressa*) and per nursery (*ex-situ* and *in-situ*). 3D photogrammetry techniques were also examined at the *in-situ* nursery site due to the rapid development of fragments into arborescent forms that was not captured by 2D imaging of growth.

Assay design and deployment

The pyramid assay design was a modified smaller version of the ones regularly used at the HCRN with three sides and a flat top, leaving space for a label (**Figure 2**). The pyramid shape

was selected because it reduces the horizontal surface upon which sediment is retained, while minimizing the 3D surface area over which corals must grow to fuse and rapidly cover the artificial substrate. Once completely covered, this design also blends well into the reef substrate which is why such coral modules were selected for outplanting by the HCRN. A polyvinyl chloride sheet (Celtec®) was cut into a form consisting of three sides and a top (**Figure 2**) and joined together by drilling holes on the edge and joining them together with zip ties. The forms were nestled upside-down into tubs of sand and a fast-setting concrete (Portland Type II cement) was poured into them. Once the concrete set the zip ties joining the forms sides were cut and the concrete pyramids were removed. All pyramids were soaked in seawater for a month to cure, and then allowed to air dry in the sun for an additional month. The labels were made on a Dymo® label maker and affixed with All-Fix two-part epoxy putty prior to the experiment began and coral fragments were attached.

Three unique *M. capitata* and three *P. compressa* parent colonies, roughly 30cm in diameter, were collected at each site from within or adjacent to the nursery (**Figure 1A, C**) resulting in six parent colonies from a combination of natural reef and coral nursery origin at each location. The standard quarantine period was also observed for the *ex-situ* nursery samples, whereby any parent colonies not already in the nursery had all epifauna removed before being placed in a quarantine tank where they were required to remain clear of aquatic invasive species (AIS), parasites or visible disease for at least one month before experimentation (**Appendix 1**). Coral predatory nudibranchs (*Phestilla* sp.) emerging during quarantine on the *P. compressa* parent colonies at the *ex-situ* nursery resulted in additional cleaning and delayed fragmentation and deployment by 2 months at this site.

The parent corals were micro-fragmented to yield seven small (≈ 1x1cm or 1cm^2 each, 7cm^2 total), three medium (≈ 1.75x1.75cm or 3cm^2 each, 9cm^2 total), and one large (≈ 3x3cm, 9cm^2 total) fragment(s) per pyramid (**Figure 2**). Each of the six unique colonies per site were fragmented into three identical replicates (A, B and C), which were then outplanted at 0, 4 and 8 months resulting in 27 assays per species (54 total) per reef outplanting site (see **Appendix 2** for detailed timeline of coral assay deployment). The assays outplanted at 0 months (T0) had no nursery time and were outplanted immediately following confirmation of solid attachment of the fragments (**Figure 1B, D**). The remaining two sets were kept in nursery conditions (**Figure 1A, C**) until outplanting the second set of 18 pyramids at the same reef sites in month 4 (T1), leaving the final replicate in the nursery. After a further 4 months (T2, 8 months since T0 was deployed) the third set was then also outplanted at the same locations.

The top (the location of the label) and all sides of the pyramid were photographed with a ruler before placing them in the nursery or outplanting site. In order to avoid effects of position on the assay, the location of each fragment size was alternated around the pyramid with a clearly visible label on the top i.e., all ‘A’ replicates had the large fragment on the upper face, the medium on the left and the small on the right, relative to the label (**Figure 2**). The pyramids were then outplanted with labels all facing the same direction, ensuring that each fragment size was exposed to all the potential variable light and water flow conditions.

In addition to recording survivorship (alive or dead), fragment size (cm^2) of all 1,188 corals was manually measured from planar scaled digital photos (**Figure 3**) using the program ImageJ (Schneider et al., 2012) at the point of fragmentation, outplanting and at the end of the experiment. The final survivorship and growth measurements were collected at the *in-situ* nursery four months after the final deployment, but were delayed due to COVID-19 lockdowns until nine months after final deployment at the *ex-situ* nursery (**Appendix 2**).

3D photogrammetric measurements:

The coral fragments at the *in-situ* nursery (**Figure 1A, B**) grew with a much higher degree of three-dimensional structure than those of the *ex-situ* nursery (**Figure 3A-D**). Therefore, we added 3D photogrammetry to estimate surface area of living coral tissue at the *in-situ* nursery, in addition to estimating growth from planar scaled digital images that was performed at both locations.

A Three-Dimensional Structure from Motion (3D SfM) photogrammetric model of coral assays at the *in-situ* nursery was constructed using Agisoft Metashape Pro v 1.5.5 (Agisoft, 2019), from approximately 500 photos taken with a Canon Rebel EOS in an Ikelite underwater housing (**Figure 3E**). 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 in Metashape Pro was run 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 0.1 cm, with an overall estimated error of 0.07 cm. The scaled 3D SfM model was exported into Cloudcompare v2.11 (GPL Software) 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 3E**). 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 (**Figure 3F**) from the SfM model by linear regression in R (RStudio, Inc., 2019).

Statistical analyses

Generalized Linear Mixed Models (glmm) were used to assess the likelihood of individual fragments surviving to the end of the experiment based on their total time in nursery and their size class (See **Appendix 3-5** for raw data and analyses). To avoid bias from fragments that did not survive their time in nursery, only fragments surviving to their designated

outplanting time were included in these analyses. A glmm was fit for each species within each nursery using the *glmer()* function from the *lme4* R package, with a binomial response defined in the model specification. Linear mixed models allowed the use of random effects to account for variation due to parent colony (genotype) and deployment pyramid within colony. Fixed effects included an interaction between experimental day outplanted (total time in nursery) and size group on the pyramid face being estimated. To ensure the reliability of glmm results we inspected model convergence as well as variance inflation using the *vif()* function from the R package *car.* Overall model performance was evaluated as adjusted marginal versus conditional R^2 using the *r.squaredGLMM()* function from the *MuMIn* R package. An example glmm model specification would be:

$$Survival \sim Day\_Outplanted*Size + (1 \mid Genotype/Pyramid)$$

Percent net growth was calculated as the percent difference between total living coral tissue area at the beginning and end of the experiment and was assessed across all fragments on each face of a pyramid. Linear Mixed Models (lmm) were fit using the R package *lme4* for both *M. capitata* and *P. compressa* datasets within each nursery, resulting in a total of four separate fitted models to estimate the effect of nursery time and size class on pyramid face net growth. Similar to models used previously to assess survivorship, these models included nested random effects for parent colony genotype and pyramid within genotype, in addition to a fixed effect interaction between experimental day outplanted and fragment size class. After model fitting a type 3 ANOVA was performed using the *Anova()* function in the R package *car* to assess the significance of marginal effects in each model. An example lmm model specification would be:

$$PercentNetGrowth \sim Day\_Outplanted * Size + (1 \mid Genotype/Pyramid)$$

5. Block assay

In addition to fragment size and nursery time, variation among colonies in response to the conditions at the outplanting site is a key factor to understand to improve restoration success, because no colony is resilient to every stress they may encounter among different environments. The pyramid assays were only capable of examining variation among 3 genotypes; therefore, we designed a second assay to specifically examine spatial variation in greater detail while accounting for high intraspecific variation in survival and growth among *M. capitata* and *P. compressa* fragments.

Ten outplanting sites throughout Kāne‘ohe Bay, ranging from 0.5 to 3.2m in depth, were selected (**Figure 4A**) to encompass the range of environmental and hydrodynamic variability seen across the bay (Bahr et al., 2015; Caruso et al. 2021). We used horizontal pre-formed concrete slabs (40x20x5cm wall cap block) as “block assays” for this purpose. Four replicate medium (≈ $3cm^2$) fragments were cut from each of nine unique and widely spaced parent colonies to ensure distinct genotypes (**Appendix 6)**, and those fragments were used to create four replicate blocks

(A, B, C, and D) per site. We used a random number generator to ensure no two positions on the assay blocks were occupied by the same genotype. All nine *P. compressa* fragments were co-located on the upper portion and all nine *M. capitata* fragments on the lower portion of each block to minimize potential for interspecific competition (**Figure 2 and 5A-C**). We also made sure that the bottom row of *P. compressa* fragments and top row of *M. capitata* fragments had at least one of each genotype (across all four replicates) to account for potential species interaction effects. Finally, two labels were attached to each assay (**Figure 2**), one on the bottom right corner and one on the top edge, to minimize potential for loss or coral overgrowth making the label illegible.

Colonies were fragmented and attached to blocks in February 2020 (**Figure 5D**), after which they were held in the HIMB *in-situ* nursery for four months. Block assays were deployed via snorkel for four months beginning in June (**Figure 5E**) and were retrieved in October 2020. Because there was minimal vertical growth, all assays were top-down planar photographed with a ruler at each time point. Twenty-eight (out of 720) fragments died in the nursery shortly after fragmentation, so they were replaced and the difference in fragmentation dates was factored into the statistical analyses. All replicates (A, B, C, D) were maintained on separate submerged racks within one nursery pen (**Figure 5D)** to ensure no one site had all assays in one area of the nursery. Each submerged rack measured approximately 1x10m and was constructed of PVC and plastic mesh with replicates arranged in alphabetic order. Survivorship and net growth (cm^2) were documented for all samples after fragmentation, before deployment, and after retrieval. As with the pyramid assays, survivorship was recorded in the field as a binary response, either alive (1) or dead (0), and net growth (cm^2) was measured from planer top down scaled digital photos in ImageJ (**Figure 5F, G**).

Statistical analyses

We define survivorship as those fragments which survived after deployment to the reef site until they were retrieved, therefore corals that died in the nursery prior to deployment were excluded. In order to incorporate both the fixed effect of 'site' and the random effect of 'genotype' (parent colony) we analyzed the binary response of survivorship using *glmm* models with a binomial (logit link) error distribution from the *lme4* package (Bates et al., 2015) in RStudio (See Appendices 7 and 8 for raw data and analyses). Similar to the pyramid assays variance inflation was inspected and overall model performance was assessed as adjusted marginal versus conditional R^2.

$$Survival \sim Site + (1|Genotype)$$

Percent net growth included only those fragments which survived to the end and was calculated as 100*(cm^2 growth at the end / cm^2 growth at the beginning), which factors in the differing initial sizes of the coral fragments that had been grown for variable periods in the nursery prior

to field deployment. We used lmm models in the *lme4* package to analyze percent net growth with site as a fixed and genotype as a random effect.

$$PercentNetGrowth \sim Site + (1 \mid Genotype)$$

Net growth for *P. compressa* was square-root transformed as required to meet assumptions of normality and homoscedasticity, but *M. capitata* did not require transformation as determined by Q-Q plots, histograms, and residuals over fitted plots. Similar to the pyramid assays after model fitting a type 3 ANOVA was performed using the *Anova()* function.

Results

1. Pyramid assays: Fragment size and nursery residence time

Fragment survival likelihood

Overall, small fragments were significantly less likely to survive to the end of the experiment (42%) compared to medium or large fragments (67% and 70%, respectively) which were not significantly different from one another (**Appendices 4-6**). GLMM models of individual fragment survivorship indicated that survivorship of *M. capitata* fragments at the *ex-situ* nursery outplanting site was significantly improved through increased time in the nursery and this effect was greatest among the smallest fragments (**Appendix 10**). By comparison, there was no significant effect of nursery time on *P. compressa* survivorship for the *ex-situ* nursery outplanting site (**Figure 6**). A large proportion of survivorship variance was attributed to the nested effect of pyramid within genotype with approximately 80.8% (40.7% marginal) for *M. capitata* and 68.2% (13.9% marginal) of variation for *P. compressa* explained (**Appendix 10**). In contrast to the *ex-situ* outplanting site, there were no significant effects of nursery time, size class, or the interaction of these effects for fragment survivorship at the *in-situ* outplanting site for either coral species (**Appendix 11**). A positive, but marginally insignificant, effect was observed for the interaction between medium size and nursery time for fragments of *Porites compressa* at the *in-situ* nursery outplanting site. For the *in-situ* outplanting site, more of the variation in survivorship is attributed to fragment genotype than to pyramid within genotype for *M. capitata*, whereas survivorship of *P. compressa* varied more with pyramid within genotype. (**Appendix 11**).

Percent Net Growth

Pyramid assays revealed that the *in-situ* and *ex-situ* outplanting experiments differed greatly in their observed effects on net growth (**Figure 4**). Because it was not possible to obtain permits for a fully reciprocal transplantation design and we have only the local outplanting site for each nursery, we cannot determine whether it is the site, the nursery design, or some interaction of both that contributed to this difference. However, neither the *in-situ* or *ex-situ* nursery duration

showed consistently dramatic improvement for net percent growth after outplanting, and only *M. capitata* showed a positive effect of both total time in the *ex-situ* nursery, and its interaction with size class on overall net growth (**Figure 6 and Appendix 12A)**. Despite some trends in the data, no such effects were significant for *P. compressa* (**Appendix 12B**). For *M. capitata* at the *ex-situ* nursery, small fragments experienced the largest net-growth benefit from increased nursery time, but this effect was reduced for the larger size classes. In contrast, neither *M. capitata* nor *P. compressa* showed a significant benefit to net growth from prolonged duration in the *in-situ* nursery (**Appendix 13**).

3D Structure from motion (SfM)

For both species, there was a strong positive relationship between surface area derived from both the 2D top-down area and 3D modeled approaches (**Figure 3F**). Both approaches of measuring the coral colonies resulted in highly similar trends and yielded comparable results according to the generalized linear model (GLM) and pairwise analyses (**Appendix 12 and 13**). We compare the two approaches and discuss relative time savings from using 3D methods at scale for future efforts (**Appendix 17**). Because the results and interpretation are unchanged between the 2D and 3D models, and only the pyramid assays showed substantial vertical growth (**Figure 3**), we opted for consistency and present the 2D measurements for both types of assays here.

Assay performance

The pyramid assay performed well in both low- and high-flow environments with 94% assay recovery at the *in-situ* nursery site (low flow) and 74% recovery at the *ex-situ* outplanting site (high flow). Pyramids were easy to handle and small enough to be mass produced and housed in either nursery. The design also makes outplanting extremely easy because of their weight which allowed for placement in sandy rocky areas at the low-flow *in-situ* outplanting site and attachment to the reef with epoxy at the high-flow *ex-situ* outplanting site. Although we did not quantify sediment accumulation directly, visual inspection confirmed that the sloped sides of the pyramids reduced issues with sedimentation while still allowing for a clearly visible label on top during outplanting (**Figure 2)**. The design provided a weighty solid substrate for fusion of coral tissue (**Figures 3A-D**) without appearing artificial, rapidly blending into the reef substrate and making some difficult to detect by the end of the experiment (**Appendix 1 C, D**). However, the small size of each face limited how long the assay could be used to assess individual survivorship, because fragments began to fuse making it hard to distinguish individuals. The compact size also limited the number of colony fragments which could be affixed, which is why the block assay was used to assess spatial variation among individuals.

2. Block assays: Outplanting site and nursery rack location

Fragment survival likelihood

Overall survivorship was 55% for *M. capitata* and 56% for *P. compressa* across all sites (**Appendix 14**). There was significant variation among the species across sites for both survivorship and growth. *M. capitata* had the lowest survivorship (28%) at site 6 and the highest survivorship (72%) at sites 2 and 10. *P. compressa* had only 42% survivorship at sites 5 and 8 to a maximum of 72% at site 7 (**Figure 4**). There was approximately twice as much variation in survivorship across individual colonies in *M. capitata* compared to *P. compressa* (36.4% vs 12.4%, respectively). For both species a larger proportion of survivorship variance was explained by the fixed effect of 'site' while a smaller proportion of variance was attributed to the random effect of 'genotype' and there appears to be an interaction because colonies that did among the best and worst at one site would reverse that trend at another. The models of *M. capitata* and *P. compressa* explained approximately 32.65% (25.2% marginal), and 11.89% (8.59% marginal) of variation in fragment survivorship, respectively (**Appendix 15**).

Percent Net Growth

The growth of both species was significantly different among sites, but *M. capitata* had more than an order of magnitude higher variation in growth (641% vs 49.5%) among individuals compared to *P. compressa* (**Appendix 16**). Percent net growth increased for both species by over 100% during the eight months, with a mean 104% increase for *M. capitata* and 129% for *P. compressa* (**Appendix 14**). Even with individual variation, there were consistent differences among sites. The lowest growth rate for *M. capitata* was at site 6 (27%), and the highest at site 2 (155%), whereas for *P. compressa* the lowest growth was seen at site 2 (78%) and the highest at site 10 (272%). Looking across the environmental gradient of the bay, with the exception of site 5, *M. capitata* tended to show the lowest growth rates (27-81%) at the central sites 3-8, and highest growth rates (123-155%) at the northern and southern ends of the bay (1-2 and 9-10). *P. compressa*, by comparison, had no obviously reduced growth regions across the bay, and only the northern sites (9-10) stand out (211-272%) with site 9 showing significantly higher net growth than other locations in the bay (**Figure 5**).

Assay performance

The block assays were easily deployed without epoxy due to the low wave energy environment in Kāneʻohe Bay, and all were successfully retrieved without loss in the field. Three top labels were lost, but in each case the backup labels remained attached. Although the flat horizontal surface appeared to retain more sediment than the sloped faces of the pyramid assay, sedimentation was minimal at most sites, and survivorship and growth were of the same magnitude between each design. However, the block design accommodated a larger number of unique colony fragments (n=18) than the pyramid assays (**Figure 2**), while still maintaining sufficient spacing for several months of growth without direct fragment-to-fragment interaction. This design allows for rapid testing of performance by individual genotypes in potential restoration locations and can help to identify and focus efforts on which species and individuals are most likely to thrive at a given location.

Discussion

Interest in active coral reef restoration and strategies to scale such efforts has increased dramatically as coral reefs continue to decline globally (Rinkevich et al. 2008; van Oppen et al. 2015; Anthony et al. 2017; Hesley et al. 2017; Voolstra et al. 2021). However, most efforts to date remain short-term, small scale, and often lack clear and achievable objectives or rigorous monitoring and reporting about whether those objectives were reached (Boström-Einarsson et al. 2020). Several have pointed out that ecological interactions are rarely considered but critical factors which can affect outplanting success (Hein et al. 2017; Boström-Einarsson et al. 2020; Ladd and Shantz 2020). Further, Hein et al. (2017) found that 88% of studies that have been published to date use growth and survival of coral fragments as the primary indicators of restoration success, and argue that a more realistic range of ecological indicators along with sociocultural, economic, and governance should all be considered when evaluating the success of reef restoration projects. One such factor is cost-effectiveness of the restoration activities and here we propose short-term assays that can help optimize restoration activities by providing information to maximize survivorship and growth while minimizing nursery and labor costs. Micro-fragmentation currently accounts for less than 5% of coral transplantation studies to date (Boström-Einarsson et al. 2020), focusing almost entirely on slower growing massive and encrusting species and predominantly using very small fragments (~ 1cm^2). These small fragments are either grown separately on a plug (part of the reskinning method; Page 2015) or attached to a module (such as a concrete block) and housed in a nursery until they fuse together prior to outplanting (Forsman et al. 2015; Page et al. 2018).

Active restoration of a reef through 'coral gardening' or 'farming' is generally considered a two-step process: first, raising coral fragments in a nursery to a size that minimizes mortality risk, before second, harvesting and outplanting them to the desired site (Rinkevich 2006). How long coral fragments need to be raised in the nursery will depend on a variety of factors such as species and growth rate, but fast-growing branching species like Acroporids are usually large enough to outplant within 1 year (Mbije et al. 2010; Horoszowski-Fridman et al. 2015). Slower growing massive and encrusting species take longer to reach equivalent sizes, so periods of up to two years in the nursery have been recommended (dela Cruz et al. 2015; Page et al. 2018). Because labor is generally the highest cost of restoration, doubling the nursery time can dramatically increase the cost of such efforts, and likely explains why it is rare to farm slower growing corals (Boström-Einarsson et al. 2020). Here we found that both branching and massive corals responded well to micro-fragmentation and outplanting and that net percent gains for both species we selected could be substantially increased over natural growth rates. We see the expected relationship between nursery residence time and increased survivorship among the smallest fragments (≈ 1cm^2) of *M. capitata* which also have the highest net growth rate overall (**Figure 6**). This growth rate is offset somewhat by higher mortality (58%) than medium (33%) or large (30%) fragments (**Appendix 9**), and culture in the *ex-situ* nursery had only a weak positive effect on net growth of medium fragments (≈ 3cm^2) of *M. capitata* but no benefit at the

in-situ nursery or for larger sizes (**Appendix 12 & 13**). Contrary to expectations, we found no evidence for size-specific benefits for either mortality or growth with nursery duration for *P. compressa* in either the *in-situ* or *ex-situ* nursery. Growing the fragments in a nursery requires space and labor that add to the total cost and reduce scalability of restoration efforts. Consequently, identifying the optimal fragment size that ensures high survivorship will be critical in reducing the need for coral source material, and reducing labor through minimal handling and nursery residence time is important to optimize cost-efficiency of restoration activities.

The primary benefit of using very small fragments is higher yield with a reduced environmental impact along with increased size-specific growth rates in comparison to larger fragments (Page et al. 2018; Forsman et al. 2006). However, predation can reduce survivorship of these smaller fragments. For example, Koval et al. (2020) found that for four species of massive corals in Florida (*Orbicella faveolata, Montastraea cavernosa, Pseudodiploria clivosa,* and *P. strigosa*), fragments < 5cm^2 experienced severe tissue damage or complete removal of fragments in the first week of deployment due to corallivorous fish. In Hawai‘i, Jayewardene et al. (2009) found coral fragments < 2cm^2 were entirely removed by corallivorous fish, but nubbins of 4cm^2 or greater were only partially consumed. Likewise, Forsman et al. (2006) found fragments 3cm^2 or larger had the highest survival and growth rates for *P. compressa* with no evidence of size specific mortality beyond that. Here, we did not observe obvious signs of fish predation (in the form of bite marks), but this will obviously vary among locations depending on the density and species of corallivores. Unfortunately, it was not possible to directly compare the *in-situ* and *ex-situ* nurseries, because reciprocal transplantation over these large distances carries risks of vectoring disease or invasive species and are not permitted in the State of Hawai‘i. The *in-situ* nursery site has similar conditions (low water flow, medium sedimentation, ~2m depth) to *the in-situ* outplanting site itself. In comparison, the *ex-situ* nursery site had vastly dissimilar conditions to idealized indoor nursery tank conditions, with high water flow, medium sedimentation, and ~5m depth. The differences among sites may account for variation in significant size-specific growth and survivorship among only the smallest *M. capitata* at the *ex-situ* nursery outplanting site. The value of field assays such as those described herein is to learn such site-specific information in advance of major restoration activities to optimize the efforts.

Pyramid assays provided a cost-effective way to test size-specific survivorship and growth under field conditions. Recovery rates were high in both low and high flow environments, and their small size took up minimal space in the nurseries and made them relatively easy to handle during outplanting. The sloped surfaces minimized accumulation of sediment from settling onto the coral fragments and the label was easily read and could be used to orientate the assays during outplanting. Their three-dimensional design provided a suitable substrate for isogenic fusion of fragments on all exposed surfaces, which does not appear artificial and rapidly blends into the reef substrate allowing modules to be left in the field if desired. This rapid overgrowth limits the duration of use however, because when colonies fuse and grow over the label it makes the modules hard to locate and area cover estimates become much more difficult. The small size also

restricts the number of genotypes which can be attached in any given assay. These shortcomings can be offset by the block assays which were purchased from a hardware store, which could make them a more accessible and cheaper option than the labor for custom made substrates in some restoration projects. Using paving blocks allowed us to test 18 genotypes, as opposed to a maximum of three on a pyramid, to assay site-specific differences in individual performance. Additionally, the weight of these blocks meant that they did not need to be attached to the reef even in moderately wave exposed reef areas, and so could be easily placed out and collected for a short-term site assessment.

While there are general trends among sites, with higher overall growth rates in the far southern (1-2) and far northern (9-10) portions of the bay (**Figure 5**), we find considerable variation among individual performance at different locations throughout the bay (**Appendix 16**). Slowly acclimating corals to conditions they will experience in the field can minimize stress and reduce predation and mortality among outplanted corals (Horoszowski-Fridman et al. 2015; Page et al. 2018). However, some traits tend to be less plastic, and corals may never acclimate to a degree that alters survivorship during transplantation (e.g., Barott et al. 2021). Acclimation through similarity to the nursery conditions could explain increased growth at sites 1 and 2 which are most similar in terms of being low energy lagoonal habitats with similar hydrodynamic regime, temperature, pH, sedimentation, and nutrient levels, but sites 9 and 10 are the most dissimilar to nursery conditions across the environmental gradient of the bay (Lowe et al. 2009a, b; Bahr et al. 2015; Caruso et al. 2021). Sites in the central portion of the bay showed lowest survivorship of *M. capitata*, whereas *P. compressa* had similar rates of survivorship across all outplanting locations (**Figure 4**). Because site-specific effects dominate and no single coral is resilient to every pressure faced in every location when outplanting, these results highlight the importance of matching effort to that spatial variation during outplanting. Therefore, rather than trying to acclimate corals to novel site conditions, assays such as these provide an alternative approach to optimize efforts to minimize mortality during restoration activities. Short-term block assays inform which species and individual genotypes have higher survivorship and growth at particular outplanting sites. Pyramid assays on the most successful individuals then allow restoration practitioners to optimize the size and spacing of fragments to maximize survivorship and growth among outplanted corals. By employing a combination of these assays over a period of 2-4 months each, restoration projects could dramatically reduce costs and improve success rates.

Conclusions

It is impossible to generalize methods for all species of corals at all sites, therefore rapid assays such as these are an important step to establish interspecies variation in the performance of variously sized fragments, as well as the role of nursery residence time, and individual performance at a given restoration site. Our study was designed to streamline outplanting practices for two encrusting species in Hawai‘i, but more generally simple assays such as these can be used on almost any reef restoration site to identify which species of coral and which individuals are most likely to survive and grow at the outplanting site and on which to focus

restoration efforts. The rapid assays we outline here are a simple and highly flexible tool to gather critical preliminary information essential to scaling large restoration projects efficiently and to maximize both the likelihood of success and cost-efficiency. Care however needs to be taken with the assays and the corals attached that they not pose unacceptable environmental risks to the outplanting habitat through overuse and unwanted introduction of invasive species. Overall, we see relatively little positive benefit of prolonged residence time in either the *in-situ* or *ex-situ* nursery. Only the smallest fragments (~1cm^2) of *M. capitata* showed a significant benefit of nursery residence time on survivorship and growth in the field following outplanting, suggesting that construction and staffing of nurseries may not pay dividends on that investment for large-scale restoration projects. However, this is also the same treatment that showed the greatest overall percent net growth gain in the experiments, highlighting the need for clear objectives for restoration activities. Thus, for a project in which the objective is to outplant in the least expensive and most cost-effective manner with the greatest coral survival outcome, for these species at these sites we would recommend direct outplanting of medium-sized fragments (~3cm^2) without any nursery care. In contrast, if the objective were to maximize growth for a high value species (such as rare or ESA-listed corals) in which starting material was limited or outplanting of large sexually reproducing colonies was the objective, then targeting ~1cm^2 fragments raised in the *ex-situ* nursery would best achieve this goal. Because the goals of each project are likely to differ as much as the species and local environmental conditions that will affect the achievement of those goals, we recommend assays such as these be undertaken to inform efforts to reduce costs and increase productivity prior to undertaking large-scale restoration activity.

Acknowledgements

We'd like to thank all the coral nursery volunteers and students who have been an integral part of the past, present and future productivity of both the HIMB and HCRN coral nurseries. In addition, we couldn't have completed this work without the fieldwork help of Mykle Hoban, Jan Vicente, and Eileen Nalley, and Mariana Rocha De Souza along with the support of rest of the ToBo lab.

References

Adjeroud M, Michonneau F, Edmunds PJ, Chancerelle Y, de Loma TL, Penin L, Thibaut L, Vidal-Dupiol J, Salvat B, Galzin R. 2009. Recurrent disturbances, recovery trajectories, and resilience of coral assemblages on a South-Central Pacific reef. *Coral reefs (Online)* 28:775–780. DOI: 10.1007/s00338-009-0515-7.

Agisoft. 2019. *AgiSoft PhotoScan Professional*. Agisoft LLC.

Anthony K, Bay LK, Costanza R, Firn J, Gunn J, Harrison P, Heyward A, Lundgren P, Mead D, Moore T, Mumby PJ, van Oppen MJH, Robertson J, Runge MC, Suggett DJ, Schaffelke B,

Wachenfeld D, Walshe T. 2017. New interventions are needed to save coral reefs. *Nature Ecology & Evolution* 1:1420–1422. DOI: 10.1038/s41559-017-0313-5.
Anthony KRN, Helmstedt KJ, Bay LK, Fidelman P, Hussey KE, Lundgren P, Mead D, McLeod IM, Mumby PJ, Newlands M, Schaffelke B, Wilson KA, Hardisty PE. 2020. Interventions to help coral reefs under global change-A complex decision challenge. *Plos One* 15:e0236399. DOI: 10.1371/journal.pone.0236399.
Bahr KD, Jokiel PL, Toonen RJ (2015) The unnatural history of Kāne‘ohe Bay: coral reef resilience in the face of centuries of anthropogenic impacts. PeerJ 3:e950
Barott KL, Huffmyer AS, Davidson JM, Lenz EA, Matsuda SB, Hancock JR, Innis T, Drury C, Putnam HM, Gates RD. 2021. Coral bleaching response is unaltered following acclimatization to reefs with distinct environmental conditions. *Proceedings of the National Academy of Sciences of the United States of America* 118. DOI: 10.1073/pnas.2025435118.
Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48. DOI: 10.18637/jss.v067.i01.
Baums, I.B. (2008). A restoration genetics guide for coral reef conservation. Molecular Ecology 17(12): 2796-2811.
Boström-Einarsson L, Babcock RC, Bayraktarov E, Ceccarelli D, Cook N, Ferse SCA, Hancock B, Harrison P, Hein M, Shaver E, Smith A, Suggett D, Stewart-Sinclair PJ, Vardi T, McLeod IM. 2020. Coral restoration–- A systematic review of current methods, successes, failures and future directions. *Plos One* 15:e0226631. DOI: 10.1371/journal.pone.0226631.
Briggs CJ, Adam TC, Holbrook SJ, Schmitt RJ. 2018. Macroalgae size refuge from herbivory promotes alternative stable states on coral reefs. *Plos One* 13:e0202273. DOI: 10.1371/journal.pone.0202273.
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. DOI: 10.1016/S0022-0981(98)00080-X.
Cabaitan PC, Yap HT, Gomez ED. 2015. Performance of single versus mixed coral species for transplantation to restore degraded reefs. *Restoration Ecology* 23:349–356. DOI: 10.1111/rec.12205.
Caruso C, Hughes K, Drury C. 2021. Selecting Heat-Tolerant Corals for Proactive Reef Restoration. *Frontiers in Marine Science* 8. DOI: 10.3389/fmars.2021.632027.
Caruso, C., de Souza, M.R., Ruiz-Jones, L., Conetta, D., Hancock, J., Hobbs, C., Hobbs, C., Kahkejian, V., Kitchen, R., Marin, C. and Monismith, S. (2021). Genetic patterns in *Montipora capitata* across an environmental mosaic in Kāne’ohe Bay. bioRxiv. DOI: 10.1101/2021.10.07.463582
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. *Scientific Reports* 7:18076. DOI: 10.1038/s41598-017-17555-z.
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. DOI: 10.1890/0012-9615(1997)067[0461:AYSOCA]2.0.CO;2.
Darling, E.S., Alvarez-Filip, L., Oliver, T.A., McClanahan, T.R. and Côté, I.M. (2012). Evaluating life-history strategies of reef corals from species traits. Ecology Letters 15(12): 1378-1386.

dela Cruz DW, Rinkevich B, Gomez ED, Yap HT. 2015. Assessing an abridged nursery phase for slow growing corals used in coral restoration. *Ecological engineering* 84:408–415. DOI: 10.1016/j.ecoleng.2015.09.042.
Delbeek JC. 2001. Coral Farming: Past, Present and Future Trends. *Aquarium Sciences and Conservation*.
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 (Online)* 32:233–238. DOI: 10.1007/s00338-012-0974-0.
Doropoulos C, Vons F, Elzinga J, ter Hofstede R, Salee K, van Koningsveld M, Babcock RC. 2019. Testing Industrial-Scale Coral Restoration Techniques: Harvesting and Culturing Wild Coral-Spawn Slicks. *Frontiers in Marine Science* 6. DOI: 10.3389/fmars.2019.00658.
Dudgeon SR, Aronson RB, Bruno JF, Precht WF. 2010. Phase shifts and stable states on coral reefs. *Marine Ecology Progress Series* 413:201–216. DOI: 10.3354/meps08751.
Edmunds PJ, Bruno JF, Carlon DB. 2004. Effects of depth and microhabitat on growth and survivorship of juvenile corals in the Florida Keys. *Marine Ecology Progress Series* 278:115–124. DOI: 10.3354/meps278115.
Edmunds PJ, Putnam HM. 2020. Science-based approach to using growth rate to assess coral performance and restoration outcomes. *Biology Letters* 16:20200227. DOI: 10.1098/rsbl.2020.0227.
Epstein N, Bak RPM, Rinkevich B. 2003. Applying forest restoration principles to coral reef rehabilitation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 13:387–395. DOI: 10.1002/aqc.558.
Ferrari R, Gonzalez-Rivero M, Mumby PJ. 2012. Size matters in competition between corals and macroalgae. *Marine Ecology Progress Series* 467:77–88. DOI: 10.3354/meps09953.
Filbee-Dexter K, Smajdor A. 2019. Ethics of assisted evolution in marine conservation. *Frontiers in Marine Science* 6. DOI: 10.3389/fmars.2019.00020.
Fletcher CH, Bochicchio C, Conger CL, Engels MS, Feirstein EJ, Frazer N, Glenn CR, Grigg RW, Grossman EE, Harney JN, Isoun E, Murray-Wallace CV, Rooney JJ, Rubin KH, Sherman CE, Vitousek S. 2008. Geology of Hawaii reefs. In: Riegl BM, Dodge RE eds. *Coral reefs of the USA*. Dordrecht: Springer Netherlands, 435–487. DOI: 10.1007/978-1-4020-6847-8_11.
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. DOI: 10.7717/peerj.1313.
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. DOI: 10.1016/j.aquaculture.2006.06.040.
Fox TR, Jokela EJ, Allen HL. 2004. The evolution of pine plantation silviculture in the Southern United States. *In: Gen. Tech. Rep. SRS–75. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. Chapter 8. p. 63-82.*
Franklin, E.C., Jokiel, P.L. and Donahue, M.J. (2013). Predictive modeling of coral distribution and abundance in the Hawaiian Islands. Marine Ecology Progress Series. 481:121-132.
Fung T, Seymour RM, Johnson CR. 2011. Alternative stable states and phase shifts in coral reefs under anthropogenic stress. *Ecology* 92:967–982. DOI: 10.1890/10-0378.1.

764 Griffin S, Spathias H, Moore TD, Baums I, Griffin BA. 2012. Scaling up *Acropora* nurseries in
765 the Caribbean and improving techniques. In: ReefBase.
766 Grottoli, A.G., Toonen, R.J., van Woesik, R., Vega Thurber, R., Warner, M.E., McLachlan, R.H.,
767 Price, J.T., Bahr, K.D., Baums, I.B., Castillo, K.D. and Coffroth, M.A. (2021). Increasing
768 comparability among coral bleaching experiments. Ecological Applications 31(4): e02262.
769 Hein, M.Y., Willis, B.L., Beeden, R. and Birtles, A. (2017). The need for broader ecological and
770 socioeconomic tools to evaluate the effectiveness of coral restoration programs. Restoration
771 Ecology 25(6): 873-883.
772 Herlan J, Lirman D. 2009. Development of a coral nursery program for the threatened coral
773 *Acropora cervicornis* in Florida. Proceedings of the 11th International Coral Reef Symposium,
774 Ft. Lauderdale, Florida, 7-11 July 2008 24: 1244-1247.
775 Hesley, D., Burdeno, D., Drury, C., Schopmeyer, S. and Lirman, D. (2017). Citizen science
776 benefits coral reef restoration activities. Journal for Nature Conservation 40: 94-99.
777 Horoszowski-Fridman YB, Brêthes J-C, Rahmani N, Rinkevich B. 2015. Marine silviculture:
778 Incorporating ecosystem engineering properties into reef restoration acts. *Ecological*
779 *engineering* 82:201–213. DOI: 10.1016/j.ecoleng.2015.04.104.
780 Hughes TP, Tanner JE. 2000. Recruitment failure, life histories, and long-term decline of
781 caribbean corals. *Ecology* 81:2250–2263. DOI: 10.1890/0012-
782 9658(2000)081[2250:RFLHAL]2.0.CO;2.
783 Huntington BE, Miller MW, Pausch R, Richter L. 2017. Facilitation in Caribbean coral reefs: high
784 densities of staghorn coral foster greater coral condition and reef fish composition. *Oecologia*
785 184:247–257. DOI: 10.1007/s00442-017-3859-7.
786 Irizarry-Soto E, Weil E. 2009. Spatial and temporal variability in juvenile coral densities,
787 survivorship and recruitment in La Parguera, southwestern Puerto Rico. *Caribbean journal of*
788 *science* 45:269–281. DOI: 10.18475/cjos.v45i2.a14.
789 Jayewardene D, Donahue MJ, Birkeland C. 2009. Effects of frequent fish predation on corals in
790 Hawaii. *Coral reefs (Online)* 28:499–506. DOI: 10.1007/s00338-009-0475-y.
791 Jury CP, Toonen RJ. 2019. Adaptive responses and local stressor mitigation drive coral
792 resilience in warmer, more acidic oceans. *Proceedings. Biological Sciences / the Royal Society*
793 286:20190614. DOI: 10.1098/rspb.2019.0614.
794 Khurana E, Singh JS. 2001. Ecology of seed and seedling growth for conservation and
795 restoration of tropical dry forest : a review. *Environmental conservation* 28. DOI:
796 10.1017/S0376892901000042.
797 Kleypas J, Allemand D, Anthony K, Baker AC, Beck MW, Hale LZ, Hilmi N, Hoegh-Guldberg O,
798 Hughes T, Kaufman L, Kayanne H, Magnan AK, Mcleod E, Mumby P, Palumbi S, Richmond
799 RH, Rinkevich B, Steneck RS, Voolstra CR, Wachenfeld D, Gattuso J-P. 2021. Designing a
800 blueprint for coral reef survival. *Biological Conservation* 257:109107. DOI:
801 10.1016/j.biocon.2021.109107.
802 Kojis BL, Quinn NJ. 2001.The importance of regional differences in hard coral recruitment rates
803 for determining the need for coral restoration. *Available at*
804 *https://www.ingentaconnect.com/content/umrsmas/bullmar/2001/00000069/00000002/art00055*
805 (accessed July 12, 2021).

Koval G, Rivas N, D'Alessandro M, Hesley D, Santos R, Lirman D. 2020. Fish predation hinders the success of coral restoration efforts using fragmented massive corals. *PeerJ* 8:e9978. DOI: 10.7717/peerj.9978.
Ladd, M.C. and Shantz, A.A. (2020). Trophic interactions in coral reef restoration: A review. Food Webs 24: e00149.
Lowe RJ, Falter JL, Monismith SG, Atkinson MJ (2009a) A numerical study of circulation in a coastal reef-lagoon system. Journal of Geophysical Research 114:C06022
Lowe RJ, Falter JL, Monismith SG, Atkinson MJ (2009b) Wave-Driven Circulation of a Coastal Reef–Lagoon System. Journal of Physical Oceanography 39:873–893
Mbije NEJ, Spanier E, Rinkevich B. 2010. Testing the first phase of the 'gardening concept' as an applicable tool in restoring denuded reefs in Tanzania. *Ecological engineering* 36:713–721. DOI: 10.1016/j.ecoleng.2009.12.018.
McGilly M. 2019. In-situ coral farms: a new source of juvenile fish recruitment? Master thesis Thesis. University of York.
Miller MW. 2014. Post-settlement survivorship in two Caribbean broadcasting corals. *Coral Reefs* 33:1041–1046. DOI: 10.1007/s00338-014-1177-7.
Mumby PJ, Steneck RS, Hastings A. 2013. Evidence for and against the existence of alternate attractors on coral reefs. *Oikos (Copenhagen, Denmark)* 122:481–491. DOI: 10.1111/j.1600-0706.2012.00262.x.
Nedimyer K, Gaines K, Roach S. 2011. Coral Tree Nursery©: An innovative approach to growing corals in an ocean-based field nursery. *AACL Bioflux* 4.
Opel AH, Cavanaugh CM, Rotjan RD, Nelson JP. 2017. The effect of coral restoration on Caribbean reef fish communities. *Marine Biology* 164:221. DOI: 10.1007/s00227-017-3248-0.
Page C. 2015. Reskinning a Reef: Mote Marine Lab scientists explore a new approach to reef restoration. *Unpublished*. DOI: 10.13140/rg.2.1.4281.0967.
Page CA, Muller EM, Vaughan DE. 2018. Microfragmenting for the successful restoration of slow growing massive corals. *Ecological engineering* 123:86–94. DOI: 10.1016/j.ecoleng.2018.08.017.
Raymundo LJ, Maypa AP. 2004. Getting bigger faster: mediation of size-specific mortality via fusion in juvenile coral transplants. *Ecological Applications* 14:281–295. DOI: 10.1890/02-5373.
Rinkevich B. 2005. Conservation of coral reefs through active restoration measures: recent approaches and last decade progress. *Environmental Science & Technology* 39:4333–4342. DOI: 10.1021/es0482583.
Rinkevich B. 2006. The Coral Gardening Concept and the Use of Underwater Nurseries: Lessons Learned from Silvics and Silviculture. In: Precht W ed. *Coral reef restoration handbook*. CRC Press, 291–301. DOI: 10.1201/9781420003796.ch16.
Rinkevich B. 2008. Management of coral reefs: we have gone wrong when neglecting active reef restoration. *Marine Pollution Bulletin* 56:1821–1824. DOI: 10.1016/j.marpolbul.2008.08.014.
RStudio, Inc. RsT. 2019. *RStudio: Integrated Development for R*. Boston, MA: rStudio, PBC.
Salinas-de-León P, Dryden C, Smith DJ, Bell JJ. 2013. Temporal and spatial variability in coral recruitment on two Indonesian coral reefs: consistently lower recruitment to a degraded reef. *Marine Biology* 160:97–105. DOI: 10.1007/s00227-012-2066-7.

Schmitt RJ, Holbrook SJ, Davis SL, Brooks AJ, Adam TC. 2019. Experimental support for alternative attractors on coral reefs. *Proceedings of the National Academy of Sciences of the United States of America* 116:201812412. DOI: 10.1073/pnas.1812412116.

Schopmeyer SA, Lirman D. 2015. Occupation Dynamics and Impacts of Damselfish Territoriality on Recovering Populations of the Threatened Staghorn Coral, Acropora cervicornis. *Plos One* 10:e0141302. DOI: 10.1371/journal.pone.0141302.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671–675. DOI: 10.1038/nmeth.2089.

Smith SR. 1992. Patterns of Coral Recruitment and Post-settlement Mortality on Bermuda's Reefs: Comparisons to Caribbean and Pacific Reefs. *American zoologist* 32:663–673. DOI: 10.1093/icb/32.6.663.

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.

Tlusty M. 2002. The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture* 205:203–219. DOI: 10.1016/S0044-8486(01)00683-4.

van Oppen MJH, Gates RD, Blackall LL, Cantin N, Chakravarti LJ, Chan WY, Cormick C, Crean A, Damjanovic K, Epstein H, Harrison PL, Jones TA, Miller M, Pears RJ, Peplow LM, Raftos DA, Schaffelke B, Stewart K, Torda G, Wachenfeld D, Putnam HM. 2017. Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology* 23:3437–3448. DOI: 10.1111/gcb.13647.

van Oppen MJH, Oliver JK, Putnam HM, Gates RD. 2015. Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences of the United States of America* 112:2307–2313. DOI: 10.1073/pnas.1422301112.

van Woesik R, Banister RB, Bartels E, Gilliam DS, Goergen EA, Lustic C, Maxwell K, Moura A, Muller EM, Schopmeyer S, Winters RS, Lirman D. 2021. Differential survival of nursery-reared outplants along the Florida reef tract. *Restoration Ecology* 29. DOI: 10.1111/rec.13302.

Voolstra, C.R., Suggett, D.J., Peixoto, R.S., Parkinson, J.E., Quigley, K.M., Silveira, C.B., Sweet, M., Muller, E.M., Barshis, D.J., Bourne, D.G. and Aranda, M. (2021). Extending the natural adaptive capacity of coral holobionts. Nature Reviews Earth & Environment 2: 747-762.

Williams GJ, Knapp IS, Maragos JE, Davy SK. 2010. Modeling patterns of coral bleaching at a remote Central Pacific atoll. *Marine Pollution Bulletin* 60:1467–1476. DOI: 10.1016/j.marpolbul.2010.05.009.

Williams SL, Sur C, Janetski N, Hollarsmith JA, Rapi S, Barron L, Heatwole SJ, Yusuf AM, Yusuf S, Jompa J, Mars F. 2018. Large-scale coral reef rehabilitation after blast fishing in Indonesia. *Restoration Ecology*. DOI: 10.1111/rec.12866.

Yap HT. 2009. Local changes in community diversity after coral transplantation. *Marine Ecology Progress Series* 374:33–41. DOI: 10.3354/meps07650.

Figure 1

Map of *in-situ* and *ex-situ* nurseries and their respective outplanting sites for the pyramid assays.

Map of O‘ahu, Hawai‘i indicating the two experimental locations, with the darker blue indicating the nurseries and the lighter blue the outplanting sites where **(A)** *in-situ* nursery at the Hawai‘i Institute of Marine Biology (HIMB), **(B)** *in-situ* outplanting reef site, **(C)** *ex-situ* nursery tanks at Ānuenue Fisheries Research Center (AFRC) **(D)** *ex-situ* outplanting reef site.

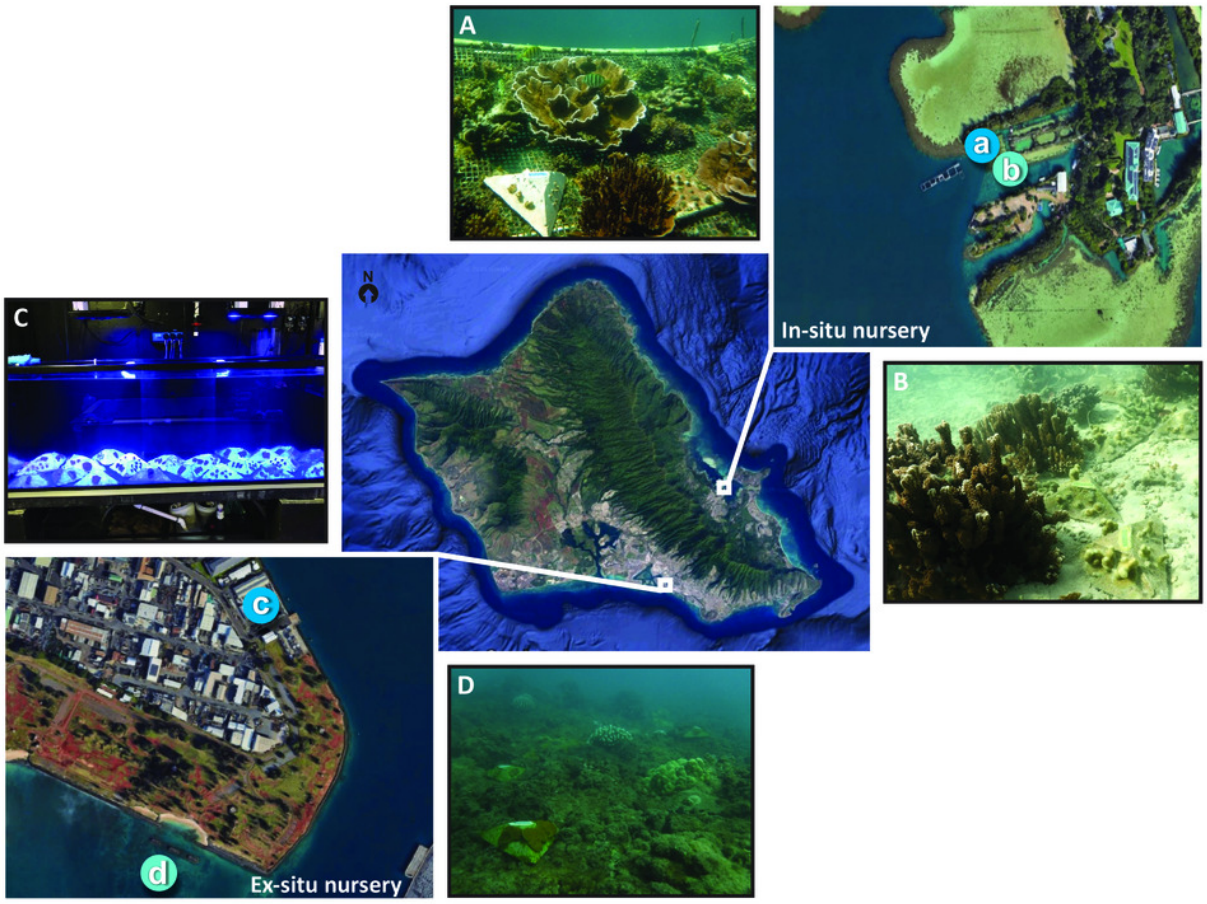


Figure 2

Pyramid and block assay design

Coral micro-fragmentation assays (where the coral fragments are represented by brown circles and labels are in grey) (**A)** pyramid assay (24Lx24Wx6H cm), with top labels providing an informative unique identifier for each pyramid assay. In order to avoid effects of position on the assay each replicate (A, B or C) rotated the location of each fragment size and were outplanted with the label facing the same direction in order to expose all size fragments to all water flow and light conditions and (**B)** block assay design, using 40Lx20Wx5H cm concrete slabs and nine *Porites compressa* fragments on top half and nine *Montipora capitata* fragments on the bottom. No two positions were occupied by the same parent colony (numbers in the circles) across the four replicates (A, B, C, and D). There were two labels for redundancy (one on the bottom right corner on the top face and one on the top edge), in case of loss or overgrowth the assay could still be identified.

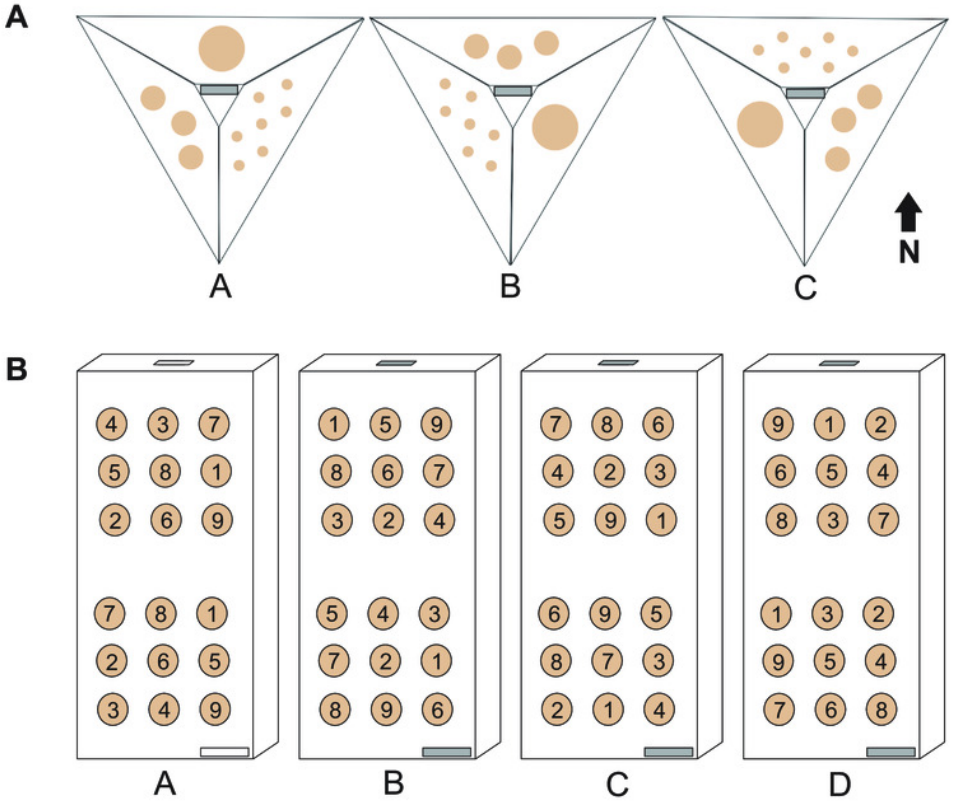

A
A
B
C
N
B
4 3 7
5 8 1
2 6 9
7 8 1
2 6 5
3 4 9
1 5 9
8 6 7
3 2 4
5 4 3
7 2 1
8 9 6
7 8 6
4 2 3
5 9 1
6 9 5
8 7 3
2 1 4
9 1 2
6 5 4
8 3 7
1 3 2
9 5 4
7 6 8
A
B
C
D

Figure 3

Examples of pyramid assay fragment growth from the *in-situ* and *ex-situ* nurseries, 3D Structure from Motion (SfM) segmentation and labeling, and the relationship between 2D and 3D SfM measurements.

Examples of *Porites compressa* **(left)** and *Montipora capitata* **(right)** medium fragments spreading horizontally and fusing and/or growing vertically on assays at both *ex-situ* (**A, B**), and *in-situ* (**C**, **D**) coral nurseries. An example of segmentation and labeling of living coral tissue on an *in-situ* nursery coral assay module for the estimation of surface area covering the complex geometry of coral colonies (**E**) and the relationship between two-dimensional (planar) area in cm^2 to surface area derived from 3D Structure from Motion (SfM) models (**F**) where light grey is *P. compressa* and dark grey is *M. capitata*.

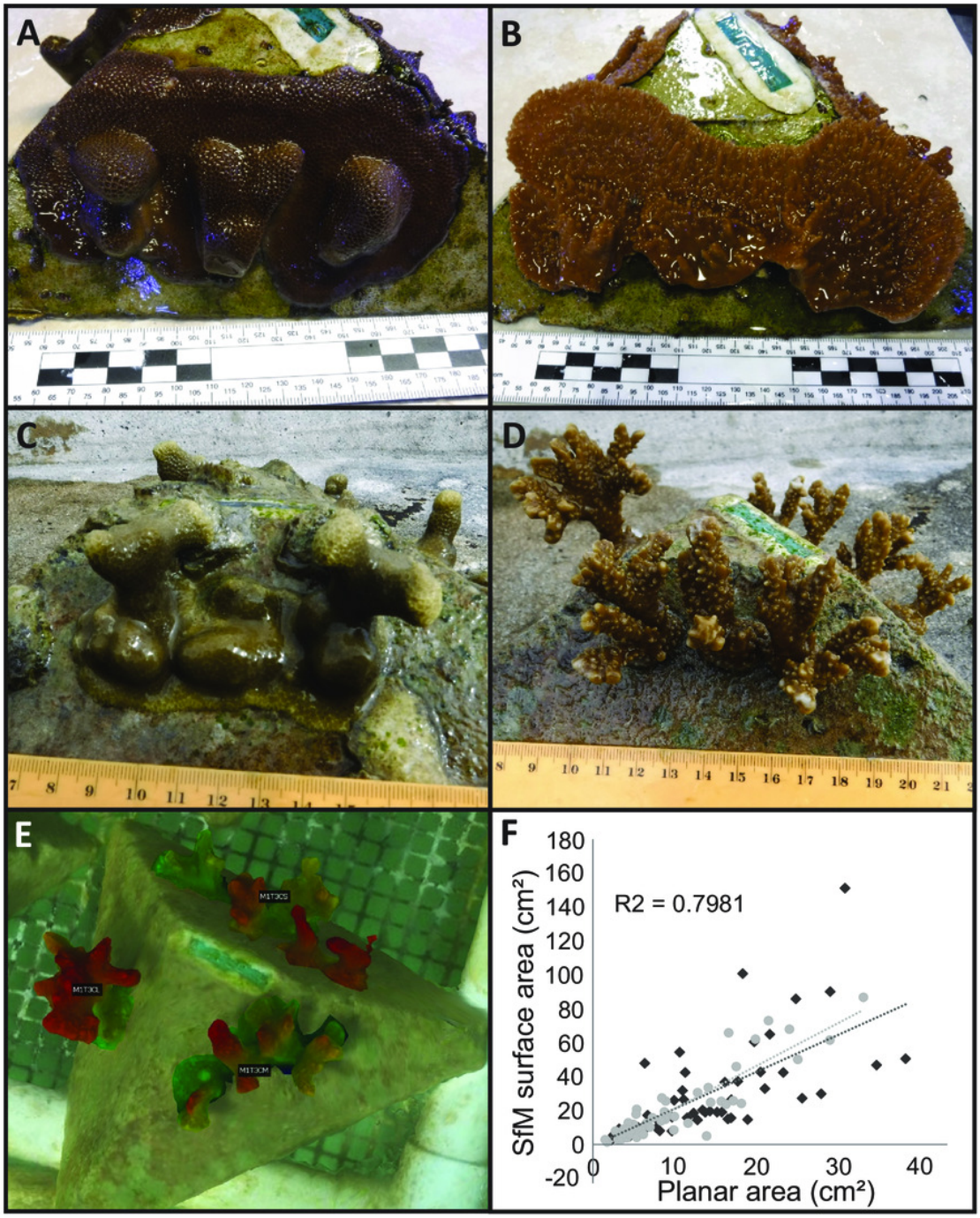

A
B
C
D
E
F
R2 = 0.7981
SfM surface area (cm²)
Planar area (cm²)
180
160
140
120
100
80
60
40
20
0
-20
0
10
20
30
40

Figure 4

Map of block assay outplanting sites within Kāneʻohe Bay, Hawaiʻi along with survivorship and growth plots.

Block assay (**A**) map of Kāneʻohe Bay, Oʻahu outplanting sites from the *in-situ* nursery (1-10), (**B**) percent survivorship (colored) and mortality (grey) of fragments after outplanting for *Montipora capitata* and *Porites compressa* across sites, and (**C**) violin plot of percent net growth across sites for *M. capitata* and *P. compressa*.

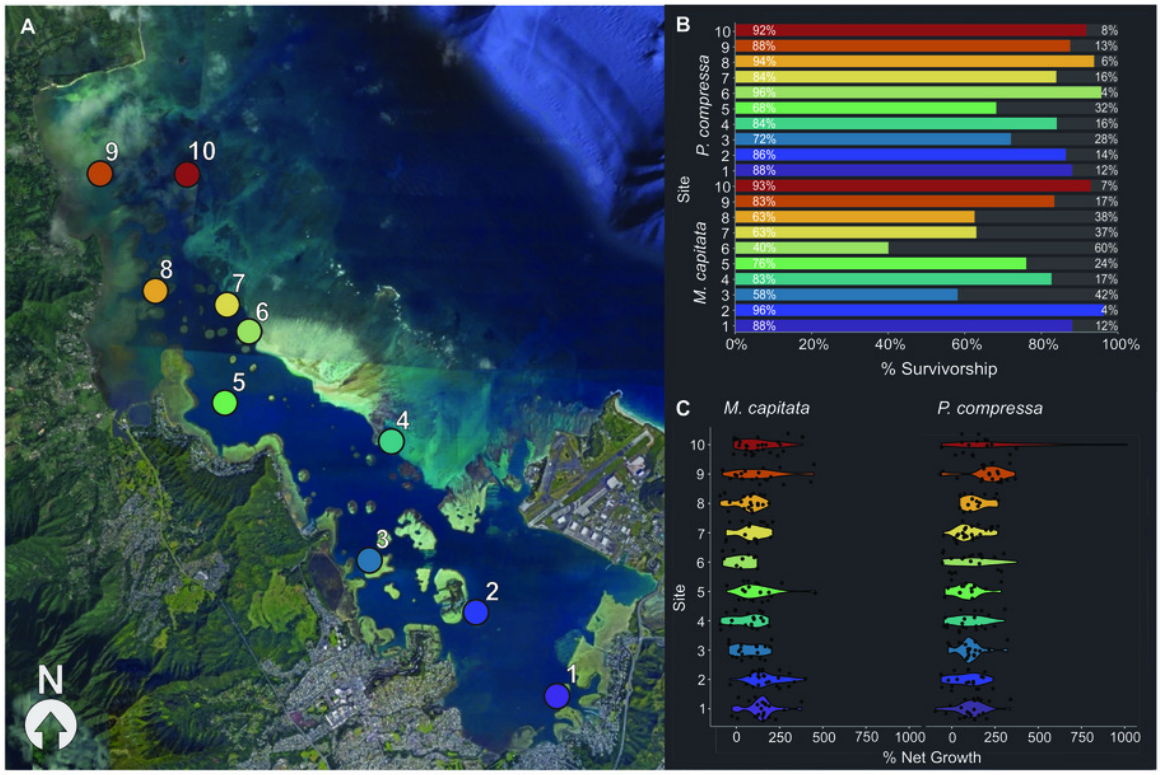


Figure 5

Example of a block assay: over time, in the *in-situ* nursery and while outplanted, along with focus on the growth of one fragment from the beginning to the end of the experiment.

Block assay design with the top row consisting of the same assay with nine *Porites compressa* fragments on top and nine *Montipora capitata* fragments on the bottom, (**A**) immediately after fragmentation, (**B**) the day of outplanting (day 112), and (**C**) the day of retrieval (day 243). Coral assays in the nursery on racks after fragmentation (**D**), an outplanted coral assay in Kāneʻohe Bay (**E**), and the same *M. capitata* fragment in imageJ used to calculate net growth (cm^2) directly after fragmentation (**F**) and then retrieval (**G**).

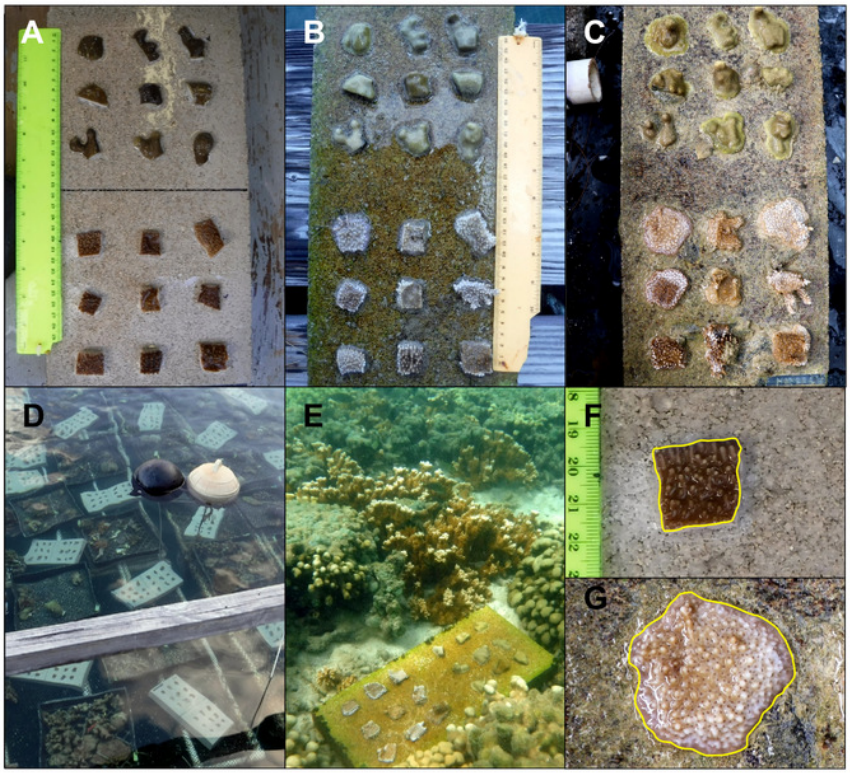
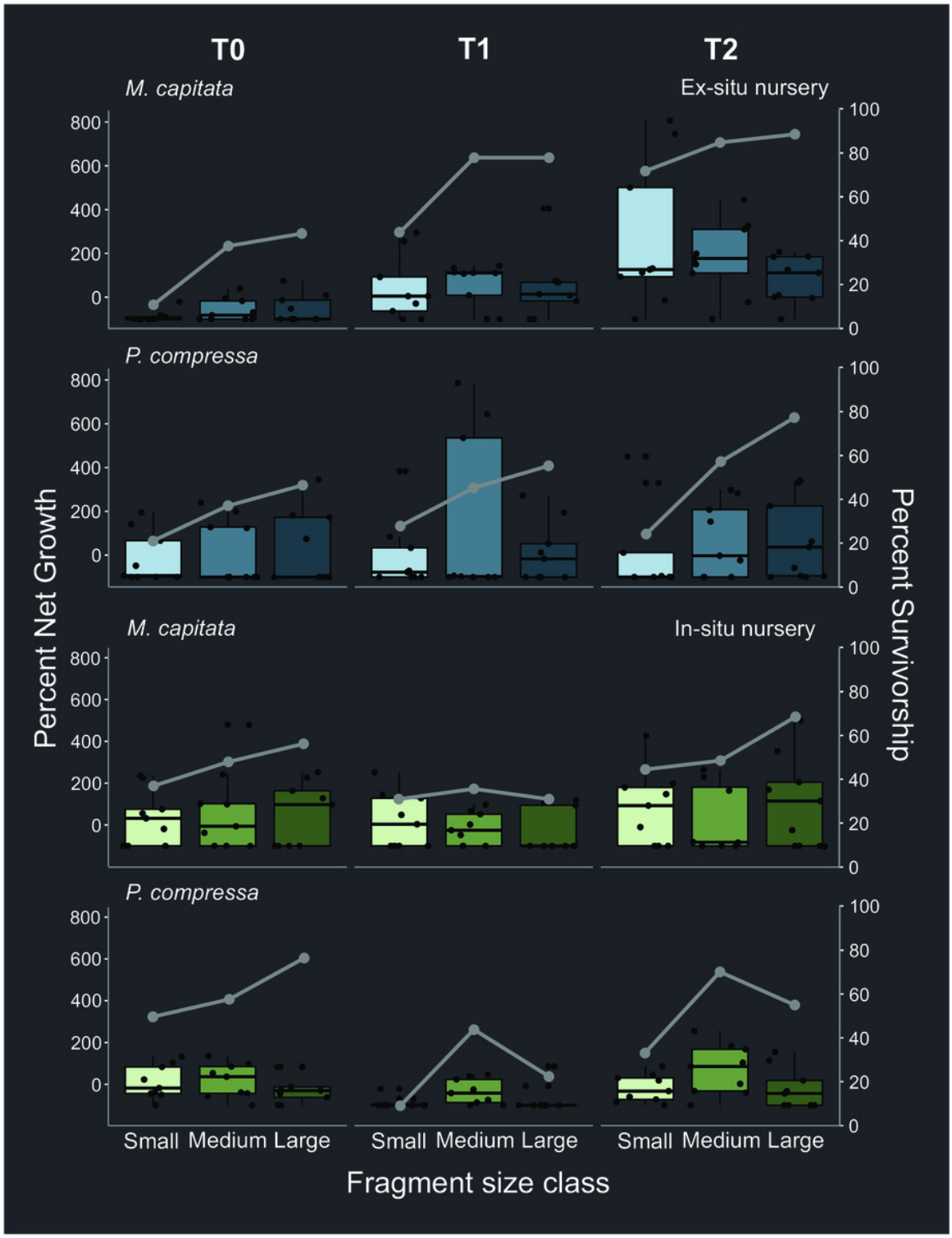

A
B
C
D
E
F
G

Figure 6

Plots visualizing pyramid assay percent net growth and percent survivorship of small, medium and large sized fragments relative to outplanting time for *Montipora capitata* and *Porites compre*ssa housed at the in-situ and ex-sit

Pyramid assay percent net growth (boxplots) and percent survivorship (lines) of *Montipora capitata* and *Porites compressa* by fragment size (small (≈ 1cm^2), medium (≈ 3cm^2) and large (≈ 9cm^2)) at the end of the experiment based on the time (T) they were outplanted (T0, 1, and 2), which were either 0, 111, and 254 days for the *ex-situ* nursery (in blue), or 0, 116 and 250 days for the *in-situ* nursery (in green).


T0
T1
T2
M. capitata
Ex-situ nursery
P. compressa
M. capitata
In-situ nursery
P. compressa
Percent Net Growth
Percent Survivorship
0
200
400
600
800
0
20
40
60
80
100
Small Medium Large
Small Medium Large
Small Medium Large
Fragment size class