

Microfragmenting for the successful restoration of slow growing massive corals

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ABSTRACT

Slow growing, massive stony corals have often been overlooked in reef-restoration activities, despite their resilience to climate change and contribution to reef framework. Techniques to effectively propagate and outplant these species have proven challenging. However, advancement in methodology may increase rates of success. In 2013, *Orbicella faveolata* and *Montastrea cavernosa* fragments were outplanted on reefs in the Florida Keys at a nearshore and offshore location, to determine whether “microfragmenting” corals, the process of creating ~1 cm² fragments, increased outplant survival and growth compared with larger fragments (16–64 cm²).

Arrays of eight microfragments were planted near one larger fragment of similar size at each location. Six replicate pairs were haphazardly placed within each ~700 m² study site. Fragments at both sites were monitored for growth and survival over 31 months, spanning two bleaching events. Initial predation occurred on microfragments, but was absent in the larger fragments. Survival and growth differed between sites, but did not differ between the larger fragments and microfragment arrays. However, excluding plots with > 40% predation at the nearshore site showed that *O. faveolata* microfragment arrays produced 10 times more tissue than traditionally used larger fragments. Results from this study suggest that if predation events are reduced, massive corals can be successfully grown and outplanted for restoration purposes.

The global decline of coral reefs is a well-documented phenomenon causing concern worldwide. Both local and global stressors are responsible for these declines and though significant efforts to reduce local pressures have occurred (Crosby et al., 2002), global impacts such as human induced climate change continue unabated. Coral bleaching, caused by the expulsion of symbiotic algae under extended thermal stress, has caused mass mortality worldwide since the 1980's (Heron et al., 2016). Increasing ocean temperatures have, and will continue to stress coral reefs (Pandolfi et al., 2011; Hoegh-Guldberg, 2007), and even if anthropogenic carbon is significantly reduced now our oceans will continue to be effected for decades to come (Pandolfi et al., 2011; Hoegh-Guldberg, 2007). Despite this, significant investment in coral restoration has occurred as reef degradation, such as in the Florida Keys, is widespread. However, species used in these restoration efforts typically represent a narrow subset of genera chosen primarily for ease of proliferation and not on performance under stress conditions (Edwards and Clark, 1999). Considering future climate scenarios, a restoration plan once focused on past conditions should become more forward looking, utilizing corals robust to climate stress (Rinkevich,

2015).

Recently, the coral gardening concept (Rinkevich, 1995; Rinkevich, 2005; Epstein et al., 2003) has become a viable coral reef restoration tool. This technique propagates corals using *in situ* coral nurseries with small amounts of wild collected stock. These corals are fragmented into small pieces and allowed to grow in size. Once grown, corals are either refragmented or are planted onto degraded reefs and monitored for growth and survival. Many studies have reported excellent initial results in both the nursery (Herlan and Lirman, 2008; Levy et al., 2010; Shaish et al., 2008) and planting phase (Hollarsmith, 2012; Putschim and Thongtham, 2008; Shaish et al., 2010). However, these efforts are rarely monitored for periods over one year and have disproportionately focused on a few genera of fast growing, “weedy species” (Shaish et al., 2010). These species are chosen because they fragment readily, have fast growth rates, and cover large areas in short periods of time (Shaish et al., 2010; Harriott and Fisk, 1988; Bowden-Kerby, 2008). Unfortunately these desirable traits are often linked to species with high susceptibility towards thermal stress events (Loya, 2001; Lirman, 2011; McClanahan, 2004), which are predicted to increase in frequency

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(Hoegh-Guldberg, 2007). Therefore, restoration efforts have been subject to significant critique, with many concluding that efforts should focus on building resistant reefs rather than recovery alone (Rinkevich, 2015; Côté and Darling, 2010).

Many massive corals throughout the Caribbean and Indo-Pacific, although slow growing and slow to recruit, are significant reef builders (Ginsburg et al., 2001) and resilient to thermal stress (Loya, 2001; Lirman, 2011; McClanahan, 2004). On the Florida reef tract, boulder corals are categorically less susceptible to high temperature stress than *Acropora cervicornis* (see Table 2 Lirman, 2011), the species used in most coral gardening activities. They are also resistant to local stressors, having formed inshore old growth reefs that receive higher anthropogenic stress, nutrients, and sedimentation than offshore locations (Wagner et al., 2010). However, the slow growth rate of massive corals has restricted the utility of these species in restoration (Krumholz et al., 2010). Those that have used massive corals have sourced material from other reefs, utilizing few large fragments (Ortiz-Prosper, 2001; Kaly, 1995) rather than mass propagating new individuals within a nursery setting (Ortiz-Prosper, 2001; Kaly, 1995; Monty, 2006), severely limiting the scale of such projects. Similarly, coral gardening has struggled to produce substantial growth and survival in massive coral species (Shafir and Rinkevich, 2010). Despite this severe bottleneck, massive corals show promise for restoration, due to high stress tolerance, and high survival rates achieved in early transplant work (Ortiz-Prosper, 2001; Clark and Edwards, 1995).

Mote Marine Laboratory has propagated massive corals in a land based nursery since 2006. Originally, Mote created ~6 cm² (or greater) fragments and grew them to a size measuring 16–64 cm² (Berzins et al., 2008) (larger fragments). These larger fragments were similar in diameter to fragments used in past transplant studies (Ortiz-Prosper, 2001; Kaly, 1995). However, a new technique has been developed for the proliferation of massive corals called microfragmentation (Page, 2013; Page and Vaughan, 2014). Microfragments are cut to ~1 cm² or less and grown to ~6 cm² prior to outplanting. This method may be amenable to restoration at scale as 6 microfragments are generated using the same broodstock material as 1 larger fragment, while having comparable survival in culture (Page unpublished data). Additionally, microfragments can be planted in arrays of the same genotype to span large areas of dead framework (as in Forsman et al., 2015), larger fragments of similar total size have a more compact footprint.

Though microfragments are prolific in culture, to be useful in large scale restoration they must demonstrate significant gains in coral cover, longterm persistence, and perform as well as larger fragments sourced from neighboring reefs. Survival bottlenecks may differ between fragment types as microfragments are smaller in size (Okubo et al., 2007). Differences may be due to limited resources for adaptation and recovery (Smith and Hughes, 1999) or consumption by predators (Jayewardene et al., 2009). However prior to being placed on the reef, microfragments are raised in ideal conditions, apart from predators and competition which may provide an advantage compared to field colonies (Horoszowski-Fridman et al., 2011). Alternatively, larger fragments acclimated to site conditions may forego excess initial predation or other consequences due to acclimation (Horoszowski-Fridman et al., 2015).

The present study tested the utility in restoration of renewably propagated massive corals using two different propagation techniques; in situ culturing of larger fragments compared with arrays of mass produced microfragments. The objectives were to i) compare survival and change in surface area (growth) after planting both microfragment arrays and larger fragments at two locations ii) identify sources that may be limiting success of planted corals (outplants) and, iii) monitor growth and survival for over two years to determine whether outplants persist longterm. The authors hypothesized that microfragment arrays would outperform single larger fragments.

1. Methods

1.1. Experimental design

Phenotypically diverse broodstock colonies of *Orbicella faveolata* and *Montastrea cavernosa* were collected in 2006 from the NOAA rescue nursery, a shallow (3 m) and turbid site located in Key West, FL. These colonies were maintained at Mote Marine Laboratory in Summerland Key. In 2010, larger fragments were cut from a subset of these colonies using a seawater-cooled tile saw (MK 101 Pro Series, MK Diamond Products inc.). Fragments were then mounted to cement bases 5–8 cm in diameter using underwater epoxy (Allfix, Cir Cut Corporation).

Microfragment arrays were cut from a separate, non-overlapping subset of these broodstock in 2012. Colonies were cut into ~1 cm² segments using a seawater-cooled diamond band saw (C-40, Gryphon Corporation). Care was taken to minimize handling and to remove excess skeleton on the bottom of the fragment, so that tissue would mount flush to artificial bases. Fragments were attached to 6.25 cm² travertine tiles (Travertine Mesh Mounted Mosaic Tile, MS International) with cyanoacrylate gel (BRS extra thick super glue gel, Bulk Reef Supply) and allowed to encrust over mounts.

Once cut, both fragment types were grown in separate, 340 L raceways fed by seawater at 2.5 lpm, sourced from a 24 m deep seawater well. Salinity was maintained at 35–37 ppt and temperature ranged with season from 22 to 27 °C. Four air stones (3 cm each) were used for water circulation and aeration within each raceway. Algae was controlled by daily siphoning and grazing by *Batillaria minima* and *Lithopoma tecta*. Raceways were covered by a canopy lined with 40% shade cloth. Conditions in raceways were high light and low turbidity. Photosynthetically active radiation during the day ranged from ~60 to 700 μmol m⁻²s⁻¹ (ModelQMSS-E, ApogeeInstrumentsInc.) peaking during midday and varying with season.

At the time of outplant, the living tissue present per larger fragment averaged 55.6 ± 18.4 cm² for *O. faveolata* and 45.4 ± 17.4 cm² for *M. cavernosa*. This was measured by calculating half the surface area of an ellipsoid as larger fragments were dome shaped. These fragments were grown for 1–2 years in the land-based nursery prior to being secured to cinder block mounts in 2011. Blocks were located both adjacent to this study's nearshore site, and 1 mile southwest of the offshore site (24.56249° N and 81.40003° W). These corals were allowed extended acclimation to field conditions before use in this study to mimic transplant work, which sources material from neighboring reefs. Microfragments were grown for 6–12 months on land prior to outplanting at study sites. At the time of outplant, *O. faveolata* and *M. cavernosa* microfragments, were 4.6 ± 1.7 cm² and 4.3 ± 1.7 cm² respectively, measured by quantifying horizontal surface area as microfragments were flat.

In May 2013 a total of 12 larger fragments and 96 microfragments per species, in apparent robust health were outplanted at a nearshore and an offshore site (Fig. 1). Outplant sites were chosen because they represented two different, yet common, reef types within the lower Florida Keys. The nearshore site was characterized by a depth of 3 m, turbid, and a substrate of dead massive corals, which perished from a 2010 cold event (Lirman, 2011). The offshore site was 6 m deep, and the substrate consisted of cavernous, dead coral pavement. These conditions are consistent with those characterized previously for nearshore and offshore reefs in the lower keys (Wagner et al., 2010; Szmant and Forrester, 1996).

At each site, 48 microfragments and 6 larger fragments were planted of each species. Microfragments were divided into 6 groups consisting of 8 replicates from the same broodstock colony. Each group of microfragments was planted onto dead reef substrate in an array 30 cm in diameter (Fig. 1). Microfragments in each array were planted approximately equidistant (~9 cm apart), and adjacent to each array (within 0.50 m) one larger fragment of the same species was also planted (Fig. 2). The bases of both larger fragments and microfragments



Fig. 1. The Nearshore site is located approximately 200 m offshore of Cook island near Big Pine Key at 3 m depth. The Offshore outplant site is located nearly 11 km offshore at 6 m depth.

were attached to dead reef using underwater epoxy (Allfix, Cir Cut Corporation). Care was taken to form a gentle slope between the coral fragment and reef substrate with epoxy.

Six array/larger fragment plots of *O. faveolata* and *M. cavernosa* were outplanted for each species at the nearshore site. The combined living tissue of *O. faveolata* in each array measured $41.8 \pm 7.1 \text{ cm}^2$, while larger fragments measured $58.1 \pm 14.6 \text{ cm}^2$. The combined living tissue of *M. cavernosa* in each array measured $36.4 \pm 9.2 \text{ cm}^2$, while larger fragments measured $34.4 \pm 13.9 \text{ cm}^2$. Fragment plots were outplanted on dead coral skeleton of the same species and were located haphazardly within a 700 m^2 area.

Array/larger fragment plots were arranged offshore, similarly to nearshore plots. Six array/larger fragment plots of *O. faveolata* and *M. cavernosa* were outplanted for each species. The combined living tissue of *O. faveolata* in each array measured $33.0 \pm 4.5 \text{ cm}^2$, while larger fragments measured $53.2 \pm 22.8 \text{ cm}^2$. The combined living tissue of *M. cavernosa* in each array measured $31.2 \pm 9.8 \text{ cm}^2$, while larger fragments measured $56.4 \pm 13.4 \text{ cm}^2$. Plots were scattered haphazardly within an 800 m^2 area. The ‘growth’ of microfragment arrays and larger fragments was compared by determining the change in surface area, using Sigma Scan Pro 5.

The surface area of each fragment type was quantified from top down photographs with 6.25 cm^2 tiles included for size reference (Fig. 2) as change in surface area occurred primarily across horizontal dead reef substrate. For microfragments, surface area was calculated by summing the living surface area of the entire microfragment array at the initial time point and subtracting it from the sum of the surface area of the final time point. To determine change in surface area of each

larger fragment the initial surface area of the larger fragment was subtracted from the surface area of the final time point. Finally, the change in surface area was divided by the initial tissue present for each array or larger fragment to account for variability between the initial size at outplanting.

Tissue loss associated with parrotfish, butterflyfish, or snail predation was quantified for each fragment at day 9 for the offshore site, and day 10 for the nearshore site (Fig. 3), by comparing the amount of tissue removed to the total footprint of each fragment in photos of each array, using Sigma scan Pro 5. Predation scars were consistent with those described by Bruckner et al (Bruckner et al., 2000). Parrotfish and butterflyfish were both observed sampling microfragments during initial outplant.

1.2. Initial growth comparisons

High mortality rates in the offshore location limited the offshore site analysis beyond the first 167 days. Additionally, omission of photographs of larger fragments taken on day 0 made them unusable for data collection. Therefore, to determine whether there were initial growth differences between arrays and larger fragments, only data collected between day 58 and day 167 was used. Kruskal-Wallis tests, with fragment type (microfragment array vs. larger fragment) as the independent variable, were used to determine differences in the overall change in surface area (i.e., amount of tissue created or lost). The change in the surface area between day 58 and day 167 was divided by the initial surface area measured for each array or larger fragment. Analyses at the nearshore and the offshore sites were conducted independently to determine if results were consistent between the two outplant sites. A Kruskal-Wallis test was also used to determine whether the change in surface area, standardized by the initial perimeter (i.e., the surface area to perimeter ratio) significantly differed between the two outplant types.

1.3. Growth at nearshore site over 2.5 years

Similar analyses were conducted only for the nearshore data in order to determine whether microfragment arrays differed in comparison to larger fragments over the entire 2.5 year study period. Kruskal-Wallis tests were used to determine whether there were differences in the overall long-term change in surface area between microfragment arrays and larger fragments, again, standardized by the amount of initial surface area of each array or larger fragment. The change in long-term surface area was also standardized by the initial perimeter to determine whether the amount of tissue produced per initial cm of perimeter significantly differed between the two outplant types

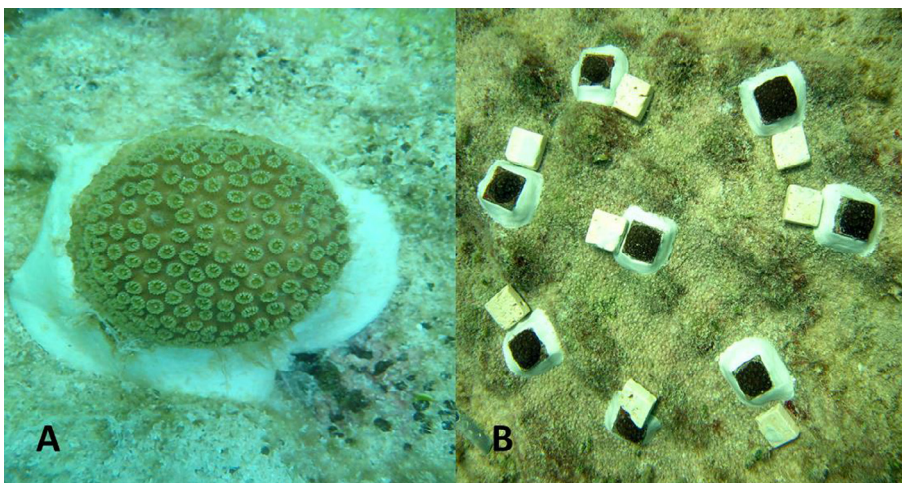


Fig. 2. Example (A) larger fragment and (B) microfragment array plot of *Orbicella faveolata*. Both fragment types were attached to dead reef using underwater epoxy. Microfragment arrays included 8 microfragments originating from the same colony. 6.25 cm^2 tiles were included in each monitoring photo to accurately quantify size throughout the study using photograph analysis software.

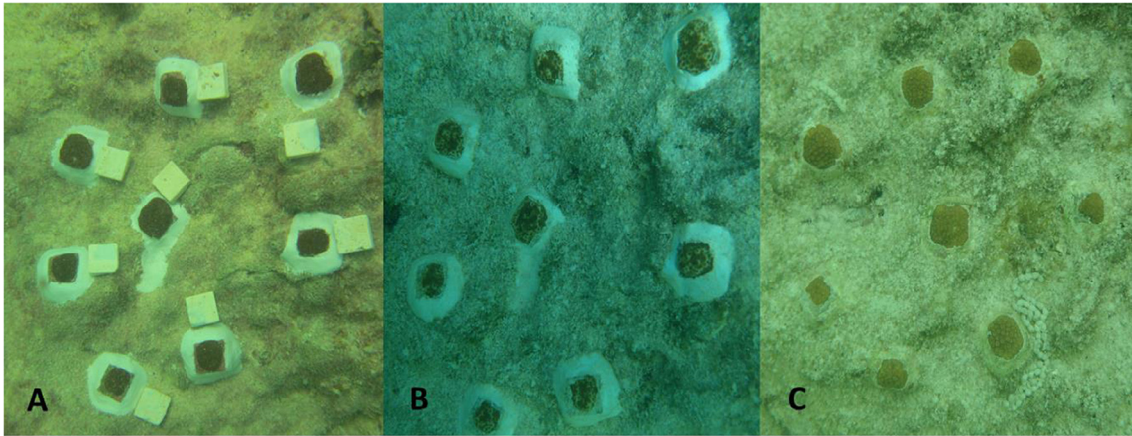


Fig. 3. During outplant (A) parrotfish and butterflyfish were observed sampling microfragment at the nearshore site, but caused no conspicuous damage. Roughly, 2 weeks after outplant (B) microfragment arrays exhibited conspicuous scouring by predators. Three months post outplant (C) microfragments appeared to have fully healed and shifted to a golden brown coloration.

2.5 years post outplant.

1.4. Survival data

Survival analysis, using a Cox proportional hazard model (Therneau, 2015) was conducted on the survival rate data to determine whether fragment type influenced overall survival. Because an array was considered the primary sampling unit, rather than each individual microfragment, arrays were considered still alive as long as one microfragment contained living tissue. A logistic regression was used to determine whether the amount of predation on each fragment effected the overall survival of each individual microfragment.

1.5. Maximum growth potential

Finally, a Student's *t*-test was used to determine whether there were differences between the maximum growth potential of *O. faveolata* microfragment arrays and larger fragments for only the nearshore pairs that did not experience heavy predation effects (i.e., < 40% loss; see below). Again, the data were standardized by the initial surface area of each fragment or array, and then by total initial perimeter to determine if there was a difference in the amount of tissue produced by fragment type per initial cm of perimeter. All statistical analyses were conducted using the program R (R Core Team, 2017).

1.6. Bleaching susceptibility

During the 2.5 years of the present study, two significant bleaching events occurred. The first was recorded in summer of 2014 and the second in summer of 2015, both of which were a result of prolonged levels of high sea water temperatures (Heron et al., 2016; Pandolfi et al., 2011). During these two time periods the peak level of bleaching of each microfragment array and larger fragment was quantified from top down photographs using a rank system from 1 to 5, where 5 represented full coloration in the fragment and 1 represented a completely white fragment, devoid of coloration. Since the data was ordinal, non-parametric Kruskal Wallis tests were used to determine whether there were differences in bleaching susceptibility between species, between years and between fragment types. A bonferoni correction was used to determine levels of significance with these multiple comparisons ($p < 0.017$).

2. Results

2.1. Initial growth comparisons

At the nearshore site, arrays of both *O. faveolata* and *M. cavernosa* grew significantly more surface area per initial cm^2 outplanted within the initial 167 days compared with larger fragments (*O. faveolata*: $X^2 = 8.308$, $df = 1$, $p = 0.004$; *M. cavernosa*: $X^2 = 7.5$, $df = 1$, $p = 0.006$; Fig. 4A,B). There was also significantly more living surface area created per cm of perimeter for *O. faveolata* at the nearshore site ($X^2 = 6.56$, $df = 1$, $p = 0.010$; Fig. 5A). The same trend was evident for *M. cavernosa*, but the data was not significantly different ($X^2 = 0.884$, $df = 1$, $p = 0.347$; Fig. 5B).

At the offshore site, arrays of both *O. faveolata* and *M. cavernosa* showed a greater loss of surface area (i.e., suffered more partial tissue mortality) compared with larger fragments although this was only significant for *O. faveolata* (*O. faveolata*: $X^2 = 6.564$, $df = 1$, $p = 0.010$; *M. cavernosa*: $X^2 = 2.7$, $df = 1$, $p = 0.100$; Fig. 6A,B). There was no statistical difference in the change in surface area per perimeter ratio for either species at the offshore site (*O. faveolata*: $X^2 = 3.103$, $df = 1$, $p = 0.078$; *M. cavernosa*: $X^2 = 0.641$, $df = 1$, $p = 0.423$).

2.2. Longterm growth at nearshore sites over 2.5 years

The average change in surface area in nearshore colonies showed that microfragment arrays produced up to 6.5 times more tissue of *O. faveolata* (microfragment array: $2.62 \pm 0.87 \text{ cm}^2$ of additional tissue per initial cm^2 ; larger fragments: $0.41 \pm 0.06 \text{ cm}^2$ of additional tissue per initial cm^2) and 2 times more tissue for *M. cavernosa* (microfragment array: $1.02 \pm 0.48 \text{ cm}^2$ of additional tissue per initial cm^2 ; larger fragment: $0.40 \pm 0.08 \text{ cm}^2$ of additional tissue per initial cm^2 ; Fig. 7). However, low sample size and large variance within the array data resulted in no significant difference in the change in surface area between fragment type for either species over the 2.5 year period (*O. faveolata*: $X^2 = 2.45$, $df = 1$, $p = 0.117$; *M. cavernosa*: $X^2 = 0.103$, $df = 1$, $p = 0.749$). There was also no significant difference detected between fragment types for the change in surface area standardized by the initial length of the perimeter (*O. faveolata*: $X^2 = 0.011$, $df = 1$, $p = 0.917$; *M. cavernosa*: $X^2 = 1.25$, $df = 1$, $p = 0.2623$).

2.3. Survival data

There were no differences in the overall rate of survival between larger fragments and microfragment arrays (*O. faveolata*: $X^2 = 0.1$, $df = 1$, $p = 0.884$; *M. cavernosa*: $X^2 = 1.6$, $df = 1$, $p = 0.199$). *O.*

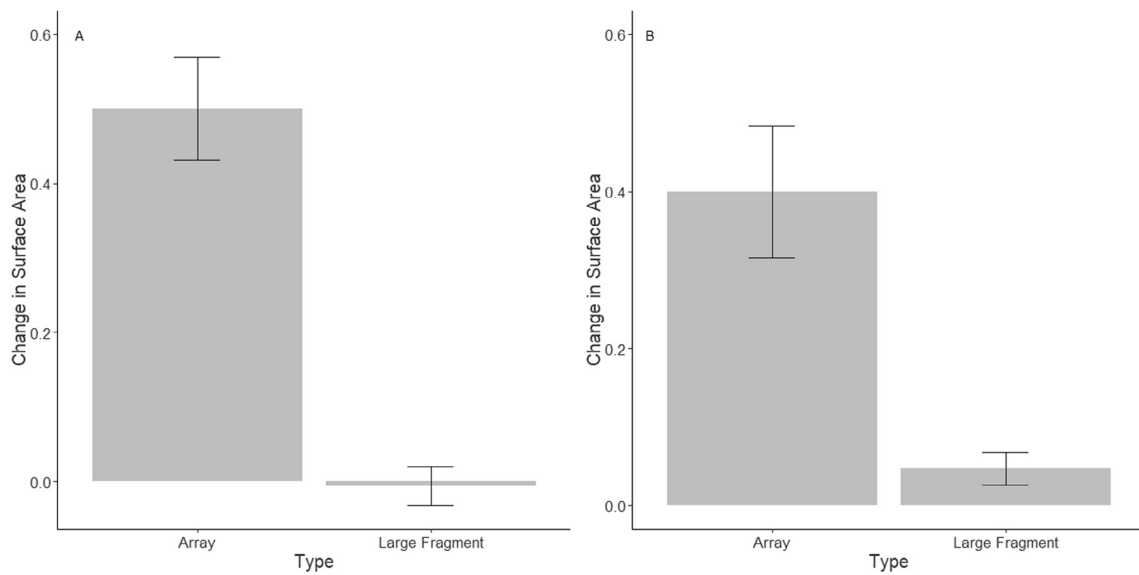


Fig. 4. Average change in surface area, per initial cm², of microfragment arrays and larger fragments of (A) *Orbicella faveolata* and (B) *Montastraea cavernosa* 167 days after outplanting within a nearshore site. Error bars represent standard error of the mean.

faveolata fragment survival rate was approximately 80% at the nearshore site and only 18% at the offshore site. Similarly, *M. cavernosa* fragments showed a 100% survival rate at the nearshore site and only a 40% survival rate at the offshore site.

N= plots not individuals so survival is of plots even if 90% of the frags were dead.
 2.4. Maximum growth potential

Heavy predation rates were observed within the offshore site compared with the nearshore site. Microfragments were more severely affected than larger fragments, suffering substantial damage for up to two weeks post outplant. One week post outplant showed that nearshore *O. faveolata* microfragments suffered 22.6 ± 22.2% damage due to predation while *M. cavernosa* suffered 0% damage. In contrast, there was little evidence of predation on larger fragments for the entirety of the study. Offshore *O. faveolata* microfragments suffered 22.2 ± 16.3% damage, while *M. cavernosa* microfragments were preyed upon more intensely, suffering 45.5 ± 16.4% damage one week post outplant. Once microfragments healed from this event (~3 months post

outplant), predation scars were not observed during subsequent monitoring events. Logistic regression showed no relationship between predation effects and survival rate within the offshore site for either coral species. There also was not a significant relationship detected between predation effects and survival rates of *M. cavernosa* at the nearshore site. However, for *O. faveolata* microfragments there was a significant influence of predation effects on survival rate for corals within the nearshore site ($Z = -3.490, p = 0.001$). When a microfragment lost over 40% of tissue from parrotfish predation there was a 0% survival rate (Fig. 8).

The maximum growth potential was also analyzed to determine if there were long-term differences between arrays and larger fragments if predation was limited. For example, when *O. faveolata* microfragments that had experienced over 40% tissue loss from predation were removed from the growth analyses (n = 2 pairs removed) the change in surface area for microfragment arrays was significantly higher than larger fragments ($t = 5.456, df = 3, p = 0.011$). The average change in surface area for microfragments was 3.38 ± 0.55 cm² per initial cm²,

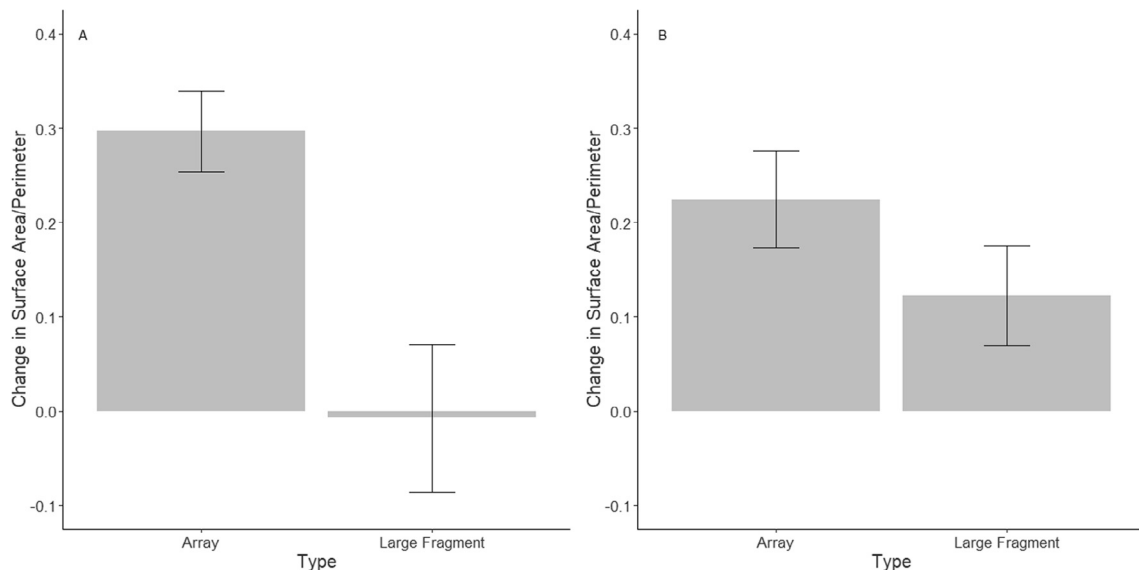


Fig. 5. Average change in surface area, per initial cm of perimeter, of microfragment arrays and larger fragments of (A) *Orbicella faveolata* and (B) *Montastraea cavernosa* 167 days after outplanting within the nearshore site. Error bars represent standard error of the mean.

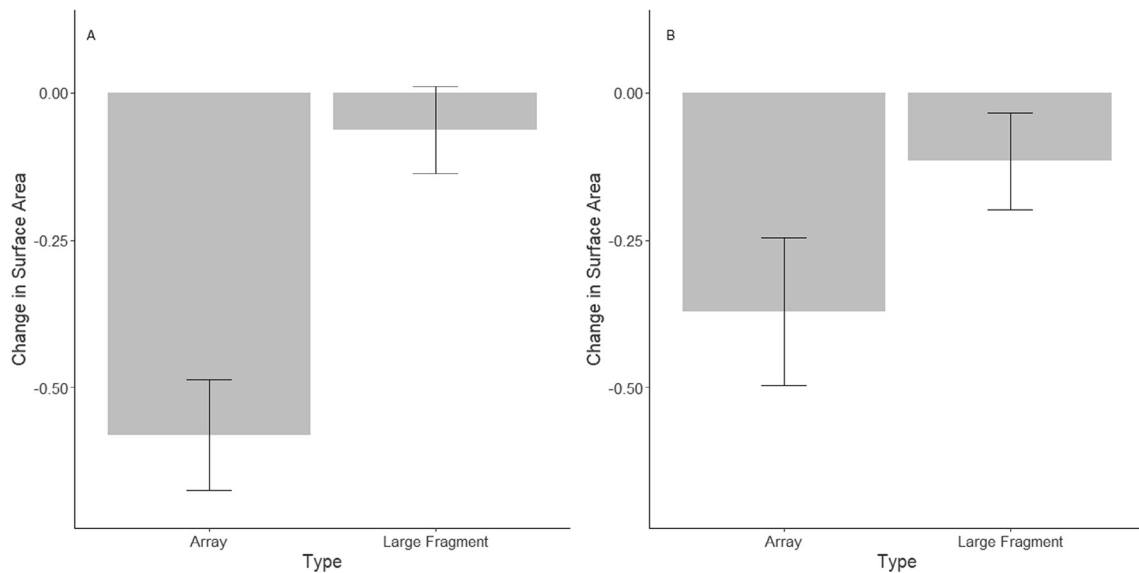


Fig. 6. Average change in surface area, per initial cm², of microfragment arrays and larger fragments of (A) *Orbicella faveolata* and (B) *Montastraea cavernosa* 167 days after outplanting within the offshore site. Error bars represent standard error of the mean.

almost 10 times more tissue than larger fragments, which only produced on average 0.35 ± 0.04 cm² per initial cm² over the 2.5 year study (Fig. 9). The amount of tissue produced per initial cm of perimeter again did not significantly differ between fragment types after the 2.5 years of outplanting ($t = 2.23$, $df = 3.6$, $p = 0.098$)

2.5. Bleaching susceptibility

Montastraea cavernosa was significantly more susceptible to bleaching with an average color index of 2 compared with *O. faveolata*, which had an index of 3 ($X^2 = 7.976$, $df = 1$, $p = 0.005$). There was no evidence of a difference in susceptibility of *O. faveolata* or *M. cavernosa* between years, suggesting these species bleached similarly in 2014 and 2015 (*O. faveolata*: $X^2 = 0.521$, $df = 1$, $p = 0.471$; *M. cavernosa*: $X^2 = 0.3313$, $df = 1$, $p = 0.069$). There was also no difference in bleaching susceptibility between fragment types of (*O. faveolata*: $X^2 = 0.016$, $df = 1$, $p = 0.900$; *M. cavernosa*: $X^2 = 0.747$, $df = 1$, $p = 0.388$).

3. Discussion

Initial growth, when standardized to initial surface area, in near-shore outplants was significantly higher for both species of microfragment arrays compared to larger fragments. This trend continued for the duration of the study; however, there was no difference between fragment types in the longterm, despite average change in surface area showing that microfragment arrays produced nearly 6.5 times more tissue for *O. faveolata* and nearly 2 times more tissue for *M. cavernosa*. This lack of significance was due primarily to high variability between arrays of each species and lack of experimental power. Distinct sources of variability include disproportionate initial predation and differences in acclimation periods between fragment types. Alternatively, differences associated with genotype may be responsible for variability in growth. However, variability in location of experimental plots and lack of replication of genets among plots precluded further exploration into its role. Survival in microfragment arrays and larger fragments was not significantly different regardless of site. Initial predation represented a

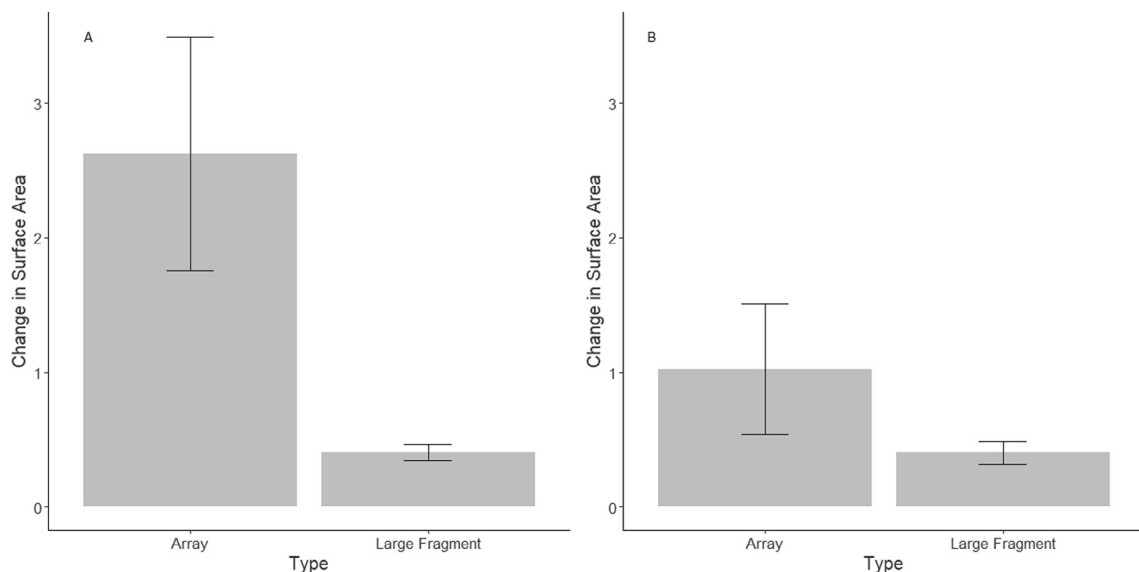


Fig. 7. Average change in surface area, per initial cm², of microfragment arrays and larger fragments of (A) *Orbicella faveolata* and (B) *Montastraea cavernosa* over the 2.5 year duration of the study. Error bars represent standard error of the mean.

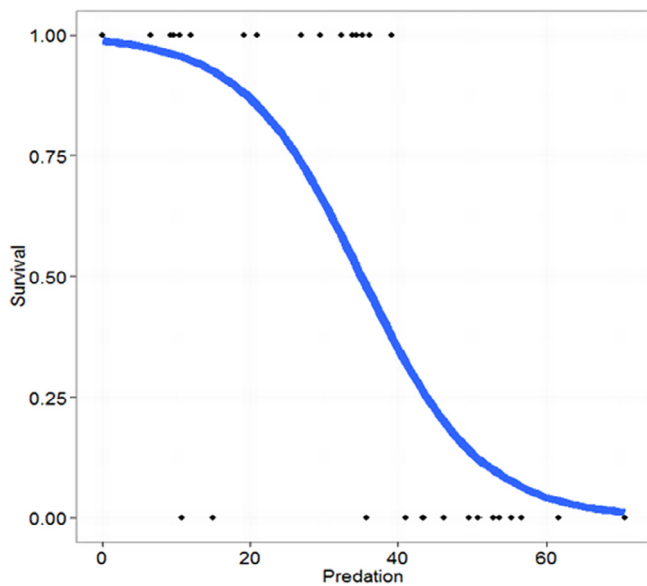


Fig. 8. Survival probability of *Orbicella faveolata* microfragments outplanted within the nearshore location under varying levels of predation. Black dots represent survival (1) or complete mortality (0) at the end of the study. The blue line represents the logistic regression line of survival probability with increasing predation effects. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

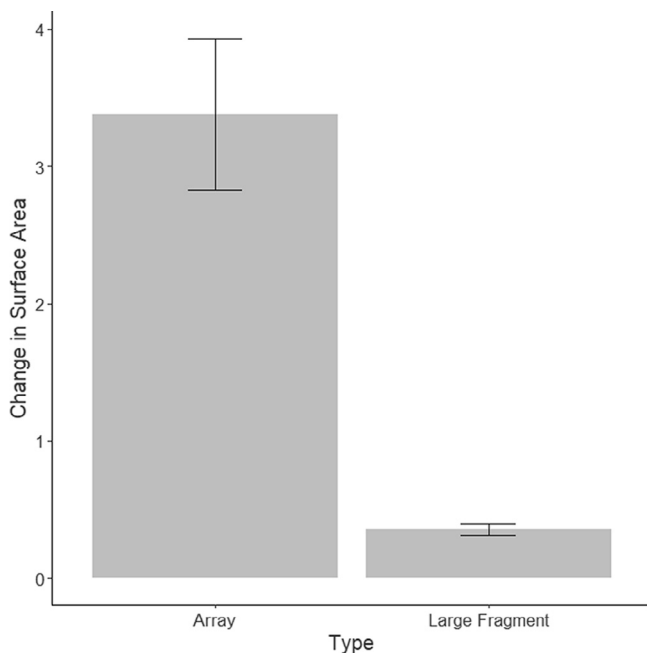


Fig. 9. Average change in surface area, per initial cm^2 , of microfragment arrays and larger fragments of *Orbicella faveolata* outplanted within the nearshore location that incurred $< 40\%$ tissue loss due to predation. Error bars represent standard error of the mean.

significant bottleneck to microfragment survival. Nearshore, *O. faveolata* suffering $\geq 40\%$ tissue loss due to predation did not survive within the present experiment. Similarly, Rotjan and Lewis (Rotjan and Lewis, 2005) found that 13% of *Porites astreoides* colonies suffered mortality after parrotfish predation was observed. Conversely, if heavily predated fragments were removed from growth comparisons between fragment types in the present study, the maximum growth potential of microfragment arrays was nearly 10 times more than larger fragments in *O. faveolata*. The rise and fall of predation on microfragments was

followed by a shift in pigmentation from dark coloration to color consistent with larger fragments, which suggests that an acclimation period may be critical when transitioning from nursery to field conditions. Horoszowski-fridman et al. (2015) found a similar spike in predation and transition in coloration in *Stylophora pistillata* after being moved from a field nursery to the reef. Additionally, the authors found that corals had lower numbers of *Symbiodinium* than nursery controls after 16 months post outplant, which may explain the observed differences in color within the present study.

There are also many inherent differences documented between captive and wild colonies, that may have contributed to differences between fragment types. Differences include calcification rates (Carlson, 2015), symbiont associations (Hartle-Mougiou, 2012), and microbial communities (Kooperman et al., 2007). Preference in corallivorous fish has been linked to superior nutrition (Bonaldo et al., 2012), difference in fouling communities (Rotjan and Lewis, 2005; Bruggemann et al., 1994), existence of chemical deterrents (Armoza-Zvuloni et al., 2016), and the clade of *Symbiodinium spp* (Rotjan and Lewis, 2006). Further research into acclimation to site conditions before outplanting may serve to increase long-term survival.

There are likely different factors that contributed to the increased growth rates recorded in nearshore microfragment arrays. In the present study, longterm growth, when standardized to initial perimeter, was not significantly different between outplant types, despite microfragment arrays having an initial perimeter averaging 4 times larger than larger fragments. Consequently, the more perimeter an outplant type had, the larger increase in surface area occurred at this site, at least over the long-term. It is also possible that differences in growth rates are in part a result of total colony size. Goreau and Goreau (Goreau and Goreau, 1960) found that calcification rates decreased as colony size increased in *Manicia aerolata* and similarly Chadwick-Furman et al (Chadwick-Furman et al., 2000) found that growth in *Fungia granulosa* decreased linearly with increasing coral size. Finally, wound creation from initial fragmentation may influence growth rates in fragment types as well. Lirman et al (Lirman, 2010) demonstrated that shortly after wound healing *A. cervicornis* display more rapid growth rates than uncut controls. Also, Goffredo and Lasker (Goffredo and Lasker, 2008) found that after clipping, colonies of *Antilloorgia elisabethae* had a higher growth rate than expected for undisturbed colonies, 4 years after clipping. These data are consistent with the results of our initial growth analyses, which suggests that microfragments produce more tissue per initial cm of perimeter compared with larger fragments, at least within the first few months after outplanting.

An aim of this study was to monitor outplants for over 2 years, to understand whether restoration with massive corals can lead to long-term persistence and an increase in coral cover. At 381 days after outplant, 18% and 40% of *O. faveolata* and *M. cavernosa* fragments, respectively, were alive within the offshore site, while 80% and 100% of *O. faveolata* and *M. cavernosa* fragments, respectively, persisted to the end of this 2.5 year study at the nearshore site. Similarly, both species demonstrated positive growth at the nearshore site. Microfragment arrays increased in surface area an average of 262% and larger fragments 41% in *O. faveolata*, while arrays increased 102% and larger fragments 48% in *M. cavernosa*. Additionally, boulder corals were selected for this study specifically for their resilience to heat stress (Loya, 2001; Lirman, 2011; McClanahan, 2004). Bleaching was observed from August to October 2014 and again from August to October 2015, when seawater temperatures rose above the average summer maximum for an extended period of time (31 °C Manzello et al., 2007). During these events, many wild corals were severely bleached, including colonies of *O. faveolata*, *M. cavernosa*, *Pseudodiploria strigosa*, and *Colpophyllia natans*. Fragments of *M. cavernosa* outplanted in the present study also bleached during both events; however, bleaching rank did not differ between years and did not differ between outplant types. *O. faveolata* fragments did not bleach and performed significantly better than *M. cavernosa* fragments. This result is in contrast to mortality rankings in

(Lirman, 2011) for each species. Fragment types did not differ in response and fragments performed equally as well between years. Although bleaching events were different from year to year, performance did not visually decline in either species. In contrast, Grottoli (2014) observed differences in color when *O. faveolata* were subject to repeat bleaching. Additionally, the authors, found that increased association with *Symbiodinium* D1a in *O. faveolata* may be linked to resilience and acclimation to repeat bleaching stress. A similar association may be present in *O. faveolata* in this study. Finally, resistance to mortality from bleaching observed here is in sharp contrast to studies performed with more sensitive species (Shaish et al., 2010) like *A. cervicornis* (Quinn and Kojis, 2006) (personal communication Erich Bartels), the species used in most restoration projects on the Florida Reef Tract.

Propagation of stony corals through microfragmentation represents a potentially renewable source of tissue for mass restoration projects. If microfragments are planted in the right location and protected from predation, the rate of production and tissue generated far exceeds that of larger fragments. Between 2009 and 2011, Mote produced an average of 130 healthy larger fragments per year using a land-based facility. From 2013 to 2015, when Mote began cutting microfragments, an average of 3646 microfragments were produced per year. In both cases, staffing consisted of one biologist and 2 interns spending approximately 20 h a week on production and maintenance (unpublished data). If one assumes that larger fragments grew to 40 cm² in a year and that microfragments grew to 6 cm², this amounts to 4420 cm² of new tissue produced by larger fragments and 18,230 cm² by microfragments, more than 4 times the amount. Although, many have demonstrated decreased survival with decreasing size in a field setting (Okubo et al., 2007; Smith and Hughes, 1999), stable *ex situ* culture conditions and proper outplanting sites can result in high microfragment survival. The lack of difference in survival of microfragment arrays and potential for orders of magnitude higher growth in this study, suggest that producing larger massive coral fragments is inefficient, whereas microfragmentation provides the mechanisms essential for the restoration of slow growing massive corals.

It is imperative that coral restoration projects consider future climate conditions when choosing candidates for restoration, as heat stress will continue to plague reefs worldwide (Pandolfi et al., 2011; Hoegh-Guldberg, 2007). Prior to this study, restoration with boulder corals was rarely attempted despite many species being resilient to heat stress (Loya, 2001; Lirman, 2011; McClanahan, 2004) and major framework builders (Lirman, 2011; Ginsburg et al., 2001). Here we demonstrate that arrays of microfragments, renewably cultured by the thousands in a land-based facility, can outperform larger fragments, growing up to 10 times faster. Nearshore, over 80% of fragments persisted 2.5 years post outplant despite enduring two consecutive bleaching events. These findings demand greater study on these much overlooked species for the active restoration of coral reefs. Future studies, should focus on understanding proper site selection, including how to overcome deleterious effects from acclimation and initial predation, as these may predict longterm persistence. Basic studies should be undertaken to determine which species are ideal candidates considering repeat heat stress as in Grottoli et al (Grottoli, 2014) and the present study. Aspects such as colony size, the role of *Symbiodinium*, and comparative performance among species are key components to this work. Additionally, studies to determine further causes for increased growth in microfragments should be undertaken to apply microfragmenting to diverse range of species.

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Author contributions

C.P. conceived of the experiment, C.P. and D.V. conducted the experiment. E.M. analyzed the results, C.P. and E.M. wrote the manuscript. All authors reviewed and edited the manuscript.

Competing financial interests

The authors declare no competing financial interests.

References

- Armoza-Zvuloni, R., Schneider, A., Sher, D., Shaked, Y., 2016. Rapid Hydrogen Peroxide release from the coral *Stylophora pistillata* during feeding and in response to chemical and physical stimuli. *Sci. Rep.* 6, 1–10.
- Berzins, I.K., 2008. Chapter 41 Aquacultured coral and restoration. *Burgers' Zoo*, pp. 375–389.
- Bonaldo, R.M., Welsh, J.Q., Bellwood, D.R., 2012. Spatial and temporal variation in coral predation by parrotfishes on the GBR: evidence from an inshore reef. *Coral Reefs* 31, 263–272.
- Bowden-Kerby, a., 2008. Restoration of threatened *Acropora cervicornis* corals: intraspecific variation as a factor in mortality, growth, and self-attachment. In: *Proc. 11th Int. Coral Reef*, pp. 7–11.
- Bruckner, A.W., Bruckner, R.J., Sollins, P., 2000. Parrotfish predation on live coral: 'Spot biting' and 'focused biting'. *Coral Reefs* 19, 50.
- Bruggemann, J.H., Vanoppen, M.J.H., Breeman, A.M., 1994. Foraging by the stoplight-parrotfish *Sparisoma viride*. 1. Food selection in different socially determined habitats. *Mar. Ecol. Prog. Ser.* 106, 41–56.
- Carlson, B.A., 2015. Organism responses to rapid change: what aquaria tell us about nature. *Integr. Comp. Biol.* 39, 44–55.
- Chadwick-Furman, N.E., Goffredo, S., Loya, Y., 2000. Growth and population dynamic model of the reef coral *Fungia granulosa* Kluzinger, 1879 at Eilat, northern Red Sea. *J. Exp. Mar. Bio. Ecol.* 249, 199–218.
- Clark, S., Edwards, A., 1995. Coral transplantation as an aid to reef rehabilitation: evaluation of a case study in the Maldives Islands. *Coral Reefs* 14, 201–213.
- Côté, I.M., Darling, E.S., 2010. Rethinking ecosystem resilience in the face of climate change. *PLoS Biol.* 8.
- Crosby, M.P., Brighthouse, G., Pichon, M., 2002. Priorities and strategies for addressing natural and anthropogenic threats to coral reefs in Pacific islands nations. *Ocean Coast. Manage.* 45, 121–137.
- Edwards, A.J., Clark, S., 1999. Coral transplantation: a useful management tool or misguided meddling? *Mar. Pollut. Bull.* 37, 474–487.
- Epstein, N., Bak, R.P.M., Rinkevich, B., 2003. Applying forest restoration principles to coral reef rehabilitation. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 13, 387–395.
- Forsman, Z.H., et al., 2015. Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. *PeerJ* 3, e1313.
- Ginsburg, R.N., Gischler, E., Kiene, W.E., 2001. Partial mortality of massive reef building corals: An index of patch reef condition. *Florida Reef Tract.* 69, 1149–1173.
- Goffredo, S., Lasker, H.R., 2008. An adaptive management approach to an octocoral fishery based on the Beverton-Holt model. *Coral Reefs* 27, 751–761.
- Goreau, T.F., Goreau, N., 1960. I. The physiology of skeleton formation in corals. iii. calcification rate as a function of colony weight and total nitrogen content in the reef coral *manicina* *Areolata* (Linnaeus). *Biol. Bull.* 118, 419–429.
- Grottoli, A.G., et al., 2014. The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Glob. Change. Biol.* 20, 3823–3833.
- Harriott, V.J., Fisk, D.A., 1988. Coral transplantation as reef management option. In: *Proc. 6th Int Coral Reef Symp.* pp. 375–379.
- Hartle-Mougiou, K., et al., 2012. Diversity of zooxanthellae from corals and sea anemones after long-term aquarium culture. *J. Mar. Biol. Assoc. United Kingdom* 92, 687–691.
- Herlan, J., Lirman, D., 2008. Development of a coral nursery program for the threatened coral *Acropora cervicornis* in Florida. *J. Hered.* 11, 1244–1247.
- Heron, S.F., Maynard, J.A., Van Hooijdonk, R., Eakin, C.M., 2016. Warming trends and bleaching stress of the World's Coral Reefs 1985–2012. *Sci. Rep.* 6, 1–14.
- Hoegh-Guldberg, O., et al., 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318, 1737–1742.
- Hollarsmith, J.A., Griffin, S.P., Moore, T.D., 2012. Success of outplanted *Acropora cervicornis* colonies in reef restoration. In: *Proc. 12th Int. Coral Reef Symp.* Cairns, Aust. 9–13 July 2012, pp. 5.
- Horoszowski-Fridman, Y.B., Izhaki, I., Rinkevich, B., 2011. Engineering of coral reef larval supply through transplantation of nursery-farmed gravid colonies. *J. Exp. Mar.*

- Bio. Ecol. 399, 162–166.
- Horoszewski-Fridman, Y.B., Br ethes, J., Rahmani, N., 2015. Marine silviculture: Incorporating ecosystem engineering properties into reef restoration acts. *Ecol. Eng.* 82, 201–213.
- Jayewardene, D., Donahue, M.J., Birkeland, C., 2009. Effects of frequent fish predation on corals in Hawaii. *Coral Reefs* 28, 499–506.
- Kaly, U.L., 1995. Experimental test of the effects of methods of attachment and handling on the rapid transplantation of corals. *CRC Reef Res. Centre*.
- Kaly, U.L., 1995. Experimental test of the effects of methods of attachment and handling on the rapid transplantation of corals. *North* 24, pp.
- Kooperman, N., Ben-Dov, E., Kramarsky-Winter, E., Barak, Z., Kushmaro, A., 2007. Coral mucus-associated bacterial communities from natural and aquarium environments. *FEMS Microbiol. Lett.* 276, 106–113.
- Krumholz, J., Barber, T., Jadot, C., 2010. Avoiding ‘Band Aid’ solutions in ecosystem restorations. *Ecol. Restor.* 28, 17–19.
- Levy, G., Shaish, L., Haim, A., Rinkevich, B., 2010. Mid-water rope nursery—Testing design and performance of a novel reef restoration instrument. *Ecol. Eng.* 36, 560–569.
- Lirman, D., et al., 2010. Propagation of the threatened staghorn coral *Acropora cervicornis*: methods to minimize the impacts of fragment collection and maximize production. *Coral Reefs* 29, 729–735.
- Lirman, D., et al., 2011. Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida reef tract and reversed previous survivorship patterns. *PLoS One* 6.
- Loya, Y., et al., 2001. Coral bleaching: the winners and the losers. *Ecol. Lett.* 4, 122–131.
- Manzello, D.P., Berkelmans, R., Hendee, J.C., 2007. Coral bleaching indices and thresholds for the Florida Reef Tract, Bahamas, and St. Croix, US Virgin Islands. *Mar. Pollut. Bull.* 54, 1923–1931.
- McClanahan, T.R., 2004. The relationship between bleaching and mortality of common corals. *Mar. Biol.* 144, 1239–1245.
- Monty, J.a., Gilliam, D.S., Banks, K., Stout, D.K., Dodge, R.E., 2006. Coral of opportunity survivorship and the use of coral nurseries in coral reef restoration. In: *Oceanogr. Fac. Proceedings, Present. Speeches, Lect.* pp. 1665–1673.
- Okubo, N., Motokawa, T., Omori, M., 2007. When fragmented coral spawn? Effect of size and timing on survivorship and fecundity of fragmentation in *Acropora formosa*. *Mar. Biol.* 151, 353–363.
- Ortiz-Prospere, A.L., et al., 2001. Planting small massive corals on small artificial concrete reefs or dead coral heads. *Bull. Mar. Sci.* 69, 1047–1051.
- Page, C.A., 2013. Reskinning a reef: Mote marine lab scientists explore a new approach to reef restoration. In: *Coral: The Reef and Marine Aquarium Magazine*, pp. 72–81.
- Page, C.A., Vaughan, D.E., 2014. The Cultivation of Massive Corals using “Micro-fragmentation” for the “Reskinning” of Degraded Coral Reefs.
- Pandolfi, J.M., Connolly, S.R., Marshall, D.J., Cohen, A.L., 2011. Projecting coral reef futures under global warming and ocean acidification. *Science* (80-) 333, 418–422.
- Putchim, L., Thongtham, N., Hewett, a., Chansang, H., 2008. Survival and growth of *Acropora* spp. in mid-water nursery and after transplanted at Phi Phi Islands , Andaman Sea , Thailand. 11th Int. Coral Reef Symp. Ft. Lauderdale, Florida 1258–1261.
- Quinn, N.J., Kojis, B.L., 2006. Evaluating the potential of natural reproduction and artificial techniques to increase *Acropora cervicornis* populations at Discovery Bay, Jamaica. *Rev. Biol. Trop.* 54, 105–116.
- R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Rinkevich, B., 1995. Restoration strategies for coral reefs damaged by recreational activities: the use of sexual and asexual recruits. *Restor. Ecol.* 3, 241–251.
- 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., 2015. Climate change and active reef restoration—ways of constructing the “reefs of tomorrow”. *J. Mar. Sci. Eng.* 3, 111–127.
- Rotjan, R.D., Lewis, S.M., 2005. Selective predation by parrotfishes on the reef coral *Porites astreoides*. *Mar. Ecol. Prog. Ser.* 305, 193–201.
- Rotjan, R.D., Lewis, S.M., 2006. Parrotfish abundance and selective corallivory on a Belizean coral reef. *J. Exp. Mar. Biol. Ecol.* 335, 292–301.
- Shafir, S., Rinkevich, B., 2010. Integrated long term mid-water coral nurseries: a management instrument evolving into a floating ecosystem. *Univ. Mauritius Res. J.* 16, 365–386.
- Shaish, L., Levy, G., Gomez, E., Rinkevich, B., 2008. Fixed and suspended coral nurseries in the Philippines: Establishing the first step in the “gardening concept” of reef restoration. *J. Exp. Mar. Bio. Ecol.* 358, 86–97.
- Shaish, L., Levy, G., Katzir, G., Rinkevich, B., 2010. Employing a highly fragmented, weedy coral species in reef restoration. *Ecol. Eng.* 36, 1424–1432.
- Smith, L.D., Hughes, T.P., 1999. An experimental assessment of survival, re-attachment and fecundity of coral fragments. *J. Exp. Mar. Bio. Ecol.* 235, 147–164.
- Szmant, A., Forrester, A., 1996. Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. *Coral Reefs* 15, 21–41.
- Therneau, T., 2015. *Package for Survival Analysis in S_ version 2.38*. Available at: <https://cran.r-project.org/package=survival>.
- Wagner, D.E., Kramer, P., Woesik, R., Van, 2010. Species composition, habitat, and water quality influence coral bleaching in southern Florida. *Mar. Ecol. Prog. Ser.* 408, 65–78.

Glossary

- Larger Fragment:** a segment of tissue severed from a stony coral colony initially measuring 6 cm² and grown to a colony size measuring 16–64 cm² prior to experimentation
- Microfragment:** a segment of tissue severed from a stony coral colony initially measuring 1 cm² or less and grown to a colony size measuring ~6 cm² prior to experiments
- Microfragment Array:** Microfragments originating from the same genotype that are spread in distinct groupings of 8 microfragments
- Ouplant:** a coral colony originating from a large fragment or microfragment that is taken from land based conditions and planted in field conditions