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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 cm^2 fragments, increased outplant survival and growth compared with larger fragments (16–64 cm^2).

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 m^2 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; Putchim 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 cm^2 (or greater) fragments and grew them to a size measuring 16–64 cm^2 (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 cm^2 or less and grown to ~6 cm^2 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 cm^2 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 cm^2 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 $m^{-2}s^{-1}$(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 cm^2 for *O. faveolata* and 45.4 ± 17.4 cm^2 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 cm^2 and 4.3 ± 1.7 cm^2 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