Title: Genotype and attachment technique influence the growth and survival of line nursery corals

Running Title: *Acropora cervicornis* line nursery

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Author contributions: ZO, DG, EG conceived the study; DG contributed project resources and funding; ZO, EG installed the nursery and monitored coral growth; ZO, EG analyzed data; EG, ZO, DG, wrote and edited the manuscript.

Abstract: The Caribbean staghorn coral, *Acropora cervicornis,* was once a dominant habitat creating coral, but its populations have declined dramatically in recent decades. Numerous restoration efforts now utilize coral gardening techniques to cultivate this species, growing colonies on fixed structures or from line/suspended nurseries. Line nurseries have become increasingly popular because of their small footprint and ease of use, replacing fixed structures in many nurseries. To evaluate the efficacy of the line technique, this study evaluated growth, condition and survivorship of *A. cervicornis* nursery colonies of three distinct genotypes grown via two line nursery techniques (suspended and direct line attachment (vertical)). Direct line

attachment of nursery colonies resulted in poor survival (43%) and growth (9.5 ± 1.33 cm/yr), while suspended culture had 100% survival and increased growth (61.1 ± 4.19 cm/yr). Suspended culture had significantly reduced disease prevalence and prevented colony predation. Suspended coral growth was also comparable to a neighboring fixed structure nursery (55.2 ± 7.86 cm/yr), and found to be as effective in propagating corals as fixed structures.

Keywords: C*oral gardening; coral restoration; floating nursery; threatened species*

Implications for practice:

1. Fixed structure nursery culture may be a better option for low budget nurseries as corals can be left to grow unmaintained for longer periods without the worry of the structure failing or sinking.
2. Diversification of culture techniques and/or nursery locations should be implemented to increase success of underperforming genotypes.
3. Nurseries should not exclude genotypes with lower productivity or survivorship, as these characteristics are not necessarily temporally or geographically consistent.
4. Nursery design should consider projections of coral growth, required harvesting in order to minimize damaging contact between overgrown colonies, and potential failures of over-weighted nursery structures.

Introduction: Coral reefs are diverse ecosystems that provide great ecological and economic value (Moberg & Folke 1999), yet they face worldwide decline driven by natural and anthropogenic impacts (Hoegh-Guldberg 1999; Hughes et al. 2003). Reef ecosystem decline has resulted in numerous efforts attempting to counteract this trend (Rinkevich 1995; Jaap 2000; Rinkevich 2005), including nursery cultivation of corals for reef restoration (Edwards & Clark 1999; Rinkevich 2000; Bowden-Kerby 2001; Lindahl 2003; Herlan & Lirman 2008; Larson 2010; Johnson et al. 2011; Young et al. 2012). In the Caribbean, losses of once-prominent *Acropora cervicornis* (staghorn coral) and *A. palmata* (elkhorn coral) have been severe (Bruckner 2002). *Acropora cervicornis'* life history characteristics (fast-growing, branching morphology, and a prominent asexual reproductive strategy) lends the species well to nursery culture (Tunnicliffe 1981; Highsmith 1982), and makes cultivating colonies via artificial propagation an attractive means of supporting restoration efforts.

Seeking potential enhancements to the efficiency of nursery operations, this study evaluated differences among multiple *A. cervicornis* genotypes grown via two popular techniques (line/floating and fixed nursery culture). With recent increased adoption of line nursery cultivation, due to ease of deployment, small footprint, and an increase in corals per unit, it is important to assess line performance against the original fixed structure technique (cinder or cement blocks) used in the Florida *A. cervicornis* nurseries (Johnson et al. 2011). Herein we will evaluate colony growth, condition and survival amongst three genotypes raised on a line nursery

and discuss how those results compare to corals raised previously on a fixed structure nursery. Based on study results recommendations for *A. cervicornis* restoration practitioners are made.

Methods: Six line nursery structures, hereafter referred to as lines, were installed at an existing *Acropora cervicornis* nursery off Fort Lauderdale, Florida in January 2011. The nursery occupied a sand channel between the nearshore ridge complex and inner reef of the region at a depth of 7 m (Walker 2012). Each nursery line (made using 3/8" polyester nautical rigging line) consisted of a 2 m horizontal line suspended 1 m above the substrate and two vertical lines (Fig. 1). Lines were secured with two helical ground anchors, and held afloat by two, 15 cm diameter buoys. Lines were parallel to each other separated by 7 m.

Each line held 24 *A. cervicornis* nursery colonies (of approximately 3 cm) for 144 total colonies. Colonies of three distinct genotypes, designated 4a, 8a, and 10a (predefined via microsatellite DNA markers (Baums et al. 2010)), were supplied from a neighboring *A. cervicornis* nursery (Larson 2010). Genotypes were selected if significant differences in growth were previously documented and adequate amounts of healthy tissue were available. Fragments with apical polyps were not used. Each genotype was replicated four times within each technique per line. Colonies were hung or attached with 18 gauge shielded wire directly to vertical lines with 10-15cm spacing (Fig. 2a & 2b). Polyp apertures faced upward for vertical colonies.

Monthly for approximately 1 year (350 days) colony survival, growth and condition were recorded. If alive, live tissue per colony was estimated (percent partial mortality). Conditions such as bleaching, disease, predation, and overgrowth were recorded as an estimated percent of the colony affected. Total linear extension (TLE) was recorded for each colony (including branches greater than or equal to 5 mm; Fig. S1) to the nearest millimeter using calipers (Larson 2010). Colonies were considered attached once tissue grew over attached wire or line.

Moderate nursery structure maintenance was conducted at each monitoring. Example maintenance included removal of hydroids overgrowing colonies, removal of fouling organisms from lines, and the rotation of suspended colonies to minimize contact. Colonies were rotated so that their growth axes were parallel to each other. Maintenance was performed evenly among lines but was neither exhaustive nor consistent among monitoring months.

Statistical analyses were performed using Statistica 13.0 © at a significance level of ± = 0.05. When data failed assumptions of normality, Shapiro-Wilks test, non-parametric tests were performed. Colony survival (chi-squared) and mean partial mortality (Kruskal-Wallis and Friedman test) were tested for technique, amongst genotype over time. Prevalence of disease was analyzed using a Kruskal-Wallis.

Colony growth was calculated by dividing the difference in TLE between monitoring events by the number of days between events including colonies that were affected by natural colony fragmentation (Larson 2010). Annual growth was calculated by dividing the difference in final colony TLE to initial colony TLE by 350. Growth was evaluated between techniques

and genotypes using Mann-Whiney U tests and within techniques and genotypes using an ANOVA with Tukey HSD post-hoc tests.

Results

Survival

By the conclusion of the project vertical colonies had significantly lower survival (43%) than suspended colonies (X^2=81.31, p<0.01); 90% of vertical colony mortality occurred during the second half of the monitoring period. Of the vertical colonies lost, the significant majority was of genotype 10a and was significantly more than 4a and 8a (X^2=8.13, p<0.05). Overall survival between genotypes was similar (pooled techniques; X^2=4.31, p>0.05; Table 1). Only one colony went missing during the experiment (vertical, 10 months in situ); therefore, both techniques were successful in terms of colony attachment.

During August and September 2011 monitoring periods, moon jellyfish (*Aurelia aurita*) were found entangled on nursery lines and were carefully removed. Recent mortality (whole colony and partial), due to colonies enveloped by jellyfish, was observed. All vertical colonies affected resulted in whole colony mortality in August (n=7) or subsequent months (n=5), no suspended colonies died.

Partial Mortality

Mean monthly vertical colony partial mortality (PM) was significantly greater than suspended for all months except July and August 2011 (Kruskal-Wallis, p<0.05; Fig. 3). Among genotypes,

10a exhibited significantly greater PM than 4a and 8a from February to May (Mann-Whitney U, $p<0.05$), and 4a in June and July (Mann-Whitney U, $p<0.05$; Fig. 3). Vertical colonies were severely impacted by hydroids and macroalgae and bivalve overgrowth and abrasion was the most frequently observed contributor to PM on suspended colonies (Fig. S2).

Disease and Predation

Rapid tissue loss (RTL) was observed on four vertical colonies in August (Fig. S3). By the following month, all four colonies (genotype 4a n=1; 8a n=2; 10a n=1) were dead. No significant difference in prevalence of disease was observed among genotypes or between techniques (Kruskal-Wallis, $p>0.05$). Predation was not observed.

Growth

After 3 months 97% of suspended and 79% of vertical colonies grew over the attachment wire. Suspended colony growth significantly exceeded vertical colony growth (Mann-Whitney U, $p<0.01$). Genotype 4a significantly outgrew genotypes 8a and 10a in both techniques (Tukey HSD, $p<0.05$; Fig. 4), and for all three genotypes the vertical colonies grew significantly more slowly than suspended (Tukey HSD, $p<0.05$; Fig. 4). Mean suspended colony growth was the fastest in August (214 days; 0.3 ± 0.03 cm/d (SE)), plateaued until the end of the year then decreased slightly (Fig. 5).

Fragment Production

Fragment production combines colony mortality and growth to represent practical nursery production. Fragment production, calculated from the difference of the final TLE to the initial

TLE per colony (percent change in colony size) divided by 5 cm provides the number of 5 cm fragments produced per nursery colony per unit time ready for either nursery expansion or outplanting. Fragment size of 5 cm was chosen as it is widely accepted by nursery practitioners as the minimum size for outplanting (Johnson et al. 2011; Griffin et al. 2012). Percent change in colony size varied with colony survival and growth, ranging between 110-1511% (Table S1). Suspended nursery colony growth after one year resulted in the highest return, 7.1 ± 0.45 SE fragments per colony which was significantly greater than vertical colony generation of 0.3 ± 0.05 fragments (Kruskal-Wallis, $p<0.05$; Fig. 6). Colonies that died (producing zero fragments) were included in this analysis to represent true fragment return, which is why it differs slightly from the growth distribution (Figs. 4 & 6). Genotype 4a colonies produced significantly more fragments than colonies of genotypes 8a and 10a (Kruskal-Wallis, $p<0.05$). Vertical colony fragment production was less than the initial colony investment per fragment for all genotypes.

Discussion: Selection of appropriate nursery culture techniques is an important decision for *Acropora cervicornis* nursery practitioners. A number of nursery techniques have been proven to be more successful than others, and herein we demonstrate that raising corals on floating structures is most successful when colonies are suspended away from direct contact of fouling organisms. Direct attachment of colonies to nursery lines resulted in significant mortality and decreased growth. Lines were installed 1 month prior to colony attachment, to assure structure stability. Minor fouling by turf algae was observed and not removed prior to attachment as we

felt that slightly fouled lines would mimic the long-term condition they would be in as part of an existing nursery. This may have contributed to increased initial partial mortality and a lower rate of colony attachment for vertical fragments. Increased vertical colony mortality in latter months when no bleaching or prominent disease outbreaks were observed, suggests mortality was a result of competition by line-fouling organisms.

Partial mortality decreased the amount of coral tissue available for nursery expansion and outplanting, and compromised colony health. The larger contact area between vertical colonies and nursery lines likely resulted in constant stress. In contrast, suspended colonies, while not completely avoiding PM, benefited by their physical separation from nursery lines and associated fouling communities. An alternative method that may increase survival while still utilizing the vertical lines is to use braided rope and insert the corals within the braid. Levy et al. (2010) attached corals with this method across three nursery designs reporting survival for branching species between 60-100%. This method reduces the portion of the colony in direct contact with the line also eliminating the need for wire. The corals used in Levy et al. (2010) were significantly larger than ours which may also contribute to increased survival (Bowden-Kerby 2001; Lirman et al. 2010).

More frequent nursery visitations should occur during jellyfish blooms. With recent jellyfish population increases (Brotz et al. 2012), such blooms may become a common occurrence. Notable jellyfish associated mortality was observed in other *A. cervicornis* line nurseries in Florida during the same season our nursery was affected (Coral Restoration

Foundation, Ken Nedimyer and Stephanie Roach, pers. comm.). Surprisingly, jellyfish entanglement was nearly non-existent on the adjacent fixed nursery less than three meters away. As such, more frequent monitoring of nursery site condition is prudent during summer months. In addition to colony mortality, the integrity of heavily stocked suspended nurseries could be compromised in high current or wave energy conditions if excessive numbers of jellyfish become entangled. This would also be of concern during algal or hydroid blooms.

The growth rate of our suspended colonies was similar to Griffin et al. (2012), but 2-times the production rate of Lirman et al. (2014) and 3-times the growth rate of O' Donnell et al. (2017). While the initial colony size in these three studies was slightly larger (4.4-5 cm) than ours, we do not feel that it is a large enough difference to cause this substantial difference in growth rates. It is possible that the difference in growth rates is partially explained by the depth of the nurseries, our nursery at 7 m was 1-3 m deeper than both Lirman et al. (2014) and O' Donnell et al. (2017), but 2-5 m shallower than Griffin et al. (2012) who found that corals grew faster at 12 m than 9m. However, it is likely that the growth differences are better explained by environmental variables such as water quality or light availability. An *A. cervicornis* reciprocal transplantation study between our nursery and an Upper Florida Keys nursery, found that corals relocated from the Keys to our nursery grew faster than the control colonies in the Keys nursery (Bliss 2015). Further supporting environmental or site factors are driving increased coral growth in Broward County.

Suspended colony growth rates in this study were comparable to growth rates on traditional fixed nursery structures in Broward County (Larson 2010), which contrasts the significantly faster growth on floating than fixed structures found by others (Lirman et al. 2014; Kuffner et al. 2017; O' Donnell et al. 2017). Three years prior to this project (2007), the same three genotypes were raised in a fixed structure nursery approximately 1 km away on the nearshore hardbottom in a depth of 5.5 m for one year (Larson 2010). Data collection methods were identical between these studies. Mean colony growth on the fixed structures (0.15 ± 0.02 SE cm/day) was similar to the suspended colonies of this study (0.17 ± 0.01 cm/day) and was dramatically greater than other published fixed nursery colony growth rates (Lirman et al. 2014; Kuffner et al. 2017; O' Donnell et al. 2017). These results may be a function of specific environmental factors associated with our fixed nursery location or general environmental factors driving faster growth rates in this region (Bliss 2015). These data, although collected at a different times, show that colonies on fixed structures and suspended from lines can have similar growth rates, and may affect nursery practitioner's structure choice. Choosing to solely use line nurseries based on the assumption that all suspended colonies have faster growth rates without evaluating the advantages of fixed structures may in fact reduce nursery efficiency. In addition, Kuffner et al. (2017) found that corals grown on floating structures had less dense skeletons than those on fixed structures, which could be problematic when outplanting back to the reef (increased colony breakage).

Based on our findings, a 15 cm minimum spacing between 3 cm colonies is appropriate for 1 year of growth to prevent damage from colony branch abrasion. Branch abrasion may have hindered suspended colony extension (growth rate plateau following August), although O' Donnell et al. (2017) reported a slower growth than ours and their colonies were suspended at a "much lower density (well over 15cm) than a practical situation" to avoid colony contact. To reduce abrasion branch grafting is a possible alternative to wider spacing and/or more frequent fragmentation. Branches of like-genotype *A. cervicornis* colonies can fuse together when grown in direct contact with one another (Gilmore & Hall 1976; Tunnicliffe 1981; Johnson et al. 2011), and suspended colonies could be fastened together to promote branch fusion, thus inhibiting mobility of neighboring colonies and reducing associated abrasion.

Genotypes considered poor or strong survivors or growers may not perform consistently in different locations or years. Overall fixed colony survival was similar to suspended colonies. However, it appears that genotypes may survive differently depending on technique. For instance, genotype 10a had low survival in the vertical orientation (29%), but experienced no mortality in the 2007 fixed structure nursery. In 2009, genotype 10a was the highest surviving genotype amongst these three genotypes when outplanted to the reef following 1 year (unpublished data). This genotype was later added (2012) to fixed nursery structures at the same site as the lines, resulting in 50% survival following 6 months (unpublished data). Genotype 4a on both the lines and 2007 fixed structures exhibited a faster growth rate than 8a and 10a. In fact, the growth rate for genotype 4a was 0.3 cm/day for both suspended and fixed colonies. The

growth rates for both 8a and 10a colonies on the fixed structures were also significantly less than 4a; however, genotype 10a grew faster suspended than fixed, whereas genotype 8a grew faster fixed than suspended.

Variability between genotypes has been studied for growth (Larson 2010; Griffin et al. 2012; Lirman et al. 2014; Lohr & Patterson 2017), bleaching (Lohr & Patterson 2017), between sites (Lirman et al. 2014), and disease resistance (Vollmer & Kline 2008). However, each study concluded that research should be extended past 1 year to determine if these traits span multiple years. Although our study did not examine the same colonies over multiple years, our data does indicate that some genotypes exhibit similar characteristics over time (e.g., genotype 4a-growth) while others are not consistent or predictable (e.g., genotype 10a-survival). Nurseries should strive to maintain the greatest genetic diversity possible, for what appears to be an optimal genotype today may not remain so tomorrow. Especially because characteristics such as relative reproductive contribution and other genetic parameters and traits have not yet been evaluated.

Predation, especially by *Hermodice carunculata*, may be greatly reduced or eliminated by suspended culture. During the duration of this study, 358 colonies on fixed structures within 3 m of the lines, were predated upon by *H. carunculata.* Prey species selection by *H. carunculata* varies with prey abundance (Berkle 2004), and as *A. cervicornis* density increased with growth, it is probable that predation would also increase, yet was never recorded on lines. Reduced predation on the line nursery was expected. In contrast to fixed structures, line

nurseries have limited predator hiding places and limited predator movement with minimal benthic habitat contact (Johnson et al. 2011; Young et al. 2012).

Suspended culture may also have reduced disease prevalence compared to fixed culture. Suspended culture may allow *A. cervicornis* colonies to better resist disease by increasing the separation of colonies from the benthos and residing up in the water column where water flow is greater. Separation of colonies from benthic biota may decrease the likelihood of disease, as benthic organisms have been shown to carry bacterial coral pathogens (Williams & Miller 2005; Miller & Williams 2007). When nursery colonies were in close proximity to benthic organisms or affected by fouling organisms the prevalence of disease was higher, 5% of vertical colonies and 3% of *A. cervicornis* colonies on neighboring fixed structures (unpublished data) were affected by disease during the time of this study.

These results and comparisons to past studies strongly indicate that *A. cervicornis* nursery colony survival and growth is affected by the habitat the nursery occupies, site environment conditions, annual environmental variability, and the nursery structure. The overall similarity in growth rates between our suspended and fixed structures is likely a function of local environment conditions promoting faster growth rates. The benefits associated with line/suspended nurseries (design versatility, efficient use of physical space and smaller benthic footprint, separation of nursery colonies from predators, etc.) make their use attractive to existing and future *A. cervicornis* nursery operations. However, colonies on fixed structures can theoretically be left to grow indefinitely and even merge or grow together, while line nursery techniques cannot support

indefinite growth (excessive colony weight, detrimental physical interaction). In many settings fixed structure nurseries may produce similar production results warranting nursery practitioners to diversify and use pilot projects to determine the best method for their region, location and genotypes.

Acknowledgements: We acknowledge funding provided by the NOAA, U.S. Department of Commerce and The Nature Conservancy under the terms of Cooperative Agreement NA09NMF4630332. Nursery installation and monitoring operations were conducted by NSU's Coral Reef Restoration, Assessment and Monitoring Laboratory. Additional thanks to Ken Nedimyer and the Coral Restoration Foundation, whose knowledge and experience were helpful in the planning and design stages of this study. The content and opinions expressed herein are those of the author(s) and do not necessarily reflect the position of the funding agencies. Research was conducted pursuant to the Florida Fish and Wildlife Conservation Commission SAL-10-1086A-SCRP.

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Table 1. Final *Acropora cervicornis* nursery colony survival by technique and genotype.

Technique	Genotype 4a	Genotype 8a	Genotype 10a	Overall
Suspended	100%	100%	100%	100%
Vertical	43%	58%	29%	44%
Overall	72%	79%	65%	

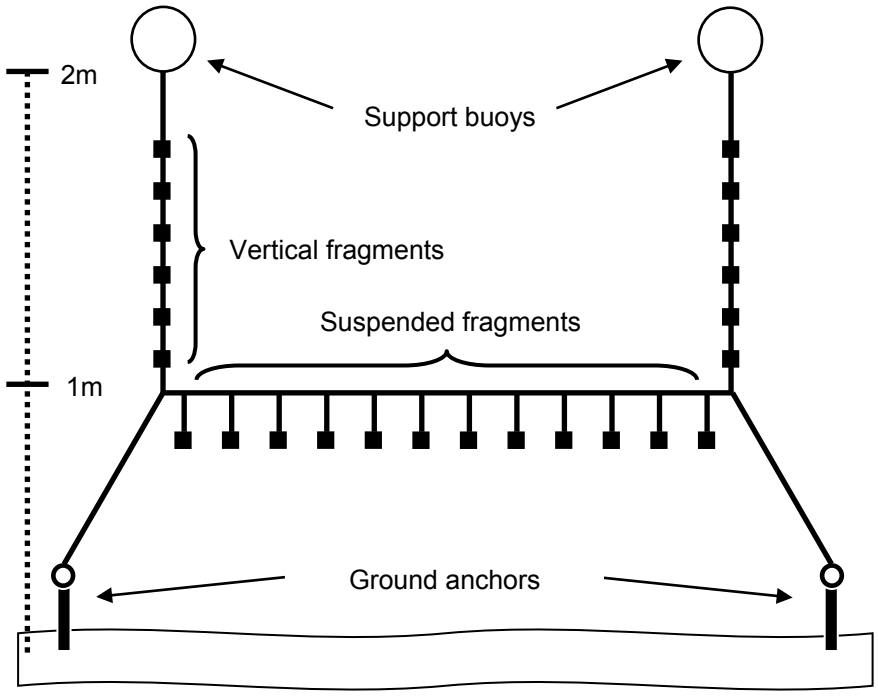

Figure 1. Diagram of nursery line. Each unit supported 12 suspended colonies and 12 vertical colonies. Fixed colonies were grown in a neighboring nursery constructed of cinder blocks.

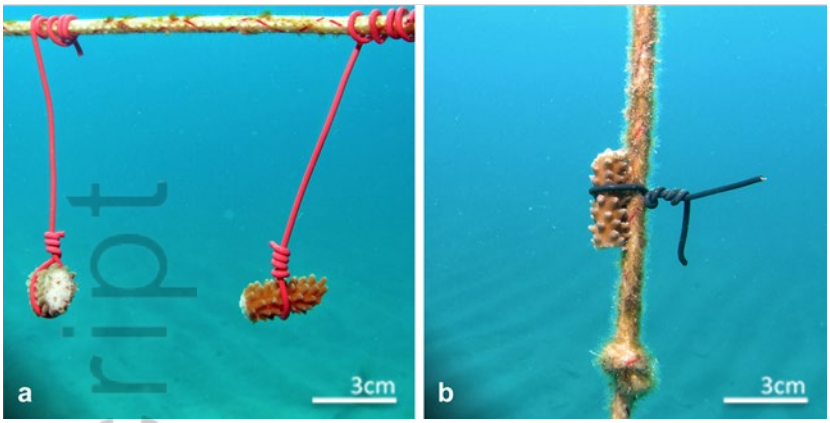

Figure 2. Newly attached *Acropora cervicornis* nursery colonies via the suspended (a) and vertical (b) techniques.

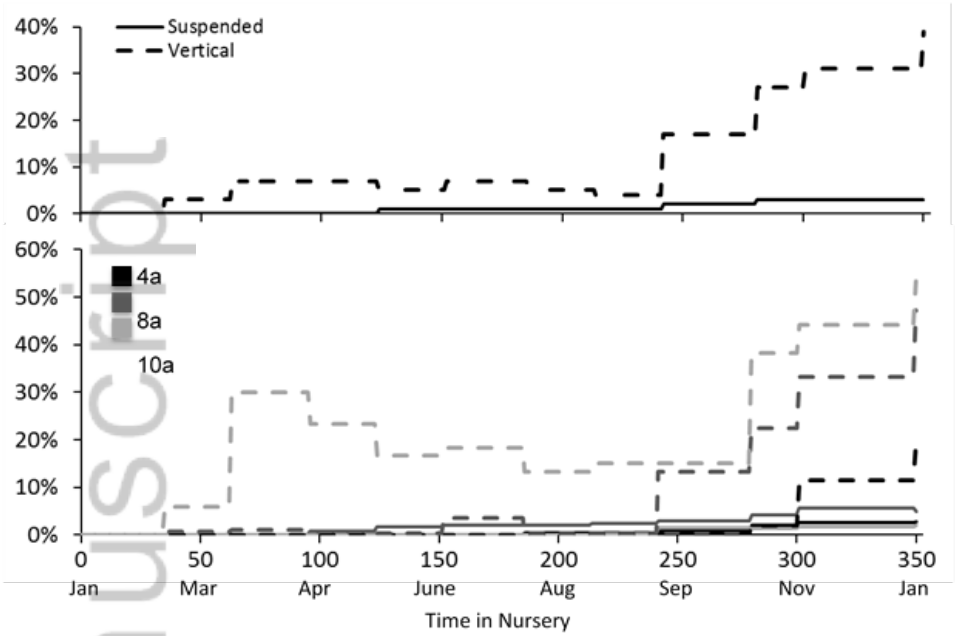

Figure 3. Mean partial mortality for suspended and vertical *Acropora cervicornis* nursery colonies (top) and by genotype within technique (bottom) over approximately 1 year. Top figure groups all genotypes together. Months below the number of days on the x-axis indicates the approximate month during which data were collected.

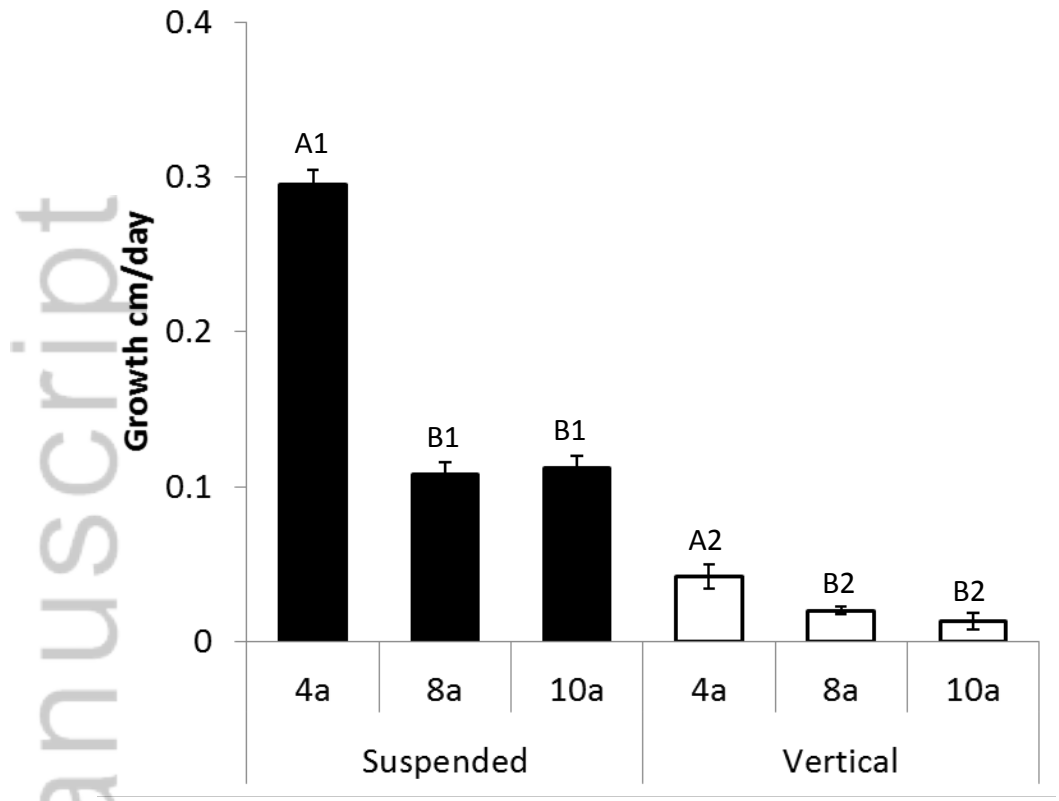

Figure 4. Mean daily nursery colony growth (± SE) by technique and genotype. Like-letters indicate statistical similarity within techniques and like-numbers indicate similarity within genotypes (Tukey HSD, ±=0.05).

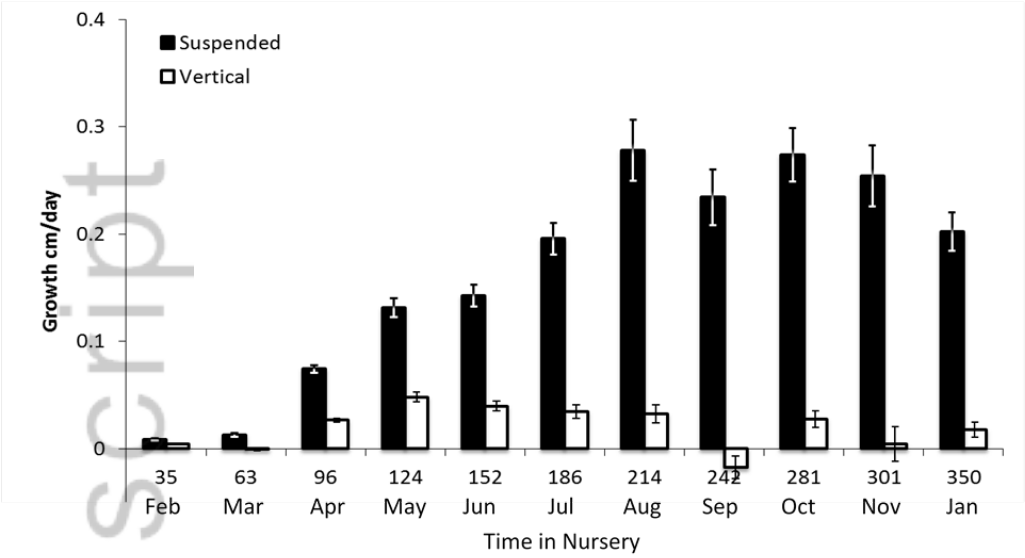

Figure 5. Mean (± SE) daily change in nursery colony size (total linear extension) across 1 year of monitoring. Rates shown were calculated for surviving colonies at each monitoring period.

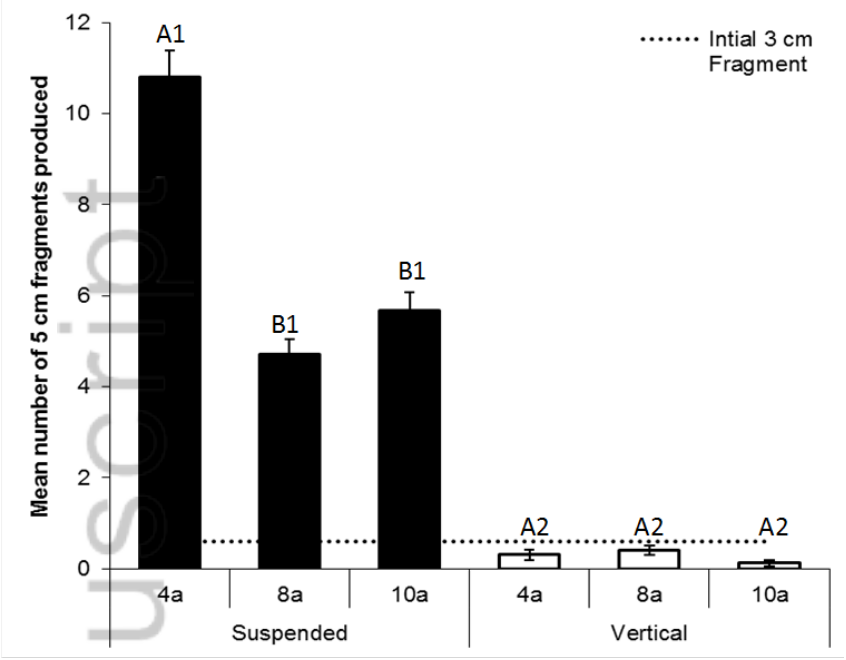

Figure 6. Mean number of 5 cm fragments produced (± SE) per colony by technique and genotype. Colonies that died (producing zero fragments) were included in this analysis to represent true fragment return. Dotted line indicates initial colony investment. Like-letters indicate statistical similarity within techniques; like-numbers indicate similarity within genotypes (Kruskal-Wallis test, ±=0.05).