

# Scaling up *Acropora* nurseries in the Caribbean and improving techniques

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**Abstract.** Over the last 2 decades, coral nurseries have evolved from small scale pilot projects to full scale production nurseries in some areas. In 2006, the T/V Margara ran- aground in Puerto Rico causing significant damage to coral reefs. Damaged fragments of *Acropora cervicornis* were safely cached in nurseries during emergency restoration. Line nurseries were eventually set up to grow additional corals for restoration. Growth rates (linear and maximum diameter) and survival of 712 colonies were monitored for one year examining the effects of genotype, depth and attachment method. The average size of each fragment when placed into the nursery was 4.4 cm. After one year, the mean annual linear growth rate was  $52.5 \pm 1.1$  cm/yr and the average maximum diameter was  $21.7 \pm 0.3$  cm. Total mortality for the year was 4.5%. Of all the materials used to attach corals to the lines, coated wire had the lowest mortality (2.6%), and cable ties had the highest mortality (12.5%). Fragments placed at deeper depths (11.2-12.4 m) had significantly higher growth rates than corals placed at shallower depths (9-10.3 m). Survival and growth rates also varied between genotypes. The line nurseries described in this study produced high survival and growth rates, required low maintenance and held up well during storm events. Given the decline in *A. cervicornis* populations in the Caribbean over the last few decades and the current focus on scaling up nurseries in the Caribbean, the results and techniques presented here are useful for the development of future nursery operations.

**Key words:** *Acropora cervicornis*, Coral nurseries, Caribbean, Line nurseries, Restoration.

## Introduction

For the past 500,000 years, *Acropora cervicornis* was one of the dominant coral reef building species in the Caribbean (Jackson, 1994). But over the last few decades, *A. cervicornis* populations have suffered a dramatic decline (>95% mortality) throughout the entire Caribbean (Aronson and Precht, 2001; Bruckner, 2002) which has led to the inclusion of this species in 2006 as “Threatened” under the Endangered Species Act. As a result of this decline, adult populations typically have low densities and genetic diversity, resulting in a reduction in genetic connectivity for this genus (Vollmer and Palumbi, 2007; Baums, 2008). *A. cervicornis* has disappeared from many reefs in Puerto Rico where they were once common (Hernández-Delgado, 2000; Weil et al., 2003). As these populations continue to decline, proactive intervention is becoming increasingly warranted (Edwards and Clark, 1998; Vollmer and Palumbi, 2007).

The life history traits of this species (fast growth rates and highly successful asexual propagation through fragmentation) make this species a prime

candidate for coral nursery programs in the Caribbean (Highsmith, 1982; Bowden-Kirby, 2008; Lirman, 2010). The purpose of coral nursery is to grow colonies in a relatively protected environment (ideally free of predators, disease, sedimentation, algae, etc.) to increase the survival and growth of the corals (Edwards, 2010). Once the corals attain a certain size, they can then be transplanted back out onto the reef or re-fragmented to expand the nursery (Rinkevich 2005). Over the last two decades, coral nursery techniques have evolved from small scale pilot projects to full scale production nurseries in some areas, and the number of nurseries has increased throughout the Caribbean using popular techniques such as line nurseries, blocks, wire mesh, and A-frames (Johnson et al., 2011). Successful coral nursery programs can be used to both increase population densities of *Acropora cervicornis* on degraded or impacted reefs as well as increase the genetic diversity of this species on various reefs to enhance successful sexual reproduction (Quinn and Kojis, 2006; Vollmer and Palumbi, 2007; Baums, 2008; Bowden-Kirby, 2008; Reyes and Schizas,

2010). Coral nurseries can also serve as a potential refuge during disease outbreaks, storms or temperature extremes or after physical impacts from storms, waves or vessel groundings (Edwards, 2010; Johnson et al., 2011).

In 2006, the T/V Margara ran- aground off the south coast of Puerto Rico causing significant damage to coral reef resources, including *A. cervicornis*. After the incident, fragments of *A. cervicornis* were placed into nurseries to safely cache the corals while emergency restoration was conducted at the site (Phase 1 of this study). Several techniques were initially used to stabilize and grow out the damaged fragments (wire mesh, concrete puddles with stakes, and line nurseries). During Phase 1, coral fragments performed best on the line nurseries. Taking advantage of the high survival and fast growth rates, additional line nurseries were set up to propagate corals during Phase 2.

The results from the 2nd phase are presented here. Growth rates (linear and max diameter) and survival were monitored in the nursery from January 2010 to January 2011 to examine the effects that genotype, depth and attachment method have on the corals. The results from this study were integral in designing the expansion of this nursery to 1,500 colonies during Phase 3 and provide useful information for nursery implementation in other areas. These colonies will eventually be used for restoration at grounding sites in the area in an attempt to accelerate recovery of the impacted areas (Rinkevich, 1995; Rinkevich 2005; Edwards, 2010).

### Material and Methods

In this study, PVC frames were used on the line nurseries to prevent the lines from sagging in the middle as the corals grew. Each line nursery had a 3 m tall by 3 m wide frame using 1.9 cm wide Schedule 80 PVC and 9 rows of monofilament line. 10 line nurseries were set up at the site with a total of 712 *A. cervicornis* fragments. There were 6 different *A. cervicornis* genotypes in this study, and each line nursery contained one genotype. Fragments were hung from the horizontal monofilament lines using a variety of materials (rubber coated wire, line, monel wire, and cable ties). Eight *A. cervicornis* fragments were hung on each line, and only one attachment method was used within each row. The attachment methods were replicated for each genotype at 0.3 m depth intervals between 9.1 m and 12.7 m deep. Line nurseries were anchored in sand and rubble channels between the reefs using helix anchors and sand screws at depths of 12–15 m. The line nurseries were held vertically by subsurface floats.

Each fragment was photographed at the start of the study (January 2010), after 5 months (June 2010) and

after 1 year (January 2011), and the depth of each row was recorded. Mortality estimates included both dead and missing colonies. Linear and maximum diameter measurements were performed using Coral Point Count with Excel Extensions version 4.0 (CPCE). Three centimeter brass clamps, used as attachment points on the monofilament lines, were used as a scale for length in each of the photographs. Measurements of maximum diameter were taken from the two furthest branch tips for each colony. To measure the overall increase in length of all branches in every direction, linear growth measurements were taken along the central axis of each branching segment of the colony similar to the methods used in several studies (Bowden-Kirby, 2001; Quinn and Kojis, 2006; Herlan and Lirman, 2008; Johnson et al., 2011). It is important to note that measurements using CPCE represent a minimum length estimate due to the possible angle of the colony when photographed or in the case of linear growth, branches that extended towards or away from the camera in the “Z” axis.

Statistical analyses proceed as follows. First, bivariate comparisons of each outcome (mortality rate, maximum diameter, and mean linear growth) were made by attachment methods, genotype, and depth. Second, multivariate regression models were fit to each outcome that controlled simultaneously for attachment method, genotype, and depth to ascertain which variables independently predicted the outcomes even after controlling for the others. Logistic regressions were used to determine if there were statistically significant differences between genotypes, depth and attachment methods for mortality and linear regression models were used to examine significant differences for maximum diameter and mean linear growth rate. All statistical analysis was done using the generalized linear model (glm) command in R.

### Molecular Analysis

1 cm<sup>2</sup> tissue samples were collected from 66 colonies for genotyping. These samples were placed in vials with 95% ethanol, stored refrigerated, and sent to Penn State University for analysis. Samples were extracted overnight using the DNeasy tissue kit (Qiagen). Genotyping of *A. cervicornis* followed Baums et al. (2005) and (2009) and included loci 166, 181, 182, 207. PCR products were visualized using an ABI 3730 (Applied Biosystems) automated DNA sequencer with an internal size standard (Gene Scan 500-Liz, Applied Biosystems) for accurate sizing. Electropherograms were analysed using GeneMapper Software 4.2 (Applied Biosystems) and alleles were scored based on amplicon size. Consistent allele scoring is paramount and was checked by two persons.

Of the 66 complete multilocus genotypes, only 5 were unique. The probability of identity for increasing

locus combinations was 0.011 (GenAlEx vers 6.41). Repeated multilocus genotypes were assigned as belonging to the same genet. The number of ramets per genet ranged from 2 to 44. Of the 5 unique genotypes identified in the lab, 4 were used during this study and were ambiguously labeled as the blue, yellow, green and brown genotype. Two of the genotypes in this study (A and B) were never analyzed in the lab so they are treated as separate genotypes.

## Results

### Average Growth and Mortality

The average size of each fragment when placed into the nursery was  $4.4 \pm 0.1$  cm. The average maximum diameter of the fragments after one year was  $21.7 \pm 0.3$  cm, and the mean annual linear growth rate was  $52.5 \pm 1.1$  cm/yr (Table 1). The monthly mean linear growth rate was significantly higher (paired T-Test;  $p < 0.0001$ ) in the last seven months ( $5.7$  cm/yr) than the first five months ( $2.6$  cm/yr). Mortality over the course of a year was  $4.5\%$ ; however mortality was higher in the first five months ( $2.7\%$ ) in comparison to the last seven months ( $1.8\%$ ). The monthly change in maximum diameter did not vary significantly (paired T-Test;  $p > 0.05$ ) between the first 5 months ( $1.3$  cm/month) and the last 7 months ( $1.5$  cm/month).

Time Period	0 – 5 Months	6 – 12 Months	One Year
Mean Linear Growth	$13.0 \pm 0.3$ cm	$39.6 \pm 1.0$ cm	$52.5 \pm 1.1$ cm
Mean Linear Growth Rate	$2.6$ cm/month	$5.7$ cm/month	$4.4$ cm/month
Maximum Diameter	$11.0 \pm 0.1$ cm	$21.7 \pm 0.3$ cm	$21.7 \pm 0.3$ cm
Change in Diameter	$1.3$ cm/month	$1.5$ cm/month	$1.4$ cm/month
Mortality	$2.7\%$	$1.8\%$	$4.5\%$

Table 1: Average mortality, linear growth, diameter and monthly growth rates for the first 5 months, last 7 months and the entire year. Standard error of the mean is included for Mean Linear Growth and Maximum Diameter.

### Bivariate results

Table 2 shows the bivariate analyses of each outcome by attachment method and genotype. As shown for attachment method, of all the materials used to attach corals to the FUCAs, coated wire had the lowest mortality ( $2.6\%$ ), and cable ties had the highest mortality ( $12.5\%$ ). Both the mean linear growth and the maximum final diameter were significantly lower for the cable tie method than all the other methods. However, there was no significant difference in linear growth (range of  $51.3 \pm 56.8$  cm/yr) between the other attachment methods (monel, coated wire and line). There was a significant difference in the final maximum diameter between using the line method ( $19.9 \pm 0.5$  cm) and monel ( $24.1 \pm 1.0$  cm). Table 2

also shows that survival (range of 94-100%) and growth rates varied between genotypes.

In terms of growth rates, the Green genotype had a significantly higher mean linear growth rate ( $90.2 \pm 4.0$  cm/yr) than all the other genotypes. Genotype A had a significantly lower mean linear growth rate ( $40.3 \pm 2.9$  cm/yr) than the other genotypes (except for Yellow). The Green and B genotypes had significantly larger maximum final diameters after one year ( $27.9 \pm 0.9$  cm and  $26.0 \pm 1.1$  cm, respectively) than the others whose values ranged from  $19.4 - 22.9$  cm.

	Mortality	Maximum Diameter (cm)	Mean Linear Growth (cm/yr)
<b>Method</b>			
Coated Wire (n=383)	2.6%	$22.7 \pm 0.4$ BC	$53.5 \pm 1.5$ B
Monel (n=72)	9.7%	$24.1 \pm 1.0$ C	$56.8 \pm 4.3$ B
Line (n=241)	8.8%	$19.9 \pm 0.5$ B	$51.3 \pm 1.9$ B
Cable Tie(n=16)	12.5%	$13.7 \pm 1.2$ A	$25.4 \pm 3.7$ A
<b>Genotype</b>			
Green (n=72)	5.4%	$27.9 \pm 0.9$ C	$90.2 \pm 4.0$ C
Yellow (n=216)	5.6%	$22.9 \pm 0.5$ B	$47.0 \pm 1.5$ AB
Blue (n=216)	5.6%	$20.1 \pm 0.5$ B	$52.1 \pm 2.0$ B
Brown (n=136)	5.8%	$19.4 \pm 0.6$ A	$52.5 \pm 2.7$ B
A (n=40)	0%	$22.0 \pm 0.9$ B	$40.3 \pm 2.9$ A
B (n=32)	0%	$26.0 \pm 1.1$ C	$56.0 \pm 4.1$ B

Table 2: Mortality, mean Linear Growth (cm/yr) and mean Maximum Diameter (cm) after one year for each Genotype and Attachment Methods used during this study. Standard error of the mean is included for Mean Linear Growth and Maximum Diameter. Different letter groups indicate significant difference ( $p < 0.05$ ) between Genotypes and Attachment Methods.

Fragments placed at deeper depths had significantly higher growth rate than corals placed at shallower depths in terms of both linear growth and diameter. Linear regression models showed that a  $0.3$  m increase in depth was associated with a  $4.2$  cm increase in growth rate ( $p < 0.001$ ) and a  $1.0$  cm increase in maximum diameter ( $p < 0.001$ ). Most depths had mortality lower than  $6\%$  although the highest mortality ( $25\%$ ) was found at the shallowest depth ( $9$  m); the logistic regression model of mortality showed that a  $1.5$  m increase in depth was association with an increased odds ratio of mortality that equaled  $2.9$  ( $p < 0.001$ ).

### Multivariate results

Table 3 shows the results for multivariate regression models of each outcome. As shown, depth continues to be a significant predictor of all outcomes even after adjusting for genotype and attachment methods. Additionally, for linear growth and diameter, the cable tie method continues to perform worse than all the other attachment methods while differences between the other 3 attachment methods are no longer statistically significant. In terms of genotypes, green genotype had significantly higher linear growth and

diameter than all other genotypes. Additionally, blue and brown genotypes had significantly lower diameter than genotype A. In terms of mortality, only coated wire was found to be significantly different from cable ties after controlling for depth. We note that since genotypes were not statistically significant for mortality, they were excluded from the multivariate model fit to mortality to improve model efficiency.

	Linear Growth Regression Coefficient (SE)	Diameter Regression Coefficient (SE)	Mortality Odds Ratio
<b>Depth</b>	4.6 (0.3) ***	1.0 (0.1) ***	1.3 ***
<b>Genotype</b>			
A	Ref	Ref	
B	-8.5 (5.7)	-1.3 (1.5)	
Blue	5.1 (4.1)	-2.6 (1.1) *	
Brown	0.0 (4.4)	-4.6 (1.2) ***	
Green	36.2 (5.1) ***	3.2 (1.3) *	
Yellow	-5.1 (4.2)	-1.2 (1.1)	
<b>Attachment Method</b>			
Cable Tie	Ref	Ref	Ref
Coated Wire	66.2 (7.0) ***	14.8 (1.8) ***	8.0 *
Line	65.3 (7.1) ***	12.8 (1.9) ***	2.2
Monel	65.4 (7.3) ***	15.2 (1.9) ***	2.0

Table 3: Results for multivariate adjusted models controlling for depth, genotype and method. \* denotes p<0.05; \*\*\* p<0.0001

## Discussion

One of the principal goals of nurseries is to maximize growth rates and minimize mortality (Edwards, 2010). Herlan and Lirman (2009) showed that mortality of *A. cervicornis* fragments larger than 4 cm in a nursery is not related to size. The average size of the fragments at the start of this study was greater than 4 cm. Previous estimates for linear annual growth rates of adult *A. cervicornis* colonies on coral reefs typically fall between 10-15 cm/yr (Shinn, 1966; Lewis et al., 1968; Gladfelter, 1984; Lirman et al., 2010) with the lower extreme being 7.1 cm/yr (Gladfelter et al., 1978) and the upper estimates being 26.6 cm/yr (Lewis et al., 1968). Using similar methods for measuring growth as other studies, the mean annual linear growth rate from the nursery in this study was 52.5 cm/yr. This is nearly double the upper estimates of growth for this species and 4-5 times greater than the average reported growth rate. In comparison with other reported nursery methods (Table 4), the linear growth rates reported in this study are significantly higher, and mortality is on the lower end. The measurements presented in this study also represent an underestimate of the actual linear growth rates due to limitations in the Coral Point Count analysis since the length of branches growing in the “Z” axis of the photos were either underestimated or not measured if they were blocked from view by the other branches. Faster growth rates and lower mortality on line nurseries may be attributed to increased water

circulation, less sedimentation, reduced predation and subsequently less disease (Edwards, 2010). Faster growth rates of fragments in line nurseries compared to blocks is because fragments placed in a horizontal position have at least two terminal ends for new growth, and as the colonies grow, they can branch out and extend in 3 dimensions rather than just the vertical axis (Herlan and Lirman, 2009).

Nursery Method	Linear Growth Rate (cm/yr)	Mortality
Blocks	6 - 18 (Herlan and Lirman, 2008)	18% (Herlan and Lirman, 2008)
	13.5 - 15.5 (Lirman et al., 2010)	81% (Quinn, 2010)
	21 - 23 (Quinn, 2010)	5 - 30% (Grablow et al., unpublished)
A-Frames	7.3 - 15.3 (Quinn, 2010)	5-60% (Bowden-Kirby, 2008) 32-96% (Quinn, 2010)
Line Nurseries	21 (Quinn, 2010) 52.5 (this study)	4.5% (this study) 0-15% (Grablow et al., unpublished)

Table 4: Published linear growth rates and mortality for different *Acropora cervicornis* nursery methods (Blocks, A-Frames and Line nurseries).

The results for mean linear growth rate and maximum diameter follow the same general patterns for depth. They had higher growth rates at 11.2-12.4 m and lower growth rates at the shallower depths (9-10.3 m). This could be explained by the fact that the donor colonies were located between 10.5-12.0 m so they are likely acclimated to that depth range.

Because of the slower growth rates at shallower depths and weight issues once the corals grow large, the line nurseries at the Margara and other sites have been modified. Now, a 1.5 m tall by 3 m wide design is being used that accommodates six rows (4 rows of monofilament and holes drilled into the 2 horizontal PVC for attachment points) with eight fragments in each row. This has allowed us to maximize growth rates on the corals, and there is no need to add additional buoys before the corals are harvested.

The line nurseries described in this study produced high survival and growth rates, required low maintenance throughout the year and held up well during storm events. The line nurseries at Margara have been exposed to multiple hurricanes over the years and repeated swells of up to 4-5 m. Little to no damage was experienced as a result of these events while other nurseries using different methods in Puerto Rico and the Virgin Islands experienced significant damage during the same storms. Given the decline in *Acropora cervicornis* populations in the Caribbean over the last few decades and the current focus on scaling up nurseries in the Caribbean, the results presented here are useful for the development of future nursery operations. These techniques, along with other previously developed nursery guidelines

can help maximize production for nurseries into the future. When possible, fragmentation should be performed when other outside stressors like high temperature are minimized (Herlan and Lirman, 2008). Fragments should also be larger than 4 cm in diameter to reduce mortality (Herlan and Lirman, 2009). It is also important to select an area with adequate water quality and circulation and high densities of herbivorous fish to reduce the level of maintenance required to clean the nurseries and provide a good environment to grow out the corals. The nursery at Margara is only visited 2-4 times a year, normally just before and after hurricane season to make any necessary adjustments, but the divers do not need to clean the line nurseries because of sufficient amounts of herbivores in the area.

In January, 2011 the first full scale outplanting was performed from this nursery. Over 1,200 colonies were outplanted to help restore several impacted reef sites in the vicinity of the nursery. The remaining colonies were used to restock the nursery with another 1,500 fragments so that the annual cycle for this nursery could continue.

Research is underway to monitor the survival of the different genotypes outplanted using a variety of methods at different depths (results after one year show 90% survival). DNA analysis will be run on genotypes A and B to see if they are separate genotypes. Additional genotypes and species will be incorporated into the nursery over time. While it is good to know which genotypes grow faster and survive better, it is important to outplant multiple genotypes to an area. The goal is not only to increase genetic diversity and population densities of *Acropora cervicornis* on degraded or impacted reefs but outplant multiple genotypes in close proximity to each other, in an effort to increase successful sexual reproduction (Quinn and Kojis, 2006; Vollmer and Palumbi, 2007; Baums, 2008; Bowden-Kirby, 2008; Reyes and Schizas, 2010)

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