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Success of outplanted *Acropora cervicornis* colonies in reef restoration

Jordan A. Hollarsmith[1], Sean P. Griffin[2], Thomas D. Moore[3]

[1] Department of Ecology, Evolution and Environmental Biology, Columbia University, New York NY, USA
[2] I.M. Systems Group, NOAA Restoration Center, 260 Guard Rd, Aguadilla, PR 00605 USA
[3]NOAA Restoration Center, 263 13th Ave South, St. Petersburg, FL 33701, USA

Corresponding author: jah2187@columbia.edu

Abstract. Populations of *Acropora cervicornis* have suffered dramatic declines throughout the Caribbean since the 1980s, leading to the listing of this species as "Threatened" under the Endangered Species Act in 2005. Due to the fast growth of this species, nurseries have been identified as a potential tool for its recovery and as a means to restore reefs that have been damaged by a variety of physical impacts. In Guayanilla, Puerto Rico, at the site of the T/V Margara grounding, three different genotypes of *A. cervicornis* were outplanted using three different attachment methods (cable ties, epoxy and stabilizing colonies in reef crevices). Colonies were monitored six months and one year after being outplanted to the reef. Data were collected on colony stability, percent tissue mortality, and overgrowth of the colony onto the reef substrate. Overall tissue mortality was low (mean 7% per colony) and only 10.9% of the colonies were missing during the course of this study. After 6 months, epoxy functioned significantly better than the cable ties, though both performed better than the method of stabilizing in crevices. There were also differences in success between genotypes. These results provide important insight for future nursery operations. While some genotypes may be more successful than others, increasing genetic diversity on the reefs is still a priority in the interest of increasing the potential for successful sexual reproduction. Whenever possible, attachment methods that increase stabilization should be used to increase the survival of transplanted corals.

Key words: Reef Restoration, Puerto Rico, *Acropora cervicornis,* Outplanting, Coral Nurseries.

Introduction

The rational for this study is to help accelerate the recovery of *Acropora cervicornis* in impacted areas by testing the most efficient and effective methods of transplantation.

Acropora cervicornis is a keystone species in the Caribbean reef system. Its high growth rate and branching morphology create topographically complex reef systems that provide ideal habitat for fish species, many of which are economically important (Lirman 1999, Luckhurst and Luckhurst 1978). Low population densities have persisted due to chronic stressors on the reef system that stem from changing land use patterns and increased shipping activity in the Caribbean (Gonzalez 2001, Helmer 2004, Garcia-Sais et al. 2008). Low genetic diversity and clonal reproduction further hampers recovery of the species by reducing the rate of sexual reproduction and likelihood of adaptation (Quinn and Kojis 2006, Garcia Reyes and Schizas 2010).

Vessel groundings are a major threat to Caribbean reefs. Groundings can result in complete loss of topographic complexity and the creation of expansive rubble fields. There is little chance of natural reef recovery due to the unstable substrate and the low levels of *A. cervicornis* larval recruitment (Bruckner and Bruckner 2001, Fox 2003, Quinn and Kojis 2006). However, the branching morphology of *A. cervicornis* is such that human restoration efforts, including reattaching coral fragments and growing fragments in nurseries, can have a significant impact on the recovery trajectory of the damaged areas (Bowden-Kerby 2008, Bruckner and Bruckner 2001, Lirman et al. 2010, Nedimyer et al. 2010, Rinkevich 2005).

This study aimed to test whether survival and performance of outplanted *A. cervicornis* differs among genotypes or fragment attachment methods used to attach the outplanted fragments. The results demonstrate the overall efficacy of the method as a means of restoring damaged reefs and as a strategy to increase *A. cervicornis* density.

Material and Methods

Corals were outplanted from the nursery to two different sites during this study: the T/V *Margara* and the LNG-C *Matthew* grounding sites. Depths range from 30 to 40 ft.

In February 2011, 45 one-year-old coral colonies with diameters ranging from 20-40 cm from three different genotypes (arbitrarily named Blue, Brown, and Yellow) were outplanted to the damaged areas at the Margara site. For each genotype, five colonies were attached to the impact site with cable ties, five with epoxy, and five were stabilized in the reef using no other materials (total n=15). Fragments of each attachment treatment and genotype were interspersed randomly within the site. In July 2011, another 24 one-year-old colonies with diameters ranging from 20-40 cm were outplanted using only epoxy at the Matthew grounding site. Of these 24 colonies, 6 of each genotype (Blue, Brown, Yellow and Green) were outplanted. All corals were transplanted within 100 m radius in similar reef habitats to eliminate effects caused by distance or different environmental parameters (Bowden-Kerby 2008, Dizon and Yap 2006). Spacing of fragments within the sites depended on substrate condition.

In the case of attachment using a cable tie, the coral was tied to a secure object – either directly to the reef or a masonry nail. When epoxy was used, it was applied to stable substrate and the coral was placed in the epoxy. The coral and attachment material were situated to minimize smothering of live tissue to prevent further mortality while still positioning live tissue as close as possible to the substrate to promote fusion via budding (Williams and Miller 2010). When no attachment materials were used, corals were stabilized by being wedged into reef cracks and crevices in an attempt to prevent movement by wave and currents. Numbered tags were attached adjacent to each outplanted colony for monitoring purposes. Each colony had 100% live tissue at the time of transplanting.

Data on survival and performance of outplanted coral were collected in June 2011 at Margara and in January 2012 at both the Margara and Matthew sites using SCUBA. Percent tissue mortality and fusion with the substrate were estimated visually. When possible, the cause of the mortality was recorded. Presence or absence of the coral was determined by locating the tag and identifying the associated coral. Stability was measured by gently applying pressure to the coral and determining range of motion (no motion was recorded as stable, any motion was deemed unstable). Data on stability, fusion, and presence were analyzed using a binomial logistic regression model to compare performance among genotype and attachment method. Data on tissue mortality were analyzed using a linear regression model. All statistical analyses were performed using RStudio.

At the Margara site, 2 out of 45 tags could not be found after 6 months (both were for the brown genotype, one was attached using epoxy and one was stabilized using no other materials) therefore no data are available for these colonies. After 1 year, 10 tags out of 45 could not be found, causing a large and unequally distributed decrease in sample size. In analysis of attachment methods, only data from the Margara site were used. Data from both sites were used to analyze genotype performance.

Results

Outplants from both the Margara and Matthew sites performed similarly in terms of tissue mortality, presence, stability and fusion (Table 1). Average tissue mortality after one year was 7%. At the Margara site, 11.4% of the colonies were missing after one year and 8.0% were missing at the Matthew site after 6 months. No colonies at either site were observed to have 100% mortality.

	Margara (n=32)	**Matthew** (n=20)	**Overall** (n=52)
Mean tissue mortality	8.4 ± SD 12%	4.8 ± SD 11%	7 ± SD 12%
Present	91 ± SD 28%	83 ± SD 38%	88 ± SD 32%
Stable	63 ± SD 49%	79 ± SD 41%	69 ± SD 46%
Fused	60 ± SD 50%	66 ± SD 48%	63 ± SD 49%

Table 1: Mean tissue mortality and percent of colonies present, stable and fused after one year at the Margara and Matthew grounding sites and overall.

After 6 months, significant differences in performance were observed among genotypes and attachment methods used at the Margara site. In general, coral colonies attached with epoxy were significantly more likely to be stable ($p=0.017$) and be fused with the substrate ($p<0.01$). The blue genotype had the highest degree of fusion ($p=0.014$) and stability ($p=0.03$). Overall tissue mortality was low, with 70% of outplants displaying no tissue mortality.

No significant differences were observed among genotypes or attachment methods in either the Margara site or the Matthew site, although analysis was inhibited by low sample size. All colonies that were stabilized were either missing a tag or loose and unattached to the substrate (Fig. 1). However, with the exception of four colonies, all stable fragments were also fused to the substrate. When only the Margara data was considered, fragments stabilized with no foreign materials experienced significantly higher levels of tissue mortality ($p<0.01$). There were no significant differences in mortality among genotypes (Fig. 2).

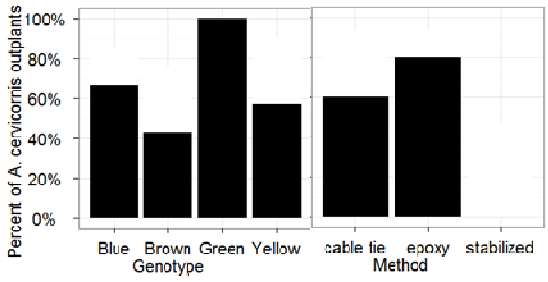

Figure 1: Percent of *Acropora cervicornis* colonies that were stable by genotype (left) and attachment method (right) after one year at both the Margara and Matthew sites.

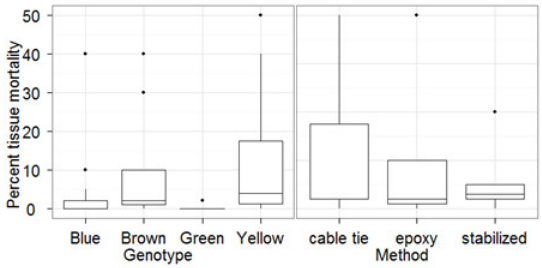

Figure 2: Percent tissue mortality of *Acropora cervicornis* colonies by genotype and attachment method after one year at both the Margara and Matthew sites. The box represents the 50% quantile; the vertical lines are the upper and lower 25% quantiles. The dots are outliers.

Discussion

Low levels of tissue mortality and no complete colony mortality one year after outplanting lend preliminary support to this method of reef restoration as a means of reef restoration and long-term growth and survival of outplanted colonies. As intermittent tissue mortality occurs naturally on healthy *Acropora cervicornis* colonies, the mean observed average mortality of 7% could be a sign of a healthy functioning colony. The 7 absent colonies that are presumed to have dislodged and washed away make up a small proportion of all outplanted coral and are not significantly associated with a particular method. Thus while differential success was observed among attachment methods and genotypes, the overall results support the restoration method outlined in this study.

After six months, both attachment method and genotype appeared to significantly influence the success of the *Acropora cervicornis* outplants. Epoxy was found to be the most effective method of attachment, whereas stabilizing the colonies using no attachment materials was the least effective. In general, the blue genotype outperformed the other genotypes in measures of stability and fusion. Significant differences were not observed after one year, though this result is likely strongly influenced by the decreased sample size.

Acroporids in both the Pacific and Caribbean have been shown to be able to reattach themselves to the substrate within 30 days if the colonies are stable (Bak and Criens 1981, Guest et al. 2011). After one year, 63% of the colonies in this study had fused with the substrate. The greater success of colonies attached with epoxy is likely due to the structural differences between epoxy and cable ties. A colony attached using a cable tie has one point of contact between the polyps and the reef substrate, providing little surface area for budding. Epoxy, however, wraps around the coral, creating more points of contact between the coral and the epoxy and thus more opportunities for polyps to form new growth onto the material.

A strong causal relationship can be observed between stability and fusion of *A. cervicornis* colonies to the substrate after both 6 months and 1 year. More colonies were found to be stable than were found to be fused with the substrate. Past studies done on various coral species have linked stability to increased budding and instability to coral mortality due to tissue abrasion and smothering (Clarke and Edwards 1995; Fox et al. 2003; Sorokin 1993). This highlights the importance of attachment methods that greatly reduce or prevent colony mobility and explains the relative success of epoxy and cable ties over attachment using no foreign materials.

It was determined that after 1 year there was no significant difference in performance of genotypes, though results were inhibited by low sample size. All genotypes had low levels of mortality and high levels of fusion with the substrate. It is important to test whether certain genotypes respond significantly differently to a given attachment method so that restoration methods can be tailored appropriately. The significant differences observed in performance among genotypes after 6 months are in line with existing literature. García Reyes and Schizas (2010) found high levels of genetic population structure among clonally reproducing species such as *A. cervicornis* in Puerto Rican reefs, even among stands in close proximity with one another. Genetic differentiation caused by long-term restriction of gene flow among populations can lead to subtle evolutionary divergences and adaptations to specific environmental conditions (Bowden-Kerby 2008).

It should be noted that although corals that quickly fuse with the substrate are important in restoration of sites completely denuded of coral such as this one, it is not necessarily an evolutionary advantage in high-density thickets. Healthy stands of *A. cervicornis* were historically common before the major population declines and were observed in the reference area surrounding the impact site. Coral colonies in high-density healthy thickets are interwoven and are secure even if they are not fused

to the reef ground. Highsmith (1982) surveyed a stand of 2105 colonies of *A. cervicornis* and found that only 47% were actually attached to the reef substrate. The remaining 53% of colonies were secured by the high degree of branching found in this species. The blue genotype was very successful in the extremely low density environment of the impact zone due to its high degree of fusion with the substrate. However, blue should not be preferentially outplanted. Given the extremely low levels of genetic diversity in Puerto Rican reefs, all genotypes should be outplanted in equal amounts to increase genetic diversity and encourage sexual recruitment (Baums 2008; Highsmith 1982; Quinn and Kojis 2006; Reyes and Schizas 2010).

Overall, only 10.9% of the colonies were missing at both sites, and no colonies were completely dead. The average tissue mortality was low (7%) for both sites. All of the colonies that were still present had significant percent cover of live coral tissue. It is not clear if the colonies that were not present were alive or dead. But these numbers indicate that overall, *A. cervicornis* was found to be an excellent candidate for reef restoration at the Matthew and Margara sites in Puerto Rico. Survival was also high in spite of the passing of Hurricane Irene in August 2011. Wide-scale outplanting efforts are resource-intensive, but they may be necessary both for the health of the reefs in vessel impact zones and for the health of *A. cervicornis* populations in general. These methods can also be used to restore *Acropora* spp. stands damaged from other causes such as severe bleaching events or disease outbreaks.

Recommendations

When possible, attachment methods such as epoxy and cable ties that increase the likelihood of stability and fusion should be used over other methods such as stabilization using no other materials. Logistics and cost, however, might not make this option feasible for all projects, and in that situation, stabilization is still relatively more successful than no action. Differences in performance among the genotypes are more difficult to incorporate into restoration work. Although some genotypes may perform better than others, it is important to consider genotypic diversity when outplanting in an attempt to increase the likelihood of successful sexual reproduction and in case there is some sort of disease outbreak, to increase the chances of survival. Future research will continue to monitor the survival of these colonies over a longer period and at other sites and at different depths.

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