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# RESTORATION OF CRITICALLY ENDANGERED ELKHORN CORAL (*ACROPORA PALMATA*) USING SEXUALLY PRODUCED RECRUITS

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## Introduction

Prior to the 1980s, the Caribbean Acroporids (i.e., *Acropora palmata* and *Acropora cervicornis*) were dominant shallow-water reef building species that provided shelter for a large variety of other reef organisms and significantly contributed to coastal protection during storms and hurricanes. In the early 1980s their abundance declined by >95% caused by a white band disease outbreak and has remained at low densities without noticeable recovery since then (*Acropora* Biological Review Team, 2005). As a result, both species were listed as "threatened" under the U.S. Endangered Species Act (Hogarth, 2006) and "critically endangered" under the IUCN Red List (Aronson et al., 2008). To facilitate these species' recovery, Caribbean-wide restoration efforts were started.

In 2010, the SECORE Foundation, in collaboration with CARMABI and the Curaçao Sea-Aquarium, launched a restoration program in Curaçao aimed at developing the techniques required to assist the recovery of depauperate *Acropora palmata* populations throughout the Caribbean. In contrast to more commonly used methods that depend on the production of offspring by fragmenting existing colonies (reviewed in Young et al., 2012), SECORE uses sexually produced offspring (i.e., more genetically diverse offspring) that are reared in nursery conditions at the Sea-Aquarium prior to their reintroduction to the reef. Since the beginning of this project, SECORE has succeeded in developing methods to culture coral larvae in a land-based nursery and has reintroduced several hundred recruits to the reef to date.

The present study was aimed at evaluating the effectiveness of the restoration techniques developed by the SECORE Foundation. The main objectives were i) to compare naturally-settled versus laboratory-reared recruits, and ii) to determine the optimal age for introduction of recruits to the reef. Coral settlers reared from gametes were introduced to the reef at various time points and their survival and growth were monitored over 6 to 11 months and compared with that of recruits kept within a land-based nursery. Since spawn quality and environmental conditions may significantly differ between spawning seasons, these objectives were investigated over a period of two consecutive spawning seasons, from August 2011 until August 2012.

## Methods

*Study location and facilities*

The study was carried out in Curaçao (12°N69°W), a relatively small island in the Southern Caribbean that still harbors healthy and dense thickets of *Acropora palmata* that spawn annually in the fall. In 2010, SECORE started a coral rearing facility at the Curaçao Sea-Aquarium consisting of five aquariums (each 2150 x 685.8 x 635 cm; 936 L volume), to which fresh seawater was continuously supplied from the nearby ocean through a 100-m pipeline. The system was specifically designed to maintain corals during their earliest life stages and to provide settled corals with a controlled environment aimed at increasing their survival compared to natural conditions.

*Gamete collection and larvae rearing*

*Acropora palmata* is a broadcast spawning species that releases gametes once or twice a year (Szmant, 1986) and for which spawning timing is well known. Larvae from this species were reared from gamete bundles collected in the field on predicted spawning nights (Fig. 1a, b, c). Fertilized eggs were reared in specially designed flow-through rearing devices (called 'kreisels'; see Hagedorn et al. 2009) in the nursery system at the Curaçao Sea-Aquarium where they completed embryogenesis (Fig 1d). After approximately 24-72 hours, larvae began to swim downward in the water column and after 4 to 7 days, they were transferred to plastic containers lined with artificial settlement substratum (i.e., tripods made of clay) and allowed to settle (Fig 1e). Young corals were then grown within the nursery (Fig 1f) and introduced to the reef.

Figure 1. Collection of spawn and coral rearing: a. Coral spawn is collected with specially designed nets into which positively buoyant sperm/egg bundles are trapped; b. At the predicted

spawning time, divers set the nets on coral colonies; c. The spawn is transported in collecting tubes and brought back to the laboratory for fertilization; d. Once fertilized, the eggs are transferred to kreisels where they will complete development; e. After a few days, the coral planulae are placed into settlement bins in which they are provided with suitable settlement substrate and water flow; and, f. Coral settlers are reared within the Curaçao Sea-Aquarium nursery. Photo credits: Paul Selvaggio and Barry Brown.

*Introduction of settlers to the reef*

To compare naturally-settled recruits with nursery-reared recruits, we first attempted to locate naturally-settled recruits on the reef using belt transects and photo quadrats. However, since no natural *Acropora palmata* settlers were found *in situ*, artificially-settled juveniles were introduced to the reef as primary polyps (i.e., 2 weeks old) in 2012. Their survivorship was monitored after 1 month, 6 months and 11 months as a proxy for this species' potential natural post-settlement success, which was compared with that of recruits from the same generation kept within the Sea-Aquarium nursery (n=30 settlement tiles, with an average of 11.4 ± 4.2 SD recruits per tile). In order to determine the optimal recruit age for introduction to the reef, coral settlers collected in August 2011 were introduced to the reef at the age of 1 week , 2 months, and 1 year old. In 2012, settlers were again introduced at an age of 1 week. Because of limited spawning in 2012, the time points of 1 month and 2 months were excluded and additional recruits from that spawning season will be out planted only at the age of 1 year (August 2013). Table 1 summarizes the planting and monitoring time points and includes all sample sizes from 2011 and 2012. All the settlement tiles seeded with recruits were transported from the coral nursery to the reef and tied to a previously secured nylon rope on the reef flat near the Sea-Aquarium, with the exception of the 1 year old recruits from the 2011 spawning which were stabilized to a wave breaker near the Sea-Aquarium using marine epoxy. The survivorship of each recruit was assessed regularly for a period of 11 months and compared with that of controls kept in the nursery.

Table 1. Summary of all planting and monitoring time points of *Acropora palmata* recruits and their respective sample sizes from 2011 and 2012. Survival rates are included for each time point and are expressed as the average percent survival and their standard deviations.

| Generation | Planting treatment | *N* (no. of tiles) | Monitoring time points (months) | Survival (Mean % ± SD) |
|---|---|---|---|---|
| 2011 | Nursery | 10 | 6 | 16.0 ± 10.0 |
| | 1 week | 40 | 6 | 6.0 ± 6.0 |
| | 2 months old | 22 | 6 | 9.0 ± 8.0 |
| | 1 year old | 8 | 6 | 100.0 ± 0.0 |
| | | | 11 | 100.0 ± 0.0 |
| 2012 | Nursery | 30 | 1 | 80.9 ± 28.7 |
| | | | 6 | 9.6 ± 10.1 |
| | | | 11 | 3.7 ± 8.3 |
| | 1 week | 30 | 1 | 65.8 ± 23.2 |
| | | | 6 | 13.8 ± 10.3 |
| | | | 11 | 11.1 ± 8.6 |

*Data analysis*

Since our data did not meet the criteria of homoscedasticity (Levene, $p<0.001$) and of normality of distribution (Shapiro-Wilk, $p<0.0001$), one-way NPMANOVA was performed to test for differences in

survival rates between the corals reared *in situ* and those kept within the nursery. The analysis was performed with Bray-Curtis distances as measure of dissimilarity among untransformed data and significance was based on *F* tests obtained with 9999 permutations. All analyses were performed with PAST 1.97 (Hammer et al., 2001).

## Results and Discussion

*Objective i) comparison of naturally-settled versus laboratory-reared recruits*

In 2012, the survivorship of recruits introduced on the reef as primary polyps (65.8% ± 23.2 SD) after 1 month was significantly lower than that of recruits within the nursery (83.7% ± 28.7 SD), ($F = 2{,}941$ $p<0.05$) (Fig. 2). In contrast, after 11 months, the recruits raised within the nursery had suffered higher mortality rates than recruits placed on the reef immediately after settlement (survival rate: 3.7 % ± 8.3 SD and 11.1 % ± 8.6SD, respectively, $F = 20.1$, $p<0.001$) indicating that, overall, the nursery conditions were suboptimal for young *Acropora palmata* corals. The high mortality rate of the artificially-reared recruits could be the result of stressful conditions occasionally experienced by the latter within the land-based nursery. For instance, between October and November 2012, the sea surface temperature (SST) reached 29.5°C on the reef and often exceeded 31.0°C in the aquaria. Previous data showed that the variability of daily mean temperature within the coral nursery is greater than on the reef adjacent to the Sea-Aquarium (SECORE, unpublished data). Thus, coral recruits kept in aquaria at the Sea-Aquarium were exposed to higher ranges of thermal differences while the reef provided them with more constant thermal conditions. Furthermore, these results show that the first 6 months after settlement of young *A. palmata* represent a crucial life stage during which mortality is highest (i.e., > 85%), both within the nursery and on the reef. Newly settled corals experience severe mortality because their small size makes them extremely vulnerable to stressors such as competition, predation, diseases, runoff and bleaching (Ritson-Williams et al., 2009). Thus, this half-year time window is critical for coral restoration programs such as SECORE.

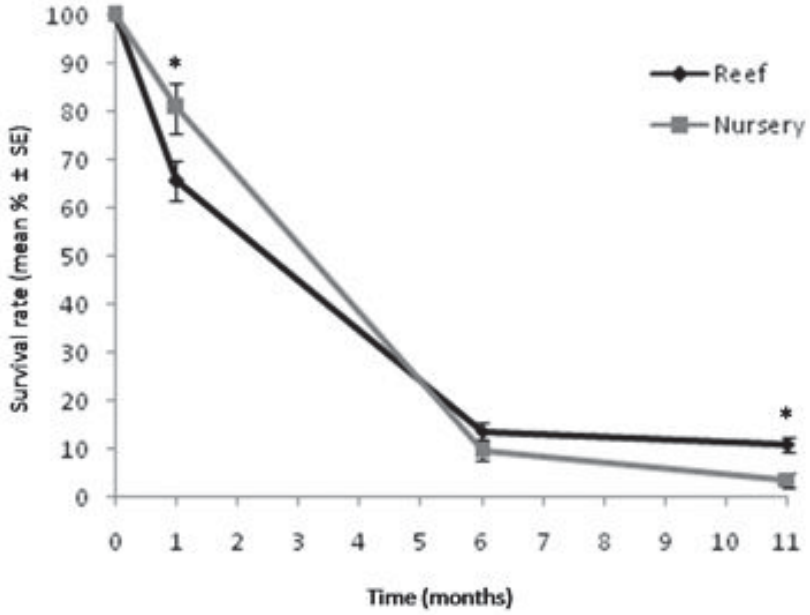


Figure 2. Average survival rates of *Acropora palmata* settlers introduced to the reef as primary polyps (black line) compared to settlers kept within the land-based nursery (grey line). Their survival was monitored after 1, 6, and 11 months. Error bars indicate standard errors (SE) and asterisks (*) indicate significant differences between the two coral populations (NPMANOVA, $p < 0.001$).

*Objective ii) determination of the optimal age/size for planting of recruits*

When planted as primary polyps, the survival rates of *Acropora palmata* settlers after 6 months in 2011 and 2012 were 5.8 % ± 6.0 SD and 13.8% ± 10.3 SD, respectively. Survival rates did not significantly increase when recruits were planted out at the age of 2 months. However, coral recruits that had spent a full year within the nursery before being introduced to the reef had a survival rate of 100% ± 0.0 SD after 11 months. Additionally, after 6 months spent on the reef, they had started growing vertically and their size was about 2 fold larger than the size of their counterparts kept within the nursery. This indicated that growth of juveniles kept for longer than a year within the nursery was somehow limited compared with those introduced to the reef.

## Conclusions

The sudden and unexpected die-off of Caribbean Acroporids in the early 1980s alongside with their dramatic decline in sexual recruitment (Vermeij et al., 2011) has prompted restoration efforts across the region to promote their recovery. The present study demonstrates that using sexually produced recruits has potential in enhancing restoration strategies for critically endangered Elkhorn coral. Indeed, the survival rates achieved after one year in the field are promising. Our results show that the first 6 months after settlement of young *Acropora palmata* represents a crucial life stage during which mortality is highest both within the nursery and on the reef, and that increased nursing periods yield higher survival rates after planting out. However, keeping recruits within a nursery for an entire year represents excessive labour and cost investments, is suboptimal for the coral recruits and is, therefore, not ideal. Alternatively, we suggest placing the settlers as primary polyps within a mid-water nursery for half a year to provide recruits with stable and natural reef conditions and allow them to achieve a bigger size class before being introduced directly to the reef substrate.

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