

Thermal Tolerance as a factor in Caribbean *Acropora* Restoration

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Abstract. Ongoing work in Belize focuses on the identification and propagation of thermally tolerant *Acropora* genotypes. The work has created in-situ gene bank nurseries of the threatened coral species: *A. cervicornis*, *A. palmata*, and *A. prolifera*. Three nursery methods were used: mesh A-frames, suspended ropes, and cement discs affixed to mesh trays. Six nurseries were established, planted with a total of 354 corals of 17 genotypes. Genetic analyses were conducted for both algal symbionts and coral hosts. All outer reef corals sampled had clade A3 symbionts while inner reef corals had a mix of clades A3 and D1. Several genotypes with clade A3 bleached severely while others did not, indicating that the coral host may be an important factor in bleaching resilience. A-frames worked well for comparing genotypes, while for increasing biomass ropes worked best for *A. cervicornis* and *A. prolifera*, and the cement disc method worked exceptionally well for *A. palmata* and *A. prolifera*. Colonies were trimmed at 7-9 months and again at 12-18 months to produce fragments for transplanting to restoration sites. Approximately four thousand second generation corals were planted to reef patches within Laughing Bird Caye National Park, where severe bleaching, disease, and two hurricanes had extirpated the *Acropora* corals. A genetically diverse *Acropora* population containing potentially thermally tolerant genotypes has now been reestablished, which in turn should facilitate the restoration of sexual processes for natural recovery.

Key words: Thermal tolerance, Coral Restoration, *Acropora cervicornis*, *Acropora palmata*, Caribbean reefs

Introduction

Climate change is a pressing long-term threat to the survival of coral reefs globally (Hoegh-Guldberg and Bruno 2010), with global warming resulting in increasingly destructive and widespread bleaching and coral mortality (Hoegh-Guldberg et al. 2007). Coral reefs are widely recognized as the most vulnerable of the planet's ecosystems to the impacts of climate change, with reefs predicted to be the first major planetary-scale ecosystem to collapse in the face of climate change. Already an estimated 19% of the world's coral reefs have been lost and a further 35% are seriously threatened (Wilkinson 2008), and one-third of all reef-building corals are considered to be at risk of extinction (Carpenter et al. 2008).

Widespread coral loss due to thermally-induced mass bleaching has already occurred (Hoegh-Guldberg et al. 2007). Caribbean reefs are particularly impacted, with lower coral cover presently than at any time in geological history (Greenstein et al. 1998). Up to 80% of live coral cover has been lost since the 1970's (Gardner et al. 2003). Two of the three most important Caribbean reef building coral species were added to the IUCN red list in 2006 as critically threatened with extinction (Hall 2006). This listing of both species of Caribbean *Acropora* corals is the first such listing for reef building corals, and so restoration

research on these species is urgently needed (Bowden-Kerby 2001, 2008). In the face of ongoing rapid coral reef degradation, restoration techniques have recently become more accepted as conservation tools (Jaap 2000; Rinkevich 2005; Baums 2008).

The only widely recognized climate change adaptation option for coral reefs thus far is to increase coral reef health through the management of stresses such as pollution, sedimentation, and overfishing (Buddemeier et al. 2004). We have explored a new coral reef adaptation option: the identification and propagation of bleaching resistant corals, followed by the transplantation of second generation coral fragments to restore reefs where thermal stress has decimated coral cover. We focus on the threatened Caribbean *Acropora* corals and their restoration to reefs where they were formerly abundant. This approach supports recent work on coral host and algal symbiont genetics and how these interactions translate to bleaching resilience and the potential for individual corals to adapt to climate change over time (Loya et al. 2001; Baker 2003, 2004; Rowan 2004; Abrego et al. 2008; Baird et al. 2010; Baums et al. 2010).

Material and Methods

Mapping of extant acroporid corals in Southern Belize began in 2006 (Carne 2008), with scoping

surveys of thermally stressed coral reefs to identify *Acropora* corals that had survived warm-water mass bleaching events. Emphasis was placed on the 'inner cayes' near Placencia as AGRRA data from 2006 (Bood 2007) showed higher coral cover versus the outer reefs, despite data showing warmer water temperatures (European Space Agency data 1985-2005). Outer reef sourced corals were included in the nurseries for comparisons.

Staghorn corals were collected as per Bowden-Kerby (2008). Elkhorn corals were trimmed by grasping the outer edge of the colony about 5-8 cm down with clean hands and pushing down or pulling up until a section snapped off. Only a fraction of each colony or thicket was taken (1-10%). Seventeen *Acropora* genotypes were collected, eleven from inner reef sites and six from outer reef sites; eight *A. cervicornis*, seven *A. palmata*, and two *A. prolifera*.

Six shallow (1-2m) nursery sites were established, five in the 'inner cayes' and one on the barrier reef. Nurseries were located in areas of good water flow but sheltered from storms; the leeward side of cayes and nestled between large massive coral colonies.

A-frame culture was set up at each of these six sites using 8 ft x 4 ft sections of 2mm gauge 20x20cm wire construction mesh. The mesh was folded to make an A-shape and painted with phosphoric acid to reduce rusting. Corals were affixed to mesh junctions using 10cm cable ties. The lowermost junctions were not planted to avoid contact with re-suspended sediments, and every other lateral junction was skipped to space the corals, leaving room for the 17 coral genotypes. Three replicate samples of each genotype were planted adjacent to each other; on the top, second and third junctions, for a total of 51 corals per frame.

The A-frames were placed on sand or seagrass and anchored with four 4-inch cement blocks. Underwater data loggers were deployed on each frame to correlate growth and bleaching with temperature regime.

Two of the nursery sites, Whipray Caye and Laughing Bird Caye National Park were expanded to include cement disc and rope cultivation methods for comparison. Cement disc culture consisted of planting the corals onto 10-15cm cement discs or "cookies", each held in place by 80 pound-test fishing line woven through four holes in each disc and into a wire mesh tray made of 1x1cm plastic coated mesh. The lines crossed diagonally from the holes, forming an X on top of each cookie, securing the corals. The completed trays were attached to a metal table constructed with 5/8 inch metal rebar.

Rope culture consisted of twisting 1/4 inch poly rope so that a hole opened up between the three major strands, inserting a small (5-15 cm) coral branch into the opening, and then releasing the twist so that the rope closed down on the coral, holding it in place.

The ropes were suspended ~1 meter above the sand or seagrass substratum, tied between two 5/8 metal bars attached to the metal table containing the cookie trays.

In addition to the corals planted in the nurseries, nineteen colonies of *A. palmata* from the outer reef had already been established in 2006 (Carne 2008) at Laughing Bird Caye National Park, the primary outplanting site of this study. The bleaching history and host/symbiont genetics of six of these corals served as an additional control for comparison.

Outplants

The outplanting site was chosen based on the success of previous transplants (Carne 2008), location in a ten-year no-take zone within the Belize Barrier Reef System World Heritage Site, and the fact that the area is the most visited tourism destination near Placencia.

For the outplanting phase, fragments of *A. cervicornis* and *A. prolifera* were trimmed from the rope nurseries at 9 and 12 months and for *A. palmata* and *A. prolifera* cement discs were outplanted in their entire form at 11 and 16 months. A total of 4168 second generation corals were planted: 3320 *A. cervicornis*, 808 *A. prolifera* and 40 *A. palmata*, all into 16 shallow (1-2.5m) subsites, spaced roughly 1-10m apart around Laughing Bird Caye. Each subsite was planted with between 41 to 1,000 second generation fragments, with one to eight genotypes per subsite. The areas covered range in size from a few meters square to 1600m². Three outplanting methods were used: ropes prepared as above and pegged into the substratum using concrete nails, individual fragments wedged into small holes in coral rock, and multiple finger-sized fragments plugged into balls of wet cement on cleaned coral rock.

Growth measurement calculations

Corals were measured for length, width at a right angle to length, and height at a right angle to the other two measurements (AGRRA methodology v. 4.0, Lang et al. 2007). These measurements when multiplied gave an inter-colony volume. Growth was calculated as day 1 divided into Day 365 volume, giving a simple estimate of relative growth.

Coral Genetics

Genetic analyses of the coral hosts and their symbionts were performed on each of the 17 *Acroporids* on the frames, and on the six *A. palmata* established previously at Laughing Bird Caye (Carne 2008). Coral host genetics (Baums et al. 2005, 2009) were done to confirm our assumption that each coral was indeed a unique coral genotype. Zooxanthellae symbiont genetics were analyzed using two methodologies. All 23 samples were run using the ITS2-DGGE methodology (Sampayo et al. 2009),

while quantitative qPCR was run on a subset of the samples to determine how much Clade A versus Clade D were present in particular corals. Bleaching during the study period was characterized as per AGRRA methodology v. 4.0 (Lang, et al. 2007).

Results

Survival of the corals on the six A-frame nurseries at one year was 87% (41 dead or missing out of 306): 81% survival for *A. palmata*, 88.9% for *A. cervicornis*, and 97.2% for *A. prolifera*. Mortality between sites ranged from a high of 35% to a low of 2%. Only one genotype exhibited higher mortality across sites (4 of 6 sites), an inner caye sourced *A. palmata*. There was no mortality at all using the rope method (0 out of >150 fragments). One-year mortality of *A. palmata* planted to cement discs secured to trays on the tables was 20.8% (5 of 24) replicates, with 0% mortality for *A. prolifera*. The disc method worked exceptionally well for both *A. palmata* and *A. prolifera*, with the cultured colonies presenting the ideal upright growth form with a substantial base for outplanting at 9-12 months.

Coral growth

Fig. 1 shows mean annual growth for *A. cervicornis*, comparing clades A and D. Fig. 2 shows the same for *A. palmata*. The relative growth figures seem exceptionally high, but in fact they represent changes in colony volume, which for *A. cervicornis* is mostly composed of empty space between the branches. Three of the fur inshore sites were distinctly superior for growth over the other sites in the study.

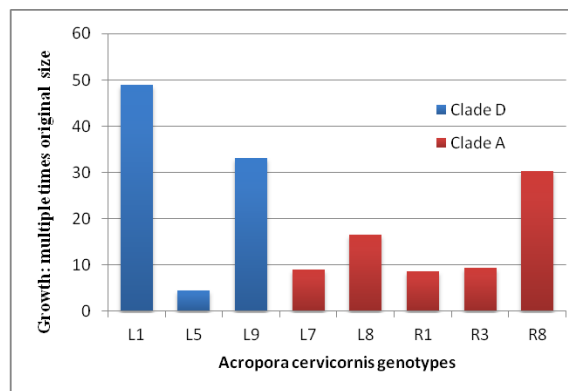


Figure 1. Mean annual growth at six nursery sites for *Acropora cervicornis* genotypes as related to dominant algal symbiont clade.

Genetics

Results of coral host genetic analyses verified that each coral represented a unique coral genotype. This included the 17 corals on the frames plus the six *A. palmata* colonies from previous transplantation work, each with a known bleaching history.

Results of algal symbiont analyses (ITS2) indicate that all outer reef corals sampled had Clade A3 symbionts, while the inner reef samples had either Clade A3 or Clade D1. The more detailed PCR analysis to identify minority clades in the corals was performed on eight samples, and each of the A3 corals tested contained small amounts of D and vice versa. One genotype had equal amounts of D and A.

To look for evidence of shuffling, the change in relative symbiont composition over time (Baker 2001, Little et al. 2005), the six *A. palmata* transplants from 2006 were sampled over the 2009 bleaching event (total of four time series), with tissue taken from bleached and non bleached areas from the two colonies that partially bleached and re-sampled after the colonies had recovered. Both ITS2 and qPCR were run and there was no evidence of shuffling.

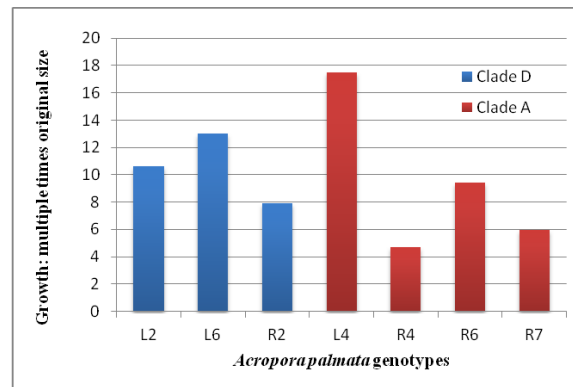


Figure 2. Mean annual growth at six nursery sites for *Acropora palmata* genotypes as related to dominant algal symbiont clade.

Annual growth rates for the various coral genotypes (Fig. 1-2), indicates that growth is more related to the coral genotype than to the symbiont clade. Fig. 3 shows the one-year growth means across all nursery sites by coral species, comparing clades and inner vs. outer reef genotypes

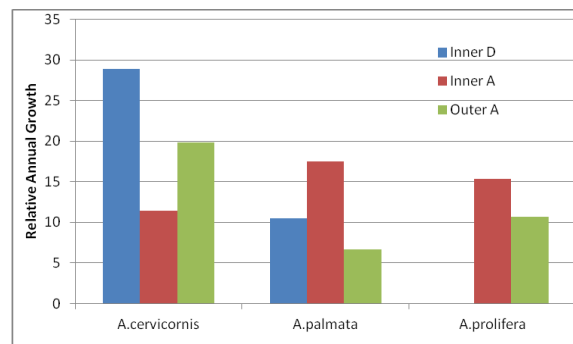


Figure 3. *Symbiodinium* clades versus mean colony growth for three species of *Acropora* from inner versus outer reefs in Belize.

Bleaching

During 2009, bleaching on the reef was milder than the 2008 event and no bleaching was observed on any of the frames, cookies, or ropes. A maximum temperature of 32.3C was recorded on 13th Sept and bleaching was recorded in October on the cement cookies at both of the shallow nursery sites; *A. palmata* (Clade A3) from the outer reefs partially bleached and the source colonies in the wild did as well. One Clade D1 *A. palmata* genotype also partially bleached.

In 2010 maximum temperatures were similar to 2009 but more prolonged, and the outer reef-sourced *A. palmata* (Clade A3) bleached consistently on all nursery frames, however several *A. palmata* with clade D also bleached. A single *A. cervicornis* genotype from the outer reef (A3) bleached on five of six frames in 2010.

In 2011 bleaching was more severe than in 2010, but bleaching rates among the coral genotypes were similar with a few exceptions: the three replicates of *A. palmata* (Clade D1) that partially bleached in 2010 were outplanted in Dec 2010 and did not bleach in 2011, while a different genotype of *A. palmata* (Clade D1) bleached. Inner reef sourced Clade A3 *A. palmata* bleached at one nursery only and out-planted replicates of that genotype did not bleach. Two *A. cervicornis* genotypes (inner reef sourced A3 and D1 clades) bleached on the LBC ropes, with the bleaching delayed until October 2011. The response of the larger *A. palmata* transplants to bleaching in these years and earlier is given in Table 1.

Table 1. Bleaching history of *A. palmata* colonies transplanted to Laughing Bird Caye National Park in 2006 versus clade. WB= wholly bleached, PB= partially bleached

Clade	2007	2008	2009	2010	2011
A	None	WB	none	none	none
A	None	WB	PB	WB	WB 90% dead
A	None	WB	PB	WB	WB
A	None	PB	none	PB	PB
A	None	none	none	PB	PB
D	None	none	none	none	All dead, disease

Outplants

Survivorship of outplants ranged between 85-97% in all but one subsite. That subsite was planted in April 2010 with 242 fragments of a single *A. cervicornis* genotype (R1, Clade A3). At one month survivorship was only 38%, and with the cause of mortality initially unclear. However, in June a disease was documented in the nurseries, which may have been "RTL" Rapid Tissue Loss or Rapid White Band Disease (Esther Peters and Steve Vollmer personal communications). The R1 genotype was the first to

succumb at all nursery locations, indicating that the disease was either present within the corals a year earlier when the work began, or that the disease was widespread in the environment and this genotype was particularly susceptible. Less than 20m away another *A. cervicornis* genotype (L9, Clade D1) had only 2% mortality and is still thriving. In Dec. 2010, 114 fragments of a different *A. cervicornis* genotype (L1, Clade D1) were planted at the site to determine if the genotype difference would be reflected in survivorship. To date, this second genotype has had only 65% survivorship, lower than any other subsite but still higher than for the R1 genotype.

Discussion

Our strategy is to create genetically diverse coral nurseries and reef restoration sites that hopefully also incorporate bleaching and disease resistant parent stock, gathering together corals from scattered remnants and growing them into sizable populations to increase the chances of fertilization and genetic recombination during spawning. It is our hypothesis that this approach will help accelerate sexually based recovery of the threatened corals while encouraging natural processes of climate change adaptation. The ultimate definition of success will be verifying the reestablishment of sexually reproductive populations to areas where they have become extirpated.

Laughing Bird Caye lost all of its *Acroporids* to two major hurricanes, bleaching and disease events. It is a no-take National Park and a World Heritage Site and thus an ideal demonstration site for the work. The high outplant survival in the site is thought to be related to a healthy ecological balance. In overfished areas of the Caribbean (Bowden-Kørby unpublished), annual mortality rates of outplants are often 100% due to *Coraliohila* snail and *Hermodice* worm predation.

Incorporating a high level of genetic diversity into restoration efforts is vital (Baums 2008, Shearer et al. 2009, Baums et al. 2010). Genetic work on coral allelic diversity (Shearer et al. 2009) has indicated that ten randomly collected parent genotypes will preserve >50% of the genetic diversity within a coral species. However, the study indicates that it requires 35 genotypes to obtain >90% of the original genetic diversity. We were initially able to collect eight confirmed *A. cervicornis* genotypes and four additional genotypes have since been found and incorporated into the ongoing work, totaling 12 genotypes. Only another eight *A. cervicornis* populations have thus far been identified in the wider Placencia Belize area (40x40km). Efforts will continue to incorporate these newly identified corals into the nurseries as resources allow. With 20 genotypes in the nurseries, well over 50% of the original genetic diversity will have been established

within the nurseries, which can serve as gene banks for further restoration and scientific work. The prospects for incorporating more than the minimum 35 recommended genotypes appears to be high for the Placencia area of Belize. Unfortunately many reefs in other areas of the Caribbean do not have anywhere near this number of surviving *A. cervicornis* genotypes (Bowden-Kerby personal observations). *A. palmata* appears to have even more genetic diversity remaining in the study area, while only thirteen distinct genotypes have been confirmed thus far, numerous colonies and populations of this species exist in the study area that were not sampled.

From the data, there was no clear evidence that algal clade differences made any difference in growth or mortality. The differences in growth rates appear to be more attributable to coral host genetics. A significant difference in growth among coral genotypes was found to be the case for several genotypes of *A. cervicornis* in Puerto Rico (Bowden-Kerby 2008), but no algal clade determinations were made. More work is required to tease out this complex relationship. The bleaching data however clearly shows that interactions between coral host and symbionts are occurring, a complex situation where supposedly tolerant D1 clades sometimes bleach adjacent to supposedly susceptible A3 clades. The data in Fig. 1-3 tend to strongly contradict the suggestion that growth is sacrificed for thermal tolerance in D clades (Sotka and Thacker 2005).

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