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Intraspecific variation in phenotype among nursery-reared staghorn coral *Acropora cervicornis* (Lamarck, 1816)

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ABSTRACT

Although genetic diversity is recognized as an important consideration for coral restoration, genotypes for use in restoration are not typically selected based on an evaluation of phenotype. Systematic documentation of phenotypic variability within coral nurseries could inform restoration efforts. To quantify differences in phenotype, ten known genotypes of *Acropora cervicornis* in an established coral nursery in the Florida Keys were selected for study. Twelve 5-cm replicate colonies of each genotype were individually tagged for identification and suspended from four identical PVC tree structures within the nursery for grow-out. Total linear extension (TLE) and number of branches were measured at approximately 45-day intervals for a period of 13 months. Buoyant weight was determined for each colony initially and after five and 13 months in order to quantify calcification. Sub-lethal bleaching was observed among experimental colonies following a natural thermal stress event, and significant differences in bleaching prevalence were present among genotypes. At the conclusion of the study, significant differences in all growth parameters were detected among genotypes. Specific growth rate across genotypes decreased following bleaching. The ratio of buoyant weight to TLE varied among genotypes and decreased with increasing TLE, suggesting a potential tradeoff between extension and skeletal density in nursery-reared *A. cervicornis*. Phenotypic variation documented in this study has implications for nursery management and may be useful in selecting genotypes for *A. cervicornis* population enhancement.

1. Introduction

The unprecedented decline in abundance of staghorn coral *Acropora cervicornis* due to a confluence of natural and anthropogenic factors (Greenstein et al., 1998; Bruckner et al., 2002; Miller et al., 2002), has resulted in a critical loss of ecosystem services for Caribbean coral reefs (Bellwood et al., 2004; Alvarez-Filip et al., 2009). In recent decades, the coral gardening method was developed and adopted throughout the wider Caribbean to culture and reestablish lost populations of *A. cervicornis* and restore their associated services (Nedimyer et al., 2011; Young et al., 2012). This method is characterized by the asexual propagation of *A. cervicornis* within in situ nurseries with the ultimate goal of conducting population enhancement on local reefs (Rinkevich, 1995; Young et al., 2012). Fragmenting portions of *A. cervicornis* donor colonies in a nursery to create new, smaller colonies can rapidly increase the amount of tissue and skeleton available for restoration purposes (M.E. Johnson et al., 2011; Lohr et al., 2015). Worldwide, hundreds of thousands of coral colonies have been farmed and gradually transplanted to natural reefs (Rinkevich, 2014). Nursery practitioners are now poised to scale up transplantation of nursery-reared colonies to match historical abundances of farmed coral species on target reefs (Rinkevich, 2014).

Although the coral gardening method has been largely successful, individual coral restoration efforts are currently small in scale (Edwards and Gomez, 2007) and can have variable outcomes (e.g. Bowden-Kerby and Carne, 2012; Ross, 2014). Sources of variability in *A. cervicornis* restoration outcomes include disease (Miller et al., 2014a), bleaching (Bowden-Kerby and Carne, 2012), breakage (Bowden-Kerby, 2008), and predation (Miller et al., 2014b). In addition, nursery-reared, outplanted colonies can vary in performance between sites with differing environmental parameters (e.g. depth, reef zone; Ross, 2014). There is also evidence to suggest that survivorship of restored *Acropora* may decline long-term (i.e. after 2 years; Bruckner and Bruckner, 2001; Ware, 2015). Given these ongoing challenges, it is apparent that, in conjunction with improvements in environmental conditions (National Marine Fisheries Service, 2015), new information is required to advance strategies for coral restoration (Rogers et al., 2015).

The role of genetics in coral restoration efforts has generated particular interest (Baums, 2008; Shearer et al., 2009). Although published

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guides for coral restoration recommend outplanting genetically diverse populations (Shearer et al., 2009; M.E. Johnson et al., 2011), selection of genotypes for outplanting is typically not based on a systematic evaluation of phenotype. Previous studies have documented differences in phenotype, particularly growth rate, among known genotypes of nursery-cultured *A. cervicornis* (Bowden-Kerby, 2008; Lirman et al., 2014). Some *A. cervicornis* genotypes have also been shown to resist infection by disease (Vollmer and Kline, 2008; Libro and Vollmer, 2016). In addition, differences in bleaching resistance among host genotypes have been documented in other scleractinian coral species (Edmunds, 1994; Baird et al., 2009; Kenkel et al., 2013).

Understanding how phenotype can vary among nursery stocks of *A. cervicornis* is critical for improving restoration outcomes given that differences in phenotype could influence the ability of outplants to cope with stressors and grow to maturity. Such information represents a necessary first step in the development of a trait-based system to select genotypes for use in population enhancement activities, which has been identified as an important strategy for improving coral restoration outcomes (Hunt and Sharp, 2014; National Marine Fisheries Service, 2015). A 13-month experiment was therefore conducted to quantify and compare linear growth, branching, calcification, and bleaching prevalence among ten known genotypes of *A. cervicornis* in an established coral nursery in the Florida Keys.

2. Materials and methods

This study was conducted within an established coral nursery operated by the Coral Restoration Foundation (CRF) located four miles offshore from Tavernier, FL. CRF maintains 150 known genotypes of *Acropora cervicornis* (CRF, unpublished data), which were identified using microsatellites developed by Baums et al. (2009). Ten genotypes were selected for use in the study based on CRF records of availability and outplanting history. Genotypes used in this study were sourced from sites within 30 km of the nursery between 1996 and 2011. These genotypes had acclimated to the nursery location for a minimum of 3.5 years prior to the initiation of this study. For each genotype, four 5-cm non-branching apical tips were clipped from each of three existing nursery donor colonies ($n = 12$ tips per genotype) to control for any intracolonial variation in genotype (Schweinsberg et al., 2015). The tree nursery method (Nedimyer et al., 2011) was used in this study due to its popularity for *A. cervicornis* grow-out at many Caribbean nurseries (e.g. Lohr et al., 2015; Meesters et al., 2015; M.E. Johnson et al., 2011). All replicate colonies ($n = 120$) were tagged for identification (genotype, donor colony, replicate) and randomized across four PVC tree nurseries installed at the same location within the source nursery using monofilament and aluminum crimps. A HOBO Pendant® data logger (Onset Computer Corporation, USA) was installed on each of two tree nurseries to continuously record temperature in situ.

Replicate coral colonies were transported from the nursery to shore and buoyantly weighed (Jokiel et al., 1978) on day 0, day 122, and day 390 at the conclusion of the experiment. Total linear extension (TLE) and number of branches ≥ 1 cm TLE for each replicate colony were also recorded in situ at the start of the experiment and then at approximately 45 day intervals throughout the duration of the study. At each interval, replicate colonies were examined for signs of disease following Miller et al. (2014a) and presence or absence of bleaching was also documented for each replicate colony. Bleaching was defined as any visible loss of pigmentation from a coral, following the Atlantic and Gulf Rapid Reef Assessment protocol version 5.4 (Lang et al., 2010). Observed loss of pigmentation was recorded in the field and verified by comparing photographs of apparently bleached replicate colonies to photographs of the same colony from the previous sampling interval. Specific growth rate was calculated for each replicate colony twice: before bleaching (the time period prior to and including the last interval at which colonies were not affected by bleaching, day 0–162) and after bleaching (the time period beginning at the first observation of bleaching up to and including the first interval at which replicate colonies were fully recovered, day 207–291). Specific growth rate was calculated as

$$SGR = \frac{\ln(f) - \ln(i)}{t} \times 100$$

where f is the final TLE and i is the initial TLE for a given colony in a given time period and t is the duration of the time period in days.

Statistical analysis was performed using R statistical software (v. 3.1.2, R Core Team, 2014). All statistical tests were conducted at a significance level of $\alpha = 0.05$. Data were assessed for normality and homogeneity of variance using the Shapiro-Wilk test and Levene test, respectively. Differences in TLE among replicate colonies sourced from varying donor colonies were tested for each genotype after 291 days of growth using ANOVA with Tukey HSD. Values for net buoyant weight and net TLE were calculated by subtracting initial values ($t = 0$ days) from final values ($t = 390$ days) and compared among genotypes using ANOVA with Tukey HSD. The relationship between net buoyant weight and net TLE was analyzed using simple linear regression. A ratio of buoyant weight to TLE was calculated for each replicate colony and compared among genotypes using a Kruskal-Wallis test with a Dunn post hoc test. The relationship between this ratio and TLE was assessed using a Pearson correlation. Final data on branch number per replicate colony were collected on day 291 and were compared among genotypes using a Kruskal-Wallis test with a Dunn post hoc test. Broad sense heritability (H^2) was calculated for TLE, buoyant weight, and branching following Császár et al. (2010) and Toker (2004). Genotypes found to differ in growth by donor colony were excluded from this analysis. Prevalence of bleaching was analyzed among genotypes using chi-squared tests, and among donor colonies within each genotype using Fisher exact tests. Specific growth rate values before and after bleaching were compared across all genotypes using a paired t-test.

3. Results

During the first 291 days of the experiment, one replicate colony was lost, and four others experienced complete ($n = 1$) or partial ($n = 3$) mortality due to unknown causes. Growth data for these colonies were excluded from analysis. No signs of active disease were observed during the study period. Between day 291 and day 390, two tree nurseries were lost. Therefore, data from the remaining 58 colonies were used for analysis of net buoyant weight and net total linear extension (TLE). Installed HOBO Pendant® data loggers were lost along with the two tree nurseries, therefore data from an adjacent National Oceanic and Atmospheric Administration monitoring station (National Data Buoy Center station MLRF1, ~6.8 km from nursery) were used to supplement available HOBO data.

Net buoyant weight ($F_{9,48} = 2.54$, $p = 0.02$) and net TLE ($F_{9,48} = 4.02$, $p < 0.001$) varied among genotypes after 390 days of growth (Fig. 1). Differences in TLE among replicate colonies originating from different donor colonies were detected for two genotypes (K2 and U41; Table 1). Buoyant weight was significantly predicted by TLE ($\beta = 0.46$, $t_{56} = 14.45$, $p < 0.001$) and TLE explained a significant proportion of variance in buoyant weight ($F_{1,56} = 208.9$, $R^2 = 0.79$, $p < 0.001$). The ratio of buoyant weight to TLE varied among genotypes ($H_9 = 17.23$, $p < 0.05$; Fig. 2) and decreased with increasing TLE ($r_{56} = -0.67$, $p < 0.001$). The number of branches per replicate colony on day 291 ranged from 8 to 30 and mean branch number varied among genotypes ($H_9 = 35.29$, $p < 0.001$). For TLE, buoyant weight, and branching, H^2 was 0.28, 0.27, and 0.25, respectively.

Sub-lethal bleaching was observed among replicate colonies on day 207 following two 10-day periods in which daily mean temperature exceeded 31 °C (Fig. 1A). Specific growth rate for all genotypes during the period preceding the bleaching event was significantly higher compared to specific growth rate during the period immediately following