Thermal history influences lesion recovery of the threatened Caribbean staghorn coral *Acropora cervicornis* under heat stress

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Abstract

Anthropogenic climate change is the biggest threat to coral reefs but reef restoration efforts are buying time for these ecosystems. Lesion recovery, which can be a determinant of colony survival, is particularly important for restored species. Here, we evaluate lesion recovery of 18 genets of *Acropora cervicornis* from Florida reefs with different thermal regimes in a temperature challenge experiment. Genets demonstrated significant variability in healing, which greatly slowed under heat stress. Only 35% of fragments healed at 31.5°C compared to 99% at 28°C. Donor reef thermal regime differentially influenced recovery between temperature treatments, with corals from warmer reefs increasing the Cox Proportional Hazard of healing by a factor of 1.74 under heat stress. These findings should encourage practitioners to utilize rapidly-healing genets, avoid fragmentation in high temperatures, and incorporate assisted relocation by moving corals from warmer to cooler reefs, where they might succeed under future climate conditions.

Introduction

Coral reef ecosystems are experiencing unprecedented declines caused by interacting stressors that include pollution, overfishing, diseases, and anthropogenic climate change (Harborne et al. 2016). At global scales, high seawater temperatures have led to increases in the extent, frequency, and duration of coral bleaching events that have affected even the most protected reefs (Eakin et al. 2019). The Caribbean acroporids, *Acropora cervicornis* and *Acropora palmata*, have undergone severe population declines of 80-90% since the 1970s or earlier, due to human pressures and a severe disease outbreak (Aronson and Precht 2001; Cramer et al. 2020). With limited population recovery, active coral propagation and reef restoration have become common tools used to help restore these taxa and their associated lost ecosystem services (Young et al. 2012).

Most restoration programs currently focus on the asexual propagation of branching corals as part of the coral gardening framework that involves the collection of fragments from wild colonies, propagation within nurseries, and the outplanting of nursery-grown corals onto depleted reefs (Lirman and Schopmeyer 2016). To enhance restoration efficacy, it is important to identify individuals with favorable phenotypic traits that can be exploited to maximize restoration success and enhance the climate resilience of restored coral populations (National Academies of Sciences, Engineering, and Medicine 2018). Phenotypic traits proposed by Baums et al. (2019) to evaluate genotype performance include: (1) partial mortality; (2) lesion healing; (3) growth rates; (4) bleaching and disease resistance; and (5) sexual reproduction. Life-history attributes such as mortality and growth (Lirman et al. 2014), disease resistance (Miller et al. 2019), bleaching resistance (Lohr and Patterson 2017) and fecundity (Vargas-Angel et al. 2005) have been well-studied for staghorn coral. However, although examined in the closely-related *Acropora palmata* (Bak 1983; Meesters and Bak 1995; Lirman 2000*)*, to date, patterns and drivers of lesion recovery, including variation by genotype, have not been evaluated for *A. cervicornis*, representing a research gap that is addressed in our study.

Wound healing is of particular interest because lesions are created throughout the gardening and natural events. Rapid healing reduces the likelihood of colonization by algae, borers, and pathogens that can cause mortality (Highsmith 1982). Thus, identifying genotypes with greater recovery potential that can be outplanted at locations where corals are more prone to fragmentation such as areas with high wave energy is a pressing restoration need. Also, since thermal stress can slow or suspend lesion recovery (Meesters and Bak 1993; Bonesso et al. 2017), it is important to evaluate the effect of high temperature on lesion repair to understand how performance might vary seasonally or under ocean warming scenarios.

Novel interventions, such as managed relocation and assisted gene flow, are currently being explored to enhance the climate resilience of reef restoration efforts (National Academies of Sciences, Engineering, and Medicine 2018). Managed relocation requires that the phenotypes of interest depend on fixed (genetic) effects that will allow these corals to maintain their phenotype after relocation or propagation within a common garden. Some coral traits, such as thermal tolerance, are commonly associated with symbiont identity (Silverstein et al. 2017), but *A.cervicornis* in Florida typically only hosts *Symbiodinium fitti* (Lirman et al. 2014). Consequentially, variation in the performance of this species in Florida is usually attributed to acclimatization or adaptation of the coral host. Previous research has found that colonies collected from different reef environments maintain differences in growth (Lirman et al. 2014) and disease resistance (Miller et al. 2019) after being held under common garden conditions. Here, we used a temperature challenge experiment to evaluate patterns of lesion recovery among staghorn genets that were originally collected from a wide range of environments across the Florida Reef Tract that vary in their thermal regime. We hypothesized that colonies from warmer environments would be able to heal lesions more rapidly under heat stress compared to colonies collected from cooler environments. Such findings would support the movement of genotypes from warmer donor reefs to cooler restoration sites that are expected to warm with anticipated climate change.

Materials and Methods

We collected 18 staghorn genets from reefs spanning >100 km of the Florida Reef Tract and cultivated them under common-garden conditions in an in-water coral nursery off Key Biscayne, Miami, Florida, USA (25.676°N, 80.098ºW; depth = 9 m, Fig. 1) for 2-6 years. Colonies were assumed to be different genets based upon distance between collection locations (Drury et al. 2016). We then assessed lesion healing rates under control and elevated-temperature conditions in a laboratory experiment. Ten 5-gallon aquaria equipped with pumps and air bubblers were used as experimental units and were split between two large tanks (2 x 0.75 x 0.25 m). Heaters placed within one of the large tanks were used to reach the desired high temperature. Replicate fragments of each genet were collected from the coral nursery, mounted onto acrylic pucks, and allowed to acclimate for two weeks at ambient temperatures in the laboratory prior to the start of the experiment. Fragments (N = 5 per genotype per treatment) contained an apical polyp and were 2.66 ± 0.40 cm (total linear extension) and 2.82 ± 0.31 cm (mean ± SD) for control and heated corals respectively. These fragments were randomly assigned to the aquaria with each genet present in each of the 10 aquaria, and fragment and aquaria positions were rotated every other day. Water changes were made every day using filtered seawater pre-heated to the desired temperature. Corals were fed every other day with Reef Chili.

Skeletal lesions were created by removing fragment tips with a band saw. Lesion areas were 0.35 ± 0.19 cm^2 and 0.32 ± 0.15 cm^2 (mean ± SD) for control and heated corals respectively. The following day, the temperature of the exposure tank was raised daily by 0.5°C increments, from 28°C to 31.5°C over 7 days, and then maintained at 31.5°C (32.03 ± 0.48°C (mean ± SD)). The ambient tank remained at 28.02 ± 0.27°C (mean ± SD) throughout the experiment. Corals were surveyed every other day following lesion generation and the amount of lesion healed was classified visually in 25% increments, from 0-100% healed (Fig. 2). To avoid excessive mortality, the experiment was terminated when no additional fragments within the warm tank were observed to heal for at least one week.

Pearson's chi-squared tests were used to test for independence of the frequency of colonies healed by treatment. To evaluate the role of thermal history in performance under temperature stress, we divided the genotypes into two groups based on the maximum monthly mean temperatures (MMMs) of the source reefs. MMM data was determined from 4-km resolution monthly sea surface temperature climatology derived from harmonic analysis of the Advanced Very High-Resolution Radiometer (AVHRR) Pathfinder Version 5.0 temperature time series data for 1982-2008 (Casey et al. 2010). Genotypes from reefs with MMM > 29.9°C were classified as "warm" environments (10 genotypes), and those from reefs with lower MMMs were classified as "cool" environments (8 genotypes) 29.9 C represents the MMM at the southern end of the "Safety Valve," series of tidal flow channels between the Ragged Keys and Key Biscayne, north of which all "cool" genotypes are located and south of which all the "warm" genotypes are located (Fig.1). We constructed a Cox Proportional Hazards model using the package "survival" in R version 1.3.959, with temperature treatment (ambient vs. heated) and donor reef grouping (warm vs. cool) as fixed effects, and the response event being full lesion recovery. We stratified the model by temperature treatment because this covariate violated the proportional hazards function assumption, and we included an interaction between temperature treatment and thermal history group because this interaction significantly improved the model ($\chi^2_{1,163}=25.1$, $p<0.05$). Because of the this interaction, we could not assume that the coefficient for thermal history from the stratified model was constant between temperature treatment groups, and determined model coefficients for these groups separately (Kleinbaum and Klein 2012). To compare genet performance between temperature treatments, we examined the quantile rank of each genet within treatment groups based on proportion of colonies healed 12 days after wounding. We also conducted a Kendall's Rank correlation test between genet proportions healed under ambient temperatures and under heat stress."

Results and Discussion

Our laboratory experiment showed that thermal stress has a strong negative influence on the recovery of skeletal lesions in *A. cervicornis*. By the end of the experiment (24 days), 64% more fragments had healed under ambient temperatures than under heat stress ($\chi^2_{1,164}$=74.66, p<0.001). While colonies exposed to the heat treatment were observed to pale, none of the fragments fully bleached, and colonies within the control group did not show signs of color loss. Our findings agree with those of Meesters and Bak (1993) who showed that corals suspend lesion recovery under bleaching conditions.

Our study also demonstrated genotype variability in lesion recovery: lesion healing rate varied significantly among genotypes under ambient conditions ($F_{17,81}$=2.66, p<0.01), but not among genotypes under heat stress because many fragments failed to heal. The proportion of colonies healed by genotype after 14 days ranged from 0-100% under ambient temperatures, and 0-60% under heat stress. No significant correlation was observed between proportion of colonies healed within ambient aquaria and that within warm aquaria by genet ($\tau_{16,18}$=0.47); however, few genets did well in both treatments (North Midchannel-B, B-AC, and Marker 9) and a few genets did poorly in both treatments (Stagreef-B and Yung's-B.)," suggesting that practitioners should evaluate genotypes within multiple environments to identify rapidly recovering individuals for restoration purposes.

In addition, we document for the first time that donor reef thermal history plays a role in staghorn recovery under heat stress, and provide evidence for thermal adaptation. There was a significant interaction between temperature treatment and thermal history in the stratified Cox model ($Z_{1,164}$=2.18, $p<0.05$). Being from a warmer donor reef significantly increased the hazard of healing by a factor of 1.74 when under heat stress ($Z_{1,81}$=1.89, $p=0.05$) while there was no apparent effect of thermal history under ambient conditions (Fig. 3, Online Resource 2). Increased thermal tolerance of individuals from warmer and variable reef environments has been documented in other coral species and regions. Morikawa and Palumbi (2019) found colonies collected from warmer environments maintained heat tolerance after 8 months in a common garden in American Samoa. Kenkel et al. (2013) found colonies that were collected from an inshore reef exposed to temperature extremes in the Florida Keys maintained thermal tolerance in a laboratory experiment with no common-garden phase, and linked these differences to significant genetic divergence.

Despite some earlier concerns (Coles and Riegl 2013), restoration scientists are now considering incorporating managed relocation into restoration efforts to promote genetic exchange and enhance survivorship (National Academies of Sciences, Engineering, and Medicine 2018; Baums et al. 2019). Our results suggest that sourcing colonies from warmer reefs and outplanting them into cooler environments that may experience temperature increases in the future would enhance lesion recovery under heat stress and could be incorporated into strategies to increase climate resilience. We also recommend avoiding fragmentation when seawater temperatures near the species' bleaching threshold. However, when fragmenting under stressful thermal conditions, practitioners can increase the likelihood of lesion recovery by selecting genotypes sourced from warmer environments. Lastly, we recommend that restoration practitioners identify rapid healers through lesion recovery tracking, and outplant these genotypes in higher abundance at restoration sites where colonies are more vulnerable to fragmentation. However, tradeoffs between regeneration and other valuable traits need to be identified and taken into consideration when selecting for this particular phenotype. Due to the importance of lesion recovery for colony survivorship, particularly in species that are routinely pruned for restoration such as *A.cervicornis*, we recommend that practitioners prioritize examining and exploiting this phenotype within their restoration programs.

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Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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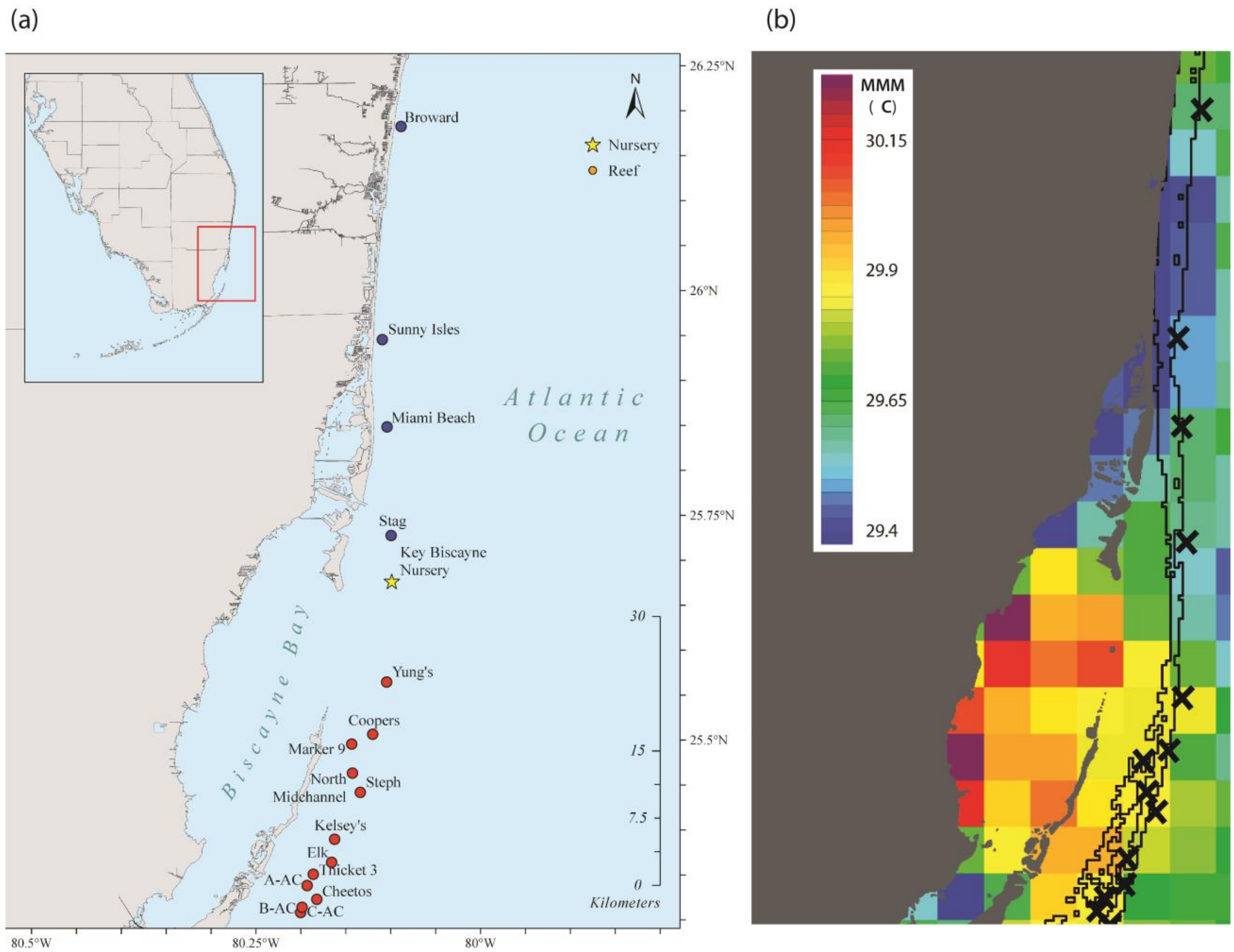

Fig. 1. (a) Key Biscayne Nursery and donor reef locations. Circle colors correspond to donor reef 4-km resolution maximum monthly mean (MMM) sea surface temperature data in which red indicates an MMM>29.9°C and blue indicates an MMM<29.9°C. (b) MMM sea surface temperatures derived from harmonic analysis of the Advanced Very High-Resolution Radiometer (AVHRR) Pathfinder Version 5.0 from 1982-2008. The Florida Reef Tract is outlined in black, and donor reef locations are indicated by black X's.

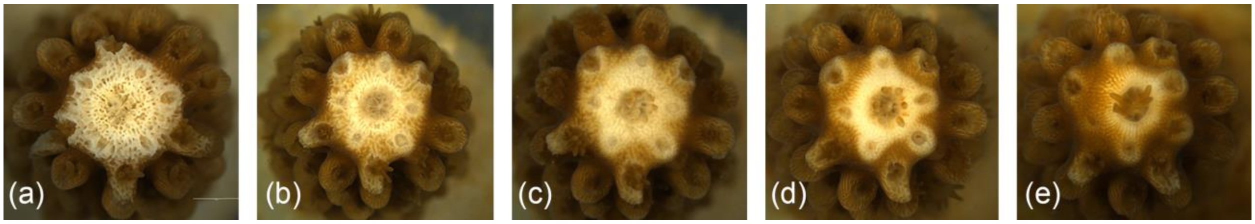

Fig. 2. Incremental lesion healing scoring system including (a) 0% healed; (b) 25% healed; (c) 50% healed; (d) 75% healed; and (e) 100% healed fragments.

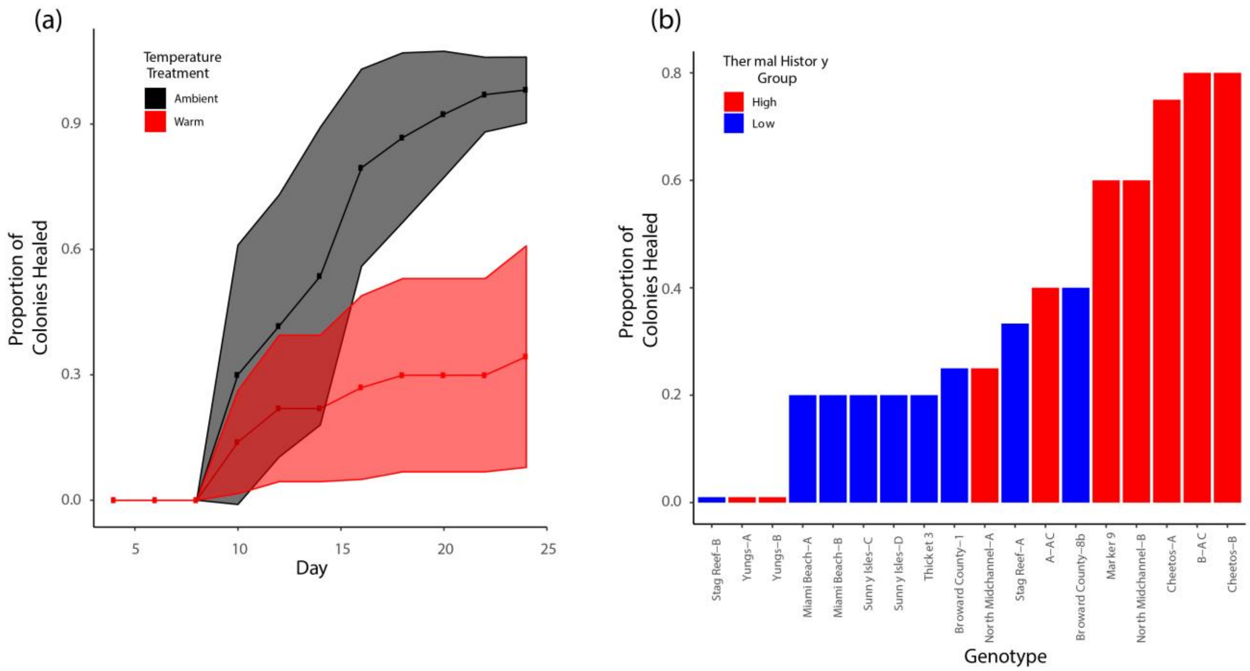

Fig. 3. Cox Proportional Hazards model cumulative hazard values for the risk of healing throughout the experiment, using separate models for the ambient temperature treatment (solid lines) and the warm temperature treatment (dashed lines), with thermal history (red indicating being from a warm reef and blue being from a cool ref) as a fixed effect.