



NOTE

Influences of genotype, phenotypes, and size characteristics on lesion recovery in Caribbean staghorn coral

Madeline L. Kaufman*, Martine D'Alessandro, Chris Langdon, Diego Lirman

Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA

ABSTRACT: Severe declines of coral cover in the Caribbean and throughout the world have led scientists and managers to invest in restoring coral reefs and determining which coral phenotypes and genotypes will maximize restoration success. *Acropora cervicornis* is the species most commonly used by restoration programs in the Caribbean, but only a handful of studies have documented its capacity for wound healing, a phenotype identified as essential for predicting genotype performance. Here, we examined the influence of genotype, lesion area, colony size, and growth, along with tissue, chlorophyll *a*, lipid, and symbiont densities, on the recovery capacity of staghorn corals in a field experiment. Colonies with smaller lesion areas, lower tissue densities, and greater chlorophyll *a* densities healed more efficiently 9 d after wound infliction. At 16 d, initial colony size was the only significant predictor variable, with smaller colonies healing more quickly. Healing rate varied significantly by genotype, ranging from 50 to 100% of fragments healed by genotype 16 d after wounding. Colony growth was not predictive of recovery rate, but wounded corals grew more slowly than non-wounded corals, providing evidence for a tradeoff between these 2 phenotypes. We encourage restoration practitioners to fragment smaller colonies and generate smaller lesions in the interest of rapid wound recovery. We also recommend that practitioners identify rapidly healing genets not from tissue assays but through lesion recovery tracking and outplant these genotypes in higher abundance at restoration sites, particularly in high-energy environments where colonies are vulnerable to fragmentation.

KEY WORDS: Coral restoration · Wound healing · Lesion recovery · Genotype variability · Regeneration · Staghorn · Growth · Tradeoff

1. INTRODUCTION

Reef restoration has emerged as a popular tool to reverse the global decline of coral reefs (National Academies of Sciences, Engineering, and Medicine 2019). One of the most common methodologies of reef restoration is coral gardening, and thousands of corals are now propagated and outplanted in the western Atlantic each year using this method (Schopmeyer et al. 2017). Florida *Acropora cervicornis*

(staghorn coral) has experienced severe declines and is the focus of restoration programs throughout the region (Boström-Einarsson et al. 2020, Cramer et al. 2020). Scientists hypothesize that outplant survivorship could be increased by matching coral phenotypes to restoration site environmental conditions and recommend that practitioners identify and restore stress-tolerant colonies (Ladd et al. 2018, National Academies of Sciences, Engineering, and Medicine 2019). Staghorn genotypes demonstrate variable

*Corresponding author: madeline.kaufman@rsmas.miami.edu

growth, calcification, and bleaching and disease susceptibility (Lirman et al. 2014, Enochs et al. 2018, Miller et al. 2019). Lesion recovery is one of the most important phenotypes for maximizing restoration success (Baums et al. 2019) but has not been widely evaluated in staghorn coral. Open wounds can expose corals to pathogens and competitive colonizers, which is of particular concern for branching species that reproduce asexually via fragmentation and are prone to breakage from marine life, wave energy, and storms (Highsmith 1982). Staghorn colonies are also wounded throughout the coral gardening process; thus, understanding the factors that influence lesion recovery can allow practitioners to increase restoration efficiency.

Coral colony and lesion size are common drivers of lesion recovery rate (Henry & Hart 2005), and biological parameters (i.e. lipid, symbiont, and chlorophyll densities) have been associated with lesion recovery in *A. muricata* (Denis et al. 2013) and staghorn coral (Lesneski 2020) but under varying environmental conditions. If consistently documented in staghorn coral, these relationships could facilitate identifying rapidly healing genets and inform restoration design to increase recovery rates. Tradeoffs must also be considered when targeting specific phenotypes for restoration, especially as regeneration competes with other processes for energy and is associated with reductions in coral growth and reproduction (Rinkevich 1996, Lirman 2000, Denis et al. 2013). Here, we evaluate the influence of genotype, lesion size, colony size, and biological properties (symbiont cell, chlorophyll, lipid, and tissue densities) on staghorn lesion recovery and explore the potential tradeoff with growth in an in-water nursery experiment.

2. MATERIALS AND METHODS

Coral colonies were collected from Florida's Coral Reef and reared in a common garden in-water nurs-

ery in Miami, Florida, for 2 to 6 yr (25.676° N, 80.098° W; depth = 9 m) (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m679p213_supp.pdf). Ten replicate colonies of 28 genotypes were suspended from experimental PVC nursery trees in June 2019 (Nedimyer et al. 2011). Lesions were generated by removing 1 branch from each colony with snips to mimic wounds generated during the restoration process and natural fragmentation events (Fig. S2). Care was taken to fragment from similar proximal–distal positions on the colonies to control for polyp age and lesion location, and initial lesion areas and colony sizes were measured (Text S1, Table S1). We monitored the colonies 9, 16, and 22 d after wounding and assigned lesions a healing score between 1 and 5 (Fig. 1). To determine if physiological properties and growth were associated with healing, we measured symbiont, chlorophyll *a* (chl *a*), tissue, and lipid densities along with growth (average annual productivity) on the same genets but separate colonies ($n = 5–6$ per genotype) (Texts S2 and S3).

Statistical analyses were run in RStudio version 1.3.959 and R version 4.0.1 ($\alpha = 0.05$). We explored genotype influence using Kruskal-Wallis rank sum tests and calculated predictor variable influence through simple linear regressions using the package *lme4*. We constructed separate models with the genotype average healing score at 9 and 16 d as a function of the following genotype averages: initial lesion area, initial colony size, annual productivity of non-wounded corals (to avoid autocorrelation between recovery and growth), and biological parameters. We also used a paired *t*-test to compare average annual productivity between wounded and non-wounded corals.

3. RESULTS

Within 9, 16, and 22 d of wound infliction, 6, 84, and 97% of colonies, respectively, had healed.

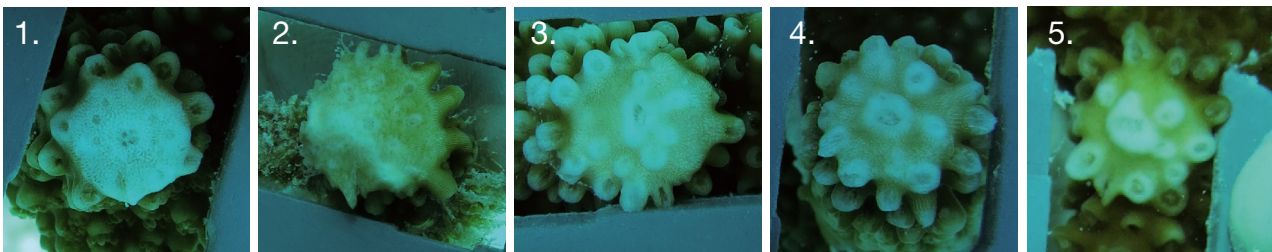


Fig. 1. Healing scores progressing from (1) minimal signs of healing, (2) tissue returning to the lesion, (3) polyps reappearing at the lesion, (4) apical tip present and fully surrounded by tissue (considered fully healed), and (5) apical tip projecting vertically

Recovery score varied significantly by genotype 9 and 16 d after wounding (Table 1, Fig. 2), and at 16 d, the percentage of colonies healed ranged from 50 to 100 % by genotype. Higher healing scores were predicted by smaller lesion areas, lower tissue densities, and higher chl *a* (cm⁻²) levels at 9 d and by smaller colony sizes at 16 d (Table 2, Fig. 3). There was no significant correlation between genotype initial lesion area and total linear extension ($t = -0.039$, $p = 0.970$). Lipid density, symbiont density, and growth were not predictive of recovery, but average annual productivity was significantly lower in wounded corals compared to non-wounded corals of the same genotype ($t = -15.61$, $p < 0.001$).

4. DISCUSSION

Staghorn is one of the fastest species to recover from small lesions (Henry & Hart 2005). The healing rates documented here (9–22 d) are comparable to those documented by Okubo (2008), who reported 100% of *Acropora muricata* colonies healing within 24 d of wounding. Staghorn lesion recovery has likely been enhanced via adaptive pressures, as branching corals depend on fragmentation for reproduction and because staghorn thickets experience frequent breakage due to the species' colony structure and abundance at shallow reef sites (Tunnicliffe 1981, Bak 1983).

The negative relationship between recovery and lesion area documented here is consistent with previous research in scleractinian corals and has been attributed to a pool of resources for regeneration

Table 1. Outcomes of Kruskal-Wallis tests comparing average healing score among genotypes 9 and 16 d after lesion generation. * $p < 0.05$; *** $p < 0.001$

Time since wounding (d)	<i>H</i>	df	Pr(> <i>H</i>)
9	57.22	27	6.03×10^{-4} ***
16	43.31	27	0.024*

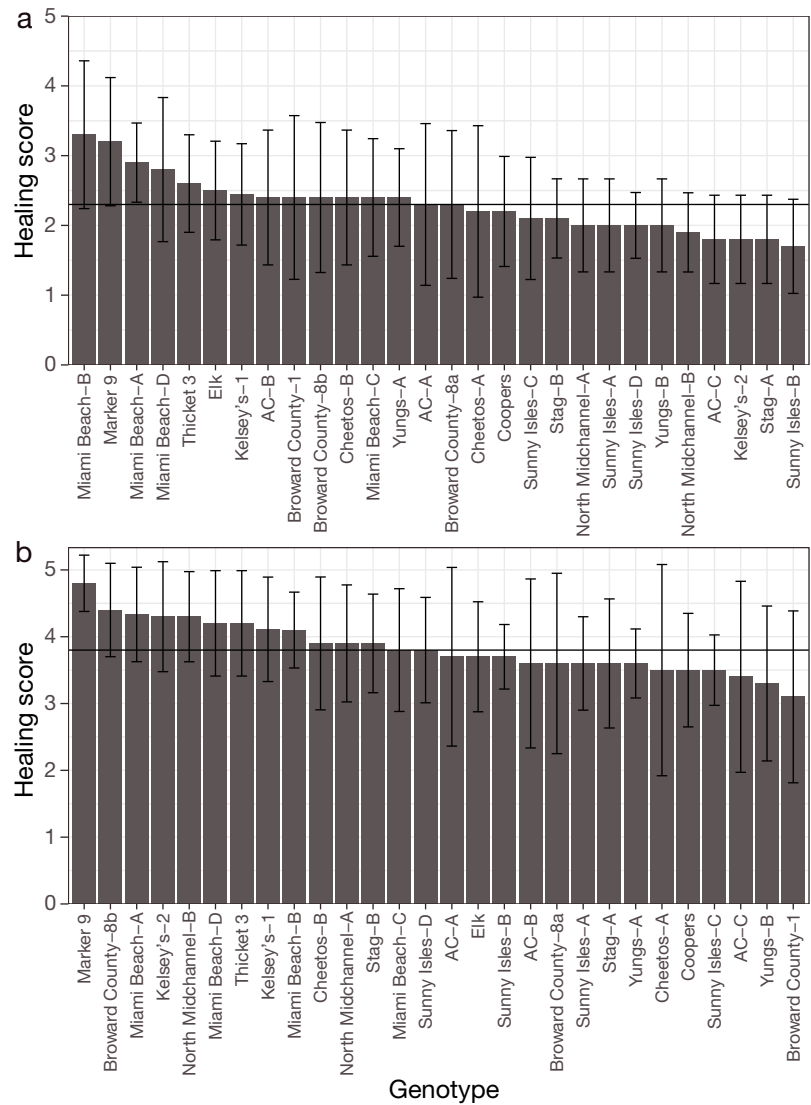


Fig. 2. Average category of healing ± 1 SD by genotype (a) 9 d after wounding and (b) 16 d after wounding. Horizontal lines indicate the average category of healing pooled across genotypes. See Fig. S1 for genotype source reef geographic locations

existing in the tissue surrounding the lesion (Meesters et al. 1994, Lirman 2000). However, the observed inverse relationship between colony size and recovery conflicts with a literature review's consensus that recovery rate increases with body size (attributed to a greater proportion of healthy tissue remaining to replenish the injured area), but the review focuses on slow-growing species without polyp dimorphism (Henry & Hart 2005). Significant differences in expression between branch bases and tips occur in staghorn genes, suggesting a strong division of labor between axial and radial polyps (Hemond et al. 2014); thus, apical lesions might not

Table 2. Outcomes of simple linear regressions modeling healing score as a function of explanatory variables 9 and 16 d after wounding. The unit for productivity is cm growth per cm of initial total linear extension. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Fixed effect	R ²	Beta	SE	T	Pr(>T)
9 d after wounding					
Initial colony size (cm)	0.101	-0.037	0.025	-1.497	0.150
Initial lesion area (cm ²)	0.281	-1.779	0.670	-2.655	0.016*
Avg. annual productivity	0.096	-0.209	0.166	-1.260	0.227
Zooxanthellae (no. cm ⁻²)	0.091	-3.958×10^{-7}	2.871×10^{-7}	-1.379	0.184
Chl a ($\mu\text{g cm}^{-2}$)	0.356	0.714	0.240	2.971	0.009**
Chl a (pg zooxanthellae cell ⁻¹)	0.007	-0.203	0.563	-0.361	0.722
Tissue density (mg cm ⁻²)	0.290	-0.730	0.269	-2.712	0.014*
Lipid density (mg cm ⁻²)	0.048	0.742	0.799	0.930	0.366
16 d after wounding					
Initial colony size (cm)	0.456	-0.067	0.017	-3.885	0.001***
Initial lesion area (cm ²)	0.042	-0.403	0.483	-0.835	0.416
Avg. annual productivity	0.033	0.091	0.114	0.802	0.433
Zooxanthellae (no. cm ⁻²)	0.152	3.310×10^{-7}	2.015×10^{-7}	-1.643	0.121
Chl a ($\mu\text{g cm}^{-2}$)	0.001	-0.028	0.222	-0.128	0.900
Chl a (pg zooxanthellae cell ⁻¹)	0.069	0.439	0.372	1.183	0.252
Tissue density (mg cm ⁻²)	0.090	20.402	0.285	-1.408	0.175
Lipid density (mg cm ⁻²)	0.102	-0.516	0.351	-1.469	0.158

benefit from colony-wide energy resources when compared to species with uniform polyps and greater pooling of resources. We might have observed faster recovery in smaller colonies because axial polyps (the site of linear extension) regenerated quickly within lesions and faster growth has been documented in smaller colonies of staghorn coral (Lirman et al. 2014). We hypothesize that wound size and the energetic resources adjacent to the lesion influence recovery immediately after wounding, and colony size effects appear later as the fragments begin to expend energy on extension.

Scleractinian corals host symbiotic zooxanthellae algae within their tissues that photosynthesize and translocate fixed carbon to the host to fuel coral calcification and growth (Muscatine 1990), and chl a is one of the primary photosynthetic pigments of zooxanthellae (Roth 2014). The positive relationship between chl a and healing rate documented here agrees with the findings of Lesneski (2020), who hypothesized that excess chl a might produce additional photosynthate to fuel recovery in staghorn coral. Moreover, photosynthetically active radiation decreases within the coral tissue (Wangpraseurt et al. 2012). Therefore, greater tissue thickness might reduce the amount of light available to fuel recovery, explaining the inverse relationship between healing and tissue density. Precoda et al. (2020) documented a negative correlation between growth and tissue density and hypothesized that investment in thicker tissue might come at the cost of growth rate, which

could also be the case for healing rate. These biological parameter influences might wane in the later stages of healing after most regeneration has occurred.

The observed slowed growth of wounded colonies adds to evidence for a tradeoff between growth and wound healing (Henry & Hart 2005). However, suppression of growth should not be of concern in staghorn restoration due to its rapid growth rate and because the species exhibits longer-term pruning vigor, whereby enhanced growth occurs in colonies over the months following fragmentation (Lirman et al. 2014). Importantly, we do not document a relationship between genotype recovery speed and growth rate, alleviating concern over how focusing on rapidly healing genotypes might result in the restoration of slower-growing colonies.

Our field study supports the findings of Kaufman et al. (2021), who documented variable healing rates among 18 staghorn genotypes in a laboratory experiment. We recommend that restoration practitioners identify rapidly healing genets through lesion recovery tracking and outplant these genotypes in higher abundance, particularly in high-energy environments where colonies are more vulnerable to fragmentation. Practitioners can increase the speed of lesion recovery in the nursery by working with smaller colonies and creating smaller lesions by pruning colonies closer to the apical tips. Importantly, these recommendations currently only apply to the nursery setting, and this experiment should be replicated at

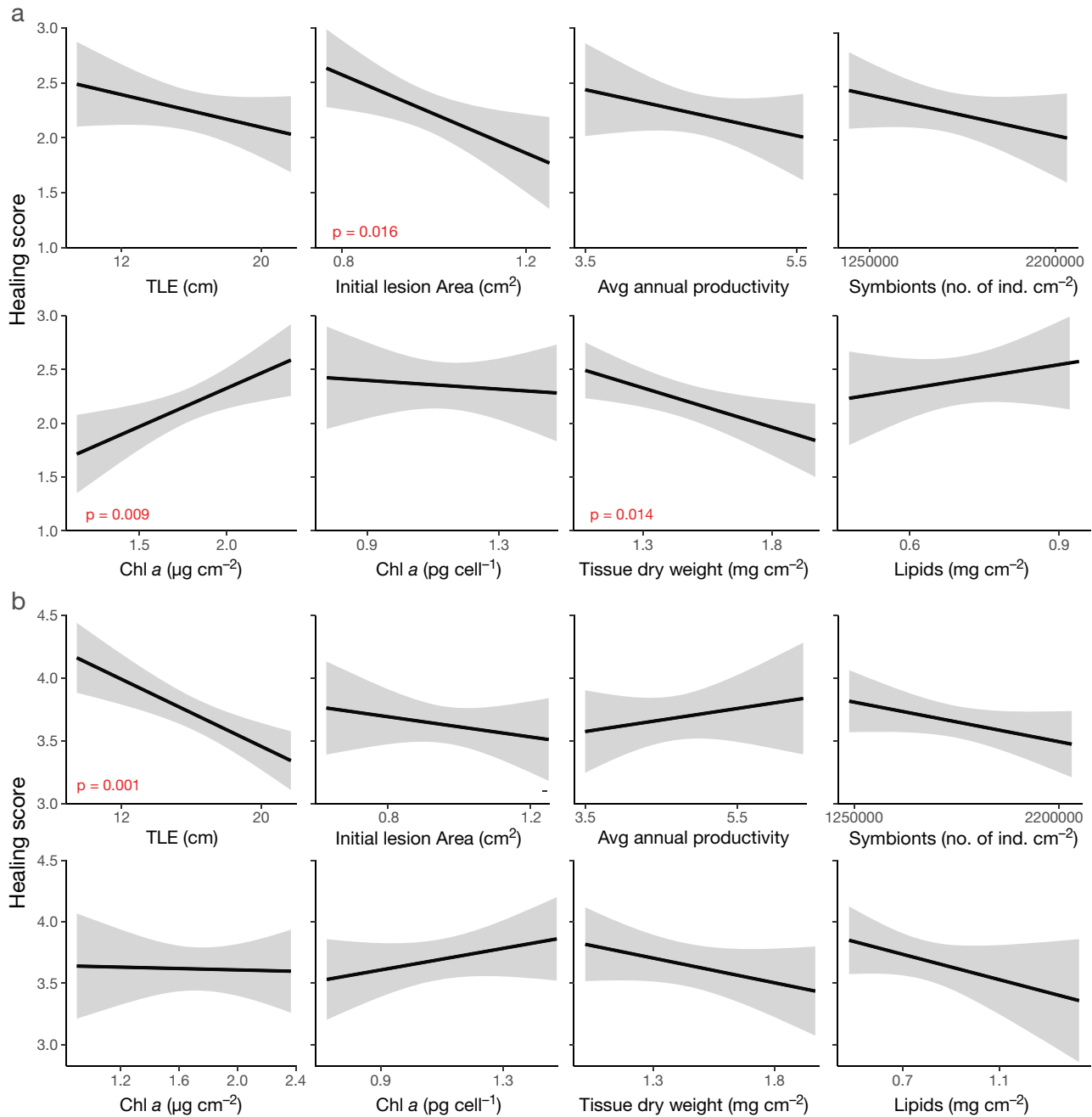


Fig. 3. Simple linear regression model predictions and 95% CIs for genotype healing score (a) 9 d after wounding and (b) 16 d after wounding as a function of explanatory variables. TLE: total linear extension. p-values of significant regression coefficients are indicated in red

restoration sites to determine if these patterns hold true on the reef, especially since van Woesik et al. (2021) found that smaller staghorn coral outplants experienced higher levels of mortality between 2012 and 2018 across the geographical subregions of Florida's Coral Reef. There is still a need for additional research examining recovery in staghorn coral, a novel field of research for this species, but there is great opportunity for restoration practition-

ers throughout the Caribbean to contribute to this important field.

Acknowledgements. We thank J. Unsworth, N. Rivas, J. Carrick, D. Hesley, and E. Watkins for their contributions to this project and A. Baker and C. Drury for reviewing this manuscript. This project was funded by NOAA's Restoration Center (award OAA-NMFS-HCPO-2016-2004840). Corals were collected under Florida Fish and Wildlife Commission Permit SAL-19-1794-SCRCP.

LITERATURE CITED

- ✦ Bak RPM (1983) Neoplasia, regeneration and growth in the reef-building coral *Acropora palmata*. *Mar Biol* 77: 221–227
- ✦ Baums IB, Baker AC, Davies SW, Grottoli AG and others (2019) Considerations for maximizing the adaptive potential of restored coral populations in the western Atlantic. *Ecol Appl* 29:e01978
- ✦ Boström-Einarsson L, Babcock RC, Bayraktarov E, Ceccarelli D and others (2020) Coral restoration—a systematic review of current methods, successes, failures and future directions. *PLOS ONE* 15:e0226631
- ✦ Cramer KL, Jackson JBC, Donovan MK, Greenstein BJ, Korpanty CA, Cook GM, Pandolfi JM (2020) Widespread loss of Caribbean acroporid corals was underway before coral bleaching and disease outbreaks. *Sci Adv* 6: eaax9395
- ✦ Denis V, Guillaume MMM, Goutx M, de Palmas S and others (2013) Fast growth may impair regeneration capacity in the branching coral *Acropora muricata*. *PLOS ONE* 8: e72618
- ✦ Enochs IC, Manzello DP, Jones PJ, Aguilar C and others (2018) The influence of diel carbonate chemistry fluctuations on the calcification rate of *Acropora cervicornis* under present day and future acidification conditions. *J Exp Mar Biol Ecol* 506:135–143
- ✦ Hemond EM, Kaluziak ST, Vollmer SV (2014) The genetics of colony form and function in Caribbean *Acropora* corals. *BMC Genomics* 15:1133
- ✦ Henry LA, Hart M (2005) Regeneration from injury and resource allocation in sponges and corals—a review. *Int Rev Hydrobiol* 90:125–158
- ✦ Highsmith RC (1982) Reproduction by fragmentation in corals. *Mar Ecol Prog Ser* 7:207–226
- ✦ Kaufman ML, Watkins E, van Hooionk R, Baker AC, Lirman D (2021) Thermal history influences lesion recovery of the threatened Caribbean staghorn coral *Acropora cervicornis* under heat stress. *Coral Reefs* 40:289–293
- ✦ Ladd MC, Miller MW, Hunt JH, Sharp WC, Burkepile DE (2018) Harnessing ecological processes to facilitate coral restoration. *Front Ecol Environ* 16:239–247
- ✦ Lesneski K (2020) Phenotypic and transcriptomic differences between colonies of staghorn coral inhabiting disparate microenvironments—implications for coral restoration. PhD dissertation, Boston University, MA
- ✦ Lirman D (2000) Lesion regeneration in the branching coral *Acropora palmata*: effects of colonization, colony size, lesion size, and lesion shape. *Mar Ecol Prog Ser* 197: 209–215
- ✦ Lirman D, Schopmeyer S, Galvan V, Drury C, Baker AC, Baums IB (2014) Growth dynamics of the threatened Caribbean staghorn coral *Acropora cervicornis*: influence of host genotype, symbiont identity, colony size, and environmental setting. *PLOS ONE* 9:e107253
- ✦ Meesters EH, Noordeloos M, Bak RPM (1994) Damage and regeneration: links to growth in the reef-building coral *Montastrea annularis*. *Mar Ecol Prog Ser* 112:119–128
- ✦ Miller MW, Colburn PJ, Pontes E, Williams DE, Bright AJ, Serrano XM, Peters EC (2019) Genotypic variation in disease susceptibility among cultured stocks of elkhorn and staghorn corals. *PeerJ* 7:e6751
- ✦ Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. *Ecosyst World* 25:75–87
- ✦ National Academies of Sciences, Engineering, and Medicine (2019) A research review of interventions to increase the persistence and resilience of coral reefs. National Academies Press, Washington, DC
- ✦ Nedimyer K, Gaines K, Roach S (2011) Coral Tree Nursery®: an innovative approach to growing corals in an ocean-based field nursery. *Aquacult Aquarium Conserv Legis* 4:442–446
- ✦ Okubo N (2008) Size-independent investment allocation to regeneration and growth of the branching coral *Acropora muricata*. *Galaxea J Coral Reef Stud* 10:83–87
- ✦ Precoda K, Hardt MJ, Baird AH, Madin JS (2020) Tissue biomass trades off with growth but not reproduction in corals. *Coral Reefs* 39:1027–1037
- ✦ Rinkevich B (1996) Do reproduction and regeneration in damaged corals compete for energy allocation? *Mar Ecol Prog Ser* 143:297–302
- ✦ Roth MS (2014) The engine of the reef: photobiology of the coral–algal symbiosis. *Front Microbiol* 5:422
- ✦ Schopmeyer SA, Lirman D, Bartels E, Gilliam DS and others (2017) Regional restoration benchmarks for *Acropora cervicornis*. *Coral Reefs* 36:1047–1057
- ✦ Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. *Proc Natl Acad Sci USA* 78: 2427–2431
- ✦ van Woesik R, Banister RB, Bartels E, Gilliam DS and others (2021) Differential survival of nursery-reared *Acropora cervicornis* outplants along the Florida reef tract. *Restor Ecol* 29:e13302
- ✦ Wangpraseurt D, Larkum AW, Ralph PJ, Kühl M (2012) Light gradients and optical microniches in coral tissues. *Front Microbiol* 3:316

Editorial responsibility: James McClintock,
Birmingham, Alabama, USA
Reviewed by: C. Whitaker Wagner Counsell and
2 anonymous referees

Submitted: December 18, 2020
Accepted: September 20, 2021
Proofs received from author(s): November 18, 2021