Vol. 570: 87–99, 2017
https://doi.org/10.3354/meps12114

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published April 27

Temperature and symbiosis affect lesion recovery in experimentally wounded, facultatively symbiotic temperate corals

E. M. Burmester[1,2,*], J. R. Finnerty[1], L. Kaufman[1], R. D. Rotjan[1,2,*]

[1]Boston University Department of Biology, 5 Cummington Mall, Boston, MA 02215, USA
[2]John H Prescott Marine Laboratory, Anderson-Cabot Center for Ocean Life, New England Aquarium, 1 Central Wharf, Boston, MA 02110, USA

ABSTRACT: The health of most reef-building corals depends upon an intracellular symbiosis with photosynthetic dinoflagellates of the genus *Symbiodinium* that is acutely sensitive to increasing ocean temperatures. However, distinguishing the individual effects of both temperature and symbiotic state on coral health is difficult to investigate experimentally in most tropical corals because the symbiosis is obligate. Here, we varied temperature (9, 18, 24°C) and symbiotic state (symbiotic, aposymbiotic) in the facultatively symbiotic, temperate scleractinian coral *Astrangia poculata* to explore the individual impact of temperature and symbiosis on wound healing, an important component of coral resilience, by determining wound size using calibrated photographs and characterizing developmental stage through the healing process over time. Symbiotic corals demonstrated a significant healing advantage over corals with lower densities of *S. psygmophilum* (aposymbiotic state), regardless of temperature. In addition, overall recovery success of both symbiotic states increased with temperature. These data suggest that a functional symbiotic relationship with *S. psygmophilum* promotes lesion recovery despite heterotrophic energy sources. Reductions in healing rate and tissue cover near the wound site under cold temperatures suggest that wound healing is compromised during the winter in these temperate corals. This study demonstrates that supplemental energy sources from symbiosis, coupled with optimal growth conditions, promote wound healing and may offer insight into factors enhancing wound recovery in tropical corals.

KEY WORDS: Coral · Recovery · Symbiosis · Temperature · Lesions

INTRODUCTION

One key determinant of coral resilience is the ability to heal from wounds. In nature, corals sustain lesions regularly, and from multiple causes, including corallivory (Kaufman 1981, Jompa & McCook 2003, Jayewardene & Birkeland 2006, Rotjan and Lewis 2008, Cole et al. 2008), algal abrasion (Coyer et al. 1993, Grace 2004), sedimentation (Nugues & Roberts 2003a,b), and hurricane activity (Bythell et al. 1993a). Tissue regeneration (by which tissue has re-grown and/or polyp body plan is reimposed) and full wound recovery (by which colony integrity is restored, including calcification) are energetically demanding (Oren et al. 2001, Henry & Hart 2005, Jayewardene et al. 2009, Lenihan & Edmunds 2010). As a result, wounding can lead to reductions in colony fecundity (Oren et al. 2001, Rotjan & Dimond 2010) and growth (Meesters et al. 1994, Jayewardene 2010, Lenihan & Edmunds 2010). Furthermore, until epithelial integrity is reestablished, wounded colonies are left with patches of bare calcium carbonate that are susceptible to overgrowth by benthic competitors such as algae and sponges (Meesters et al. 1996, 1997, Diaz-Pulido & McCook 2002, Jompa & McCook 2003, Rotjan & Lewis 2005). Such overgrowth may inhibit the recovery of coral tissue and overall colony growth (River & Edmunds 2001).

*Corresponding authors: eburmest@bu.edu, rrotjan@bu.edu

Therefore, the pace of healing and regeneration is critical to coral survival.

Healing rate can be influenced by a number of factors, including coral species (Bak & Steward-Van Es 1980), the size of the lesion (Meesters et al. 1996), and the ratio of wound perimeter to surface area (Meesters et al. 1997). Infrequent high-energy traumas tend to cause larger injuries, whereas due to their greater frequency, the fine-scale wounds that result from smaller disturbances (such as fish bites) can account for a greater loss of live tissue than large-scale wounds (Hughes & Jackson 1985, Bythell et al. 1993b, Rotjan & Lewis 2006). Therefore, it is particularly important to understand the factors that impact how rapidly corals heal from these small lesions.

Environmental stress also influences the rate of healing and regeneration (Fisher et al. 2007). For example, temperature-induced bleaching (Meesters & Bak 1993, Rotjan et al. 2006) and high sedimentation (Meesters et al. 1994, Cróquer et al. 2002) impair healing capability in corals. Because of their importance to coral resilience and sensitivity to environmental conditions, wound recovery and regeneration have been used as metrics to assess colony health in the field (Meesters et al. 1994) and in the laboratory (Work & Aeby 2010), and to forecast the ability of a coral to survive prevailing environmental conditions (Downs et al. 2005).

While lesion healing has been studied extensively in the field in a variety of species (reviewed by Henry & Hart 2005, and for example: Nagelkerken & Bak 1998, Denis et al. 2011, Cameron & Edmunds 2014) as well as across a range of intrinsic and extrinsic factors (e.g. Van Veghel & Bak 1994, Nagelkerken et al. 1999, Kramarsky-Winter & Loya 2000, Edmunds 2009), few studies have directly studied the impact of symbiosis on the healing process. In tropical corals, photosynthetic endosymbiotic alga of the genus *Symbiodinium* can provide up to 95% of the coral host's energy (Muscatine 1990); however, environmental stress (such as rising temperatures or changes in salinity) can decouple this obligate symbiosis, resulting in 'bleaching' (Hoegh-Guldberg 1999, Rowan 2004). This bleached state is not stable because of the resulting nutritional deficiency, and those colonies that are unable to shift or regain symbiotic partners will die (Grottoli et al. 2006, Baker et al. 2008). Because it is difficult to maintain tropical corals in a bleached state, and because high temperatures result in bleaching, it is difficult to experimentally distinguish the impacts of bleaching and temperature on wound healing (Henry & Hart 2005), although this is of particular interest to coral conservation (Edmunds & Lenihan 2010).

The northern star coral *Astrangia poculata* (= *A. danae*; Peters et al. 1988) is an ideal organism to decouple the effects of temperature and symbiosis because its distribution spans a wide range of temperatures and it exhibits a facultative symbiosis with *Symbiodinium*. *A. poculata* is a temperate species whose native range extends along the US east coast, from Florida and the Gulf of Mexico to Rhode Island (RI) (Dimond & Carrington 2007, Thornhill et al. 2008). In nature, *A. poculata* may be found in symbiosis with *S. psygmophilum* (Lajeunesse et al. 2012), but colonies may also be found in an aposymbiotic (relatively low density of *S. psygmophilum*) state in the same habitat with symbiotic colonies (Dimond et al. 2013). Even within a colony, symbiont densities can vary markedly among polyps, resulting in mixed or mottled phenotypes (Fig. 1). Regardless of symbiont state, all colonies of *A. poculata* rely heavily on heterotrophy as a source of energy (Szmant-Froelich & Pilson 1980). The symbiotic state of the colony is a function of zooxanthellae expulsion rates, i.e. aposymbiotic colonies actively maintain a very low density of *S. psygmophilum* through high expulsion rates (Dimond & Carrington 2007). Polyps of *A. poculata* remain functionally aposymbiotic until *S. psygmophilum* density reaches or exceeds 10^6 cells cm^{-2}, after which polyps appear consistently brown throughout the body column (Dimond & Carrington 2007). As a result, these functionally aposymbiotic colonies differ significantly from bleached corals, as the low density of *Symbiodinium* sp. within host cells is (1) not indicative of stress and (2) not the result of a breakdown in symbiotic pathways between alga and host. Thus, unlike corals in obligate symbioses, qualitative differences in symbiont density exist as stable states in nature. Additionally, the sympatric overlap of different symbiont states provides a natural experiment for exploring the relative roles of symbiont and host on different biological functions. Previous studies have exploited the facultative symbiosis of *A. poculata* to investigate the effects of the coral–algal symbiosis on coral health, including the effect of symbiont density on coral nutrition (Szmant-Froelich & Pilson 1980, 1984), resistance to ocean acidification (Holcomb et al. 2010, 2012), post-sedimentation recovery (Cohen et al. 2002), calcification and metabolism (Jacques & Pilson 1980, Cummings 1983, Jacques et al. 1983), and physical parameters of wound recovery (DeFilippo et al. 2016).

The objective of this study was to determine the effects of temperature and symbiont state on wound recovery in coral colonies. We conducted controlled laboratory experiments to investigate tissue recovery in naturally occurring symbiotic (high density of *S.*

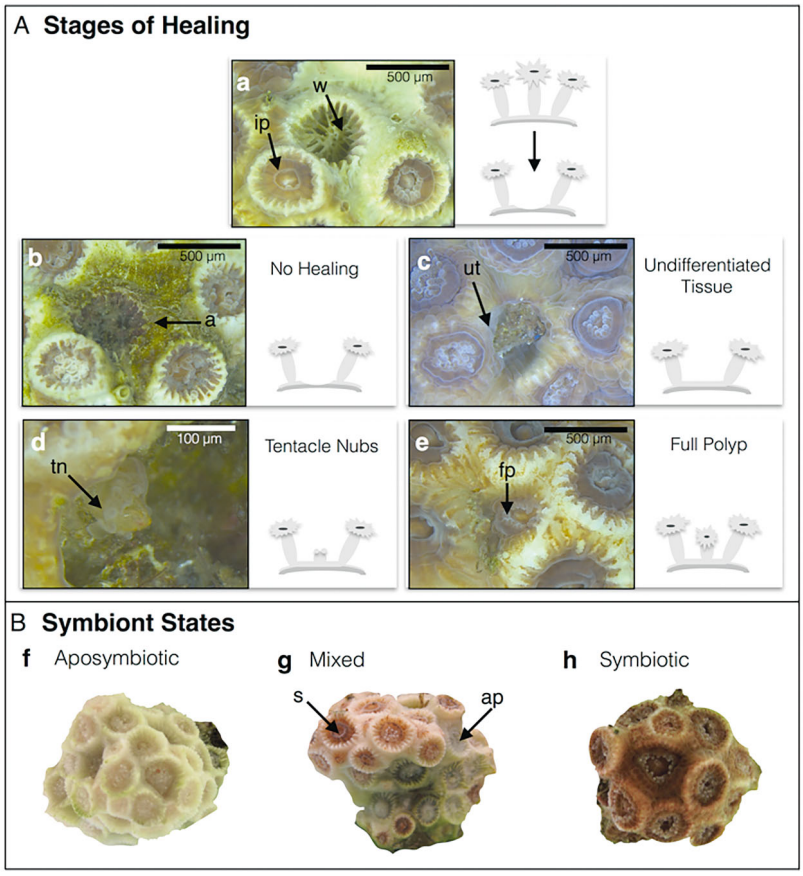

Fig. 1. (A) Stages of wound healing as demonstrated in *Astrangia poculata*. a: Experimentally-induced bare skeleton wound (w) from initial polyps (ip); b: no demonstration of recovery with wound site covered in algae (a); c: formation of undifferentiated tissue (ut) from wound edges (as shown) or from within calice; d: formation of tentacle nubs (tn); e: re-establishment of full polyp (fp) with fully functional tentacles. (B) Symbiont states in *A. poculata*. f: Aposymbiotic colonies appear white; g: mixed symbiont colonies have a range of symbiotic (s) and aposymbiotic (ap) polyps; h: fully symbiotic colonies appear brown

psygmophilum) and aposymbiotic (low density or absence of *S. psygmophilum*) colonies of *A. poculata* at 3 environmentally relevant temperatures characteristic of winter (9°C), summer (18°C), and a temperature above this coral's natural range (24°C). Several healing metrics indicate that symbiotic corals exhibit significantly greater healing ability at 18 and 24°C.

MATERIALS AND METHODS

Collection and husbandry

Colonies exhibiting a range of symbiont densities were collected from depths of 6 to 10 m at Fort Wetherill State Park in Jamestown, RI (41° 28′ 40″ N, 71° 21′ 34″ W) from late spring through early fall 2014. As described by DeFilippo et al. (2016), specimens were housed in a flow-through aquarium system at the New England Aquarium. Seawater was filtered using a protein skimmer and UV treatment, and water quality was measured weekly. Tanks were illuminated for 10 h d^{-1} using T5 HO fluorescent lighting fixtures (Hamilton Technology, Aruba Sun T5-V Series). Photosynthetically active radiation (PAR) was kept constant at an average of 37.5 ± 10.1 µmol m^{-2} s^{-1}. Corals were fed daily with frozen copepods (JEHM) directed to all polyps within each colony using a turkey baster. All colonies were acclimated to 18°C for at least 2 wk prior to commencing healing trials. Throughout the duration of the initial acclimation and the experimental trials, all colonies were cleaned weekly using a soft nylon brush to remove algae and forceps to remove epibionts (e.g. polychaetes). Only bare skeletal regions (i.e. no live tissue) were brushed in this manner to avoid inflicting additional wounds or tissue damage. Colonies were assigned symbiont state as described by Dimond & Carrington (2007) and demonstrated by Dimond et al. (2013) and DeFilippo et al. (2016), where symbiont state was determined visually using color as a proxy for chlorophyll density. Additionally, to ensure this method accurately represented our system, a random subset of photos of 20 symbiotic and 20 aposymbiotic colonies were analyzed for approximated chlorophyll density using the protocol of Dimond & Carrington (2007). On average, the approximated chlorophyll densities for symbiotic colonies (0.45 ± 0.04 µg cm^{-2}, mean ± SE) were significantly higher than those of aposymbiotic colonies (0.13 ± 0.02 µg cm^{-2}; ANOVA, $F_{1,38} = 53.4058$, $p < 0.0001$).

Experimental setup and temperature manipulation

Symbiotic and aposymbiotic colonies were sorted into pairs for treatment. Colony pairs were randomly placed into treatment groups, controlling for size between treatments. Sizing was based on colony mass, a measurement of whole colony mass after

the removal of excess water from bare skeletal regions. This measurement exhibited a high degree of precision; 5 separate mass measurements were performed on each of 37 colonies, and the mean variation in measurements performed on the same individual was 0.69%. Overall, average colony mass was 6.32 ± 0.27 g (SE). Paired colonies were placed adjacent to one another on a submerged platform in a randomized fashion in order to control for tank microclimate (lighting, flow, etc.). However, each pair of colonies was kept at a distance of 6–10 cm to avoid direct interaction. Three temperature treatments were tested: 9°C (within the natural range for winter), 18°C (within the natural range for summer), and 24°C (outside the typical temperature range for this site). Experimental temperatures were chosen based on environmental data for the area (NOAA Tides & Currents, Newport, RI: site 8452660), where sea surface temperatures ranged from 0.5 to 23°C during the 2014 calendar year. To account for seasonality and to accommodate the limitations of our system (i.e. tank space, ability to manipulate multiple temperatures simultaneously), 9°C treatments were performed in the winter months and 24°C treatments were performed in the summer and early fall. For continuity between seasonal experiments and to control for captivity duration, 2 trials of the 18°C group were carried out: 1 in winter and 1 in summer. Winter and summer 18°C trials were then compared to determine any potential changes due to season, captivity duration, or husbandry (there were none). Therefore, at 18°C, 40 colonies of each symbiont state were sampled; meanwhile, 20 colonies of each symbiont type were sampled at 9 and 24°C. At 18°C, 1 wounded aposymbiotic colony was lost during routine tank cleaning (non-mortality), reducing the sample size of that group to 39 colonies (and a total of 159 colonies overall). Corals in the 9 and 24°C tanks were subjected to a temperature increase or decrease from 18°C at a rate of 1°C every 12 h. Colonies in these tanks were acclimated at their final experimental temperatures for 1 wk prior to wounding.

Wounding

All experimental corals were wounded in a consistent fashion: a single polyp, centrally located in the colony, was removed using a scalpel, and the calyx was cleared of tissue using a Waterpik (Fig. 1) before the immediate surrounding skeleton was filed to a uniform basal skeletal height using a diamond-coated file, followed by a final cleaning with a Waterpik. These lesions were designed to mimic destructive forces that remove both tissue and skeleton (i.e. predation, mechanical injury, storm damage, etc.), but were inflicted in such a way to create as uniform a wound as possible between replicates. Colony mass for each colony was measured before and after wounding to determine wound size (via change in mass).

Lesion recovery

Lesions were photographed using a Leica M165FC stereomicroscope immediately after wounding (Day 0) and at 10 time points post-wounding (5, 10, 15, 20, 25, 30, 40, 50, 60, and 75 d). Photographs from Day 0 were used to calibrate post-wound photos to ensure consistent magnification as well as colony angle and position. Photographs were scored for whether or not colonies exhibited signs of healing (i.e. new tissue within the wound site) as well as the developmental state of new tissue (undifferentiated tissue, tentacle nubs, or full polyp; Fig. 1A [panels c–e, respectively]). These 3 stages were chosen because they can be identified unambiguously, and they represent important developmental landmarks on the way towards full healing, which begins with the formation of undifferentiated tissue, followed by initiation of tentacle formation, and concludes with the formation of a full polyp that has the ability to feed (as evidenced by tentacular contraction; Fig. 1A). Achievement of this final developmental landmark was recognized as the indicator of healing success. Wound surface area was determined for Days 0 and 75 using hand-drawn area tools that are part of the Leica M165FC software. Three measurements were taken for each photo, and the wound surface area was estimated as their average. The percent change in wound surface area was calculated as final wound area minus the initial wound area divided by the initial wound area.

Colony mass

Bare skeletal regions of each colony were thoroughly cleaned, avoiding live tissue, with a soft nylon brush and forceps prior to each measurement of mass to prevent confounding colony growth with algal growth. The difference between the initial post-wound mass (Day 0) and final mass (Day 75) was

used to calculate a healing mass differential, i.e. the gain or loss of mass over the healing process. We also determined the mass of the wound itself by subtracting the colony's post-wound mass from its pre-wound mass. Two specimens were removed from the analysis (1 each from the 9°C symbiotic and aposymbiotic groups) because their colony mass at the time of wounding had not been accurately recorded.

Photosynthetic efficiency

In order to test for symbiont performance, photosynthetic efficiency (maximum quantum yield: F_v/F_m) was measured for each colony at 3 time points over the course of the experiment (0, 30, and 60 d) using a Walz JUNIOR-PAM pulse-amplitude modulated fluorescence meter. All readings were taken between 11:00 and 13:00 h on each day to reduce diel fluctuations between readings across time points. As described by DeFilippo et al. (2016), colonies were acclimated to darkness for 30 min in a closed dark container and then transferred individually to a glass beaker with 5–7 cm of seawater stored within a dark box to reduce light exposure during measurement. For each measurement, polyps were first exposed to 6 s of far-red illumination to determine minimal fluorescence while dark-adapted (F_0). They were then exposed to 0.6 s of a saturating pulse (10 000 µmol m^{-2} s^{-1}) to determine maximal fluorescence (F_m). The change in fluorescence between F_0 and F_m (ΔF) was divided by maximal fluorescence (F_m) to calculate maximum quantum yield (F_v/F_m; Suggett et al. 2010). Readings were taken with a light fiber held approximately 1 mm from the oral groove of 3 haphazardly selected polyps per colony, and the resulting average maximum quantum was used to represent the colony.

Documentation of altered symbiotic state

We visually inspected microscope photos from Day 75 to qualitatively assess whether any of the polyps within a 2-polyp radius of the wound had undergone a change in symbiont density as evidenced by a shift in color. Shifts in symbiont density were quantified as the percentage of polyps changing symbiont state between Day 0 and Day 75; only polyp-level shifts from aposymbiotic to symbiotic were observed. Such shifts became apparent when individual polyps in a colony would develop clusters of brown spots (i.e. symbiosomes) detectable under the microscope.

Statistics

Healing initiation (proportion of colonies having developed any of the 3 landmark stages of healing) and healing success (proportion of colonies having regenerated new full polyps) were analyzed using generalized logistic mixed models (GLMMs) in the lme4 package in R. Logistic models were compared using Akaike's information criterion (AIC) scores, and a reduction in AIC of at least 2 was required to accept a given model over another or to validate the inclusion of a new variable in the model (Burnham & Anderson 2002). In the case of 2 models whose AIC scores differed by less than 2, the simpler model was chosen (Burnham & Anderson 2002). Odds ratios were generated using exponentiated estimates. The effects of symbiont state and temperature on colony mass and surface area were compared using Laplace-approximated generalized linear mixed models on the nlme package in R (R Core Team 2013, Pinheiro et al. 2017). In order to test for the potential effect of colony size and wound size on recovery and colony mass, we included wound mass, initial mass, and initial wound surface area as fixed effects in addition to symbiont state and temperature in the analyses of healing initiation, healing success, wound closure, and colony mass. The impacts of added fixed, interaction, and random effects were determined using a forward and reverse stepwise approach (using AIC for logistic models and likelihood ratio tests for linear models). Additionally, tank assignment was designated as a random effect in all generalized logistic and linear models to control for potential consequences of multiple colony pairs in the same tank.

In order to test for changes in photosynthetic efficiency over time, maximum quantum yield was analyzed between symbiont states and temperatures over time using a restricted maximum liklihood (REML)-fitted GLMM in the lme4 package in R (R Core Team 2013, Bates et al. 2015). Individual coral identity was nested within tank in order to control for changes in an individual across time points and to account for potential confounds of tank housing. Model selection criteria were based on reductions in AIC in the same manner as logistic model selections.

Fisher's exact tests and ANOVAs were used to determine the effect of shifts in symbiont density on the relative ratios of healing ability and stage, and relative means of colony mass and surface area recovery, respectively, for aposymbiotic corals at 18 and 24°C. No colonies at 9°C showed signs of shifts in symbiont density, and this group was therefore excluded from this analysis.

RESULTS

Healing initiation and success

Overall, there was a significant impact of symbiosis on the healing process (Fig. 2). Adjusted for tank grouping, symbiotic corals (40 out of 80 colonies) were 2.4 times as likely as aposymbiotic (25/79) corals to have initiated healing (developed new tissue of any kind) at the wound site (Table 1), and 5.8 times as likely to have successfully completed healing (reached the full polyp stage) by the end of the trial (15/80 symbiotic; 3/79 aposymbiotic; Table 2). Healing initiation also increased with temperature (Fig. 2): with random effects, the odds ratio of exhibiting any healing at 24°C (23/40) was 4.7 times greater than at 9°C (9/40) and 1.6 times greater than at 18°C (33/79), while the odds ratio for corals at 18°C was 2.9 times that of corals kept at 9°C. None of the 9°C colonies developed full polyps by the end of the trial; however, among those colonies that did successfully complete the healing process, the odds ratio of complete healing at 24°C (10/40) was 3.2 times greater than at 18°C (8/79). Consistent with these observations, both symbiont state and temperature were found to be significant predictors of healing initiation in a GLMM analysis (Table 3). However, neither the interaction between these 2 variables nor the added fixed effects of mass, wound mass, and wound surface area significantly improved the model (AIC 208.6, Table 3). Likewise, only symbiont state and temperature were found to be significant predictors of full polyp formation (i.e. healing success) in corals at 18 and 24°C (AIC 68.64, Table 4).

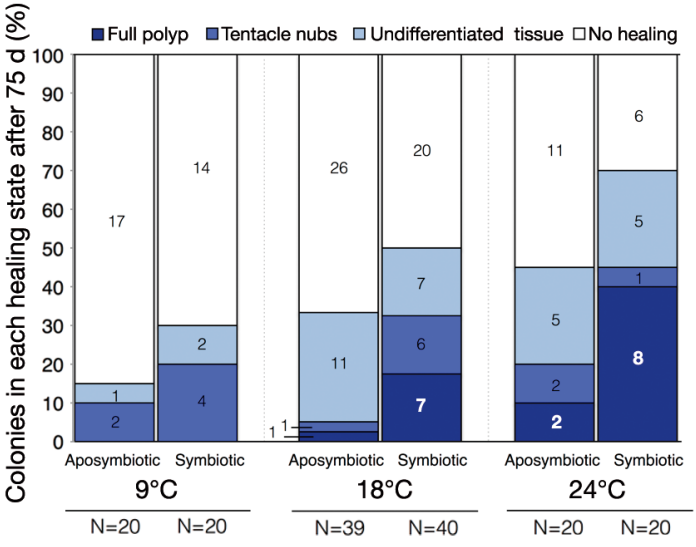

Fig. 2. Proportion in each healing stage of symbiotic and aposymbiotic colonies of *Astrangia poculata* maintained at 3 temperatures (9, 18, 24°C) 75 d post-wounding. Numbers in each column represent total number of colonies at that healing stage. Cumulative areas in blue delineate colonies that have demonstrated signs of healing, while bars in dark blue indicate only those colonies that have achieved successful healing through the formation of a complete polyp

Table 1. Odds ratios (with 95% CI) for fixed effect comparisons of healing ability of *Astrangia poculata*

Effect	Odds ratio (95% CI)
Symbiont state	2.370 (1.205, 4.663)
9°C: 18°C	2.856 (1.033, 7.898)
9°C: 24°C	4.664 (1.459, 14.910)
18°C: 24°C	1.633 (0.6325, 4.215)

Table 2. Odds ratios (with 95% confidence interval, fixed effect comparisons of healing success (polyp formation) of *Astrangia poculata*

Effect	Odds ratio (95% CI)
Symbiont state	5.769 (1.346, 24.732)
Temperature	3.253 (0.927, 11.418)

Table 3. Laplace-approximated generalized mixed logistic regression for healing ability of *Astrangia poculata* (AIC = 208.6). S: symbiotic; A: aposymbiotic. For fixed effects, symbols indicate significance considered at $^{**}p < 0.001$, $^{*}p < 0.05$

Effect	Estimate	SE	*Z*	p
Rooted under aposymbiotic and 9°C conditions				
Intercept	−1.7812	0.4876	−3.653	0.0003**
Symbiont state (S)	0.8627	0.3452	2.499	0.0124*
18°C	1.0496	0.5189	2.023	0.0431*
24°C	1.5399	0.5929	2.597	0.0094*
Rooted under symbiotic and 18°C conditions				
Intercept	0.1311	0.3193	0.411	0.6813
Symbiont state (A)	−0.8627	0.3452	−2.499	0.0124*
9°C	−1.0496	0.5189	−2.023	0.0431*
24°C	0.4903	0.4839	1.013	0.3109

Table 4. Laplace-approximated generalized mixed logistic regression for healing success (rooted under aposymbiotic and 18°C conditions) (AIC = 68.64). S: symbiotic. For fixed effects, symbols indicate significance considered at $^{**}p < 0.001$, $^{*}p < 0.05$, $^{+}p < 0.01$

Effect	Estimate	SE	*Z*	p
Intercept	−2.4522	0.7477	−3.280	0.0010**
Symbiont state (S)	1.7525	0.7427	2.360	0.0183*
Temperature (24°C)	1.1796	0.6406	1.842	0.0655+

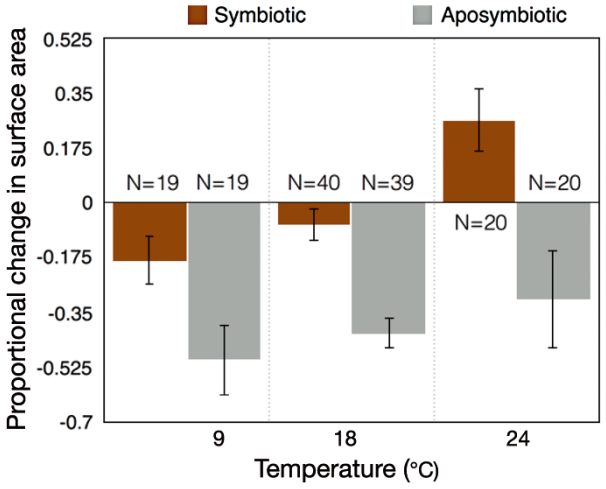

Fig. 3. Proportional change in wound surface area on *Astrangia poculata* after 75 d normalized over the initial wound surface area. Values were measured from aligned photographs by dividing the final wound surface area on Day 75 by the initial wound surface area on Day 0. Negative values indicate an increase in wound size, while positive values indicate a decline in wound size. Error bars are SE

Wound closure

On average, the inflicted wounds were (mean ± SE) 38.4 ± 1.2 mm^2 in area and accounted for a loss in mass of 0.14 ± 0.01 g. With the exception of symbiotic corals housed at 24°C, colonies, on average, experienced an increase in wound surface area, representing an expansion of the wound site (Fig. 3). All (100%) symbiotic colonies experienced a decrease in wound size at 24°C, while none (0%) of the colonies (regardless of symbiont density) experienced such a decline at 9°C. More symbiotic colonies (32.5%) saw reductions in wound size at 18°C than did aposymbiotic colonies (18%). A similar proportion of aposymbiotic colonies were able to restore any lost surface area at 18°C (18%) and 24°C (15%). The proportion of tissue loss was lower in symbiotic corals (group mean, M = 0.098) than aposymbiotic corals (M = 0.195). Temperature also had a significant effect on wound surface area (Table 5), as corals in warmer water recovered a higher proportion of tissue than those in cooler water (M_9 = −0.349, M_{18} = −0.137, M_{24} = 0.046). Again, symbiont state and temperature were significant contributors to the best mixed model (Table 5), although the effect of temperature was reduced (p = 0.0918).

Colony mass

Except for the 24°C symbiotic group, colonies lost mass (g) over time (Fig. 4). Symbiotic colonies (M = −0.004 g) lost less mass than did aposymbiotic colonies (M = −0.409 g). Similarly, corals kept at higher temperatures experienced less tissue loss than colonies at lower temperatures, with the colonies at 24°C experiencing a slight gain in mass on average (M_9 = −0.345 g, M_{18} = −0.242 g, M_{24}= 0.021 g; Fig. 4). Both symbitont state and temperature had a significant impact on colony mass, but there was no significant interaction between these 2 variables (Table 6). Colony mass, wound mass, and initial wound surface area had no significant effects.

Table 5. Laplace-approximated generalized linear mixed model for wound surface area (AIC = 218.8). S: symbiotic; A: aposymbiotic. For fixed effects, symbols indicate significance considered at $^{**}p < 0.001$, $^{*}p < 0.05$, $^{+}p < 0.01$

Effect	Estimate	SE	df	*t*	p
Rooted under aposymbiotic and 9°C conditions					
Intercept	−0.5382	0.1138	136	−4.7289	<0.0001**
Symbiont state (S)	0.3977	0.0670	136	5.9463	<0.0001**
18°C	0.0908	0.1347	19	0.6737	0.5086
24°C	0.2822	0.1588	18	1.7761	0.0918+
Rooted under symbiotic and 18°C conditions					
Intercept	−0.0497	0.0860	136	−0.5774	0.5646
Symbiont state (A)	−0.3977	0.0670	136	−5.9463	<0.0001**
9°C	−0.0908	0.1347	19	−0.6737	0.5086
24°C	0.1914	0.1404	19	1.3631	0.1888

Photosynthetic efficiency

Regardless of symbiont state, measurable levels of photosynthesis were observed in all treatment groups (Fig. 5). Symbiotic colonies had higher values for maximum quantum yield (F_v/F_m), with the exception of symbiotic colonies kept at 9°C (which demonstrated photosynthetic efficiency levels similar to those of aposymbiotic corals). Aposymbiotic colonies, on the other hand, demonstrated consistently similar F_v/F_m values regardless of temperature. The data show a slight significant decline in photochemical efficiency for symbiotic corals over time, but a slight significant increase for aposymbiotic corals. The preferred model included only symbiont state and the interactions between symbiont state and temperature as well as symbiont state and time (Table 7). Based on our model selection criteria (requiring a reduction in AIC of at least 2 to permit the inclusion of new fixed effects), there was little support for the inclusion of other fixed effects (temperature and time) or interactions of fixed effects (temperature/time).

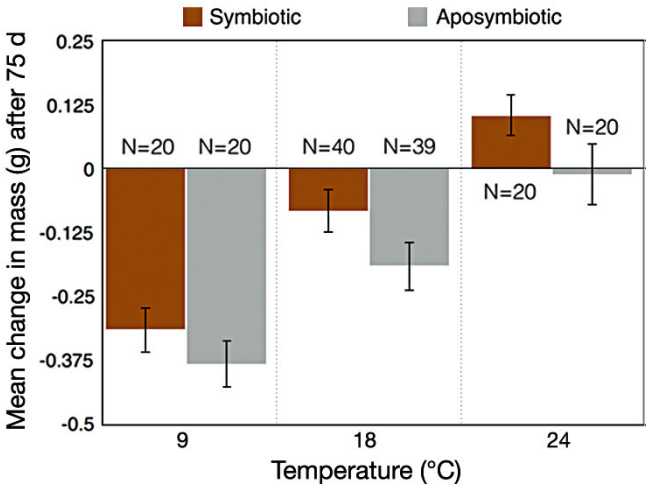

Fig. 4. Mean change in *Astrangia poculata* colony mass 75 d after wounding. Values were calculated by subtracting the final mass of each colony from its post-wound mass. Error bars are SE

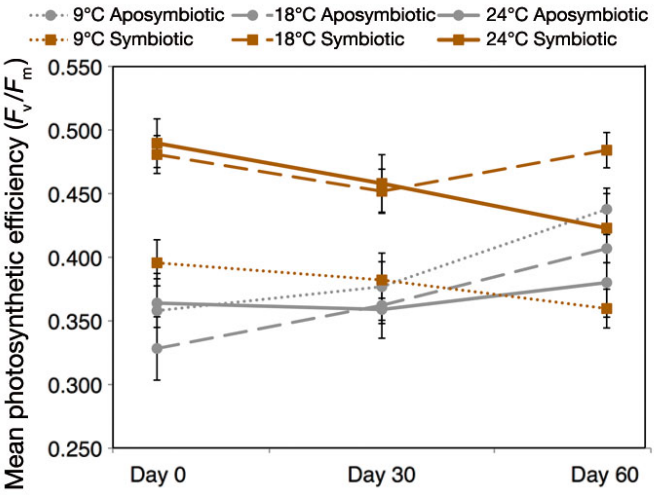

Fig. 5. Mean photosynthetic efficiency (F_v/F_m) as measured via pulse-amplitude modulated fluorescence (Walz JUNIOR-PAM). Error bars are SE. For all 9°C and 24°C groups, N = 20; for 18°C symbiotic corals, N = 40; and for 18°C aposymbiotic corals, N = 39

Table 6. Laplace approximated generalized linear mixed model for growth (AIC = −8.535). S: symbiotic; A: aposymbiotic. Symbols indicate significance considered at $^{**}p < 0.001$, $^{*}p < 0.05$

Effect	Estimate	SE	df	*t*	p
Rooted under aposymbiotic and 9°C conditions					
Intercept	−0.2686	0.0569	133	−4.7214	<0.0001**
Symbiont state (S)	0.1073	0.0322	133	3.3317	0.0011*
18°C	0.1700	0.0673	19	2.5258	0.0206*
24°C	0.2364	0.0794	19	2.9784	0.0077*
Rooted under symbiotic and 18°C conditions					
Intercept	0.0087	0.0426	133	0.2040	0.8387
Symbiont state (A)	−0.1073	0.0322	133	3.3317	0.0011*
9°C	−0.1700	0.0673	19	−2.5258	0.0206*
24°C	0.0664	0.0698	19	0.9516	0.3533

Symbiont state switching

At 18 and 24°C, some polyps of aposymbiotic corals were found to switch symbiont states, as indicated by pockets of high *Symbiodinium psygmophilum* density along the body column or oral groove. This phenomenon was significantly more likely in colonies at 24°C (15/20; 75%) than in colonies kept at 18°C (18/39; 46.15%; 1-tailed Fisher's exact test; n = 59, p = 0.0319). However, among these corals, there was no significant effect of symbiont switching on healing initiation (2-tailed Fisher's exact test; n = 59, p = 0.4232), colony mass ($\bar{x}_{switch}$ = −0.1244 g; $\bar{x}_{non\text{-}switch}$ = −0.1313 g; n = 59, $F_{1,57}$ = 0.0075, p = 0.9310), or wound closure ($\bar{x}_{switch}$ = −0.441; $\bar{x}_{non\text{-}switch}$ = −0.303; n = 59, $F_{1,57}$ = 1.1012, p = 0.2984). It should be noted that no aposymbiotic colonies ever completely switched states where every polyp in the colony transitioned from white to brown.

DISCUSSION

Overall, our data suggest that symbiotic colonies exhibit a clear advantage over aposymbiotic colonies with respect to single-polyp wound healing. At all temperatures, a greater proportion of symbiotic corals developed new tissue at the wound site and went on to develop full polyps. Likewise, regardless of temperature, symbiotic corals lost less mass and less tissue area at the lesion site after wounding (to the point of partially to fully closing lesions at 24°C). Therefore, symbiotic corals proved more successful at resisting wound expansion and achieving wound closure for single polyp lesions. These results are consistent with previous studies, which suggested that symbiotic colonies of *Astrangia poculata* may be able to recover a higher proportion of multipolyp wounds from small pockets of residual tissue (DeFilippo et al. 2016). Additionally, wounding experiments on another temperate and facultatively symbiotic coral, *Oculina patagonica* (Fine et al. 2001), demonstrated a similar advantage to symbiosis, whereby the percentage recovery of lesions was greater in unbleached colonies than partially bleached or fully bleached (which did not recover at all) colonies (Fine et al. 2002).

Table 7. REML-fitted generalized linear mixed model for photosynthetic efficiency (AIC = −678.4). S: symbiotic; A: aposymbiotic. Significance (*) assumed from *t*-values (df = 452) at $p < 0.05$

Effect	Estimate	SE	*t*
Rooted under aposymbiotic and 18°C conditions			
Intercept	0.3292	0.0220	14.950*
Symbiont state (S)	0.1569	0.0228	6.877*
Symbiont state (A): Temperature (9°C)	0.0342	0.0370	0.925
Symbiont state (S): Temperature (9°C)	−0.0926	0.0254	−3.652*
Symbiont state (A): Temperature (24°C)	−0.0048	0.0328	−0.145
Symbiont state (S): Temperature (24°C)	−0.0044	0.0260	−0.171
Symbiont state (A): Time	0.0012	0.0003	4.239*
Symbiont state (S): Time	−0.0005	0.0003	−1.940*
Rooted under aposymbiotic and 9°C conditions			
Intercept	0.3634	0.0318	11.414*
Symbiont state (S)	0.1569	0.0228	6.877*
Symbiont state (A): Temperature (18°C)	−0.0342	0.0370	−0.925
Symbiont state (S): Temperature (18°C)	0.0926	0.0254	3.652*
Symbiont state (A): Temperature (24°C)	−0.0390	0.0413	−0.942
Symbiont state (S): Temperature (24°C)	−0.0881	0.0298	2.958*
Symbiont state (A): Time	0.0012	0.0003	4.239*
Symbiont state (S): Time	−0.0005	0.0003	−1.940*

The positive effect of symbiosis on healing observed here could be attributed to a number of benefits conferred to the corals by their symbiotic partners, including (1) higher energy reserves and tissue content at the time of wounding, (2) added energy availability during healing due to active photosynthesis, or (3) potential direct symbiont contribution to the healing pathway (Fine et al. 2002). Because we also found that aposymbiotic polyps can switch partially or fully to a symbiotic state — and that these switches did not significantly impact the colony's ability to heal or grow — it is possible that symbiotic advantage accrues from a long-term association. Alternatively, a related study on the facultatively symbiotic *O. patagonica* (Fine et al. 2002) determined that carbon can be preferentially translocated to recovering tissue from a distance of 4–5 cm away; however, translocation only occurs in fully unbleached (and not partially bleached, 30–80%) colonies. This suggests a bleaching threshold (or a minimum density of *Symbiodinium* spp.) below which colonial integration and resource translocation is disrupted. There could also be an energetic cost to symbiont acquisition that overcomes the potential immediate energy gain from photosynthesis (Hill & Hill 2012). In tropical corals, which rely more heavily on photosynthesis to supply their nutritional needs, this disparity in healing ability between the symbiotic and aposymbiotic states could have significant impacts on coral health, particularly after bleaching. Even after returning to a full symbiont load, the diminishment of energetic reserves that results from a prolonged period of bleaching could undermine wound recovery for an extended period of time (Meesters & Bak 1993). This could compound the impact of bleaching and elevate post-bleaching mortality rates.

While our study does not directly address the possible advantages of a facultatively symbiotic life history, it does raise some important questions about the costs, benefits, and dynamics of the coral–algal symbiosis. While symbiosis clearly enhanced healing initiation, wound closure, and the development of full polyps in the current study, naturally occurring, aposymbiotic colonies of *A. poculata* are abundant in the field (Dimond et al. 2013). Furthermore, while symbiont state can be manipulated, switching between symbiont states does not appear to be common in nature (Dimond & Carrington 2008). The prevalence of aposymbiotic colonies in nature therefore suggests a cost to maintaining a high density of *Symbiodinium* that outweighs the observed advantages of the symbiotic state on colony health and recovery. Dimond et al. (2013) suggested that this cost may be most pronounced at cold, winter temperatures when colonies are dormant. In winter, all colonies experience a net loss of tissue, but aposymbiotic colonies lose less tissue than symbiotic colonies (Dimond et al. 2013). Thus, while symbiotic colonies have enhanced healing potential in summer, they may also need to compensate for greater tissue loss during the winter.

Environmental temperature had a direct effect on tissue loss and replacement in this experiment, regardless of symbiotic state. At 9°C, a natural winter temperature, both aposymbiotic and symbiotic colonies experienced greater tissue loss at the wound site and lost more total mass over time than at 18°C (a temperature more typical of summer) or 24°C (a temperature not typically encountered by *A. poculata* in Rhode Island). Similarly, the smallest statistical difference between symbiotic and aposymbiotic colonies occurred at 9°C, as colonies of both symbiont states had low rates of wound recovery; these cold-exposed colonies were less likely to develop new tissue at the wound site, and no colonies were able to successfully complete the regeneration process. These results are consistent with previous studies that found temperature to be a major driver of calcification and growth in both obligate and facultative

zooxanthellate and azooxanthellate corals (Jacques et al. 1983, Miller 1995, Marshall & Clode 2004, Edmunds 2005, Dimond et al. 2013). However, it should be noted that growth and regeneration may be unrelated or even competing life traits, particularly in times of stress (Denis et al. 2013). The drop in healing initiation, healing completion, and colony mass likely derives from a significant decline in photosynthetic efficiency with decreasing temperature as well as a potential decrease in oxidative respiration below 11.5°C (Jacques et al. 1983). Previous studies (Jacques et al. 1983, Dimond et al. 2013) have noted a state of metabolic dormancy in *A. poculata* below 10°C, characterized by the retraction of polyps into their calices and the inability to feed. Interestingly, in our study, all colonies at 9°C were actively feeding over the course of the experiment, potentially explaining the ability of some colonies to exhibit some healing despite their potentially reduced metabolism and lack of energetic input from photosynthesis. Additionally, while 9°C represents a typical winter temperature for this habitat, it is far from the lowest temperature encountered by wild colonies of *A. poculata* (0.3°C, NOAA Tides & Currents, Newport, RI: site 8452660). Therefore, as documented by Dimond et al. (2013), it is possible that quiescence could have a significant and inverse impact on healing with regards to symbiont state than observed in this study.

In contrast to 9°C, colonies at 24°C demonstrated the greatest wound recovery, despite experiencing a temperature outside the typical range of their natural habitat (which ranged from 0.3 to 23°C in 2014 [the year of this study]). Therefore, for this coral, these data support that survivability and, therefore, habitat range are more likely being limited by exposure to cold, winter temperatures than prolonged exposure to very warm summer temperatures (Dimond et al. 2013).

One hypothesis for the observed increase in healing and survivability by symbiotic corals is increased photosynthetic efficiency. Over time, there was an increase in photosynthetic efficiency in aposymbiotic colonies. Surprisingly, there was an inverse relationship with temperature (i.e. corals at 9°C experienced the greatest rise in photosynthetic efficiency). However, this is very likely due to the fact that, in colder temperatures, colonies tended to lose more live tissue and, subsequently, bare skeletal scars were covered in adventitious algae. Additionally, the limited Gain settings on the JUNIOR-PAM may obscure fluorescence values when chlorophyll densities are low. Cold-treated symbiotic corals exhibited photosynthetic efficiency levels similar to aposymbiotic corals, but again, it is difficult to determine how much of this photochemical activity was generated by *S. psygmophilum* versus other potentially non-symbiotic photosynthetic organisms. *S. psygmophilum* is a cold-tolerant species whose photosynthetic efficiency peaks between 18 and 25°C and reaches a minimum (but does not cease altogether) at 10°C (Thornhill et al. 2008). Following this pattern, symbiotic corals at 18 and 24°C demonstrated higher levels of photosynthetic activity, although warm-treated corals (24°C) seemed to experience a reduction in photosynthetic efficiency over time.

A. poculata is a gonochoristic coral (Peters et al. 1988), and production of gametes occurs at warmer temperatures. Given the cost of producing gametes, and the generally greater cost of producing eggs relative to sperm (Holcomb et al. 2012), it is possible that corals at warmer temperatures might experience a trade-off between gamete production and healing, and this trade-off might differ between males and females. However, because gametes can only be identified and quantified using destructive methods, we were unable to investigate the potential impact of gamete production on recovery without producing additional wounds, which would have confounded our experiments. We hypothesize that (1) wounds may confer a loss in fecundity, (2) gamete production may incur a cost to lesion recovery, or (3) reproductive dynamics promote a synergistic loss for both tissue recovery and fecundity (Rinkevich 1996).

Wound healing is a dynamic process impacted by a number of different environmental and biological factors. In addition to symbiotic state and temperature, lesion healing is affected by the perimeter to surface area ratio of the wound (Van Woesik 1998) as well as by the size of the lesion (Rotjan & Lewis 2008) and the size of the colony (Meesters et al. 1996). However, the influences of these physical parameters may vary by species and developmental timing (Bak 1983, Meesters et al. 1992, 1997, Oren et al. 1997, Van Woesik 1998). One previous study found that 'Phoenix Effect' recovery, whereby tissue is regenerated from within the calice rather than across wound edges, is the primary mode of recovery in *A. poculata* (DeFilippo et al. 2016). A similar pattern of recovery was observed in the experimentally wounded colonies in our trials. The wounds inflicted in this experiment most closely resembled those created through colony breakage or corallivory, where regions of both skeleton and tissue are removed. In the field, colonies of *A. poculata* are most often abraded by foliose algae. Colony morphology is significantly impacted (flattened) by repeat interactions in regions with high

macroalgal density (Grace 2004). While no predators have yet been characterized in the field for this coral, its ability to recover from such extremes (deep and well cleared of residual tissue) demonstrates its utility as a laboratory model for these studies.

Here, we have demonstrated that wound recovery is affected by symbiont state and temperature in a facultatively symbiotic coral. Comparable experiments cannot be conducted in tropical corals because elevated temperatures induce bleaching, and tropical corals cannot exist in a stable aposymbiotic state. By allowing us to decouple 2 key parameters that are conflated in tropical corals, *A. poculata* can provide unique insight into how particular biological and environmental states or stressors may impact coral health. While the study of temperate corals offers limited direct comparisons to tropical corals in their metabolism and physiology, it is likely that core mechanisms of the molecular stress response machinery are deeply conserved. Additionally, we can exploit the wide environmental tolerances of this temperate coral to investigate how a range of stressors and their intensities can affect coral health under non-lethal conditions. Indeed, determining the threshold for lethal stress is a critical area of study; using temperate corals in controlled laboratory studies may yield valuable insights into the effects of synergistic stressors, and the relative role of symbiosis.

Acknowledgements. This work was funded by the PADI foundation, the Boston University Marine Program, and the New England Aquarium. We thank Kiki Ballotti, Georgie Burruss, Nicholas Lawrence, Samantha Pelletier, Aaron Pilnick, and Ryan Schosberg for assistance with photography and data collection. We are also grateful to Tasia Blough, Julio Camperio-Ciani, Gregory Coote, Lukas DeFilippo, Corbin Kuntz, Georgia Luddecke, Katrina Malakhoff, Jessie Matthews, and Matthew Tohl for their roles in the husbandry and maintenance of coral specimens. We appreciate the input of the anonymous reviewers that contributed to the improvement of this manuscript.

LITERATURE CITED

Bak RPM (1983) Neoplasia, regeneration and growth in the reef-building coral *Acropora palmata.* Mar Biol 77: 221–227

Bak RPM, Steward-Van Es Y (1980) Regeneration of superficial damage in the scleractinian corals *Agaricia agaricites* f. *purpurea* and *Porites astreoides*. Bull Mar Sci 30: 883–887

Baker A, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: an ecological assessment of long-term impacts, recovery trends and future outlook. Estuar Coast Shelf Sci 80:435–471

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting l-inear mixed-effects models using lme4. J Stat Softw 67: 1–48

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York, NY

Bythell JC, Bythell M, Gladfelter EH (1993a) Initial results of a long-term coral reef monitoring program: impact of Hurricane Hugo at Buck Island Reef National Monument, St. Croix US Virgin Islands. J Exp Mar Biol Ecol 172:171–183

Bythell JC, Gladfelter EH, Bythell M (1993b) Chronic and catastrophic natural mortality of three common Caribbean reef corals. Coral Reefs 12:143–152

Cameron CM, Edmunds PJ (2014) Effects of simulated fish predation on small colonies of massive *Porites* spp. and *Pocillopora meandrina.* Mar Ecol Prog Ser 508:139–148

Cohen AL, Owens KE, Lane GD, Shimizu N (2002) The effect of algal symbionts on the accuracy of Sr/Ca paleotemperatures from coral. Science 296:331–333

Cole AJ, Pratchett MS, Jones GP (2008) Diversity and functional importance of coral-feeding fishes on tropical coral reefs. Fish Fish 9:286–307

Coyer JA, Ambrose RF, Engle JM, Carroll JC (1993) Interactions between corals and algae on a temperate zone rocky reef: mediation by sea urchins. J Exp Mar Biol Ecol 167:21–37

Cróquer A, Villamizar E, Noriega N (2002) Environmental factors affecting tissue regeneration of the reef-building coral *Montastraea annularis* (Faviidae) at Los Roques National Park, Venezuela. Rev Biol Trop 50:1055–1065

Cummings C (1983) The biology of *Astrangia danae*. PhD dissertation, University of Rhode Island, Kingston, RI

DeFilippo L, Burmester EM, Kaufman L, Rotjan RD (2016) Patterns of surface lesion recovery in the northern star coral *Astrangia poculata.* J Exp Mar Biol Ecol 481:15–24

Denis V, Debreuil J, De Palmas S, Richard J, Guillaume MMM, Bruggemann JH (2011) Lesion regeneration capacities in populations of the massive coral *Porites lutea* at Réunion Island: environmental correlates. Mar Ecol Prog Ser 428:105–117

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

Diaz-Pulido G, McCook LJ (2002) The fate of bleached corals: patterns and dynamics of algal recruitment. Mar Ecol Prog Ser 232:115–128

Dimond J, Carrington E (2007) Temporal variation in the symbiosis and growth of the temperate scleractinian coral *Astrangia poculata.* Mar Ecol Prog Ser 348:161–172

Dimond J, Carrington E (2008) Symbiosis regulation in a facultatively symbiotic temperate coral: zooxanthellae division and expulsion. Coral Reefs 27:601–604

Dimond J, Kerwin AH, Rotjan R, Sharp K, Stewart FJ, Thornhill DJ (2013) A simple temperature-based model predicts the upper latitudinal limit of the temperate coral *Astrangia poculata.* Coral Reefs 32:401–409

Downs CA, Woodley CM, Richmond RH, Lanning LL, Owen R (2005) Shifting the paradigm of coral-reef 'health' assessment. Mar Pollut Bull 51:486–494

Edmunds PJ (2005) The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Barrier Reef. Oecologia 146:350–364

Edmunds PJ (2009) Effect of acclimatization to low tempera-

ture and reduced light on the response of reef corals to elevated temperature. Mar Biol 156:1797–1808
Edmunds PJ, Lenihan HS (2010) Effect of sub-lethal damage to juvenile colonies of massive *Porites* spp. under contrasting regimes of temperature and water flow. Mar Biol 157:887–897
Fine M, Zibrowius H, Loya Y (2001) *Oculina patagonica*: a non-lessepsian scleractinian coral invading the Mediterranean Sea. Mar Biol 138:1195–1203
Fine M, Oren U, Loya Y (2002) Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica.* Mar Ecol Prog Ser 234:119–125
Fisher EM, Fauth JE, Hallock P, Woodley CM (2007) Lesion regeneration rates in reef-building corals *Montastraea* spp. as indicators of colony condition. Mar Ecol Prog Ser 339:61–71
Grace S (2004) Ecomorphology of the temperate scleractinian *Astrangia poculata*: coral-macroalgal interactions in Narragansett Bay. PhD dissertation, University of Rhode Island, Kingston, RI
Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440: 1186–1189
Henry L, Hart M (2005) Regeneration from injury and resource allocation in sponges and corals — a review. Int Rev Hydrobiol 90:125–158
Hill M, Hill A (2012) The magnesium inhibition and arrested phagosome hypotheses: new perspectives on the evolution and ecology of *Symbiodinium* symbioses. Biol Rev Camb Philos Soc 87:804–821
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866
Holcomb M, McCorkle D, Cohen AL (2010) Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata.* J Exp Mar Biol Ecol 386:27–33
Holcomb M, Cohen A, McCorkle D (2012) An investigation of the calcification response of the scleractinian coral *Astrangia poculata* to elevated pCO_2 and the effects of nutrients, zooxanthellae and gender. Biogeosciences 9:29–39
Hughes TP, Jackson JBC (1985) Population dynamics and life histories of foliaceous corals. Ecol Monogr 55:141–156
Jacques TG, Pilson MEQ (1980) Experimental ecology of the temperate scleractinian coral *Astrangia danae* I. Partition of respiration, photosynthesis and calcification between host and symbionts. Mar Biol 60:167–178
Jacques TG, Marshall N, Pilson MEQ (1983) Experimental ecology of the temperate scleractinian *Astrangia danae*: effect of temperature, light intensity and symbiosis with zooxanthellae on metabolic rate and calcification. Mar Biol 76:135–148
Jayewardene D (2010) Experimental determination of the cost of lesion healing on *Porites compressa* growth. Coral Reefs 29:131–135
Jayewardene D, Birkeland C (2006) Fish predation on Hawaiian corals. Coral Reefs 25:328
Jayewardene D, Donahue MJ, Birkeland C (2009) Effects of frequent fish predation on corals in Hawaii. Coral Reefs 28:499–506
Jompa J, McCook LJ (2003) Coral-algal competition: macroalgae with different properties have different effects on corals. Mar Ecol Prog Ser 258:87–95
Kaufman L (1981) There was biological disturbance on Pleistocene coral reefs. Paleobiology 7:527–532
Kramarsky-Winter E, Loya Y (2000) Tissue regeneration in the coral *Fungia granulosa*: the effect of extrinsic and intrinsic factors. Mar Biol 137:867–873
Lajeunesse TC, Parkinson JE, Reimer JD (2012) A genetics-based description of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two dinoflagellates symbiotic with cnidaria. J Phycol 48:1380–1391
Lenihan HS, Edmunds PJ (2010) Response of *Pocillopora verrucosa* to corallivory varies with environmental conditions. Mar Ecol Prog Ser 409:51–63
Marshall AT, Clode P (2004) Calcification rate and the effect of temperature in a zooxanthellate and an azooxanthellate scleractinian reef coral. Coral Reefs 23:218–224
Meesters EH, Bak RPM (1993) Effects of coral bleaching on tissue regeneration potential and colony survival. Mar Ecol Prog Ser 96:189–198
Meesters EH, Bos A, Gast GJ (1992) Effects of sedimentation and lesion position on coral tissue regeneration. Proc 7th Int Coral Reef Symp 2:681–688
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
Meesters EH, Wesseling I, Bak RPM (1996) Partial mortality in three species of reef-building corals (*Scleractinia*) and the relation with colony morphology. Bull Mar Sci 58: 838–852
Meesters EH, Pauchli W, Bak RPM (1997) Predicting regeneration of physical damage on a reef-building coral by regeneration capacity and lesion shape. Mar Ecol Prog Ser 146:91–99
Miller MW (1995) Growth of a temperate coral: effects of temperature, light, depth, and heterotrophy. Mar Ecol Prog Ser 122:217–225
Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Coral reefs: ecosystems of the world, Vol 25. Elsevier, New York, NY, p 75–87
Nagelkerken I, Bak RPM (1998) Differential regeneration of artificial lesions among sympatric morphs of the Caribbean corals *Porites astreoides* and *Stephanocoenia michelinii*. Mar Ecol Prog Ser 163:279–283
Nagelkerken I, Meesters EH, Bak RPM (1999) Depth-related variation in regeneration of artificial lesions in the Caribbean corals *Porites astreoides* and *Stephanocoenia michelinii*. J Exp Mar Biol Ecol 234:29–39
Nugues MM, Roberts CM (2003a) Partial mortality in massive reef corals as an indicator of sediment stress on coral reefs. Mar Pollut Bull 46:314–323
Nugues MM, Roberts CM (2003b) Coral mortality and interaction with algae in relation to sedimentation. Coral Reefs 22:507–516
Oren U, Benayahu Y, Loya Y (1997) Effect of lesion size and shape on regeneration of the Red Sea coral *Favia favus.* Mar Ecol Prog Ser 146:101–107
Oren U, Benayahu Y, Lubinevsky H, Loya Y (2001) Colony integration during regeneration in the stony coral *Favia favus.* Ecology 82:802–813
Peters EC, Cairns SD, Pilson MEQ, Wells JW and others (1988) Nomenclature and biology of *Astrangia poculata* (= *A. danae*, = *A. astreiformis*) (*Cnidaria*: *Anthozoa*). Proc Biol Soc Wash 101:234–250
Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) nlme: linear and nonlinear mixed effects models. R package version 3.1-130. https://CRAN.R-project.org/package=nlme
R Core Team (2013) R: a language and environment for sta-

tistical computing. R Foundation for Statistical Computing, Vienna

Rinkevich B (1996) Do reproduction and regeneration in damaged corals compete for energy allocation? Mar Ecol Prog Ser 143:297–302

River GF, Edmunds PJ (2001) Mechanisms of interaction between macroalgae and scleractinians on a coral reef in Jamaica. J Exp Mar Biol Ecol 261:159–172

Rotjan RD, Dimond JL (2010) Discriminating causes from consequences of persistent parrotfish corallivory. J Exp Mar Biol Ecol 390:188–195

Rotjan RD, Lewis SM (2005) Selective predation by parrotfishes on the reef coral *Porites astreoides*. Mar Ecol Prog Ser 305:193–201

Rotjan RD, Lewis SM (2006) Parrotfish abundance and selective corallivory on a Belizean coral reef. J Exp Mar Biol Ecol 335:292–301

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol Prog Ser 367:73–91

Rotjan RD, Dimond JL, Thornhill D, Leichter J, Helmuth B, Kemp D, Lewis SM (2006) Chronic parrotfish grazing impedes coral recovery after bleaching. Coral Reefs 25: 361–368

Rowan R (2004) Coral bleaching: thermal adaptation in reef coral symbionts. Nature 430:742

Suggett DJ, Borowitzka MA, Prášil O (eds) (2010) Chlorophyll *a* fluorescence in aquatic sciences: methods and applications. Developments in applied phycology, Vol 4. Springer Science+Business Media, New York, NY

Szmant-Froelich A, Pilson MEQ (1980) The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia danae* Milne Edwards and Haime, 1849. J Exp Mar Biol Ecol 48: 85–97

Szmant-Froelich A, Pilson MEQ (1984) Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral *Astrangia poculata*. Mar Biol 81:153–162

Thornhill DJ, Kemp DW, Bruns BU, Fitt WK, Schmidt GW (2008) Correspondence between cold tolerance and temperate biogeography in a western Atlantic *Symbiodinium* (*Dinophyta*) lineage. J Phycol 44:1126–1135

Van Veghel MLJ, Bak RPM (1994) Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis*. III. Reproduction in damaged and regenerating colonies. Mar Ecol Prog Ser 109:229–233

Van Woesik R (1998) Lesion healing on massive *Porites* spp. corals. Mar Ecol Prog Ser 164:213–220

Work TM, Aeby GS (2010) Wound repair in *Montipora capitata*. J Invertebr Pathol 105:116–119

Editorial responsibility: Peter Edmunds, Northridge, California, USA

Submitted: September 28, 2016; Accepted: March 9, 2017
Proofs received from author(s): April 8, 2017