Lesion regeneration rates in reef-building corals *Montastraea* spp. as indicators of colony condition

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ABSTRACT: Regeneration rates of coral lesions reflect the ability of colonies to repair damage and therefore can be useful indicators of coral health and environmental conditions. We quantified regeneration rates of boulder corals *Montastraea* spp. at four, 6 m deep patch reefs within Biscayne National Park (BNP) and the upper Florida Keys National Marine Sanctuary (FKNMS), and along a 3 to 18 m depth transect in FKNMS. Coral lesions (approx. 2 cm^2) created during sampling for cellular-diagnostic analysis were monitored quarterly in 2001 and 2002, and in February 2003. Regeneration was a dynamic process, continuing longer than previously reported (>300 d after lesion formation). Geographic location was the strongest factor affecting regeneration rate at our study sites. Lesion regeneration differed significantly among 6 m deep sites; sites offshore from John Pennekamp Coral Reef State Park (Algae Reef and White Banks) consistently had the highest regeneration rates, with colonies exhibiting exponential declines in lesion size and a high percentage of completely healed lesions. Along the depth gradient, corals at the 3 m site regenerated significantly faster than corals at 6, 9, and 18 m. These results suggest that corals sampled at FKNMS 6, 9 and 18 m sites and BNP were in poor physiological condition or were exposed to suboptimal environmental conditions, as evidenced by highly variable and overall low regeneration rates, a low percentage of healed lesions, and a high occurrence of breakage or Type II lesions (lesions that increased in size by merging with areas of denuded tissue on the colony).

KEY WORDS: Regeneration · Indicators · Lesion · *Montastraea* spp. · Coral · Partial mortality · Damage

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INTRODUCTION

Most corals are colonial organisms; a colony can experience partial mortality in which a portion remains alive while another portion dies. When a disturbance produces a lesion (partial mortality), the exposed coral skeleton becomes vulnerable to invasion by sessile organisms such as algae, resulting in the lesion increasing in size. Alternatively, healing can occur if tissue regeneration processes are not impeded (Kawaguti 1937). Lesions that fail to heal completely within about 2 mo are likely to become permanent patches of mortality (Meesters et al. 1994).

Percent mortality of coral colonies is a useful gauge of reef condition (Ginsburg et al. 2001) because it can point to a recent or chronic disturbance (Lang 2003) and can influence colony growth and reproduction (Meesters et al. 1994, Van Veghel & Bak 1994, Lirman 2000a). Williams (1994) proposed using coral lesions as indicators of environmental stress because they are a generalized response to a range of disturbances, are independent of reef type, and can be monitored by resource managers easily and inexpensively. Williams (1994) noted that the frequency of coral lesions varies among sites, with polluted sites having more lesions than relatively unpolluted sites. Quantifying colony

damage and recovery rates also are essential for predicting demographic changes in coral populations (Bak & Meesters 1999).

In corals, lesion regeneration begins with growth of an undifferentiated tissue layer created by the coenenchyme and polyps surrounding the lesion (Bak et al. 1977). After about 2 wk, polyps begin to develop in the new tissue (Meesters et al. 1994) and secrete thecal walls and a basal plate. These give rise to numerous radially arranged calcareous partitions (septa), which project inward and support the polyp mesenteries. Pigmentation and zooxanthellae return at the end of the regeneration process (Bak et al. 1977, Kramarsky-Winter & Loya 2000). Coral regeneration rates can vary with species (Kawaguti 1937, Bak et al. 1977, Nagelkerken & Bak 1998) and are influenced by lesion characteristics including the type of injury and its initial size, perimeter and shape (Meesters et al. 1994, 1997b, Oren et al. 1997, Lirman 2000b, Hall 2001), and colony characteristics such as size (Kramarsky-Winter & Loya 2000, Oren et al. 2001). Under normal conditions, lesion size decreases exponentially; deviations from this response suggest resource limitation (Meesters et al. 1997b), stress due to environmental conditions (Lester & Bak 1985, Meesters & Bak 1993, Meesters et al. 1993, Mascarelli & Bunkley-Williams 1999, Croquer et al. 2002, Fine et al. 2002) or competition (Hall 2001).

The present study was part of a long-term project in the Florida Keys testing the use of an integrated molecular biomarker system in corals (Downs et al. 2000, 2005, Fauth et al. 2003). Here we compare the ability of star boulder corals (*Montastraea* species complex) within 2 marine protected areas, to regenerate biopsy-induced lesions. Lesion regeneration rates were assessed to characterize coral condition at these sites. Three specific questions were addressed: (1) Do regeneration rates differ among sites, seasons or years? (2) Do regeneration rates vary with depth? (3) Do regeneration rates vary with lesion parameters (e.g. initial lesion size, perimeter, shape) or colony characteristics (e.g. morphotype/species, size, % mortality)? In future papers, we will relate coral regeneration rates to ecological and cellular indicators to further identify potential sources of stress at our study sites.

MATERIALS AND METHODS

Study sites. We assessed reef condition at community and colony scales at 1 patch reef (Alina's Reef) in Biscayne National Park (BNP), and 4 patch reefs and 2 fore reef sites in the upper Florida Keys National Marine Sanctuary (FKNMS), as part of an ongoing study of coral ecophysiology (e.g. Downs et al. 2000,

2005, Fauth et al. 2003). These 7 sites (Fig. 1) comprised both a latitudinal transect with 4 sites at 6 m depth and a depth transect (Key Largo, KL, 3 to 18 m) and were chosen in consultation with resource managers to reflect gradients in environmental conditions. Algae Reef (AR) and White Banks (WB) were adjacent to the extensive John Pennekamp Coral Reef State Park, with intact coastal hammock, mangroves and seagrass beds. Key Largo 6 m (KL 6 m) was located offshore from the most urbanized coastline of Key Largo, from which natural vegetation has been removed, natural topography has been altered to maximize waterfront properties, and coastlines are lined with seawalls. This site lies along the route that recreational boaters and commercial dive operators take to reach popular Molasses Reef and other outer reefs in the Upper Keys reef tract. Molasses Reef is 'the most heavily visited reef in the Upper Keys for diving' (FKNMS website). Biscayne National Park (BNP) is offshore from urban
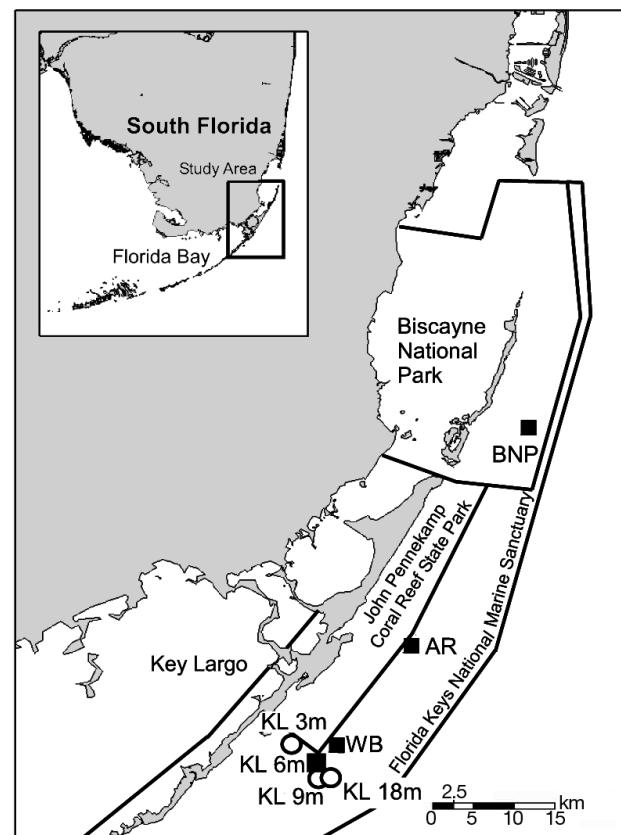

Fig. 1. Study sites. ■: 6 m sites, comprising patch reefs Key Largo (KL) 6 m (25° 01.09′ N, 80° 23.84′ W), White Banks (WB) (25° 02.23′ N, 80° 22.50′ W), Algae Reef (AR) (25° 08.80′ N, 80° 17.60′ W), and Alina's Reef in Biscayne National Park (BNP) (25° 23.19′ N, 80° 09.78′ W); O: along depth gradient, comprising 2 patch reefs, KL 3 m (25° 02.45′ N, 80° 25.44′ W) and KL 6 m, and 2 fore reefs, KL 9 m (25° 00.15′ N, 80° 23.63′ W) and KL 18 m (25° 00.21′ N, 80° 23.02′ W)

Miami, Florida, which is potentially influenced by the extensive agricultural area south and west of Miami that drains into Biscayne Bay. BNP also is located in proximity to a nuclear power plant and major landfill site.

Benthic community assessments. In March 2002, a dive was made at each 6 m site to assess the benthic organisms using the rapid assessment methods described by Lang (2003). At each site, a 10 m transect line was placed just above a haphazardly selected area of reef surface and live coral cover was determined by estimating the amount of living coral directly beneath the line. For each coral >10 cm in diameter lying beneath the transect, we recorded species, maximum diameter and height, and percent recent and old mortality. 'Recently dead' was defined as any non-living parts of the coral in which the corallite structures were still intact or covered by a thin layer of algae or fine mud (Lang 2003). 'Long dead' was defined as any non-living parts of the coral in which the corallite structures were either gone or covered by organisms that were not easily removed (Lang 2003). Because of differences in reef types, we could not use this method for comparisons along the depth gradient.

Lesion regeneration. Between June 2001 and February 2003, we collected tissue samples (creating a lesion) approximately quarter-yearly (February/March, June, August, October/November) from the same 5 colonies at each site. Previous studies showed that quarterly sampling was adequate to detect changes in coral physiology as a result of seasonal and stressor variation (Downs et al. 2000, Fauth et al. 2003). We preferentially chose *Montastraea faveolata* for this study but sampled the morphotypes *M. annularis* and *M. franksi* when *M. faveolata* was not available. A single morphotype was not found at all study sites: We sampled *M. faveolata* at all sites except KL 18 m, *M. annularis* at KL 6 m and WB, and *M. franksi* at KL 9 and 18 m, WB, and AR. We estimated size (maximum diameter) and percent partial mortality of each colony at the start of the study. We measured diameter (live and dead areas) in planar view perpendicular to the axis of growth to the nearest 1 cm using a meter stick. Partial mortality was visually quantified by estimating the percentage of dead area from above in planar view as recommended by Lang (2003).

We removed coral tissue and skeleton using a leather punch, which created a circular lesion 1 to 2 cm^2 in area and 3 mm deep. Experimental lesions were always completely surrounded by live tissue. We immediately filled the hole with clay (Roma plastilina, medium grey; Blick Art Materials) to fill the void produced by removing the underlying skeleton and limit intrusion of fouling and bioeroding organisms (Fig. 2). Use of clay filler was a decision made by park managers when permitting biopsy of these corals for molecular biomarker analysis. Clay provided corals with a flat surface over which to regenerate tissue but, as seen in our study, did not prevent fouling or bioerosion. However, the regeneration rates reported in this study may represent maximal rates due to a possible reduction in biofouling. We then photographed each lesion using a Nikonos V 35 mm camera with a close-up adapter and frame, calibrating measurements with a 4.5 mm long bar. We re-photographed each lesion during subsequent quarterly samplings to observe changes in size over time (Fig. 2). We scanned photographs to digital images and used image-analysis software (Image Pro™) to calculate area (A) and perimeter (P) of all lesions that remained completely surrounded by live tissue (Type I lesions: Meesters et al. 1997a).

If a lesion enlarged, thereby merging with an area of the colony that lacked tissue (Type II lesions: Meesters et al. 1997a), we conservatively assumed no change in lesion size for that sampling date and removed it from further analyses because subsequent changes in area were unconstrained. When lesions merged with other
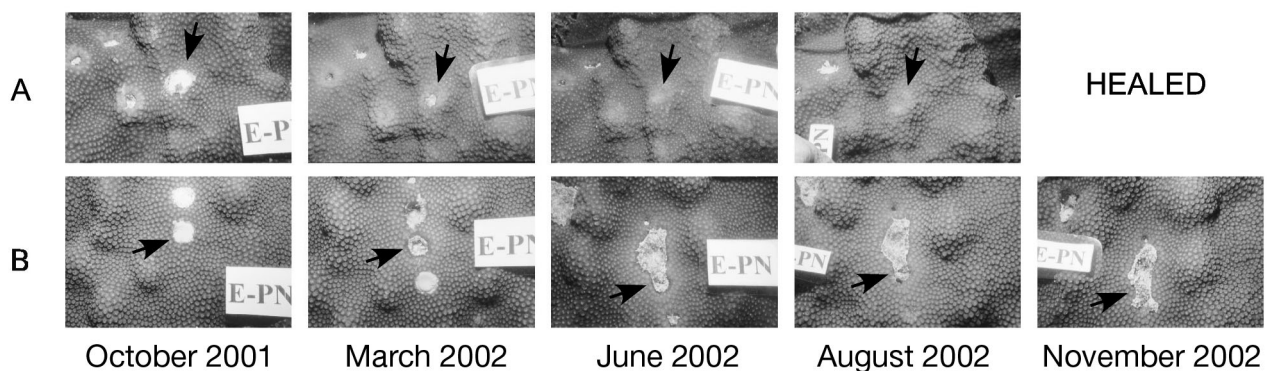

Fig. 2. *Montastraea* spp. Examples of lesions at 6 m sites between October 2001 and November 2002 showing 2 extremes. (A) Algae Reef (lesion completely healed by June 2002); (B) Alina's Reef in BNP (lesion joined with other sampling lesions in June 2002 and became covered with turf algae). Black arrows point to relevant lesions

created lesions (Fig. 2B), we calculated their area as $A_L = A_T/n$; where A_L is the area of the lesion used for further analyses, A_T is the total area of all lesions joined together, and n is the total number of lesions joined together. This calculation provided a conservative estimate of lesion area increase. In the few cases where initial lesion size was unavailable due to camera malfunctions, we substituted the average initial lesion size for that sampling period. When photographs of final lesion size were unavailable, we used *in situ* measurements to calculate lesion area and perimeter using the equation for an ellipse, $A_L = \pi ab$, and $P_L = 2\pi \cdot \sqrt{[(a^2 + b^2)/2]}$, where A_L is the area of the lesion, P_L is the perimeter of the lesion, and a and b are one-half of lesion length and width, respectively. Larger lesions resulted from breakage of the coral skeleton, which was often highly bioeroded. Because of the effect of initial lesion size on regeneration, we removed lesions >3.4 cm^2 from further analyses.

Data analysis. *Benthic community assessments:* We used 1-way ANOVA followed by the Tukey-Kramer Honestly Significant Difference (HSD) test to determine if sites differed significantly in live coral cover and coral colony density. Data on coral diameter and height, and recent and old mortality did not meet the normality assumptions of ANOVA. For these data, we used Kruskal-Wallis followed by Wilcoxon rank-sum tests to test for differences among sites.

Lesion regeneration: Data were analyzed in 2 groups: (1) by sites at 6 m depth along the NE–SW traverse (BNP, AR, WB and KL 6 m), and (2) by sites along the depth gradient (KL 3, 6, 9 and 18 m). The KL 6 m site was common to both groups (Fig. 1). We examined lesion changes in 3 different ways to answer specific questions:

• Did lesion size decrease exponentially with time (cm^2 d^{-1}) and did this differ among sites and seasons? Can deviations from this model be used as an indicator of stress? We used least-squares regression to fit an exponential model of regeneration with an asymptote as recommended by Meesters et al. (1994, 1997b):

$$y = y_0 + ae^{-b \cdot \text{time}}$$

where y_0 is the asymptote, a is the amount of tissue regenerated, and b is the slope of the curve. We only applied the exponential model to lesions with a minimum of 1 yr of observations.

• Did regeneration rates differ among sites and seasons? We calculated rates for 2 different periods: short-term (45 to 154 d) and quasi-annual (319 to 376 d). The shortest period monitored reflects a time frame similar to that in previous regeneration studies (e.g. Meesters et al. 1994, 1997b, Van Veghel & Bak 1994). We calculated the quasi-annual rates to determine how lesion size changed over multiple seasons. For each lesion,

we calculated the amount of tissue regenerated or lost (ΔT) as ΔT = % change in lesion size $\cdot$ initial lesion size/time. We standardized regeneration to initial lesion perimeter (P) because this influenced regeneration rates. We used repeated-measures MANOVA to determine whether standardized regeneration rates ($\Delta T:P$) differed among sites, seasons and their interactions. We checked model assumptions (e.g. sphericity, homogeneity of variances, normality and independence) using residual plots. In cases where the sphericity assumption was not met, we applied a univariate (unadjusted epsilon) approach. To interpret effects detected by MANOVA, we used 1-way ANOVA followed by the Tukey-Kramer HSD test. We regressed residuals of the regeneration-rate model against lesion (A, P and shape [P:A]) and colony (species, size, % mortality) parameters to determine if they affected regeneration rates. Any colony characteristics that explained significant variation in residuals were used as covariates in the MANOVA model. We also regressed quasi-annual regeneration rates against short-term rates to determine if monitoring for short time periods could be used to predict long-term trends.

• Were lesions among all sites capable of completely healing and did the number of Type II lesions differ among sites and seasons? We used *G*-tests of independence with William's correction (Sokal & Rohlf 1995) to determine if the number of lesions that closed completely or progressed into Type II lesions differed among sites.

We performed non-linear regression using Sigma-Plot 2000 (Systat Software) and all other statistical analyses using JMP Version 3.2 (SAS Institute), with α = 0.05 for all hypothesis tests.

RESULTS

Community data

Mean percent live coral cover and coral colony density were low at all 6 m sites; both were highest at AR and lowest at KL 6 m (coral cover: ANOVA $F_{3,13}$ = 11.4, p < 0.0007; coral colony density: ANOVA $F_{3,26}$ = 3.7, df = 3, p < 0.03; Table 1). Colonies at AR were significantly larger (maximum diameter) than colonies at WB and KL 6 m; colonies at BNP were also significantly larger than colonies at KL 6 m (χ^2 = 9.2, df = 3, p < 0.03; Table 1).

Regeneration model

After removing lesions >3.4 cm^2 from further analyses, initial lesion area ranged from 0.75 to 3.02 cm^2

Table 1. *Montastraea* spp. Comparison of benthic parameters at four 6 m patch reefs from 10 m transects using Atlantic Gulf and Rapid Reef Assessment protocol (Lang 2003). Data are means (±SE); values not bearing same superscript are significantly different (p < 0.05). Site abbreviations here and in Tables 2 & 3 as in Fig. 1 legend

Site	n	Colonies (no. m^{-1})		Live coral cover (%)		Coral height (cm)		Coral diam. (cm)		Recent mortality (%)		Old mortality (%)	
KL 6 m	7	0.64 [A]	(0.07)	7 [A]	(1)	11 [A]	(3)	21 [A]	(5)	3 [A]	(2)	11 [A]	(4)
WB	8	0.71 [AB]	(0.07)	9 [A]	(1)	13 [A]	(1)	19 [AB]	(2)	2 [A]	(1)	8 [A]	(2)
AR	8	0.94 [B]	(0.10)	16 [B]	(1)	23 [A]	(4)	40 [C]	(9)	2 [A]	(1)	12 [A]	(3)
BNP	8	0.64 [A]	(0.05)	8 [A]	(1)	25 [A]	(6)	37 [BC]	(8)	4 [A]	(2)	19 [A]	(8)

with a mean (±SE hereafter) of 1.75 (±0.04) cm^2 (n = 136) for the 6 m sites and from 0.68 to 3.32 cm^2 with a mean of 1.80 (±0.05) cm^2 (n = 128) along the depth gradient.

Lesion size decreased exponentially over time at AR, WB and the KL 3 m site (Fig. 3) as indicated by large r^2 values and slopes (Table 2). With few exceptions, lesion size at BNP, and KL 6, 9 and 18 m either changed little or, in some cases, increased over time (Fig. 3). Lesions on corals at these sites deviated from the expected decay model and fit to either an exponential growth (increase in lesion size) model or a reduced model, as indicated by low r^2 values and slopes (Table 2).

Short-term regeneration rates (45 to 154 d)

Short-term regeneration rates (Δ T:P) ranged from −40 to 65 × 10^{-4} cm d^{-1} with a mean of 13 ± 1 × 10^{-4} cm d^{-1} (n = 136) at 6 m sites and from −43 to 91 × 10^{-4} cm d^{-1} with a mean of 13 ± 2 × 10^{-4} cm d^{-1} (n = 127) along the depth gradient.

Mean short-term regeneration rates differed significantly among the 6 m sites (repeated-measures MANOVA: site effect $F_{3,10}$ = 10.6, p < 0.002; Fig. 4A), but not among species, seasons, or their interactions. Mean short-term regeneration rates at AR were significantly higher than at the other 6 m sites, and short-term regeneration rates at WB were significantly higher than at KL 6 m and BNP (AR: 23 ± 2 × 10^{-4} cm d^{-1}; WB: 15 ± 2 × 10^{-4} cm d^{-1}; KL 6 m: 7 ± 2 × 10^{-4} cm d^{-1}; BNP: 6 ± 2 × 10^{-4} cm d^{-1}; Tukey's HSD test). Short-term re-
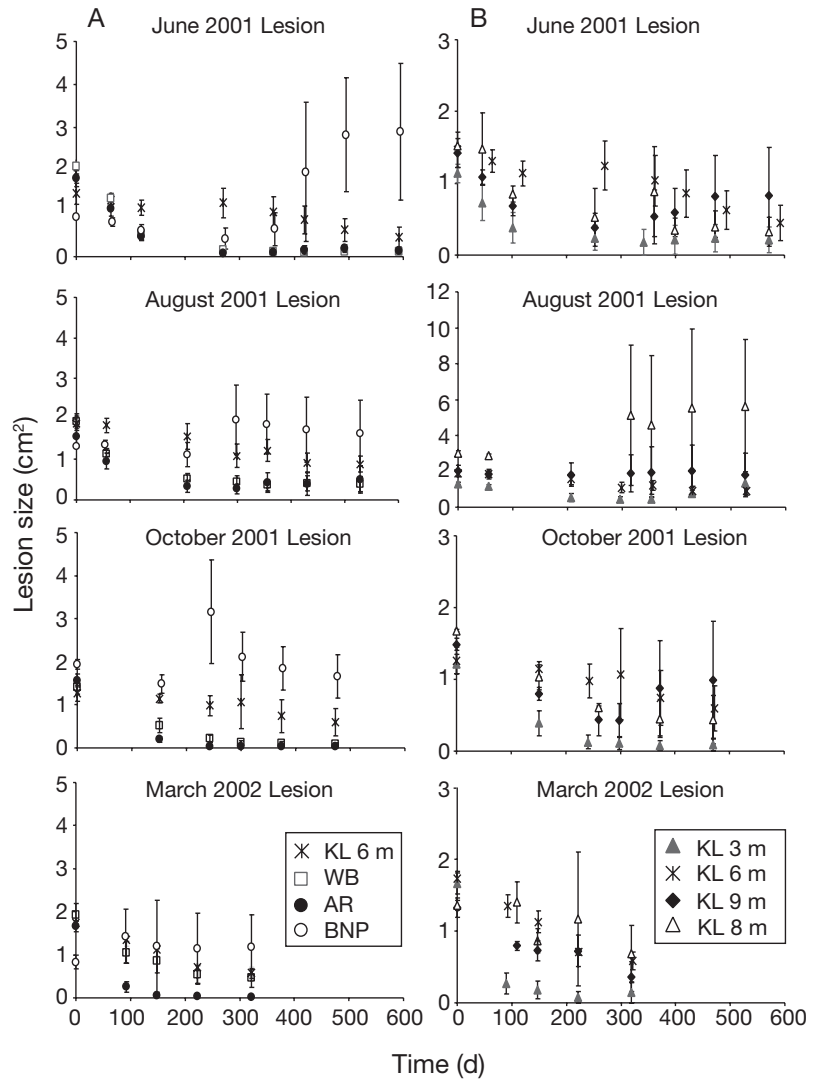

Fig. 3. *Montastraea* spp. Mean (±SE) lesion size over time for each season between June 2001 and March 2002 (A) at 6 m sites and (B) along depth gradient. Merging of 2 sampling-induced lesions occurred at KL 9 m (in March 2002), at KL 18 m (in February 2003), and at BNP (in June 2002 and August 2002). An additional lesion joined with the previously merged lesions at BNP in October 2002. Lesions that progressed into Type II lesions or lesions for which data were removed for other reasons (breakage or initial size >3.4 cm^2: see 'Materials and methods') were not included in means. Note expanded *y*-axis in graph showing lesion regeneration along depth gradient in August 2001. Site abbreviations here and in subsequent figures as in Fig. 1 legend

Table 2. *Montastraea* spp. Mean (±SE) r^2 values of the 5 colonies for the regression decay model, $y = y_0 + ae^{-b \cdot \text{time}}$; zero r^2 value was assumed for all lesions that did not fit this model. Last 2 columns show overall mean and slope (*b*) (cm^2 d^{-1} × 10^{-2})

Site	Jun 2001– Jun 2002	Aug 2001– Aug 2002	Oct 2001– Nov 2002	Mar 2002– Feb 2003	Overall mean	Slope
KL 3 m	0.95 (0.03)	0.52 (0.22)	0.94 (0.05)	0.98 (0.01)	0.85 (0.07)	2.1 (0.7)
6 m	0.55 (0.18)	0.61 (0.15)	0.33 (0.18)	0.88 (0.07)	0.59 (0.09)	0.3 (0.1)
9 m	0.58 (0.19)	0.39 (0.24)	0.55 (0.23)	0.72 (0.24)	0.55 (0.11)	0.1 (0.1)
18 m	0.56 (0.22)	0.37 (0.22)	0.72 (0.24)	0.71 (0.24)	0.59 (0.11)	0.4 (0.2)
WB	0.97 (0.01)	0.98 (0.01)	0.98 (0.02)	0.90 (0.07)	0.96 (0.02)	1.2 (0.2)
AR	0.96 (0.01)	0.80 (0.20)	1.00 (0.00)	1.00 (0.00)	0.93 (0.05)	1.7 (0.3)
BNP	0.23 (0.23)	0.36 (0.22)	0.37 (0.22)	0.21 (0.20)	0.30 (0.10)	0.2 (0.2)

generation rates also differed significantly along the depth gradient (repeated-measures MANOVA: site effect $F_{3,8} = 4.4$, p < 0.05; Fig. 4B) but not with season or depth × season interactions. Short-term regeneration rates at KL 3 m were significantly faster than at

Fig. 4. *Montastraea* spp. Mean (±SE) regeneration rates standardized to initial lesion perimeter (ΔT:P) for each season, from one sampling event to next, (A) among 6 m sites, and (B) along depth gradient. Rates calculated between June and August 2001 (54 ± 13 d), August and October 2001 (56 d), October 2001 and March 2002 (153 ± 2 d), March and June 2002 (91 ± 1 d), June and August 2002 (48 ± 13 d), August and November 2002 (74 d) and November 2002 and February 2003 (99 ± 1 d)

KL 6 m (19 ± 3 × 10^{-4} cm d^{-1} vs. 7 ± 2 × 10^{-4} cm d^{-1}, respectively, Tukey's HSD test) but not at KL 9 and 18 m.

Species differences partially explained variation (<7 %) in the residuals of the regeneration-rate model for the 6 m sites (period 45 to 154 d), with *Montastraea annularis* having higher regeneration rates than *M. franksi* (1.3 ± 0.3 × 10^{-4} cm d^{-1} vs. 0.8 ± 0.4 × 10^{-4} cm d^{-1}, respectively). However, this was largely a result of an uneven distribution of morphotypes among sites, and the species effect was not significant when added as a covariate to the regeneration-rate model. Species differences did not significantly explain variation in the residuals along the depth gradient. Initial lesion size, perimeter, and shape explained less than 10 % of the residual error in the regeneration (T:P) model at the 6 m sites ([A] $r^2 = 0.09$, p = 0.002; [P] $r^2 = 0.06$, p < 0.01; [P:A] $r^2 = 0.09$, p = 0.002). Residuals were positively correlated with both initial lesion size and perimeter, and negatively correlated with P:A. Along the depth gradient, lesion size, perimeter and shape were independent of model residuals.
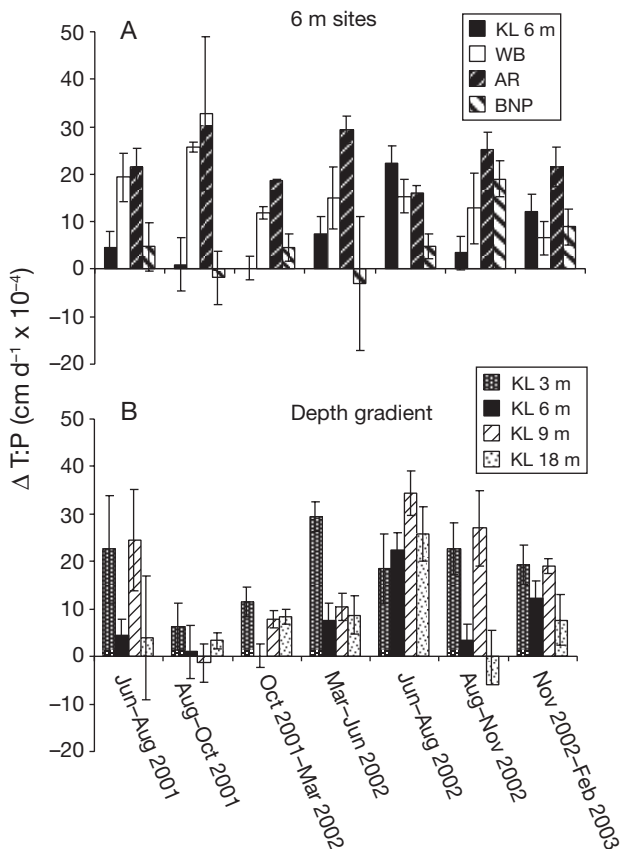
Quasi-annual regeneration rate (319 to 376 d)

At the 6 m sites, mean quasi-annual regeneration rates differed among sites (repeated-measures MANOVA: site effect $F_{3,12} = 14.8$, p = 0.0002), season ($F_{3,36} = 11.2$, p < 0.0001), and with the site × season interactions ($F_{9,36} = 4.2$, p < 0.0009). Corals at AR and WB regenerated significantly faster than corals at KL 6 m and BNP between June 2001 and 2002 (ANOVA: $F_{3,15} = 7.3$, p < 0.003; 9 ± 1 × 10^{-4} cm d^{-1} and 9 ± 0 × 10^{-4} cm d^{-1} vs. 3 ± 1 × 10^{-4} cm d^{-1} and 2 ± 3 × 10^{-4} cm d^{-1}, respectively, Tukey HSD, Fig. 5A). Corals at BNP regenerated significantly slower than corals at WB between August 2001 and 2002 (ANOVA: $F_{3,14} = 3.4$, p < 0.05; –4 ± 5 × 10^{-4} cm d^{-1} vs. 8 ± 1 × 10^{-4} cm d^{-1}, respectively, Tukey HSD) and corals at AR between October 2001 and 2002 (ANOVA: $F_{3,14} = 4.8$, p < 0.01; 0 ± 3 × 10^{-4} cm d^{-1} vs. 8 ± 1 × 10^{-4} cm d^{-1}, respectively, Tukey HSD). Corals at AR also regenerated signif-

icantly faster than colonies at KL 6 m and BNP between March 2002 and February 2003 (ANOVA: $F_{3,15} = 5.3$, $p < 0.01$; Fig. 5A; $21 \pm 5 \times 10^{-4}$ cm d^{-1} vs. $7 \pm 1 \times 10^{-4}$ cm d^{-1} and $2 \pm 5 \times 10^{-4}$ cm d^{-1}, respectively; Tukey HSD). Short-term regeneration rates were significantly correlated with quasi-annual trends ($r^2 = 0.37$, $p = 0.0001$; regression equation: ΔT:P [annual] = 0.29 ΔT:P [short] + 2.3).

Mean regeneration rates varied significantly along the depth gradient (repeated-measures MANOVA: site effect $F_{3,10} = 4.1$, $p = 0.04$), with season ($F_{3,8} = 38.3$, $p < 0.0001$), and the season × site interactions ($F_{9,19.6} = 5.6$, $p < 0.0007$; Fig. 5B). Regeneration rates at KL 3 m exceeded those at all other sites between March 2002 and February 2003 (ANOVA: $F_{3,15} = 5.3$, $p < 0.02$; $18 \pm 4 \times 10^{-4}$ cm d^{-1} vs. $7 \pm 1 \times 10^{-4}$ cm d^{-1}, $6 \pm 2 \times 10^{-4}$ cm d^{-1} and $4 \pm 2 \times 10^{-4}$ cm d^{-1}, respectively; Tukey HSD). Along the depth gradient, short-term regeneration rates explained little variation in quasi-annual trends ($r^2 = 0.10$, $p = 0.007$; regression equation: ΔT:P [annual] = 0.12 ΔT:P [short] + 3.3) due to high variability, especially among colonies at the KL 9 and 18 m sites.

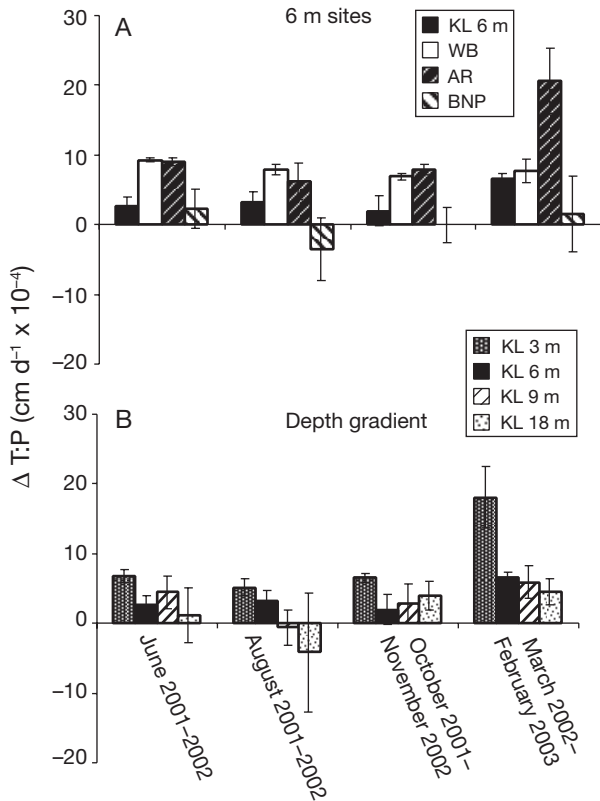Fig. 5. *Montastraea* spp. Mean (±SE) regeneration rates (ΔT:P) standardized to initial lesion perimeter for each season between June 2001 and March 2002, from time of sampling until following year (A) among 6 m sites, and (B) along 3 to 18 m depth gradient. Rates calculated between June 2001 and 2002 (357 ± 10 d), August 2001 and 2002 (355 ± 1 d), October 2001 and November 2002 (374 ± 2 d) and March 2002 and February 2003 (321 ± 2 d)

Healed and Type II lesions

Coral colonies at AR completely healed significantly more lesions (30%) than colonies at the other 6 m sites ($G_{adj} = 15.8$, df = 3, $p < 0.005$). Along the depth gradient, significantly more lesions healed completely at 3 m depth (31%) than at other depths ($G_{adj} = 12.8$, df = 3, $p < 0.01$; Fig. 6). These results indicate significant heterogeneity among sites in healing.

Of a total of 171 lesions created at the KL 3 m and all 6 m sites combined, only 2 merged with other lesions to become Type II lesions (Table 3). In contrast, at the
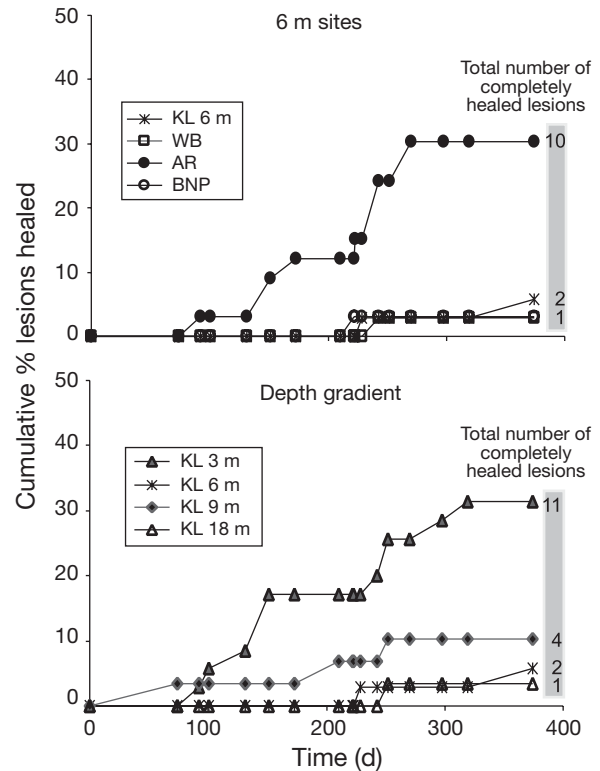

Fig. 6. *Montastraea* spp. Cumulative percentage (%) of lesions completely healed over time (A) at 6 m sites, and (B) along depth gradient. In the shaded region number adjacent to each line is total number of completely healed lesions at the relevant site

Table 3. *Montastraea* spp. Percentage of healed and Type II lesions (no longer enclosed by living tissue) at each site. Total number of lesions was <35 at KL 9 and 18 m, AR, and BNP due to breakage during sampling (see 'Materials and methods')

Site		Total no.	— Healed —		— Type II —	
			No.	%	No.	%
KL	3 m	35	11	31	0	0
	6 m	35	2	6	1	3
	9 m	29	4	14	6	21
	18 m	29	1	3	9	31
WB		35	1	3	0	0
AR		33	10	30	0	0
BNP		33	1	3	1	3

deepest sites (KL 9 and 18 m combined), 26% of lesions merged to become Type II lesions (G_{adj} = 12.2, df = 3, p < 0.01; Table 3). In 2 cases, lesions joined with another sampled lesion before merging with partial mortality on other parts of the colony, becoming Type II lesions. In all other cases, lesions joined with partial mortality naturally formed on the colony and were often associated with increases in algae, thereby forming Type II lesions.

DISCUSSION

Following recommendations of Williams (1994) and utilizing the extensive work of Bak, Meesters and coworkers (e.g. Meesters et al. 1997b), we evaluated lesion regeneration as an indicator of coral-colony condition at 7 reefs in BNP and FKNMS. Using this bioindicator, we successfully detected significant differences among sites in mean lesion regeneration rates. While most previous studies of regeneration monitored lesions for 60 to 150 d, we followed regeneration for up to 595 d, which allowed us to observe changes in recovery trends that might be missed by a study of shorter duration. Short-term regeneration rates were useful predictors of longer-term regeneration rates among 6 m sites, but explained little variation along the depth gradient. Monitoring long-term regeneration appears necessary when comparing coral colonies living in different reef types/depths. Long-term regeneration rates were time-dependent whereas short-term regeneration rates were not.

Coral lesions regenerate at a rate determined by the number of polyps surrounding each lesion (Meesters et al. 1997b, Oren et al. 1997, Lirman 2000b) and normally follow an exponential-decay model with an asymptote at full healing (Meesters et al. 1994, 1997a, Lirman 2000b). We found that changes in lesion size were dynamic and site-dependent and often deviated from the expected exponential-decay model. Some lesions that initially increased in size later regenerated, and other lesions that initially began to regenerate later increased in size, especially at sites with high algal growth (e.g. BNP, and KL 9 and 18 m). If lesions with a P:A ratio >2 cm^{-1} were able to fully regenerate (Meesters et al. 1997a), then most lesions in our study should have healed completely. However, only 13% (n = 229) fully regenerated. The largest lesion (2.0 cm^2) that fully regenerated did so after 243 d; after 151 d, this lesion had regenerated 78% of its area to a size of 0.45 cm^2. Most lesions that healed completely regenerated most (>70%) of their area within 151 d, but complete healing often required 1 yr or longer. One lesion that completely healed after 270 d increased 66% in size in the first 56 d before beginning to regenerate.

Our study confirms that regeneration can continue for 1 yr or more and that lesions that do not initially regenerate (or even increase in size) can regenerate later if conditions become favorable.

6 m sites

Mean lesion regeneration rates varied significantly among sites at the same depth, suggesting that lesion regeneration may be a useful indicator of variation in environmental conditions. Of the 6 m sites, corals consistently had the highest regeneration rates at AR, which is adjacent to the extensive John Pennekamp Coral Reef State Park. Colonies at AR had significantly more completely healed lesions than the other 6 m sites. AR also had the highest live coral cover, with relatively large colonies. Lesions at the other site adjacent to the state park (WB) also regenerated exponentially but many failed to heal completely, leaving these corals susceptible to fouling organisms. Partial coral mortality of the community was lowest at this site. In contrast, KL 6 m, located offshore from the most urbanized coastline of Key Largo, had low regeneration rates and overall live coral cover. Corals from the site in Biscayne National Park, offshore from urban Miami, had the lowest regeneration rates; lesions there often increased in size. Large increases in lesion size at BNP were often associated with seasonal increases in algae (e.g. June 2002), which sometimes resulted in lesions merging together. During our study, BNP corals had poor lesion recovery and also exhibited mortality elsewhere on the colonies. Mean partial mortality of the coral community was highest at this site. In 2000, *Montastraea* colonies at BNP experienced a severe oxidative and protein denaturing stress, probably due to chemical contaminant exposure (Downs et al. 2005). The colonies we sampled were generally large in size, with substantial contiguous areas of living tissue, suggesting that the stressor(s) causing poor lesion recovery and partial mortality were probably recent, within the last 10 to 15 yr or less. Therefore, if stresses can be identified and alleviated at this site, these large coral heads may survive.

Responses of other reef organisms (e.g. white grunts and foraminifers) at these sites are consistent with observations of lesion regeneration. Downs et al. (2006) compared biomarker levels in white grunts *Haemulon plumieri* at BNP, WB and KL 6 m, finding evidence for a toxic response to a xenobiotic at BNP. Concentrations of pesticides in grunt livers were highest at KL 6 m and lowest at WB (Downs et al. 2006). Using abundances of reef-dwelling foraminifers, Hallock (2000) proposed that host algal symbionts can

indicate whether environmental conditions support calcifying organisms dependent upon algal symbioses; Fisher et al. (unpubl. data) found that abundances of such foraminifers were lowest at BNP and KL 6 m and highest at WB and AR.

Depth gradient

Mean regeneration rate also varied among depths, but results depended on how long lesions were monitored. When monitored for <1 yr (45 to 154 d), shallow-water (3 m) corals regenerated significantly faster than corals at 6 m but not those at 9 and 18 m. KL 3 m also had significantly more healed lesions than other sites along the depth gradient. For lesions sampled in March 2002 and monitored for approximately 1 yr, shallow-water corals (3 m) showed higher regeneration rates than all deeper-water corals (6 to 18 m) along the Key Largo transect. Nagelkerken et al. (1999) observed that deeper-water corals typically receive less radiant energy and therefore may have lower carbon reserves than corals in shallow water. However, this does not explain why KL 9 and 18 m had mean short-term regeneration rates similar to those of the shallowest site. Also, differences in mean regeneration rates were not seen between the KL 6 m and the deeper sites.

Regeneration rates of corals from KL 9 and 18 m were highly variable. Lesions that initially decreased in size often later increased in size, and overall live coral cover at these sites was low (<7%). These *Montastraea* colonies were bioeroded by clionid sponges, making them susceptible to breakage and resulting in greater patchiness of live tissue, possibly reducing the coral's ability to recover from damage. Type II lesions developed more frequently in corals from these deeper sites. Many lesions joined with dead regions that were unrelated to our sampling. In 2 cases, the entire colony died; 1 each at KL 9 and 18 m.

Lesion growth was often associated with increased algal turf, particularly thick turfs mixed with fine sediments. Hall (2001) reported that regeneration was negatively correlated with algal settlement and cover (particularly macroalgae), which requires large energy expenditure by corals to overgrow. In our study, algal turfs and macroalgae fluctuated in abundance, possibly associated with seasonal changes (as in Lirman & Biber 2000). Some lesions at our sites regenerated when algal biomass declined, but later increased in size as algae grew, shading and possibly killing polyps surrounding the lesions. Particularly in spring and summer, we observed dark reddish cyanobacterial blooms that formed thick mats on the bottom and overgrew portions of these corals.

Comparisions among all study sites

All sites we sampled had relatively low coral cover (<20%) and appeared to be experiencing stress (Fisher et al. unpubl. data). Connell (1997) observed that chronically stressed reefs were less likely to recover from acute or physical disturbances than reefs that were not chronically stressed. We observed that coral colonies along developed portions of the coastline (i.e. BNP, and KL 6, 9 and 18 m) were the least capable of recovering from further damage and mortality. Colonies at sites offshore from John Pennekamp State Park (AR and WB) recovered from damage despite exposure to potential stressors (e.g. photic stress, contaminants). Although KL 3 m is along the same portion of coastline as KL 6, 9, and 18 m, lesion recovery and coral condition (36 ± 13% live coral cover) at this site was good. Other studies also have found that Florida's inshore patch reefs appear to be in better condition and have higher coral cover relative to offshore reefs (Beaver et al. 2005). Corals at KL 9 and 18 m bleached in 1999, while those at KL 3 and 6 m did not (Fauth et al. 2003).

Effect of colony and lesion characteristics

Colony size and previous partial tissue mortality (%) did not significantly affect regeneration rates, probably because colony size was not small enough to limit resources allocated to regeneration (Oren et al. 2001). Once regeneration rate was standardized to perimeter, which is a measure of coral tissue available for re-growth in the surrounding margin (Meesters et al. 1994), lesion area explained only a small percentage of the variation in regeneration rate. Colony morphotype did not have a significant effect on regeneration rate, but since the 3 types were not evenly sampled among sites this could have confounded the results. However, low variation among colonies within sites containing different species suggests that morphotype was not a major factor affecting regeneration. Taxonomic differences between these morphologies remain uncertain (Lopez et al. 1999, Fukami & Knowlton 2005).

CONCLUSIONS

To standardize comparisons of lesion regeneration rates, we recommend (1) monitoring lesions of a similar size and perimeter, (2) comparing sites similar in depth and habitat type (i.e. patch reef, fore reef), and (3) monitoring lesions for more than 1 yr because many lesions may require >200 d to heal. We also recommend recording the percentage of healed lesions and the occurrence of Type II lesions.

Based on observations of lesion regeneration rates, we conclude that coral colonies under relatively favorable environmental conditions (e.g. AR, WB, and KL 3 m) will consistently have high regeneration rates, with lesion sizes decreasing exponentially over time, a high percentage of healed lesions, and a low occurrence of Type II lesions. Likewise, we conclude that less favorable conditions (e.g. KL 6, KL 9 and 18 m, and BNP) can be identified by lesions that exhibit little regeneration, or high variability including increases in lesion size (overall low regeneration rates), low percentage of healed lesions, a high occurrence of Type II lesions, and a high percentage of breakage (indicative of bioerosion). Causes for differences in coral condition at small spatial scales deserve further investigation.

Regeneration rates of coral lesions reflect the ability of colonies to repair damage and therefore can be useful, inexpensive indicators of reef coral condition or of environmental conditions. A caveat of this indicator is that it is not capable of separating effects of coral health versus external environmental factors on lesion regeneration rate. More expensive assays can then be applied to distinguish between stressor types at sites where coral regeneration is compromised.

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