

Assessment of Resilience in *Montastrea faveolata* Inside and Outside of a Marine Park

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Abstract

Lesion regeneration (wound healing) was used as a proxy for assessing organismal resilience in the coral, *Montastrea faveolata*. Potential effects of wounding on quantum yield determined by PAM fluorometry were also examined. In a mesocosm experiment, surface lesions were imaged daily over 15 days to develop healing curves. Quantum yields were obtained daily using a PAM fluorometer (2 mm fiberoptic). Results demonstrated that 50% healing occurred in 5-6 days. Lesion healing was compared between an unprotected location near Lee Stocking Island (Exuma, Bahamas) and in the Exuma Cays Land and Sea Park using coral cores deployed to field arrays at each site. Lesions, images and PAM measurements were made on day 0 and again 5-7 days later. These procedures were repeated in the winter, spring and summer of 2007 and winter, 2008. There were no seasonal differences in healing rates or between genotypes. No consistent effects of site, time of year or wounding were seen on quantum yields of surrounding tissue, however, newly formed tissues had significantly lower yields than measured in undamaged tissues. Healing was significantly greater at the Park site in the winter of 2007 but there were no site differences in spring or summer.

Key words: corals, organismal resilience, coral stress, wound healing

Introduction

There is a broad consensus that reef corals are threatened by a variety of anthropogenic and natural stressors. This has increased interest in assessing organismal stress and resilience. Some approaches, such as measuring coral growth, are inexpensive and simple but usually take considerable time for results to be obtained. Other methods are more rapid and can also provide very specific information. These include measurement of photosynthetic performance and/or competence, determination of calcification rates and a broadening array of cellular biomarkers. Drawbacks include higher costs, technical expertise requirements or access to a suitable laboratory.

Several investigators have examined lesion regeneration (wound healing) in response to environmental conditions such as turbidity and sedimentation (Lester and Bak 1985; Meesters et al. 1992; Cróquer et al. 2002), bleaching (Meesters and Bak 1993; Mascarelli and Bunkley-Williams 1999; Fine et al. 2002) or pollution (Williams 1994). Results from these and other studies (Bak et al. 1977; Meesters et al. 1994; 1997; Nagelkerken et al. 1999; Oren et al. 2001) suggest that wound healing reflects overall physiological condition and response to the environment. These findings led to proposals (Meesters and Bak 1994; Williams 1994) and projects

(Fisher et al, 2007) to use lesion regeneration as a measure of coral condition.

In addition to environmental conditions and species (Bak and Van Es 1980; Nagelkerken and Bak 1998; van Woesik 1998; Nagelkerken et al. 1999), results from previous studies have demonstrated that lesion size and shape have a profound effect on healing rates (Meesters et al. 1994, 1997; Oren et al, 1997; Loya 1997; van Woesik 1998; Lirman 2000; Hall 2001). Large lesions heal more slowly, are more susceptible to enlargement by grazers (Cróquer et al. 2002) or colonization by epibionts (van Woesik 1998; Hall 2001), in some cases preventing complete closure (Mascarelli and Bunkley-Williams 1999; Cróquer et al. 2002) or allowing establishment of disease such as black-band (Cróquer et al. 2002).

To be useful for comparing coral resilience over time and space, the lesion regeneration method should minimize impact on corals and be standardized with respect to species, colony size and environment, lesion characteristics and measurement. Here we present and propose a Coral Resilience Assay that meets these requirements and is rapid, inexpensive and requires little technical expertise. The method is applied to examine whether corals in the Exuma Cays Land and Sea Park, a well-established marine protected area, have greater resilience than in unprotected areas.

Methods

Mesocosm Experiment

Four 1" diameter cores were extracted from each of four *Montastrea faveolata* colonies using a carbide core bit. Colonies had been maintained in a 16,000-liter tank 23-28 months, thus all had been exposed to similar environmental conditions. The mesocosm experiment described here was also conducted within this tank. Cores were mounted to 2" square plastic PVC or ABS plates using epoxy (Quick Fix 2300; Progressive Epoxy Polymers, Inc.) and allowed to recover for several days.

Digital plan images of each core were recorded (Nikon 3100; 2048x1536 pixels) with a 1-cm scale and pulsed-amplitude modulated fluorometry (Walz Diving PAM) measurements performed. These tasks were accomplished using the CORAL Resilience Assessment System (CORAS; see Mueller 2008 for details and images). The CORAS is a submersible platform that allows repeatable positioning of the cores in three axes (X-Y-Z). Using a 2-mm optic fiber, two PAM measurements were made at each of the four cardinal points (N, E, S & W). The first was 3 mm from the area to be wounded (see below) and the second 5 mm distal to the first. Angle of the fiber optic was 45 and it was held 3 mm above the coral surface. Procedures were conducted at mid-day and corals were not dark adapted, thus, yield measurements are apparent.

On 9/24/06, a 5/16" carbide end mill was used to make a ~0.5 cm² wound in the center of three cores from each colony to a depth of 2-3 mm; the fourth core was left as a control. All cores were re-imaged and PAM measurements made immediately following wounding. All were imaged daily until 10/10/06, 16 days after wounding. Wound areas in each image were calculated using SigmaScan Pro (V.5). The PAM measurements described above were conducted daily 9/24-28/06 then on 9/30/06 and 10/2/06. On 10/9/06, PAM measurements were made in just two locations on each coral, on a portion of healed lesion and on tissue that had not been injured. Temperature and conductivity (salinity) were recorded at 10 min. intervals (Alec Electronics).

Site Comparison with Cultured Corals

Twelve 1" cores were removed from six cultured *M. faveolata* colonies (different from those in the previous experiment), mounted on PVC plates and secured to six PVC field arrays (Fig. 1). The six colonies were each represented by two cores on each array; the exact cores and their positions within each array were randomly selected. Three arrays were installed Jan., 2007 at Jeep Reef (EX01) in the Exuma Cays Land and Sea Park (ECLSP; a no-take area) and

three at North Norman's Reef (EX02) near Lee Stocking Island (unprotected). Both sites were on sand near patch reefs with similar depths (6-7 m). One array at each site was instrumented with a conductivity-temperature logger and a depth recorder (Alec Electronics).

Over the following year, coral responses to wounding were seasonally examined (winter 2007, spring 2007, summer 2007 and winter 2008). Coral cores were retrieved from the arrays and brought to a shore-side seawater system overnight. They were imaged and PAM measurements conducted. After wounding, these procedures were repeated and the cores returned to the field arrays for 5-7 days. They were again retrieved for imaging and PAM measurements. Procedures were similar to that described above with the following differences: images were made with a higher resolution camera (Sea & Sea DX8000; 3264x2448 pixels), four (rather than 8) PAM measurements were made per core at each of the cardinal points and wounds were made with a 5/16" stainless steel rod. Instruments were serviced and downloaded during each assessment period.

Site Comparison with In Situ Corals

Wound healing assessments were conducted in February and March/April 2008 near Warderick Wells (WW) in the ECLSP and in Elizabeth Harbour (EH) near George Town, the largest population center in the Exumas (Feb. April, and May, 2008). Within both study areas, four mature *M. faveolata* colonies of similar size and condition were haphazardly selected at each of four similar patch reefs (16 colonies within each study area). Four wounds per colony (at least 10 cm apart) were made on the colony top surface with a 5/16" punch. Wounds were imaged (Canon SD800; 3072x2304 pixels) with a 1-cm scale and repeated 5 days later. Wound areas were assessed using Coral Point Count with Excel Extensions V 3.5 (Kohler and Gill 2006).

Results

Mesocosm Experiment

The end mill wounds were somewhat ragged, thus, the day after the wounding (9/25/06), when tissue had sloughed off, was used as the basis for initial lesion size. Thus, the number of days shown on the graphs is 15 rather than 16 days. Fig. 1 shows an example of the healing observed the experiment. The first wounds healed 100% by day 13 and by the end of the experiment (day 15), five of the 12 wounds had completely healed. Three months later (data not shown), all but two cores had completely healed.

The healing of the three replicate cores was averaged to represent each colony and the four colonies averaged to produce grand means for each day. The recovery curve for all cores over the initial 15 days is shown in Fig. 2. The 50% lesion recovery time was just over 5 days.

No effects of wounding were observed on the apparent quantum yields of tissues proximal (~3 mm) or distal (~8 mm) to the lesion. However, newly healed tissues had significantly lower apparent quantum yields than the adjacent undamaged tissues.

Site Comparison with Cultured Corals

Over the 1-year of this experiment, tissue loss resulted in morbidity that precluded making all four PAM measurements or complete mortality of the coral core. Based on the initial 72 cores, the morbidity rate over the year was 35% and the mortality 14%. Corals fared most poorly during the period (8/07-1/08) prior to the last assessment. Factors responsible could include maximal temperatures of 31-32° C at both sites (8/07) and Tropical Storm Noel (11/07) that produced salinities as low as 30 ppt (EX02; 34 ppt at EX01).

Normalized healing (% of initial lesion day⁻¹) at all sites in all seasons are compared in Fig. 3. Because no field measurements were made in the Fall, the mesocosm experiment results (“LSI”) are shown for comparison. With the exception of healing at EX02 in the Winter of 2007, averaged healing at all locations throughout the year was typically 9-11 % day⁻¹. The only significant site difference was in Winter, 2007 where wounds at EX01 healed faster than at EX02.

Overall, there were no seasonal effects. ANOVA results indicate that there were no genotypic differences. Apparent quantum yield (F_v/F_m) values typically ranged from 0.4-0.6 under subdued mid-day lighting. No significant differences were attributable to sites, season or genotype.

Site Comparison with In Situ Corals

Regeneration of lesions on *M. faveolata* colonies in situ were generally similar to those seen on the 1” cores in the previous experiments. During the February 2008 assessment, coral lesions at the Warderick Wells sites healed significantly faster than those in Elizabeth Harbour (Fig. 4). The mean healing rate for all colonies (n=16) at the four WW sites was 11.74±3.09 % day⁻¹ and for the EH sites 7.63±3.72 % day⁻¹. A repeat of the procedures in March/April on the same colonies did not show a significant difference between the study areas (WW: 8.51±3.78 % day⁻¹; EH: 7.28±3.30 % day⁻¹). The regeneration rates at the WW sites were lower during the second assessment but not significantly so.

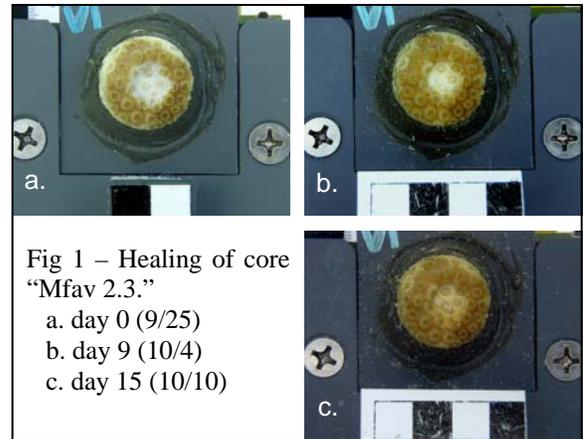


Fig 1 – Healing of core “Mfav 2.3.”
a. day 0 (9/25)
b. day 9 (10/4)
c. day 15 (10/10)

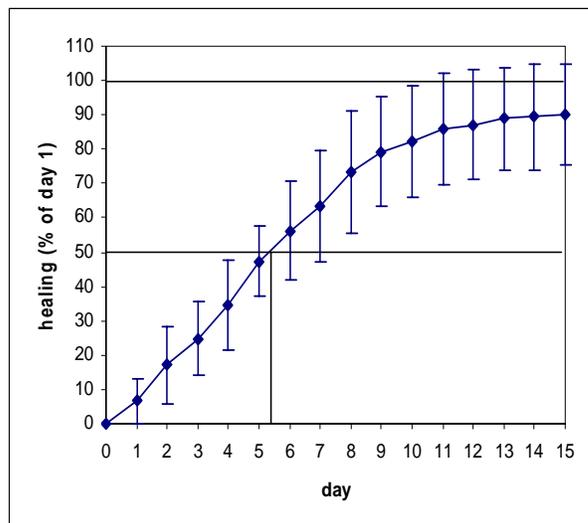


Fig. 2 – % healing of all corals (mean ± SD; n=4).

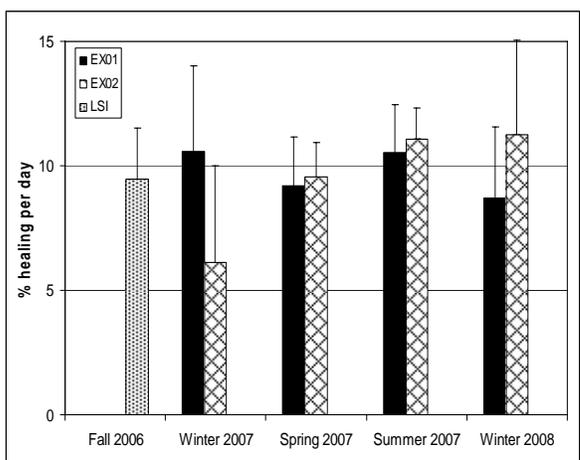


Fig. 3 – Wound healing at field sites (n=6; EX01 & EX02) and in the mesocosm experiment (n=4; Fall, 2006). Mean ± SD.

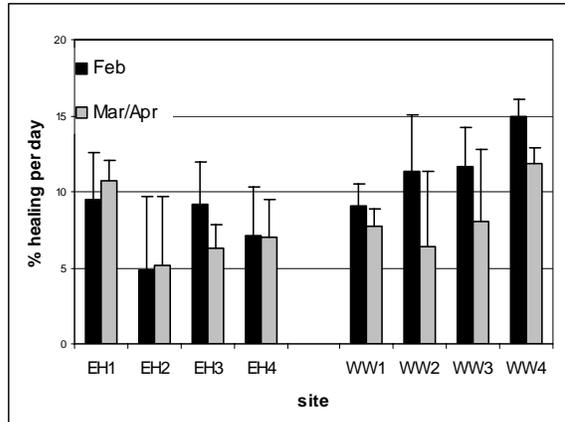


Fig. 4 – % healing of *in situ* colonies at Elizabeth Harbour (EH) and Warderick Wells (WW) sites in Feb. and Mar./April, 2008. Bars represents the mean \pm SD of 4 colonies (each with 4 lesions) at each patch reef site.

Discussion

The Coral Resilience Assay has been applied to corals in several experimental settings: cores derived from cultured parents in mesocosm- and field-based experiments and colonies *in situ* on patch reefs. The Assay resulted in consistent tissue regeneration times. Results showed generally faster tissue regeneration than in other studies with corals within the *Montastrea annularis* complex, some of which founds tissue regeneration times requiring months (Mascarelli and Bunkley-Williams 1999; Cróquer et al. 2002; Fisher et al. 2007).

Using a small (0.5 cm²) lesion maximizes the possibility that the lesion will completely heal, thus preventing establishment of epibionts or disease, and allows for results to be obtained quickly. A five-day regeneration period was found to be suitable for producing enough tissue to provide a good signal-to-noise ratio but not so long as to allow complete lesion healing that would preclude quantitative assessment

M. faveolata was selected as a standardized species because of its importance to Western Atlantic reefs and its broad distribution across the province. In addition to broad distribution, *M. faveolata* is commonly found on all reef types encountered in the area and across a wide depth range. The surface of *M. faveolata* was found conducive to producing uniform lesions.

Field experiments with cores and *in situ* colonies did not find consistent differences inside or outside of the ECLSP. Two of the six assessments described (1 of 4 with cores; 1 of 2 with *in situ* colonies), did find significantly faster healing rates inside the ECLSP. Although both of these cases were in the winter months, no seasonal trends were apparent as other

assessments in winter found no differences. Examination of apparent quantum yields did not reveal any effects of the lesions on adjacent tissues or seasonal effects. However, newly-formed tissues over the lesion did have significantly lower apparent quantum yields than adjacent, undamaged tissues.

To obtain useful results, the Coral Resilience Assay must be applied with special attention paid to making lesions of consistent type, size and depth (Oren et al. 1997; Hall 2001). In designing a field project, data from others strongly indicate that lesion position on the colony is important (Cróquer et al. 2002) and that colonies should be of similar size (Meesters et al. 1997) and depth (Nagelkerken et al. 1999; Fisher et al. 2007).

The Coral Resilience Assay is relatively inexpensive and all necessary equipment, excluding a computer for image analysis, can be obtained for less than \$500.00. Suitable image analysis software (CPCe) is available as a free download from the National Coral Reef Institute, Nova University, Dania, FL (www.nova.edu/ocean/cpce/). Scientists and resource managers are encouraged to employ this standardized lesion protocol across the Western Atlantic Province to examine coral resilience and potential stress within and between regions. A booklet with detailed descriptions of the methods and data analysis methods is available from Conservation International (www.conservation.org).

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