PERIODIC ENDOLITHIC ALGAL BLOOMS IN *MONTASTRAEA FAVEOLATA* CORALS MAY REPRESENT PERIODS OF LOW-LEVEL STRESS

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ABSTRACT

Cores from the scleractinian coral *Montastraea faveolata* (Ellis and Solander, 1786) *sensu* Weil and Knowlton (1994) from the Mesoamerican Reef possess obvious green bands, sometimes occurring annually, but more often at less frequent intervals. Bands are remnant concentrations of the endolithic green alga *Ostreobium* spp. that grow parallel to and below the living coral tissue. We dated green bands in 58 *M. faveolata* cores collected from four sites on the Mesoamerican Reef. We found that the bands are not related to coral skeletal growth reductions, but instead are caused by algal blooms within the coral skeleton. We hypothesize that the blooms occur during periods of coral paling (the partial loss of pigmentation), during which more light penetrates through the translucent coral tissue into the coral skeleton. This hypothesis is supported by observations of discontinuous banding within the skeleton, the patchiness of pigment loss in living corals, and ecological observations of endolithic algal blooms in living bleached corals. At three sites, there was a significant increase in green band occurrence over time, which suggests that coral paling may have increased over the last several decades.

Endolithic algae of the genus *Ostreobium* occur naturally in the skeleton of many coral species (Lukas, 1974), including *Montastraea faveolata* (Ellis and Solander, 1786), living just below the tissue layer. Where endolithic algae are highly concentrated, they appear in the coral skeleton as green bands. Typically, multiple bands occur throughout the *M. faveolata* coral skeleton (Fig. 1A). All green bands except the topmost are composed of degrading pigments and are not living (Kanwischer and Wainwright, 1967). However, organic compounds are well-preserved in massive coral skeletons, probably due to anoxia and slow diffusion rates (Ingalls et al., 2003). Therefore, though the deeper bands are no longer living, they do not fully degrade and in this study were qualitatively just as vividly pigmented as the living bands. Although several studies have attempted to explain the existence of green bands of endoliths in coral skeleton, no consensus has been reached. For instance, Odum and Odum (1955) suggested that the endoliths may live in mutualistic symbiosis with the coral, much like zooxanthellae. Lukas (1973) suggested that the endolithic algae grow upwards with the coral, and that during periods of slow coral growth, the algae accumulate into a dense band, with which Le Campion-Alsumard et al. (1995) agreed. An alternative view was presented by Highsmith (1981) who suggested that bands might represent blooms of endoliths due to improved growth conditions for the algae.

Endolithic algae blooms have been observed under bleached coral tissues in *Oculina patagonica* (De Angelis, 1908) (Fine and Loya, 2002) and *M. faveolata* (Rodríguez-Román et al., 2006). Coral bleaching is a stress response characterized by a reduction in the coral's symbiotic zooxanthellae, or zooxanthellae pigmentation, and on large spatial scales is usually caused by thermal stress (Brown, 1997). Other studies have shown that photosynthates synthesized from endolithic production can be translo-
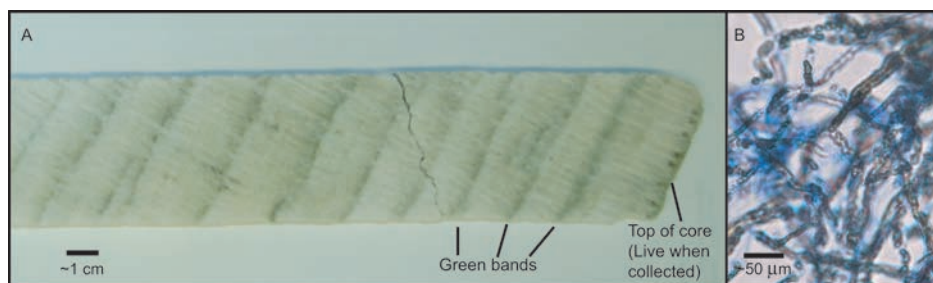
Figure 1. (A) Plain light photo of slab removed from core (from the Sapodilla Cayes) showing green bands. (B) Photomicrograph of stained endolithic algae from a decalcified coral sample.

cated to the coral host (Schlichter et al., 1995; Fine and Loya, 2002) suggesting that this alternate carbon source might allow some coral species to survive better than others during bleaching events (Fine and Loya, 2002; Rodríguez-Román et al., 2006). An increase in endolith abundance may also help corals to recover from bleaching by reducing skeletal reflectivity and associated light stress on recovering zooxanthellae (Rodríguez-Román et al., 2006). Pigment from zooxanthellae normally absorbs > 95% of ambient photosynthetically active radiation (Schlichter et al., 1997; Magnusson et al., 2007), so the loss of pigment can stimulate growth of the endolithic algae due to increased access to light (Fine et al., 2005; Rodríguez-Román et al., 2006). Indeed, under natural bleaching conditions, Fine et al. (2005) found that endolithic algae in *Montipora monasteriata* (Forsskål, 1775) were able to photoacclimate to increased light, although sudden increases in light did lead to photoinhibition.

We investigated the occurrence of green banding in 58 coral cores from the Mesoamerican Reef which were collected primarily for skeletal growth rate analyses. The ubiquitous nature of banding in all the cores we collected inspired us to assign dates to individual bands in each core to determine whether they all occurred at the same time across the reef, and were therefore caused by some external forcing, or were randomly distributed in space and time, controlled by intrinsic factors in each coral head. In addition, because we had analyzed skeletal growth rates, we were able to test whether the green bands were caused by reduced coral growth rates or instead represent algal blooms.

Materials and Methods

A total of 92 *M. faveolata* coral cores were collected from four sites on the Mesoamerican Reef in 2006 and 2007 (Turneffe Atoll, the Sapodilla Cayes, Utila, and Cayos Cochinos, Fig. 2, Table 1). Cores were collected between 2.5- and 13-m depth on the fore-reef in spur-and-groove habitat using a hand-held pneumatic drill with a 5 cm diameter core barrel and carbide teeth. Cores ranged from approximately 20–120 cm in length; with annual extension rates of about 8 mm, these covered approximately the previous 25–145 yrs. Drill holes were filled with pre-cast concrete plugs to facilitate recovery of the coral colonies. Once drilled, the living coral tissue was removed using a Waterpik on site and cores were rinsed in fresh water and air-dried. A slab was removed from the middle of each core using a carbide-tipped double-bladed table saw for x-ray analysis. Cores were kept in drawers to deter fading of the green bands, which occurs when algal pigments are exposed to sunlight. Endolithic algae were identified to the genus *Ostreobium* by microscopic inspection of stained decalcified material (Fig. 1B). The alga *Phaeophila dendroides* has been observed in other coral species (e.g., Titlyanov
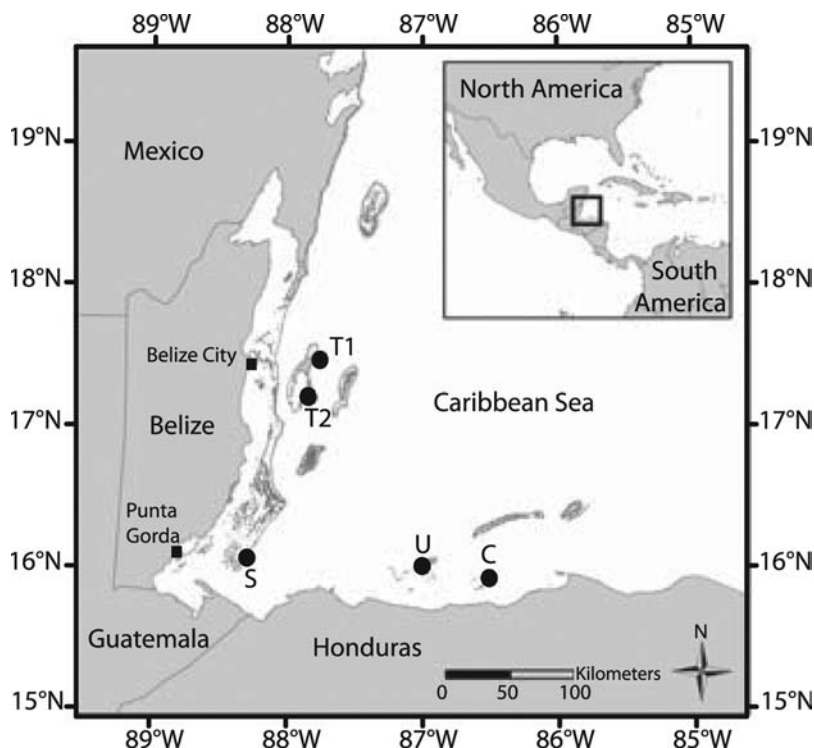
Figure 2. Map of the Mesoamerican Reef showing locations of coral collections as black circles. Dark grey denotes reef, light grey denotes land areas. T1, T2 = Turneffe Atoll (4 cores from T1, 13 from T2), S = Sapodilla Cayes, U = Utila, C = Cayos Cochinos.

et al., 2008) and might also have been present in low quantities in our samples, though it was not found in any of the bands that we inspected.

Coral slabs were x-rayed to reveal annual density banding, which was used to assign dates to green bands (Knutson et al., 1972). Fifty-eight cores were chosen for this study based on the clarity of annual bands in their x-rays (14 from Turneffe, 12 from Utila, 10 from Cayos Cochinos, and 22 from the Sapodilla Cayes). Endoliths grow up to 0.5 cm below the living coral tissue in *Porites* spp. (Le Campion-Alsumard et al., 1995) and *M. monasteriata* (Magnusson et al., 2007). The depth of endolith growth within *M. faveolata* has not yet been quantified, however, skeletal isotopic analyses across green bands indicate that they probably form just below the living tissue in *M. faveolata* (A. Hartmann, Scripps Institution of Oceanography, unpubl. data), and therefore, green bands were assigned dates under this assumption. Including a time-lag in case the bands actually formed up to 0.5 cm deeper in the skeleton did not significantly change our results, so only the former situation is included here. Time series of green band occurrence were constructed as the percentage of cores with a green band in each

Table 1. Coral core collection site locations, with dive site name or nearby caye and coordinates.

Site	Dive site name	Coordinates
Turneffe 1	Dog Flea Caye	17°29′59″N, 87°45′30″W
Turneffe 2	Harry Jones	17°18′25″N, 87°48′04″W
Sapodilla	Frank's Caye, Northeast buoy	16°07′45″N, 88°14′59″W
Utila	Diamond Caye	16°03′52″N, 86°57′30″W
Cayos Cochinos	Pelican Point, Peli 2	15°58′41″N, 86°29′06″W

individual year (Fig. 3). We tested whether the observed proportion of green bands could have occurred due to random change by comparing our data to a Poisson distribution using a chi-squared test. The location of green band occurrence, whether within the high-density or low-density portion of each annual band, was also noted. In addition, we quantified the number of bands which were not continuous across a 5 cm wide coral core.

To test whether green bands occur due to periods of diminished coral growth, coral extension rates (cm yr^{-1}) and annual density (g cm^{-3} yr^{-1}) were separated into two groups: those with a green band and those without a green band. Due to non-normality of the data, a Mann-Whitney U test was used to test for significant differences in coral growth rates between the green and non-green groups. Note that due to extreme growth suppression in all cores after the 1998 mass bleaching event in corals from the Sapodilla Cayes and Utila (Carilli et al., 2009a), only data through 1997 were used.

Logistic regression analysis was used to determine whether there was a significant change in the probability of green band occurrence over time. First, a linear model was used to describe the probability of green band occurrence with time. The best-fit parameters of the model were then estimated using the Nelder-Mead optimization algorithm in R. A likelihood ratio test was used to determine whether the slope for the model was significantly different than zero.

Results

In total, 774 green bands were identified in the samples used for this study. There was no significant difference in coral extension or density in years with or without green bands (Fig. 4; Mann-Whitney U test: P = 0.44 and 0.11, respectively). In our sample set, 64% of the bands were found within the high-density portion of a skeletal band which forms during the late summer and early fall (Cruz-Piñon et al., 2003). 11% of the green bands identified were discontinuous across the 5 cm width of the cores and either ended partway across a core or shifted abruptly to a slightly different depth in the core.

Within each core, the mean proportion of years with a green band out of all total years at all sites was 0.26 ± 0.09 (mean ± SD), indicating that green bands occur, on average, about once every 4 yrs. Green bands were significantly less common at Turneffe Atoll (0.18 ± 0.06) than at the Sapodilla Cayes (0.26 ± 0.08) (Mann-Whitney U test: P = 0.004).

Time series of the occurrence of green bands for all the cores from a given site show that green bands are more common in some years than others, and that the distribution of green bands is not random (Chi-squared test: P < 0.001). Some years (such as 1980 and 1985) show an abundance of green bands at all sites, but other years (such as 2004) show high green band incidence only at a single site (Fig. 3). The logistic regression determined that the proportion of cores containing a green band increased over time (i.e., time was a significant predictor of green band occurrence, P < 0.05) both among all sites pooled and within all individual sites aside from Utila (Fig. 3). The oldest core in our sample set extends back to 1869, and includes a green band in that year.

Discussion

We found no significant relationship between coral growth rates and the occurrence of green banding. Therefore, the occurrence of green bands must be a result of algal blooms. Other studies have shown that endolithic algae bloom under bleached
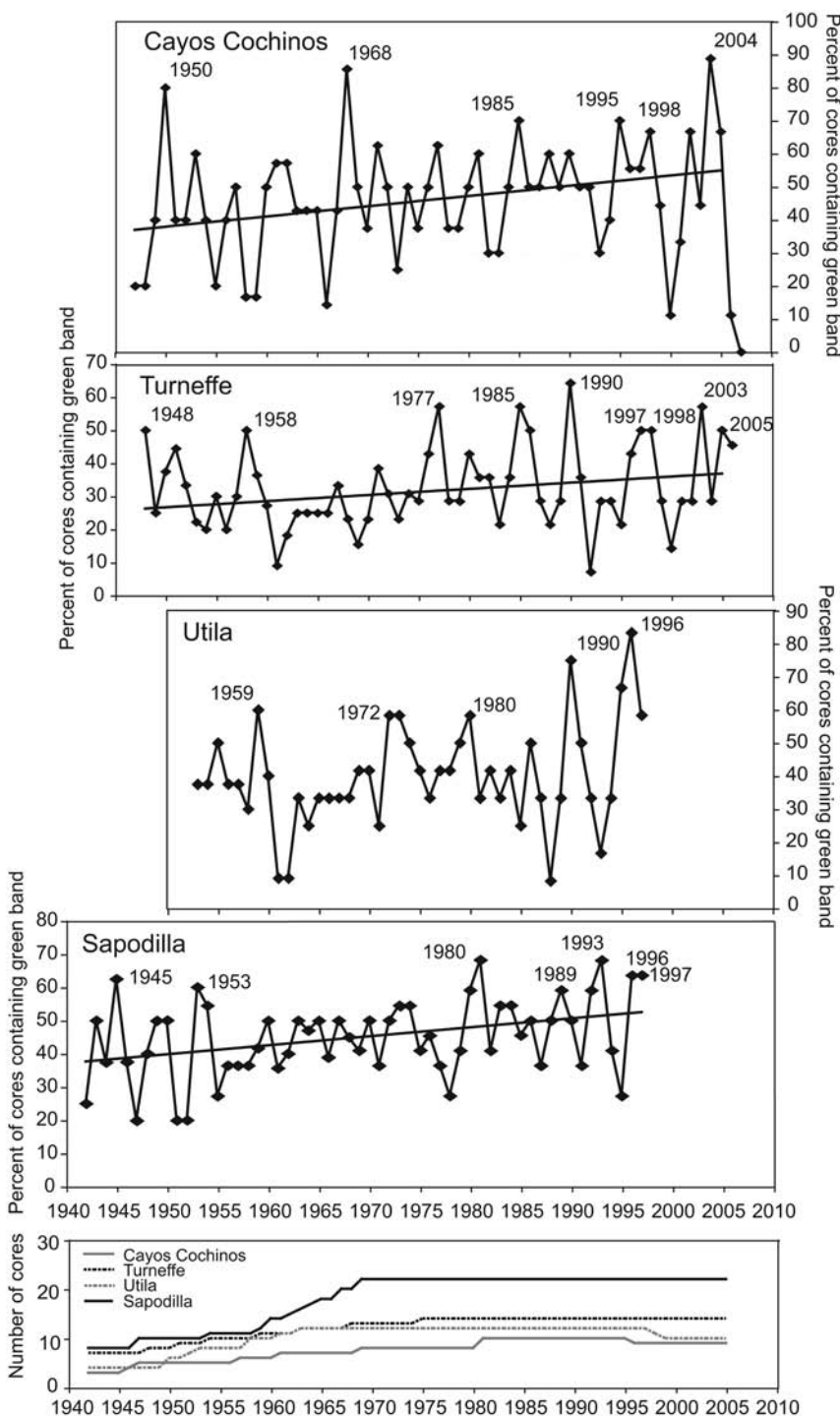
Figure 3. Percentage of cores from each site with green bands in a given year (diamonds), and fitted values from the logistic regression model (straight line). For clarity, chronologies are plotted with at least eight cores, except for Cayos Cochinos, which is shown with at least five cores. Bottom panel: the number of cores comprising each chronology above.
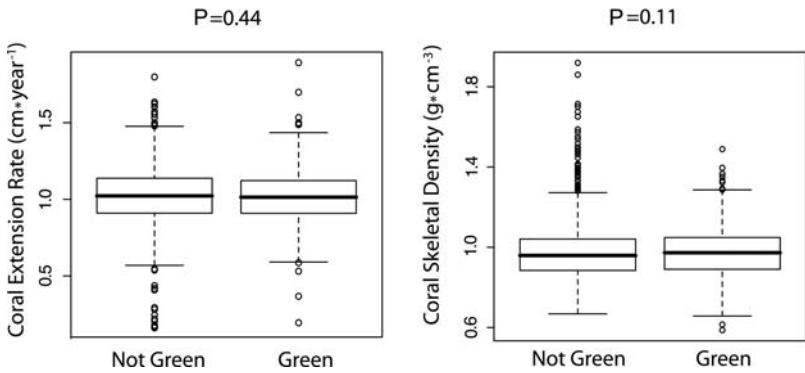
Figure 4. Boxplots of skeletal extension and density for years without green bands and years with green bands. There is no significant difference in either parameter during years with or without a green band (P-values calculated by the Mann-Whitney U test).

coral tissues (Fine et al., 2002; Rodríquez-Román et al., 2006). The lack of widespread occurrence of green bands during known years of mass coral bleaching, however, indicates that green bands are not a simple proxy for mass coral bleaching. Severe, large-scale bleaching events occurred on the Mesoamerican Reef in 1995 (McField, 1999) and 1998 (McField, 2000). However, coral cores from the Sapodilla Cayes and Utila do not have a high incidence of green bands in these years, and Turneffe Atoll cores only have a high incidence of green bands during 1998, not 1995. These between-site differences may stem from regional-scale differences in the severity of bleaching onset, though no significant spatial trends were observed during the mass bleaching event in 1995 (McField, 1999) or recovery after the 1998 mass bleaching event assessed in 1999 (Kramer and Kramer, 2000). In addition, we found green bands deep in the coral cores, some of which are as old as 1869, but no large-scale bleaching was reported on the Mesoamerican Reef until the 1990s. Fine et al. (2005) showed that endolithic algae became photoinhibited when exposed quickly to increased light, but that during slow loss of zooxanthellae, endoliths were able to photoacclimate and continue photosynthesis. These authors suggested that more resistant corals, which may take longer to bleach, could allow photoacclimation of the endolithic algae. Since *M. faveolata* is one of the first coral species to show signs of bleaching (Fitt and Warner, 1995), during severe bleaching years, the loss of zooxanthellae may be so rapid that the endolithic algae are not able to photoacclimate, and no bloom occurs. Though green bands are therefore unlikely to be useful proxies for severe bleaching events (probably due to photoinhibition), we suggest that they instead represent periods of low-level stress, and may be a more sensitive proxy than skeletal growth rates for low-level stress. While skeletal growth tends to have clear anomalies associated with severe bleaching events (e.g., Leder et al., 1991; Carilli et al., 2009a), more subtle changes might not be recorded.

Coral bleaching is a generalized response to stress and can occur at a range of severity, from slight to complete loss of pigmentation (Brown, 1997; Fitt et al., 2000; Siebeck et al., 2006). *Montastraea* corals often display a patchy loss of some pigmentation (Fig. 5), referred to here as "paling" (Gates, 1990; Rowan et al., 1997). Indeed, *M. faveolata* normally experience seasonal fluctuations in zooxanthellae and chlorophyll-a density, even during years not recognized as a bleaching event (Fitt et al., 2000). Rowan et al. (1997) attributed patchy paling in *M. faveolata* to differential
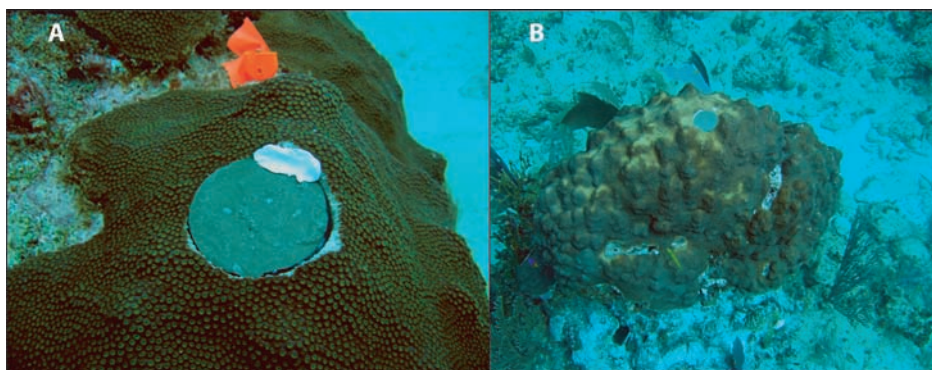
Figure 5. (A) *Montastraea faveolata* colony with normal pigmentation. (B) *M. faveolata* colony with significant paling on the top surfaces.

loss of zooxanthellae *Symbiodinium* clades with low thermal susceptibility. We suggest that green bands form when the pigmentation of coral tissue drops sufficiently to promote endolith blooms within the underlying coral skeleton.

Several observations suggest that green bands are related to paling. First, the discontinuous nature of some green bands (11% of the bands observed here) is consistent with the observed patchy distribution of paling in the coral tissue. Second, endolith blooms have been observed under bleached tissue in the corals *O. patagonica* (Fine and Loya, 2002) and *M. faveolata* (Rodríguez-Román et al., 2006). Hence, ecological observations of patchy and frequent paling in *M. faveolata* provide a clear mechanism for the formation of green bands. Third, the observations of very old green bands from the late 1800s suggest that the process responsible for creating bands is not a new phenomenon, unlike mass coral bleaching, but reflects a long-standing, if episodic, process. Furthermore, green bands are not randomly distributed in time and synchronicity of banding between coral heads indicates that green band formation is not strictly controlled by processes unique to individual coral heads. However, a purely extrinsic force such as a spike in nutrients is also unlikely, as there are no years where 100% of corals exhibit a green band.

We did test for relationships between green bands and a number of physical forcing factors and found no significant relationships; however, it is possible that the scale of the forcing measures (for instance sea surface temperatures from a 1×1 degree cell) were too coarse. Another alternative explanation for green band formation could be tissue retraction, which may allow more light to penetrate through the coral tissue without any change in zooxanthellae density (Brown et al., 1994). However, tissue retraction appears to occur more often in response to physical stress such as subaerial exposure (Brown et al., 1994) or mechanical abrasion by macroalgae (Coyer et al., 1993), both highly unlikely to occur in the corals studied here from water depths over 3 m (with a very small tidal amplitude) and the top-middle of large hemispherical heads. Paling is therefore the most parsimonious explanation for the formation of green bands.

The between-site differences in years with a high proportion of cores with a green band indicate that mostly local-scale instead of regional-scale forcing factors are driving the endolithic algae blooms. These sites experience a range of local stressors, with land-based runoff (sedimentation and nutrients) highest at the Sapodilla Cayes,

followed by Cayos Cochinos, Utila, and Turneffe Atoll (Prouty et al., 2008; Carilli et al., 2009b). Recent work has shown that corals experiencing increased local stress such as runoff are more susceptible to bleaching (Wooldridge, 2009; Carilli et al., 2009c) and recover to normal growth rates more slowly after bleaching (Carilli et al., 2009a). Of the sites studied here, a metric of stress on reef communities involving quantitative assessments of local human population size, fishing pressure and both sediment and nutrient runoff demonstrated that Turneffe Atoll experiences the lowest level of local stress (Carilli et al., 2009a). Perhaps because of this lower background stress, Turneffe also has the lowest overall levels of green band occurrence. We suggest that spatial differences in background stress contribute to between-site differences in the frequency and timing of paling and green band formation. Our findings suggest that green bands can be used as a proxy for coral paling, or generalized coral stress.

Even excluding the period since the globally and regionally severe bleaching event in 1998, we found a statistically significant trend toward increasing frequency of green banding toward the present. If we are correct that green bands can be used as a proxy for coral paling, this trend suggests that paling has increased over the past century, possibly pointing to an overall increase in low level stress within Mesoamerican *M. faveolata.* We speculate that the increased incidence of paling may reflect the overall history of stress on reef corals better than does the occurrence of severe bleaching events or periods of partial mortality.

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