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DEPTH RELATED DISTRIBUTION AND ABUNDANCE OF MICROBORING ORGANISMS: ST. JOHN, US VIRGIN ISLANDS

JONATHAN ROGERS, University of Wisconsin - Oshkosh Research Advisor: Eric Hiatt

INTRODUCTION

The production of calcium carbonate is largely a function of sunlight availability, as dependent on water depth and clarity (Hudson, 1981). There is a tendency to assume then, that reefs will build faster in shallower waters. However, this is a naive prediction that is not the case. For a long time, the natural degradation of substrate was excused from studies of reef accretion. Some studies, however, that examine more than just initial calcification, show the importance of destructive variables such as bioerosion, as a major player in development of the reef framework (Stearn et al., 1977; Hubbard et al., 1990). Sediment that is created by bioeroders, transported down the interior and re-cemented constitutes a large part of coral-reef structure (Glynn, 1987).

Following the death of a coral colony, microbioeroders are among the first to contribute to post-mortem degradation (Perry and Hepburn, 2008). Among these microscopic organisms are: fungi, cyanobacteria, algae and some microsponges (e.g. Glynn, 1973). Most microorganisms that erode into coral colonies, are constrained by many of the same variables that control coral growth, in particular, light levels that are sufficient to allow for photosynthesis and respiration. For different organisms, the depth in which they may tunnel to changes according to light availability; as a result, light availability controls the water depth in which they can effectively bore (Chazottes et al., 1995). Thus, there should be a zonation pattern for microborers much like the one for photosynthetically dependent corals. Characterizing this pattern for the infaunal communities across a sampled depth gradient in Lameshur and Haulover Bays (see Fig. 1 in Hubbard and Parsons-Hubbard, this volume) is the primary goal of this study.

METHODS

After a catastrophic bleaching event that occurred within the study area in the summer of 2005, over 90% of the reef corals were killed, and bioeroders have since heavily bored into the dead substrate. Samples were collected from 11 transects in two modern reef complexes at Haulover Bay (three transects) and Little Lameshur Bay (8 transects) (see Table 1 in

Sample	Depth (m)	Diameter				Morphology		
		<1micron	1-3microns	4-7microns	8-15microns	Branching	Bulbous	Filamentous
		Abundance (%)						
Н08-6 Тор	4.9							
Н14-22 Тор	6.1	20%	55%	20%	5%	40%	35%	25%
Н15-5 Тор	7.6	0%	90%	10%	Х	35%	15%	50%
T15	7.6	10%	65%	22%	3%	30%	10%	60%
Т16-2 Тор	10.1	7%	85%	8%	Х	25%	30%	45%
T17	11.6	5%	60%	30%	5%	35%	15%	50%
T12	12.2	5%	75%	20%	х	40%	5%	55%
т06	14	0	80%	15%	5%	50%	20%	30%
Т07-5 Тор	15.5	15%	70%	5%	10%	65%	12%	23%
T19-3 Crypti	18.3	10%	85%	5%	Х	55%	5%	40%
T20-1 Top	19.2	3%	90%	7-8%	Х	70%	15%	25%

Table 1. The size (width) and morphologies of microboring traces present in samples along a depth gradient of 4.9m-19.2m water depth.



Figure 1. Graphs showing the size and shape of dominate microborers in this study. Graph A represents changes in microboring diameters with depth. The graph shows that all sizes of microborings are present across the entirety of the sample gradient; 1-3um is shown to be the typical microborer size, as its' percent abundance is high across 6.1m-19.2m water depth. Graph B shows the prevalence of microboring morphologies that are present (as percentages) at depths between 6.1m-19.2m water depth. The graphs shows that branching morphologies are increasingly abundant with increased water depth; the highest concentration (represented by %) of branching morphologies is in the deepest sample collected at 19.2m water depth. Filamentous and bulbous forms do not discern a trend relating percent abundance and water depth, i.e. water depth at which the coral colony exists does not predict microboring shape.

Hubbard and Parsons-Hubbard, this volume). Each transect extends for ten-meters and is characterized by having at least fifty-percent hard ground with little or no coral cover. All transects are orientated

at N30°E and occur at water depths ranging from 4.9m to 19.2m. Samples were chosen with the aid of bimonthly to annual video surveys provided by the US National Park Service (NPS) (see Fig. 2A in Hubbard and Parsons-Hubbard, this volume), who has been monitoring these permanent transects since 2005. This allowed us to determine approximate time of death. In total, seventy-three dead and four live colonies of *Montastraea annularis* were collected.

In the laboratory, samples were cut into slabs, generally parallel to the transect. If the colony died over an extended period of time, cuts were made to include each represented time period. Samples used for analysis of microborings were chosen to compliment related projects on macroboring (Witcher, this volume) and biont encrustation (McElroy, this volume). A parallel study to quantifu removal of available carbonate by microbioeroders was undertaken by Neal (this volume). Individual slabs were soaked in bleach (5%) and hydrogen-peroxide to remove organic tissue matter. Small chips were cut from the tops and sides of each slab, dried overnight, impregnated with a blue epoxy resin under vacuum, and polymerized at 800-100oC and 120 psi for 12-24 hours.

Chips were subsequently cut parallel to the axis of growth to give a cross-sectional view of the exposed inner coral growth surface. A subsample of chips for the study on infaunal distributions by depth, were etched in 10% nitric acid for 20 minutes, to remove the calcium carbonate, leaving behind a preserved cast of the microboring network. Samples were dried overnight and coated with gold-palladium under vacuum. Under scanning electron microscope (SEM), images showing the geometry of microborings were taken of eleven samples to investigate associated assemblages of organisms, in relation to the depth in which the sample was collected. Images were enhanced using the program Fireworks MX 2004 to improve grayscale contrast. Analysis of each photomicrograph by modal percentages was done to characterize the depth ranges for various microborers. General identification (cyanobacteria, algae and fungi) of microbes was guided by the following constraints: size, shape (bulbous or filamentous), mode of branching and associated accessory structures (Budd and Perkins, 1980). Scale determination and calibra-



Figure 2. Morphologies of each major microborer type. The top two images (A and B) show algae as straight to sinuous in shape with local swelling; the images also show algae as smooth and textured forms. The arrow in (A) shows swelling and points to an example of Ostreobium sp.; the arrow in (B) shows texture in resin cast. The middle images (C and D) show fungi forming thick interwoven networks and rectilinear branching (see arrow). The bottom images (E and F) show different morphologies of cyanobacteria.

tion was aided by the use of ImageJ64 provided by the National Institute of Health (NIH).

RESULTS

Euendolithic organisms are defined as organisms able to etch into hard (mainly carbonate) substrates (Glaub et al., 2001). Microborings are the product of this active penetration into hard substrate by microorganisms. These euendolithic organisms attack substrates predominantly by chemical means (e.g. Chazottes et al, 2009). By boring in this manner, they leave behind traces of casts comprised of unique features that are taxonomically characteristic. The analysis of the preserved resin casts in the SEM images from this study is used to characterize morphological features that characterize each major microboring group.

This study does not identify individual species of microbial organisms, but instead classifies them only as fungi, algae, and cyanobacteria. Images were compared to published results (Konigshof and Glaub, 2004; Nothdurft, 2009; Radtke, 1993; Radtke et al., 2010). Groups were distinguished on the basis of their size (width) and shape of boring cast, mode of branching, overall characteristic patterns, and associated accessory structures (cf. Budd and Perkins, 1980).

The shapes and widths of the dominate microborers are represented in Figure 1a and 1b, and are generated from the data found in Table 1, which summarizes differences in boring size, widths and morphologies at depth among the samples. The first graph (Fig. 1a) compares percent of microborer by size, and water depth. Size categories of the microborings include: $<1\mu$ m, 1-3 μ m, 4-7 μ m and 8-15 μ m. The graph shows that each of these microborer diameters is ubiquitous at water depths of 4.9m-19.2m; the graph also shows that 1-3µm is the predominate size, as it's percent abundance at each depth is greater than the other size categories. The second graph (Fig. 1b) compares percent of microborer by shape, and water depth. The graph represents percentages of branching, bulbous, and filamentous forms found between 6.1m-19.2m water depth. The r2-value of the branching form shows that this morphology increases with increased water depth. A relationship between that of filamentous and bulbous forms with respect to water depth is indiscernable.

Algae are the most abundant form of bioeroder across the entire sample set. Most often they occur as straight to sinuous branches that commonly curve and locally swell, up to 5μ m- 7μ m in diameter at these swelling points (Fig. 2a). They also occur as irregular forms, and may be smooth or textured in resin cast when viewed under SEM (Fig. 2b). Cast diameter generally ranges from 1μ m to 5μ m, but can be up to 8μ m in diameter. Bifurcations are common with the algal forms found in this study, with the branch generally stemming from the centers of wider regions.

Fungal forms are common in all samples collected in the range of 4.9m to 19.2m water depth, but are most abundant in those collected in deeper water. Their filaments form rather thick interwoven networks (Fig. 2d) and they sometimes exhibit rectilinear branching (Fig. 2c). The widths of the fungal microborings exhibit a wide range of boring cast diameters, ranging from less than 1um up to $12\mu m$, and likely change as a function of which species is present.

Cyanobacteria *Hyella* and *Mastigocoleus* (Fig. 2e and 2f) are likewise present across the entire depth range of samples, but are noticeably more abundant in samples collected from the shallower transects (e.g., Haulover Bay, and T15-17 in Lameshur Bar). Sample H15-5 (1 of 2), which is the second shallowest sample in the study, holds the heaviest concentration of cyanobacteria. Below 11.6m they become less abundant than the still dominant algal and fungal forms of microbes; however, they are still present.

DISCUSSION

The size of microborings examined in this study do not show a relation to the water depth in which the sample was collected from. As Figure 1a shows, all size categories of microborings from $<1\mu$ m up to 15μ m, are prevalent between the range 6.1m-19.2m of water depth. The occurrence of each size present at each depth, may be explained by which specie of microborer is present with regards to favorable conditins. The morphologies of microborings with respect to water depth, likewise, do not show a strong



Figure 3. Shapes of microborings representative of a typical shallow-water assemblage versus a typical deep-water assemblage. The scale on both images is 100um. (A) was collected from a water depth of 4.9m and is discriminated by long filamentous borings that occur as straight to sinuous in form. (B) was collected from a water depth of 19.2m and is discriminated by terminus branching borings.

relation. Although, it may be discerned that samples collected from deeper water depths are characterized by branching morphologies, as Figure 1b shows that there is an increase in branching forms with increased water depth.

The endolithic organisms described in this study generally occupy wide bathymetric ranges (i.e all groups share the same distribution ranges). This may be explained in the fact that the depths from which the samples in this study were collected are well within the photic zone; therefore light availability may not be the dominate control. If our study had sampled corals from deeper water depths, we might expect to see stronger zonation patterns. The bathymetric zonation and distribution of organisms are controlled by light penetration and the organisms' ability to perform photosynthesis and respiration (e.g. Golubic et al., 1975). This constrains the limit to which a zonation may be made for each bioeroder, for they all receive sunlight sufficient enough to maintain a balance of photosynthesis and respiration. However, although each microbe exists throughout the entire sampled gradient, each group was found to be more abundant within a restricted range of water depth. These zones of greatest abundance's may suggest some light dependence and 'zonation' and allow for an endolithic assemblage by depth to be ascribed.

The euphotic zone extends from the supratidal zone down to 360m water depth (Chazottes et al., 1995). Our study sampled in place dead corals of Montasraea annularis at depths from 4.9m-19.2m water depth. There is still plenty of light present to perform photosynthesis at 19.2m, and thus all major microboring groups are found in even the deepest sample. However, the high occurrence of cyanobacteria present in samples collected from shallow depths (e.g. Haulover Bay and T15) may suggest that cyanobacteria is more sensitive to changes in light intensity within the water column (cf. Budd and Perkins, 1980). Modern cyanobacteria depend on light driven metabolism, and therefore prefer the well-illuminated portions of the water column (Glaub et al., 2001). This study shows that cyanobacteria are most abundant in samples collected in the range of 4.9m-11.6m water depth, and decrease in those collected in deeper water. This suggests that the influence of cvanobacteria decreases as a contributor to bioerosion with increased water depth (with fungi and algae as competitors).

Each described organism exists throughout the whole range of collected samples, but a higher concentration of cyanobacteria, relative to algal and fungal forms (shallower to deeper, respectively) reflects the level of sunlight necessary for each form to thrive. Findings of cyanobacteria being highest in the shallowest

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of collected samples sits well with past studies on bathymetric endolithic zonations, as vertical patterns appear to be related primarily to light penetration (cf. Budd and Perkins, 1980; Golubic et al., 1999). On the basis of having a higher abundance of cyanobacteria in the shallower samples, and conversely a higher abundance of fungal forms in deeper waters, it seems that if our study had included samples taken from the lower photic zone, we might find an increasing dominance of fungi; possibly reaching a threshold in which this would be the only form of microbe contributing to coral reef post-mortem degradation.

Based on the SEM image analysis in this study, we cannot discriminate a bathymetric model of infaunal communities for the sites at Haulover and Lameshur Bay. A sampling from a wider depth range would allow for better assessment of distinct depth zonation. Although zonations of microbial life are not explicitly answered, the traces of microbial euendolithic organisms in this study do provide a distribution range of organisms that bore into and colonize in situ coral colonies following their death.

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