

# PROCEEDINGS OF THE TWENTY-EIGHTH ANNUAL KECK RESEARCH SYMPOSIUM IN GEOLOGY

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## QUANTIFYING THE MICRO- AND MACRO- BORING COMMUNITIES IN CORAL GARDENS, BELIZE

GABRIELA GARCIA, Oberlin College

Research Advisor: Dennis K. Hubbard, Oberlin College

### INTRODUCTION

The majority of the carbonate in modern reefs is produced by corals. Once the coral dies, the carbonate skeleton is immediately occupied by algae, followed by a host of infaunal borers seeking shelter. Grazing organisms grind away substrate as they feed on algae while infaunal bioeroders break down carbonate, producing large quantities of sediment. The interiors of most Caribbean reefs are dominated by sediment and not in-place coral (Hubbard et al. 1998), highlighting the importance of bioerosion in reef building.

Since the late 1970s, coral cover on Caribbean reefs has declined dramatically (Gardner et al 2003; Jackson et al. 2014). In central Belize, branching *Acropora cervicornis* was among the hardest hit species along lagoon patch reefs (from 70% in 1986 to nearly 0% in 1993; Aronson and Precht 1997). This species is an important Caribbean reef builder and its loss has had significant impacts on both carbonate production and the complexity of reef structure.

Recent studies have highlighted the importance of the changing relationship between carbonate production and bioerosion on reefs (Eakin 2001; Alvarez-filip et al. 2009). Perry (2012) has provided a protocol that integrates bioerosion into monitoring.

Most bioerosion studies have used artificial substrates to experimentally determine the rate of bioerosion by borey. Notable exceptions used naturally occurring colonies of *A. cervicornis* and *Agaricia tenuifolia* (Lescinsky et al. 2008) and *Orbicella annularis* (Whitcher, 2012) for which the time of death was reasonably constrained. Like many earlier studies,

these focused on macro-bioerosion by worms, molluscs and sponges.

This study adds the dimension of micro-boring, using dead *A. cervicornis* samples collected from three patch-reefs transects near Ambergris Caye, Belize (Fig. 1 in Greer et al., This volume). It examines the relationship between micro- and macro-boring and estimates the time of death for the Ambergris Cay samples using rates determined elsewhere in Belize (Lescinsky et al 2008). Understanding the relationship between micro and macro-boring, as well as the relative timing of each can help us to understand the post-mortem pathways of corals in central Belize. It also serves as a way to constrain the time of death for *A. cervicornis* samples from these patch reefs and allows us to tie their demise to specific events (e.g., the bleaching event in 1998). Combined with data from other parts of the project, this may help determine whether these reefs have been affected by recent stresses or are truly refuge sites for this important Caribbean species.

### METHODS

#### Sampling and Preparation

Forty dead *A. cervicornis* branches were collected from transects 2, 4 and 5 in a lagoon patch-reef complex near Ambergris Cay (Fig. 1: Greer, this volume). Three kinds of samples were collected: mostly-dead branches with living tissue along the tip (20 “live tip” samples); completely dead but still-attached branches from the base of the patch reef (10 “framework” samples) and unattached rubble near the base of the reef (10 “rubble” samples).

All samples were photographed in the field while they were still fresh and bionts could be distinguished. In the lab, each branch was cut into 2-cm subsamples (“discs” in Figure 1a). High-resolution photographs were taken before and after cutting, and the two faces of each “disk” were scanned at 2400 dpi (Fig. 1b). Selected “discs” were impregnated with epoxy resin, first under vacuum and then at high pressure (~150 psi) to fill all open spaces in the coral. In 14 of the impregnated “live tip” samples, the disk surfaces at 0, 4, 8 and 12 cm from the edge of the living tip were polished flat, etched for 6 seconds with 5% HCl and carbon coated for SEM examination. This was repeated for 3 of the “rubble” samples.

### Micro-boring

SEM images were taken of each prepared face at 1500X. Initially, images were taken at four equally spaced cardinal points on the margins of each disk (Fig. 1b). Due to low variability in boring densities among the replicates for the “live tip” samples, this was reduced to two opposing cardinal points per sample. This procedure was followed for the micro boring analysis in the three rubble samples.

Bioerosion was quantified along the edge of a natural cavity (i.e., intra-skeletal structure, not a bioerosion gallery) with full access to sunlight and free-flowing water, but also protected from grazing, since the cavity was intact (Fig 1c). The freeware program CPC 4.1 was used to point count boring density in the SEM images. A box 80- $\mu\text{m}$  long by 10- $\mu\text{m}$  deep was set at the coral surface and CPC placed 25 random points within that area (Fig. 1d). Each point was classified as “previous carbonate surface” [0], “boring” [1], or void space [not numbered]. This process was repeated to a depth of 70  $\mu\text{m}$  beneath the coral surface. Analyses were completed on 38 discs from 12 branches.

### Macro-boring

To qualitatively assess the degree of macro-boring, all the samples were visually assigned to 1 of 5 categories that reflect an increasing intensity of macro-boring from 1 (no borings) to 5 (extremely altered). Macro-boring density was quantified in one disk from the central portion of six branches that represented all “taphogrades” (proxies for macroboring intensity).

Approximately 200 points were classified on high-resolution scans of each disk using the same categories as above. Three disks came from “live tip” samples (taphograde 1 and 2); three others came from “rubble” samples (taphogrades 3-5).

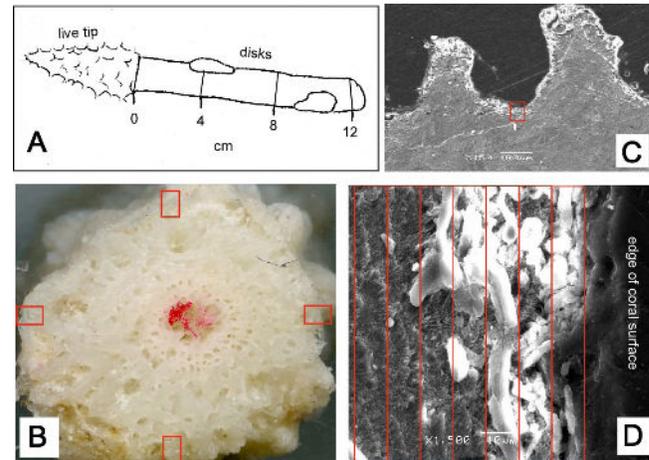


Figure 1. Sample processing A) Initial cutting of the coral branch into 4 disks, which are 4 cm apart. Each number marks the area of the disk cross section. B) The surface of a disk cross section with the four replicates in the cardinal directions. C) SEM image of a natural cavity in the skeleton. Transect location is marked in red. Note 100  $\mu\text{m}$  scale. D) SEM image of a full transect for point counting analysis, each red rectangle represents a 10 micron wide section. Note the 1500  $\mu\text{m}$  scale.

## RESULTS

In the “live tip” samples, total micro-boring within the outer 70  $\mu\text{m}$  ranged from  $8.63 \pm 7.95\%$  to  $29.67 \pm 17.41\%$  (Fig. 2). While this generally decreased toward the tip of the *A. cervicornis* branch, the variability was high, and the micro-boring density at the base reached an average of 30% (Fig. 2). Micro-boring density generally falls below 10% by 60 microns beneath the skeletal surface (Fig. 3). Micro-boring values were consistently higher in the “rubble” samples ( $63.66\% \pm 21.06\%$ , Fig. 4c).

The three types of samples represent a gradient in macro-boring intensity (Fig. 4). The “living tip” samples were lightly bored, while the rubble samples were most heavily altered (Fig. 4b). Micro- and macro-boring intensity were positively correlated, with the more highly micro-bored pieces also being the more highly macro-bored (i.e., the rubble samples in Figure 4c). It is important to note that, in the

“rubble” samples, micro-boring intensity was still at ca. 30% at the 70- $\mu\text{m}$  limit of our measurements (Fig. 4d). Thus, the total micro-bioerosion for the rubble samples would have been even higher had counting continued beyond 70  $\mu\text{m}$ .

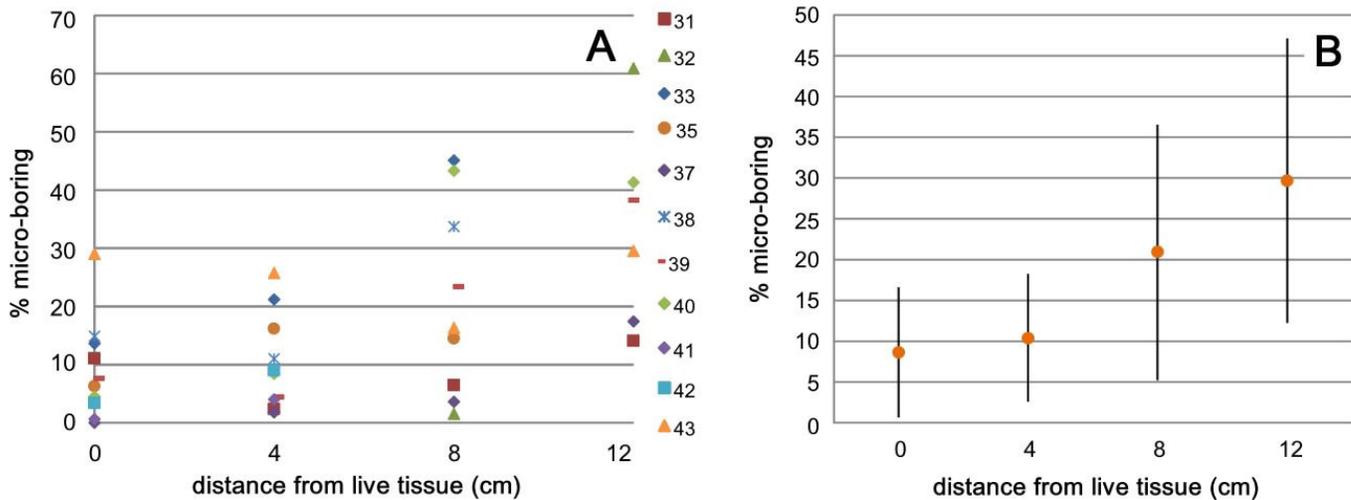


Figure 2. Percent of micro-boring versus distance from live tip near the branch tip. A) Each point is the average for the outer 70  $\mu\text{m}$  measured in the two SEM images for one sample (i.e. disk). B) Mean and standard deviation for all subsamples at 4-cm intervals along the branches.

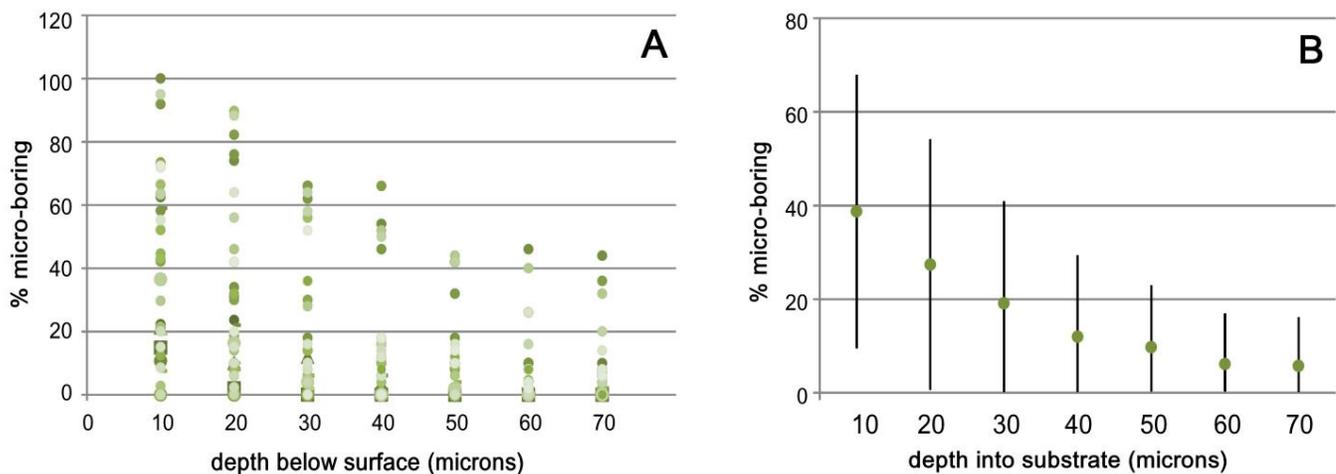


Figure 3. Micro-boring intensity versus depth below the coral surface. A) Averages for the “north” and “south” images for each sample. B) Mean and standard deviation for micro-boring in all “live tip” samples at 10- $\mu\text{m}$  intervals from the surface.

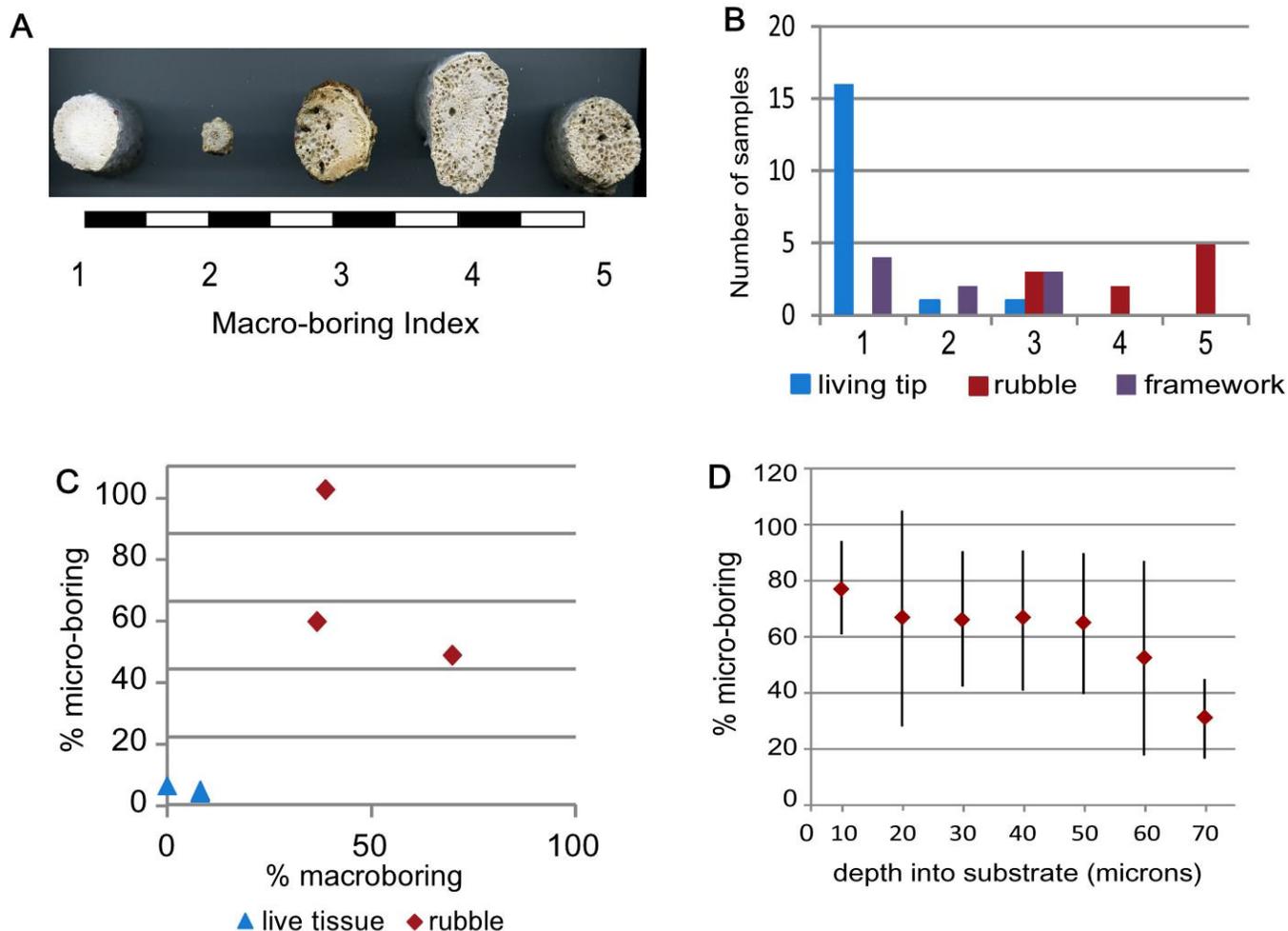


Figure 4. A) Photographs of typical samples from each macro-boring taphograde. B) Distribution of sample types across taphogrades. C) Percent macro-boring in three “live tip” samples (taphograde 1 and 2: samples 31; 37; 41) versus three “rubble” samples (taphogrades 3-5: samples 12; 16; 18). D) Percent micro-boring versus depth below the coral surface averaged from 3 “rubble” samples (12, 16, 18). Mean and standard deviation for micro-boring in samples at 10- $\mu$ m intervals from the surface. Note overlap of the two living tip samples at 36% and 38% micro-boring respectively

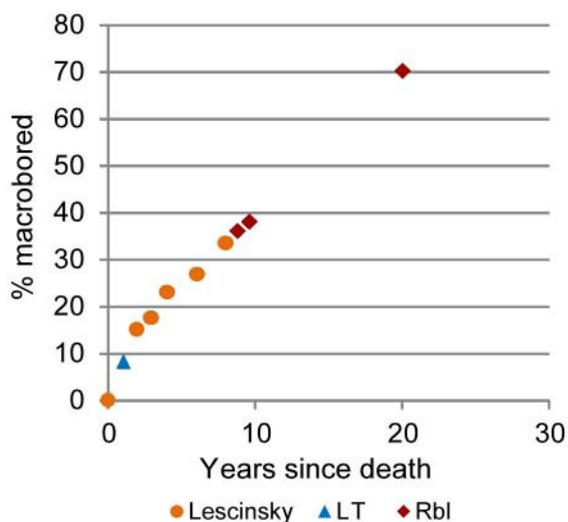


Fig. 5. Time model for age of two “live tip” (LT) and three “rubble” samples (Rbl) in Figure 4b based on data from Lescinsky (2008). The modeled time of death was calculated by subtracting the age of each sample from the date of collection (summer 2014).

## DISCUSSION

### Patterns of Bioerosion

The percent micro-boring in the “live tip” samples increased toward the base of the branch (Fig. 3), suggesting that the part of the branch farther from the tip has been exposed longer. The near absence of macro-borers suggests that their activity begins much later than that of the micro borey. While micro-boring algae cover as much of the outer surface as they can, they are unable to penetrate much deeper than 70  $\mu\text{m}$  into the coral skeleton, at least initially (Fig. 3).

The decrease in micro-boring toward the living tip suggests that death started closer to the base of the branch and proceed toward the tip. This is consistent with White Band Disease (WBD), a known pathogen of *A. cervicornis*. It is also the opposite pattern of fire worm predation, which begins at the tip of the coral. All “live tip” samples were collected from unshaded areas, precluding the possibility of progressive die-off toward the tip as the reef patch expanded.

WBD spreading rates up the branch range from 0.5 to 2.0 cm /day (Precht 2012; Gil-Agudelo et al. 2006). Based on these values, it would have taken between 6 (2 cm/day) and 24 days (0.5 cm/day) for WBD to spread along the 12-cm interval analyzed in the “living tip” samples. The gradient in microbioerosion has persisted since the coral branch died, and it is worth trying to quantify that timing. If this pattern is quickly overprinted by macro- and micro- bioerosion, then its presence would suggest relatively recent death of the samples. Because death appears to be a recent event, the living tip might have succumbed a few days after sampling. If the die off was recent, micro-boring started almost immediately after death and reached the measured density levels in the coral quite soon after the death of the coral. No sign of WBD disease was seen in any of the living tips.

Regardless of the absolute age, the occurrence of micro-boring suggests that it occurs early and before macro-boring (Fig. 4b). The increased micro-boring levels in the rubble suggest that it continues as macro-boring takes hold later in the process, and then both processes subsequently continue in tandem (Fig. 4c).

Because measurements were taken in sites protected from grazing, its relationship to micro-boring cannot be constrained. However, these samples either have the elevated corallites still present or have been encrusted in ways that would preclude significant grazing. Further analyses of the “framework” samples could shed light on whether micro-boring facilitates the entrance of macro-boring into the coral skeleton, as well as their interactions over time.

### Bioerosion Rates and Time of Death

Lescinsky et al (2008) determined bioerosion rates for *A. cervicornis* rubble in southern Belize after widespread die-off in a bleaching event in 1998. If these rates are similar to what has occurred near Ambergris Cay, then bioerosion in the dead portion of the “live tips” samples has been active for less than 2 years (Fig. 5). Based on this time model, the rubble samples are 9, 10 and 20 years old, suggesting times-of-death of 2004, 2003 and 1995, respectively. The estimated years coincide with strong El Niño years, which might indicate bleaching events in this site. Further analysis is required to clarify this relationship. Also, these modeled estimates are near the younger end of the radiometrically determined rubble ages (Butch, this volume). Nevertheless, they are generally consistent.

It is important to consider that the macro-boring bioerosion rates of the Lescinsky et al (2008) study were initially high, and after 3-4 years slowed down. This study used the estimates from years 0-2 for the live tip samples, and years 2-8 for the rubble samples, in order to be as accurate as possible (Fig. 5). The maximum intensity of the pieces collected in the Lescinsky study reached about 40% macro-bioerosion, while one of the samples was 65% bored, which is well outside of the data range used for rate estimation. Although there might be a threshold of maximum density for *Cliona*, i.e., the collapsing point of the more bored substrates as suggested by Lescinsky, these estimates show that highly bored coral pieces can still hold some structural integrity well beyond 40% boring. The fact that our rubble samples were often at least 3 cm in diameter and heavily encrusted might also play a role in the preservation of the substrate.

It is possible that some *A. cervicornis* branches have “resheeted”. Although signs of widespread resheeting were seen in the field, no signs of recent resheeting were seen in any of the samples analyzed. This suggests that many of these corals might have had more uninterrupted growth instead of multiple resheeting instances in their recent past. Nevertheless, no obvious signs of ongoing WBD were observed, therefore the timing of the living tips and their relationship to events recorded in the coral skeletons cannot be absolutely constrained. Further analysis of the framework samples might shed light on the transitional between the two end members of the boring spectrum.

Future studies could aim to quantify the amount of dead tips and rubble pieces in the reef to understand how much coral might have died in the bleaching event of 1998. The rate of live vs. dead vs. infected tips could also be analyzed over time through transect pictures, to investigate if this ratio has shifted over time. These data could be used in turn calculate an approximation to the amount of carbonate that the borings are responsible for eating away, making the carbonate budget for this reef more precise. It would also be interesting as well to look at branches that have certainly not died from WBD, but of bleaching or something similar instead, to see if that type of death reflects an even rate of micro-boring. If these investigations were to corroborate the results from this study, it might mean that, despite being affected by ENSO events, these corals have an unusual resistance to WBD. The reasons as to why these reefs might be resistant to such pathogen, and yet still vulnerable to bleaching event might be hiding in unexplored aspects of the reefs.

## CONCLUSIONS

Based on the finding thus far from this study, the following conclusions are offered:

- 1) Microborings come in very soon after death, while macroboring has a slower initial rate.
- 2) Both of these processes keep increasing and eventually continue in tandem.
- 3) Microboring increases also in depth of substrate

over time. This pattern is also reflected in the transition of the macroboring index. However, *A. cervicornis* rubble can have enough structural stability to exist at 69% boring.

- 4) The “live tip” samples have died recently, and the microboring pattern in them suggests that there might be WBD in this reef. The years of death for the analyzed samples seem to coincide with warm ENSO events.

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