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SARAH HOLMES, Beloit College Research Advisor: Carl Mendelson

INTRODUCTION

Coral reefs are rich in biodiversity and function through a wide range of mechanisms that make their ecosystems flourish. Reefs, however, are easily damaged when subjected to stresses that adversely affect important systems within the reef. In the past 40 years, reefs have experienced a major decline across the globe due to temperature change, sunlight penetration, and a variety of other factors, including salinity, disease, eutrophication, storm activity, sedimentation, and fossil-fuel pollution (Knowlton, 2001; Greer et al., 2009; Aronson and Precht, 2001). Sunlight penetration is important because the zooxanthellae that live in mutualistic symbiosis with their coral hosts are photosynthetic and therefore require sufficient sunlight for growth (Baker, 2011). Those corals adapted to a narrow range of sea surface temperature (SST) for optimal reef development may become stressed by a small rise or drop in SST. As critical framework-building corals die off, they are replaced by fleshy macroalgae, which change ecosystem dynamics and restrict regrowth of these corals.

Acroporid reefs-especially those in the Caribbean Sea-have experienced a major decline. This makes it important to better understand optimal environmental ranges for acroporids. Some of these corals, including those investigated here, have been able to flourish despite rising temperatures, and studying what they have experienced in the recent past can give insight to their optimal ranges. In order to do this, we need to find better ways to extract

paleoenvironmental information from acroporids. This study aims to determine how to effectively sample these important branching corals in order to extract paleoenvironmental information at various resolutions.

Environmental proxies

This study focuses on better understanding the paleoenvironmental record preserved in Acropora cervicornis that are thriving under current conditions of oceanic warming and environmental change. Determining the thermal tolerance of this species may contribute to our understanding of the role of temperature in reef collapse; moreover, we may be able to better predict the fate of coral reefs experiencing environmental fluctuations.

Coral skeletons allow us to investigate environmental changes through geochemical signatures that are preserved within their aragonite framework. Proxies such as stable isotopes of carbon and oxygen can give insight into environmental conditions during the formation of coral skeletons (Porter et al., 1989; Swart et al., 1996; Grotolli, 2000; Grotolli and Eakin, 2007).

 δ^{13} C can be used as a proxy for photosynthesis by zooxanthellae, which preferentially utilize the lighter 12 C, leaving relatively more 13 C to be incorporated into the coral skeleton. This system results in higher δ^{13} C during times of increased photosynthesis (Porter et al., 1989; Swart et al., 1996; Grotolli, 2000). Amount of photosynthesis may be affected by many factors, including cloud cover, water clarity, or sedimentation.

 δ^{18} O is a proxy for both SST and salinity. As SST increases, δ^{18} O in the coral skeleton will decrease due to temperature-dependent kinetic fractionation. Temperature decrease will have the opposite effect. Increased rainfall can decrease salinity and δ^{18} O in ocean water. Therefore, a relative depletion of δ^{18} O could be the result of either a rise in temperature or decrease in salinity (Gratolli and Eakin, 2007). A relative enrichment in δ^{18} O can result from decreased SST, increased salinity, or ocean-water evaporation (Porter et al., 1989; Gratolli and Eakin, 2007).

Sampling Strategies

Relatively little research has been focused on the stable isotope systematics of branching corals, such as *A. cervicornis*. Many workers have extracted meaningful data from massive corals (Porter et al., 1989; Cohen et al., 2004; Grottoli, 2007), but thus far we have not been able to extract the same extent of information from branching corals. Branching corals are important because their growth strategy differs from that of massive corals (Sadler et al., 2014), which could affect their isotopic signatures.

Additionally, their faster growth rate could potentially provide a much higher-resolution environmental record. Goals of this study are to determine the best way to sample *A. cervicornis* for meaningful paleoenvironmental information, to attempt to determine what stable isotope data are reflecting at different sample resolutions and methods, and to better constrain the controls on stable isotope proxies for branching corals.

METHODS

Field methods

Coral rubble and dead coral framework were collected around the edges of transects T1 and T5 at Coral Gardens (Fig 1., Greer et al., this volume) where the greatest variability in depth of coral framework and rubble was exposed. The edges of acroporid stands offered easy access to the base of dead framework material. Samples were culled with bias given to those with well-preserved aragonite cores to promote higherquality sample material and to avoid errors associated with geochemical contamination.



	δ ¹³ C (‰ VPDB)					δ ¹⁸ Ο (‰ VPDB)			
Sampling Strategy	Minimum	Maximum	Mean	Range		Minimum	Maximum	Mean	Range
Low- resolution	-0.07	3.28	1.36	3.25		0.47	3.60	2.25	3.13
Longitudinal	0.623	3.34	2.38	2.72		2.18	4.12	3.06	1.94
Transverse	0.23	3.50	1.96	3.27		1.72	3.68	2.87	1.95

Figure 1. Methods for sampling strategies. Black arrow beside coral branch (left) indicates direction of coral growth. Longitudinal samples were sampled up the axial corallite every 5 mm. Transverse samples were sampled from core to edge of branch every 2.5 mm. 2-3 low-resolution samples per branch were collected up the branch. Combination *low-resolution samples are* a combination of each set of low-resolution samples from a coral branch. Table compares sampling strategies based on minimum, maximum, mean, and range values of data from $\delta^{13}C$ and $\delta^{18}O$ analyses of Coral *Gardens samples from transect* T5 and Pit A. Data from Sample A from T5 are used for both longitudinal and transverse values.

A second set of samples was collected from Excavation Pits A and B. These samples were collected down through dead patches of *A. cervicornis* at increasing depths with the aim of collecting samples from a large span of ages. Material was bleached, dried, and transported to laboratory facilities.

Laboratory Methods

Small, cuboidal pieces ($\leq 3.5 \text{ cm}^3$) were prepared from coral samples with a rock table saw and crushed into a fine powder using a mortar and pestle. Select samples from each transect and from both excavation pits were mounted on thin section slides and examined using an X-ray diffractometer (XRD) to determine aragonite composition. Low-resolution (LR) samples (taken from 2-3 locations per branch) and combination low-resolution (CLR) samples (taken as a combination of the LR samples from each branch) (Fig. 1) were collected. Select coral branches from T5 (Sample A and Sample B) were cut with a rock table saw along the growth axis (longitudinal) as well as perpendicular to the growth axis (transverse) (Fig. 1). Samples were powdered using a modified Dremel with a dental drill bit attachment. The longitudinal sections were sampled every 5 mm up the axial corallite; transverse sections were sampled every 2.5 mm from the axial corallite to the edge of the branch.

Powdered samples were analyzed using a Gas Bench – Isotope Ratio Mass Spectrometer at Washington and Lee University. Isotopic values were corrected using a VPDB standard.

RESULTS

XRD data identified pure aragonite for samples chosen for geochemical characterization. δ^{13} C values of LR samples from Pit A (Fig. 2) range from 0.07 ‰ to 3.18 ‰ and CLR samples range from 0.19 ‰ to 2.16 ‰. Data are tightly clustered with no apparent trends. δ^{18} O values of LR samples from Pit A (Fig. 2) range from 0.47 ‰ to 3.60 ‰ and CLR samples range from 1.93 ‰ to 3.64 ‰. Data are tightly clustered with no apparent trends. The δ^{13} C/ δ^{18} O ratio of all samples from Pit A show poor correlation (Fig. 2); LR samples have an r² value of 0.015 and the CLR samples have an r² value of 0.0052. LR sample averages from each branch are compared to the CLR samples (Fig. 3).

Data from samples along longitudinal sections were plotted as three-point averaged δ values as a function of increasing distance up the growth axis (Fig. 4). δ^{13} C values of Sample A from T5 range from 0.63 ‰ to 3.34 ‰, and δ^{18} O values range from 2.18 ‰ to 4.12 ‰. δ^{13} C values of Sample B from T5 range from 1.19 ‰ to 3.04 ‰, and δ^{18} O values range from 2.65 ‰ to 4.05 ‰. Transverse data plots (Fig. 5) show a general depletion in δ^{13} C from the core to the edge of the branch, ranging from 0.23 ‰ to 3.50 ‰, and a slight depletion in δ^{18} O, ranging from 1.72 ‰ to 3.68 ‰.



Figure 2. $\delta^{13}C$ and $\delta^{18}O$ values in relation to order of collection (shallowest to deepest) of Coral Gardens samples from Pit A. Order of collection is not representative of time. Blue diamonds indicate low-resolution samples. Red squares indicate combination low-resolution samples. (A) $\delta^{13}C$ values. (B) $\delta^{18}O$ values. (C) $\delta^{13}C/\delta^{18}O$ ratio. Note poor correlation coefficients: r^2 value of low-resolution samples is 0.015 and r^2 value of combination low-resolution samples is 0.0052.



Figure 3. $\delta^{18}O$ and $\delta^{13}C$ values from samples collected at Coral Gardens from Pit A as a function of order of collection of lowresolution sample averages and combination low-resolution samples. Red values indicate $\delta^{13}C$ data. Blue values indicate $\delta^{18}O$ data. Squares indicate combination low-resolution samples. Triangles indicate averages of low-resolution samples. 11th through 34th sample collected are not included because only sets of low-resolution samples that had corresponding combination low resolution samples were graphed to focus on differences between the two data values.

DISCUSSION

Data from LR samples show a scatter of δ^{13} C and δ^{18} O values that are tightly clustered with no apparent additional trends. This strategy is potentially useful to characterize longer-term trends through time. LR sample data suggest that there are no long-term patterns apparent from these corals. The tight cluster could be the result of samples from Pit A representing a brief time span. Additionally, only 2 to 3 LR samples were taken per branch; therefore, this strategy would be appropriate for capturing longer-term reef trends rather than information on individual coral branches.

The LR samples and CLR samples display trends that varied from each other. If CLR samples were truly representing an even mix of each branch's LR samples, then they should theoretically be identical to the average of the branches' LR samples. However, when CLR samples are compared to the LR sample averages (Fig. 3), no correlation is evident. This could indicate that an error occurred in the sampling method, such as a lack of sufficient powder mixing. Could CLR data be representative of bulk sampling? Cohen et al. (2004) found that a bulk sample (a sample crushed together as a whole) will reflect the more enriched winter SST of the branch. If CLR samples were truly bulk samples and reflecting a winter SST, then we would expect the data to be consistently more enriched in δ^{18} O than the LR samples from the same branch.



Figure 4. $\delta^{13}C$ and $\delta^{18}O$ values from Coral Gardens samples collected at transect T5 of longitudinal samples in relation to distance sampled up the growth axis. Red values indicate $\delta^{13}C$ and blue values indicate $\delta^{18}O$. Data are smoothed using a three-point average. (A) Sample A. (B) Sample B.



Figure 5. Box and whiskers plots of $\delta^{13}C$ and $\delta^{18}O$ data from transverse samples in respect to distance from core of Sample A from transect T5 from Coral Gardens. Data of 0-mm through 7.5-mm from core distances represent a total of 6 samples per box plot. The plot of samples collected 10-mm from core represents a total of 3 samples. (A) $\delta^{13}C$ values. (B) $\delta^{18}O$ values.

Data, however, show that this is not the case (Fig. 2); therefore, they are likely not representative of bulk sampling or winter SST.

A more precise sampling method was employed for longitudinal and transverse samples to address higherresolution changes in the environment of individual corals. The data from these sampling strategies display cyclic trends rather than a tight cluster. Because of this I conclude that the longitudinal and transverse data capture information on environmental or biological trends. It is important to note that though we are sampling using growth path as a relative time analogue, we do not have a robust age control. The linear extension rate of *A. cervicornis* in T5 ranges from 1.98 cm/yr to 16.8 cm/yr, with a mean growth rate of 10.2 cm/yr. Throughout the year, the linear extension rate of *A. cervicornis* can vary, often based on SST (Gladfelter et al., 1978), and individual branches may grow considerably faster or slower than these values.

Sample A from T5 is interpreted to reflect close to 2 years based on cyclicity of the δ^{18} O and δ^{13} C. δ^{18} O data of Sample B from T5 also show apparent cyclicity that may be representative of a little over one year, but the δ^{13} C values show data that are less easy to interpret. Based on trends from the longitudinal sampling strategy, data suggest that sampling up the axial corallite can provide information related to annual cycles.

Now we can focus on investigating whether the transverse sampling strategy shows consistent data from core to edge of a coral branch, or if there is variation in the isotopic signature. If transverse data show ranges similar to those of longitudinal strategy, this could possibly serve as a cheaper, faster sampling strategy. The transverse samples show δ^{13} C values that are relatively (and relatively consistently) more enriched in δ^{13} C at the axial core of the corallite than the outer edges. δ^{18} O values show relatively heavier values at the core than at greater distances, but the range of values at each distance also overlaps. Only 3 samples were used for the data sampled at the 10-mm distance from core, as opposed to the 6 samples used at all other distances. This could result in an error as fewer data points could cause the range of data to decrease at the location with fewer samples. A 1°C SST increase is noted as corresponding to about a 0.22 ‰ decrease in δ^{18} O (Grottoli, 2001). If δ^{18} O data are showing an accurate depletion of 0.68 % from core to edge, then the data could be representing an environmental change. However, if transverse data have the same range as the longitudinal sampling strategy, then both methods potentially capture the same temperature range and could be used to gather SST range information from coral branches. Correlations between degree of photosynthesis and δ^{13} C is less known, but with a difference of 1.0 ‰ between median values of samples taken at

the core and those taken at 7.5-mm from core, data could potentially represent a change in the isotopic signature.

If isotopic values are consistently depleted from core to edge, this could be the result of calcification patterns during thickening. Gladfelter (1982) describes two phases of *A. cervicornis* skeleton growth: primary precipitation of the coral framework and secondary thickening and strengthening of the skeleton including infilling of skeletal canals. Juillet-Leclerc et al. (1996) found that *A. formosa* morphologies that have a greater abundance of primary aragonite result in a greater ¹⁸O enrichment. They found that faster growth rate correlates with less infilling and a higher abundance of primary framework, suggesting that rapid elongation is associated with enriched δ^{18} O values. If this is true, growth rate could account for the slight isotopic depletion from the core to edge.

A data comparison of different sampling strategies (Fig. 1) shows that the LR samples have a greater range in both δ^{18} O and δ^{13} C than the longitudinal and transverse samples. This indicates that LR samples are capable of capturing a great range of environmental conditions of a reef. This is likely because this strategy samples many different years in the life of the reef rather than individual branches, showing greater fluctuations over a single year. The δ^{18} O data show transverse and longitudinal samples share nearly identical ranges. This could mean that both strategies capture similar environmental histories and could account for the overlapping ranges seen in the transverse data. This potentially tells us that if we are interested in finding a range in temperature, we can use the transverse sampling method in place of the longitudinal strategy. However, there is other important information that can be gained from longitudinal sampling, such as timing of annual cycles or possibly growth rate. There is a 0.50 ‰ difference between the δ^{13} C ranges of the longitudinal and transverse samples. This could mean that the two sampling strategies for carbon may represent different environmental histories. What this difference is should be further investigated.

This study shows that different sampling resolutions can provide different information about acroporids. It is important to note that not only sampling resolution,

but also location sampled from in the skeleton may be important. LR sampling can provide a range of reef conditions over a larger time span. For information on individual branches, high-resolution (transverse and longitudinal) sampling may capture comparable SST ranges. Transverse sampling may be a quicker option than longitudinal sampling; however, longitudinal sampling was found to be the best strategy for capturing annual variations and cyclicity. Caution should be taken when sampling because although ranges from high-resolution sampling methods are comparable, there may be variation from core to edge. Further research using paleoenvironmental data should be done to better develop strategies to investigate the optimal environmental ranges of A. cervicornis, which could potentially provide important information on past and future coral reef health.

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