Evaluation of Amino Acid Racemization Variability in Quaternary Corals

Noelle J. Van Ee[1], Katherine E. Cooper[2], Darrell S. Kaufman[2], Gregor P. Eberli[1]

[1]Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL 33149 USA
[2]Amino Acid Geochronology Laboratory, Northern Arizona University, Flagstaff, AZ, 86011 USA
Corresponding author: nvanee@rsmas.miami.edu.

Abstract. Four Quaternary sea-level cycles are recorded in coral cores from Glover's Reef, a Caribbean atoll. Due to extensive meteoric diagenesis, U-series and C-14 dating techniques have failed to provide pre-Holocene ages for these cores; however, preliminary results show that the extent of amino acid racemization (AAR) increases monotonically down core and has potential to provide independent age control. Of particular interest is a lowstand exposure horizon through a cluster of amino acid racemization values, which suggests that the two youngest Pleistocene sequences are similar in age, possibly even sub-stages of Marine Isotope Stage 5e. To assess the limitations of the technique, we explored the natural variability in AAR using different pre-treatment and subsampling strategies on four coral species. Eight different amino acids were measured in 29 subsamples from a 60 cm section of Pleistocene *Montastraea cavernosa*. Corallite and coenosteum skeletal material were analyzed separately and samples were sub-divided to compare different preparation methods. Using only coenosteum material and not bleaching samples prior to analysis results in the least intra-sample variability. Of the eight amino acids analyzed, only aspartic acid, glutamic acid, and valine had strong down core trends ($R^2 \geq 0.75$). Cross-plots of the extent of AAR in aspartic and glutamic acids show strong correlations and correct stratigraphic ordering for both *Montastraea annularis* and *Acropora palmata* samples, although the species have distinct racemization rates. Future work will use dated corals from nearby localities in Belize to calibrate the rate of AAR and assess the resolution of the technique in the late Pleistocene.

Key words: Amino acid racemization, Quaternary, Coral, Variability.

Introduction

Recent studies have proposed a 5-15 m sea-level drop within the last interglacial in the Red Sea and elsewhere (Siddall et al. 2003; Thompson and Goldstein 2005). These studies generate questions about how platform stratigraphic architecture responds to high frequency and amplitude sea-level changes, but pre-requisite to this work is the ability to precisely date the fluctuations within Marine Isotope Stages (MIS). Amino acid racemization (AAR) is a Quaternary dating technique that has demonstrated the ability to attain a resolution of 5-10 ky in Pleistocene strata from tropical sites without the sensitivity to aragonite preservation of U-series dating (Miller and Brigham-Grette 1989). AAR is also inexpensive, rapid, and requires very little fossil material for analysis. Researchers in the Bahamas have used whole-rock and *Cerion*-based amino stratigraphy to differentiate between an early and late MIS 5e (Hearty and Kaufman 2000, 2009). In contrast, work on AAR in Pleistocene corals has not been published since the 1970s when it was largely discredited because the majority of results violated stratigraphic superposition (Husseini 1973; Wehmiller et al. 1976). However, recent studies are contributing improvements in the understanding of the factors that influence AAR, analytical techniques, and data screening (Ingalls et al. 2003; Penkman et al. 2008; Kosnik and Kaufman 2008) as well as demonstrating that the extent of AAR correlates with age in corals over historical time scales (Goodfriend et al. 1992; Nyberg et al. 2001). These developments suggest that AAR may be a powerful tool for dating Quaternary corals, providing that the inherent variability within coral skeletons is understood and best practices are followed.

Four Quaternary sea-level cycles are recorded in coral cores from Glover's Reef, a Caribbean atoll (Fig. 1). Due to extensive meteoric diagenesis, U-series and C-14 dating techniques have failed to provide ages for these cores; however, preliminary results show that the extent of AAR increases monotonically down core and has potential to provide independent age control (Fig. 2). Of particular interest is a lowstand exposure horizon through a cluster of AAR values, which suggests that the two youngest Pleistocene sequences are similar in age, possibly even sub-stages of MIS 5e. To assess the limitations of the technique, we explored the natural variability in AAR using

different pre-treatment and subsampling strategies on four coral species from two genera.

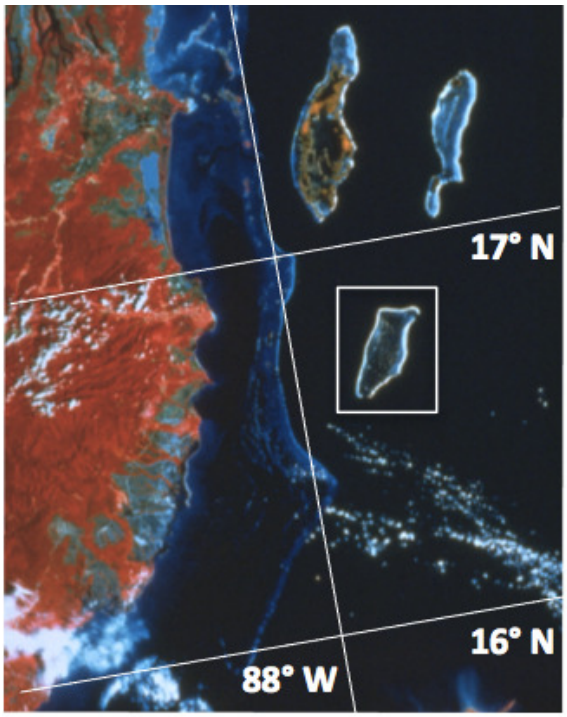

Figure 1: Location of Glover's Reef, Belize. Glover's Reef (white square) is located seaward of the Belize Barrier Reef, approximately 45 km offshore. Land is green and red. Shallow water and reefs are light blue. Image modified from Figure 1A, Appendix D of Harris and Kowalik (1994).

Material and Methods

Twenty individual fossil corals were collected from five drill sites on Glover's Reef, Belize. Glover's Reef (87°48'W, 16°50'N) is an offshore atoll with water temperatures ranging from 27 to 29 °C. Species include: *Acropora palmata*, *Acropora cervicornis*, *Montastraea annularis*, and *Montastraea cavernosa*. Holocene corals dated using radiocarbon range in age from 200 to 10,194 years before present (BP) (Table 1). Radiocarbon dating was performed by Beta Analytic Inc. of Miami, Florida and calibrated to calendar years using INTCAL04 Radiocarbon Age Calibration. The standard marine reservoir correction ($\Delta R = 0$ yr) was applied with no adjustment for local effects. U-series dating occurred at the IFM-GEOMAR laboratory in Kiel, Germany according the method of Fietzke et al. (2005). Older corals are estimated to be late Pleistocene in age. Collection depth for all corals ranged from 1.75 to 14.7 meters below sea level (mbsl). Each individual coral sample was divided into subsamples using a diamond wheel Dremel® rotary tool. Except in rare cases where not enough material was present or where otherwise noted, each coral sample was divided into 4-5 subsamples and AAR results are reported as an average with an associated variance. Subsamples were cut perpendicular to the coral growth axis and areas with signs of boring, encrusting, or other indications of alteration were avoided.

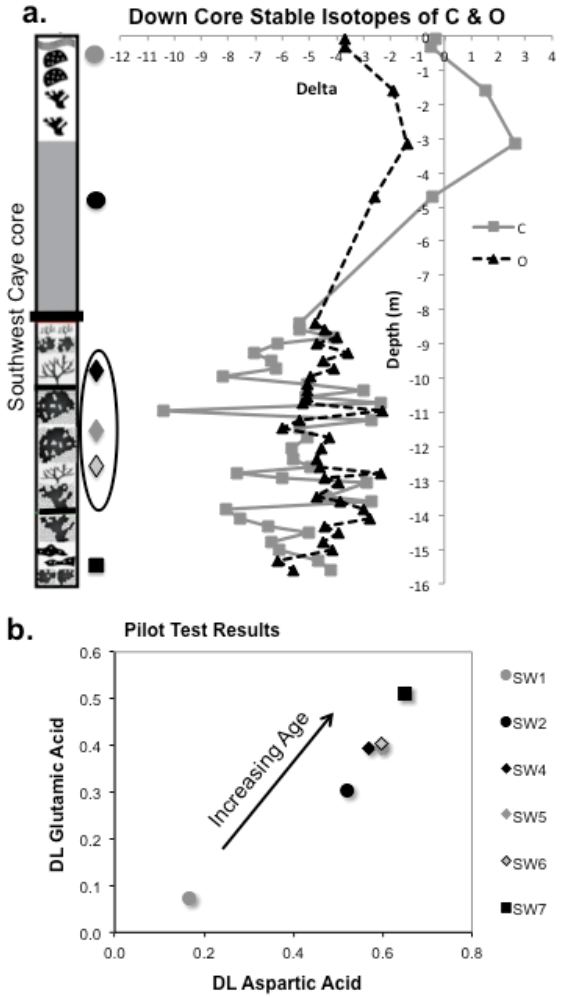

Figure 2: Preliminary results from the Southwest Caye core. A) Negative carbon isotope and positive oxygen isotope values in excursions mark two exposure horizons beneath the Holocene – Pleistocene boundary at ~8.5 mbsl. B) The extent of AAR in aspartic acid and glutamic acid increases monotonically down the core. A cluster of amino acid D/L values (diamonds) spans a stratigraphic exposure horizon. This suggests that the two youngest Pleistocene sequences are similar in age, perhaps even sub stages of MIS 5e.

All subsamples were cleaned thoroughly according to standard procedure using deionized distilled water (DDI), repeated sonication, and an acid leach (Wehmiller and Miller 2000). Subsamples loaded in DDI-filled test tubes were placed in a sonicator bath for 1-2 minutes, rinsed with DDI water, and replaced into the sonicator bath until the test tube water remained clear after sonication. Subsamples were then rinsed and decanted 3-5 times with DDI.

Subsamples then underwent a 33% weight leach using 2M HCl and were rinsed 3-5 more times with DDI water. Finally, subsamples were dried under a laminar flow hood prior to being hydrolyzed for amino acid analysis.

Liberation of the total hydrolysable amino acid (THAA) population of the amino acid pools was accomplished using 7M HCl under N_2 at 110 °C for 6 hr. Analysis was performed using an integrated Agilent 1100 HPLC with separation by a reverse-phase column packed with a C_{18} stationary phase and detection by fluorescence. A full description of the hydrolysis procedure, chromatographic instrumentation, and separation of amino acid enantiomers can be found in Kaufman and Manley (1998). Eight amino acids were measured: Aspartic acid, glutamic acid, serine, alanine, valine, phenylalanine, isoleucine, and leucine. Additional methods for different trials specific to this study are described in the following sections.

Sample	Lab ID	Species	Depth (m)	Age (ky)
MaSW1	Beta-283713	*M. annularis*	0.2	0.20 ± 0.06*
MaSW2	Beta-283714	*M. annularis*	4.7	6.42 ± 0.05
SW-1	XRDSW-1	*A. palmata*	1.75	3.24 ± 0.02
ER1-1	XEDER1-1	*A. palmata*	9.18	5.31 ± 0.03
NR9.2m	Beta-263591	*A. cervicornis*	9.20	6.17 ± 0.05
MC-1	XRDMC-1	*A. palmata*	6.17	7.13 ± 0.04
OR2-1	XRDOR2-1	*A. cervicornis*	4.57	10.19 ± 0.07

Table 1: Radiometrically dated Holocene corals used for AAR analysis. The ages for NR9.2m, MASW1 and MASW2 were determined using C-14. All other ages were determined using the U-series method. For the correction of detrital Th230, a Th230/Th232 activity ration of 0.6 ± 0.2 was used. Depth is reported in meters below sea level. *Not calendar calibrated.

Sampling Different Coral Skeletal Structures

Hearty and Kaufman (2009) have shown that routinely sampling the same location on gastropod shells can reduce the variability between samples from the same stratigraphic level; the same concept can be applied to corals. The coral skeleton can be broadly divided into two parts: the corallite, which houses the individual polyps, and the coenosteum, which is the common surface of the corallum/colony between the corallites. In order to assess the variability within a single coral associated with different skeletal components, a Pleistocene *Montastraea cavernosa* sample AAR031 was divided into 29 subsamples. Subsamples differentiated between the corallite and coenosteum material along six transects, each 10 mm apart (Fig. 3a). Transects were oriented perpendicular to the coral's growth axis so they were roughly time-equivalent.

Bleaching Prior to Hydrolysis

Bleaching prior to hydrolysis removes all but the intra-crystalline fraction of amino acids. The intra-crystalline faction is the fraction of amino acids encapsulated within mineral crystals of the skeletal material. In order to assess the effect of bleaching on racemization values in Holocene and Pleistocene samples, duplicates of subsamples were bleached according to the procedure outlined in Penkman et al. (2008). This procedure involves grinding samples to less than .425 mm diameter then immersing the samples in 10-12% sodium hypochlorite (NaOCl) solution for 48 hr.

Results

To construct an AAR age model, differences in amino acid DL ratios are attributed to age (using depth as a reasonable proxy since sampled corals were in growth position) The exponential correlation between DL (right/left chiral forms) aspartic acid and depth is R^2 = 0.95 for *Montastraea* corals in the Southwest Caye core from Glover's Reef. However, the variability within a single sample, such as AAR031, sometimes extends across the range of several other samples (Fig. 3b).

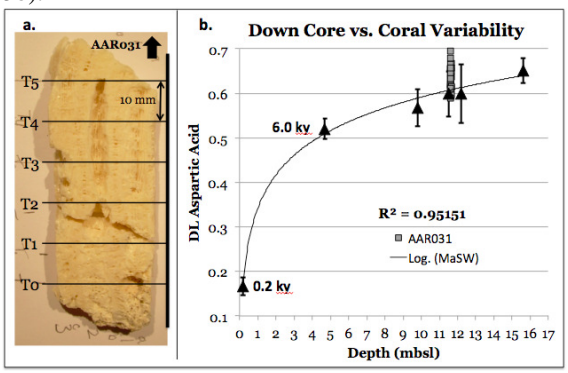

Figure 3: Intra-coral variability. A) Sampling scheme for AAR031 (Pleistocene *Montastraea annularis*). Three corallites and three sections of coenosteum were subsampled along each of the six time-equivalent transects. B) Although the correlation between DL aspartic acid and depth is very high (R^2 = 0.95), the variability within the single sample AAR031 extends across the range of several Pleistocene samples. Holocene samples are shown with their age.

Corallite vs. Coenosteum in Sample AAR031

Separate analysis of corallite and coenosteum material along several time-equivalent transects within a single coral show that the difference between average DL ratios is significant (p = 0.047) only for aspartic acid. The DL aspartic acid values from coenosteum material are lower with approximately half the variability among subsamples (based on the standard

deviation) (Fig. 4). Results for each transect are reported as averages with the standard deviation shown as error bars.

Effect of Bleaching

Bleaching of Pleistocene *M. cavernosa* subsample duplicates (AAR031) significantly changed DL ratios only for the amino acid valine (Fig. 5a). For valine, bleaching resulted in consistently lower DL values but with the same variance as unbleached duplicate subsamples. In *M. annularis* subsamples, the difference between bleached and unbleached duplicates was significant for DL ratios of aspartic acid, glutamic acid, and alanine ($p \leq 0.05$), with bleached samples consistently returning lower DL values than unbleached samples of the same age (Fig. 5b). Bleaching of *A. palmata* samples resulted in weaker exponential relationships between depth and DL ratio for all amino acids except isoleucine. The strongest exponential relationships between DL ratio and depth were in unbleached *A. palmata* subsample duplicates for aspartic acid, glutamic acid, and valine, $R^2 = 0.85$, 0.88, and 0.75, respectively (Table 2).

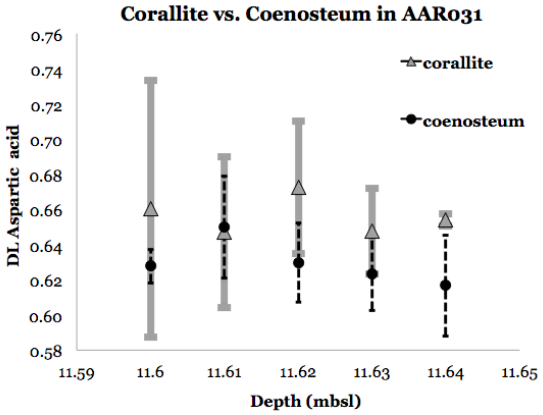

Figure 4: The average aspartic acid DL ratio is significantly different for corallite versus coenosteum material. Coenosteum material typically has lower DL ratios and half the standard deviation compared with corallite material.

Variability between Coral Species

While Husseini (1973) reported that the amino acid composition of several coral species is not species-specific, we found that the relationship between amino acid DL ratios and time/depth depends strongly on species (Fig. 6). When DL ratios for glutamic and aspartic acid are plotted against each other, the samples should fall in stratigraphic order; however, this is not the case when multiple coral species are plotted together. For example, an *A. cervicornis* from 15.9 mbsl plots with *M. annularis* and *M. cavernosa* samples from 11-12 mbsl but should show a different ratio (Fig. 6). Likewise, the *A. palmata sample* (15.5 mbsl) from the oldest Pleistocene level, P3, plots far above the *M. annularis* sample with the same depth and age. Instead, the 15.6 mbsl *M. annularis* sample plots nearly on top of an *A. palmata* sample from 13.6 mbsl. If kept separate, both *A. palmata* and *M. annularis* samples conform to a monotonic trend of increasing amino acid ratios with time/depth.

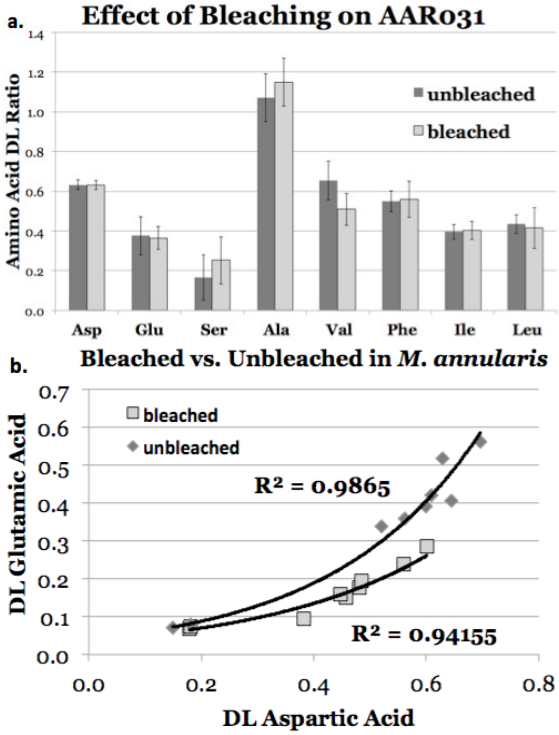

Figure 5: The effect of bleaching on amino acid DL ratios in two *Montastraea* species of coral. A) For Pleistocene *M. cavernosa* sample AAR031, a significant difference in DL ratios between bleached and unbleached only occurred for valine. B) Bleached *M. annularis* samples consistently return lower DL values than their unbleached counterparts.

Discussion

The exponential correlation between DL aspartic acid and depth ($R^2 = 0.95$) in Pleistocene *M. annularis* AAR031 suggests that AAR has good geochronological potential for corals, if intra- coral variability can be reduced and inter-species age models are avoided (Fig. 3). Although only significant for DL aspartic acid, measuring only coenosteum material is good practice because it reduces the intra-sample variability in an amino acid that is strongly positively correlated with depth.

Penkman et al. (2008) showed that bleaching reduces inter-sample variability in fossil mollusks; however, a similar effect is not observed in fossil corals. Rather, bleaching generally results in weaker linear correlations between amino acid DL ratios and depth in subsamples than their unbleached duplicates.

Therefore, we do not recommend that fossil corals be bleached prior to analysis.

While amino acid DL ratios in both *A. palmata* and *M. annularis* plot in stratigraphic sequence, the relationship between depth and amino acid ratios is species-dependent and multiple coral species should not be mixed within a single age model. Furthermore, our preliminary results indicate that *A. cervicornis* samples should not be utilized for geochronology because they plot out of stratigraphic order for both the Holocene and Pleistocene specimens. This is more probably related to diagenesis than bio mineralization since the reversals are larger in the Pleistocene than the Holocene and closely related *A. palmata* samples plot in stratigraphic order. Caution should be used when interpreting AAR-based age models in corals that do not take into consideration the variability introduced by different species, sampling, and preparation methods.

Amino Acid	**R^2 (Correlation with depth)**
Aspartic acid	0.85
Glutamic acid	0.78
Alanine	0.38
Valine	0.76
Phenolylalanine	0.05
Isoleucine	0.001
Leucine	0.04

Table 2: Correlation of amino acid DL ratios with depth in Holocene and Pleistocene *A. palmata* samples.

Future work will endeavor to use the improved understanding of the effect of sampling techniques and preparation methods to construct calibrated age models for Glover's Reef and the Belize Barrier Reef with resolution to conclusively document suborbital sea-level fluctuations in the late Pleistocene. The results of this study are widely applicable to researchers working to construct geochronologies in tropical and subtropical reef environments were extensive diagenesis has rendered other options unfeasible.

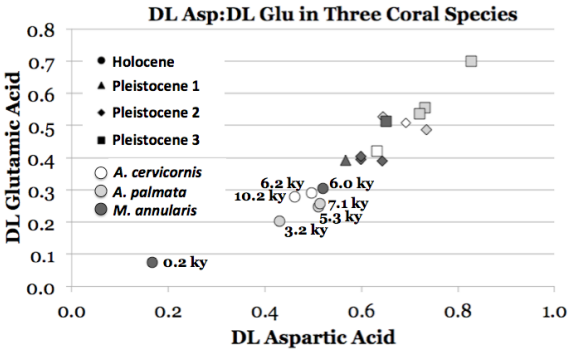

Figure 6: DL glutamic vs. DL aspartic acid for *M. annularis*, *A. palmata*, and *A. cervicornis*. Progressively older Pleistocene sequences are marked 1, 2, and 3. *A. cervicornis* samples plot out of order and *M. annularis* and *A. palmata* coral species appear to have different racemization rates.

Acknowledgement

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