TESTING THE REPRODUCIBILITY OF CARBON AND NITROGEN ISOTOPIC RECORDS IN BAMBOO CORAL FROM SOUTHEASTERN UNITED STATES

An Undergraduate Research Scholars Thesis

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TABLE OF CONTENTS

ABSTRACT

Testing the Reproducibility of Carbon and Nitrogen Isotopic Records in Bamboo Coral from Southeastern United States

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Theoretical Framework

Analysis of bulk carbon and nitrogen from deep-sea corals is a common way scientists have interpreted past local oceanic environmental conditions. Previous work using isotopic records proxies for high resolution paleoceanogaphic reconstruction over decadal timescales have assumed these isotopic records are reproducible. Deep-sea corals are a relatively new archive and in part owing to the difficulty of obtaining samples using submersibles or ROVs, different proxy records have been assumed to be reproducible. Here we test whether carbon and nitrogen isotopic records in bamboo coral from the Southeast United States are reproducible.

Project Description

In this research project, deep-sea bamboo coral collected off the southeastern coast of the United States (SE USA) will undergo isotopic ratio analysis to test for reproducibility. This species of coral is a part of the family *Isididae*. It is an important archive for understanding deep-water variability and past oceanic conditions considering that the proteinaceous gorgonin internodes are deriving the carbon from particulate organic matter (POM) recently exported out of the surface

waters while the carbonate nodes are deriving their carbon from the *in-situ* dissolved inorganic carbon (DIC) pool at the depth at which the corals were living (Schiff et al., 2014, Roark et al., 2005). Isotopic analysis will measure the $\delta^{15}N$ and $\delta^{13}C$ ratios found within the corals proteinaceous gorgonin internodes. The reproducibility information gathered in this study can potentially be used as validation for using isotopic records as a proxy for oceanographic environmental variability at a high resolution over time.

Thesis Statement

Isotopic records are a product of an environmental mechanism and can be used as tracers of oceanographic environmental variability if the records are reproducible. In order to interpret these isotopic records in bamboo corals as recorders of environmental changes within the SE USA, the carbon and nitrogen signals must be reproducible.

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INTRODUCTION

Deep-sea coral (DSC) communities are globally distributed making them a valuable tool for paleoceanographic reconstruction, biogeochemical, and climate change research such as studies in the Gulf of Alaska, Tasmanian coast, and California Margin have shown (Roark et al., 2005, Sherwood et al., 2009, Schiff et al., 2014). Scientists, who work with the skeletons of DSC specimens, use them because they serve as biological archives that record information about the local ecosystem. Several studies have measured the bulk carbon and nitrogen isotopic variability from the proteinaceous gorgonian internodes as well as the carbon and oxygen isotopes in the calcium carbonate nodes. However, there are few studies for reproducibility of these isotopic records in bamboo corals.

Bamboo coral is known as a mystery coral given that the speciation of bamboo coral is undefined and subject to revision (Ross and Nizinski, 2007). Though they are known to have a life-span of up to 400 years, little is known about their ecology (Thresher et al., 2004, Sherwood et al., 2009). These two facts argue for the need to test the reproducibility of bulk carbon and nitrogen isotopic records found in Bamboo coral, family *Isididae* genus *Keratoisis*.

Study Location

The bamboo specimen, JACKJSL05-4700-BAM1, was live-collected in 2005 off the coast of southeastern Florida in the Jacksonville Lithoterms at 560 m depth as seen in Figure 1 (30° 30' 50.754", -79° 39' 36.102"). The term "lithoterm" stems from the abundant amount of *Lephelia* and *Enallopsammis* coral that cover the hard carbonate mounds. In this area, there is a moderate abundance of bamboo coral and they are distinguishable by their branching morphology (Ross and Nizinski, 2007). Bamboo corals are characterized by the eight pinnate tentacles on each polyp and have proteinaceous internodes (Sherwood et al., 2014, Sherwood et al., 2009). Since exported organic matter is housed in the coral endoskeleton, gorgonin $\delta^{15}N$ and $\delta^{13}C$ values can be measured and compared to surface water environmental variability over time potentially indicating changes in primary production and nutrient sources in surface water depth (Roark et al., 2005, Schiff et al., 2014).

Figure 1 Map of study area with coral sampling location labeled.

The SE USA is home to DSC communities and diverse marine species that can be found at about 30° to 31° N and 200 to 800 m depths. Numerous ROV and submersible dives have explored this area and have noted the most dominant deep-sea coral to be *Lophelia pertusa* and black coral. The mobile macrofauna and deep-sea coral habitats are valuable for commercial fishing and scientific research. The interaction of the Gulf Stream and Atlantic Ocean in this area provides nutrient rich water created by cold-water upwelling that can result in high phytoplankton concentrations which could potentially influence the trophic levels of the coral (Sedberry et al., 2004, Sherwood et al., 2014). Testing the reproducibility of bamboo coral collected from this region will allow us to determine if this particular genus of coral can serve as a proxy for past oceanic conditions across decadal or even subdecadal timescales (Roark et al., 2005).

SECTION I

REPRODUCIBILITY IN ISOTOPIC RECORDS

Reproducibility is a fundamental step and a key aspect in any scientific process but is sometimes limited in paleoceanographic proxy development studies but because of cost and access to samples. Independently verifying the signals measured from this coral specimen is necessary when beginning to use them for biogeochemical research. Testing for reproducibility also provides a level of transparency. In this study, a high degree of reproducibility will demonstrate the utility of isotopic signatures for bamboo coral collected off SE USA. The goal of this project is to validate and verify that this genus of coral found at this location is fit for isotopic analysis from which the results will be used to reconstruct local environmental change. The results from this project can potentially strengthen the use of carbon and nitrogen isotope analysis in bamboo corals. One other study tested for reproducibility in bamboo coral from SE USA but it was of trace element profiles (Ba, Mg, Sr, Mn, U, Pb) (Sinclair et al., 2011).

There are few studies on bulk isotope analysis for bamboo coral *Keratoisis* and DSC specimens of the family *Isadidae* and *Coralliidae* in comparison to the number studies of aragonite skeletons of scleractinian corals (Chaabane et al., 2016). Studies on bamboo coral from different geographic locations have used $\delta^{15}N$ values of living tissue and underlying gorgonin to propose a nutrient source control and associate the results to trophic dynamics in the region from which the coral specimens were collected after demonstrating some level of reproducibility. For example, bamboo coral that was collected on Tasmanian seamounts ran duplicate samples and the differences between the duplicates averaged 0.11‰ (n=11) (Sherwood et al., 2009). The low percent

difference indicates that the duplicate sample values were largely reproducible. No studies have been made for bamboo coral collected near SE USA.

SECTION II

MATERIALS AND METHODS

The primary goal of this research project is to measure bulk carbon and nitrogen of two internodes from the same bamboo specimen and compare the two records to test for reproducibility. The two internodes are from different branches of the specimen and are assumed to be a reflection of the same time interval because the specimen was collected alive and the distances from the internodes to the base are the same. This means that starting from the outermost layers the two internodes were depositing their skeleton at the same time.

Coral Sampling

To isolate the organic internodes from the body of the coral specimen a diamond band saw was used. Two internodes were cut at approximately the same distance from the base of the specimen (Figures 2-4). A coral disk is primarily proteinaceous material but will have calcite from the corals skeleton. Two organic internodes were sectioned from the bamboo specimen to immerse in 4% (v/v) HCl and dissolve the calcite fraction. After 4 days of dissolution, the coral disks were transferred to glass petri dishes filled with deionized water (18Ω). Using a microscope, the coral disk was bisected to release the pressure of the concentric rings. A scalpel and tweezers were used to peel the internodes with the thickness of each peel being approximately 250-500 µm. There were a total of 35 samples after completely peeling internode 1A. A total of 36 peels were collected for internode 2A. The samples were stored in hexagonal petri dishes and were transferred into vials. The samples were rinsed 3 times with deionized water to get rid of any remaining HCl solution and then dried at 45°C for 72 hours.

Figure 2 Internode 1A sampling from the left branch of JACKJSL05-4700-BAM1

Figure 3 Bamboo coral specimen JACKJSL05-4700-BAM1

Figure 4 Internode 2A sampled from the right branch of JACKJSL05-4700-BAM1.

Elemental Analyzer

For bulk total organic carbon and nitrogen analysis, approximately 500 µg of each sample were weighed and placed in tin capsules. Combustion and reduction of the organic samples was done with a Carlo Erba NA 1500 Elemental Analyzer with a Costech Zero-Blank Autosampler coupled to a Thermo Scientific DELTA^{plus}XP isotope ratio mass spectrometer (IRMS). Rice, methionine, acetanilide, and USGS 40 and 41 are used as reference standards when running the samples. Nitrogen isotope values are reported in the standard notation $\delta^{15}N$. Carbon values are reported in the standard notation as well, $\delta^{13}C$. Instrument precision for the $\delta^{15}N$ and $\delta^{13}C$ was ±0.15‰ (1 sigma) in the Stable Isotope Geoscience Facility based on the repeat running of standards. Values for nitrogen and carbon are reported in per mil (‰) units and are relative atmospheric N_2 and CO_2. All IRMS work, including preparation and data processes was done in house at the Stable Isotope Geoscience Facility at Texas A&M University.

δ^{15}N isotope ratios are calculated with the following equation:

$$\delta^{15}\mathrm{N} = \left[\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{sample}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{standard}} - 1\right] \times 1{,}000 \qquad (1)$$

δ^{13}C isotope ratios are calculated with the following equation:

$$\delta^{13}\mathrm{C} = \left[\frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{sample}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{standard}} - 1\right] \times 1{,}000 \qquad (2)$$

SECTION III

RESULTS AND DISCUSSION

Stable Isotope Variation

I tested for reproducibility in the bamboo coral by comparing the isotope signatures in Internode 1 with those in Internode 2. Plots of bulk $\delta^{13}C$ and $\delta^{15}N$ verses the distance from the edge of the internode appear to be largely reproducible with most of the variability within error (Figure 5). Bulk nitrogen values range from 8.8‰ to 10.4‰, while bulk carbon values range from -16.5‰ to -15.0‰ (Table 3). Values of bulk $\delta^{13}C$ averaged ±15.50‰ for internode 1 and ±15.72‰ for internode 2 (Table 1). Values of bulk $\delta^{15}N$ averaged 9.64‰ for internode 1 and 9.41‰ for internode 2 (Table 2). The average for bulk carbon is similar to the average found in bulk carbon (-16.01 ± 0.2‰) in a bamboo coral genus Isidella collected from Monterey Canyon (Schiff et al., 2014). The average for bulk nitrogen is consistent with the average $\delta^{15}N$ measured in DSC from in the Tasmanian seamounts (Sherwood et al., 2009). Raw data can be found in Table 4.

Measured $\delta^{15}N$ values appear reproducible over the early parts of the records but appear less reproducible over the later parts of the record where growth peels were measurably thicker. The same can be said for $\delta^{13}C$ values. There is variability between the carbon signatures of sample peels closest to the edge for internode 1 and 2. The largest amount of variability for $\delta^{13}C$ is from ~3.5-5.8 mm and ~6.5-7.2 mm. The amount of variability is moderately the same for $\delta^{15}N$ within this distance (Figure 5). However, the overall trend does not significantly fluctuate. Comparing the differences between the maximum and minimum values of $\delta^{13}C$ and $\delta^{15}N$ for internode 1 and 2 shows that there is small difference in range of ~1‰ (Table 1).

Table 1 Maximum, minimum, and differences of $\delta^{13}C$ and $\delta^{15}N$ for internode 1 and internode 2.

Internode 1	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Internode 2	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Internode 1 and 2	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Max_1	17.36	9.91	Max_2	16.42	10.53	Max_1-Max_2	0.94	0.62
Min_1	14.98	8.80	Min_2	15.2	8.88	Min_1-Min_2	0.22	0.08
$Difference_1$	2.38	1.11	$Difference_2$	1.22	1.65	$Difference_1$ – $Difference_2$	0.73	0.54

Table 2 Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± standard deviation) for peels from the Bamboo specimen, Internode 2 (n = 36). The second row is the standard error of bulk carbon and nitrogen.

$\delta^{13}C$ (‰)	**$\delta^{15}N$ (‰)**
-15.7 ± 0.66	9.41 ± 0.73
0.11	0.12

Table 3 Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± standard deviation) for peels from the Bamboo specimen, Internode 1 (n = 35). The second row is the standard error of bulk carbon and nitrogen.

$\delta^{13}C$ (‰)	**$\delta^{15}N$ (‰)**
-15.8 ± 0.68	9.51 ± 0.69
0.14	0.12

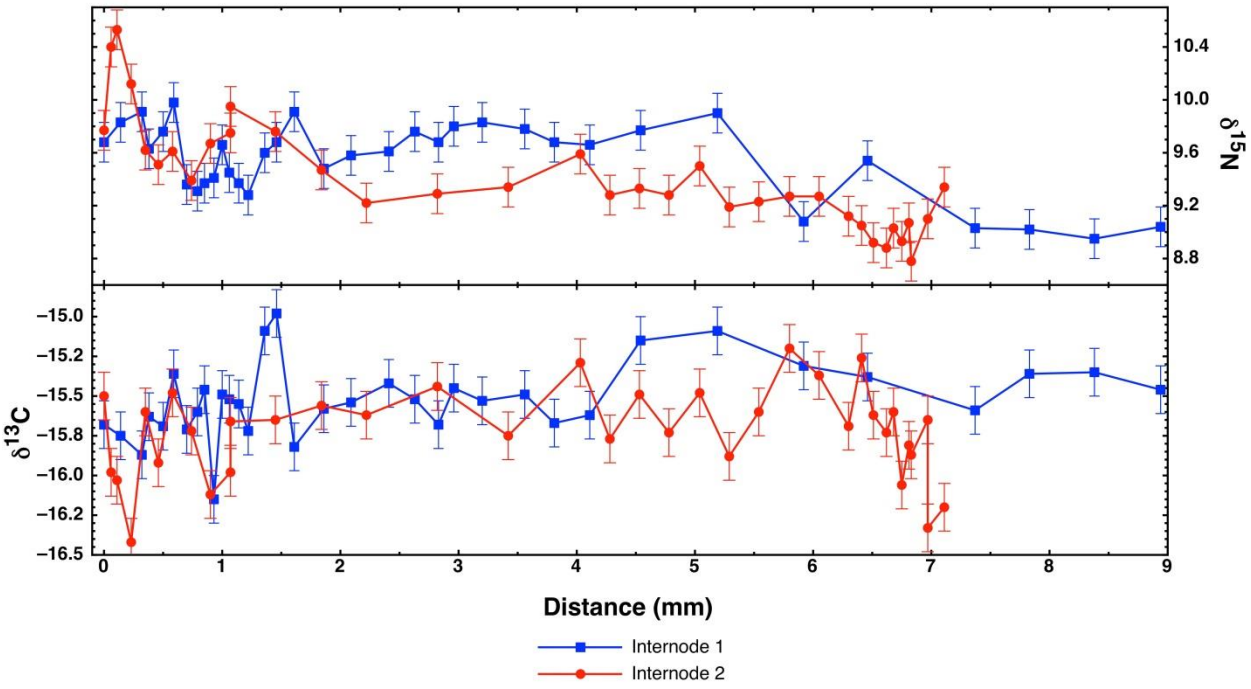

Figure 5 Bulk carbon and nitrogen values (‰) for the bamboo specimen, internode 1 and internode 2, vs the distance from the edge (mm).

Table 4 Isotope data ($\delta^{13}C$ and $\delta^{15}N$) for the Southeastern United Stated Bamboo Coral (*Keratoisis* sp.)

Sample 1A n = 35	Distance from edge (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample 2A n = 36	Distance from edge (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
1.1	0.00	-17.36	9.41	**1**	0.00	-15.50	9.77
1.2	0.14	-16.78	9.62	**1.1**	0.06	-15.98	10.40
1.3	0.32	-16.99	9.60	**1.2**	0.11	-16.03	10.53
1.4	0.38	-16.88	9.62	**2**	0.23	-16.42	10.12
1.51	0.50	-16.64	9.41	**2.1**	0.35	-15.60	9.62
1.52	0.59	-16.28	9.93	**2.2**	0.46	-15.92	9.51
1.53	0.70	-16.36	9.65	**2.3**	0.58	-15.48	9.61
1.54	0.79	-16.57	9.37	**3**	0.74	-15.72	9.39
1.55	0.85	-16.33	8.80	**3.1**	0.90	-16.12	9.67
1.56	0.93	-16.15	9.41	**3.2**	1.07	-15.98	9.75
1.57	1.00	-15.67	9.69	**3.2**	1.07	-15.66	9.95
1.58	1.06	-15.99	9.51	**4**	1.45	-15.65	9.76
1.59	1.14	-15.46	9.18	**4.01**	1.84	-15.56	9.47
1.6	1.22	-15.53	9.18	**4.1**	2.22	-15.62	9.22
1.61	1.36	-15.09	9.60	**5**	2.82	-15.44	9.29
1.62	1.46	-14.98	9.68	**5.1**	3.42	-15.75	9.34
1.63	1.61	-15.82	9.91	**5.2**	4.03	-15.29	9.59
1.64	1.86	-15.58	9.48	**6.01**	4.28	-15.77	9.28
1.65	2.09	-15.54	9.58	**6.02**	4.53	-15.49	9.33
1.66	2.41	-15.42	9.61	**6.03**	4.78	-15.73	9.28
1.67	2.63	-15.52	9.76	**6.11**	5.04	-15.48	9.50
1.68	2.83	-15.68	9.68	**6.12**	5.29	-15.88	9.19
1.69	2.96	-15.45	9.80	**6.13**	5.54	-15.60	9.23
1.7	3.20	-15.53	9.83	**6.14**	5.80	-15.20	9.27
1.71	3.56	-15.49	9.78	**6.2**	6.05	-15.37	9.27
1.72	3.81	-15.67	9.68	**6.21**	6.30	-15.69	9.12
1.73	4.11	-15.62	9.66	**7**	6.41	-15.26	9.05
1.74	4.54	-15.15	9.77	**7.1**	6.51	-15.62	8.92
1.75	5.19	-15.09	9.90	**7.2**	6.62	-15.73	8.88
1.8 R	5.92	-15.31	9.08	**8**	6.68	-15.60	9.03
1.81 R	6.46	-15.38	9.54	**8.1**	6.75	-16.06	8.93
1.82 R	7.37	-15.59	9.03	**8.2**	6.81	-15.81	9.07
1.83 R	7.83	-15.36	9.02	**9**	6.83	-15.87	8.78
1.84 R	8.38	-15.35	8.95	**10**	6.97	-15.65	9.10
1.85 R	8.94	-15.46	9.04	**10**	6.97	-16.33	9.10
				11	7.11	-16.20	9.34

Linear Age Model and Growth Rate

I calculated an experimental linear age model using the following equation and an independent growth rate estimate of 55 μm/year from a previous study on bamboo corals from the SE United States (Sinclair et al., 2011). The calculated age of the internodes suggest that the bamboo coral is approximately 129-163 years old (Table 5). A linear age model and a growth rate establishes a chronology which assigns a date for the carbon and nitrogen signals measured in each peel. This is important since radiocarbon measurements were not made in this study. Both internodes were sectioned off from the same bamboo specimen but from different branches. As mentioned before we are assuming that this specimen is depositing its skeleton at the same rate, therefore the isotopic records from the two internodes should be reflective of one another.

Following radiocarbon dating, each peel will have an assigned year. With that we can then correlate the growth peels from internode 1 and internode 2 with one another and reduce the amount of variability seen in Figure 5 and 6. Furthermore observing trends within a timescale will allow for us to make assumptions about possible cyclical changes or food sources changes such as those made in previous studies (Chaabane et al., 2016, Sherwood et al., 2014, Sherwood et al., 2009, Roark et al., 2005, Thresher et al., 2004, Thresher et al., 2009, Hill et al., 2011) Comparing the growth rate of this bamboo coral to known growth rates from around the world may also contribute to what we know about POC export rates (Roark et al., 2005). Variability in carbon and nitrogen can be further investigated with a known date and traced to possible environmental mechanisms. By applying this age model to the isotopic records measured for the bamboo coral specimen, distance no longer is a place holder for time.

Table 5 **Estimated dates for sample peels using a linear age model (growth rate = 55 µm/year)**

Sample 1A n = 35	Internode 1 Calculated Age = 163	Sample 2A n = 36	Internode 2 Calculate age =129
1.1	2005	**1**	2005
1.2	2002	**1.1**	2004
1.3	1999	**1.2**	2003
1.4	1998	**2**	2001
1.51	1996	**2.1**	1999
1.52	1994	**2.2**	1997
1.53	1992	**2.3**	1994
1.54	1991	**3**	1992
1.55	1990	**3.1**	1989
1.56	1988	**3.2**	1986
1.57	1987	**3.2**	1986
1.58	1986	**4**	1979
1.59	1984	**4.01**	1972
1.6	1983	**4.1**	1965
1.61	1980	**5**	1954
1.62	1979	**5.1**	1943
1.63	1976	**5.2**	1932
1.64	1971	**6.01**	1927
1.65	1967	**6.02**	1923
1.66	1961	**6.03**	1918
1.67	1957	**6.11**	1913
1.68	1954	**6.12**	1909
1.69	1951	**6.13**	1904
1.7	1947	**6.14**	1900
1.71	1940	**6.2**	1895
1.72	1936	**6.21**	1890
1.73	1930	**7**	1889
1.74	1922	**7.1**	1887
1.75	1911	**7.2**	1885
1.8 R	1897	**8**	1883
1.81 R	1888	**8.1**	1882
1.82 R	1871	**8.2**	1881
1.83 R	1863	**9**	1881
1.84 R	1853	**10**	1878
1.85 R	1842	**10**	1878
		11	1876

Equation for linear age model:

$$\boldsymbol{Age\ Model} = \frac{\boldsymbol{Year\ collected - Distance\ from\ edge}}{\boldsymbol{growth\ rate}} \qquad (3)$$

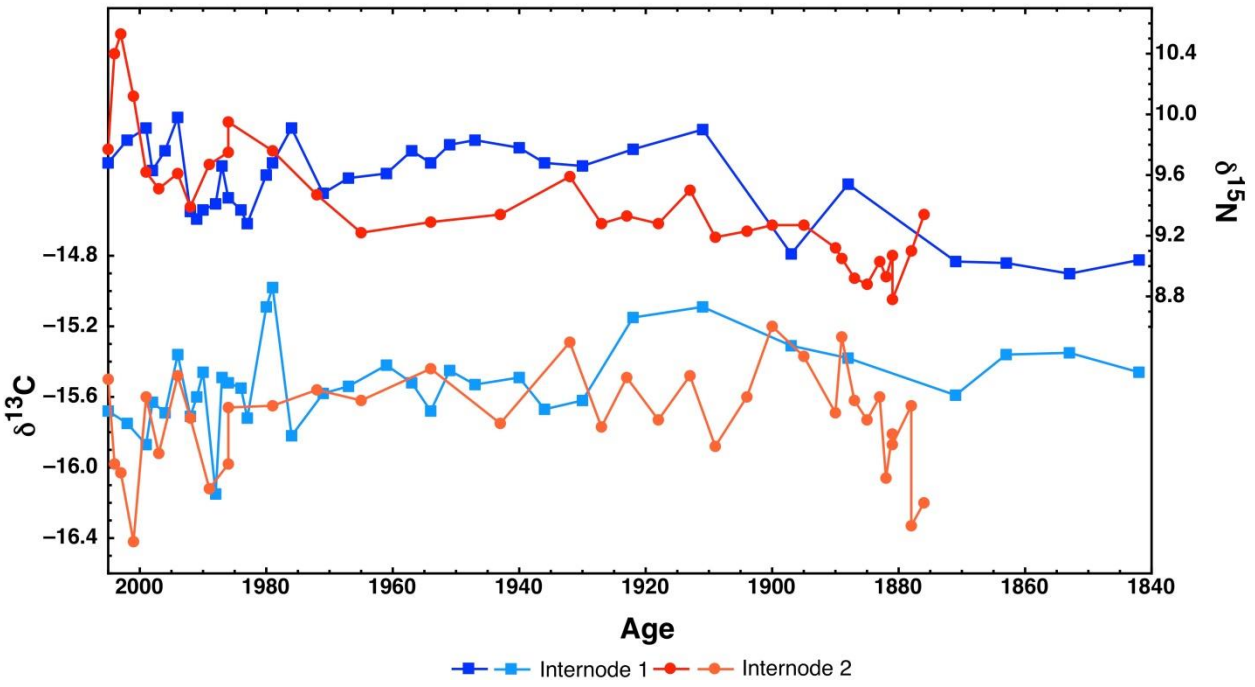

Figure 6 Age model of bulk carbon and nitrogen values (‰) for internodes 1 and internode 2.

Radiocarbon Dating for Future Work

By measuring the radiocarbon content in the gorgonin internodes of the bamboo coral specimens, a true linear age model of the coral is possible (Roark et al., 2005, Sherwood et al., 2014). A linear age research project would put the isotope records into a temporal context assuming constant linear growth. Once a date is assigned to the isotopic records, variations within the signatures can be analyzed from the year the bamboo coral began growing to 2005 when it was collected. Further local environmental condition assumptions can also be made. A temporal context for these $\delta^{15}N$ and $\delta^{13}C$ signatures will provide more opportunities for analytical work.

Radiocarbon weighs more than carbon. Both carbon and radiocarbon occur naturally in the atmosphere. However, the decay rate compared to nitrogen is different. The amount of radiocarbon is small and is constant. This makes it a reliable tool for dating, hence radiocarbon dating. In the 1950's and 1960's above-ground nuclear bomb testing increased the amount of radiocarbon in the atmosphere. This increase in carbon 14 acts as a marker. It is used in what is known as the bomb-spike analysis. Nuclear weapons testing started in 1957 and peaked in 1970, so by knowing the growth rate of coral between these years and the date it died, a linear growth rate can be calculated. A spike in carbon 14 is reflective of the artificial productive of radiocarbon. It is used because it suggests that recently exports particulate organic carbon (POC) is the source of carbon for coral specimens (Roark et al., 2005) A percentage of uncertainty comes from the assumption that coral grows at a constant rate. Radiocarbon dating is used regardless of uncertainty because recently exported particulate organic carbon is a source of carbon for bamboo coral. Noticeable peaks in Therefore there is are a reliable source in dating coral with an accuracy of a few years (Roark et al., 2005).

CONCLUSION

Reproducibility is possible for carbon and nitrogen isotopic records in bamboo coral collected from the southeastern coast of the United States. Bulk carbon and nitrogen values were similar to isotopic values found in bamboo coral from previous studies. Radiocarbon dating, however, is necessary in order to determine the growth rate of the specimen and its age. This would also reduce the amount of variability seen amongst the $\delta^{13}C$ and $\delta^{15}N$ values for internodes 1 and 2. A growth rate provides a timeline from 2005, when it was live-collected, to when it first began growing. Coupling the timeline and the observed trends will then allow us to make conclusions about the conditions near the Jacksonville Lithoterms using bamboo coral.

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