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## Deep-water antipatharians: Proxies of environmental change

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#### ABSTRACT

Deep-water (307–697 m) antipatharian (black coral) specimens were collected from the southeastern continental slope of the United States and the north-central Gulf of Mexico. The sclerochronology of the specimens indicates that skeletal growth takes place by formation of concentric coeval layers. We used <sup>210</sup>Pb to estimate radial growth rate of two specimens, and to establish that they were several centuries old. Bands were delaminated in KOH and analyzed for carbon and nitrogen stable isotopes. Carbon values ranged from -16.4% to -15.7%; the oldest specimen displayed the largest range in values. Nitrogen values ranged from 7.7% to 8.6%. Two specimens from the same location and depth had similar <sup>15</sup>N signatures, indicating good reproducibility between specimens.

Keywords: corals, nitrogen, carbon, stable isotopes, <sup>210</sup>Pb dating.

#### INTRODUCTION

Deep-water black corals (Antipatharia) have substantial potential as proxy records of historical oceanographic and biogeochemical changes (Grange and Goldberg, 1994). Their long life spans, wide geographic distribution, and large depth range (Grigg, 1965; van der Land and Opresko, 2001) suggest that they may provide environmental information in geographic locations and for periods of time not available from other sources.

The semirigid antipatharian skeleton is composed of chitin complexed with proteins (Goldberg et al., 1994). Antipatharians feed on particulate organic matter (POM) composed of detritus, marine snow, and plankton in the water column (Grigg, 1965). The transfer of POM from surface waters to the antipatharian organic skeleton indicates a link between the isotopic composition of the skeleton and surface oceanographic and biogeochemical processes affecting the constitution of POM (Heikoop et al., 1998, 2002).

Studies of growth ring structure and formation in shallow-water antipatharians suggest that the skeleton is formed of concentric coeval rings such that the inner rings of a branch are the oldest and the outside rings are the most recently formed (Goldberg, 1991; Grange, 1985a, 1985b; Grange and Goldberg, 1994). Similar growth in deep-water specimens would allow us to develop radial growth chronologies of the skeleton.

In this paper we document the sclerochronology of these deepwater corals using <sup>210</sup>Pb analysis. We test skeletal treatment with KOH to delaminate bands and analyze skeletal stable isotopic composition to obtain proxy records of environmental change.

#### **Study Sites**

Samples from three study sites were selected for analysis (Fig. 1). The Jacksonville lithoherm (Paull et al., 2000) site is at  $\sim$ 550–650 m

depth on the Florida-Hatteras Slope  $\sim 180$  km off Jacksonville, Florida. The Stetson Banks site is 380 km off Savannah, Georgia, at 650–700 m depth. Both sites are influenced by the Gulf Stream (Paull et al., 2000; Stetson et al., 1962). The third site was the Viosca Knoll area in the north-central Gulf of Mexico,  $\sim 100$  km east of the Mississippi River Delta, at 300–530 m depth (Schroeder, 2002).

#### **METHODS**

#### **Sample Collection**

Four antipatharian specimens (Fig. 2) were collected (Table 1) using the Johnson Sea-Link submersible during two research cruises in 2004, 9–15 June (continental slope; southeastern U.S.) and 31 July–1 August (north-central Gulf of Mexico) (Fig. 1). Specimens were tentatively identified as *Leiopathes glaberrima* (Esper) (= *Antipathes glaberrima*) based on branch pattern and size, although further taxonomic validation is needed (D. Opresko, 2005, personal commun.). After collection, cenosarc was picked off with forceps. The specimens were then rinsed in seawater and air-dried on deck.

#### Imaging

In the laboratory, 1-cm-thick cross sections were cut from the base of each specimen with a wafering blade attached to a Dremel Multipro drill. Polished 30  $\mu$ m and 100  $\mu$ m cross sections were cut adjacent to the thick sections. Digital photographs of the thin sections were taken under compound microscope with a Leica DFC300 camera to examine banding patterns. Small portions of the thick section were placed in 2 g of KOH in 50 mL of Milli-Q water for 4 h and band separation was examined using a scanning electron microscope (SEM).



Figure 1. Location of antipatharian collection sites on southeastern U.S. continental slope at Jacksonville lithoherms, Stetson Bank, and Viosca Knoll.

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Figure 2. Video frame grab of black coral colony observed at 561 m at Jacksonville lithoherm site.

#### Skeletal <sup>210</sup>Pb Dating

We subsampled 4-mm-thick cross sections from the bases of specimens A9902 and A8601 into three equal concentric regions labeled, from the outside to the inside, A9902-A, A9902-B, and A9902-C, and A8601-A, A8601-B, and A8601-C, respectively. Subsamples were cut with a wafering blade attached to a Dremel drill to obtain a minimum 0.5 g of material, cleaned three times ultrasonically with Milli-Q water for 10 min to ensure removal of all particles not incorporated into the skeleton, and then dried overnight at 40 °C. After cleaning, subsamples were handled with clean forceps at all times to prevent contamination. Subsamples (>0.5 g) and  $^{209}$ Po spike (~3 dpm) were dissolved in a mixture of two-thirds concentrated HCl and one-third concentrated HNO<sub>3</sub> on a low-temperature hot plate until completely dissolved and dried. The dried mixture was redissolved in 0.5 N HCl; <sup>210</sup>Po was plated onto a silver disc from the solution at  $\sim 80$  °C for several hours. The <sup>210</sup>Po activity was counted in an Alpha Counter EGG-ORTIC 476 according to methods of Ruiz-Fernández et al. (2003).

There are three potential sources of <sup>210</sup>Pb to the antipatharian skeleton: the excess (unsupported) fraction, the supported fraction, and the in-growth fraction. The <sup>210</sup>Pb has low solubility, thus it adheres to particulate matter in the water column. The relatively slow radial growth rates of antipatharians (based on estimates from shallow-water specimens; Grange and Goldberg, 1994) provide sufficient time for particulate matter to adhere to the outside layer of the skeleton during formation; this is the source of unsupported <sup>210</sup>Pb<sub>ex</sub> to the skeleton. The <sup>210</sup>Pb<sub>ex</sub> fraction can be used to determine the age of a subsample; however, first the other two sources of <sup>210</sup>Pb must be accounted for. Detrital particles in the water column are also a source of supported <sup>210</sup>Pb, which comes from the decay of <sup>238</sup>U. The in-growth <sup>210</sup>Pb fraction comes from the in situ decay of <sup>226</sup>Ra taken up from seawater during skeletal formation. The supported and in-growth fractions are assumed to be 0; this is discussed in the following.

The subsamples for <sup>210</sup>Pb analysis were taken along a timedependent growth axis from the external and youngest subsample A to the internal and oldest subsample C for both specimens. The distance from the average point of each subsample along the radial growth axis to the outside edge of the section (time = 0) was plotted against measured <sup>210</sup>Pb activity. The asymptote of the curve for specimen A9902 is essentially 0; therefore, we can assume that supported <sup>210</sup>Pb and ingrowth <sup>210</sup>Pb is 0 and thus does not need to be accounted for in this specimen. The natural log of <sup>210</sup>Pb activity for each subsample was then taken to convert the curve into a straight line in which the natural logarithm of the y-intercept represents initial activity. For specimen A9902 the trend equation is y = -0.07563x + 1.8788 (R<sup>2</sup> = 0.9993)

TABLE 1. SPECIMEN COLLECTION LOCATION AND DIAMETER AT BASE

Specimen	Location	Lat/Long (°N/°W)	Dive #	Depth (m)	Diameter (mm)*
A8601	Jacksonville Lithoherm	30°30.1′, 79°39.2′	4684	593	14.0
A8401	Jacksonville Lithoherm	30°30.8′, 79°39.7′	4684	561	8.3
A9902	Stetson Banks	31°50.7′, 77°36.6′	4699	679	5.8
AG002	Viosca Knoll	29°06.4′, 88°23′	4744	307	11.2

and initial activity  $(A_0) = 6.84$  dpm/g. This calculation assumes that initial <sup>210</sup>Pb<sub>ex</sub> is constant in time and space.

The age of specimen A9902, based on the radioactive decay of <sup>210</sup>Pb in the coral skeleton, was calculated using:

$$A_{ex} = A_0 e^{-\lambda t}, \tag{1}$$

where  $A_{ex}$  is the activity measured at time (*t*),  $A_0$  is the initial activity at t = 0, and  $\lambda$  is the decay constant of <sup>210</sup>Pb ( $\lambda = 0.0311$  yr<sup>-1</sup>) (Druffel et al., 1990). The initial activity calculated above was then substituted into Equation 1 along with the measured excess activity to determine specimen age.

#### **Band Separation and Analysis**

A 5-mm-thick cross section from the base of each specimen was placed in a solution of 4 g of KOH in 50 mL of water for ~1 week, resulting in band delamination (Fig. 3). Bands were separated under a light microscope using forceps, working from the outside of the section toward the center. After removal, each band was rinsed three times in Milli-Q water and dried overnight at 40 °C. Subsamples (0.7-0.8 mg) were taken from each band and analyzed for  $\delta^{13}C$  and  $\delta^{15}N$ . Analysis was performed on an Isoprime Mass Spectrometer in continuous flow with a Carlo Erba Elemental Analyzer. Isotopic abundances are reported in the standard delta notation versus Vienna Peedee belemnite (Coplen, 1994) and atmospheric nitrogen (Mariotti, 1984) for carbon and nitrogen, respectively, where  $\delta^{13}C$  or  $\delta^{15}N = [(R_{sample} - R_{standard})/$  $R_{standard}$ ] [1000 %] and  $R = {}^{13}C/{}^{12}C$  or  ${}^{15}N/{}^{14}N$ . Analyses were made against internal laboratory standards and the international standards USGS-25 and IAEA C6 sucrose. Instrumental precision of the mass spectrometer is  $<\pm 0.1\%$  for  $\delta^{13}C$  and  $\pm 0.2\%$  for  $\delta^{15}N$  based on repeated analysis of standards. Precision, and thus reproducibility, for the organic antipatharian skeleton was measured for two bands using five random replicate subsamples from each band. The combined analytical precision of both bands for  $\delta^{13}$ C was  $\pm 0.04\%$ . For  $\delta^{15}$ N, the combined precision was  $\pm 0.17\%$ . The effect of KOH treatment on stable isotope composition is negligible (Williams, 2005).



Figure 3. Cross section of specimen A8601 after KOH treatment.

TABLE 2. SUBSAMPLE AGE AND EXTRAPOLATED SPECIMEN AGE

Subsample	Distance (mm)	Activity (dpm/g)*	Calculated age (years) <sup>†</sup>	Extrapolated age (years)§
Specimen A9902				
A	0.46	2.47 (±0.03)	33 (±1)	
В	1.43	0.35 (±0.01)	95 (±2.5)	
С	2.40	0.04 (±0.004)	163 (±1)	
Radius	2.90		198 (±2)	198
Specimen A8601				
A	1.65	0.27 (±0.02)		
В	3.95 -	-0.01 (±0.005)		
С	6.25	0.009 (±0.04)		
Radius	7.00			483
Specimen A8401				
Radius	4.30			290
Specimen G002				
Radius	5.60			386

*Note*: Concentric, coeval subsamples were subsampled from the most recent growth (A) to the oldest growth (C). The error represents instrumental imprecision in measuring <sup>210</sup>Pb activity and the resulting calculated age.

\*Activity represents <sup>210</sup>Pb<sub>ex</sub> activity.

<sup>†</sup>Calculated age determined from the decay of <sup>210</sup>Pb<sub>ex</sub> activity.

<sup>§</sup>Extrapolated age based on growth rate from specimen A9902 (0.0145 mm yr<sup>-1</sup>).

#### **RESULTS AND DISCUSSION**

#### **Dating and Growth Rates**

These corals are long-lived and slow-growing organisms. Results and extrapolated age calculations are provided in Table 2. The <sup>210</sup>Pb activity yielded ages of 33, 95, and 163 yr for subsamples A9902-A, A9902-B, and A9902-C, respectively, and an overall age of 200 yr for a specimen with a diameter of 5.8 mm at the base. The activity of <sup>210</sup>Pb (dpm/g) for subsamples A9902-A, A9902-B, and A9902-C was 2.47 ( $\pm$ 0.03), 0.35 ( $\pm$ 0.01), and 0.041 ( $\pm$ 0.004), respectively. For subsamples A8601-A, A8601-B, and A8601-C the <sup>210</sup>Pb (dpm/g) activity was 0.27 ( $\pm$ 0.02), -0.013 ( $\pm$ 0.005), and 0.0094 ( $\pm$ 0.004).

Specimen A9902 grew 0.97 mm in 62 yr from A9902-A to A9902-B, resulting in a growth rate of 0.015 mm yr<sup>-1</sup> and 0.97 mm in 68 yr from A9902-B to A9902-C, with a growth rate of 0.014 mm yr<sup>-1</sup>. The average growth rate was 0.0145 mm yr<sup>-1</sup>. The average measured radius of the specimen was 2.9 mm; therefore, the total age of the specimen is 200 yr. A major assumption with this method of dating is constant growth rates. Growth-rate estimates from subsamples A9902-A to A9902-B and from A9902-B to A9902-C were very similar, suggesting that the average growth rate did not change significantly between the first and second half of the specimen's life. This assumption is supported by a comparison of growth rates between specimens A9902 and A8601. Subsample A8601-A plotted on the same line as subsamples from A9902 (y = -0.07563x + 1.8788) calculated from plotting the natural log of <sup>210</sup>Pb activity versus distance from the subsample center to the outside edge of the cross section. This indicated a similar growth rate between specimens. Subsamples A8601-B and A8601-C were ignored because <sup>210</sup>Pb activity was essentially zero.

If average radial growth rates of deep-water *L. glaberrima* are approximately constant between specimens, this suggests by extrapolation that specimen A8601 is 480 yr, A8401 is 290 yr, and specimen AG002 is 390 yr old. Whatever the error in such extrapolation, it seems certain that each specimen is >100 yr old. Other long-lived cnidarians have been reported: the zoanthid *Gerardia*, formed of a layered proteinaceous skeleton, may reach ages of 1800 yr ( $\pm$ 300 yr) (Druffel et al., 1995).

#### Banding

Examination of 30  $\mu$ m thin sections from all specimens under light microscope revealed banding in *L. glaberrima*, visible as banding couplets of different optical density (Fig. 4). The optically dark bands



Figure 4. Cross section of specimen A8601 under light microscope.

ranged in width from 0.002 to 0.018 mm; the optically lighter bands ranged from 0.003 to 0.022 mm. Specimen A8601 contained 460 bands, specimen A8401 contained 310, specimen A9902 contained 200, and specimen AG002 contained 230. Visual examination of thick sections polished and partially treated with KOH displayed thin layers of skeleton, 25–40  $\mu$ m, tightly packed together. SEM photographs of deep-water *L. glaberrima* suggested that growth occurred by regular cyclic formation of a thin layer of skeleton. This growth pattern is similar to *Gerardia*, which was originally classified as an antipatharian (Druffel et al., 1995). In contrast, *A. fordensis* grows with lighter and darker regions, and studies suggest that the optically darker bands are annual (Grange and Goldberg, 1994), the optically lighter bands being formed of cement layers (Goldberg, 1991).

The factors influencing formation of the skeletal growth layers in *L. glaberrima* are not known. Physico-chemical factors potentially causing band formation in *A. fiordensis* were reviewed in Grange and Goldberg (1994); however, no correlations were found between band formation and surface or underwater light levels, salinity, or temperature. In deep-water gorgonians, variation in the structure of the skeleton may be related to food availability (Sherwood, 2002). Under nutrientrich conditions, the animal forms protein-rich organic skeleton, whereas calcite forms under nutrient-poor conditions (Sherwood, 2002). A similar mechanism could be present in the antipatharians, such that the skeleton is formed under nutrient-rich conditions, but under nutrient-poor conditions skeletal formation ceases.

#### **Stable Isotopes**

Nitrogen isotope values for the specimens ranged from 7.70% to 8.61% (Table 3). The  $\delta^{15}N$  signature of deep-water antipatharians is determined by their probable food source, which is believed to be falling organic matter from surface waters. The  $\delta^{15}N$  specimens A8601 and A8401 were collected from comparable depths (593 and 561 m, respectively) at the Jacksonville lithoherms. They displayed similar  $\delta^{15}N$  values, suggesting a similar food source, while specimen A9902, from a deeper area (679 m) and farther offshore (Fig. 1), is depleted in <sup>15</sup>N (7.70%). The  $\delta^{15}N$  signature of sinking particles decreases with depth (Altabet et al., 1991), which may account for the variation between specimens A8601-A8401 and A9902. Specimen AG002, from the Gulf of Mexico, was collected from the shallowest depth (307 m) and has an intermediate mean <sup>15</sup>N value (8.02%). The  $\delta^{15}N$  of organic particulate matter also reflects phytoplankton and nitrate concentration

TABLE 3. MEASURED  $\delta^{13}C$  AND  $\delta^{15}N$  ISOTOPIC ABUNDANCES

Specimen	Depth (m)	Age (years)	Mean δ¹³C (‰)	±SE	Range (‰)
A8601 A8401 A9902 G002	593 561 679 307	483 290 198 386	-15.66 -16.31 -16.14 -16.39	0.05 0.02 0.06 0.03	2.51 1.57 1.42 0.74
Specimen	Depth (m)	Age (years)	Mean δ¹⁵N (‰)	±SE	Range (‰)
A8601 A8401 A9902 G002	593 561 679 307	483 290 198 386	8.61 8.46 7.70 8.02	0.02 0.07 0.15 0.19	3.65 2.56 2.56 3.36

dynamics (Wada and Hattori, 1976; Altabet and Francois, 1994). The northern Gulf of Mexico receives high fluxes of nitrate fertilizer ( $\delta^{15}$ N from -4% to 4%; Kendall, 1998) from the Mississippi-Atchafalaya watershed (Goolsby et al., 2001). The input of <sup>15</sup>N-depleted material relative to antipatharian specimens and anthropogenic alteration of nitrogen dynamics can account for the intermediate <sup>15</sup>N value of specimen AG002 relative to the deeper Atlantic specimens.

Carbon isotope values for the four specimens ranged from -15.66% to -16.39% with a variety in range of values from 0.74%to 2.51% (Table 3). Similar to nitrogen, carbon isotopes in the skeleton likely are determined by the food source, organic matter. The average  $\delta^{13}$ C value for southeastern U.S. specimens was  $-16.04\% \pm .04\%$ (Table 3), although specimen A8601 from the Jacksonville lithoherms had notably lower values  $(-15.66\% \pm .05\%)$  than the other two southeastern U.S. specimens: A8401 (-16.31% ± .02%) and A9902  $(-16.14\% \pm .06\%)$ , and the Gulf of Mexico specimen AG002  $(-16.39\% \pm .03\%)$ . The  $\delta^{13}$ C values for A8401 and A9902 were similar: 1.6% and 1.4%, respectively, while AG002 was half as much (0.74%) and A8601 was twice as much (2.5%). This suggests that  $\delta^{13}C_{POM}$  has been very variable over the past 500 yr in the Atlantic, possibly as a reflection of changing atmospheric carbon dioxide concentrations, and that carbon dynamics have been more stable in the Gulf of Mexico over a similar period of time.

This is the first study of possible environmental signals recorded in skeletons of antipatharians. Their wide distribution in the world's oceans, coupled with their slow growth rate, suggests that they may provide data from a range of depths on the time scale of hundreds of years.

#### CONCLUSIONS

Deep-water antipatharians are slow growing, with estimated radial growth rates of 0.0145 mm  $yr^{-1}$ , far slower than those recorded from warm and temperate shallow-water antipatharians.

Stable isotope results are reproducible among specimens from the same location, indicating that antipatharians have potential as proxy records.

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