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Late Holocene radiocarbon and aspartic acid racemization dating of deep-sea octocorals

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Abstract

Primnoa resedaeformis is a deep-sea gorgonian coral with a two-part skeleton of calcite and gorgonin (a fibrillar protein), potentially containing long-term records of valuable paleo-environmental information. For various reasons, both radiocarbon and U/Th dating of these corals is problematic over the last few centuries. This paper explores aspartic acid racemization dating of the gorgonin fraction in modern and fossil specimens collected from the NW Atlantic Ocean. Radiocarbon dating of the fossil specimen indicates a lifespan of 700 ± 100 years, the longest yet documented for any octocoral. Gorgonin amino acid compositions were identical in the fossil and modern specimens, indicating resistance to organic diagenesis. Similar to bone collagen, the fibrillar protein of gorgonin may impose conformational constraints on the racemization of Asp at low temperatures. The rate of racemization of aspartic acid (D/L-Asp) was similar to previously published results from an 1800 year old anemone (*Gerardia*). The age equation was: age (years BP 2000 AD) $= [(\text{D/L} - 0.020\ (\pm.002))/.0011\ (\pm.0001)]^2 (r^2 = 0.97, p < .001)$. The error in an age estimate calculated by D/L-Asp was marginally better than that for ^{14}C dating over the most recent 50–200 years, although the dating error may be improved by inclusion of more samples over a broader time range. These results suggest that D/L-Asp dating may be useful in augmenting ^{14}C dating in cases where ^{14}C calibrations yield two or more intercept ages, or in screening samples for further ^{14}C or U/Th dating.

1. Introduction

Deep-sea octocorals often have a two-part skeleton of calcite and gorgonin, a tough, horny protein. Based on Δ^{14}C, δ^{13}C and δ^{15}N evidence, the calcite fraction is derived from dissolved inorganic carbon (DIC) at depth, while the gorgonin is derived from recently exported particulate organic matter (POM; Griffin and Druffel, 1989; Druffel et al., 1995; Heikoop et al., 2002; Roark et al., 2005; Sherwood et al., 2005a). The gorgonian species *Primnoa resedaeformis* has concentric rings in its axial skeleton that form annually (Andrews et al., 2002; Sherwood et al., 2005b). This may be true in other octocoral species as well (Thresher et al., 2004). Together, these observations suggest that high temporal-resolution, geochemical-based climate reconstructions of both surface and intermediate/deep-water processes may be made from the skeletons of deep-sea octocorals (Heikoop et al., 2002; Thresher et al., 2004; Sherwood et al., 2005a,c).

Obtaining specimens of deep-sea coral for study is not a trivial matter. Financial and logistical demands may render sampling expeditions very difficult. On the other hand, many large, priceless specimens are often available from fishermen and museums. In recent years, we have obtained about 20 large specimens of *P. resedaeformis* from long-line fishermen in Nova Scotia. Some of these specimens may contain several hundreds of year's worth of environmental records in their skeletons (Risk et al., 2002). Unfortunately, most of the large specimens were collected dead. The greater apparent availability of dead vs. live specimens in recent decades may be the result of increasing habitat destruction by the dragger fishing fleet since the 1950s (Watling and Norse, 1998; Hall-Spencer et al., 2002). In addition, the

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skeletons of *P. resedaeformis* may contain death/re-growth surfaces representing hiatuses in skeletal accretion (Sherwood, 2002).

Since accurate chronology is a fundamental requirement in proxy reconstructions, absolute dating methods are required to exploit the potential climate signals contained in these skeletons. Carbon-14 dating has been used to date fossil octocorals. Risk et al. (2002) used ^{14}C to determine a lifespan of 320 years in a fossil specimen of *P. resedaeformis* dredged from Georges Bank. Druffel et al. (1995) used ^{14}C to determine a lifespan of 1800 years in *Gerardia*, dubbing it the ''Bristlecone pine of the deep-sea''. *Gerardia* is an anemone, not a coral, but it forms a tough organic endoskeleton much like the gorgonin in *P. resedaeformis*. A major problem with ^{14}C is the inability to resolve dates over the period ca. 1650–1950 AD because of local variations in the marine reservoir effect and the possibility for multiple calibrated ages. Uranium-series dating may also be used to date deep-sea octocorals (Thresher et al., 2004). However, uncertainty in the extent of initial thorium contamination, and low uranium concentration in calcite presents a major problem with this method.

This paper explores the use of amino acid racemization to date the skeletal gorgonin fraction of *P. resedaeformis*. The rate of equilibration of D- and L-handed isomers (racemization) after death of an organism depends on age and temperature. If temperature remains constant, which is more or less expected for the deep-sea, the increase in the D-isomer may be used to calculate age (e.g., Goodfriend, 1992). The approach used here was to calibrate the racemization rate of aspartic acid (Asp) in known-age samples, then to evaluate whether D/L-Asp would be useful in dating specimens over the last 400 years where ^{14}C dating is problematic.

2. Methods

Live-collected and fossil colonies of *P. resedaeformis* were used in this study. Both were collected from the Northeast Channel, a submarine canyon located SW of Halifax, Canada (approximately 65°40′W/42°00′N), between 250–475 m depth. The live specimen (DFO2002-con5) was collected by trawl in the summer of 2002 (Sherwood et al., 2005c). The fossil specimen (Fossil-95; Fig. 1) was collected by fishermen using long-line gear in 1995. Samples were air-dried following collection. The bases of colony trunks were sectioned with a rock saw, and the sections were ground and polished on a diamond lap wheel and photographed with a digital camera in macro mode.

Radiocarbon and AAR analyses were performed on the gorgonin fractions of Fossil-95 and DFO2002-con5. To isolate the gorgonin, the sections were placed in 0.5 M HCl until complete dissolution of calcite. This took up to 3 weeks. Gorgonin layers were separated by peeling them apart with tweezers and scalpel under a binocular microscope. The position of each layer was carefully marked on the digital photographs while sampling. Samples were

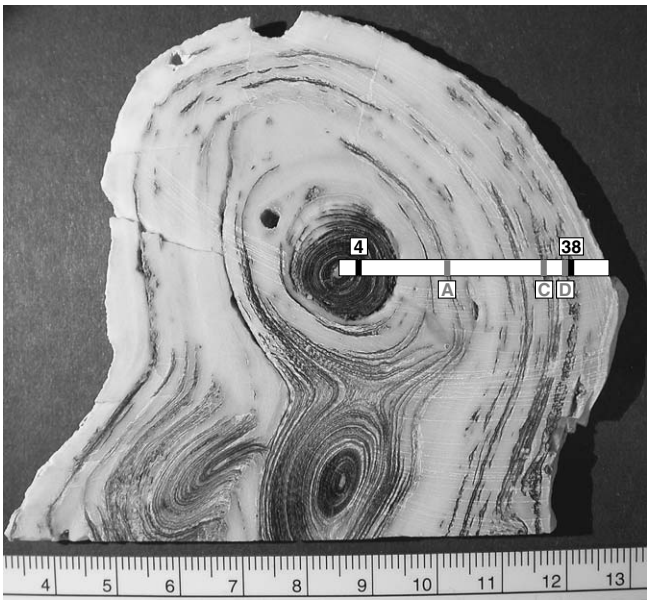

Fig. 1. Image of specimen Fossil-95 viewed in cross-section. Black bars and letters show samples isolated for ^{14}C dating. Grey bars and letters show samples isolated for amino acid analysis. Scale on ruler in cm.

placed in 5 mm polyethylene vials with 0.5 M HCl for an additional 48 h, triple rinsed in Milli-Q water and dried for 48 h in a low temperature oven (~50 °C).

Samples for radiocarbon analysis were combusted in individual quartz tubes and reduced to graphite in the presence of iron catalyst. $\Delta^{14}C$ was determined on graphite targets at the Center for AMS. Results include a background and $\delta^{13}C$ correction and are reported as $\delta^{14}C$ according to Stuiver and Polach (1977).

Amino acid analyses were performed by reverse-phase high performance liquid chromatography with pre-column derivatization following the procedure in Kaufman and Manley (1998). First, samples were dissolved in 7 N HCl over the course of 7 days with intermittent 20 min intervals in an ultrasonic bath. This expedited dissolution but did not allow the samples to reach a high enough temperature to induce racemization. Samples were hydrolyzed in 7 N HCl, sealed under N_2 and heated to 110 °C for 6 h. The hydrolysates were then desiccated under a N_2 atmosphere at 80 °C for ~20 min, and then rehydrated in 0.01 M HCl with known quantity of L-homo-arginine (L-hArg) for use as an internal standard. Although initial analyses of Asp yielded poor peak separation, a slower mobile-phase flow rate increased resolution and the subsequent analyses were considered reliable. Analytical error, as determined by the range in sample replicates, was 2–4%.

3. Results and discussion

3.1. Skeletal chronology

Skeletal chronology in the younger colony, DFO2002-con5, was reported earlier (Sherwood et al., 2005b).

Chronology was established on the basis of growth ring counts performed by three amateur counters, and validated by the timing of increase in bomb radiocarbon. The samples used here for amino acid measurements ranged from 1926 to 2002 years AD.

Radiocarbon ages of the fossil specimen, Fossil-95 (Fig. 1), were calibrated with the program CALIB (Stuiver and Reimer, 1993, version 5) using the Marine04 calibration (Hughen et al., 2004). A correction for local variation in the marine reservoir effect, ΔR, was applied (i.e., the difference in reservoir age between the local region of interest and the model ocean; Stuiver and Braziunas, 1993). Values of ΔR for Northwest Atlantic shelf waters are available rom the Marine Reservoir Correction Database (http://radiocarbon.pa.qub.ac.uk/marine); however, to more accurately correct ^{14}C ages for the Northeast Channel, ^{14}C measurements from known-age specimens of *P. resedaeformis* were used. These measurements span the period 1924–2002 and include both pre- and post- nuclear bomb values (Sherwood et al., 2005b). The pre-1950 values were used to calculate ΔR (e.g., Surge et al., 2003), which averaged 128 ± 35 years (mean $\pm$ SD; Table 1). The large range in ΔR reflects the scatter in pre-bomb $\Delta^{14}C$, and suggests the influence of different water masses over short timescales. The Northeast Channel is located near a sharp gradient between northward Labrador Current waters and southward subtropical gyre waters, which are likely to differ in pre-bomb $\Delta^{14}C$. For comparison, the Marine Reservoir Correction Database reports ΔR estimates of 94 ± 22 years (Georges Bank) and 95 ± 24 years (southern Grand Banks).

Separate annual rings isolated from near the inner and outer skeletal regions of Fossil-95 (Fig. 1) yielded ΔR-corrected model ages of 1700 ± 75 and 1125 ± 65 years BP, respectively, (Table 2; 1σ age ranges). The corresponding radial growth rate between samples is 0.06 ± 0.01 mm yr^{-1}. This value is very close to the 0.04 mm yr^{-1} reported earlier in a fossil colony from Georges Bank (Risk et al., 2002). Applying this growth rate from the center to the extreme edge of the colony yields a lifespan of 690 ± 120 years. To our knowledge, this is the longest documented lifespan of any deep or shallow-water octocoral.

3.2. Amino acid composition of the tissue and gorgonin

The results of all 10 amino acid analyses are summarized in Table 3. Following Goodfriend (1997), the percent mass of the organic fraction accounted for by the amino acids was calculated by multiplying the mean molecular weight of each amino acid (weighted by the % composition of each amino acid) by the total molar concentration of all 13 quantified amino acids (Table 3). Values ranged from 70% to 100% of the sample mass. In two analyses (AAL #10864 and AAL #10868) amino acid concentrations were systematically lower; on this basis these were judged unreliable and excluded from all subsequent calculations. Thus, the 13 quantified amino acids accounted for, on average, 78%, 87% and 84%, respectively, of the mass of tissue, modern gorgonin and fossil gorgonin.

Fig. 2 compares amino acid profiles in the tissue and modern and fossil gorgonin. Compared with gorgonin, the tissue was higher in threonine (Thr), glutamic acid (Glu), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr) and phenylalanine (Phe), and lower in aspartic acid (Asp), serine (Ser), glycine (Gly),

Table 1
Calculation of local marine reservoir correction from known-age samples of *P. resedaeformis*

Sample	Ring No.	Ring year[a] (years AD)	Pre-1950 age (years)	CAMS #	$\Delta^{14}C$ (‰)	^{14}C age (years BP 1950)	Model age[b] (years BP 1950)	ΔR (years)
DFO2002-con5A1	37	1948 (4)	2	97103	−80 (3)	620 (30)	469	151
DFO2002-con5A1	43	1942 (5)	8	97104	−72 (3)	545 (30)	460	85
DFO2002-con5A1	50	1934 (6)	16	97105	−77 (3)	595 (30)	457	138
DFO2002-con5A1	58	1924 (6)	26	97106	−72 (3)	550 (30)	451	99
NED2002-371A	58	1947 (2)	3	111330	−82 (4)	630 (35)	464	166
							Mean	128
							SD	35

1 SD errors in brackets.
[a] Average from three amateur ring counters (Sherwood et al., 2005b).
[b] Marine04 calibrated age (Hughen et al., 2004).

Table 2
Radiocarbon ages for the fossil *P. resedaeformis* specimen

Sample	Distance (mm)[a]	CAMS #	$\Delta^{14}C$ (‰)	^{14}C age (years BP 1950)	Model age[b] (years BP 1950)
Fossil95-D2-38	4.5	111138	−196 (3)	1700 (35)	1125 (65)
Fossil95-D2-4	37	111137	−249 (3)	2245 (40)	1700 (75)

1 SD errors in brackets.
[a] Distance from skeletal margin.
[b] ΔR-corrected Marine04 calibrated age.

Table 3
Summary of amino acid abundance and D/L data

Sample ID	Sample type	Distance (mm)[a]	Age (years AD)	AAL ID	Amino acid abundance (μmol/mg)													% wt	D/L -Asp	D/L- Glu	D/L -Val
					Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Arg	Total AA			
DFO2002-con5																					
1	Tissue	0	2002[b]	10859	0.84	0.44	0.55	0.76	0.33	0.57	0.48	0.16	0.42	0.59	0.24	0.36	0.31	6.05	78	.026	.022	0
2	Gorgonin	0.1	2000[b]	10860	1.49	0.41	1.04	0.65	1.36	1.71	0.37	0.07	0.24	0.25	0.24	0.10	0.84	8.74	103	.022	.011	0
10	Gorgonin	3.9	1981[b]	10861	1.07	0.31	0.70	0.47	0.91	1.17	0.33	0.05	0.21	0.18	0.15	0.08	0.62	6.24	74	.027	.014	0
19	Gorgonin	6.1	1967[b]	10862	1.45	0.40	0.94	0.63	1.26	1.58	0.43	0.06	0.27	0.23	0.17	0.10	0.80	8.34	98	.031	.015	0
30	Gorgonin	9.0	1951[b]	10863	1.21	0.31	0.79	0.52	1.02	1.30	0.29	0.04	0.18	0.19	0.13	0.08	0.63	6.71	79	.052[d]	.011	0
38	Gorgonin	10.4	1940[b]	10864	0.68[d]	0.18[d]	0.45[d]	0.30[d]	0.56[d]	0.72[d]	0.17[d]	0.02[d]	0.11[d]	0.12[d]	0.07[d]	0.05[d]	0.36[d]	3.77[d]	44[d]	.025	.010	0
44	Gorgonin	12.6	1927[b]	10865	1.25	0.35	0.82	0.55	1.06	1.36	0.36	0.04	0.23	0.22	0.13	0.09	0.66	7.15	84	.034	.014	0
Fossil-95																					
A	Gorgonin	19	500[c]	10866	1.02	0.36	0.80	0.53	1.00	1.20	0.38	0.07	0.25	0.24	0.23	0.10	0.69	6.86	82	.06	.014	0
C	Gorgonin	11	760[c]	10867	1.10	0.38	0.80	0.55	1.03	1.22	0.42	0.08	0.27	0.23	0.28	0.10	0.75	7.19	86	.062	.016	0
D	Gorgonin	4	820[c]	10868	0.67[d]	0.23[d]	0.48[d]	0.34[d]	0.61[d]	0.73[d]	0.26[d]	0.04[d]	0.17[d]	0.14[d]	0.14[d]	0.07[d]	0.45[d]	4.32[d]	52[d]	.062	.015	0

[a] Distance from skeletal margin.
[b] Age determined by counting annual rings (Sherwood et al., 2005b).
[c] Age determined by applying linear growth rate, as calculated from ΔR-corrected ^{14}C ages from Table 2.
[d] Unreliable data; excluded from subsequent calculations.

alanine (Ala) and arginine (Arg). These differences probably relate to the different protein compositions, which is reflected in different C:N values between tissue and gorgonin (7 vs. 3, respectively; Sherwood et al., 2005a).

Compared with the modern gorgonin, the fossil gorgonin had slightly lower amounts of Asp and Ala, and higher amounts of Val, Tyr and Arg (Fig. 2). These differences probably reflect original variability at the time of formation, since there were no differences in the least stable amino acids Thr, Ser, and Met. A similar result was found in the organic endoskeleton of an 1800 year old *Gerardia* (Goodfriend, 1997).

Fig. 3 shows ontogenetic trends in amino acid abundances in the modern gorgonin. There were no significant increases or decreases with age of the layer (regression statistics were insignificant for all 13 amino acids). Taken together, the similarity between modern and fossil gorgonin in: (1) % mass accounted for by amino acids, (2) amino acid profiles, and (3) the lack of ontogenetic trends in the modern gorgonin argue for little or no organic diagenesis for at least ca. 1500 years. These findings are consistent with gorgonin being one of the toughest known proteins (Goldberg, 1976).

Gorgonin is thought to be composed of a fibrillar protein supported in a proteinaceous matrix (Lewis et al., 1992). The fibrillar protein was characterized earlier as aromatically cross-linked collagen (Goldberg, 1974, 1976; Szmant-Froelich, 1974). More recently, gorgonin has been characterized as a polyphenol-containing fibrillar protein (H. Ehrlich., private communication). Independent evidence for a fibrillar protein in *P. resedaeformis* is provided here by scanning electron microscopy (Fig. 4; Sherwood, 2002). The voids left by decalcification in acetic acid reveal the fibrillar nature of the remaining organic material. The individual fibres measure approximately 200 nm in diameter, similar to the proteinaceous fibres observed in shallow-water gorgonians (Lewis et al., 1992). Although the exact nature of the gorgonin in *P. resedaeformis* will have to await more detailed study, the presence of fibrillar protein has a significant implication for D/L dating, as discussed below.

3.3. D/L ratios

D/L ratios were measured for Asp, Glu and Val (Table 3 and Fig. 5). For Asp, there was no difference in D/L between modern tissue and gorgonin. There was a significant difference between modern and fossil gorgonin, as expected from the rapid racemization rates of Asp (Goodfriend, 1992). For Glu, D/L was higher in fossil gorgonin than in modern gorgonin; but the difference was not significant, consistent with slower racemization of Glu. Tissue D/L-Glu was nearly twice that of gorgonin. The reason for this is not clear. D-Val could not be resolved in any of the samples.

Fig. 6A shows D/L-Asp vs. age for the gorgonin fraction of *P. resedaeformis*. Thirty percent of the total increase

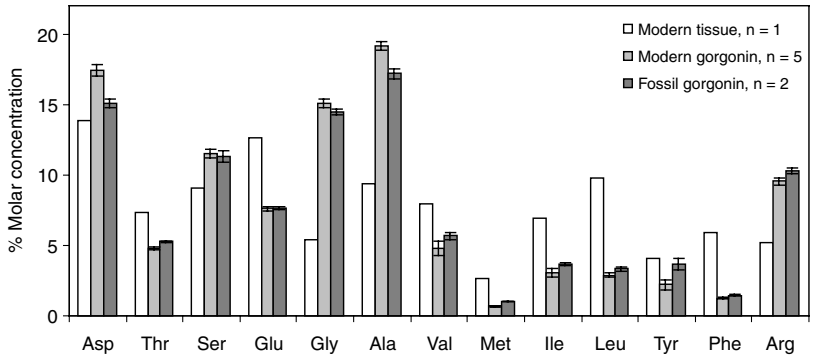

Fig. 2. Amino acid composition of the tissue and gorgonin (modern and fossil) fractions of *P. resedaeformis*. Error bars are ±1 SD.

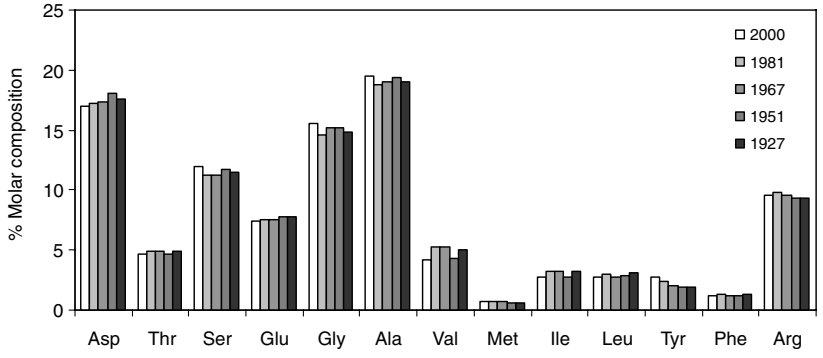

Fig. 3. Ontogenetic trends in amino acid composition of the modern gorgonin. Each bar corresponds to an individual annual ring isolated from the skeleton, as indicated by year. Regression analysis indicated no significant increases or decreases in any of the 13 amino acids with age.

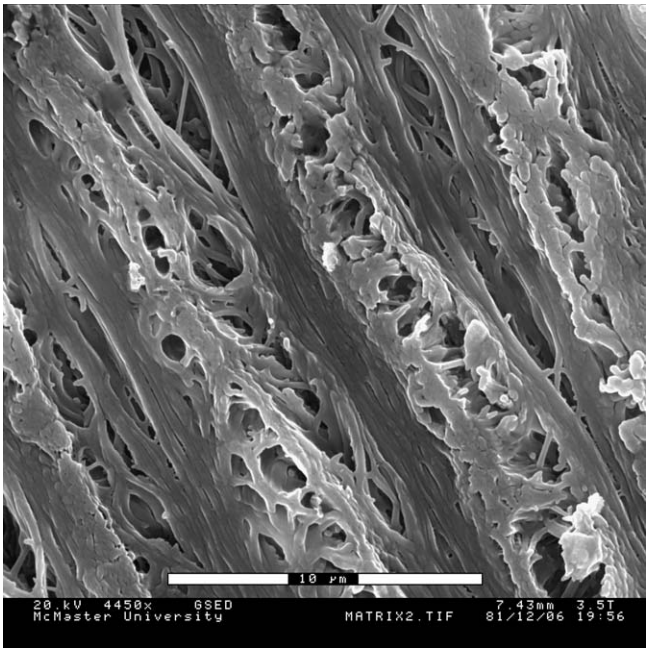

Fig. 4. SEM image of acetic acid-etched *P. resedaeformis* viewed in cross-section. The four repeating diagonal bands are sub-annual bands (Risk et al., 2002; Sherwood, 2002). Fibrillar protein material is oriented both parallel and perpendicular to the plane of view.

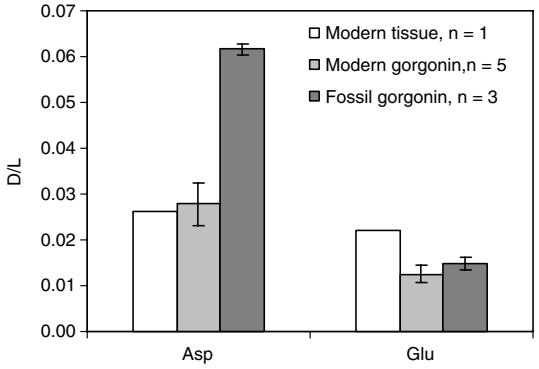

Fig. 5. D/L composition of the tissue and gorgonin (modern and fossil) fractions of *P. resedaeformis*. Error bars are ±1 SD.

occurred over the most recent 75 years, while the remaining 60% occurred over the remaining 1400 years. This convex-upward pattern is consistent with Asp racemization kinetics in other organisms (e.g., Mitterer and Kriausakul, 1989; Goodfriend, 1991). Asparagine (Asn) is converted to Asp during the hydrolysis step of sample preparation (Brinton and Bada, 1995), so the measured increase in D-Asp actually represents the combined responses of Asp + Asn (Goodfriend, 1991). Both amino acids are

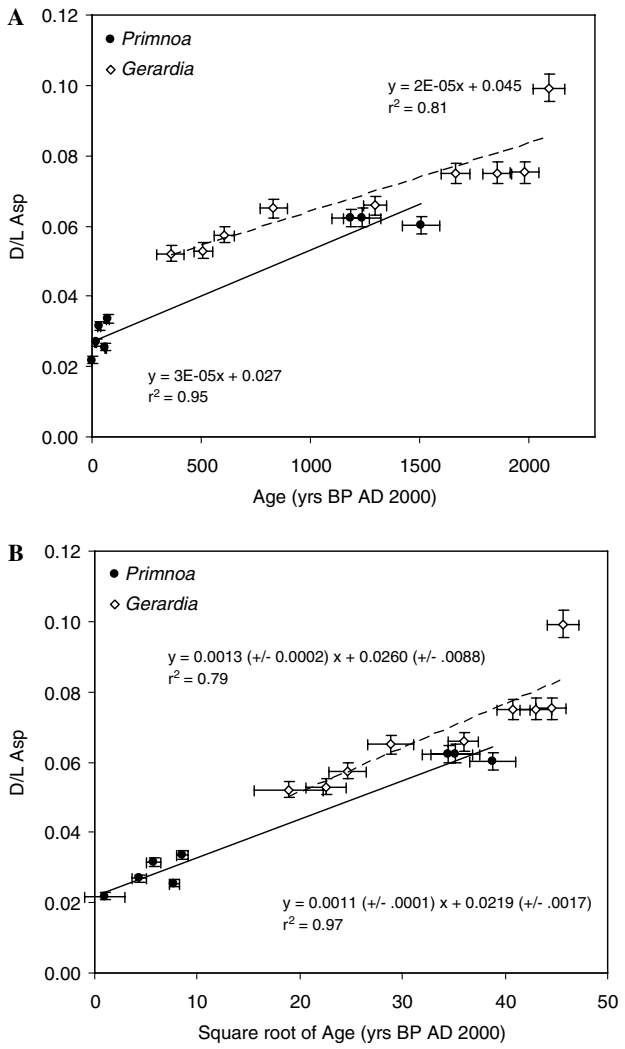

Fig. 6. D/L-Asp vs. calendar age (A) and square root of calendar age (B) for *P. resedaeformis* and *Gerardia*. *Gerardia* ^{14}C ages originally reported in Druffel et al. (1995) have been calibrated and corrected for marine reservoir effect (see text). Error bars are ± 1 SD.

thought to racemize via deamination to an aminosuccinyl residue (Asu; Geiger and Clarke, 1987). Racemization in the Asu residue is some 5 times higher than in free Asp (Radkiewicz et al., 1996). Collins et al. (1999) attribute the non-linear racemization kinetics to the greater tendency for Asn, as compared with Asp, to form Asu residues. Therefore, the earlier phase of rapid racemization in *P. resedaeformis* (Fig. 6A) is likely dominated by Asn, while the later phase is dominated by Asp.

The data for *Gerardia* collected at ~600 m on Little Bahama Bank are also shown in Fig. 6A. Values of D/L-Asp for this specimen are from Goodfriend (1997) and corresponding radiocarbon ages are from Druffel et al. (1995). To maintain consistency in radiocarbon dating, the ^{14}C ages originally reported in Druffel et al. (1995) were corrected with the Marine04 calibration and a ΔR value of 36 ± 14 (Florida and Bahamas regional average from the Marine Reservoir Correction Database). The D/L values are similar between *P. resedaeformis* and *Gerardia*, although we caution that Goodfriend (1997) used a different sample preparation procedure in obtaining his D/L data, and the organic endoskeleton in *Gerardia* is not mineralized. The lower slope for *Gerardia* (Fig. 6A) probably reflects the lack of data over the most recent centuries, where Asp racemization is generally fastest.

To linearize racemization rates over the time period of interest, we tested a variety of transformation functions of D/L (i.e., ln[(1+D/L)/(1−D/L)]; D/L raised to an exponent > 1) and time (square root of time). In all cases, coefficients of determination (r^2) ranged from 0.91 to 0.97 (*P. resedaeformis*) and 0.64 to 0.86 (*Gerardia*). In Fig. 6B, the results for D/L vs. the square root of age are shown because r^2 values are reasonably good both for *P. resedaeformis* (0.97) and *Gerardia* (0.79) and it is easier to visualize the spread of values along the regression across the entire time period. While D/L-Asp values are ~0.007 higher in *Gerardia*, the slopes and intercepts of the two regressions are within 1 standard error of each other. On this basis, we assume that the fit for *P. resedaeformis* reliably estimates D/L values between the two extremes in age represented by the present data. This is an empirical calibration and there is no a priori reason to choose one linearization over another. Recently, Collins et al. (1999) and Collins and Riley (2000) have challenged the practice of treating the net racemization as a "black box", in favor of a modeling approach that accounts for the underlying kinetics of hydrolysis, racemization and decomposition separately. The purpose of the present study, however, was simply to test whether D/L-Asp could be used to resolve ages in the time period ca. 100–400 years BP.

Goodfriend (1997) used heating experiments to derive Arrhenius parameters for Asp racemization in *Gerardia*. From his results, the calculated age of the colony was only 250 ± 70 years, in conflict with the 1800 years determined by ^{14}C (Druffel et al., 1995). Goodfriend (1997) offered four explanations for the conflicting age estimates: (1) possible incorporation of older, ^{14}C-depleted carbon sources in the *Gerardia* skeleton; (2) Non-linearity in the Arrhenius relationship, such that the rate at ambient temperature cannot be reliably extrapolated from rates calculated at high experimental temperatures; (3) Variation in rates among the different layers of skeleton; (4) Change in ambient temperature during the lifetime of the organism. Goodfriend (1997) discussed each of these possibilities at length and largely discounted the importance of the latter two explanations.

Measurements of bomb radiocarbon in the recent layers of *Gerardia* (Druffel et al., 1995) as well as in several other species of deep-sea gorgonian (Griffin and Druffel, 1989; Roark et al., 2005; Sherwood et al., 2005b) suggest that the proximate source of carbon to the organic endoskeleton is recently exported particulate organic matter (POM). Since bomb radiocarbon was measured in the older (i.e., mature) layers of *Gerardia*, as well as in younger layers (<50 years) of modern specimens of other gorgonians (Sherwood et al., 2005b), there is no evidence that

the organic endoskeleton incorporates a ^{14}C-depleted carbon source, such as DIC, dissolved organic carbon or suspended POM found at depth, at any point in the organism's lifetime. This is also supported by the similarity of $\delta^{13}C$ and $\delta^{15}N$ in the same modern and fossil specimens of *P. resedaeformis* presented here (Sherwood et al., 2005a). On the other hand, the ultimate source of carbon could potentially be upwelled DIC or DOC with a depleted ^{14}C signature, upon which POM may grow. The radiocarbon ages measured in *Gerardia* increased linearly with distance from the edge of the skeleton (Druffel et al., 1995). To explain the apparent 1800 year lifespan in terms of feeding on ^{14}C-depleted POM would require a smooth decrease in upwelling intensity over time, a scenario we find highly unlikely. Therefore, we suggest that Goodfriend's (1997) explanation 1, above, can safely be discounted.

Collins et al. (1999) called into question the practice of estimating racemization rates in collagen by extrapolating from high experimental temperatures. This is because denaturation temperatures (approximately 70 °C) occur between ambient and experimental temperatures (Collins et al., 1999). Below the denaturation temperature, the triple helical structure of collagen imposes a severe conformational constraint on the deamination of Asp + Asn to form Asu, and therefore on the racemization of Asp and Asn. There is little or no racemization within the helical structure of collagen itself, except at the frayed ends of the helix (Collins et al., 1999). Although the organic endoskeleton of *Gerardia* has not been characterized by modern sequencing techniques, it is likely to be a fibrillar protein of some sort, and subject to similar conformational constraints on racemization. The heating experiments on *Gerardia* (Goodfriend, 1997) likely overestimate racemization rates, resulting in younger apparent age (Collins et al., 1999). Thus, the 1800 year age estimate from ^{14}C is probably correct. Similar to *Gerardia* and bone collagen, the fibrillar protein in *P. resedaeformis* may impose a conformational constraint upon Asp + Asn deamination. The observed racemization probably reflects racemization at the degraded ends of protein fibrils, and/or within the associated non-fibrillar protein matrix.

Racemization rate is dependent on temperature as well as time. The present experiment was not designed to evaluate temperature effects, and if the assumption that gorgonin behaves like collagen is correct, then extrapolating racemization rates from Arrhenius parameters determined at high experimental temperatures may be problematic. However, if we assume that racemization kinetics are similar in *P. resedaeformis* and *Gerardia*, then a preliminary evaluation may be made by comparing the two D/L-Asp vs. square root of age relationships (Fig. 6B). No useful information is provided by the intercepts, or by the fact that *Gerardia* D/L values average 0.007 higher, because a certain amount of racemization is induced during hydrolysis in the laboratory, and in this case, preparation methods differed. On the other hand, the slopes should not be affected by laboratory procedure. For *P. resedaeformis*, ambient temperature was 7 °C (250–500 m average, area SS29 from the Oceanographic Database, Bedford Institute of Oceanography; http://www.mar.dfo-mpo.gc.ca/science/ocean.database). For *Gerardia*, the ambient temperature was 12.5 °C (Griffin and Druffel, 1989). Since the slopes were within 1 standard error of each other, differences in racemization rate over a temperature of 6.5 °C could not be determined. However, since racemization rate is taxon-specific, it is possible that a taxon effect compensates for, and masks, a temperature effect.

Taking a different approach, the standard error of the regression for *P. resedaeformis* (Fig. 6B) was 0.0032, or about twice the analytical error in D/L-Asp measurements. Much of this variability may be due to annual-decadal temperature fluctuations, which can exceed ± 2 °C in the Northeast Channel between 250–500 m. It is probable that Little Ice Age temperatures at this depth were persistently cooler because of southward penetration of the Labrador Current; this phenomenon characterized the 1960s (MERCINA, 2001), when NE Channel bottom temperatures were about 2.5 °C cooler than the 1950–2000 average. In the analysis below, we assume that the errors in the regression coefficients (Fig. 6B) reflect temperature effects, and that these temperature effects are accounted for by the overall simulated dating error.

3.4. Prospects for D/L-Asp dating of *P. resedaeformis*

The purpose of this study was to evaluate whether D/L-Asp could be used to date recent (<400 years) colonies of *P. resedaeformis*. From Fig. 6B, the relationship between D/L-Asp and time is:

$$\text{Age (years BP 2000 AD)} = [(\text{D/L} - 0.020(\pm.002))/.0011(\pm.0001)]^2 \quad (r^2 = 0.97,\ p < 0.001) \tag{1}$$

where the errors in brackets are $1\sigma_x$. Using a spreadsheet, the error in an age calculation was estimated with a Monte Carlo simulation over 1000 iterations (e.g., Kaufman, 2003). Values of the slope and intercept in Eq. (1) were applied to specific values of D/L. Each iteration used values drawn at random from normal probability distributions defined by the mean and error in each variable. The $1\sigma_x$ error was used for slope and intercept. Since D/L analyses were not performed on co-eval samples, the 2σ analytical error (±8%) was chosen to reflect inter-sample variability in D/L. The 1σ error in a D/L age estimate was calculated for a range of different ages (Fig. 7). For example, the error for a 100 year old sample is ± 60 years. For comparison, Fig. 7 also shows local model ^{14}C dating errors, using a ΔR value of 128 ± 35 years, over the same time period. The error associated with a D/L age is marginally better than that of ^{14}C over the last 100–200 years, or where model ^{14}C ages are invalid (50–95 years BP AD 2000). Prior to 200 years BP, ^{14}C dating provides more precise model ages, while over the most recent 50 years, bomb-^{14}C may be used

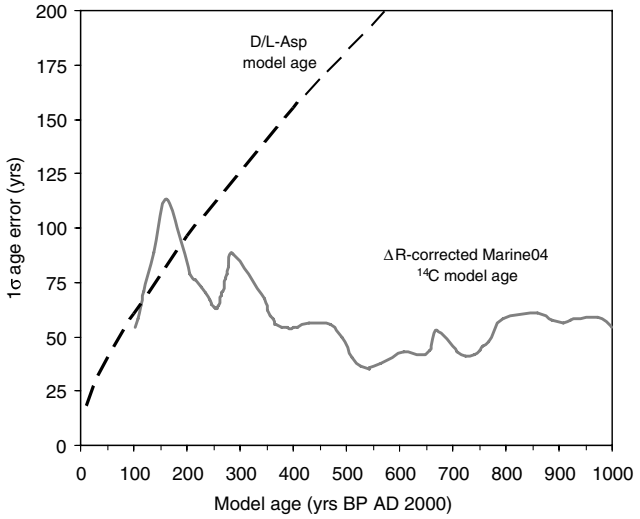

Fig. 7. Comparison of 1 SD dating errors associated with D/L-Asp and ^{14}C dating methods. D/L-Asp errors calculated with Monte Carlo simulation applied to Eq. (1) (see text). Marine model ^{14}C age errors calculated with Calib 5.0, using a ^{14}C age error $\pm$ 35 years and a ΔR correction of 128 $\pm$ 35 years. Model ^{14}C ages <95 years BP 2000 AD are invalid.

to constrain age with a high level of precision (Sherwood et al., 2005b). This comparison is limited by the fact that for model ^{14}C ages, ΔR is assumed to be constant over time. On the other hand, some of the error associated with the D/L age model itself depends on ^{14}C error, as well as analytical error and the effect of variable temperature.

From these preliminary results, the prospects for dating deep-sea coral by D/L-Asp racemization over the last 400 years do not look promising. However, analyses of more samples spanning a greater range of ages may better constrain the age equation, and thereby improve upon dating error. Rates of D/L racemization may also be further constrained by analyzing the fibrillar protein or protein matrix components of gorgonin separately. D/L-Asp dating of *P. resedaeformis* may be useful in screening samples for further ^{14}C or U/Th dating, since D/L determinations are relatively inexpensive and can be performed on minute quantities of sample material. Another approach to using D/L-Asp dating is where a ^{14}C calibration yields two or more intercepts. The D/L-Asp data might clearly discern which of the intercepts is more likely.

4. Conclusions

1. Radiocarbon dating of a large fossil specimen of *P. resedaeformis* indicates a lifespan of 700 $\pm$ 100 years, the longest documented lifespan of any deep or shallow-water octocoral.
2. There were no ontological or long-term trends in amino acid abundances of gorgonin over the last 1500 years. This indicates that the gorgonin may be a durable, long-term archive of paleo-environmental information.
3. The increase in D/L-Asp with time is non-linear, as expected from previous studies on other organisms. Similar to bone collagen, the fibrillar protein of gorgonin may impose conformational constraints on the racemization of Asp at low temperatures.
4. Compared with ^{14}C dating, D/L-Asp dating of *P. resedaeformis* may offer marginally better age constraint over the last 50–200 years, but more analyses are required to refine the D/L-Asp vs. time relationship.

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