

Aspartic acid racemization dating of Holocene brachiopods and bivalves from the southern Brazilian shelf, South Atlantic

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Abstract

The extent of racemization of aspartic acid (Asp) has been used to estimate the ages of 9 shells of the epifaunal calcitic brachiopod *Bouchardia rosea* and 9 shells of the infaunal aragonitic bivalve *Semele casali*. Both taxa were collected concurrently from the same sites at depths of 10 m and 30 m off the coast of Brazil. Asp D/L values show an excellent correlation with radiocarbon age at both sites and for both taxa ($r^2_{\text{Site 9 } B. \text{ rosea}} = 0.97$, $r^2_{\text{Site 1 } B. \text{ rosea}} = 0.997$, $r^2_{\text{Site 9 } S. \text{ casali}} = 0.9998$, $r^2_{\text{Site 1 } S. \text{ casali}} = 0.93$). The Asp ratios plotted against reservoir-corrected AMS radiocarbon ages over the time span of multiple millennia can thus be used to develop reliable and precise geochronologies not only for aragonitic mollusks (widely used for dating previously), but also for calcitic brachiopods. At each collection site, *Bouchardia* specimens display consistently higher D/L values than specimens of *Semele*. Thermal differences between sites are also notable and in agreement with theoretical expectations, as extents of racemization for both taxa are greater at the warmer, shallower site than at the cooler, deeper one. In late Holocene marine settings, concurrent time series of aragonitic and calcitic shells can be assembled using Asp racemization dating, and parallel multi-centennial to multi-millennial records can be developed simultaneously for multiple biomineral systems.

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Introduction

Amino-acid racemization/epimerization techniques are an exceptionally useful and versatile tool of Quaternary geochronology (e.g., Wehmiller and Miller, 2000). They are attractive because they can be applied to a wide range of biomineralized fossils, subfossils and modern material, including skeletal material of marine organisms (e.g., mollusks, corals, brachiopods), remains of terrestrial organisms (e.g., ostrich eggs, plants, bones, teeth), soils, and other biogenic materials (e.g., Goodfriend et al., 2000). In particular, marine invertebrates are an important target for high-resolution geochronological studies in

the late Quaternary because shells collected from modern seafloors and coastlines tend to represent age mixed (time-averaged) assemblages that may potentially provide nearly complete multi-centennial to multi-millennial time series for the Holocene, and in some cases, the Pleistocene (e.g., Flessa and Kowalewski, 1994; Wehmiller et al., 1995; Meldahl et al., 1997; Kowalewski et al., 1998; Carroll et al., 2003).

This project focuses on marine benthic shelly remains from open habitats of the southern Brazilian shelf and provides a comparative radiocarbon-based assessment of aspartic acid (Asp) racemization dating methods for two very different shelled invertebrates (calcitic epifaunal brachiopods and aragonitic infaunal bivalves) collected concurrently at two sites that differ in depth and may have had different thermal histories. Using independent radiocarbon calibrations for each site and each shell type, several important aspects of the amino acid geochronology are explored quantitatively.

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First, this study compares amino acid dating methods for two different biomineral systems (the aragonitic infaunal bivalve mollusk *Semele casali* versus the calcitic epifaunal brachiopod *Bouchardia rosea*) collected simultaneously from the same sampling sites and same shelly assemblages. Bivalves and brachiopods have been important producers of marine shell beds since the early Paleozoic. Many previous researchers have noted that differences may exist in apparent rates of racemization across genera, families, and higher taxonomic groups (Lajoie et al., 1980; Miller and Hare, 1980; Andrews et al., 1985; Goodfriend et al., 1997; Wehmiller and Miller, 2000). This analysis is an explicit attempt to quantitatively contrast racemization rates between sympatric organisms representing different biomineral systems. Such direct comparisons allow insight into the diagenetic histories of two major groups (bivalves and brachiopods) that are among the most important taxa represented in the fossil record.

Second, multiple studies showed that marine aragonitic bivalves can be dated reliably using amino acid racemization methods (e.g., Wehmiller et al., 1995; Goodfriend et al., 1997; Kowalewski et al., 1998). However, calcitic brachiopods have remained virtually untested, with an exception of one recent study (Carroll et al., 2003), which demonstrated that A/I (D-alloisoleucine/L-isoleucine) ratios can be used to

date shells of the brachiopod *Bouchardia rosea* from the Brazilian Atlantic Coast. Here, we attempt to test the applicability of Asp D/L values for dating brachiopods, thereby providing comparisons of results for two amino acid ratios determined on the same two species of interest (*B. rosea* and *S. casali*) from the same collection sites (Sites 1 and 9, Fig. 1).

Last, racemization rates are known to be temperature dependent, and thus amino acid geochronologies may vary notably across sites with different thermal histories. To test if this effect can be observed in environments with subtle thermal (bathymetric) differences, samples of both species targeted in this analysis were collected from two sites that may have differed slightly in their late Holocene thermal history.

The independent per-site, per-species radiocarbon calibrations of Asp racemization carried out in this study should allow us to evaluate these issues rigorously and evaluate the practical applicability of the Asp racemization method for dating large numbers of marine invertebrate shells collected from modern seafloors. This study will permit the development of simultaneous time series for multiple biomineral systems from the same depositional systems, thus offering a high-resolution geochronological framework for future geochemical, paleoenvironmental, and paleoecological studies.

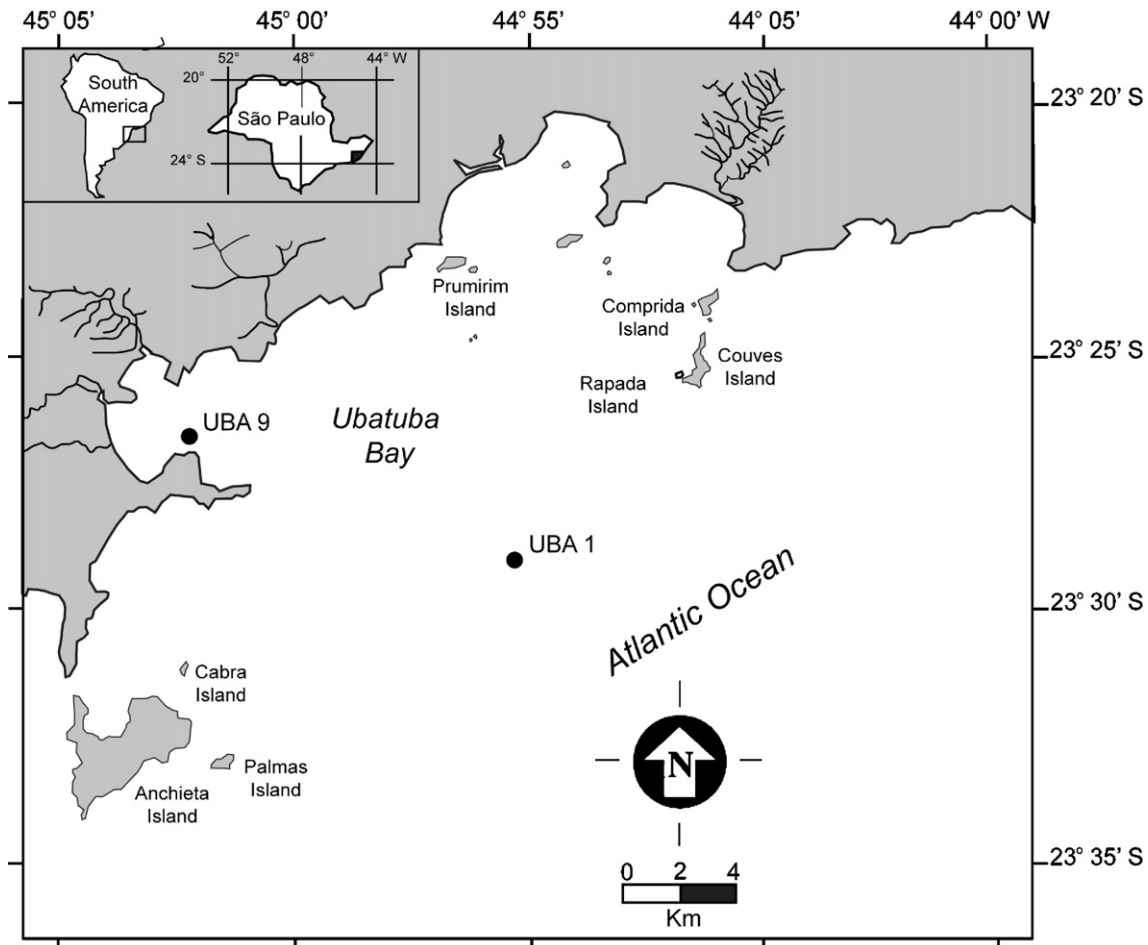


Figure 1. Map of the study area and collection sites.

Methods

Specimens were collected from two sites situated at Ubatuba Bay, a small embayment off the coast of the state of São Paulo, Brazil (Fig. 1), located on the inner shelf in the Southeast Brazilian Bight Marine Province (Campos et al., 1995; Mahiques et al., 2004). The bay is characterized by humid tropical climate, shallow depths (0–30 m within the sampled area), full marine salinity (34–35 ppm) and warm water masses (mean annual temperature ~24°C) (Mahiques, 1992, 1995; Mahiques et al., 1998; Mantelatto and Fransozo, 1999). The area is fed by the South Brazil Current, a warm, western boundary current, and within the bay is dominated by Coastal Water (Campos et al., 1995). The eastward-opening bay is protected from the dominant S–SW winds and high-energy waves (Mahiques et al., 1998). The bay's substrate is dominated by fine-grained (silt and very fine sand) terrigenous sediments, terrestrial organic matter (Burone, 2002) and coarse biogenic constituents (brachiopods, mollusks, echinoids, bryozoans, and foraminifers) (Mahiques et al., 1998; Mantelatto and Fransozo, 1999). Rhynchenelliform (terebratulid) brachiopods, overwhelmingly dominated by *Bouchardia rosea*, are locally abundant (Simões et al., 2004), and a diverse molluscan fauna is also present. Further details on Ubatuba Bay hydrodynamics, sedimentation, sediment geochemistry and faunal content can be found in Emilson (1959), Matsuura (1986), Castro Filho et al. (1987), Mahiques (1992, 1995), Sanches (1992), Mantelatto and Fransozo (1999), Burone (2002) and Muniz (2003).

Samples were collected in conjunction with an ongoing project of the Marine Ecology Group of São Paulo State University. Specimens were collected at two sites (10 m and 30 m, Fig. 1) using a Van Veen grab sampler (1/40 m²), which collects unconsolidated surficial deposits (the uppermost several centimeters of the substrate) which represent a time-averaged, or temporally mixed assemblage (e.g., Walker and Bambach, 1971). Physical parameters at each sampling site (number of specimens analyzed, average temperature and salinity) were recorded (Table 1), and all brachiopod and mollusk specimens recovered from each grab were retained. Temperature measurements were conducted through the calendar year in 2000 and included three bottom water measurements per month at each sampling site, ranging over the year from 19.3 to 25.2°C at the shallow site and 18.0–21.7°C at the deep site, with an average temperature difference between the two sites of 2.6°C.

Table 1
Sampling site data

Site	9	1
Depth	10 m	30 m
Latitude	23° 26' 41"	23° 28' 53"
Longitude	45° 02' 07"	44° 55' 21"
Temp °C	22.0	19.4
Salinity ‰	34.0	35.0
No. analyzed shells: <i>Bouchardia</i>	5	4
No. analyzed shells: <i>Semele</i>	3	6

Temperatures are averages of monthly bottom water measurements from 2000.

Each specimen was prepared for radiocarbon and amino acid analyses using a uniform, standard methodology. First, shell fragments (18.9–132.0 mg in weight) were taken from posterior portions of each shell, when possible, using wire snips. This controlled sampling location was used to minimize intra-shell variability in amino acid ratios, known to occur in mollusks and brachiopods vertically across shell layers with different microstructure and/or spatially within a given shell layer (e.g., Goodfriend et al., 1997; Carroll et al., 2003). All shells were cleaned following routine procedural methods (e.g., Goodfriend, 1987), which consisted of a surficial cleaning performed on shell fragments using dental tips attached to a Dremel® rotary tool, followed by ultrasonication and etching with dilute HCl. In the case of brachiopods, mechanical cleaning of *B. rosea* specimens also served to remove the outer primary layer of the shell, so that all analyses were only performed on the thickest, secondary layer. Lab preparations were performed at George Washington University, Virginia Tech, and the University of Delaware.

Analysis of the extent of racemization of aspartic acid was conducted at the University of Delaware using an Agilent 6890 Gas Chromatograph equipped with a 25-m Chirasil-Val column and flame ionization detector. A representative chromatogram can be found in Wehmiller and Miller (2000). Sample pretreatment involved hydrolysis of the dissolved sample in 6N HCl for 20 h at 100°C, desalting with HF, and preparation of the N-TFA isopropyl derivative of the amino acid mixture. Under normal operating conditions, D/L values for six or seven amino acids are obtained with this procedure. However, for this study only Asp D/L values are reported because the other amino acid D/L values are generally too low to calculate consistently from sample to sample due to the young age of the samples, and thus they offer little age-resolution information. D/L values were calculated from the peak–area ratios obtained from at least two chromatograms of each sample derivative when possible. A small proportion of chromatograms were rejected due to low concentrations of amino acids in the derivative, resulting in small aspartic peaks. In such cases, the sample derivative was concentrated and always successfully rerun. Integration of peak areas was accomplished using both HP3390 integrators and Chemstation integration software. The typical coefficient of variation for multiple measurements of Asp D/L values on a single derivative is <3%. Analyses of inter-laboratory comparison samples (Wehmiller, 1984) during the period of the present study revealed no systematic drift of the overall laboratory results. During the interval 2001–2004, which includes the period in which the reported analyses were completed, mean and standard deviation D/L Asp values for ILC A and ILC C powders were 0.418 (0.024) and 0.903 (0.039) ($n = 41$ and 35, respectively).

A total of eighteen specimens from the two species, including both sites and representing a broad range of Asp D/L values, were selected for radiocarbon analysis at the NOSAMS Lab (Woods Hole) where ¹⁴C was measured by Accelerator Mass Spectrometry (see <http://www.nosams.whoi.edu/nosams.html> for procedural details).

Table 2
Sample calibration data

Sample	Delaware lab no.	Asp D/L	No. injections	NOSAMS ¹⁴ C Lab no.	δ ¹³ C	¹⁴ C age (yr B.P.)	Calibrated age (yr B.P.)
<i>B. rosea</i> , Site 9 (10 m)							
91018	2003020	0.098	2	OS-42387	1.08	(1.103556 F Modern)	0
91006	2003034	0.230	1	OS-42386	1.44	480 ± 30	80 (43–119)
BB15*	CH126, 241	0.340	2	OS-26545	1.92	1250 ± 30	784 (726–829)
BB14*	CH125, 304	0.438	2	OS-26544	1.68	2790 ± 45	2522 (2433–2612)
91032	2003026	0.534	1	OS-42388	1.31	3870 ± 35	3825 (3759–3888)
<i>B. rosea</i> , Site 1 (30 m)							
13022	2003210	0.269	1	OS-42383	2.15	835 ± 30	465 (439–494)
13010	2003199	0.413	1	OS-42378	2.40	2800 ± 35	2539 (2461–2613)
13006	2004035	0.600	3	OS-43395	1.85	6130 ± 90	6550 (6441–6654)
13002	2003024	0.599	1	OS-42377	1.11	6230 ± 45	6669 (6611–6734)
<i>S. casali</i> , Site 9 (10 m)							
91071	2003106	0.071	1	OS-42389	1.47	(1.111611 F Modern)	0
91062	2003104	0.172	1	OS-39672	2.14	745 ± 35	378 (324–425)
91075	2003141	0.317	2	OS-42453	2.21	2770 ± 30	2484 (2389–2562)
<i>S. casali</i> , Site 1 (30 m)							
13077	2003086	0.075	2	OS-39670	2.34	375 ± 35	0
13081	2003088	0.083	2	OS-42384	2.20	555 ± 30	183 (138–247)
13087	2003138	0.119	2	OS-42385	2.12	590 ± 25	222 (147–163)
13088	2003139	0.184	2	OS-39671	2.76	1130 ± 30	676 (645–703)
13075	2003131	0.222	2	OS-43396	2.47	1350 ± 50	883 (818–941)
13071	2003082	0.273	2	OS-39673	2.07	3050 ± 35	2808 (2754–2846)

Samples marked with an asterisk were analyzed at George Washington University. Calibrated ages were calculated using Calib 5.0 (Stuiver et al., 2005) using the SHCal04 and marine04.14c databases (Hughen et al., 2004; McCormac et al., 2004), assuming a mean marine reservoir age of 408 ± 18 yr (ΔR 8 ± 17). Median age and 1-sigma age ranges are shown for calibrated specimen ages.

Shell ages were calibrated to calendar years using CALIB (Stuiver et al., 2005; version 5.0), and error estimates for radiocarbon were based on information provided by NOSAMS. Age values were determined by the SHCal04 (Southern Hemisphere) and marine04.14c databases (Hughen et al., 2004; McCormac et al., 2004), assuming a mean marine reservoir age of 408 ± 18 yr (ΔR 8 ± 17) recently established through an extensive empirical analysis by Angulo et al. (2005). Calibrated ages are reported in Table 2 as median age with 1-sigma age ranges.

Results

Nine specimens of *B. rosea* and nine of *S. casali* have paired AMS-radiocarbon and Asp D/L results, as summarized in Table 2. Raw Asp D/L values plotted against calibrated ¹⁴C age show initially rapid increases in D/L Asp, followed by a slowing in the apparent rate in older samples. It has often proven

convenient to identify a transformation that results in a linear plot of Asp racemization against time (e.g., Goodfriend and Stanley, 1996; Goodfriend et al., 1996; Manley et al., 2000). Many workers have employed the simple “parabolic kinetic” equation $D/L = k t^{0.5}$ (k = constant, t = time; Mitterer and Kriausakul, 1989) for the analyses of results like those presented here. However, a power transformation (e.g., Goodfriend et al., 1996) was chosen as the most appropriate transformation for Asp D/L values in this data set. As live-collected specimens were not available for heating experiments, the single most appropriate transformation for each taxon (D/L Asp^{2.6} for *B. rosea* and D/L Asp^{3.1} for *S. casali*) was identified by iteratively raising the power of D/L Asp until maximum r^2 values were obtained while maintaining a positive y intercept (D/L ratio). Taxon-specific transformations are used consistently for all specimens as if deduced by heating experiments, and not adjusted on a per-site basis. Transformations show a

Table 3
Calibration information for all sites and taxa, including the calibration equation number (from text), slope and intercept of the calibration regression line

Site	Species	Equation	Slope	Intercept	Adjusted r^2	P	f	RMSE
9	<i>B. rosea</i>	1	6.46E-05	0.038	0.97	0.001	156.2	265.4
1	<i>B. rosea</i>	2	4.66E-05	0.051	0.997	0.001	889.4	178.0
9	<i>S. casali</i>	3	3.68E-05	0.010	0.9998	0.006	9751.8	19.2
1	<i>S. casali</i>	4	2.50E-05	0.011	0.93	0.001	70.2	270.1

Calibration statistics, including adjusted r^2 , P value, f statistic and root mean square error for the regression of D/L aspartic values against shell age are also noted for all sites and taxa.

high significant linear correlation ($r^2 > 0.93$ in all cases) with the calibrated ^{14}C age of the specimen (Table 3), suggesting that Asp is a good predictor of age within this system for each site and each species. It is noteworthy that regardless of the particular transformation chosen, results and conclusions that follow below were not significantly altered.

The CALIB-calibrated radiocarbon estimates and the resulting Asp D/L calibrations are summarized in Table 2 and Figure 2. For data pooled across sites (Fig. 2), Asp ratios of both brachiopods and bivalves show high and significant ($r^2_{B. rosea} = 0.98, P < 0.001$ and $r^2_{S. casali} = 0.86, P = 0.0002$) linear correlations. Note that regression equations and statistics were computed using the program SAS (Version 9.1) with the dependent variable (D/L Asp values) on the y axis and calibrated shell age on the x axis. Equations were transformed to provide a formula for calculating shell age from D/L Asp values Eqs. (1)–(4). Statistics do not vary regardless of which variable is considered dependent or independent.

Calibration plots for *B. rosea* grouped by site (Fig. 3A) yield the following regression equations:

$$\text{(Site 9) Age} = (\text{Asp}^{2.6} - 0.01266)/4.633\text{E} - 05 \quad (1)$$

$$\text{(Site 1) Age} = (\text{Asp}^{2.6} - 0.01043)/3.824\text{E} - 05 \quad (2)$$

Calibration plots for *S. casali* grouped by site (Fig. 3B) yield the following regression equations:

$$\text{(Site 9) Age} = (\text{Asp}^{3.1} - 0.00013503)/1.137\text{E} - 05 \quad (3)$$

$$\text{(Site 1) Age} = (\text{Asp}^{3.1} - 0.00065487)/6.44\text{E} - 06 \quad (4)$$

The calibrations are valid for the studied taxa from each sampling site within the approximate age ranges of the radiocarbon dated specimens, or less than around 3000 yr in age for *S. casali* and 7000 yr for *B. rosea*.

A comparative plot of calibration curves for brachiopods and bivalves (Fig. 2) suggests that the two species differ

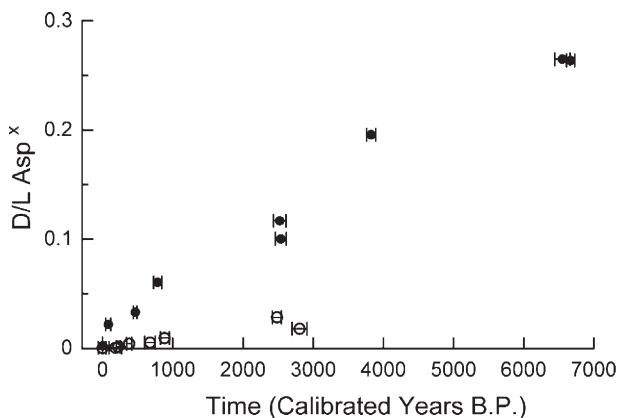


Figure 2. Plot of aspartic acid ratios for *B. rosea* ($\text{D/L Asp}^{2.6}$, closed circles) and *S. casali* ($\text{D/L Asp}^{3.1}$, open circles) graphed against (median) calibrated radiocarbon age (years B.P.) and 1-sigma age ranges. Racemization rates are significantly higher for *B. rosea* as compared to *S. casali* (see text for more details).

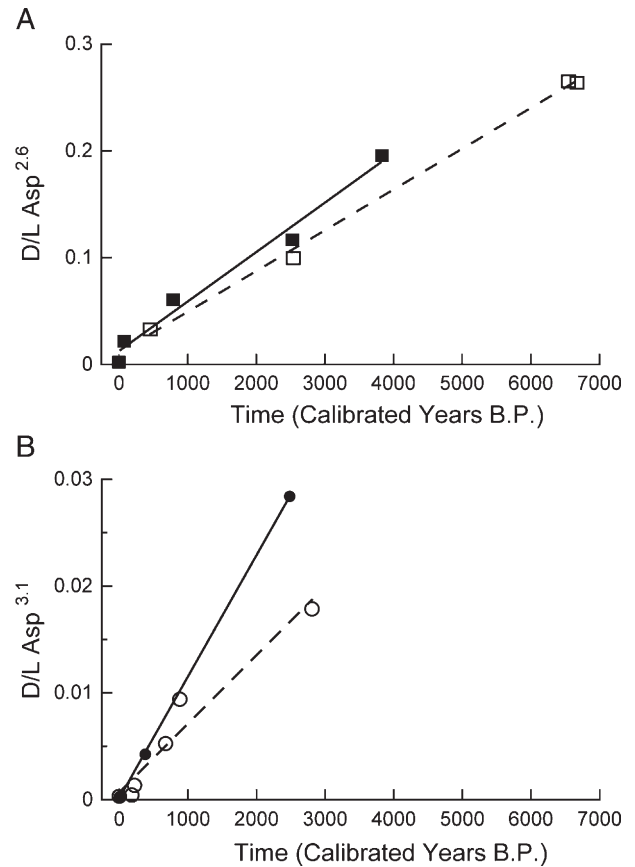


Figure 3. Plot of aspartic acid ratios for (A) *B. rosea* and (B) *S. casali* graphed against calibrated radiocarbon age (years B.P.). For both plots, sampling Site 9 (10 m, bold line) and Site 1 (30 m, dashed line) are plotted separately. Site-specific calibrations show that samples from Site 9 racemize at a faster rate than samples from Site 1.

notably in racemization rates. First, the intercept is significantly higher ($t = 14.81, P < 0.0001$) for brachiopods (0.011 vs. $2.68\text{E}-4$) suggesting that the initial Asp ratio (primarily reflecting artificial racemization induced during sample processing—see Gillard et al., 1991) is greater for *B. rosea*. Moreover, the slope of the racemization curve is visibly steeper for brachiopods (the difference between the two slopes is statistically significant; $t = 7.32, P < 0.0001$). Consequently, regardless of the calibrated age, the Asp ratios are always higher (by a factor of 1.2 to 2.6) for *B. rosea* relative to *S. casali* of the same age.

Figure 3 also suggests that racemization rates differ between the two collection sites. This difference is consistently shown by both taxa: both brachiopod and bivalve shells from Site 9 (10 m) appear to have racemized at faster rates than samples from Site 1 (30 m). Differences between calibrated slopes ($t = 4.51, P = 0.006$) and intercepts ($t = 3.48, P = 0.02$) are statistically significant between collection sites for *S. casali*, but only significant for intercepts between collection sites for *B. rosea* (slopes: $t = 2.18, P = 0.08$; intercepts: $t = 7.41, P = 0.0007$).

Carroll et al. (2003) showed that D-alloisoleucine/L-isoleucine (A/I) epimerization ratios in the brachiopod *B. rosea* could be used successfully to estimate the numerical shell age via

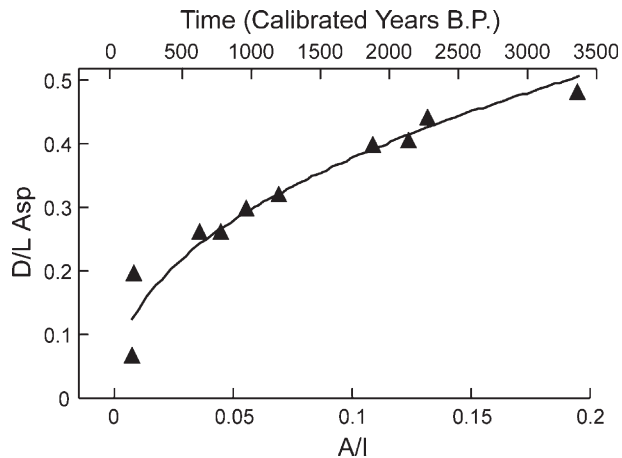


Figure 4. Cross-validation data using D/L Asp values plotted against A/I values obtained for the same set of *B. rosea* specimens (A/I data analyzed using High Performance Liquid Chromatography (HPLC) after Carroll et al., 2003).

radiocarbon calibration. Here, specimens analyzed by Carroll et al. (2003) were re-analyzed for Asp ratios. The A/I and Asp ratios show a high and positive correlation with one another (Fig. 4), and A/I ratios display slower racemization rates than the Asp ratios.

Discussion

The tight positive correlation between D/L aspartic acid values and calibrated radiocarbon ages observed consistently for both species at each of the two targeted sites (Figs. 2 and 3) indicates that Asp racemization techniques provide reliable means for dating late Holocene invertebrate shells collected from present-day seafloors, as noted in many previous studies, including for microfossils (Hearty et al., 2004). Particularly encouraging is the successful calibration of aspartic acid chronology in calcitic brachiopods, which, to our knowledge, have never been dated with Asp D/L values before. The good correlation of the racemization ratios between A/I (Carroll et al., 2003) and Asp ratios (Fig. 4) in conjunction with their independently derived ^{14}C calibrations indicate that both of these amino acids can be used reliably for amino acid racemization dating of *B. rosea* (see also below). Admittedly, the analysis by Carroll et al. (2003) and this study both target the same brachiopod species from a single region. It is desirable, therefore, to further test the utility of amino acid racemization dating for other brachiopod species in other depositional and climatic settings. The only other amino acid racemization study on brachiopods that we are aware of (Torres et al., 1997) dealt with fossils that span beyond the range of radiocarbon calibration, preventing those authors from rigorous testing of the dating reliability of amino acid racemization techniques. In addition, whereas Asp D/L values can clearly provide reliable dating means, the results of this study also provide useful quantitative insights into several problems that may weaken the utility and reliability of the amino acid racemization techniques.

Reservoir age

First, it is worth exploring the assumption of the reservoir age (408 ± 18 yr with a ΔR of 8 ± 17 yr, see also above). Whereas the assumed reservoir age is supported by an extensive regional compilation of reservoir estimates (Angulo et al., 2005), previously published marine reservoir estimates for the region have been quite variable, ranging from 204 ± 44 to 720 ± 40 yr (Stuiver et al., 1998; Nadal de Masi, 1999; Eastoe et al., 2002; Angulo et al., 2005), possibly reflecting temporal variability in regional ocean upwelling (Eastoe et al., 2002). These reservoir ages appear to cluster into two groups: one yielding a mean reservoir correction of 220 ± 20 yr and the other at 515 ± 10 yr (Eastoe et al., 2002). The value of 408 ± 18 yr used here was derived by Angulo et al. (2005) as representative of the majority of the shells dated in the region (see Angulo et al., 2005, for discussion).

However, the sensitivity of our age estimates to changes in the postulated reservoir age is worth examining because of the variability in published reservoir ages. A sensitivity analysis (Fig. 5) indicates that changing the reservoir age shifts the intercept of the calibration curve, with no significant change in slope of the calibration trends (reservoir age 408 vs. 204: $t = 0.10$, $P = 0.93$; reservoir age 408 vs. 540: $t = 0.68$, $P = 0.52$; reservoir age 204 vs. 540: $t = 0.78$, $P = 0.46$) with numerical age estimates progressively becoming older as the assumed reservoir age is decreased. Thus, changing the assumed reservoir age would affect the numerical age estimates of Asp dated shells. However, such changes would *not* alter the estimated rates of racemization (both the shape and the slope of a given calibration curve remains virtually the same as the reservoir age is changed; Fig. 5). The stability of the calibration curve implies that neither age differences between shells, nor the relative position of dated shells when arrayed into a time series are affected by changes in the assumed reservoir age. The only effect of assuming the incorrect reservoir age is thus a shift in numerical age estimates. In the worst-case scenario, this shift could be as high as 200–300 yr for more recent specimens and

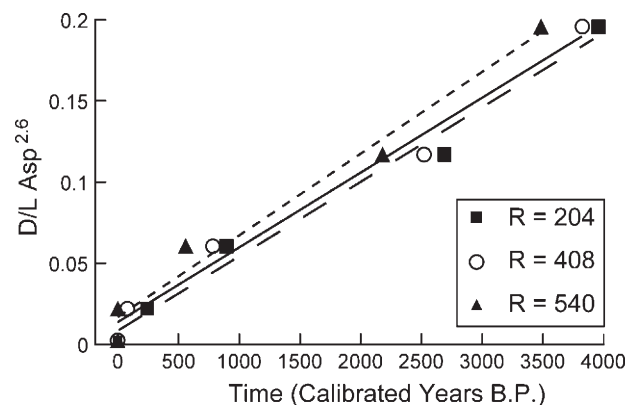


Figure 5. Radiocarbon calibration plots for *B. rosea* samples (Site 1) simulating the minimum (squares, Eastoe et al., 2002), average (circles, Angulo et al., 2005) and maximum (triangles, Eastoe et al., 2002) published reservoir ages for the collection area. Recalibration shifts the ages of specimens to older values with a decrease in the estimated reservoir age.

even greater for specimens thousands of years old. It is therefore advisable to monitor regional estimates of reservoir age and update the calibration equations whenever significant errors in regional estimates of reservoir effect are conclusively demonstrated. Pre-bomb collected shells from our sampling sites were not available for analysis. However, the extent of racemization of *B. rosea* specimens plotted against uncalibrated radiocarbon ages were used to estimate a reservoir age of 560 yr in Carroll et al. (2003). This estimate is well within the range of published regional reservoir ages (Angulo et al., 2005).

Variability due to age estimation of modern shells is also problematic. Shells too young for radiocarbon calibration may actually be variable in age, from recently dead (collected in 2000, or – 50 yr B.P.) to collected in 1950 (0 yr B.P.) for this dataset. The fraction modern (F14C) value (Table 2) was used on the CALIBomb web site <http://calib.qub.ac.uk/CALIBomb/frameset.html> in attempt to calibrate modern shells (Reimer et al., 2004). Modern *B. rosea* specimen 91018 from Site 9 (10 m) yielded a one-sigma age range of either 1958.68 (September) to 1959.01 (January) or 1996.67 (September) to 1996.94 (December). Modern *S. casali* specimen 91071 from Site 9 (10 m) yielded a one-sigma age range of either 1958.74 (September) to 1959.04 (January) or 1995.68 (September) to 1996.94 (December). *S. casali* specimen 13077 from Site 1 (30 m) with uncalibrated radiocarbon age 375 ¹⁴C yr B.P. was too young for calibration after reservoir corrections were made and too old for use with the CALIBomb program, so is thus likely closer in age to the year 1950 than 2000. In the calibration of amino acids against calibrated shell age, a 50-yr offset in modern shell ages yields an offset in calibrated age between 179 yr (D/L Asp = 0.98) and 195 yr (D/L Asp = 0.44) for modern specimens of *B. rosea* from Site 9 (10 m). This error is equivalent to or less than the error produced by minor shifts in reservoir age. Negative shells ages are also graphically and conceptually difficult to handle, and thus the standard age of 0 cal yr B.P. was chosen to represent all modern specimens.

Taxonomic effects

The notable differences in overall rates or extents of racemization between the two species provide compelling evidence that it is critical to calibrate amino acid D/L values separately for each taxon that is being studied. As in many previous studies of both Holocene and Pleistocene racemization geochronology (e.g., Wehmiller, 1982; Andrews et al., 1985; Wehmiller and Miller, 2000; Hearty et al., 2004) this study provides direct evidence that the variation in apparent racemization rates can be very significant for different taxa collected from the same site. Not only does *B. rosea* racemize significantly faster than *S. casali*, but, in fact, the difference in racemization is quite dramatic (e.g., a 3615-yr-old *S. casali* shell (D/L Asp = 0.3) from Site 1 would appear 870 yr old if the *B. rosea* calibration curve from the same site were applied). The data acquired in this study do not allow us to rigorously evaluate the causative factors responsible for such dramatic differences in racemization rates—most likely, though, these are due to differences in shell mineralogy and protein structures between

aragonitic bivalves and calcitic brachiopods. Note that the mode of life is unlikely to be the cause here, considering that it would be more likely that it were infaunal bivalves that experienced slightly cooler thermal history, and racemized more slowly, than epifaunal brachiopods, especially at the shallow site which is more likely to be affected by solar insolation.

Bathymetric effects

This study suggests that even minor bathymetric differences, including sites representing the same regional and environmental settings, may result in significant disparities in racemization rates. For both taxa, samples from Site 9 (10 m) show greater racemization rates than samples from the deeper Site 1 (30 m), significantly so for *S. casali* and despite the fact that the two sites are located in the same bay and differ in depth by only 20 m (Fig. 1). These consistent offsets suggest differences in the late Holocene thermal histories between the two sites, with the more nearshore site being slightly warmer than the more offshore one. This is to be expected: as reported above, currently, the average annual temperature difference between the two sites is ~3°C (2.6°C; data for the year 2000). The direction of the offset is predictable theoretically. First, the proximal, shallower Site 9 should be warmer on average because of the relatively stronger effects of solar insolation at shallower depths. Second, the cooling effect of water masses brought in by the shelf-break upwelling, which operates intermittently in the region (e.g., Campos et al., 1995; Kowalewski et al., 2002), should be more manifested in the deeper, more offshore Site 1. Most importantly, neither of the sites is believed to have been subaerially exposed (or notably variable in depth) during the late Holocene, as the sea level is believed to have been relatively stable in the region (~1 m fluctuations) during the last 3000 yr (Angulo et al., 1999; Lessa et al., 2000; Baker et al., 2001). If the bathymetric temperature difference observed today for the two sites persisted into the past, it would be substantial enough to produce the observed difference in racemization rates between collection sites. Finally, in the case of this specific study, the effects of thermal differences are much less pronounced than taxonomic effects. Indeed, data pooled within each species across the two collection sites (Fig. 2) provide calibration curves with respectable r^2 values for both taxa ($r^2_{B. rosea} = 0.98$, $P < 0.0001$; $r^2_{S. casali} = 0.86$, $P = 0.0002$). However, the calibration curves carried out separately for each collection site offer a better fit (for both species r -square values are equal or higher for per-site calibrations than for pooled data; see Table 3), and thus, a more precise and accurate calibration.

Shell transport

Given observed thermal differences between nearby sites, the tight D/L Asp vs. calibrated age correlation within each site has an interesting corollary. Although episodic winter storms may generate some shell transport, such processes are unlikely to have been substantial enough to homogenize the distribution of shells across the bay. The high per-species correlations within each site and the drop in those correlations when data are pooled across sites both indicate that no substantial post-mortem shell transport affected the surficial shell assemblages throughout the

late Holocene. In other words, subtle thermal differences across nearby sites can only have been detected if the majority of shells were preserved in place, and not mixed spatially by offshore–onshore or lateral transport. This interpretation is further supported by a highly patchy distribution of shells (likely reflecting original patchiness in biological populations). This is also consistent with many taphonomic studies that suggest that out-of-habitat post-mortem shell transport is rarely a factor even in higher-energy environments (e.g., Kidwell and Flessa, 1995; Flessa, 1998; Martin, 1999; Behrensmeyer et al., 2000).

Comparison of racemization rates between amino acids

Finally, the comparison of Asp ratios against the A/I ratios of Carroll et al. (2003) (Fig. 4) shows that in the case of *B. rosea* (no A/I data are available for *S. casali*) aspartic acid racemizes at a much faster overall rate than isoleucine, thus potentially providing better time resolution for late Holocene brachiopod shells. This is consistent with other studies for other organisms that all demonstrate the very rapid initial racemization of aspartic acid compared with other amino acids (e.g. Goodfriend, 1992). Once high values of Asp D/L are achieved, the overall rate of Asp racemization falls and is often comparable to that of other amino acids generally considered as “slow racemizers” (e.g., Manley et al., 2000; Wehmiller and Miller, 2000). As importantly, the consistency of these two techniques suggests that they can be used jointly to enhance their calibrations, cross-check their performance against one another, and maximize their dating range (see also Goodfriend, 1992).

Conclusion

In summary, whereas various complicating factors may limit the utility of amino acid racemization techniques, the per-site, per-species ^{14}C calibrations employed here provided a successful strategy for developing reliable geochronological calibrations for both species and both sites. Consequently, the calibrated Asp D/L values provided an effective and inexpensive way to assemble extensive time series of both brachiopods and bivalves. This study illustrates the powerful opportunity provided by Holocene marine shell assemblages for developing multi-centennial to multi-millennial geochronological frameworks concurrently for calcitic and aragonitic biomineral systems. Future use of sensitive reverse-phase (RPLC) analytical methods (e.g., Kaufman and Manley, 1998; Hearty et al., 2004) for high-resolution analysis of small samples should additionally enhance the utility of amino acid dating for detailed chronological analysis of many different types of shell assemblages.

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