



Long-Term Resilience of Late Holocene Coastal Subsistence System in Southeastern South America

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Abstract

Isotopic and molecular analysis on human, fauna and pottery remains can provide valuable new insights into the diets and subsistence practices of prehistoric populations. These are crucial to elucidate the resilience of social-ecological systems to cultural and environmental change. Bulk collagen carbon and nitrogen isotopic analysis of 82 human individuals from mid to late Holocene Brazilian archaeological sites (~6,700 to ~1,000 cal BP) reveal an adequate protein incorporation and, on the coast, the continuation in subsistence strategies based on the exploitation of aquatic resources despite the introduction of pottery and domesticated plant foods. These results are supported by carbon isotope analysis of single amino acid extracted from bone collagen. Chemical and isotopic analysis also shows that pottery technology was used to process marine foods and therefore assimilated into the existing subsistence strategy. Our multidisciplinary results demonstrate the resilient character of the coastal economy to cultural change during the late Holocene in southern Brazil.

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Introduction

The Brazilian coast encompasses a wide range of tropical and sub-tropical ecosystems that have sustained human populations from the middle Holocene to the present day. The large shell mounds, or sambaquis, are a distinctive feature of this coastline, testament to large-scale exploitation of marine resources, from ~8,000 to ~1,000 calibrated years before present (cal BP). In southern Brazil some sambaquis reached more than 35 m high and contained hundreds of burials, post holes and faunal remains testifying the development of a complex social panorama [1]. The exploitation of aquatic (mostly marine) resources was an important subsistence activity at these sites [2] and must have drawn people to the coast. However indirect evidence reveals that the contribution of plants also appears to be important [3–8]. Sambaquis containing freshwater and land snail shells are also found along the courses of rivers and their distribution penetrates some distances inland. These "Riverine sambaquis" are the same age or even older than their coastal analogues (~10,000 to

~1,000 cal BP) [9] and occasionally finds of marine fauna at these riverine sites suggest some connection to the coast [10], [11], [12].

A dramatic change is seen in the archaeological record at ~1,500 cal BP with the abrupt cessation of large shell mound formation [13]. At this time it is thought that new populations from the southern highlands (known as the Taquara/Itararé tradition) [14] expanded to the coastal lowlands [15], likely driven by rapid population growth, increasing of social interaction and intensification in food production, involving maize and exploitation of pine forest (*Araucaria angustifolia*) [16]. The appearance of Taquara/Itararé pottery along the southern coast of Brazil therefore may mark a key turning point in exploitation of rich coastal ecotones, as prehistoric groups gained the knowledge and technology to develop new economic practices. However the extent to which the transmission (or imposition) of this new subsistence system transformed the indigenous coastal economy, and its capacity to adjust, persist and maintain its fundamental properties, is still a matter of debate [17].

While there is some evidence for consumption of new cultigens like maize by Taquara/Itararé groups [7] and increased

consumption of terrestrial resources [18], marine fauna continue to be found at high abundance [19]. Similarly, while pottery is often assumed to be associated with the processing of new produced and foraged foods, with parallels in the southern Brazilian Highlands [16], there is no direct evidence of what it was used for. Our understanding of the diet and subsistence economy of the prehistoric inhabitants of coastal Brazil has been largely limited to traditional archaeological information, based on faunal, botanical and artefactual remains. In particular, the contribution of marine and terrestrial foods to the diet of both pre-ceramic and ceramic coastal populations of this region still remains largely unknown [11], [20], [21] and only few studies have considered the use of ceramics during this period [22], [23]. As a result, the impact of new economic and technological strategies on coastal adapted hunter-gatherers societies is not yet understood.

Here we report the results of an integrated study into the dietary variability of coastal and inland sambaqui populations. We analysed the stable carbon and nitrogen isotope composition of human bone collagen, a technique widely used to reconstruct paleodiets, and particularly for distinguishing marine versus terrestrial diets [24], [25]. We determined the stable carbon isotope signature of individual amino acids from bone collagen to identify different macronutrient constituents of diet [26–28]. We assessed the potential of bone mineral for isotope analysis in order to provide information on whole diet [24]. Finally we considered additional information regarding the diet of ‘incoming’ ceramic producing groups through the analysis of the organic contents of their pottery [29–31]. Hansel et al. [22] and Hansel and Schmitz [23] have already shown the potential for retrieving lipids from pottery in coastal Brazil, but here we report the first compound-specific isotopic analysis of these artefacts.

Archaeological Setting

The archaeological records include four middle and late Holocene sites located in southeast (São Paulo) and southern (Santa Catarina) regions of Brazil (Fig. 1). These consist of one inland riverine site (Moraes) and two coastal sambaquis (Jabuticabeira II, Piaçaguera), along with a recently excavated ceramic coastal site (Galheta IV), dated to the time of the expansion of the highland groups to the coast. The sambaqui sites dated to the preceramic period show a wide range, but well-established chronology (~6,700 to ~1,700 cal BP) and offer a unique opportunity to elucidate dietary variability between coastal and inland mound builders. They also offer a valuable isotopic baseline for assessing changes in subsistence strategies associated with the spread of pottery technology (~1,500 cal BP). Preliminary stable isotope studies have already been carried out on human remains from some of these sites (Moraes, Jabuticabeira II) [11], [21] as well as other coastal sites in southern Brazil [20], [32]. Here we undertake the analysis of 106 human remains with the aim of greatly expanding the dietary isotope record for this region.

Moraes (MRS) is situated in the Ribera do Iguape Valley, at ~35 km from the São Paulo coast. The site forms a mound with a conspicuous concentration of land snail shells dated between 6,775–6,499 and 5,289–4,887 cal BP [33]. The mound was used mainly for funerary purposes [34] and 55 human individuals were recorded in the deposit [34], [35], along with a consistent amount of faunal remains mostly represented by terrestrial mammals, followed by amphibians, birds, and only few reptiles and fish bones [10], [11].

Piaçaguera (PCG) is located in the south-east coastal plain of São Paulo state, at ~12 km from the present day shoreline. PCG similarly provided a stratigraphic succession containing more than 80 human individuals, artefacts and faunal remains dated to

5,894–5,326 and 5,887–5,314 cal BP [35], [36]. Fish and molluscs dominate the assemblage, followed by some crustaceans, a few terrestrial mammals and carbonized seeds [35–38].

Jabuticabeira II (Jab-II), at ~7 km from the present day shoreline at Santa Catarina, is one of the best-studied sambaqui sites of Brazil [21], [39]. The stratigraphic sequence is composed of alternating layers of marine shells and fish bones [21]. Numerous human burials and the absence of compelling domestic activities point to a ceremonial function of the site from 3,137–2,794 to 1,860–1,524 cal BP [13], [40].

Finally, Galheta IV (G-IV) is a funerary site overlooking the Atlantic Sea, at ~20 km north of Jab-II, in Santa Catarina [41]. The site yielded 8 adult human burials, some of them partially cremated, associated with abundant marine faunal remains (fish, sea mammals, seabirds) and artefacts, including potsherds of Taquara/Itararé tradition. G-IV was ^{14}C dated between 1,304–1,140 and 913–739 cal BP, and it dates to the expansion of inland ceramic producers (southern Jê speakers) to the coast.

Material and Methods

Human and Faunal Remains: Sampling Procedure and Ethical Statement

A total of 106 human individuals (different age and sex) from MRS, PCG, Jab-II and G-IV were sampled for isotopic analysis. In order to build a faunal isotopic reference for the region, 36 animal bone remains (terrestrial mammals, sea mammals, birds and fish) from these four sites were also selected for isotopic analysis (Tab. 1). Human samples were obtained almost entirely from the ribs, whereas fauna samples were from a range of different skeletal elements. All necessary permits were obtained for the described study, which complied with all relevant regulations of the Instituto do Patrimônio Histórico e Artístico Nacional – IPHAN (protocols n° 01506.00407/2012-14, 01506.001516/2006-47 and 01510.000047/2003-37). Archaeological materials used in this study are stored at the University of York (UK), Biology S-Block.

Isotopic analyses were also conducted on modern fish ($n = 10$) caught using traditional fishing techniques and acquired at the central market of Florianópolis (Santa Catarina, S. Brazil). The use of modern fish specimens was carried out in strict accordance with the recommendations of the Brazilian Institute of Environment and Renewable Natural Resources – IBAMA. No *In Vivo* experiment was developed, thus ARRIVE guidelines (Animal Research: Reporting *In Vivo* Experiments) for this study is not applicable. The transport of modern specimens to the University of York was approved under the Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES-IBAMA (protocol n° 113508). Modern fish collagen $\delta^{13}\text{C}$ were corrected (+1.14%) for the global decrease of atmospheric $\delta^{13}\text{C}$ values [42]. We also incorporated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from Plens [11] and De Masi [20], who report collagen isotopic composition of archaeological faunal remains from MRS and other coastal sites of southern Brazil.

Assessing Bone Collagen and Mineral Preservation

Bulk collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ derive primarily from dietary protein, although macronutrients (carbohydrates, lipids) may variably contribute to collagen carbon, particularly for the non-essential amino acids in collagen [43]–[45]. The $\delta^{13}\text{C}$ of bone apatite instead reflects the total dietary pool of carbon ingested [46]. Therefore the combination of collagen and apatite $\delta^{13}\text{C}$ has been shown to give information on main energy and protein consumed several years prior to death [47], [48]. In archaeological

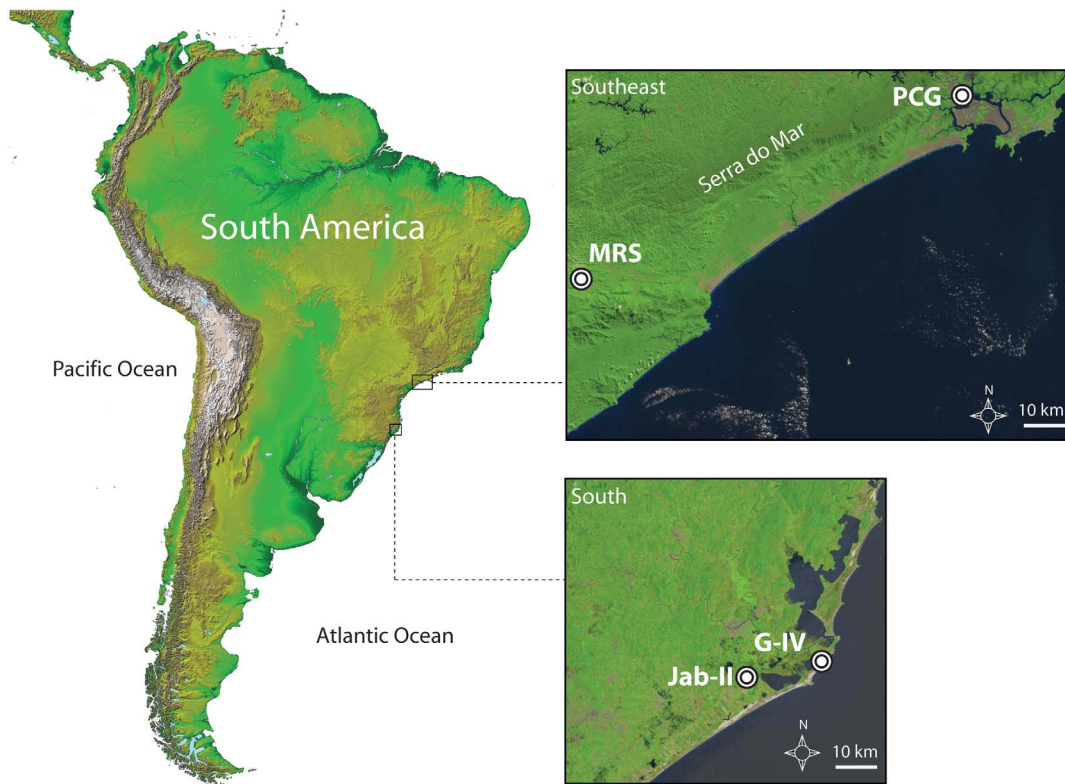


Figure 1. Geographic position of the analysed archaeological records. Location of the study area showing the archaeological sites from southeast (PCG, MRS) and south (Jab-II, G-IV) regions of Brazil. Satellite imagery from USGS (EarthExplorer) and NASA (Shuttle Radar Topography Mission).

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contexts, however, the burial environment may impact the physical and chemical composition of bones in different ways [49], particularly through loss of collagen and alteration of bioapatite and the stable isotope signature of this fraction [50].

Assessment of collagen preservation in both human and faunal remains was carried out following the criteria proposed by van Klinken [51]. In addition, Raman spectroscopy studies were performed on randomly selected human bone samples from MRS ($n = 9$), Jab-II ($n = 9$), G-IV ($n = 4$) and PCG ($n = 10$) to assess diagenetic change to the mineral fraction, with modern lamb bone being used as a control [52]. To optimise the quality of the Raman spectra, the samples of bone were flattened and smoothed by gentle rubbing with fine-grade diamond paper. The Raman spectra were collected from the samples using an HORIBA XploRa instrument at 532 nm laser wavelength and under $\times 100$ magnification in confocal mode ($NA = 0.9$, with 2400 g mm⁻¹ grating). Five spectra were collected from each bone specimen using 1 s laser exposure at ~ 3.5 mW power at the sample, with each measurement averaged over 40 spectral acquisitions. The spectra were collected over 4 spectral windows to achieve a total spectral range of 200–3200 cm⁻¹. The software package IGOR Pro 6.32 was used to average, baseline correct, and analyse the Raman spectra using Gaussian peak-fitting procedures, with the ν_1 carbonate peak (at ~ 1070 cm⁻¹) de-convoluted according to published protocols [53].

Collagen Extraction and Isotope Analysis

Collagen preparation follows the protocol described in Craig et al. [54]. Before isotopic analysis, lipids were removed from modern fish bones with dichloromethane:methanol (2:1, $\times 3$).

Between 300 and 500 mg of cleaned human and animal bones were used for collagen extraction. Samples were agitated in 8 ml of 0.6 M hydrochloric acid at 4°C to demineralize. Once demineralization had occurred the samples were removed from the acid and washed with ultrapure water three times. The samples were gelatinised in pH 3 hydrochloric acid and maintained for forty-eight hours at 80°C. The gelatinised samples were then ultrafiltered and a >30 kDa fraction was lyophilised. Duplicates (1 mg) were measured using a continuous flow isotope ratio mass spectrometry Thermo Finnigan Delta Plus XL in the Department of Archaeological Sciences of the University of Bradford (UK), to determine the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The results are reported using the delta scale in ‰ relative to internationally accepted standards, V-PDB and AIR respectively. Analytical error, calculated from repeated measurements of each sample and measurements of the bovine control from multiple extracts, was $<0.2\%$ (1σ).

Analysis of Individual Amino Acids in Collagen

Stable carbon isotope analyses were performed on collagen amino acids isolated from randomly selected coastal individuals from Jab-II ($n = 10$) and G-IV ($n = 7$). Approximately 1 mg of collagen was hydrolysed under vacuum in amino acid free 6 M hydrochloric acid (1 ml) at 110°C for 24 hours. After hydrolysis the samples were dried in a rotary vacuum concentrator and stored at -20°C until analysis. Prior to isotopic analysis, the samples were redissolved under sonication in MilliQ water with the addition of an internal standard (2-amino-isobutyric acid).

Instrumental analysis was carried out using Thermo Scientific Liquid chromatography isotope ratio mass spectrometry (LC-

Table 1. Bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of faunal remains.

Site	Taxon	Vernacular name	$\delta^{13}\text{C}\%$	$\delta^{15}\text{N}\%$	%C	%N	C:N	Col wt%	Source
MRS	<i>Tayassu</i> sp.	Peccary	-21.0	+8.6	27.0	9.6	3.3	0.5	This study
MRS	<i>Tayassu</i> sp.	Peccary	-21.3	+6.7	37.4	13.4	3.3	1.4	This study
MRS	<i>Tayassu</i> sp.	Peccary	-23.5	+9.2	35.6	12.7	3.3	1.5	This study
MRS	<i>Tayassu</i> sp.	Peccary	-22.4	+8.7	40.5	14.9	3.2	1.5	This study
MRS	<i>Mazama</i> sp.	Brocket	-23.0	+9.6	31.6	11.2	3.3	0.6	This study
MRS	<i>Mazama</i> sp.	Brocket	-24.6	+8.4	41.0	14.7	3.2	1.5	This study
MRS	<i>Cuniculus paca</i>	Lowland paca	-20.8	+7.9	42.5	14.4	3.4	1.2	This study
MRS	<i>Cuniculus paca</i>	Lowland paca	-20.6	+8.7	41.7	14.8	3.3	1.0	This study
MRS	<i>Alouatta</i> sp.	Howler monkeys	-21.5	+7.9	26.0	9.3	3.3	3.3	This study
MRS	<i>Alouatta</i> sp.	Howler monkeys	-22.5	+8.6	43.0	15.5	3.2	1.4	This study
MRS	<i>Tayassu</i> sp.	Peccary	-22.3	+6.5	-	-	-	-	[11]
MRS	<i>Alouatta</i> sp.	Howler monkeys	-21.6	+6.3	-	-	-	-	[11]
PCG	Selachimorpha	Shark	-11.8	+15.2	29.2	9.8	3.5	3.0	This study
PCG	<i>Euphractus sexinctus</i>	Six-banded armadillo	-20.3	+13.3	40.5	13.7	3.4	1.9	This study
PCG	<i>Alouatta</i> sp.	Howler monkeys	-22.5	+6.9	56.4	19.8	3.3	2.0	This study
Jab-II	<i>Trichurus lepturus</i>	Hairtail	-11.1	+12.8	41.1	14.8	3.2	3.5	This study
Jab-II	<i>Lobotes surinamensis</i>	Tripletail	-10.5	+14.5	41.9	14.6	3.4	2.3	This study
Jab-II	<i>Pogonias cromis</i>	Blackdrum	-9.8	+12.6	41.2	14.3	3.4	3.4	This study
Jab-II	Ariidae	Sea catfishes	-9.2	+15.5	42.1	15.1	3.3	2.7	This study
Jab-II	Pomacanthidae	Angelfishes	-10.8	+13.4	41.5	14.4	3.4	1.6	This study
Jab-II	Cetacea	Undeter	-10.8	+16.1	39.5	13.8	3.3	2.2	This study
Jab-II	Aves	unknown bird	-19.5	+7.2	42.3	15.0	3.3	4.8	This study
G-IV	Aves	unknown seabird	-12.5	+17.4	43.3	15.7	3.2	6.6	This study
G-IV	Aves	unknown seabird	-9.8	+18.9	44.3	16.0	3.2	6.2	This study
G-IV	Aves	unknown seabird	-13.6	+17.9	42.7	15.5	3.2	4.6	This study
S Brazil	<i>Arctocephalus tropicalis</i>	Subantarctic fur seal	-11.0	+16.0	-	-	-	-	[20]
S Brazil	<i>Arctocephalus australis</i>	S American fur seal	-11.4	+16.4	-	-	-	-	[20]
S Brazil	<i>Eubalaena australis</i>	Southern right whale	-15.2	+6.9	-	-	-	-	[20]
S Brazil	<i>Spheniscus magellanicus</i>	Magellanic penguin	-11.2	+14.5	-	-	-	-	[20]
S Brazil	Selachimorpha	Shark	-9.5	+16.0	-	-	-	-	[20]
S Brazil*	<i>Macrondon ancyodon</i>	King weakfish	-11.8	+13.0	60.0	22.4	3.1	11.0	This study
S Brazil*	<i>Epinephelus marginatus</i>	Dusky grouper	-11.1	+16.0	44.1	16.9	3.0	19.4	This study
S Brazil*	<i>Micropogonias furnieri</i>	whitemouth croaker	-11.3	+13.9	43.5	16.4	3.1	13.5	This study
S Brazil*	<i>Peprilus paru</i>	American harvestfish	-15.0	+13.0	49.9	14.7	4.0	11.1	This study
S Brazil*	Mugilidae	Mulletts	-12.5	+11.5	41.5	15.5	3.1	12.8	This study

Table 1. Cont.

Site	Taxon	Vernacular name	$\delta^{13}\text{C}\%$	$\delta^{15}\text{N}\%$	%C	%N	C:N	Col wt%	Source
S Brazil*	<i>Cynoscion acoupa</i>	Acoupa weakfish	-9.0	+11.3	43.3	16.4	3.1	10.7	This study
S Brazil*	<i>Urophycis</i> sp.	Brazilian codling	-10.9	+14.2	41.0	15.6	3.1	13.0	This study
S Brazil*	<i>Coryphaena</i> sp.	Common dolphinfish	-13.3	+9.2	41.9	15.8	3.1	16.1	This study
S Brazil*	<i>Pomatomus saltatrix</i>	Blue fish	-11.9	+15.3	55.7	20.8	3.1	7.8	This study
S Brazil*	<i>Xiphias gladius</i>	Swordfish	-12.1	+9.9	70.9	26.2	3.2	11.5	This study

Bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are also reported from previous studies [20], [11] and for modern (*) fish from the south Brazilian coast, after correction for the decrease of $\delta^{13}\text{C}$ values of atmospheric CO_2 . doi:10.1371/journal.pone.0093854.t001

IRMS) system consisting of an Accela 600 pump connected to a Thermo Scientific LC Isolink and a Delta V Plus Isotope Ratio Mass Spectrometer housed at the La Trobe Institute for Molecular Sciences (LIMS, La Trobe University, Melbourne, Australia). LC-IRMS analysis of single amino acid fractions of collagen hydrolysates were carried out using a three phase LC/IRMS method similar to that described in Smith et al. [55], with the exception that a Primesep A (SCIELC) column (2.1×250 mm, 100 \AA , 5 \mu m) was used. This is a narrower column to that used by Smith et al. [55] and thus lower LC flow rates were used. Conditioning runs were made at $110 \text{ \mu L min}^{-1}$ using phases ratios of 85B:15C–95B:5C, analytical runs were made using flow rates of $60\text{--}80 \text{ \mu L min}^{-1}$ and oxidation reagent flow rates were set at $35 \text{ \mu L min}^{-1}$ each. Approximately 10 \mu g of amino acid hydrolysate (on column) was used for each run. Amino acid peak $\delta^{13}\text{C}$ values were measured against CO_2 gas pulses throughout the run ($\delta^{13}\text{C}_{\text{VPDB}} = -2.8\%$) calibrated against international standard USGS-40 L-Glutamic Acid ($\delta^{13}\text{C}_{\text{VPDB-LSVEC}} = -26.4 \pm 0.04\%$). In house standard runs were made during the sample runs to monitor measurement quality.

Organic Residues Analysis

Molecular and isotopic analysis of organic residues absorbed into porous vessels or preserved in surface deposits offer valuable information concerning pottery use [29], [30], [54], [56–58]. Lipids were extracted (and methylated in one-step) from 14 potsherds according to protocols reported in Craig et al. [31], [59]. Briefly, after cleaning the surface, methanol (4 ml) was added to powdered ceramic samples (70 to 240 mg) and the mixture was sonicated for 15 min and then acidified with concentrated sulphuric acid (800 ml). The acidified suspension was heated in sealed tubes for 4 h at 70°C and then cooled, and lipids were extracted with n-hexane ($2 \text{ ml} \times 3$). The extract was dried under a gentle flux of nitrogen and internal standard (n-hexatriacontane) was added before the direct analysis by gas chromatography/mass spectrometry (GCMS) at the University of York (UK).

Stable isotopic analysis of n-hexadecanoic ($\text{C}_{16:0}$) and n-octadecanoic ($\text{C}_{18:0}$) acids from 11 extracted lipid samples were performed using a gas chromatograph (GC) coupled to a combustion isotope ratio mass spectrometry (GC-C-IRMS) at the University of Liverpool (UK) following the protocol reported in Craig et al. [59]. Instrument precision on repeated measurements was 0.2% (s.e.m.).

Charred residues of food were preserved in the internal part of 6 ceramic potsherds. Samples (3–7 mg) were removed and subsamples (1 mg) selected for carbon and nitrogen isotopic analysis at the University of Bradford by using the same IRMS procedure as for bone collagen [56].

Statistical Analysis

The proportional contribution of different food sources to human diet (based on stable isotope values) was estimated using a Bayesian mixing model in SIAR V4 (Stable Isotope Analysis in R) [60]. The bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of faunal remains (Tab. 1) was used in the model calculation after adding trophic enrichments of 1% and 4% for carbon and nitrogen respectively [61]. Bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were also analysed using the parametric One-Way Anova test in the software PAST 2.13 [62], after checking for normal distribution (Shapiro-Wilk) and using a statistical significance probability threshold of $\alpha = 0.05$. Available radiocarbon ages were calibrated with OxCal 4.2, using the Southern Hemisphere curve SHCal04 [63], [64].

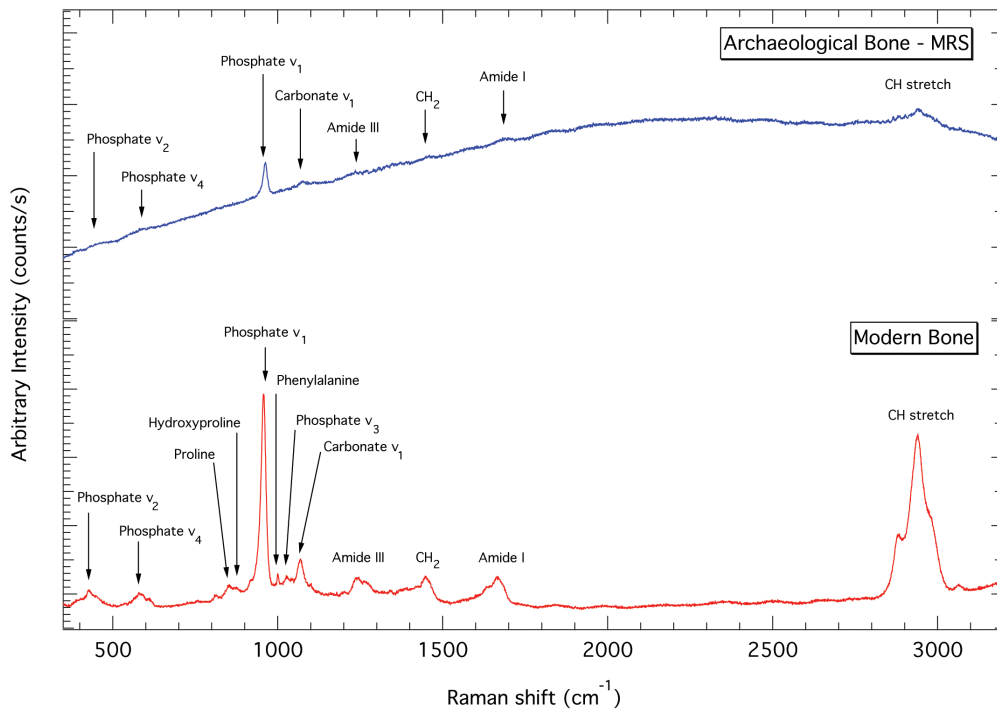


Figure 2. Bone diagenesis. Examples of Raman spectra of an archaeological bone spectrum (MRS) and a modern lamb bone without baseline correction showing the Raman band assignments of the key peaks. All peaks are identified as per the literature [68] and [112], with results being comparable to those described in Edwards et al. [69].
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Results

Bone Apatite and Collagen Preservation

Raman analysis revealed substantial alteration to the mineral phase of all of the archaeological samples that were analysed (Tab. S1, Fig. 2). Substitution of non-biogenic carbonate for biogenic

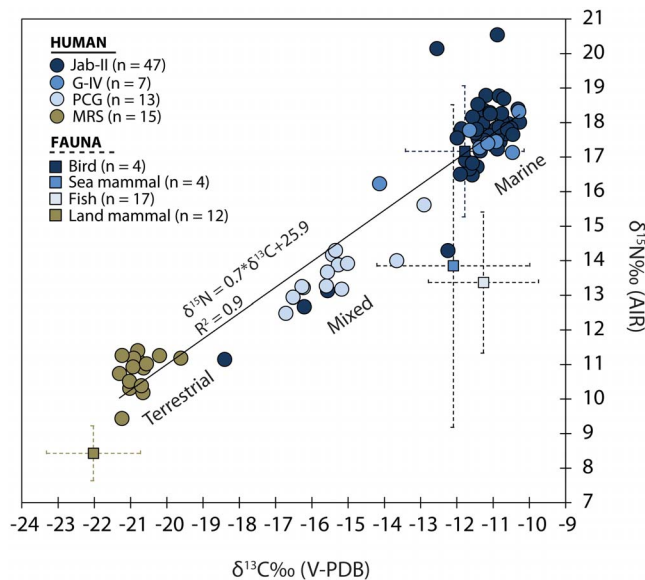


Figure 3. Bulk collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Distribution of human and faunal values from Jabuticabeira II (Jab-II), Galheta IV (G-IV), Piaçaguera (PCG) and Moraes (MRS). Fish values also include modern specimens.
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phosphate, or reduction in biogenic phosphate, is indicated by the increased carbonate v_1 (C) to phosphate v_1 (P) intensity ratios in archaeological bones compared to the modern control [65], [66]. An increase in crystallinity due to mineral alteration in the archaeological specimens is expected [49], [67] and confirmed in our Raman data by a decrease in the full width at half maximum (FWHM) of the v_1 P band (at $\sim 957\text{ cm}^{-1}$) relative to the modern bone sample [65], [66], [68]. Increasing crystallinity in diagenetically altered bone has been linked to the loss of collagen [49], which is further corroborated in our data by reduced CH/P ratios compared to the modern bone sample. Here, CH refers to the collagen band (i.e. CH stretching) at $\sim 2933\text{ cm}^{-1}$. The CH/P trend is also in agreement with the Raman spectroscopy results of Edwards et al. [69], which demonstrated reduced intensity in the collagen modes in human bones collected at JAB-II. Finally, the large fluorescent background in the archaeological samples (Fig. 2) is attributed to spectral emission from luminescent ions incorporated into the bone lattice due to diagenetic alteration [65].

Despite widespread alteration to the bone mineral phase, most of human burials (82 out of 106) matched the criteria for adequate collagen preservation [51]. Collagen yield and C:N ratios individuals from MRS ($n = 15$), PCG ($n = 11$), Jab-II ($n = 47$) and G-IV ($n = 6$) range from 0.9 to 7.7 wt% and from 3.2 to 3.5 respectively (Tab. S12). Three individuals from PCG and one from G-IV contain $<1\%$ collagen but still had acceptable C:N ratios and their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are coherent with the other humans. Similarly, most of the animal samples from the study sites (25 out of 36) had adequate ($>1\%$) collagen yields (Tab. 1). Of these, one peccary (*Tayassu* sp.) and one brocket (*Mazama* sp.) from MRS show collagen yield of 0.5 and 0.6 wt% respectively, but again the C:N matches the criteria for unaltered collagen. In summary, whilst the bone mineral fraction is unlikely to preserve a vital isotopic signal, the collagen yield and C:N composition

Table 2. Average bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of humans, including isotopic variability ($\Delta\delta\%$) and the number of individual analysed.

Site	$\delta^{13}\text{C}\%$	$\Delta\delta^{13}\text{C}\%$	$\delta^{15}\text{N}\%$	$\Delta\delta^{15}\text{N}\%$	N
MRS	-20.8 ± 0.4	1.7	$+10.8\pm 0.5$	2.0	15
PCG	-15.4 ± 1.0	3.8	$+13.7\pm 0.8$	3.1	13
Jab-II	-11.5 ± 1.5	8.1	$+17.4\pm 1.6$	9.4	47
G-IV	-11.4 ± 1.2	3.8	$+17.4\pm 0.6$	2.1	7

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suggests acceptable collagen preservation for the majority of the samples [51], [70].

Bulk Collagen Stable Isotope Analysis

Terrestrial fauna comprehensively show average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of $-22.0\pm 1.2\%$ and $+8.4\pm 1.7\%$ respectively (Tab. 1). Some variability is observed in carbon isotopes at MRS and may be a result of hunting in different environments. The aquatic fauna, modern and archaeological fish, exhibits average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of $-11.1\pm 1.5\%$ and $+13.5\pm 1.8\%$ respectively. Sea mammals show average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of $-12.3\pm 1.9\%$ and $+12.9\pm 4.5\%$. Seabirds show average $\delta^{13}\text{C}$ of $-13.3\pm 3.7\%$ and the highest

average $\delta^{15}\text{N}$ value among faunal remains, $+15.2\pm 4.8\%$, pointing to the consumption of higher trophic levels marine resources.

Human $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values show strong positive linear correlations ($r=0.95$; $R^2=0.92$; $p<0.001$; Tab. S2) and fall between the end-points derived from correcting the observed marine and C3 terrestrial fauna for isotopic fractionation (Fig. 3). Therefore the human isotope values can be largely explained by direct routing of both carbon and nitrogen from dietary protein to collagen, which implies that the diets contained sufficiently high protein [71]. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differ significantly among sites ($p<0.001$; Tab. 2). Lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were observed in inland individuals from MRS, as opposed to higher

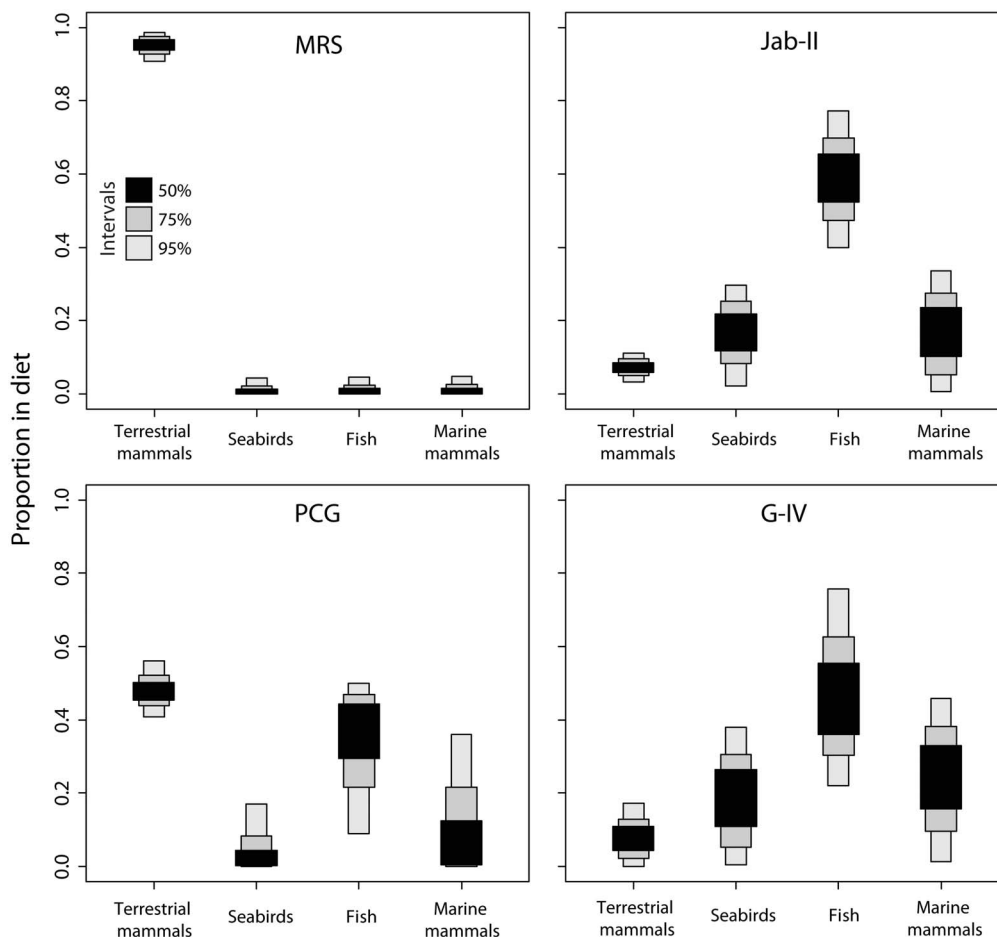


Figure 4. Bayesian-derived proportion of protein sources for archaeological site according to 95%, 75% and 50% of the dataset. Overlap of land mammal and fish in PCG show that human diet was based on marine as well as terrestrial items. This is not observed at MRS, Jab-II and G-IV.

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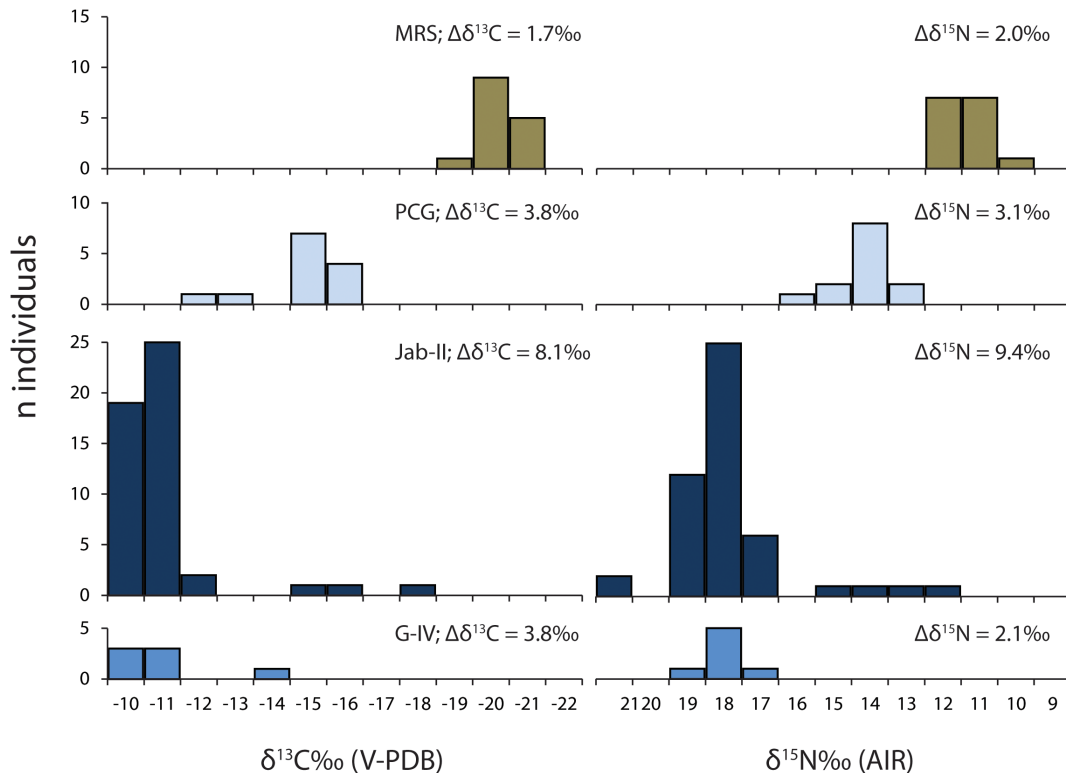


Figure 5. Bulk collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variability ($\Delta\delta$) in inland and coastal populations. Note the large isotopic variability in humans from Jab-II.

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values of coastal pre-ceramic and ceramic individuals from Jab-II and G-IV, in agreement with preliminary isotopic studies [11], [21]. Individuals from PCG, instead, exhibit $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values consistent with a mixed marine/C3 terrestrial diet.

The proportional contribution of different marine and C3 terrestrial animal resources to each individual's diet can be crudely

estimated through linear interpolation between the marine and terrestrial end-members. We also used a Bayesian model which generates possible dietary solutions from multiple dietary source categories. This model predicts that inland peoples acquired > 90% of their protein from C3 terrestrial resources, whereas people on the coast were assimilating protein mainly from fish, up to 80%, along with some contribution from seabirds and sea mammals (Fig. 4). A large isotopic variability, however, was detected in coastal groups, in particular at Jab-II (Fig. 5) but it seems not to be related to sex and age (Tab. S3).

It is important to note that these estimations refer to the protein contribution to total dietary protein (by dry weight) and not the contribution to total diet. In this case, the bulk isotopic analyses are highly insensitive to the other dietary components, such as carbohydrate and lipid, which must have been consumed to some extent in order to avoid protein poisoning [72].

Carbon Stable Isotope Analysis of Single Amino Acids

Stable carbon isotope values were obtained from 15 amino acids corresponding to 97.5% of the carbon atoms in collagen (Tab. S4). Mass balance calculations were used to estimate the $\delta^{13}\text{C}$ of whole collagen from the measured individual amino acid $\delta^{13}\text{C}$ values. These estimated values were strongly correlated with the observed bulk $\delta^{13}\text{C}$ values ($R^2 = 0.98$) and the offset between the estimated and observed measurements was <1% in all cases. The $\delta^{13}\text{C}$ of individual amino acids were strongly and positively correlated with both the $\delta^{13}\text{C}$ ($R^2 = >0.8$) and $\delta^{15}\text{N}$ ($R^2 = >0.7$) values of whole collagen for both marine and C3 terrestrial consumers, confirming that both dispensable and non-dispensable amino acids were largely derived from a dietary protein source.

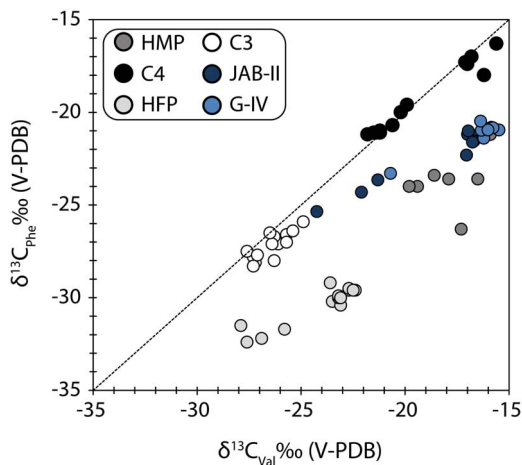


Figure 6. A biplot of phenylalanine and valine $\delta^{13}\text{C}$ values. The biplot distinguishes two dietary groups at Jab-II and G-IV; those with mixed marine/C3 terrestrial animal diets and those consuming high marine protein (HMP). Data from Honch et al. [28] are reported for comparison and include C4, C3 and high marine (HMP) and freshwater protein (HFP) consumers.

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carbohydrate marker in palaeodietary contexts warrants further testing.

Organic Residue Analysis from Pottery at G-IV

Molecular and isotopic compositions of adsorbed organic residues indicate that pottery vessels were used for the processing of marine products, along with plants and other animal resources. Overall the lipid preservation was poor, and the majority of samples contained only low levels ($<0.5 \mu\text{g mg}^{-1}$) of palmitic and stearic acids. Nevertheless one sample (G18E) has a lipid profile consisting of medium- and long-chain saturated (C_{14} – C_{24}) and monounsaturated ($\text{C}_{18:1}$ – $\text{C}_{22:1}$) fatty acids, isoprenoid fatty acids (4,8,12-trimethyltridecanoic acid and phytanic acid) and long-chain (C_{18} – C_{22}) ω -(*o*-alkylphenyl) fatty acids (Fig. 8, Tab. S5). Such a profile is characteristic of degraded aquatic oils, as established on contemporaneous pottery from this region [22]. A similar lipid distribution was found in G16P but with lesser preservation of ω -(*o*-alkylphenyl) fatty acids, preventing a clear confirmation of aquatic oils. This sample and G24P contain a series of triterpenes (m/z 189, 218) revealing the presence of plant resins which were also found in Taquara/Itararé pottery assemblages and were interpreted as a waterproofing coating [23]. Finally, one sample (G22P) had low quantities of very long chain fatty acids (up to $\text{C}_{28:0}$), traces of long chain dicarboxylic fatty acids (C_{22} – C_{24}) and isomers of the C_{18} ω -(*o*-alkylphenyl) fatty acid only. The latter compound is formed by heat alteration of polyunsaturated C_{18} fatty acids, which is consistent with a plant contribution in the profile. It also contains an unusually high concentration of $\text{C}_{12:0}$ which in this context could be derived from palm kernel oil [74], [75], although the difference in the fatty acid distribution as the various biomarkers present suggest a complex mixture of lipids from different origins.

The $\delta^{13}\text{C}$ values (both $\text{C}_{16:0}$ and $\text{C}_{18:0} \geq -25\%$) of medium chain-length *n*-hexadecanoic ($\text{C}_{16:0}$) and *n*-octadecanoic ($\text{C}_{18:0}$) acids from 7 out of 11 pottery samples are within the range of marine oils reported in previous studies (Tab. S5). Lower $\delta^{13}\text{C}$ values, in contrast, match those observed in modern pottery vessels used to process freshwater and non-ruminant animal fats and oils [30], [31]. There was no evidence for the processing of ruminants in pottery [59] despite the presence of cervids (*Mazama* sp., *Ozotoceros bezoarticus*) in the faunal assemblages [41]. Biomarkers associated with maize processing, e.g. *n*-dotriacontanol, were absent in all the vessels studied although there are doubts whether these would accumulate or preserve in sufficient quantities to allow identification [76].

Charred surface residues show $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranging from -22.6% to -25.8% and from $+6.7\%$ to $+12.7\%$ respectively (Tab. S6). Samples enriched in ^{13}C and depleted in ^{15}N may tentatively indicate some contribution of C4 plants [77]. However the correlation between C4 plants (e.g. maize) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of charred deposit are not straightforward [78], [79], and our $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results are also consistent with those observed in coastal areas of Northern Europe resulting from the processing of aquatic resources [30]. In spite of the complexity underlying food crust isotopic signatures, compound specific isotopic data from the same potsherds reinforces the interpretation that marine foods principally contributed to the isotopic signal of these charred deposits.

Discussion

Marine and C3 terrestrial animals were the main sources of protein for coastal and inland sambaqui builders of S. Brazil between $\sim 6,700$ and $\sim 1,700$ cal BP. The isotopic gradient from

the inland to the coast suggests the existence of confined catchment areas and/or selective targeting of specific resources [80–82], which is a common feature amongst sambaqui builders [11], [13], [20], [21] and other coastal populations in South America [81]. There is no isotopic evidence for the contribution of freshwater resources, which is consistent with the very low frequency of these remains in both mainland and coastal sites [11], [21]. Interestingly, some individuals at Jab-II have an unusually high intake of C3 terrestrial proteins, denoting some degree of population variability on the coast. Although the lack of significant isotopic differences between sexes and age is consistent with pervasive food sharing among these populations, the intra-population $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variability at Jab-II may point to the presence of non-local individuals, as observed in other preceramic coastal populations [18], perhaps assimilated into the group through post-marital residential practices [83]; however isotopic variability may also be associated to food restrictions among members of the community [84].

Our results also revealed that the peoples at G-IV relied substantially on marine resources, to the same extent as the pre-ceramic coastal adapted populations. The isotopic results of diets at coastal sites are broadly supported by the rich archaeozoological evidence dominated by marine resources [21], [41], [85]. Collagen from individuals at Jab-II and G-IV are amongst the most enriched in ^{13}C and ^{15}N in the eastern coast of South America between $\sim 8,000$ and $\sim 1,000$ cal BP [20], [21], [81], [86–97].

The carbon isotope signature of individual amino acids indicate a minor contribution of plants to the diet of some individuals, including those that post-date the adoption of pottery on the coast. The occurrence of mortars and plant macro-remains at several, but not all, sites [1], [4], [5], [98], along with variable degree of caries, starch grains in dental calculus [5], [6] and dental wear [99] also indicate that plants made a contribution to the diets of some coastal groups at this time [1], [3], [100]. Furthermore preliminary studies have also successfully extracted phytoliths and starch grains from charred deposits of pottery from G-IV, and one individual provided amino acid (alanine) $\delta^{13}\text{C}$ values suggestive of a larger intake of C3 carbohydrates. However, the bulk collagen isotope analysis indicates that plants were unlikely to be major dietary staples for these coastal groups, rather the diet was protein-rich and oriented toward marine resources.

The continuity in coastal exploitation is further supported by molecular and isotopic results from organic residues preserved in pottery. These data attest to the use of pottery for the processing of animal products, including marine organisms. Hansen and Schmitz [23] achieved similar results from coeval sites in southern Brazil, revealing that pottery was commonly used for the manipulation of marine resources. Combined results therefore indicate that the adoption of pottery in coastal areas is not directly connected with the imposition of food production and did not affect the proportional contribution of marine resources to the diet of coastal populations. Small ceramic vessels had most likely a ritual and symbolic utility [16], [41]. Therefore, the molecular and isotopic analyses provide new direct evidence of the importance of marine resources in symbolic spheres. This is an interesting finding as it may have been assumed that novel or exotic cultivated plants would have had a more ritual and symbolic role in cuisine and therefore would have been more visible in the pottery contents.

Conclusion

Maritime adaptations sustained South American pre-Columbian populations since the late Pleistocene [101] and stable isotope studies reveal the crucial role of aquatic resources to several

Holocene coastal groups (e.g. [86–92]), even during the intensification of food production (e.g. [82], [102–108]). However these direct lines of evidence are strongly biased towards archaeological records along the western and south-eastern coast of South America. Here we have extended the information to the subtropical Atlantic rainforest coast of Brazil. The isotope results show that it is highly unlikely that these coastal populations relied on plant carbohydrate as a major dietary source. Instead, we demonstrate the strong dependence of marine animal resources, despite the decline of monumental shell mound building and the arrival of a new subsistence strategy at ~1,500 cal BP, involving domesticated plants and pottery technology, from inland areas. Therefore our results imply that the productive maritime economy was highly resilient to social and cultural change. It remains to be assessed if ceramic producing populations on the coast were directly descended from indigenous coastal foragers or immigrants from the highlands who, having reached the coast, oriented their economy toward aquatic resources. However the resilient character of this subsistence system is further expressed by its flexibility. Rather than transforming the coastal economy, as observed in the Atlantic coasts of Europe [109], [110], the adoption of pottery was incorporated into marine focused subsistence strategies [30]. These results emphasize how the archaeological record offers a unique and exceptional opportunity to illuminate the longstanding trajectory of New World maritime adaptations, which still today play a pivotal role to coastal populations in Latin America [111].

Supporting Information

Table S1 Carbonate ν_1 (C) to phosphate ν_1 (P) intensity ratios, full width at half maximum (FWHM) of the phosphate ν_1 (P) band and organic (C-H stretch) to phosphate ν_1 (P) intensity ratios determined for each averaged spectrum as a function of the sample type. Samples are ordered from youngest to oldest with data acquired using the same Raman confocal settings across all samples. Modern lamb bone is justified as an appropriate control due to the similarities in sheep and human bone as per the RS study of Rehman et al. [52]. (DOCX)

Table S2 Bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of humans. Also show the age class or the relative age (young, adult) and the sex (F: female, M: male). (DOCX)

Table S3 One-way ANOVA showing a general lack of significant isotopic differences between sexes and age at MRS, Jab-II and PCG. Data from G-IV was not sufficient to be tested statistically. Because of the limited information about the age, individuals from MRS and PCG were sorted out in two

categories: <36 and >36 years old. At Jab-II, individuals belonging to the age class 11–20 years old show higher $\delta^{15}\text{N}$ values (mean $19.1 \pm 1.0\%$, $n = 3$) than individuals belonging to the age class 36–50 yrs (mean $17.3 \pm 0.9\%$, $n = 13$). (DOCX)

Table S4 Collagen amino acid $\delta^{13}\text{C}$ values for humans from Jab-II ($n = 10$) and G-IV ($n = 7$). (DOCX)

Table S5 Ceramic sherds selected for lipid analysis by GCMS and GC-c-IRMS. FA (Cx:y) - fatty acids with carbon length x and number of unsaturations y, br -branched chain acids, phy- phytanic acid, TMTD - 4,8,12-trimethyltridecanoic acid. APFA (Cn) - ω -(o-alkylphenyl) alkanolic acids with carbon length n. tr - trace. DCx - α,ω -dicarboxylic acids with carbon length x. P - interior, E - exterior. Aquatic oils are interpreted from the presence of isomers of APFA (C₂₀ or C₂₂) and at least one isoprenoid fatty acids (pri, phy or TMTD). Resins are interpreted from the presence of triterpenes. Plant oils are interpreted from the presence of long chain fatty acids, dicarboxylic acids and the presence of isomers of C₁₈ APFA. A high abundance of C_{12:0} could be consistent with Palm Kernel. Aquatic (marine) fats are defined on the isotopic characteristics of the C₁₆ and C₁₈ saturated fatty acids. (DOCX)

Table S6 Bulk isotope characteristics of charred deposits from the interior of potsherds from G-IV. (DOCX)

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Author Contributions

Conceived and designed the experiments: ACC MC OC CS AM YH. Performed the experiments: ACC AL ME AM RARP AG. Analyzed the data: ACC AL ME OC AL CS AM YH RARP AG. Contributed reagents/materials/analysis tools: MC OC AG YH PADD LF VW CP SE DF. Wrote the paper: ACC MC AL YH RARP CS PADD LF VW CP SE DF OC.

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