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Food and diet of the pre-Columbian mound builders of the Patos Lagoon region in southern Brazil with stable isotope analysis

Ingrid Chanca[1,2*], Caroline Borges[3], André Carlo Colonese[4,5], Kita Macario[1], Alice Toso[5], Maria Fontanals-Coll[4], Roberto dos Anjos[6], Marcelo Muniz[6], Renan Pereira[6], Sahra Talamo[7,8], Rafael Guedes Milheira[9]

1 Laboratório de Radiocarbono, Instituto de Física, Universidade Federal Fluminense, Av. Litorânea s/n, 24210-346, Niterói, RJ, Brazil. ichanca@id.uff.br, kitamacario@id.uff.br

2 Max Planck Institute for Biogeochemistry, Hans-Knoell-Str. 10, 07745, Jena, Germany. ichanca@bgc-jena.mpg.de

3 Departamento de História, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros s/n, 52171-900, Recife, PE, Brazil. caroline.borges@ufrpe.br

4 BioArCh, Department of Archaeology, University of York, York, YO10 5DD, United Kingdom. maria.fontanals-coll@york.ac.uk

5 Department of Prehistory and Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain. andrecarlo.colonese@uab.cat, alice.toso@uab.cat

6 Laboratório de Mudanças Climáticas e Ambientais, Instituto de Física, Universidade Federal Fluminense, Av. Litorânea s/n, 24210-346, Niterói, RJ, Brazil.
rmeigikos@id.uff.br, marcelocostamuniz@id.uff.br, renanpcardoso@gmail.com

7 Department of Chemistry G. Ciamician, Alma Mater Studiorum, Bologna University, Via Selmi 2, I-40126 Bologna, Italy. sahra.talamo@unibo.it

8 Max Planck Institute for evolutionary Anthropology, Deutscher Platz 6, D-04103, Leipzig, Germany. sahra.talamo@eva.mpg.de

9 Departamento de Antropologia e Arqueologia, Universidade Federal de Pelotas, Coronel Alberto Rosa 154, 96010-160, Pelotas, RS, Brazil. milheirarafael@gmail.com

*corresponding author

ABSTRACT

A constant and intense debate in South American archaeology stands on the origin and patterns of food production of ancient populations and the correlation of both aspects with the demographic growth. Some studies associated the population growth with the shift from foraging to agricultural practices in hotspots on the continent. This association has been confronted by a number of studies performed in the recent years that reconstructed dietary patterns for several tropical and subtropical areas of eastern South America. However, there is still a lack of information on the diet for Late Holocene populations in the wetlands of the Pampa Biome. In order to access the paleodiet of earthen-mound builders from southern Patos Lagoon (Brazil), this study combined bulk collagen stable carbon and nitrogen isotopes analysis of faunal and human remains with Bayesian Stable Isotope Mixing Models. The paleodiet of 20 human and one dog remains from six sites was reconstructed. The Bayesian mixing model on collagen $\delta^{13}C$ and $\delta^{15}N$ values revealed variable subsistence strategies with high consumption of marine/estuarine protein for some individuals. Other individuals' diets rely on terrestrial/freshwater resources as protein sources. In southeastern South America, such patterns reinforce the resource-rich aquatic environment as a facilitator for the endurance of mixed economies. Our results also show an intra-site dietary variability that supports an argument of the Patos Lagoon surroundings as a "hub of interactivity". Besides, our results challenge the conventional view of the mound builders as highly transients.

KEYWORDS: Cerritos; Paleodiet; Stable Isotopes; Bone collagen; Bayesian mixing model

HIGHLIGHTS

- The diet of Late Holocene mound builders of southern Brazil had been poorly known
- Mound builders may have attained large populations without relying on agriculture
- Stable isotopes coupled with Bayesian Mixing Models were used to investigate diet
- Results reveal considerable dietary variability with emphasis on marine resources

Introduction

Differing positions on the origin and changing nature of food production economies spark some of the most contentious debates in South American archaeology. Middle-Late Holocene population growth has been often associated with the shift from foraging to predominant agricultural practices in cultural hotspots on the continent (Goldberg et al., 2016). However, in several tropical and subtropical areas of eastern South America, the importance of agriculture to demographic growth and the establishment of sedentary populations during the Late Holocene is subject to contention. A growing body of evidence suggests that in these regions agriculture, if defined as a "*provisioning system based primarily on the production and consumption of domesticated resources*" (Smith, 2001; Zeder, 2015), did not develop until recent times (Iriarte et al., 2017; Neves, 2013). Instead, the prevailing subsistence economies were mixed, based on varying combinations of plant gathering and cultivation (both wild and domesticated), forest management, hunting and fishing. This supported population growth reduced territorial mobility and the development of long-lasting cultural practices during most of the Late Holocene (De Blasis et al., 1998; Fausto and Neves, 2018; Iriarte et al., 2017; Neves, 2013; Ottalagano and Loponte, 2017).

Subtropical and tropical coastal areas of southeastern South America preserve archaeological evidence of mixed economies that successfully persisted throughout the Middle and Late Holocene, and over extensive geographic areas. Shell mounds and middens of the Brazilian coast (locally known as sambaquis) are examples of sites built by coastal-adapted populations who attained relatively large demographic sizes and developed relatively complex social organizations without relying on domesticated plants. Their economic systems were based on the exploitation of aquatic organisms (Bastos et al., 2014; Colonese et al., 2014; Fossile et al., 2019; Pezo-Lanfranco et al., 2018b), along with a range of terrestrial plant resources, including some cultivars in more recent times (Pezo-Lanfranco, 2018; Scheel-Ybert and Boyadjian, 2020; Wesolowski et al., 2010), and the occasional hunting of land mammals (da Rocha Bandeira, 2015). In agreement with contemporary evidence from wetland areas in eastern South America (Iriarte et al., 2017), the picture that emerges from coastal sites in Brazil is one where aquatic environments offered a competitive edge to mixed economies in tropical and subtropical areas during the Late Holocene. However, considerable uncertainty still remains about the extent that coastal resources were used by adjacent populations, such as the flora and fauna of the wetlands of the Pampa biome, and the relative contribution of marine and terrestrial food items to their overall subsistence economy.

The Pampa biome and the La Plata basin in Brazil, Uruguay, and Argentina were occupied by groups who built earthen-mounds during the Late Holocene, known as *cerritos* (Bonomo et al., 2017, 2011b; Iriarte, 2006; Iriarte et al., 2004). Archaeobotanical data from sites in the La Plata basin reveal that these groups cultivated domesticated crops (e.g. maize, beans, squash) *ca.* 4,000 years ago (Iriarte et al., 2004). More recent groups also cultivated domesticated crops in the Paraná River delta and exploited wild faunal and plant resources (Bonomo et al., 2011a, 2011b). This is confirmed by recent stable isotope analysis on human individuals from earthen-mounds in this region, which indicated diets with a predominant consumption of C_3 plants complemented by the hunting of wild animals (Bonomo et al., 2017). By contrast, very little is known about the subsistence economy and diet of groups who occupied the Patos Lagoon in Southern Brazil. This ecosystem is particularly productive in terms of aquatic and terrestrial animals and zooarchaeological analysis suggests that fish may have been a major source of protein for groups in this region (Chim, 2016; Milheira et al., 2019a; Schmitz, 1976).

To reconstruct the paleodiet of Late Holocene human remains from a number of earthen-mounds in the southern Patos Lagoon (Figure 1), this study combined bone bulk collagen stable carbon and nitrogen isotope analysis with Bayesian Stable Isotope Mixing Models (BSIMMs). BSIMMs express dietary contribution probability distributions while accounting for multiple sources of uncertainty (Fernandes et al., 2014; Parnell et al., 2013; Phillips et al., 2014), therefore offering more robust dietary estimates than simple raw data comparison. Stable isotope analyses were also performed on dentine serial sections to obtain information on childhood and adolescent diets from a few individuals. Each section portrays the average diet of 6 – 8 months of the individual's life, making this technique a powerful tool for highlighting dietary variability with greater temporal resolution. We aimed to assess the relative contribution of marine and terrestrial/freshwater food sources, including plants, to the diet of mound builders and to contribute to debates on the nature of subsistence strategies in eastern South America during a period of increased social complexity and cultural networking.

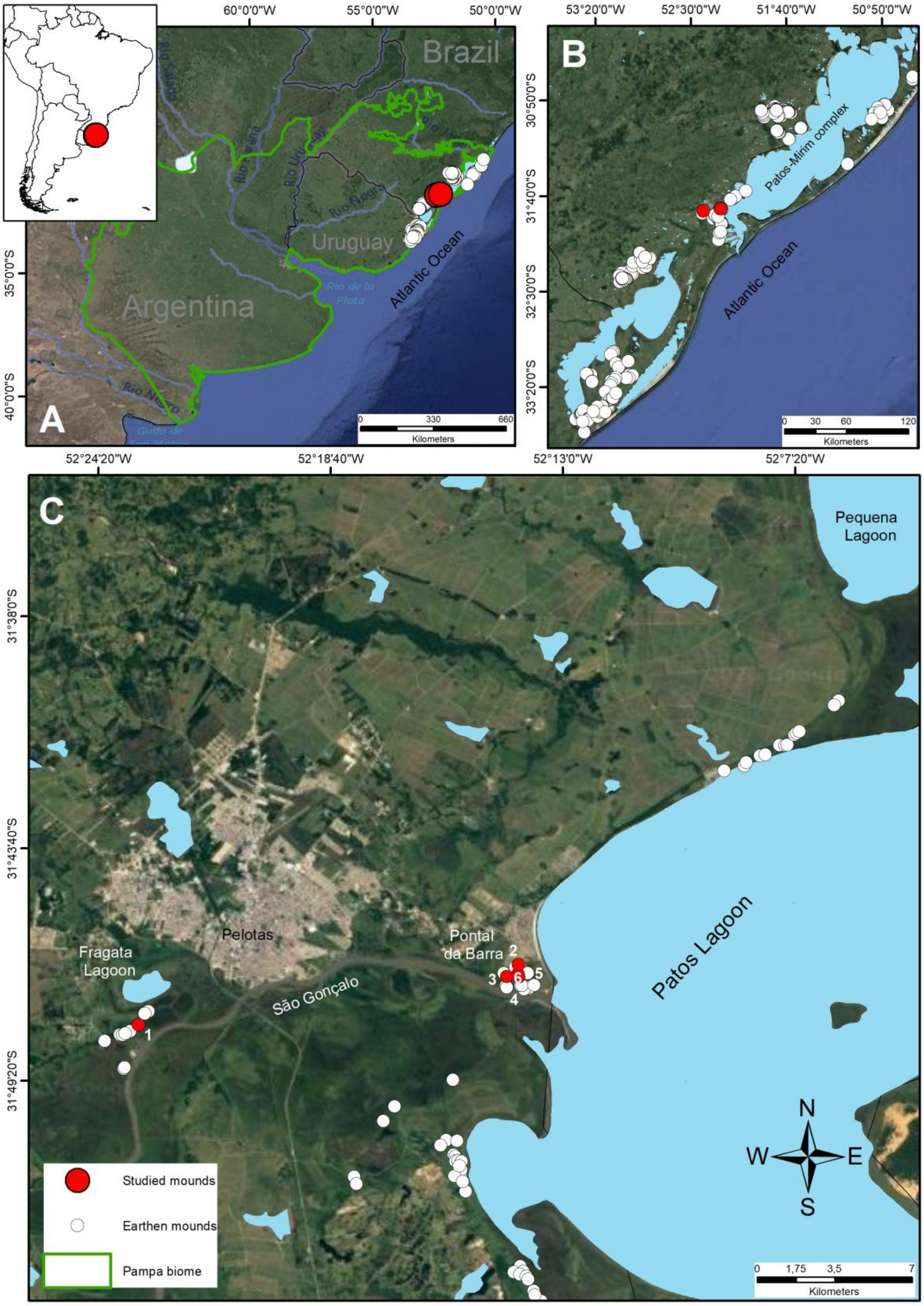

Figure 1: A) Earthen-mounds in the Pampa biome, around the Patos-Mirim complex in southern Brazil and northern Uruguay. The red dots indicate the sites studied; B) detailed location of earthen-mounds in

the southern Patos-Mirim complex; C) distribution of the earthen-mounds at Fragata Lagoon (1, PSGLF-02 – Fragata Lagoon) and Pontal da Barra (2 - PSG-01; 3 - PSG-02; 4 - PSG-03; 5 - PSG-06; 6 - PSG-07).

***Cerritos*, the earthen-mounds of the Pampa biome**

Cerritos in southern Brazil, Uruguay and Northeast Argentina have been associated with distinctive activities, including temporary camps, residential households, and cemeteries (Figure 2). The height of the mounds varies between 30 cm to 7 m, and they are commonly articulated to adjacent built structures including micro-reliefs (mounds less than 30 cm tall), elongated platforms, borrow pits, tracks, pathways and artificial lakes that compose archaeological complexes situated in flooded environments (Bracco, Del Puerto and Inda 2008; López Mazz y Pintos 2000; Milheira and Gianotti 2018). The chronology of the sites spans the Middle Holocene to the colonial period, from *ca.* 4700 to 200 yr cal BP (Bracco et al. 2000; Bracco, Del Puerto and Inda 2008; Iriarte 2003, 2006; Lópes Mazz 2001; Gianotti 2015; Milheira and Gianotti 2018), but most sites in the Patos Lagoon region were formed between 2400 and 800 years ago (Schmitz 1976; Milheira et al 2017; Milheira et al 2019a).

Figure 2: A) Panoramic view of *Cerrito Lagoa Pequena* 01 at the Pequena Lagoon complex; B) *Cerrito* Moreira 01 at the Fragata Lagoon complex; C-D) *cerritos* PSG-02, PSG-05, PSG-06, PSG-07 at the Pontal da Barra; E) Site Pavão 01 with "boomerang" shape associated with the *cerrito* Pavão 02, located by the São Gonçalo channel, at the Patos Lagoon; F) Human mandible associated to a pendant made of dolphin tooth and ceramic from *cerrito* PSG-02; G) Detail of the pendant; H) ceramic shard with fish remains from the *cerrito* PT-02, on Feitoria Island in the Patos lagoon; I) Human mandible with intensive dental wear

from *cerrito* PSGLF-02, at the Fragata Lagoon; J) fish and ceramic remains from *cerrito* PT-02, on Feitoria Island.

Beginning 2400 years ago, the sites in the region became more numerous, associated with elements of social complexity, such as clusters of monumental sites, technology changes, resource intensification and broad territoriality (Iriarte 2006; Moreno 2014; Bracco, Del Puerto and Inda 2008; Del Puerto 2015; Gianotti 2015). This time interval coincides with more specialized hunting strategies with the management of wild species (Moreno 2014). Domesticated and managed plants, including maize (*Zea mays*), squash (*Cucurbita* sp.), beans (*Phaseolus* sp.), tubers (*Canna* sp. and *Calathea* sp.), and palm nuts (*Butia odorata* and *Syagrus romanzoffiana*), have been recorded since *ca.* 4000 cal years BP (Iriarte et al., 2004), but became more intensively incorporated to the economy 1600 years ago (Iriarte 2006, Del Puerto 2015, del Puerto and Campos 1999, del Puerto and Inda 2008, Milheira and Gianotti 2018). The earliest evidence of domestic dog (*Canis lupus familiaris*) in the region, often associated with human burials, is also dated to around 1600 years ago (Prates et al 2010; Acosta & Loponte 2011a, 2011b; López Mazz et al 2018; Milheira et al. 2016a).

Fish is typically considered a complementary source of animal protein, which was composed primarily of cervids and small rodents such as the *Cavia aperea* (Pintos 2000; Moreno 2014). However, in regions such as the Patos Lagoon, fishing is attested at many sites (Schmitz 1976, Schorr 1975, Milheira et al. 2016b, Chim 2016, Calippo 2000, Ulguim 2010, Ulguim 2018), and evidence of it dominates the zooarchaeological assemblages at Pontal da Barra (Milheira et al. 2019a). The most abundant taxa are marine catfish (*Genidens planifrons, Genidens barbus,* and others of the Ariidae family), drums, from the Sciaenidae family, notably the whitemouth croaker (*Micropogonias furnieri*) and the black drum (*Pogonias cromis*). Freshwater species are scarce and only represented by trahira (*Hoplias* sp.) and swamp eel (*Synbranchus* sp.). A great diversity of marine catfish and drums is also observed in the Patos Lagoon and adjacent marine areas today (Garcia et al., 2003). Aquatic birds, freshwater turtles and terrestrial mammals, mainly small rodents, carnivores and deer, were also exploited in the area.

Pontal da Barra: environmental and archaeological background

The Patos Lagoon (30°S and 32°S) is the world's largest choked lagoon and one of the most voluminous water-bodies in southern Brazil, with an area of *ca.* 10227 km^2 (Garcia et al., 2003). It is located between Rio Grande do Sul state and the east coast of Uruguay and is connected with the Mirim Lagoon through the 75 km long São Gonçalo channel forming the Patos-Mirim complex or Patos Lagoon complex (Simon and da Silva, 2016). The lagoon is considered an important nursery and feeding ground for marine species and, despite the overexploitation of fish stocks, fisheries remain highly productive (Haimovici and Cardoso, 2016). The potential for catches attracted indigenous populations since pre-colonial times, as attested by more than 400 *cerritos* (Milheira et al., 2019b), shell mounds (Ribeiro and Calippo, 2000) and Guarani sites (Milheira and Wagner, 2014) with evidence of exploitation of aquatic resources.

In the municipalities of Pelotas and Capão do Leão, at the southern tip of the Patos Lagoon, the team of the project "Archaeology and Indigenous History of the Pampas" has surveyed 43 earthen-mounds in the wetlands of the Pontal da Barra, Fragata Lagoon, and Pequena Lagoon. The excavations at six of these sites provided the most robust chronological information for *cerritos* in southern Brazil to date, with radiocarbon dates ranging from 2177 to 754 cal BP (Milheira et al., 2019a, 2017). These sites are circular and elliptical-shape mounds, no more than 1.20 m tall, usually located in riparian forests along rivers and lagoons. The combination of radiocarbon dating and analysis of the material culture and faunal remains suggests that the long-term occupation might be compatible with the development of semi-sedentary villages. A complex stratigraphic record is a distinctive feature of these sites. The archaeological deposits are dark, rich in faunal and charred plant remains and artifacts including a vast number of pottery sherds and at times human bones. Human remains always appear disarticulated, highly fragmented and spatially scattered, following the same distribution of other materials. Although the nature of these funerary patterns is unclear, the consistency of radiocarbon dates strongly indicates that *in-situ* manipulation of bodies or secondary burials occurred during the construction of the sites (Milheira et al., 2019a).

Material and Methods

Collagen extraction

Bone samples of faunal and human remains were collected from six different sites: PSG-01, PSG-02, PSG-03, PSG-06, PSG-07, and PSGLF-02. Sites with the abbreviation PSG are located at the Pontal da Barra, while PSGLF refers to those at the Fragata Lagoon. Both are located along the southern Patos Lagoon (Figure 1C).

Fifty samples were submitted to stable isotope analysis of carbon and nitrogen in three different laboratories: the Environmental Isotope Laboratory (EIL) of the University of Arizona (USA), the Radiocarbon Laboratory (MPI-EVA) of the Max Planck Institute for Evolutionary Anthropology (Germany), and the BioArCh facilities of the University of York (UK). Bulk collagen was successfully extracted from 26 faunal and 19 human samples, totaling 45 collagen samples. Permissions were granted for the isotopic analyses in York, Germany and Arizona by the Brazil's National Institute of Historic and Artistic Heritage (IPHAN, protocol numbers 01512.000265/2019-19, 01450.008639/2015-92 and 01450.000343/2016-12).

Human remains consisted of teeth (n = 4), fragments of cranial bones (n = 14) and one metacarpal (n = 1) (Table 1). Faunal remains represent the most frequent taxa at the sites, including several species of marine-estuarine fishes (*Genidens barbus*, *Genidens planifrons*, *Cynoscion virescens*, *Micropogonias furnieri*, *Pogonias cromis*), freshwater fishes (*Hoplias* sp. and *Synbranchus* sp.), semi-aquatic fauna such as freshwater turtles (*Trachemys dorbigni*), Anseriforme birds, medium-sized rodents (*Myocastor coypus*), terrestrial fauna such as Argentine giant tegus (*Salvator merianae*), Pampas deer (*Ozotoceros bezoarticus*), medium-sized carnivores and small rodents (*Cavia aperea*) (Table 2).

At the EIL of the University of Arizona, collagen was extracted using a modified version of the Law and Hedges (1989) method. Samples were gelatinized at 70 °C, collagen was then filtered using autovials (0.45 µm glass microfiber filter) and lyophilized (Law and Hedges, 1989).

At the MPI-EVA, collagen was extracted following Talamo and Richards (2011) . After surface cleaning with an abrasive jet of aluminium oxide 50 µm, samples were crushed and ca. 700 mg was used for collagen extraction. Samples were demineralised in 0.5 M HCl for several days at approximately 4 ºC, rinsed with 0.1 M NaOH for 30 min at room temperature, gelatinised in 0.001 M HCl at 70 ºC for 48 h and then filtered in ultrasonically pre-cleaned Ezee and VIVASPIN 15 filters before freeze-drying. Samples were weighed into tin capsules (0.45 – 0.55 mg) for determination of δ^{13}C and δ^{15}N values.

At the BioArCh facilities (University of York) the bones were cleaned using a Sand Blaster 80060 Master Problast 3 to remove solid and potential contaminants. Collagen was then extracted following a modified Longin method (Brown et al., 1988) and details can be found in previous studies (Craig et al., 2010). In short, shards of bones (*ca.* 200 to 300 mg) were demineralised using 0.6 M HCl at 4 °C for several days, then rinsed with ultrapure H_2O (milli-Q®) and gelatinised with 0.001 M HCl at 80 °C for 48 h. The supernatant containing the collagen was filtered using Polyethylene Ezee filters (Elkay Laboratories Ltd., 9 mL, pore size 60 – 90 µm) and then filtered using 30 kDa Amicon® Ultra-4 centrifugal filter units (Millipore, MA, USA). Samples were then frozen for 24 – 48 h at -20 ºC, lyophilized, and weighed into tin capsules (0.5 mg per duplicate) for stable isotopic analysis.

Site	Sample ID	FRUITS ID	Anatomical identification	δ^{13}C	δ^{15}N	wt C%	wt N%	C/N	Coll yield (wt%)	Reference
PSG-01	116.01.290	BA1	Cranial bone	-13.1	14.7	35.2	12.4	3.3	4.6	This study*
PSG-01	116.01.297	BA2	Cranial bone	-16.4	11.0	16.0	5.8	3.2	2.1	This study*
PSG-01	116.01.309	MP2	Cranial bone	-13.0	15.5	46.6	16.8	3.2	4.1	This study**
PSG-02	111.7.999	BA3	Cranial bone	-21.1	7.1	38.7	13.5	3.3	2.3	This study*
PSG-02	111.7.783	MP1	Cranial bone	-17.6	10.2	44.7	15.9	3.3	6.2	This study**
PSG-02	111.7.1014	MP3	Cranial bone	-21.3	7.4	44.3	15.7	3.3	3.9	This study**
PSG-02	111.7.780	MP4	Cranial bone	-14.5	14.4	43.9	15.8	3.2	5.7	This study**
PSG-02	111.11.1482	UG1	Femur	-12.2	5.8	-	-	-	-	Milheira et al. 2017b***
PSG-03	41.03.178	BA4	Cranial bone	-10.7	16.8	22.9	8.2	3.3	1.5	This study*
PSG-06	114.01.029	BA5	Tooth premolar	-12.8	15.4	43.3	15.8	3.2	2.7	This study*
PSG-06	114.01.103	AA1	Tooth molar	-12.5	18.3	39.6	13.5	3.4	6.6	This study****
PSG-06	114.02.429	MP6	Cranial bone	-12.1	17.0	45.9	16.5	3.2	3.9	This study**
PSG-07	115.04.189	BA6	Cranial bone	-11.9	15.1	27.8	10.0	3.3	2.5	This study*
PSGLF-02	122.6.501	BA7	Cranial bone	-15.9	13.0	35.1	12.6	3.2	3.0	This study*
PSGLF-02	122.6.759	BA8	III metacarpal	-16.6	12.4	42.2	14.8	3.3	4.4	This study*
PSGLF-02	122.6.772	BA9	Cranial bone	-15.9	13.3	35.6	12.9	3.2	3.2	This study*
PSGLF-02	122.5.525	BA10	Tooth 2º premolar	-16.8	13.2	43.2	15.9	3.2	2.2	This study*
PSGLF-02	122.6.752	BA11	Tooth canine	-14.1	16.2	44.7	16.3	3.2	3.1	This study*
PSGLF-02	122.06.779	MP7	Cranial bone	-16.1	12.5	46.7	16.9	3.2	1.5	This study**
PSGLF-02	122.05.481	MP5	Cranial bone	-16.3	12.3	46.2	16.6	3.2	7.5	This study**

Table 1: Stable isotope values of bulk collagen of human individuals from cerritos in Patos Lagoon. Stable isotope analyses were performed at the BioArCh facilities of the University of York (*), at the Radiocarbon facilities of the Max Planck Institute for Evolutionary Anthropology (**), the University of Georgia's Center for Applied Isotope Studies (***), University of Arizona AMS Laboratory (****). Teeth δ^{13}C and δ^{15}N values

analyzed at the University of York represent median values from incremental dentine analysis (see table S1). Atomic C/N errors of ±0.1.

Taxa	**Class**	**Anatomical identification**	**Site**	**Sample ID**	**$\delta^{13}C$**	**$\delta^{15}N$**	**wt C%**	**wt N%**	**C/N**	**Coll yield (wt%)**	**Reference**
Synbranchus sp.	Actinopterygii	Dentary	PSGLF-02	122.6.755	-22.4	7.5	29.9	10.8	3.2	3.7	This study*
Hoplias sp.	Actinopterygii	Premaxilla	PSGLF-02	122.6.753	-18.1	10.2	34.2	11.9	3.4	3.2	This study*
Genidens barbus	Actinopterygii	Dentary	PSG-07	115	-5.7	10.9	42.4	14.9	3.3	1.8	This study*
Genidens barbus	Actinopterygii	Cleithrum	PSG-07	115	-13.3	12.4	42.7	14.5	3.4	1.5	This study*
Genidens barbus	Actinopterygii	Cleithrum	PSG-07	155	-11.6	14.2	23.8	8.5	3.3	2.4	This study*
Genidens planifrons	Actinopterygii	Supra-occipital process	PSG-02	111.1.255	-11.4	15.9	15.3	5.0	3.6	2.2	This study**
Cynoscion virescens	Actinopterygii	Premaxilla	PSG-07	243	-10.3	14.1	23.6	8.5	3.2	3.0	This study*
Micropogonias furnieri	Actinopterygii	Vertebrae	PSG-02	111.1.255	-11.8	10.8	41.3	14.0	3.4	1.2	This study**
Micropogonias furnieri	Actinopterygii	Premaxilla	PSG-07	115	-11.5	15.7	30.6	10.5	3.4	2.4	This study*
Micropogonias furnieri	Actinopterygii	Premaxilla	PSG-07	115	-10.7	14.8	32.1	10.9	3.4	1.2	This study*
Pogonias cromis	Actinopterygii	Dentary	PSG-02	111.1.255	-12.9	13.2	32.6	10.8	3.5	2.0	This study**
Pogonias cromis	Actinopterygii	Premaxilla	PSG-07	115	-12.1	10.9	32.7	11.1	3.4	1.2	This study*
Estuarine fish	Actinopterygii	Fin spine	PSG-02	111.8.1083	-11.2	10.6	41.8	13.8	3.5	2.7	This study**

Trachemys dorbigni	Reptilia	Carapace	PSG-02	111.8.1083	-11.4	11.4	36.9	12.4	3.5	2.3	This study**
Trachemys dorbigni	Reptilia	Plastron	PSGLF-02	122.6.744	-12.7	12.1	24.7	9.1	3.2	2.8	This study*
Trachemys dorbigni	Reptilia	Plastron	PSGLF-02	122.6.663	-14.9	11.2	38.7	13.9	3.2	7.8	This study*
Salvator merianae	Reptilia	Dentary	PSG-02	111.1.512	-16.0	9.4	43.2	14.6	3.5	8.4	This study**
Aquatic Bird (Anseriformes)	Aves	Tibiotarsus	PSG-02	111.8.1083	-24.9	7.9	27.6	9.2	3.5	3.9	This study**
Cavia aperea	Mammalia	Mandible	PSG-02	111.1.255	-18.0	8.9	40.2	12.8	3.7	1.6	This study**
Cavia aperea	Mammalia	Mandible	PSG-07	115	-19.7	8.4	32.4	11.2	3.4	3.0	This study*
Myocastor coypus	Mammalia	Mandible	PSGLF-02	122.6.763	-21.1	4.7	31.1	11.2	3.2	3.8	This study*
Myocastor coypus	Mammalia	Mandible	PSGLF-02	122.6.721	-18.8	3.1	36.7	12.9	3.3	3.6	This study*
Myocastor coypus	Mammalia	Mandible	PSGLF-02	122.06.766	-17.7	5.2	41.6	14.0	3.5	4.9	This study**
Canis lupus familiaris	Mammalia	Tooth	PSG-07	115.04.046/BT1DOG	-11.4	10.1	-	-	3.1	1.1	Guedes Milheira et al. 2017a***
Medium sized carnivore	Mammalia	Ulna	PSG-07	155	-15.2	10.8	46.4	16.9	3.2	4.3	This study*
Ozotoceros bezoarticus	Mammalia	Mandible	PSGLF-02	122.06.664	-20.9	4.1	42.5	14.5	3.4	3.6	This study**
Ozotoceros bezoarticus	Mammalia	Calcaneus	PSGLF-02	122.6.726	-20.4	5.1	37.0	13.5	3.2	7.1	This study*

Table 2: Stable isotope values of bulk collagen of faunal remains from cerritos in Patos Lagoon. Stable isotope analyses were performed at the BioArCh facilities of the University of York (*), at the University of Arizona AMS Laboratory (**) and the Beta Analytic, USA (***). Atomic C/N errors of ±0.1.

Collagen extraction of incremental dentine samples

Stable isotope analyses were conducted on collagen samples sequentially obtained from full longitudinal root sections to explore trends in infant feeding practices (King et al., 2017; Pezo-Lanfranco et al., 2020; Sellen and Smay, 2001; Tsutaya and Yoneda, 2015). A sequential isotope approach to dentine tissue is particularly useful in the case of *cerritos* due to the paucity of complete human remains at these sites. Collagen was extracted from 52 sequential dentine samples prepared at the BioArCh from three human teeth following Beaumont and Montgomery (2015). The teeth samples cover distinct time intervals: one premolar (which could not be identified further; 114.01.029 from PSG-06) formed from approximately 1 to 14 years of age, one second premolar (122.5.525 from PSGLF-02) formed from approximately 2 to 14 years, and one canine (122.6.752 from PSGLF-02) formed from 4 months to 15 years (AlQahtani et al., 2010). Sequential isotope values from these samples do not provide information on the early stage of breastfeeding.

Teeth were placed under a sandblaster to remove all exogenous debris from the enamel surface and were then cut in half longitudinally using an IsoMet1000 Precision Cutter to expose the enamel-dentine junction (EDJ). Once the majority of the surrounding enamel had been mechanically removed, the remaining mineral phase was demineralised in 0.6 M HCl for 2 – 4 weeks in a laboratory rocker. The demineralised tooth was cut into 1 mm transversal sections, in an occlusal-apical direction, taking note of the crown-root junction (CRJ). Collagen extraction of each section followed the aforementioned method of bone collagen extraction at BioArCh, commencing in the gelatinisation step that was conducted for 24 h at 80 °C. Dentine collagen was not filtered or ultra-filtered as these are unnecessary steps due to the prior removal of exogenous debris and high collagen yields (Beaumont and Montgomery, 2016). The age of formation for each section was estimated using the Beaumont and Montgomery (2015) chart for tooth development, noting age at dentine initiation and age at apex completion for each tooth. To calculate the age per increment, the age at dentine initiation was subtracted from the age at apex completion for each tooth. This value was divided by the number of dentine sections obtained during the sample preparation to calculate an average duration for each dentine section. An age at formation was finally calculated for each dentine section adding the average duration of each increment to the age at initiation until reaching the age of apex completion (Supplementary information 1). This method allows investigating changes in diet over several years depending on the time of teeth formation, at a resolution of approximately 6 – 7 months in our samples.

Stable isotope analyses

In Arizona, bone collagen (1 mg) was combusted to produce CO_2 and N_2 in a continuous flow isotope ratio mass spectrometry (Finnigan Delta PlusXL) coupled with an elemental analyzer (Costech). Standardisation was based on acetanilide for elemental concentration, Polyethylene foil (IAEA CH-7) and Graphite powder (USGS-24) for δ^{13}C, and ammonium sulfate (IAEA-N-1 and IAEA-N-2) for δ^{15}N. Precision is better than ± 0.08 for δ^{13}C and ± 0.2 for δ^{15}N (1σ), based on repeated internal standards.

At York, bulk collagen extracted from the bone (1 mg) and sequential dentine samples (0.5 mg) were combusted to obtain CO_2 and N_2 with a Sercon GSL system attached to a Sercon 20-22 mass spectrometer

(Sercon, Crewe, UK). At York, the overall uncertainties in the measurements of each sample were calculated based on the Kragten method (Kragten, 1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials (these are expressed as one standard deviation). International standards for caffeine (IAEA-600), ammonium sulphate (IAEA-N-2), and cane sugar (IA-Cane) were used as reference materials in each analytical run. Precision was better than 0.2 ‰ (1σ) for both δ^{13}C and δ^{15}N values.

Bayesian mixing model and Statistical Analysis

Bayesian Stable Isotope Mixing Models (BSIMMs) in FRUITS 3.1 (Fernandes et al., 2014) were used to estimate the relative contribution (%) of different food sources to human diets. The models account for the uncertainties in consumer and food isotopic values, macronutrient composition, diet-to-consumer isotopic offsets, and dietary routine. Specifically, the model estimates the relative caloric contribution from food groups to diet, and the relative caloric contribution from the protein-only component of food groups to diet. The model is based on dietary proxies of humans and multiple food sources and accounts for macronutrient fractions and routing, as well as uncertainties in dietary inferences. The dietary proxies used in model estimations were δ^{13}C and δ^{15}N values, with the uncertainty set at 0.5 ‰. The δ^{13}C and δ^{15}N values of a previously analysed human femur of PSG-02 (Milheira et al., 2017) and of a domestic dog from PSG-07, directly dated to 1720 ± 30 BP (Guedes Milheira et al., 2017), were also modelled. Four food sources with their respective macronutrient compositions were considered: terrestrial/freshwater animals (protein, lipids), marine/estuarine animals (protein, lipids), C_3 plants (protein, carbohydrates), and C_4 plants (protein, carbohydrates).

The average δ^{13}C and δ^{15}N values of macronutrients (protein and lipids) of fauna were estimated from the average δ^{13}C and δ^{15}N values of contextual faunal remains recovered from sites at Pontal da Barra. For terrestrial/freshwater fauna (n = 12), the average δ^{13}C and δ^{15}N values were respectively -19.3 ± 2.7 ‰ and +7.7 ± 2.6 ‰. For marine/estuarine fauna (n = 14), the average δ^{13}C and δ^{15}N values were -11.6 ± 2.0 ‰ and +12.3 ± 1.9 ‰. The average δ^{13}C and δ^{15}N values of plant protein and carbohydrates were estimated from published δ^{13}C and δ^{15}N values of C_3 and C_4 plants possibly available in the study area. For C_3 plants, we used the average δ^{13}C and δ^{15}N values of modern fruits (n = 30), roots (n = 5) and heart of palm (n = 13) of -29.2 ± 3.0 ‰ and +1.1 ± 2.0 ‰, respectively collected in national parks in the southeastern Atlantic Forest between 2010 and 2012 (Galetti et al., 2016) but also present in the Pampa biome (Pillar et al., 2009). For C_4 plants, we used the average δ^{13}C and δ^{15}N values of modern *Sporobolus montevidensis* (n = 10), a grass in the Poaceae family that is dominant in the saltmarshes of the Patos Lagoon (Garcia et al., 2007), and archaeological maize (n = 6), *Zea mays*, from Argentinian contexts (Peixoto, Costa, 2004; Abreu et al., 2006; Gil et al., 2006, 2010; Claudino et al., 2013). The δ^{13}C values of modern plants were corrected for the Suess effect depending on the years of collection (+1.7 ‰ to 2 ‰) (Hellevang and Aagaard, 2015).

For each food source the average δ^{13}C and δ^{15}N values of the nutrient fraction (protein, carbohydrate, lipids) were estimated using fractionations previously reported (Fernandes et al., 2015, 2012). For terrestrial mammals the offsets are −2 ‰ ($\Delta^{13}C_{\text{protein-collagen}}$), −8 ‰ ($\Delta^{13}C_{\text{lipids-collagen}}$) and +2 ‰ ($\Delta^{15}N_{\text{protein-collagen}}$). For fish these are −1 ‰ ($\Delta^{13}C_{\text{protein-collagen}}$), −7 ‰ ($\Delta^{13}C_{\text{lipids-collagen}}$)

and +2 ‰ ($\Delta^{15}N_{protein-collagen}$). For both C_3 and C_4 plants, the offsets are −2 ‰ ($\Delta^{13}C_{bulk-protein}$) and +0.5 ‰ ($\Delta^{13}C_{bulk-lipids}$), and assume that the $\delta^{15}N$ value of plant protein was the same as the average bulk plant $\delta^{15}N$ value. A conservative uncertainty between 1 ‰ and 3 ‰ was used for offsets.

The model assumed that nitrogen isotopes were exclusively sourced from proteins (100%), but carbon isotopes represent 74 ± 4 % and 26 % of protein and energy (lipids, carbohydrates) respectively due to *de novo* synthesis of non-essential amino acids (Fernandes et al., 2012). A concentration-dependent and routed model was considered. Diet-to-collagen $\delta^{13}C$ offsets (+5 ± 0.5 ‰), and diet-to-collagen $\delta^{15}N$ offset (+5.5 ± 0.5 ‰) were taken from Fernandes et al. (2012). Dietary estimations were constrained for a conservative acceptable range of protein intake of >5 % and <45 % of total calories according to physiological studies (Fernandes et al., 2014).

Box and whiskers plots summarizing the posteriors of dietary estimates generated using FRUITS were created using the R statistical software environment (version 4.0.3) and the R package ggplot2 (version 3.3.2).

Results

Bone collagen stable isotopes

Collagen was successfully extracted from 90 % of the samples (n = 50) and from 91 % of the sequential dentine samples (n = 52). Extracted collagen had $C:N_{atomic}$ ratios ranging from 3.1 to 3.7 and collagen yields spanning from 1.5 wt% to 7.5 wt% (Tables 1 and 2). The agreement with established quality criteria for archaeological and palaeontological bone collagen (Ambrose, 1990; DeNiro, 1985; Szpak, 2011; van Klinken, 1999) indicates an overall good preservation of these bone samples.

The $\delta^{13}C$ and $\delta^{15}N$ values of terrestrial fauna and freshwater fish remain, excluding one dog sample, ranged from -24.9 ‰ to -15.2 ‰ and from +7.9 ‰ to +10.8 ‰ respectively. Most of the $\delta^{13}C$ values can be associated with C_3 plant ecosystems, but their high values (e.g. -15.2 ‰) indicate some contribution of C_4 plants to diets (Powell et al., 2012). The $\delta^{13}C$ and $\delta^{15}N$ values of marine and estuarine fish remains ranged from -14.9 ‰ to -5.7 ‰ and from +10.6 ‰ to +15.9 ‰ respectively, in agreement with values reported for modern specimens from the Patos Lagoon (Garcia et al., 2007). Therefore, the first group was defined as terrestrial/freshwater animals, while the latter as marine/estuarine animals.

Bone collagen $\delta^{13}C$ and $\delta^{15}N$ values of human remains ranged from -21.3 ‰ to -10.7 ‰ and from +5.8 ‰ to +18.3 ‰ respectively, showing remarkable intra-site (especially at PSG-02) and inter-site variability (Figure 3). Considering the associated error of replicated $\delta^{13}C$ and $\delta^{15}N$ values (Pestle et al., 2014), the results suggest that in most cases the human bone samples analyzed here are from distinct individuals. However, this might not be the case for some samples from PSGLF-02 and PSG-02. The comparable $\delta^{13}C$ and $\delta^{15}N$ values and atomic characteristics of samples 122.06.779, 122.05.481 and 122.6.759 from PSGLF-02 suggest they may be from the same individual. This might be the case for samples 122.6.501 and 122.6.772, which are also from PSGLF-02. Similarly, samples 111.7.999 and 111.7.1014 from PSG-02 could potentially represent one single individual. To summarise, a minimum of thirteen individuals are possibly represented by our human samples.

The high human δ^{13}C and δ^{15}N values detected at PSG-01, PSG-03, PSG-06, PSG-07 indicate that their diets were dominated by marine proteins such as marine/estuarine fish. By contrast, the lowest δ^{13}C and δ^{15}N values of samples from PSG-02 are consistent with diets primarily based on terrestrial/freshwater resources from C_3 ecosystems, including terrestrial animals, plants and potentially freshwater fish. Human individuals with intermediate δ^{13}C and δ^{15}N values found at PSGLF-02, but also at PSG-01 and PSG-02 can be associated with mixed C_3 terrestrial/freshwater and marine/estuarine diets. Only one individual, an outlier from PSG-02, had less negative δ^{13}C value and lower δ^{15}N value, which might be indicative of substantial consumption of C_4 plants (e.g. maize).

The wider distribution of δ^{13}C and δ^{15}N values and their moderate positive linear correlation ($R^2 = 0.46$) reflect a protein gradient between the end-members of C_3 terrestrial/freshwater and marine/estuarine diets. A much stronger positive linear correlation ($R^2 = 0.89$) is obtained when the individual outlier from PSG-02 with C_4-derived δ^{13}C value is removed. As suggested for other coastal populations in southern Brazil, this high correlation could imply substantial routing of carbon and nitrogen from dietary protein to collagen due to sufficient amounts of protein in their diets (Colonese et al., 2014).

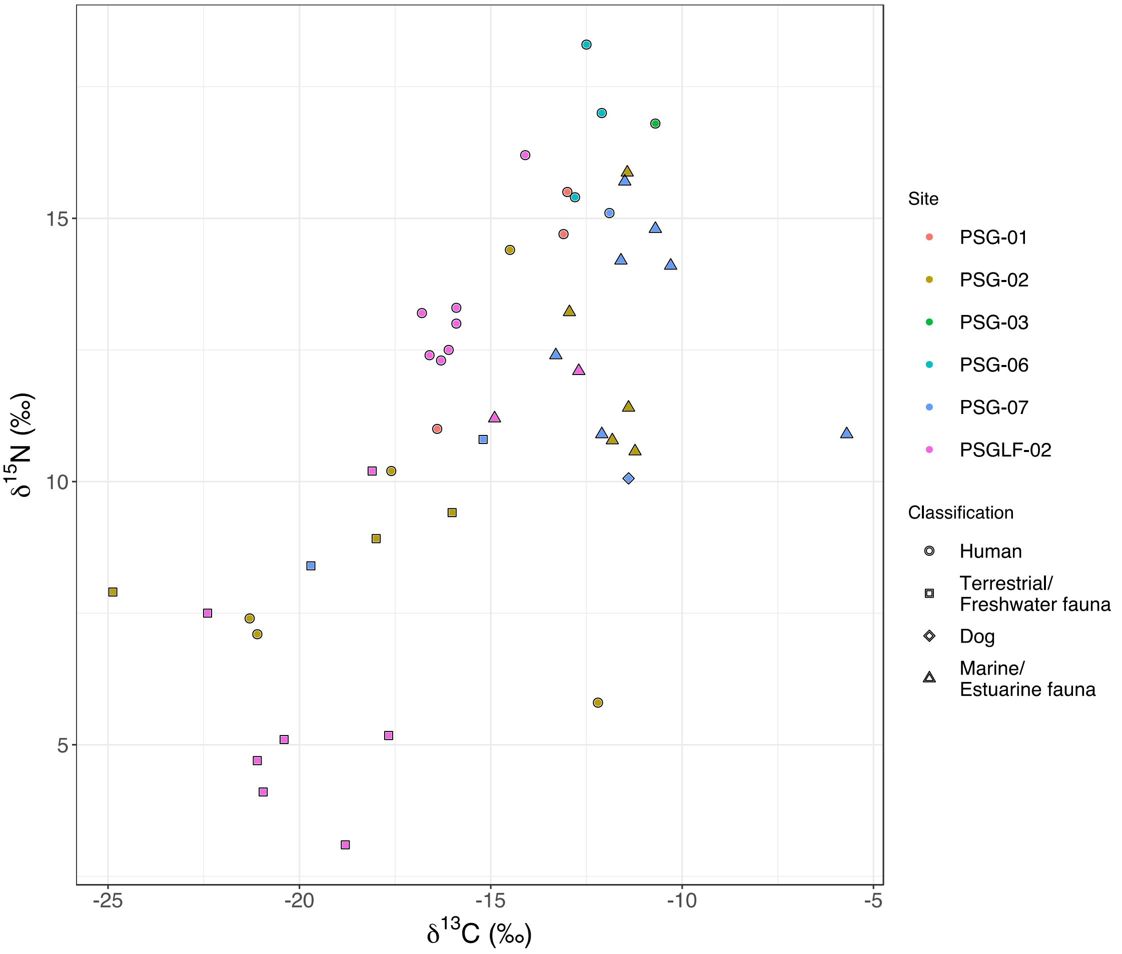

Figure 3: δ^{13}C and δ^{15}N values of human and animal samples from the sites studied.

Sequential dentine collagen stable isotopes

The δ^{13}C and δ^{15}N values of the sequential dentine collagen reflect infant and young adult diets about which very little is known for these populations. The sequential sampling resolution varied with the teeth and in general corresponded to ages between *ca.* 3 and 14 years for 114.01.029, 1.5 to 14.5 years for 122.6.752 and 3 to 14 years for 122.5.525 (Supplementary information 1). The age-spans of teeth formation did not capture the early stages of breastfeeding and possibly also weaning. Examples from foragers and horticulturalists (Tsutaya and Yoneda, 2013), including data from Late Holocene coastal populations from southern Brazil (Pezo-Lanfranco et al., 2018a), show that weaning can vary among populations but often occurred around 3 years of age. The lack of sex identification also prevented us from addressing the gender dimension of sequential dentine isotope data. For example, sex-biased differences could reflect distinct parental investment (Cronk, 2007).

The δ^{13}C and δ^{15}N values ranged from -14.7 ‰ to -11.2 ‰ and from +14.0 ‰ to +17.2 ‰ respectively for 114.01.029 ($n = 16$; PSG-06); -16.0 ‰ to -11.9 ‰ and from +15.0 ‰ to +19.9 ‰ respectively for 122.6.752 ($n = 23$; PSGLF-02); -17.7 ‰ to -14.5 ‰ and from +12.2 ‰ to +15.0 ‰ respectively for 122.5.525 ($n = 18$; PSGLF-02). The differences among the teeth were statistically significant for both δ^{13}C (Shapiro-Wilk normality test $p = 0.150$, One way ANOVA $p < 0.05$) and δ^{15}N (Shapiro-Wilk normality test $p = 0.078$, One way ANOVA $p < 0.05$). Proteins from marine resources dominate the isotopic compositions of 122.6.752 and 114.01.029, more so than for 122.5.525, which in turn had a large intake of C_3 plants and terrestrial/freshwater animals. In all samples decreasing δ^{13}C values suggest higher incorporation of terrestrial protein sources, such as C_3 plants and animals, during childhood. The results thus indicate that dietary differences were not restricted to adult life, but also occurred among individuals during their childhood. Samples 122.6.752 and 122.5.525, both retrieved from PSGLF-02, are examples of individuals with contrasting diets since the early stage of their lives.

Bayesian Stable Isotope Mixing Models

The estimates obtained from BSIMMs on bone collagen isotopic values reveal considerable variability in the caloric and protein contributions to diet from distinct food sources (Figures 4 and 5, supplementary information 2). The majority of calories were supplied by C_3 plants (median 31.4 %, Q1 15.9 %, Q3 50 %), followed by C_4 plants (median 21.3 %, Q1 10.7 %, Q3 35.4 %), terrestrial/freshwater resources (median 22.2 %, Q1 9.5 %, Q3 39 %) and to a lesser extent marine/estuarine resources (median 10.3 %, Q1 3.4 %, Q3 22.3 %) (Figure 4). Marine/estuarine animals were the main sources of dietary proteins (median 31.4 %, Q1 12.4 % and Q3 53.4 %), followed by terrestrial/freshwater resources (median 28.8 %, Q1 13.5 %, Q3 48.8 %) and C_3 plants (median 13.6 %, Q1 5.1 % and Q3 29 %), and finally C_4 plants (median 8.4 %, Q1 3.4 %, Q3 17.3 %) (Figure 5).

Figure 4: Boxplots of the contribution of calories from different sources to the individuals diet from all sites analysed here.

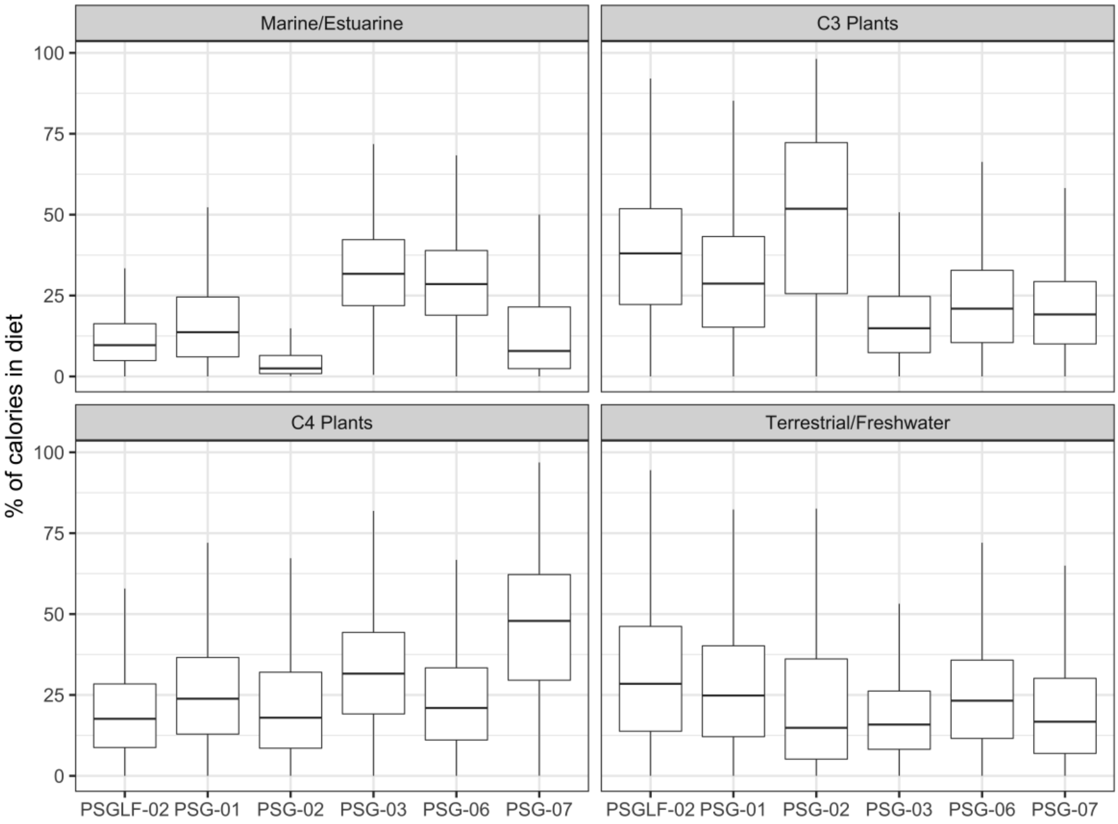

Figure 4: Boxplots of the contribution of calories from different sources to the individuals diet from all sites analysed here.

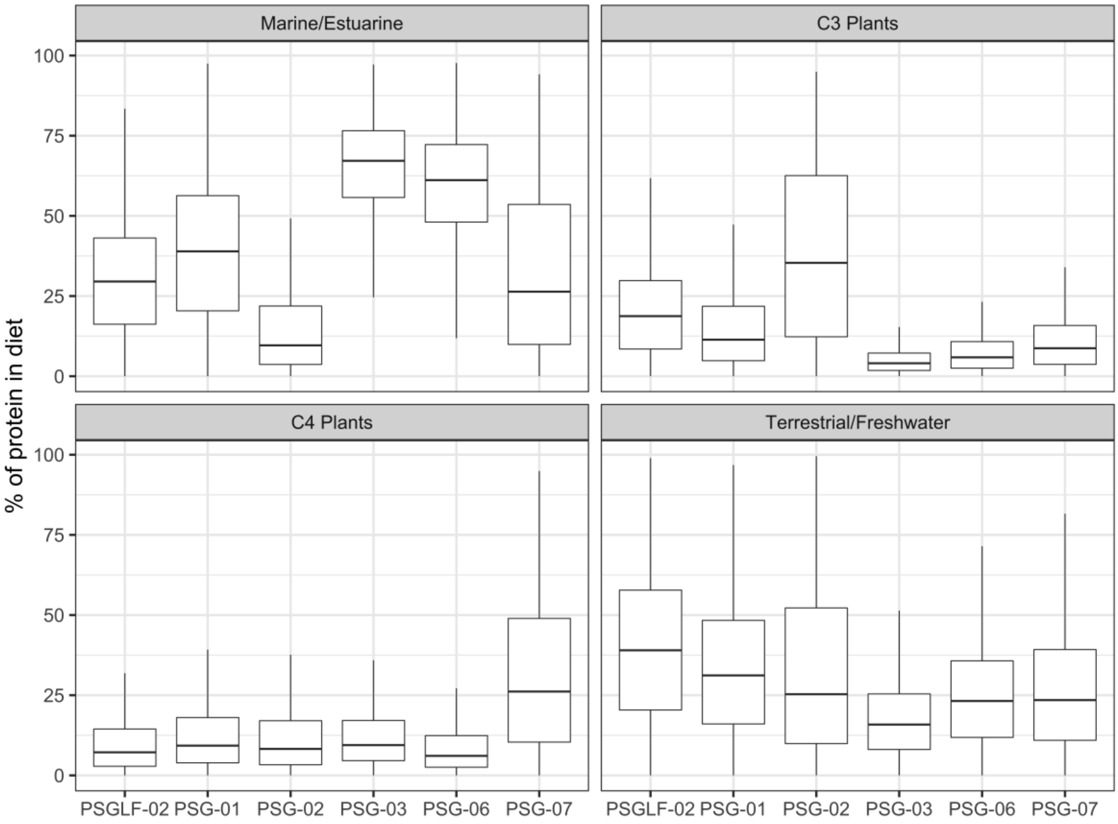

Figure 5: Boxplots of the contribution of protein from different sources to the individual diets from all sites analysed here.

The domestic dog from PSG-07 was the modelled sample with the highest prevalence of C_4 contribution to the diet, at 60% of caloric intake and 48% of protein. Previous studies have shown considerable similarities in stable isotope values between human and associated dog remains (Burleigh and Brothwell, 1978; Cannon et al., 1999; Guiry, 2013, 2012). This could be reasonably expected if dogs had access to food isotopically similar to those ingested by humans, for example through scavenging, handouts, and consumption of human feces (Cannon et al., 1999), although several factors complicate direct analogies (Guiry, 2012). Here, the dog had an average diet that contrasted with most human individuals. Only one human individual (UG1), from a different archaeological context (PSG-02), had stable carbon isotopic composition indicating substantial consumption of C_4 plants. If the *canine surrogacy approach* is applied, the evidence suggests that both the dog and this human individual may have not been locals, but originated from areas where C_4 plants (e.g. maize) constituted a significant dietary component.

Discussion

Stable isotope analysis detected considerable variability in $\delta^{13}C$ and $\delta^{15}N$ values among individuals from Pontal da Barra, including among individuals from the same site. The application of BSIMMs, although with some overlapping of outputs from distinct food groups, indicate different dietary regimes with variable dependence of marine/estuarine and terrestrial/freshwater food resources. The diversified economic system was mostly oriented towards the exploitation of estuarine and marine resources for protein acquisition, complemented by terrestrial and freshwater animals. C_3 plants emerged as prominent sources of calories and may have involved several wild (*Butia odorata*, *Siagrus romanzoffiana*, *Canna* sp. *and Calathea* sp.) and domesticated crops (*Cucurbita* sp., *Phaseolus* sp.), as widely documented in earlier and contemporary sites in areas farther south (Bonomo et al., 2017, 2011b; Iriarte, 2006; Milheira and Gianotti Garcia, 2018). According to model estimates, C_4 plants were also consumed and possibly involved maize, as attested to in culturally related sites dated back to 4000 years ago (Iriarte et al., 2004).

It is worth noting that there is no evidence for a chronological trend in human $\delta^{13}C$ and $\delta^{15}N$ values. Although the modelled radiocarbon dates from fish otoliths, charcoals, and human and dog bone samples from the sites span from 2177 to 754 cal BP, the most intense human activities occurred from 1800 to 1200 cal BP (Milheira et al., 2017) with no apparent correlation with individual dietary outputs. The detection of dietary variability in a confined geographic area such as the Pontal da Barra could instead indicate social interactions between groups having isotopically distinct diets and the absorption of non-local residents (e.g. postmarital residence practices). Dietary variability during childhood as revealed from sequential isotope data also indicates that groups subsisted on isotopically distinct diets, with variable degrees of marine and terrestrial sources of protein and calories. Individuals with a greater reliance on marine/estuarine resources may have been permanent residents or with residential mobility confined mostly along the coast. Those with a higher reliance on freshwater/terrestrial resources might have been occasional/intermittent residents, or newcomers integrated with these coastal communities or families. This could be the case of the human individual and the dog, which were outliers and had diets that included a substantial amount of C_4 plants, possibly maize. With the data in hand, the disarticulated nature of human remains prevented an understanding of the age and gender dimension of these differences. However, intra-site dietary variability has also been reported for some Late Holocene sites on the Atlantic Forest coast of Southern Brazil (Bastos et al., 2015, 2014; Colonese et al., 2014; Pezo-Lanfranco et al., 2018b).

The results reinforce the argument that Pontal da Barra was a central place for social networking, involving groups having distinct subsistence economies, as previously argued by Milheira et al. (2019b) based on spatial analysis of the sites. The region could be conceived as a “hub of interactivity”, with permanent residents exploiting estuarine and wetland resources year-round. The stable isotope results challenge the conventional view that mound builders were highly mobile hunter-gatherers pursuing resources between the Pampas biome and the coast (Schmitz 1976). Rather, the relatively high consumption of marine/estuarine fish by some of these individuals would allow us to characterize them as local fishers, who exploited a range of economically important ecological niches on the coast, through hunting, plant gathering and management, and possibly also cultivation.

A growing body of evidence suggests that access to resource-rich aquatic environments may have offered competitive advantages to mixed economies in southeastern South America during the Middle and Late Holocene and that the intensification of agriculture was not a necessary condition for population growth and reduced residential mobility in this region. Examples of this are seen in the Paraná River delta (Apollinaire and Bastourre, 2016; Bonomo et al., 2019), the La Plata Basin (Iriarte et al., 2017), and tropical and subtropical coastal areas of Brazil (Pezo-Lanfranco et al., 2018b; Scheel-Ybert and Boyadjian, 2020). To some groups, the importance of aquatic resources transcended nutritional values and extended to symbolic and ideological spheres, as demonstrated by the sophisticated stone sculptures (zooliths) of southern Brazilian sambaquis that have also been found in *cerritos* (Prous, 2018, 1974). The stable isotope data from mound builders of the southern Patos Lagoon corroborate this regional picture and reveal that distinct forms of mixed economies not only interacted in Pontal da Barra but contributed to shaping the cultural landscape of this region during the Late Holocene.

Conclusion

This study combined the analysis of bulk collagen stable carbon and nitrogen isotopes with Bayesian Stable Isotope Mixing Models (BSIMMs) to reconstruct the paleodiet of Late Holocene human remains from a number of earthen-mounds in the southern Patos Lagoon. Our results revealed considerable dietary variability among mound builders. The high $\delta^{13}C$ and $\delta^{15}N$ values detected in some individuals reflect diets dominated by marine proteins such as marine/estuarine fish. However, there is evidence of diets primarily based on terrestrial/freshwater resources from C_3 ecosystems, including terrestrial animals, plants and potentially freshwater fish, as well as samples with intermediate $\delta^{13}C$ and $\delta^{15}N$ values related to mixed C_3 terrestrial/freshwater and marine/estuarine diets. The presence of individuals with different diets suggests that Pontal da Barra was a central place for social networking between resident and non-resident individuals. This study reinforces the view that resource-rich aquatic environments offered conditions for the endurance of mixed economies in southeastern South America during the Late Holocene.

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