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Elucidating pre-columbian tropical coastal adaptation through bone collagen stable isotope analysis and bayesian mixing models: insights from Sambaqui do Moa (Brazil)

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Keywords:

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ABSTRACT

Over the last decades, the sambaquis from the Saquarema region (Rio de Janeiro, Brazil) have been subjected to systematic archeological investigations aimed at elucidating the lifestyles of Holocene populations that inhabited these coastal environments. The present study is an extension of these investigations and aims to elucidate the dietary information of 11 human individuals excavated from Sambaqui do Moa through the analysis of bone collagen stable carbon and nitrogen isotope compositions and Bayesian mixing models. The results reveal that marine and brackish fauna played dominant roles as sources of dietary proteins for all the individuals, while C_3 plants were an important contributor to their overall diet, supporting the view that these groups had a mixed economy. Although all individuals presented a reasonably homogeneous diets, the isotopic results revealed noticeable differences between the two occupation phases, suggesting a dietary shift through time. There were also small differences between males and females. Further dietary studies on sambaquis from Saquarema are important to refine and expand our understanding of subsistence strategies in tropical coastal environments of eastern South America.

Palabras clave:

sambaqui;
isótopos estables;
modelos de mezcla Bayesianos;
dieta;
arqueología brasilera.

RESUMEN

Durante las últimas décadas, los sambaquis de la región de Saquarema (Río de Janeiro, Brasil) han sido objeto de investigaciones arqueológicas sistemáticas destinadas a dilucidar el estilo de vida de las poblaciones del Holoceno que habitaban estos entornos costeros. El presente estudio es una extensión de estas investigaciones, y tiene como objetivo traer información dietética de 11 individuos humanos excavados en Sambaqui do Moa a través del análisis de composiciones de isótopos estables de carbono y nitrógeno de colágeno óseo combinado con modelos de mezcla Bayesianos. Los resultados revelan que la fauna marina y salobre desempeñó un papel dominante como fuentes de proteínas dietéticas para todos los individuos, pero las plantas C_3 contribuyeron de manera importante a su dieta total, lo que respalda la opinión de que estos grupos tenían una economía mixta. Aunque todos los individuos presentaron una dieta razonablemente homogénea, los resultados isotópicos indican pequeñas diferencias entre las dos fases de ocupación, lo que sugiere un cambio en la dieta. También hubo pequeñas diferencias entre hombres y mujeres. Es importante realizar más estudios dietéticos sobre los sambaquis de Saquarema para perfeccionar y ampliar nuestra comprensión de las estrategias de subsistencia en los entornos costeros tropicales del este de América del Sur.

1- Introduction

Artificial shell mounds known as Sambaquis are commonly found along the tropical and subtropical coast of Brazil and are unique evidence of long-term human adaptation to coastal environments in eastern South America. Since the 19th century the understanding of the construction of Sambaquis have shifted over time, directly influencing interpretations about the nature of the sites and the groups that built them (Lima, 1999-2000; Gaspar et al., 2008, 2014). Recent theoretical contributions associate most sambaquis with funerary and ceremonial practices, and interpret the material culture embedded in them (*e.g.* shells, fish bones, lithics, plant remains and bone tools) as the product of the intentional deposition aimed at increasing the monumentality and the ritual connotation of the sites, and less as the result of ordinary domestic activities (Gaspar et al., 2014; Klökler, 2014). Significantly, marine

and brackish organisms (fish, shellfish, marine mammals, sea birds) were constants in sambaqui architecture and have played a role in the intricated symbolic and utilitarian spheres of these groups, pointing to a strong relationship between humans and aquatic ecosystems. This relationship brings forth relevant questions regarding the economy and dietary practices of these groups. Zooarchaeological and archaeobotanical analyses have shown that the economy of sambaqui groups was built substantially on a variety of fish (bony and cartilaginous), along with marine invertebrates, terrestrial and marine mammals, birds and plants (Figuti, 1993; Kneip, 1994; Costa et al., 2012; Guimarães, 2013; Boyadjian & Eggers, 2014; Boyadjian et al., 2016; Scheel-Ybert, 2018), with more recent studies highlighting possible management and cultivation of plants (Scheel-Ybert et al., 2003; Scheel-Ybert & Boyadjian, 2020). Osteological analyses of human individuals have also provided crucial evidence of dietary practices and other aspects related to lifestyle. In particular, stable carbon and nitrogen isotope analysis has contributed to clarifying the role of marine organisms to diet, showing that most individuals relied primarily on fish from coastal environments as sources of dietary proteins (De Masi, 2009; Colonese et al., 2014; Bastos et al., 2014; Oppitz et al., 2018; Pezo-Lanfranco et al., 2018a). So far, however, most sambaqui isotopic studies were performed on sites located in Southern Brazil, mainly in Santa Catarina state, where marine productivity is high and thus a relatively heavy reliance on marine resources could be expected. By contrast, little is known from coastal areas further north, particularly in tropical environments. The present study combines bone collagen stable carbon and nitrogen isotope analysis of human individuals from Sambaqui do Moa, located in Saquarema in the state of Rio de Janeiro, with Bayesian mixing models. The aims of this work are to determine the individual diets of these individuals, and to expand our understanding of pre-Columbian socio-ecological trajectories in coastal areas of tropical Brazil during the Late Holocene.

2- Archaeological context

Saquarema is a municipality located on the coast of the state of Rio de Janeiro, southeastern Brazil. With an average land elevation of 2 m above sea level, the area lies within a humid tropical climate with an average temperature of 23.2 ºC and thermic amplitude around 5 ºC. The city is located between the Atlantic Ocean and the Serra do Mar mountain range, with a lagoon complex, fed by both fluvial and oceanic waters, along its coastline covering an area of 23.8 km^2. In addition to the beaches and lagoons, Saquarema also encompasses mangroves, dunes, sandbank vegetation, and the Atlantic Forest. The different ecosystems in the region favor a rich and diversified fauna and flora (Kneip et al., 1997; Silveira, 2001; Borges, 2009).

The first archaeological studies in Saquarema date from the early 1930s, and become more systematic a several decades later, during the 1980-90s, being led by the archaeologist Lina Kneip from the National Museum of the Federal University of Rio de Janeiro (Beltrão, 1976; Kneip et al., 1991; Guida, 2019). 19 sambaquis were found in Saquarema, generally located around the lagoons, spanning an occupation of approximately 5,000 years. The earliest site is Sambaqui de Itaúnas, dated between 6,631 and 6,304 BP, and the most recent, Sambaqui da Pontinha, providing radiocarbon dates ranging from 1,810 to 1,542 BP (Barbosa-Guimarães, 2011; Guida, 2019). The end of the sambaquis in the region was the result of processes of cultural modification that lasted 1,000 years and that are associated with a series of factors, including environmental changes and contact with ceramist groups from the interior (Barbosa-Guimarães, 2011). The Sambaqui do Moa (Figure 1), located near the eastern shore of the Saquarema lagoon complex (coordinates 22º55'44" S and 42º29'07" W), formed a 2 m high oval shape, with an area of 2,800 m^2. The site was excavated in two different periods; first in 1988 coordinated by Lina Kneip, and then in 1998 by Maura Imazio da Silveira (Kneip & Machado, 1993; Silveira, 2001). A total of 169 m^2 were excavated, 49 m^2 during the first excavation and 120 m^2 during the second (Kneip & Machado, 1993; Barbosa-Guimarães, 2011).

All skeletal remains analyzed in the present study are from the 1988 excavation, and contextual information can be found below. According to Kneip and Machado (1993), the excavation revealed two main archaeological deposits, each one presenting a depth of 30 to 40 cm and both with funerary and combustion structures. The conventional radiocarbon dates obtained are 3,610 ± 190 BP for layer I, and 3,960 ± 200 BP for layer II. Both dates were sampled from shells; however, it was not specified if the species were associated with a marine or brackish environment. In order to calibrate these dates, we considered both samples to have a marine provenance and applied the MARINE20 curve (Heaton et al., 2020) at 100 % with a ΔR = -54 ± 101, obtained by the average ΔR value of the 10 closest reference points (from latitude 22.82° S to 23.95° S) (Alves, 2015; De Masi, 1999; Eastoe, 2002). Layer I presented results of 3,986 to 2,850 cal. BP, and layer II from 4,491 to 3,304 cal. BP at 95% confidence interval.

A total of 61 individuals were excavated from Sambaqui do Moa during the 1988 and 1998 campaigns, with 33 of individuals excavated during the first

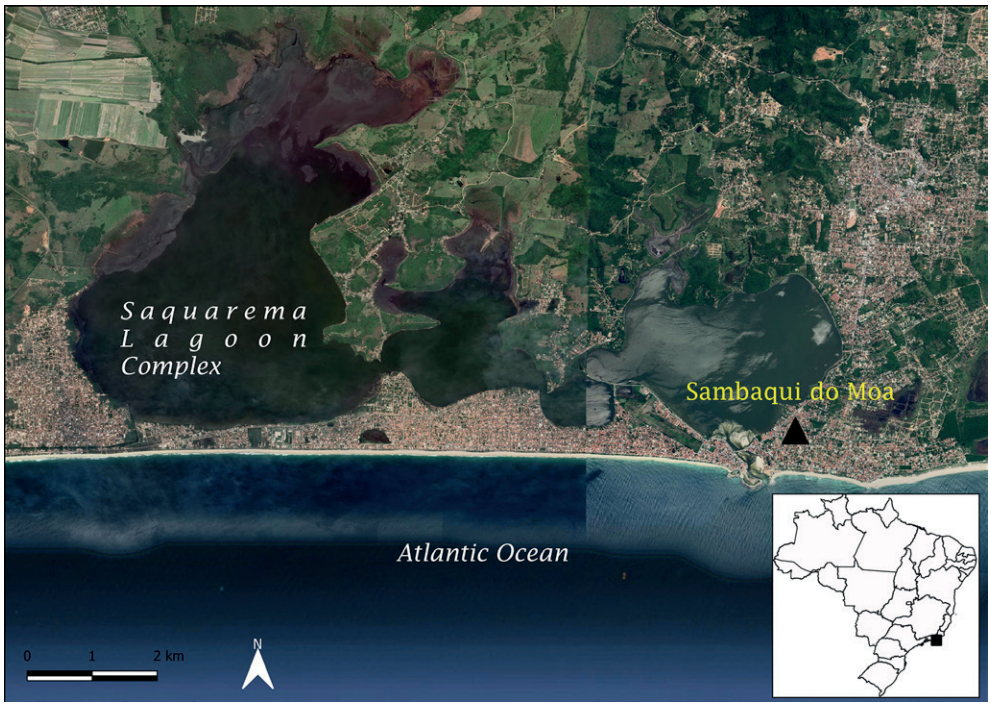

Figure 1. Locality of the Sambaqui do Moa in Saquarema, Rio de Janeiro state, Brazil. Map created using QGIS software, version 3.2. Satellite image by Google, acquired through QuickMapServices plugin for QGIS.

intervention. According to Kneip and Machado (1993), 96% of the first intervention burials were primary depositions and 4 % secondary. Most individuals were buried in dorsal decubitus or ventral decubitus positions, while two were found in a semi-flexed position. Most burials from both layers presented reddish-colored clay sediment involving the skeletal remains. Some burials also presented grave goods such as polished and flaked stone axes, and a few mollusk species found exclusively in relation to burials, like *Lyropectem nodosus*, *Cypraea zebra* and *Tonna galea*.

The identification of the faunal remains revealed 92 taxa, and while most of them are of bivalves (24), including for example *Anomalocardia brasiliana*, *Ostrea* sp. and *Lucina pectinate*, and several gastropods (21), there was a significant diversity of other groups: 14 taxa of fish, including elasmobranchs, 15 mammals, 11 birds, three reptiles and one amphibian (Kneip, 1994; Silveira, 2001; Guida, 2016). The fauna collected from Sambaqui do Moa suggest that a range of ecosystems (sea, mangrove, lagoon, river, sandbank, and Atlantic Forest) were exploited to meet utilitarian, ritual and funerary demands.

Although no palaeobotanical studies have been performed on material from Sambaqui do Moa, studies on charcoal remains collected from two other sambaquis from Saquarema (Sambaqui da Beirada and Sambaqui da Pontinha) identified species related to the Atlantic Forest, sandbank vegetation and mangrove, including some plants potentially used as food (*e.g. Dioscorea* sp.). While the information regarding plant use in Saquarema are still scarce, there is clear evidence that sambaqui groups from southern Brazil were accessing plants such as yams, palm trees, sweet potato, some fruits and even maize in some later sites, as evidenced by starch grain and phytolith analyses on dental calculus (Wesolowski, 2007; Wesolowski et al., 2010; Boyadjian et al., 2016).

Sambaqui do Moa also presented several lithic, bone and shell artifacts that were related to foraging and food processing, such as axe blades, points, scrapers, and hooks (Kneip, 1994). Ceramic fragments were also found in the upper portion of the most recent archaeological layer (layer I), but their chronology is uncertain. The ceramics presented no texture or decoration and were associated with groups from the interior (Tradição Una) that occupied the region in a later period (Kneip, 1994; Barbosa-Guimarães, 2011).

Previous osteological analyses, and more specifically oral health analyses, on human individuals from Sambaqui do Moa indicated that the individuals presented a high frequency of severe dental wear, a low frequency of dental caries, and a high prevalence of dental calculus, all of which that point to an abrasive, high protein diet with low carbohydrate consumption (Machado & Kneip, 1994; Guida, 2019). This pattern is also found in most sambaquis from southern Brazil, supporting the hypothesis of a high consumption of marine fauna, especially fish. The oral health studies

from Moa also revealed differences between male and female individuals, with periapical cavities and *ante mortem* tooth loss occurring more frequently in women (Guida, 2019). As both processes can be related to oral trauma (Dias & Tayles, 1997; Hillson, 2008; Waldron, 2008), these results suggest that females from Sambaqui do Moa had a more intense use of the buccal apparatus compared to males, which can be associated to food processing and paramastigatory activities. Musculoskeletal stress markers analyzed on individuals from Moa also indicated different physical activities between males and females (Rodrigues-Carvalho & Mendonça de Souza, 2005).

3- Material and methods

3.1 Collagen extraction and stable isotope analysis

The human skeletal remains excavated from Sambaqui do Moa were part of the Biological Anthropology collection of the National Museum of the Federal University of Rio de Janeiro, Brazil. All the bone samples were collected prior to the tragic fire of 2018, that severely damaged the building as well as the osteological collections. For isotopic analysis, we selected approximately 1 gram of rib fragments from 11 individuals from the 1988 excavation. Children were excluded from the study to avoid any breastfeeding interference with the isotopic results. Sex and age were previously estimated by Kneip and Machado (1993). A total of five males, five females and one individual with unidentified sex were selected for analysis.

All rib fragments were physically cleaned with a toothbrush and a scalpel with distilled water, and then sonicated for 20 minutes. Collagen extraction followed Bastos et al. (2014, 2015), adapted from Ambrose (1990). Initially the samples were immersed in 0.1 M NaOH for 24 hours to remove humic acids. After rinsing with deionised water, the samples were demineralized in 0.25 M HCl for at least 72 hours, they were rinsed again and followed by another treatment with 0.1 M NaOH for 24 hours. Lipids were removed with a mixture of chloroform, methanol and water (2:1:0.8) for 24 hours. After rinsing, the collagen pseudomorphs were dried overnight in an oven at 60°C. The samples were stored individually in centrifuge microtubes.

The isotopic analyses were performed at the Geochronology Laboratory of the University of Brasilia, using a MAT Delta V Plus (Thermo Finnigan) mass spectrometer coupled to a Flash 2000 CHN analyzer. Around 0.6 to 0.8 mg of collagen was weighted and placed in tin capsules. For each sample, the carbon and nitrogen percentage and C:N molar ratio were determined to monitor the collagen preservation. The $\delta^{13}C$ e $\delta^{15}N$ were calibrated using NIST 8542 ($\delta^{13}C$ = -10.33 ‰ ± 0.02), USGS41 ($\delta^{13}C$ = 36.47 ‰ ± 0.22; $\delta^{15}N$ = 47.75 ‰ ± 0.63) and an internal standard ($\delta^{13}C$ = -40.88 ‰ ± 0.09; $\delta^{15}N$ = -0.13 ‰ ± 0.2). The data were generated in V-PDB for carbon and atmospheric (AIR) for nitrogen ratios.

3.2 Statistical analysis and Bayesian stable isotope mixing models

Bayesian mixing models express food source contribution probability distributions in consumer tissues that incorporate uncertainties related to: 1) differences in food macronutrient composition and their respective isotopic values; 2) the varying degrees of routing of dietary compounds to consumer tissue; and 3) diet-to-tissue isotopic offsets (Fernandes et al., 2015; Phillips et al., 2014). Here we used Bayesian mixing models in FRUITS 3.1, selecting a concentration-dependent and routed model. We assumed that nitrogen isotopes were 100% sourced from proteins, while carbon isotopes derived mostly from proteins (74 ±4%) but also from carbohydrates and lipids (26%) (Fernandes et al., 2012). The model assumptions and parameters were essentially the same as those used by Toso et al. (2021). The isotopic composition of macronutrients in the faunal baseline were calculated from the average bulk collagen $\delta^{13}C$ and $\delta^{15}N$ values of the terrestrial mammals, freshwater fish and marine-brackish resources (fish, sea mammals) reported by Toso et al. (2021) for Babitonga Bay. We also used the following fractionations between macronutrient isotope composition - for terrestrial mammals: -2‰ ($\Delta^{13}C_{protein\text{-}collagen}$), -8‰ ($\Delta^{13}C_{lipids\text{-}collagen}$) and +2‰ ($\Delta^{15}N_{protein\text{-}collagen}$); freshwater fish: -1 ‰ ($\Delta^{13}C_{protein\text{-}collagen}$), -7‰ ($\Delta^{13}C_{lipids\text{-}collagen}$) and +2‰ ($\Delta^{15}N_{protein\text{-}collagen}$); marine-brackish fish and sea mammals: -1 ‰ ($\Delta^{13}C_{protein\text{-}collagen}$), -7‰ ($\Delta^{13}C_{lipids\text{-}collagen}$) and +2‰ ($\Delta^{15}N_{protein\text{-}collagen}$). For plants we used the average $\delta^{13}C$ (-29.2 ± 3.0‰) and $\delta^{15}N$ (+1.1 ± 2.0‰) values of modern fruits (n = 30), roots (n = 5) and palm-heart (n = 13) collected in national parks in the southeastern Atlantic Forest between 2010 and 2012 (Galetti et al., 2016). Plant $\delta^{13}C$ values were corrected for the Suess effect (+2 ‰) using the $\delta^{13}C$ value of the atmosphere in 2010 (-8.4 ‰) (Hellevang & Aagaard, 2015). Plant macronutrient $\delta^{13}C$ values were then corrected for the offsets of -2 ‰ ($\Delta^{13}C_{bulk\text{-}protein}$) and +0.5 ‰ ($\Delta^{13}C_{bulk\text{-}carbohydrate}$). We assumed that the $\delta^{15}N$ value of plant protein was the same as the average bulk plant $\delta^{15}N$ value. A conservative uncertainty of 1 ‰ was used for the $\delta^{13}C$ and $\delta^{15}N$ values of macronutrients. Diet-to-collagen $\delta^{13}C$ offset (+4.8 ± 0.5 ‰) and $\delta^{15}N$ offset (+5.5 ± 0.5 ‰) were taken from Fernandes et al. (2016). Dietary estimations considered a conservative acceptable range for protein intake of >5% and

<45% (Fernandes et al., 2014). Box and whiskers plots summarizing the posteriors of FRUITS estimates (Markov chains) were generated using the R statistical software environment (version 4.0.3) and the package ggplot2 (version 3.3.2) (Wickham, 2016), with McGill et al (1978) variations of Box Plots. The horizontal lines correspond to the median, and the hinges to the 25th (Q1) and 75th (Q3) percentiles. The whiskers extend from the hinge to the smallest or largest observation greater than or equal to -1.5 * IQR (interquartile range) or less than or equal to +1.5 * IQR, respectively; outliers were excluded.

4- Results

The isotopic data of all eleven individuals analyzed from Sambaqui do Moa are shown in Table 1.

All rib fragments presented a good level of collagen preservation. The wt% C and wt% N ranged from 31.6 % to 40.2 % and 10.5 % to 13.3 %, respectively, with C:N ratios between 2.9 and 3.2, consistent with intact collagen according to well established criteria (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). The δ^{13}C values ranged from -15.6 ‰ to -10.7 ‰ (mean -12.8 ‰ ± 0.36) and the δ^{15}N values between +15.0 ‰ and +17.3 ‰ (mean +16.3‰ ± 0.22). Comparing the δ^{13}C and δ^{15}N values of human bone collagen with that of marine, freshwater and terrestrial faunal remains published by Toso *et al.* (2021) for southern Brazil (Babitonga Bay), the results indicate that humans acquired most of their dietary proteins from marine organisms, such as fish. This is broadly confirmed by the Bayesian mixing models (Figure 2), which revealed that marine resources contributed to the largest portion of dietary proteins (median 63.3%), followed by terrestrial mammals (median 13%), C_3 plants (median 10.1%), and to a lesser extent freshwater fish (median 8.6%) (Figure 2). Regarding the dietary

Burial/individual no.	Layer	Sex	Age	δ^{13}C	δ^{15}N	%C	%N	C:N
1	I	Male	30-35	-13.29	16.54	35.89	12.31	2.9
2a	I	Female	20-22	-15.58	15.68	35.37	11.98	3.0
2b	I	Unknown	Adult	-12.90	15.88	33.85	11.38	3.0
5	I	Male	39-44	-13.74	17.30	36.43	11.31	3.2
7	II	Female	20-22	-12.01	15.27	34.07	11.37	3.0
10	II	Male	30-35	-12.12	16.38	34.69	12.04	2.9
11	II	Male	27-30	-12.46	17.11	40.27	13.35	3.0
20	II	Female	18-19	-12.48	15.05	31.62	10.53	3.0
22	II	Male	Adult	-10.68	15.94	33.82	11.62	2.9
23	II	Female	Adult	-12.98	16.85	35.17	11.73	3.0
26	Unknown	Female	Adult	-12.88	16.85	37.72	12.74	3.0

Table 1. Bone collagen δ^{13}C and δ^{15}N values of 11 individuals from Sambaqui do Moa, with age and sex estimations, and the archaeological layer where the burials (individuals) were found.

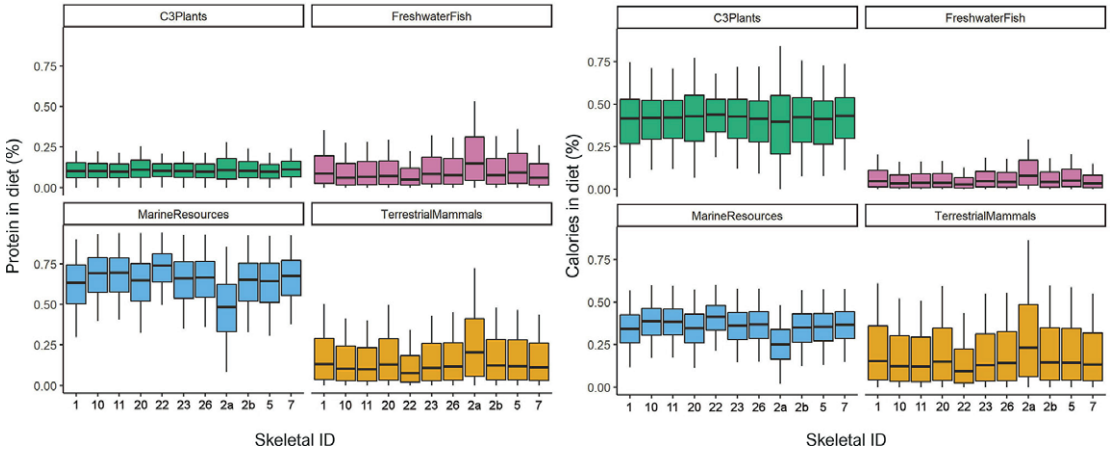

Figure 2. Box and whiskers plots summarizing the probability distribution of the relative protein and calories contributions from different food sources to the diets of the analyzed individuals (Skeletal ID) from Sambaqui do Moa. The horizontal lines correspond to the median and the hinges to the 25th [Q1] and 75th [Q3] percentiles.

calories (broadly equivalent to the whole diet in the model outputs (Fernandes et al., 2014), the model outputs indicated that C_3 plants played an important role (median 42%), but marine resources were also significant (median 35.6%), followed by terrestrial mammals (median 14.2%) and freshwater fish (median 4.3%).

Comparing both sexes (Figure 3), there was no significant difference in the $\delta^{13}C$ values (Mann-Whitney test, $p < 0.6761$) and $\delta^{15}N$ values (Mann-Whitney test, $p < 0.20869$). However, the two lower nitrogen isotope values (15.27 ‰ and 15.05‰) were from female individuals (burials 7 and 20). Also, the most negative $\delta^{13}C$ value (-15.58 ‰) was from a female individual (burial 2a). These results might suggest some small differences in the diet between men and women at Sambaqui do Moa, as also observed in other sites in southern Brazil (Colonese et al. 2014; Bastos et al., 2015; Pezo-Lanfranco et al. 2018a), even though the results were not statistically significant. Interestingly, significant differences were observed between the $\delta^{13}C$ values of the individuals buried in layer 1 and those in layer 2 (Mann-Whitney test, $p < 0.025181$), although no significant differences were seen for the $\delta^{15}N$ values (Mann-Whitney test, $p < 0.74912$). Essentially, individuals buried in layer 1 had lower $\delta^{13}C$ values compared to the earlier archaeological occupation represented by layer 2, which can be taken as indicative of a discrete dietary shift toward a more terrestrial diet over the time. Comparing both periods, Bayesian mixing models also indicate some differences in the food sources between the individuals buried in the two archaeological layers (Figure 4).

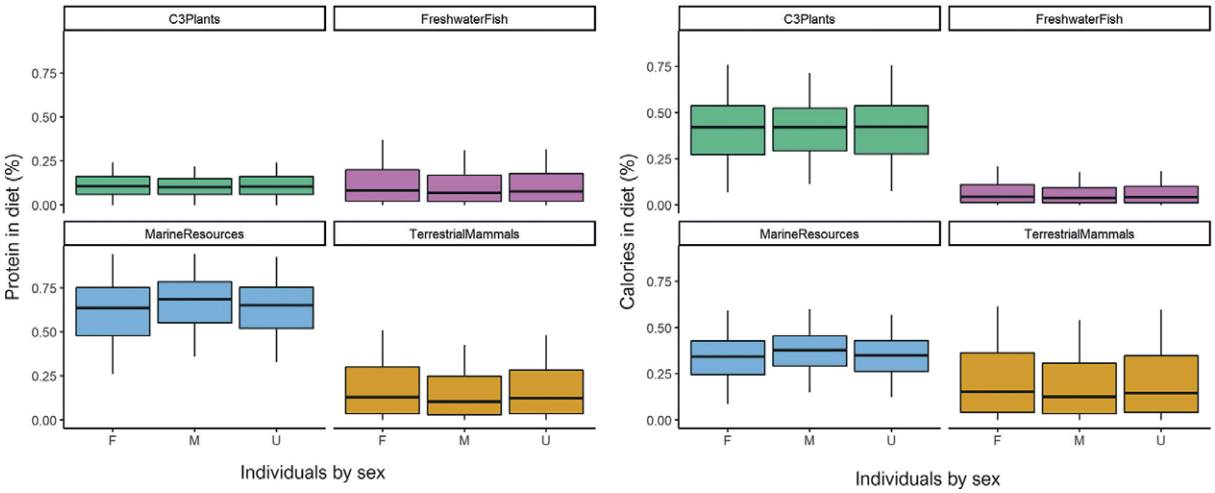

Figure 3. Box and whiskers plots summarizing the probability distribution of the relative protein and calories contributions from different food sources to the diets of the females (F), males (M) and unidentified (U) individuals from Sambaqui do Moa. The horizontal lines correspond to the median and the hinges to the 25th [Q1] and 75th [Q3] percentiles.

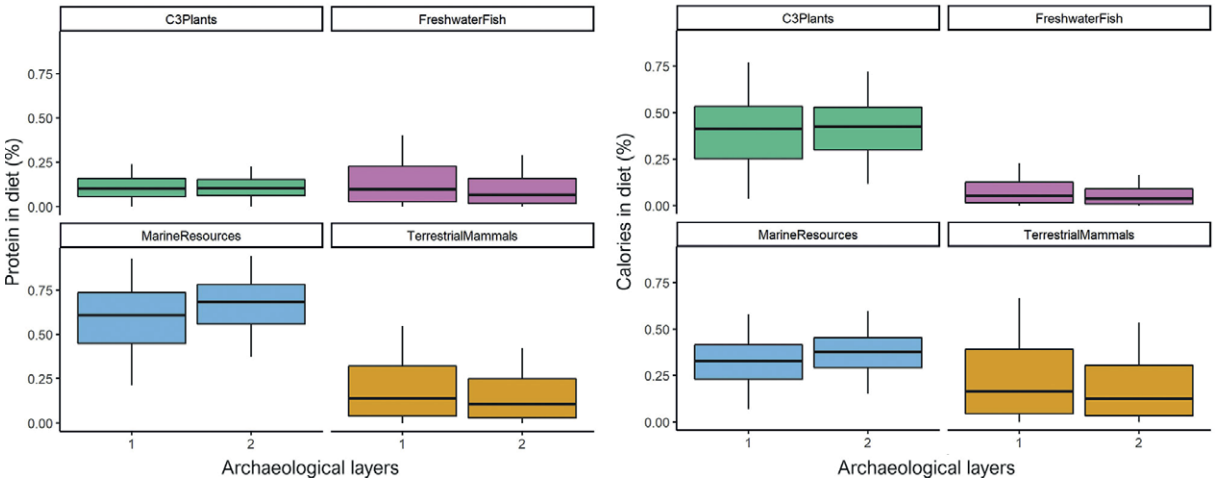

Figure 4. Box and whiskers plots summarizing the probability distribution of the relative protein and calories contributions from different food sources to the diets of the individuals from Sambaqui do Moa excavated from layer 1 versus those from layer 2. The horizontal lines correspond to the median and the hinges to the 25th [Q1] and 75th [Q3] percentiles.

5- Discussion

Carbon and nitrogen stable isotope data combined with Bayesian mixing models can provide a wider perspective on subsistence strategies of past human groups, estimating the contribution of different food sources available for the studied population. Regarding the 11 individuals from Sambaqui do Moa, the results reveal that most of their dietary proteins were secured from marine fauna, as previously seen in individuals from southern areas of the Atlantic Forest and the Pampa biome of Brazil (Bastos et al., 2014, 2015; Chanca et al., 2021; Colonese et al., 2014; Oppitz et al., 2018; Pezo-Lanfranco et al., 2018a, 2018b; Toso et al., 2021). This is also supported by the faunal record recovered from the site. For instance, from 92 faunal species recovered during the excavation of Sambaqui do Moa, 52% are marine and include several economically important fish species, such as the whitemouth croaker (*Micropogonias* sp.) and members of the Ariidae family (Guida, 2016). Also, the less expressive contributions of terrestrial mammals and freshwater fish revealed by the isotopic and mixing model results are consistent with the zooarchaeological findings from Sambaqui do Moa.

Overall, the individuals from Sambaqui do Moa did not present significant intra-site variability in diet, as they all showed a similar proportion of the different food sources. Nevertheless, the subtle differences between individuals from layer 2 (early) and 1 (later) suggest some dietary changes were occurring, notably an increase in a terrestrial-based diet in more recent times. However, the nature of such change remains unclear. The stratigraphic information provided by Kneip (1994) does not support any significant differences between the identified layers that corroborate a more terrestrial diet during the later occupation. The presence of ceramic fragments in the uppermost part of the site would not be associated with the burials as they are culturally related to groups that occupied the region much later. An alternative to an increased intake of terrestrial resources could be a change in the stable carbon isotope composition of the local food web. For example, long-term consumption of brackish resources in low salinity waters could hypothetically shift the $\delta^{13}C$ of the consumer's tissue without necessarily altering the $\delta^{15}N$ values. Such a scenario could potentially result from significant coastal environmental changes, and increased exposure to freshwater circulation. Stable carbon and nitrogen analysis of contextual marine faunal remains would be necessary to validate this hypothesis.

The isotopic data also point to small differences between sexes. The relatively lower $\delta^{15}N$ values from two female individuals is indicative of a slightly lower trophic level diet, probably related to either a more terrestrial diet or more marine organisms from lower trophic levels than most males. Differences between males and females were also found in oral health studies. According to Guida (2019), female individuals from Sambaqui do Moa presented a higher degree of tooth wear than males, with severe wear frequencies of 53.7% and 43.3%, respectively. In addition, males also showed a higher frequency of light wear when compared to females, 30% against 13.2%. Frequency and prevalence of other physiopathologies, such as *ante mortem* tooth loss and periapical cavities, were also higher for females, but these results were not statistically significant. Therefore, both isotopic and oral health studies point towards the existence of small differences in the diets of males and females. Similar results have been obtained for sites in the southern Brazil and tentatively interpreted as food restrictions among members of the community and/or the presence of non-local individuals assimilated through post-marital residential practices (Colonese et al., 2014; Pezo-Lanfranco et al., 2018a). Further excavations and isotopic studies of sambaquis in Saquarema can enlighten more aspects of these complex groups in the region.

Other than the strong reliance on marine and brackish organisms as sources of proteins, the Bayesian mixing models highlighted the relevance of plants as energetic sources for the analyzed individuals. The isotopic data regarding dietary calories indicate that C_3 plants were an important contributor to the total diet of the Sambaqui do Moa population. This scenario is compatible with recent archaeobotanical studies in other sambaquis, where the presence and distribution of some wild and domesticated plants, such as yams and palms, suggest that these human groups were variably engaged in food production, including plant management and cultivation for a range of purposes (food, medicine, mortuary practices, artifact production, etc.) (Sheel-Ybert & Boyadjian, 2020). In addition, previous studies combining stable isotopes and Bayesian mixing models have shown that plants were important dietary constituents to Late Holocene coastal populations along the Brazilian coast (Chanca et al., 2021; Colonese et al., 2020; Pezo-Lanfranco et al., 2018a; Toso et al., 2021), and in some cases plant consumption was intensified to levels similar to horticulturists (Pezo-Lanfranco et al., 2018a). Our study supports the emerging consensus that Sambaqui builders developed distinct mixed economies, variably founded on both fishing-hunting-gathering and horticulture (Scheel-Ybert, 2000; Boyadjian et al., 2016; Pezo-Lanfranco et al., 2018a; Sheel-Ybert & Boyadjian, 2020).

Our understanding of the isotope ecology of Brazilian sambaquis is strongly biased towards individuals

recovered in subtropical regions, typically below 23 ° latitude south (Toso et al., 2021). Here, we extend this knowledge to individuals adapted to tropical coastal environments, more precisely in the Saquarema region. We have shown that the general dietary patterns observed in the southern subtropical regions (*e.g.* reliance on marine resources, plant consumption) are also observed in our study area, reinforcing the view that coastal environments were crucial for the successful development of locally adapted mixed economies in southern South America.

6- Conclusion

Over the last decades there has been an increase in archaeological studies focusing on sambaqui populations that inhabited the Brazilian coast. In this vein, stable isotopes are contributing to elucidate some key aspects of the diet and overall economy of these groups, which is more recently understood as highly diverse and complex. The results herein, from individuals from Sambaqui do Moa, one of the few sambaquis studied in southeastern Brazilian, illustrated part of this complexity, showing a high reliance on marine food sources but also the important role of plants, supporting the hypothesis of a mixed economy. It is worth noting that a great part of the interpretation of the Sambaqui do Moa isotopic results has been done using Bayesian mixing models, which relies on sampling a significant amount of different food sources that would be accessible to the studied population. While there has been some relevant work on building an archaeological fauna isotopic database for the coast of Santa Catarina state, this data is still lacking for the southeast region of Brazil, including the coast of Rio de Janeiro state. Therefore, the development of a southeastern isotopic archaeological fauna database would improve the accuracy of the dietary models for the sambaquis and other groups that inhabited the region in the past. Lastly, regarding the skeletal remains of Sambaqui do Moa and some other sites from Saquarema, they were part of the Biological Anthropology collection of the National Museum and were severely damaged by the fire that affected the main building of this institution in 2018. This tragedy compromised any future studies of these (and many other) osteological series, including the isotopic analysis of the other individuals from Moa which could clarify issues such as dietary differences between male and females and between the occupation periods. While new archaeological excavations in Saquarema could address some of these issues, the studies performed with the skeletal remains that were affected by the fire now belong to a different perspective, forming part of the memory of this important Brazilian archaeological heritage.

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