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# Stable isotopes and diet in complex hunter-gatherers of Paraná River Basin, South America

**Flavia Ottalagano[1] · Daniel Loponte[1]**

**Abstract** This paper discusses isotopic analyses carried out on human bone samples corresponding to complex hunter-gatherers from the Paraná River basin in northeastern Argentina. Based on the $\delta^{15}N$ and $\delta^{13}C$ values obtained, the dietary patterns of 23 individuals recovered from pre-Hispanic archaeological sites of the Late Holocene are characterized. These sites are associated with the archaeological unit generically called Goya-Malabrigo, which is identified in South America over the entire middle basin of the Paraná River and on a part of the lower river. The values obtained show diets based on depleted $\delta^{13}C$ proteins linked to the $C_3$ photosynthetic pathway, which is in turn consistent with the isotopic values detected in the main food sources of these human groups: freshwater fishes and continental mammals. The $\delta^{15}N$ values and the results of a multivariate model also indicate a low consumption of plant foods. Although archaeobotanical information from the area has reported maize and other cultigens, the diet of the individuals studied was based on animal proteins, especially on freshwater fish. Moreover, the intake of maize was not isotopically detectable in the human bones analyzed.

## Introduction

The middle and lower Paraná River basin was occupied at least within the time span of 2400–500 years BP by human groups whose economic base was centered on hunting, fishing, and gathering. These activities were framed within a context of substantial and increasing social and technological complexity, with numerous adaptations driven by human population density. These societies developed certain features such as central residential camps with some stability, cemeteries used for generations, storage systems, territoriality, ownership of territories and productive patches, intensification in the exploitation of the environment, and development of a complex trade network (Ceruti 2003; Loponte and Acosta 2006; Loponte 2008). Among the intensification-oriented behaviors, we must emphasize the extensive use of pottery vessels to process food and enhance the ability to extract nutrients by boiling (Acosta 2005; Loponte 2008). The use of pottery for this purpose was sufficiently intense that almost all of the pottery recorded shows evidence of fatty acids associated with vegetable and/or animal foods (Naranjo et al. 2010; Ottalagano 2013; Pérez et al. 2013). These populations also developed a complex kit of bone and stone artifacts, including a sophisticated weapon system that included the bow and arrow, hand-held spears, spear-throwers, bola stones, detachable harpoons, and fishing nets as well as traps (Buc 2010; Ceruti 2003; Loponte and Acosta 2006; Loponte 2008).

Freshwater fishes were an important part of the diet, especially towards the end of the Late Holocene. The subsistence systems also included the exploitation of deer (*Blastocerus*

✉ Flavia Ottalagano
flaviaott7@gmail.com

Daniel Loponte
dloponte@inapl.gov.ar

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto Nacional de Antropología y Pensamiento Latinoamericano (INAPL), 3 de febrero 1387, Buenos Aires (C1426BJN), Argentina

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*dichotomus*; *Mazama gouazoubira*; *Ozotoceros bezoarticus*), freshwater clams, and small-to-medium rodents (*Myocastor coypus*, *Cavia aperea*) (Acosta 2005; Acosta et al. 2010, 2013a, b; Arrizurieta et al. 2010; Barboza and Píccoli 2013; Bonomo et al. 2011a; Ceruti and González 2007; Cione and Tonni 1978; Loponte and Acosta 2004; Loponte 2008; Loponte et al. 2012; Musali 2010; Nóbile 1993; Ottalagano et al. 2015; Pérez Jimeno 2007; Salemme et al. 1987; Salemme and Tonni 1983; Santiago 2004; Sartori and Colasurdo 2012; Tonni et al. 1985, among others). Also, one large rodent (*Hydrochaeris hydrocaheris*) was exploited, although seemingly only marginally since the archaeological representation is small, and hunting of this animal largely appears to have had a symbolic meaning, especially in the Lower Paraná River basin (Acosta 2005; Loponte 2008; Salemme 1987). Agroforestry of local palms and the development of sporadic and small gardens have also been postulated (Acosta and Ríos Román 2013; Loponte 2008). In relation to these activities, the archaeological presence of phytolith and starch grains assignable to species such as *Cucurbita sp.* (squash), *Prosopis* sp. (algarrobo), *Phaseolus* sp. (beans), *Ipomea* sp. (sweet potato), *Zea mays* (maize), and palms (*Syagrus romanzoffiana*) has been reported (Acosta et al. 2013b; Bonomo et al. 2011a, b, c; Colobig and Ottalagano 2015; Cornero and Rangone 2015; Sánchez et al. 2013; Zucol and Loponte 2008).

One of the archaeological units corresponding to complex hunter-gatherers in the region is generally known as "Goya-Malabrigo" (Ceruti 2003; Rodríguez 2001; Serrano 1946, 1972), and although this unit is still somewhat ill-defined, its main diagnostic feature is the presence of pottery with zoomorphic appendages, usually representing different types of birds (especially parrots). These figures may have constituted symbols of group identity within a context of growing social complexity (Ottalagano 2013). On numerous occasions, archaeologists studying the region have argued for the possibility that these societies had developed small-scale horticultural practices, often based on ethnohistorical sources but also as suggested by certain archeological features (Ceruti 2003; Loponte 2008; Rodríguez 2001; Serrano 1972). Archaeobotanical identification of micro-remains from *Zea mays* and other cultigens such as *Cucurbitaceae*, *Cannaceae*, and *Phaselous* on ceramic and stone artifacts corresponding to this archaeological unit (e.g., Bonomo et al. 2011a, b, c; Colobig and Ottalagano 2015; Cornero and Rangone 2015; Sánchez et al. 2013) has recently led some authors to suggest the existence of a mixed economy, which would imply a significant degree of dependence on plant products (Bonomo et al. 2011a, b). However, the current data are insufficient to allow assessment of the existence of dependency on cultivated resources in "Goya-Malabrigo" contexts.

Over three decades ago, stable isotope analysis of carbon and nitrogen began to be used to infer human dietary patterns (e.g., Ambrose and DeNiro 1986; DeNiro and Schoeninger 1983; Van der Merwe and Vogel 1978; Vogel and Van der Merwe 1977). Unlike archaeofaunal, archaeobotanical, or fatty acid analysis, isotopic studies provide a direct source of data on human diets. This is because the isotopic composition of human tissues is derived from the daily food intake of the individual and then remains constant after death. Therefore, measurement of isotopic values facilitates the identification of different resources present in the diet and the ratios of their consumption. In the lower Paraná River basin, isotopic studies began over a decade ago in relation to other archaeological units (Acosta and Loponte 2001; Loponte and Acosta 2007; Loponte 2008; Loponte et al. 2015), with only one study related to Goya-Malabrigo contexts (Loponte and Kozameh 2009). In that analysis, no evidence of maize consumption was found, at least as isotopically detectable.

The aim of the present paper is therefore to introduce and discuss new $\delta^{15}N$ and $\delta^{13}C$ isotopic data obtained from human remains recovered from archaeological sites associated with the Goya-Malabrigo archaeological unit, located in the middle and lower Paraná areas, in order to characterize the dietary patterns existing there and to assess any eventual dependence upon plant foods. To improve the analytical strength of this analysis, we have also incorporated the values obtained from human remains recovered from Guaraní sites located in the lower Paraná River basin. These last contexts belong to Amazonian horticulturist groups, who are known to have had a mixed diet with a significant intake of maize and other cultivated plants as well as consumption of animal food products derived from hunting and fishing activities (Loponte and Acosta 2013; Loponte et al. 2015). These data thus provide local isotopic values for an economy that had a significant horticultural component.

## Environment

The study area comprises a section of the middle Paraná River basin as well as its lower section, covering a total length of 700 km along the watercourse (Fig. 1). The Paraná is an extensive, high-volume South American river that originates from rainfall in the tropics that is mostly produced by the South American summer monsoon (SASM), which is associated with the intertropical convergence zone (ITCZ) (Gan et al. 2004; Vuille et al. 2003; Zhou and Lau 1998). The tropical environment of the headwaters extends as a vector of moisture and temperature along the river basin, allowing for development of the southernmost subtropical wetland in South America, with the presence of a multi-layered riparian forest with a continuous 8–15-m canopy, associated with riverine prairies highly fragmented by streams (Cabrera 1976; Kalesnik et al. 2008; Roesler and Agostini 2012). In the middle Paraná, this wetland environment is narrow and

**Fig. 1** Location of the archaeological sites included in this study: *1*, Arroyo Las Mulas I; *2*, Arroyo Arenal I; *3*, La Palmera II; *4*, Los Tres Cerros I; *5*, Los Marinos; *6*, Escuela 31
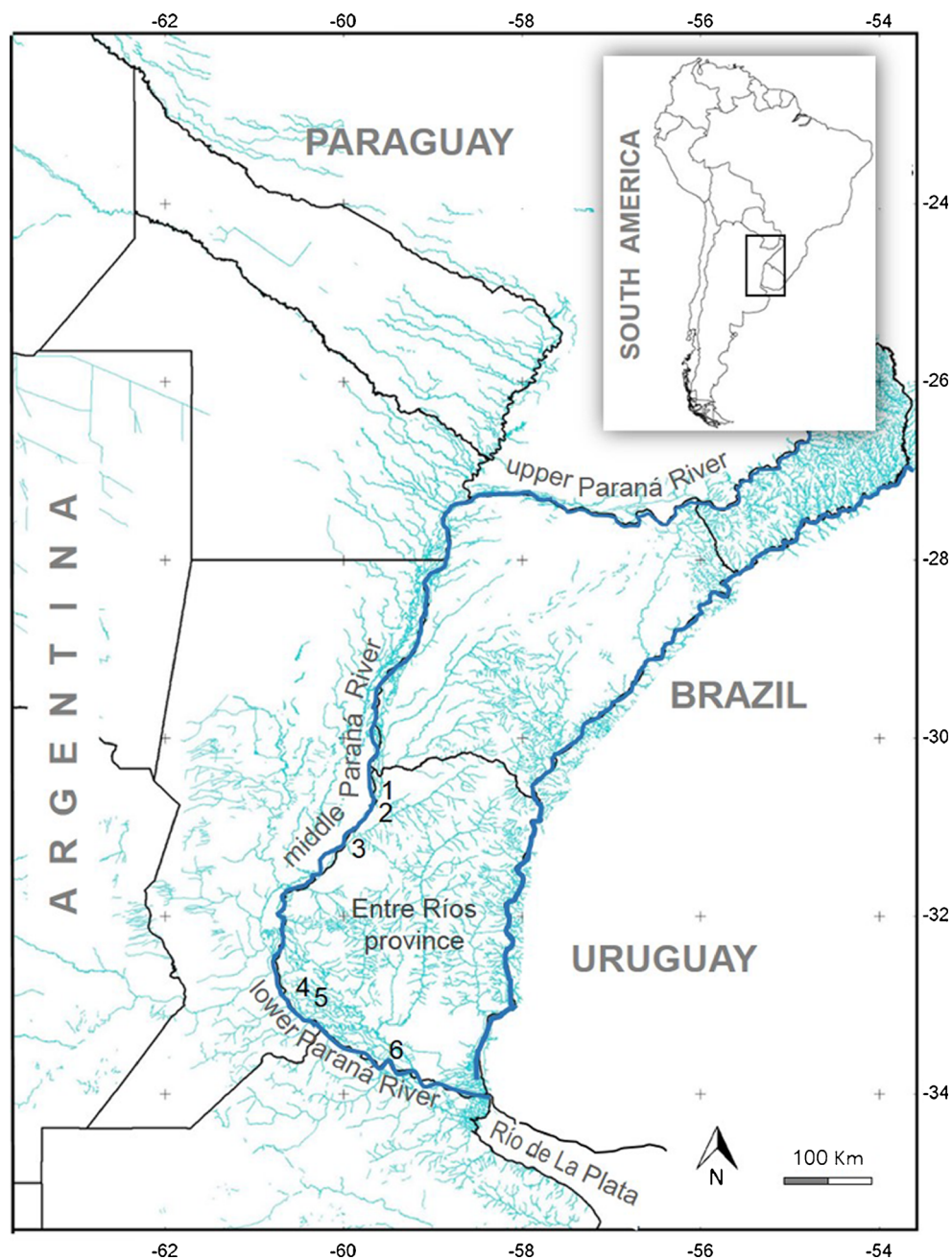


circumscribed to the river's banks and the small islands within it. In the lower Paraná, however, for a distance of 320 km, the wetlands become 60-km wide on average, cutting across the temperate environment of the Pampean steppe. This facilitates the dispersal of flora and fauna and extends the humid conditions from more northern areas, creating a giant azonal and hierarchical patch of resources (Burkart et al. 1999; Loponte 2008). Most of the vegetation cover and the main dietary energy sources in the Paraná fluvial system have a $C_3$ pattern (Madanes et al. 2013; Marchese et al. 2014), and therefore virtually all of the region's fishes and semi-aquatic and terrestrial mammals also possess a $C_3$ pattern. In Table 1, we present

the isotopic data available for the main preys considered in this paper. The data come mostly from archaeological samples except those corresponding to capybara, which are modern. Due to the Suess effect, which is defined as a global decrease in the $^{13}C$ content of atmospheric carbon dioxide, primarily because of fossil fuel burning over the last 150 years that started with the industrialization process (Francey et al. 1999; Indermühle et al. 1999), we applied a time-dependent correction of $-0.005‰$ per year between 1860 and 1960 and $-0.022‰$ per year since 1960 (Chamberlain et al. 2005) until 2013, when the modern samples were

**Table 1** Isotopic values of the main preys exploited along the middle and low Paraná River by pre-Columbian human populations

| Scientific name | Common name | N | $\delta^{13}C_{co}$ (‰) | $\delta^{13}C_{ap}$ (‰) | N | $\delta^{15}N$ (‰) | Weight (kg) | Source |
|---|---|---|---|---|---|---|---|---|
| *Ozotoceros bezoarticus* | Pampas deer | 3 | $-21.1 \pm 1.1$ | $-10.9 \pm 1.5$ | 3 | $5.2 \pm 0.4$ | 18–25 | (1) (2) |
| *Myocastor coypus* | Coypu | 7 | $-20.1 \pm 1.6$ | $-9.1 \pm 3.1$ | 2 | $5.4 \pm 1.5$ | 4–8 | (1) (2) |
| *Blastocerus dichotomus* | Marsh deer | 24 | $-21.1 \pm 1.4$ | $-10.3 \pm 1.4$ | 12 | $6.2 \pm 0.6$ | 90–150 | (2) (5) |
| Osteichthyes | Catfishes | 2 | $-17.8 \pm 0.3$ | $-9.8 \pm 0.5$ | 2 | $5.8 \pm 0.4$ | 1–5 | (1) |
| *Mazama* sp. | Brocket | 2 | $-23.8 \pm 1.1$ | | 2 | $9 \pm 0.8$ | 15–20 | (4) |
| *Hydrochaeris hydrochaeris* | Capybara | 19 | $-14.0 \pm 2.7$ [a] | | 9 | $4.8 \pm 0.6$ | 30–80 | (5) (2) |
| Mean | | | $-19.7$ | 9.1 | | 6.0 | | |
| Median | | | $-20.6$ | 10.0 | | 5.5 | | |
| Stand._dev. | | | 3.4 | 0.7 | | 1.5 | | |
| Coeff._var. (%) | | | 17.1 | 7.6 | | 25 | | |

Sources: (1) Loponte (2008); (2) Loponte et al. (2015); (3) Loponte and Corriale (2012); (4) Colonese et al. (2014); (5) Corriale and Loponte (2015)
[a] $\delta^{13}C$ corrected (+1.66‰ added to published modern capybara values to correct for Suess effect)

collected. This correction provides an approximate value of $\delta^{13}C$ for comparing ancient and modern ecosystems.

## Stable isotopes

As mentioned above, one of the key aspects for understanding the success of isotopic studies is that this type of data provides information about an individual's diet for the last 7–10 years of life, especially when these analyses are carried out on bone tissue (Ambrose 1993). Numerous studies have shown that $\delta^{13}C$ values measured in an individual's collagen ($\delta^{13}C_{co}$ from here forward) are enriched by about 5‰ compared with the diet. Thus, herbivorous diets based on resources with a $C_3$ photosynthetic pathway show values of approximately $-21‰$, while monoisotopic $C_4$ diets present values of around $-7‰$ (DeNiro and Epstein 1978a, b; Koch 2007; Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Vogel and Van der Merwe 1977). In the inorganic fraction ($\delta^{13}C_{ap}$ from here forward), monoisotopic diets present values of $-13.5$ and $-3‰$ and below, respectively (Kellner and Schoeninger 2007; Lee-Thorp and Van der Merwe 1987; Sullivan and Krueger 1981).

Various studies have also shown that carbon values from collagen more closely reflect the content of the protein intake in the diet, while those from apatite have a closer relationship with the total diet (protein + fat + carbs) (Ambrose and Norr 1993; Katzenberg 2008; Krueger and Sullivan 1984; Tykot 2006). This leads to the conclusion that differences between the two carbon sources ($\delta^{13}C_{co-ap}$, or $\Delta^{13}C$ from here forward) largely reflect the plant component of the diet. This model developed by Krueger and Sullivan (1984) is used as an indicator of the trophic level of diets and/or to identify marine components in them (Ambrose and Norr 1993; Ambrose et al. 1997, 2003; Ambrose and Krigbaum 2003;

Harrison and Katzenberg 2003; Lee-Thorp et al. 1989; Loftus and Sealy 2012; Norr 1995; Pate 1995; Rand et al. 2013; Roksandic et al. 1988; Waterman et al. 2015; White et al. 2006). Larger differences are typical of herbivores, with mean values of $6.8 \pm 1.4‰$, while carnivorous diets are below $4.0 \pm 1.0‰$ (Ambrose and Norr 1993; Ambrose and Krigbaum 2003; Clementz et al. 2009; Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Loftus and Sealy 2012). However, there are no absolute values for demarcating thresholds, and instead these should be defined empirically according to the particular context analyzed (Ambrose and Norr 1993; Kellner and Schoeninger 2007). These thresholds also depend upon the combination of photosynthetic proteins and carbohydrates ingested (Ambrose and Norr 1993; Ambrose and Krigbaum 2003; Ambrose et al. 2003; Cormie and Schwarcz 1996; Kellner and Schoeninger 2007; Metges et al. 1990; Stevens et al. 2008; Van Klinken et al. 1994). However, the spacing of values related with trophic levels has been supported empirically in many studies, and these analyses are reinforced when used in conjunction with nitrogen values. In fact, the $\delta^{15}N$ increases at a rate of 3–4‰ as the body incorporates foods of higher trophic levels (Bocherens and Drucker 2003; Hedges and Reynard 2007; Schoeninger and DeNiro 1984). Thus, the combination of both carbon and nitrogen isotopes is used to estimate the trophic position of individuals (Ambrose et al. 1997, 2003; Ambrose and Norr 1993; Froehle et al. 2010, 2012; Loftus and Sealy 2012; Tykot 2006; Warinner and Tuross 2009).

Based on laboratory experiments, Kellner and Schoeninger (2007) have argued that isotopic analysis is more reliable if regression lines are used on both values of $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$. Some authors have suggested that this model has methodological limitations in relation to metabolic aspects and the intake of other nutrients that are not included in the analysis of controlled diets, as well as a lack of consideration

of nitrogen values (Froehle et al. 2012; Loftus and Sealy 2012; Warinner and Tuross 2009). To overcome some of these problems, Froehle et al. (2012) developed a multivariate model incorporating the $\delta^{15}N$ values from calculation of discriminant functions, which are then plotted in clusters of known diets. This allows more accurate evaluation of the archaeological diets, especially those that incorporate maize (a $C_4$ plant), which has played an especially role in isotopic studies (Ambrose et al. 2003; Balasse et al. 1999; Boyd et al. 2008; Calo and Cortes 2009; Gheggi and Williams 2013; Gil et al. 2010; Hart et al. 2011; Froehle et al. 2012; Killian et al. 2012; Laguens et al. 2009; Metcalfe et al. 2009; Rand et al. 2013; Scherer et al. 2007; Slovak and Paytan 2009; Smalley and Blake 2003; Staller et al. 2006; Stronge 2012; Tykot 2002, 2004, 2006; White et al. 2006).

## Materials and methods

In this paper, we included isotopic values for 23 individuals recovered from six Goya-Malabrigo archaeological contexts (Fig. 1). These sites are located along the middle and lower Paraná River, within a chronological range between $460 \pm 50$ and $1807 \pm 47$ years $^{14}C$ BP (Table 2). Human remains from the Arroyo Las Mulas I and Arroyo Arenal I sites were recovered by Ceruti (2003), while those from La Palmera II and Escuela 31 sites were excavated by Ottalagano et al. (2015) and by Loponte (unpublished work), respectively. For the isotopic analysis, fragments of long bones, ribs, and skull were selected, which were seen as preferable as elements that offer a higher proportion of cortical tissue and since they are more suitable for collagen extraction (Ambrose 1993; Falabella et al. 2007). We also preferably considered burials with a high level of integrity, although in a few cases we also had to resort to isolated human remains recovered from the archaeological layers. Most of the analyses were performed in the USA, mainly at the SIRFER laboratory at the University of Utah, although others were carried out at the University of Georgia (CAIS) and at the University of Arizona (Environmental Isotope Laboratory). A few samples were analyzed at the Institute of Isotope Geology and Geochronology in Buenos Aires (INGEIS). We have incorporated into our analysis four isotopic values of $\delta^{13}Ccol$, $\delta^{13}Cap$, and $\delta^{15}N$ previously published by Loponte and Kozameh (2009) and two $\delta^{13}Cco$ values obtained as part of the AMS dating performed at the NSF Arizona AMS Lab (Table 2). There are six direct AMS radiocarbon dates available from different individuals. The rest of the samples were associated chronologically with their dated contexts (Table 2).

## Results and discussion

Carbon values for individuals associated with the Goya-Malabrigo archaeological unit show a $C_3$ monoisotopic trend, with depleted $\delta^{13}Cco$ values with a mean of $-19.4 \pm 1.6\%_o$ with low variability (CV = 8.2 %) (Table 2). Similarly, the average $\delta^{13}Cap$ of $-13 \pm 1.2\%_o$ (CV = 9 %), although we currently have a small sample for apatite analysis, reflects an almost pure monoisotopic diet (Kellner and Schoeninger 2007; Sullivan and Krueger 1981; Lee-Thorp and Van der Merwe 1987), while the high levels of nitrogen ($11.8 \pm 1.1\%_o$; CV = 9.2 %) indicate a substantially carnivorous diet (Ambrose 1993) (Table 2). Thus, the $\delta^{13}C$ values found in the individuals analyzed correspond more to $C_3$ protein intake rather than consumption of $C_3$ plants.

Regarding spatial variations between the two analyzed sectors of the basin, there is little difference in the confidence intervals. The values from collagen are slightly enriched in the samples from the middle Paraná (CI = $-19.9\%_o$, $-18.2\%_o$) compared to those from the lower Paraná (CI = $-21.0\%_o$, $-18.9\%_o$). In turn, nitrogen values are similar (CI = 11.6–12.3‰ in middle Parana, CI = 11.0–13.4‰ in lower Paraná, excluding the anomalous value of individual EIL-1021; including this value the LL is 9.5‰, see Table 2).

The samples can also be grouped into three chronological blocks to assess the impact of plant resources in the diet, including maize: an early period ($1807 \pm 47$ to $1732 \pm 50$ $^{14}C$ BP); an intermediate period ($1056 \pm 47$ to $950 \pm 120$ $^{14}C$ BP); and a late period ($775 \pm 85$ to $460 \pm 50$ $^{14}C$ BP). The first has a value of $\bar{x}_3 = \delta^{13}Cco$ $-20.1 \pm 1.10\%_o$, the second $\bar{x}_{12} = -19.4 \pm 1.3\%_o$ (CI = $-20.3\%_o$, $-18.6\%_o$), and the last $\bar{x}_8 = -19.1 \pm 0.7\%_o$ (CI = $-20.7\%_o$, $-17.5\%_o$). There are therefore no significant differences seen among these values (Fig. 2). The nitrogen values, in turn, show an increase in the intake of animal-derived protein over time (Fig. 3), which could indicate an intensification of fishing-based resource exploitation, a trend that has been verified in other archaeological units in the area based on faunal analysis (Loponte et al. 2012).

The mean carbon values from both collagen and apatite show hardly any fractionation with respect to the average values for prey. However, a more detailed analysis is required here. The large rodent known as capybara (*Hydrochaeris hydrocaheris*), which shows the most enriched diet of all of the prey considered, has a very low representation in the faunal assemblages: At the Arroyo Las Mulas I site, it is practically absent (Serrano 1946); at the La Palmera II site, it makes up only 0.5 % of the NISP (Ottalagano et al. 2015); and at the Escuela 31 site, it is observed in an equally low

**Table 2** Isotopic values for individuals recovered from Goya-Malabrigo sites

| | Lab. Code | Site | Lat | Age | Sex | $\delta^{13}C_{co}$ | $\delta^{13}C_{ap}$ | $\delta^{15}N$ | C/N | $^{14}C$ YBP | Context |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MIDDLE PARANÁ | Sirfer 13-8009 | A. Arenal I | 30º | adult | M? | -17.9 | | 12.1 | 2.9 | 625 ± 46 | |
| | Sirfer 13-8007 | A. Arenal I | 30º | adult | F | -18.3 | | 11.9 | 3.3 | | 625 ± 46 |
| | Sirfer 13-8008 | A. Arenal I | 30º | adult | M | -15.9 | | 12.6 | 2.9 | | 625 ± 46 |
| | Sirfer 13-7997 | A. Las Mulas I | 30º | n/d [a] | n/d | -21.3 | | 11.8 | 3.1 | | |
| | Sirfer 13-7998 | A. Las Mulas I | 30º | adult | F? | -20.3 | | 12.5 | 2.9 | | |
| | Sirfer 13-7999 | A. Las Mulas I | 30º | adult | M? | -20.1 | | 12 | 2.9 | | |
| | Sirfer13-8000 | A. Las Mulas I | 30º | n/d | n/d | -19.9 | | 11.7 | 3.3 | | |
| | Sirfer 13-8001 | A. Las Mulas I | 30º | n/d | n/d | -20.8 | | 11.1 | 2.9 | | 950 ± 120 |
| | Sirfer 13-8002 | A. Las Mulas | 30º | n/d | n/d | -18.1 | | 13.2 | 3.2 | | |
| | Sirfer 13-8003 | A. Las Mulas I | 30º | n/d | n/d | -17.2 | | 11.9 | 3.3 | | |
| | Sirfer 13-8004 | A. Las Mulas I | 30º | n/d | n/d | -20.2 | | 11.5 | 2.9 | | |
| | Sirfer 13-8005 | A. Las Mulas I | 30º | adult | F? | -20 | | 11.6 | 2.9 | | |
| | Sirfer 13-8006 | A. Las Mulas I | 30º | n/d | n/d | -17.1 | | 11.9 | 2.9 | | |
| | AIE 33597 | La Palmera II | 31º | adult | n/d | -19.4 | | 10.7 | 3.3 | | |
| | Sirfer 13-8010 | La Palmera II | 31º | adult | n/d | -19.1 | | 12.3 | 2.9 | 1056 ± 47 | |
| LOWER PARANÁ | AA 93218 | Los Tres Cerros I | 32° | n/d | n/d | -22.8 | | | n/d | 775 ± 85 | |
| | UGA 3303 | Los Marinos | 32° | adult | M | -19.16 | -14.3 | 12.7 | 2.9 | | 460 ± 50 |
| | UGA 3304 | Los Marinos | 32° | adult | M | -19.86 | -14.6 | 12.3 | 2.9 | | 590 ± 60 |
| | UGA 3305 | Los Marinos | 32° | adult | F | -20.73 | -13.0 | 12.7 | 2.9 | | |
| | UGA 3306 | Los Marinos | 32° | adult | F | -18.56 | -11.9 | 12.8 | 2.9 | | 660 ± 70 |
| | AA103644 | Escuela 31 | 33° | adult | F | -18.2 | | | n/d | 1807 ± 47 | |
| | EIL-1021 | Escuela 31 | 33° | adult | n/d | -22.0 | -12.2 | 8.0 | 3.1 | 1764 ± 46 | |
| | EIL-1022 | Escuela 31 | 33° | adult | n/d | -20.3 | -12.1 | 10.5 | 3.3 | 1732 ± 50 | |
| DESCRIPTIVE STATISTICS | N | | | | | 23 | 6 | 21 | | | |
| | Mean | | | | | -19.4 | -13.0 | 11.8 | | | |
| | Median | | | | | -19.9 | -12.6 | 11.9 | | | |
| | 25_prcntil | | | | | -20,3 | -14.4 | 11.6 | | | |
| | 75_prcntil | | | | | -18.2 | -12.0 | 12.6 | | | |
| | Stand._dev. | | | | | 1.6 | 1.2 | 1.1 | | | |
| | Coeff._var. (%) | | | | | 8.2 | 9.0 | 9.2 | | | |

The isotopic values from the Los Marinos site were taken from Loponte and Kozameh (2009) and those from the Los Tres Cerros I site from Bonomo et al. (2011c). The radiocarbon dates for the Los Marinos, Arroyo Las Mulas I, and La Palmera II sites were taken from Kozameh and Brunás (2013), Ceruti (2003), and Ottalagano (2014), respectively

*n/d* no data

proportion (detailed quantification does not exist for the rest of the sites considered).[1] Brocket deer (*Mazama* sp.) must also be considered to be an occasional resource, as these remains are either scarce or not recorded at sites in the area (see Acosta 2005; Arrizurieta et al. 2010; Bonomo et al. 2011c; Loponte 2008; Nóbile 1993; Ottalagano et al. 2015).

Excluding these two taxa mentioned, the average $\delta^{13}C_{co}$ for the most common preys is $-20.0 \pm 1.5$‰, a magnitude that

also shows hardly any fractionation compared to the human remains analyzed ($-19.4 \pm 1.6$‰), where the expected difference between the isotopic values of prey and predator collagen ranges from 0 to 2‰ (Bocherens and Drucker 2003). In turn, for this discussion, what is really crucial are the isotopic values for the most abundant fishes from the Paraná River system. While Table 1 shows the average for two omnivorous Siluriformes taxa (*Pterodoras granulosus* and *Pimelodus* sp.) recovered from two archaeological sites in the lower Paraná, the faunal evidence indicates that the most heavily exploited species was *Prochilodus lineatus*, a bottom-feeding species that represents more than 50 % of the fish biomass of the Paraná River and which is considered to be a key species for the fluvial system (Loponte 2008). This characiform has

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[1] An NISP of $n = 7$ for *Hydrochaeris hydrocaheris* is recorded for the Arroyo Arenal I site (Nóbile 1993); however, the percentage this represents with respect to the total sample is not specified.
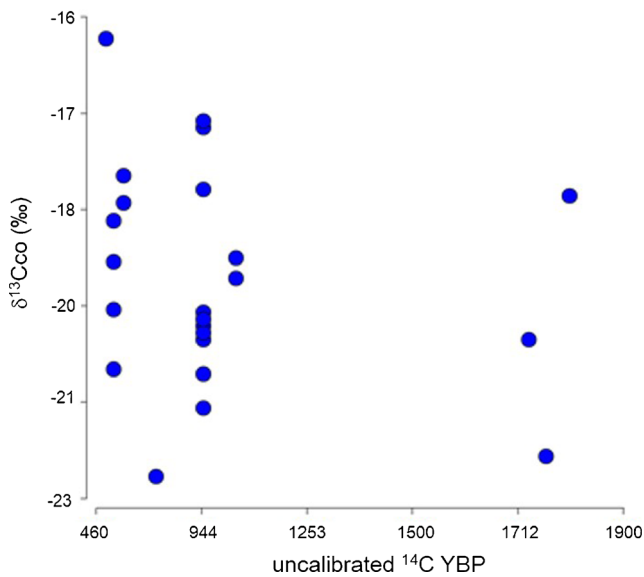
**Fig. 2** Temporal trends for the carbon values from collagen



**Fig. 3** Temporal trends for the nitrogen values

extremely thin and fragile bones and is therefore notably under-represented in the archaeological record because of problems with preservation and identification (Acosta 2005; Loponte 2008; Musali 2010). A study performed on this species in northern areas of the Paraná basin yielded values for muscle tissue ranging between $\delta^{13}$Cco $-24.8 \pm 3$ and $-31.5 \pm 2.6\%_o$ (Lopes et al. 2007), with an average of $-27.5\%_o$ (Suess-corrected $\delta^{13}$C $-26.1\%_o$).[2] Another analysis conducted by Marchese et al. (2014) on individual specimens captured in the middle Paraná yielded a similar average ($-27.3 \pm 3.1\%_o$, Suess-corrected $\delta^{13}$C $-25.7\%_o$).[3] Given that all of these values obtained from muscle tissue are depleted by 1.1–1.6‰ with respect to bone collagen in fishes (Sholto-Douglas et al. 1991), an approximate mean of $\delta^{13}$C $= -24.5 \pm 3\%_o$ can be considered as an accepted average value for bone collagen.[4] Other fishes that are also common in the archaeological sites of the region, such as *Leporinus obtusidens*, *Pimelodus maculatus*, *Hoplias malabaricus*, *Salminus brasiliensis*, *Ageneiosus brevifilis*, and *Pseudoplatystoma reticulatum* (Acosta 2005; Arrizurieta et al. 2010; Loponte 2008; Loponte et al. 2012; Musali 2010), show a depleted $\delta^{13}$C average of $-26.7 \pm 1\%_o$ in muscle tissue (calculated based on Saigo et al. 2015: Table 2), and thus a value of $-25.3\%_o$ can be considered for bone collagen (Suess-corrected $-23.7\%_o$).

Bulk value of collagen of fish ($-24.1\%_o$)[5] therefore provides monoisotopic $C_3$ signals as do the other preys ($-20.1\%_o$), but substantially depleted, and this may help to understand the carbon levels found in the human samples. Some authors (i.e., Bocherens and Drucker 2003) have suggested that to understand the values of collagen of the predators, it is necessary to consider all the significant preys in the archaeological record. Although we do not know exactly the proportion between terrestrial mammals and fish resources in the diet of individuals analyzed, considering an average between the two types of resources, we can explain the values of collagen registered in humans within the ranges that are reasonable to understand the observed isotopic fractionation between preys and humans ($-22.1$ vs. $-19.4\%_o$, respectively), including here probably also some minor intake of $C_3$ plants.

A similar situation occurs in the case of nitrogen. The human samples analyzed show enriched $\delta^{15}$N values ($\overline{x} = 11.8 \pm 1.1\%_o$) that cannot be explained by consumption of the preys listed in Table 1 ($\overline{x} = 6 \pm 1.5\%_o$). Although is also likely to other species with depleted $\delta^{13}$Cco values contributing in a significant manner to human subsistence, these human nitrogen levels could be explained in part by high consumption of fish. Indeed, *P. lineatus* shows an average of $9.13 \pm 0.82\%_o$ (in muscle) (Marchese et al. 2014) for individual fish captured in the middle Paraná and between $10.7 \pm 2.2$ and $11.9 \pm 0.7\%_o$ for specimens obtained in the upper Paraná (Lopes et al. 2009), for an average of $10.6 \pm 1.2\%_o$ for this species. Thus, the nitrogen value for *P. lineatus* would be around $8.3 \pm 1.2\%_o$ in

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[2] Fish were caught between 1999 and 2004 (Lopes et al. 2007). Thus, we take the year 2001 as an average for calculating the Suess effect, following Chamberlain et al. (2005).

[3] Samples were collected between November 2009 and December 2010 (Marchese et al. 2014); therefore, we consider the Suess effect until 2010.

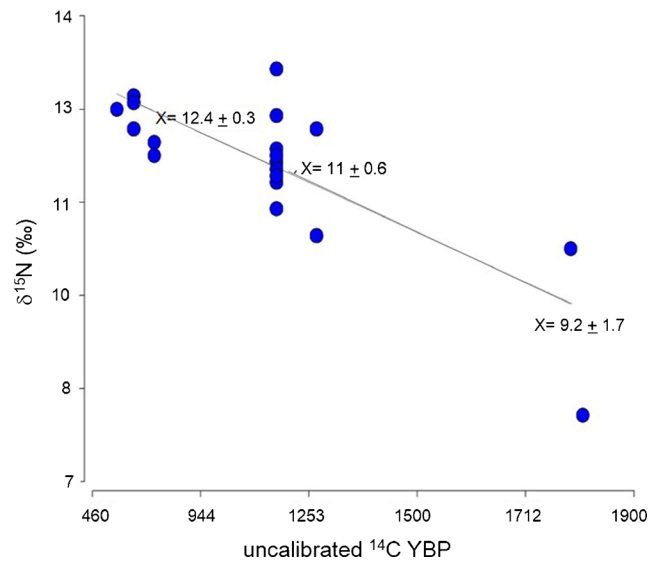[4] Taken an average of $\delta^{13}$C $-25.9\%_o$ (Suess correction) plus 1.35‰ (average 1.1–1.6) for bone collagen.

[5] Averaging corrected values for shad ($-24.5\%_o$) and corrected values for other fishes ($-23.7\%_o$).

bone collagen since it is depleted by 2.3–2.4‰ compared to muscle tissue (Sholto-Douglas et al. 1991). The remaining fish species mentioned above, which are present in regional archaeofaunal assemblages, show an average of $10.4 \pm 1.1‰$ (calculated on the values obtained from the dorsal muscle, Saigo et al. 2015: Table 2), averaging ~8‰ in bone collagen (see also Bocherens and Drucker 2003; Hedges and Reynard 2007; Schoeninger and DeNiro 1984). Considering the nitrogen level in preys listed in Table 1 (6‰) and other fishes (~8‰), an average of 7‰ is an approximate value for nitrogen signal intake for humans. The resulting isotopic fractionation (~4.8‰) is within the known range between predators and preys (Bocherens and Drucker 2003).

Therefore, the enriched nitrogen values and depleted carbon levels observed among the hunter-gatherer individuals analyzed in the present study can be explained within the framework of a diet with a strong emphasis on fish consumption and, certainly, limited direct consumption of plant resources.

It is estimated that human individuals with bone collagen values ranging between −17.0 and −14.0‰ correspond to a mixed diet, which could have been made up of 25–50 % $C_4$ plants and/or $C_4$ plant-based animal protein (Coltrain and Leavitt 2002). The only individual who falls within these parameters comes from Arroyo Arenal I site ($\delta^{13}C co = -15.9‰$, SIRFER 13-8008 in Table 2). However, this individual has a high nitrogen value, similar to the rest of the population, also suggesting in this case a limited intake of plant products.

The general trend towards a $C_3$ diet based on animal resources is clearly observed when the values from the human individuals analyzed from Goya-Malabrigo sites are plotted together with those for local herbivorous and omnivorous preys and human individuals recovered from Guaraní sites of the area (Amazonian horticulturalists), whose diets actually included a substantial element of cultigens, including maize (Figs. 4 and 5). To further understand the incidence of maize within these two populations, and for comparative purposes, we have also plotted the values published by Sommerville et al. (2013) for individuals from the southern Maya lowlands throughout the Classic Period, where there was a substantial dependence upon corn[6] (Fig. 4).

If the discriminant functions developed by Froehle et al. (2012) are used to consider the nitrogen values, the Goya-Malabrigo individuals analyzed fall into the box of diets composed of 100 % protein and energy intake related with a $C_3$ photosynthetic pathway. In contrast, the Guaraní samples fall into an intermediate position between the box for $C_3/C_4$

energy and protein intake and the box representing the highest consumption of C4 energy (Fig. 6). There is also an isotopic gap seen when these two populations are plotted by their means plus/minus one standard deviation (Fig. 7). Finally, the carnivorous diet of the human individuals recovered at Goya-Malabrigo sites is also quite clear when a typical carnivore from the area (the jaguar, *Pantera onca*) is added to the plot (Fig. 7).

The isotopic signals observed in the analyzed individuals corresponding to the Goya-Malbrigo archaeological unit, as well as data provided by archaeobotanical studies in relation to these groups (see Bonomo et al. 2011a, b, c; Colobig and Ottalagano 2015; Cornero and Rangone 2015; Sánchez et al. 2013), are consistent with the observations of sixteenth-century travellers in reference to the post-Hispanic societies then occupying the Paraná River areas where the highest concentrations of Goya-Malabrigo sites have now been recorded. That is, a low incidence of vegetable foods, use of small quantities of maize, and the importance of animal resources, especially fishes. For example, one historical source remarks: "… They have certain lagoons that have large fisheries, and they take out fish and store them for later" (Fernández de Oviedo y Valdés 1944:195, own translation). However, no such clear references seem to exist in relation to the storage of maize or other crops. In some cases, historical references indicate that the local people only ate "meat and fish" (Schmidl [ca. 1531] 1948:71), whereas in other references it is mentioned that in addition to these resources, the local population also engaged in small-scale consumption and/or cultivation of maize, squash, sweet potatoes, and beans (García de Moguer [1528] 1908:245; Ramírez [1528] in Madero 1939:384). In this sense, they could be seen as complex foragers with low-level food production (cf. Keeley 1988; Kelly 1995; Smith 2001; Zeder 2012) as has been suggested on several occasions in regional archaeological studies from throughout the twentieth century (see a summary in Loponte 2008).

An economic base focused on animal resources also clearly has a correlation with other aspects of the bioarchaeological record. For example, it is known that consumption of cultivated plants, especially maize, has a multiplier effect on the level of dental caries seen in individuals (Larsen 1984; Larson 1995, 1997; Rose et al. 1984; Turner 1979). In the mortuary context of the Los Marinos site, where three dates confirm an antiquity close to the time of European contact, the individuals studied have very low levels of dental caries, typical of non-agricultural populations (Kozameh and Barbosa 1996; Mazza and Barrientos 2012). The same applies to the Escuela 31 site, where none of the three individuals recovered have even a single instance of dental caries. This is especially remarkable considering that these individuals also show the lowest nitrogen levels of the samples analyzed in the present study. Studies currently in progress regarding the bioarchaeological record from other sites referred to in this

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[6] We considered the average reported by Somerville (2013:1544) from a total of 102 samples, which grouped together different time periods and which includes both commoners and elite individuals, ensuring greater variability in the comparative sample.

**Fig. 4** Values for $\delta^{13}C_{co}$ versus $\delta^{15}N$ for individuals from societies with different economic systems, complex hunter-gatherers (Goya-Malabrigo), horticulturalist economy (Guaraní), and agriculture-based societies (Maya), along with the main preys exploited along the middle and lower Paraná River by pre-Columbian human populations
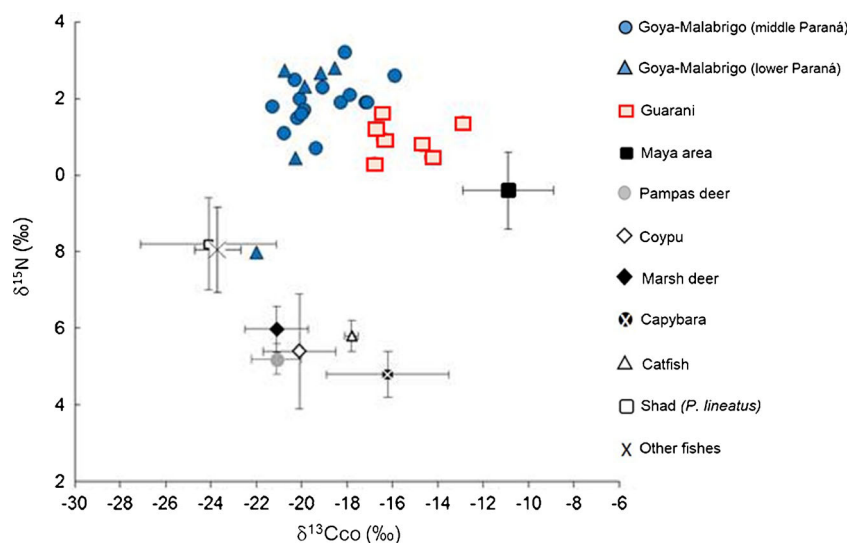


article should allow for a more detailed understanding of these aspects.

## Final remarks

The results presented and discussed in this article indicate that human individuals recovered from sites corresponding to the archaeological unit called Goya-Malabrigo had a monoisotopic $C_3$ diet. In virtually all of the samples examined, maize was not isotopically detectable, indicating that this crop had a marginal to null impact on the local diets. The carbon and nitrogen levels show a preponderantly carnivorous diet, most likely based on freshwater fishes, and secondly on terrestrial species with depleted $\delta^{13}C_{co}$ values, such as coypu and some ungulates. The nitrogen levels detected are even higher than

those observed for complex hunter-gatherers who are associated with other archaeological units from the southernmost part of the Paraná River basin (Loponte 2008). Furthermore, the isotope values recorded in the individuals studied also clearly differ from those obtained from local horticulturist groups corresponding to the Guaraní archaeological unit, which had a mixed diet that undoubtedly included maize consumption. These data lead to questioning the hypothesis that crop plants played a significant role for the groups labeled as Goya-Malabrigo, as has been proposed by some authors based on archaeobotanical record (Bonomo et al. 2011a, b). The first exploration of this issue by isotopic data, presented in this paper, suggests on the contrary that they could not be classified as societies with a mixed economy. Rather, isotopic information obtained on individuals analyzed in this study seems to indicate an economic base focused on animal resources.
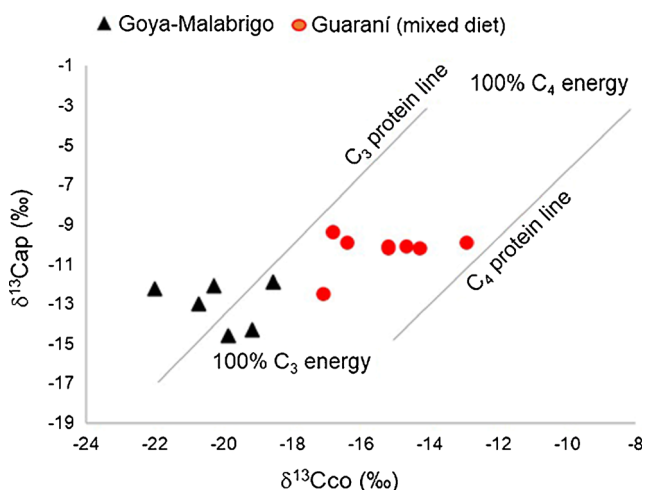


**Fig. 5** Values for $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$ following the model proposed by Kellner and Schoeninger (2007) for individuals with a horticulturalist economy (including maize) (Guaraní) and complex hunter-gatherers (Goya-Malabrigo). It should be noted that the graph is based on the six apatite values currently available
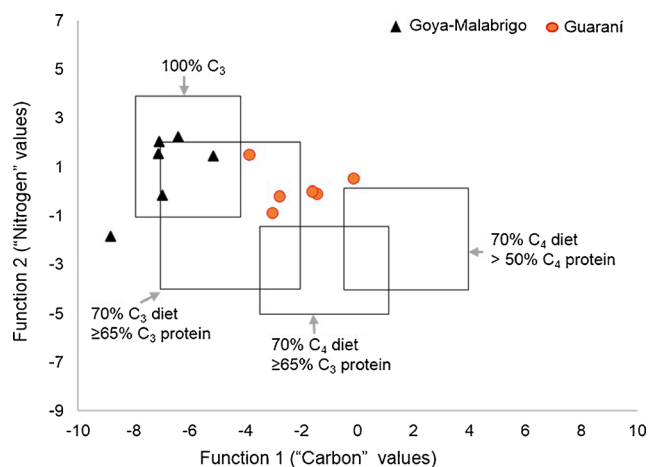


**Fig. 6** F1 and F2 discriminant function values from individuals recovered at Goya-Malabrigo and Guaraní sites, plotted against previously generated dietary clusters (see Froehle et al. 2012)
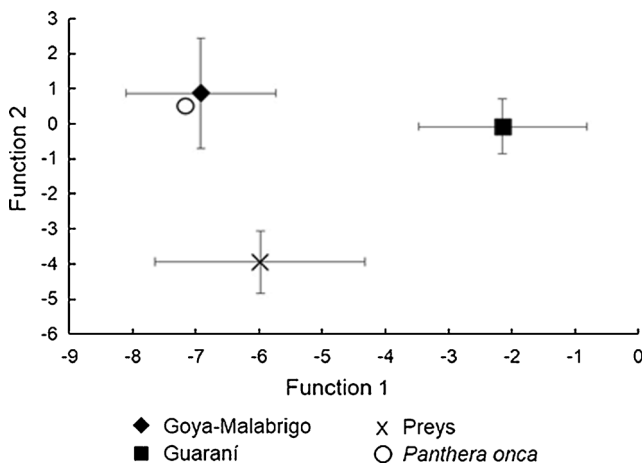
**Fig. 7** Means of discriminant function values (after Froehle et al. 2012) for individuals recovered from Goya-Malabrigo and Guaraní sites. The value for *Panthera onca* (jaguar) was taken from Loponte et al. (2015)

This interpretation of low or possibly null importance of cultivated plants and vegetable foods, as established by the isotopic analysis, mainly through $\delta^{15}N$ and $\delta^{13}C$ values, is not contrary to the detection of phytoliths and starch grains as reported by archaeobotanical studies, since this evidence could be associated with only a low level of processing and/or consumption of cultigens. Temporal analysis of the samples also suggests that there was no existing trend towards the incorporation of maize or other cultigens over time. To the contrary, our analysis essentially points to an isotopically stable diet for the chronological period of 1800 ±47 to 460±50 $^{14}C$ year BP, with a slight trend towards increased consumption of animal protein during the later portion of this range. New samples currently under analysis should soon allow isotopic characterization of the diets of these groups to be expanded and should lead to a better understanding of their spatiotemporal variability.

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