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The role of the artificial ponds for the conservation of mammals in the state of Santa Catarina

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ABSTRACT

Man has profoundly modified the upper valley of the Uruguay River and its basin. The plains of these valleys and the lower areas of the hills have been modified for agricultural production, leaving small patches of wild forest on the tops of the hills, where wildlife takes refuge. These less modified sectors generally lack water. Therefore, the wild mammals must descend to the bottom of the valleys to drink. However, there are numerous fence lines between the hills and the rivers and streams which prevent the fauna access to these watercourses, so they ingest water from artificial ponds present in the agricultural establishments instead, which is reflected in the high values of $\delta^{18}O$ observed in the bone bioapatite of local wild mammals. This finding highlights the importance of artificial reservoirs distributed in the agricultural landscape of Santa Catarina for the preservation of wildlife and the importance of their sanitary monitoring to prevent the transmission of diseases from livestock to wildlife.

Keywords: artificial ponds, Oxygen-18, Santa Catarina, stable isotopes, wildlife preservation.

O papel dos reservatórios artificiais de água para a conservação dos mamíferos no estado de Santa Catarina

RESUMO

O homem tem modificado profundamente a região abrangida pela bacia do alto rio Uruguai. As planícies deste rio e as partes baixas dos morros têm sido modificadas para a produção agrícola, deixando pequenas parcelas de mata natural, principalmente nas partes altas dos morros, onde se refugia a vida silvestre. Estes setores menos modificados, geralmente, carecem de água, fazendo com que a fauna se desloque até o fundo dos vales para beber. Contudo, existem diferentes obstáculos entre os morros e os rios e córregos, que impedem o acesso diário da fauna aos cursos d' água, por isso acabam bebendo dos reservatórios artificiais presentes nos estabelecimentos agrícolas, o que se expressa nos altos valores de $\delta^{18}O$ observados na bioapatita óssea dos mamíferos selvagens locais. Estes dados destacam a

importância dos reservatórios artificiais distribuídos na paisagem agrícola de Santa Catarina, para a preservação da vida silvestre, bem como a importância que tem a vigilância sanitária para prevenir a transmissão de enfermidades dos animais domesticados na vida silvestre.

Palavras-chave: conservação, isotopos estáveis, oxigenio-18, reservatórios artificiais, Santa Catarina.

1. INTRODUCTION

In the upper valley of the Uruguay River in the State of Santa Catarina, the wildlife is sheltered in remnant patches of native forest. These areas were left out of agricultural production due to their steep slopes and often rocky soils. On the contrary, the cultivated fields practically occupy all the bottoms of the valleys, and the slopes of the hills suitable for intensive and mechanized agriculture. All these fields, which are privately owned, have numerous and different lines of fences that prevent the local fauna from reaching the bottom of the valleys where the streams and rivers run, becoming a serious problem for survival, since the tops of the hills, due to their positive topography, practically lack surface water. The wildlife takes refuge during most of the day in those preserved areas of the tophills, where they feed, and during the twilight or night hours they descend to drink water. Since in general they cannot access the valley bottoms due to the fences, they use the artificial water reservoirs that farms build for livestock, fish production or crop irrigation in the midway between the tops of the hills and the bottom of the valleys. It is quite usual for the owners of the region to report the presence of wild animals in the surroundings of these artificial ponds. This wildlife dynamic is known by the farmers, but it was never investigated. Confirmation of this dynamic would be extremely important since these artificial water sources would become key elements for the conservation of wildlife. This would also highlight the need to monitor the quality of the water in these reservoirs to prevent the transmission of diseases from livestock to wildlife.

In this study we determine if the autochthonous mammals (Figure 1) really depend on the artificial ponds in the highly modified landscape of the Upper Uruguay River. To achieve this goal, we analyzed the values of $\delta^{18}O$ obtained in the bone tissue of 17 mammals from 12 species. If mammals use artificial ponds instead of water from rivers and streams, their $\delta^{18}O$ values should be higher than if they were drinking from the latter. Artificial ponds have higher values of $\delta^{18}O$ than rivers and streams because they are ponds of water with less recharge and no runoff. This stagnant water undergoes a greater evaporation process than river water, raising the concentration of oxygen-18 (Dansgaard, 1964; Gat, 1995; 1996; Kendall and Caldwell, 1998; see next section).

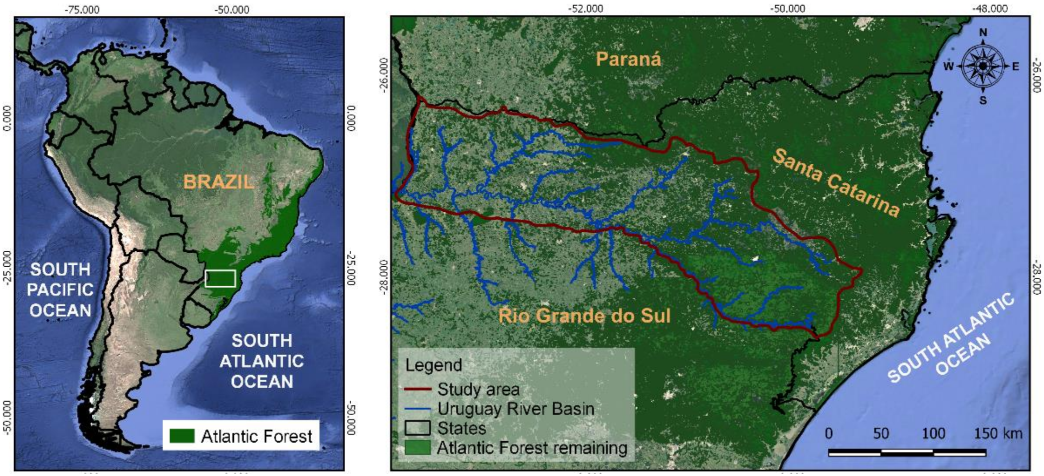

Figure 1. Area of study.

2. THE APPLICATION OF ^{18}O FOR THE STUDY OF MOBILITY

2.1. General aspects

Oxygen-18 is widely used to study the mobility of organisms since ^{18}O values of the tissues and environmental water have a linear relationship (Knudson, 2009; Longinelli, 1984; Luz *et al.*, 1984; Luz and Kolodny, 1985). The atoms of oxygen in the bioapatite of the bones are incorporated from body water, which in mammals is formed mostly from drinking water (Bryant and Froelich, 1995; Kohn, 1996; Kohn and Cerling 2002; Podlesak *et al.*, 2008; Sponheimer and Lee-Thorp, 1999). The bioapatite precipitates in the skeletons through a thermodynamic process which is equilibrated with body temperature. Mammals are homeothermic organisms with a constant body temperature of ~ 37°C. Mammals are also mostly frequent or obligate drinkers, that is, they ingest water through direct ingestion of surface water. Approximately 70 to 80% of the molecular water in mammalian bioapatite comes from direct water intake (Bryant *et al.*, 1996; Clementz and Koch, 2001; Kohn and Dettman, 2007; Longinelli, 1984; Luz and Kolodny, 1985). Thus, the ^{18}O values in the bioapatite of mammals present a narrow range linearly related with ^{18}O values of the local sources of water. The compact tissue of the bones constitutes a reservoir of isotopic information, since this tissue averages the isotopic signals of several years of food and water intake of individuals (Cox and Sealy, 1997; Hedges *et al.*, 2007; Lamb *et al.*, 2014; Pollard *et al.*, 2012).

Some precautions must be considered when using δ^{18}O values from mammalian bones. For instance, several studies have pointed out that body mass has some influence in ^{18}O values. However, most of the results obtained in mammals with different weight show narrow ranges or even no statistical differences (see different positions in Bryant and Froelich, 1995; Cerling *et al.*, 2004; Clementz and Koch, 2001; Crowley *et al.*, 2015; D'Angela and Longinelli, 1990; Longinelli, 1984; Longinelli *et al.*, 2003; Luz *et al.*, 1984; Kohn, 1996; Loponte and Ottalagano, 2022). Feeding habits are a recognized source of variability, not only between the different guilds, but also internally among them (*e.g.* herbivores: frugivores vs. grazers or browsers, and between faunivores: carnivores vs. Insectivores, etc.) (Lee-Thorp, 2008; Luyt and Sealy, 2022, among others).

Carnivorous mammals have also been the subject of some debate regarding their oxygen-18 values. Some authors have pointed out that they present more negative values because they acquire most of the water through food. In this case, the explanation given is that their foods have depleted δ^{18}O values (Luyt and Sealy, 2022; Sponheimer and Lee-Thorp, 1999; 2001). However, this is an untested hypothesis. Another more likely explanation is that they drink more frequently when water is available, and especially from free-flowing water (Luyt and Sealy, 2022; Sponheimer and Lee-Thorp, 1999). Therefore, the relationship between apatite oxygen-18 values and environmental water values in carnivores is a topic that still needs to be properly evaluated.

2.2. The local landscape

The upper course of the Uruguay River crosses the Atlantic Forest from east to west. The latter is a conglomerate of 15 ecoregions formed by tropical and subtropical forests and, to a lesser extent, different types of high altitude grasslands (above 1000 masl) (Olson *et al.*, 2001). This region receives between 1700 and 2400 mm/year of rains which came from the Intertropical Convergence Zone (ITCZ), especially during the southern summer, and from the extratropical cyclonic activity of the South Atlantic Convergence Zone (SACZ), mainly during winter. This hydrological regime was established several millennia ago (Cruz *et al.*, 2006; Gan *et al.*, 2004; Zhou and Lau, 2001). These two sources of water vapor have different ^{18}O values; the first is more negative than the second, and both water sources mix in a wide region that extends from the Uruguay River Basin to the south and east, reaching the Rio de la Plata River, establishing a particular ecozone of ^{18}O, where the organisms average the values of both sources of meteoric water. Several $\delta^{18}O$ values were obtained from bone apatite of pre-Columbian mammals in this region, ranging from -2.5 ‰ to -0.5 ‰ (V-PDB) (Loponte *et al.*, 2016; Loponte and Ottalagano, 2022). This range was established as the expected natural model of this ecozone for mammals. New analysis carried out with other pre-Columbian samples from Rio Grande do Sul and the lower Uruguay River show consistent data with this range (Carbonera *et al.*, 2022; Loponte *et al.*, 2022).

The local landscape in the upper course of the Uruguay River presents a large number of rivers and streams with steep slopes, which generate a mountain water regime. After precipitating, the water drains quickly. There are practically no natural lakes in the region. The current human colonization has privileged the occupation of the bottoms of the valleys where the topography is flatter, where the landscape is used for intensive and mechanized agriculture. This capitalist production process has incorporated much of the slopes of the local hills, which are generally gentle and allow the use of large machines for crop production. The natural environment in these two sectors has been completely modified, where the Upper Paraná Forest has been replaced by open fields. As we have pointed out in the introduction to this study, most agricultural establishments in these areas have one or more ponds that are used to store water for raising livestock, irrigating crops or raising fish. These ponds are recharged in many different ways, including rains, water pumps that draw infiltrated or stored water, or by artificial canals that carry water from nearby streams. These bodies of water are subjected to an evaporation process, which increases the concentration of ^{18}O in the ponds, because ^{16}O requires less energy to be evaporated (*cf.* Dansgaard, 1964; Gat, 1995; 1996; Kendall and Caldwell, 1998). Since the water is stagnant in the ponds, the evaporation rate in these reservoirs is higher than in free-flowing water. This process is highly variable because it depends on multiple factors. Among them, the initial volume of water present in the ponds, the frequency of use and recharge, and the origin of the water used to fill them. Therefore, these man-made reservoirs are expected to have highly variable $\delta^{18}O$ values relative to each other and, on average, higher than streams and meteoric water.

The tops of the hills generally have steep slopes and rocky soils. For this reason they are not used for agriculture, preserving the original forest and becoming the last refuges for wildlife in the region. Because they are small patches with positive topographies, they generally do not have water sources. The wildlife, especially mammals, rest and feed during the day in these patches of denser vegetation, and descend to the bottom of the valleys to drink water, especially during nights. Due to the fences, as well as the more immediate encounter of these artificial ponds, they drink water directly from these reservoirs. Many of these artificial ponds are often only a few hundred meters from these feeding and refuge areas (Figure 2). Therefore, if this were indeed the case, they incorporate water with higher values of ^{18}O than expected in the natural model on a regular basis. It should also be noted that in some areas the fauna can use the simultaneous intake of water from the streams to a greater or lesser extent, which increases the variability of the isotopic signals of the ingested water.

3. MATERIALS AND METHODS

3.1. Samples

Most of the samples were obtained from the bones of mammals run over at various points on the state and federal highways of the state of Santa Catarina. A small portion of the samples were acquired through local residents at the same time, who donated bone fragments from animals that died of natural causes on their properties. Each owner identified the species by its external characteristics, and simultaneously, these donated bones were compared with the biological collections of the Museu de Ciências da Universidade Comunitária da Região de Chapecó (Museum of Sciences of the Community University of Chapecó Region; Unochapecó) to verify the accuracy of the taxonomic determinations. The samples were recovered within an east-west transect, parallel to the course of the upper Uruguay River (Figure 3). In all cases, small fragments of compact bone tissue were removed. The species correspond mostly to mammals which acquire most of their body water by direct intake of water (Figure 4). The body weights of the species included in this study vary between 300 grams, as in the case of *Cavia fulgida* to 50 kg for *Hydrochoerus hydrochaeris*. The weights of each species were taken from Nowak and Walker (1999) and International Union for Conservation of Nature (IUCN, 2022). For each sample, the date of collection, geographic coordinates, altitude and distance from the sea were recorded (Table 1).

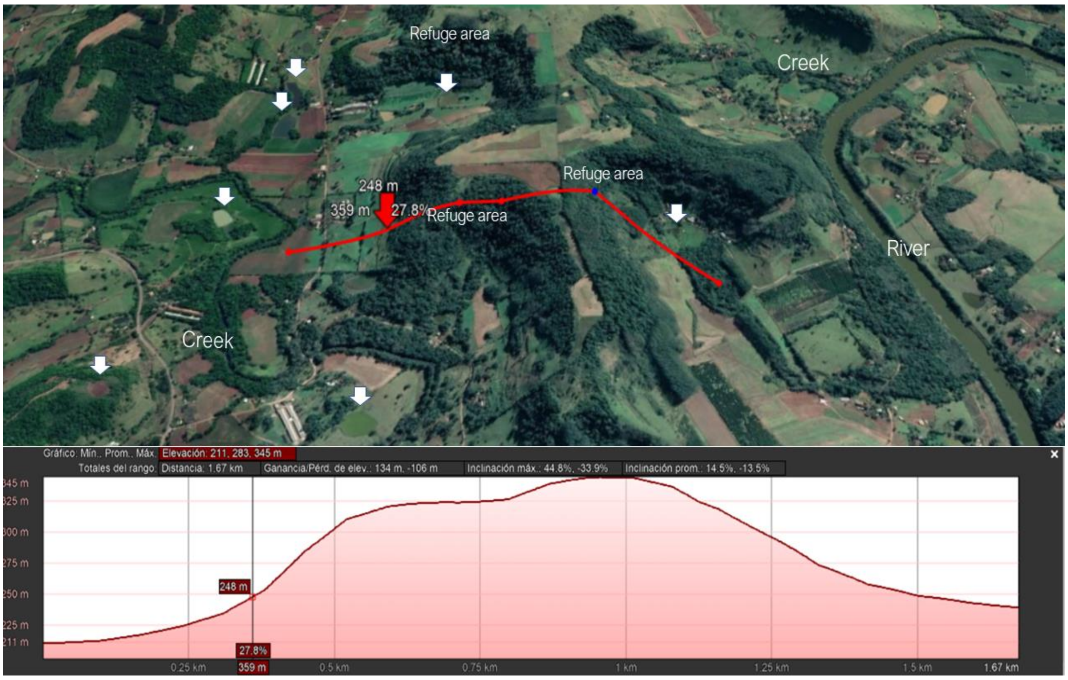

Figure 2. Location of refuge areas of the wildlife in a typical area of the Uruguai Basin, in Mondai County. The artificial ponds are marked with white arrows. The red line indicates the altitude profile shown in the lower image.

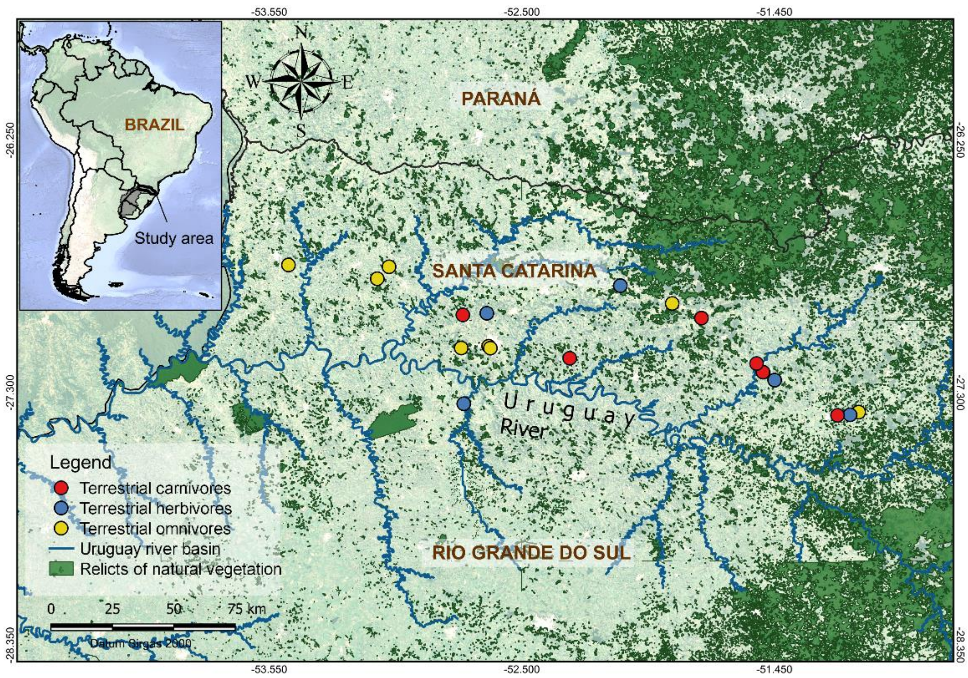

Figure 3. Sample collection areas.

Figure 4. Species included in this study. 1: *Hydrochoerus hydrochaeris*. 2: *Tamandua tetradactyla*. 3: *Leopardus wiedii*. 4: *Leopardus guttulus*. 5: *Cerdocyon thous*. 6: *Didelphis* sp. 7: *Sapajus nigritus*. 8: *Cavia fulgida*. 9: *Procyon cancrivorus*. 10: *Nasua nasua*. 11: *Mazama* sp. 12: *Euphractus sexcintus*.

3.2. Isotopic analyses

The bone fragments were mechanically cleaned until the bone was free of soft tissue observed with the naked eye. After that, each fragment was cleaned with water and neutral soap and gently dried in the oven. The samples were packed to be sent to the laboratory. The isotopic analysis was performed at the Environmental Isotope Laboratory in the Department of Geosciences at the University of Arizona. In this facility, the surfaces of the bones were scraped until both sides of external layers were removed. The samples were pulverized and treated with 2% NaOCl to eliminate organic matter and finally they were submerged in a 0.1 M acetic acid bath, rinsing three times in the centrifuge with distilled water. Subsequently, they were subjected to a reaction with phosphoric acid under vacuum at 70°C with Ag. Isotopic content was measured with a KIEL-III carbonate preparation device coupled to a Finnigan MAT-252 gas mass spectrometer. For the calibration of the O isotopic measurement, the standards NBS-19 and NBS-18 were used. The analytical precision obtained is ± 0.08 ‰. $\delta^{18}O$ values are expressed as the difference ($\delta^{18}O$) with the V-PDB standard (Coplen, 1994).

Table 1. Species included in this study, areas and environmental data.

Scientific name	Common Name	County	Z	East	North	Altitude (masl)	Rainfall (mm)	Temp (°C)	Distant to sea (km)
Mazama gouazoubira	Gray-brocket	Nonoai	22J	327763	6974000	-	1900	18	378
Mazama sp.	Brocket deer	Campos Novos	22J	486612	6970113	920	1800	16	251
Mazama nana	Dwarf brocket deer	Cordilheira Alta	22J	336697	7015840	542	2000	18	515
Hydrochoerus hydrochaeris	Capibara	Ponte Serrada	22J	391698	7029152	1444	2100	16	339
Cavia fulgida	Cavy	Erval Velho	22J	455567	6986016	861	1900	17	278
Tamandua tetradactyla	Southern tamandua	Vargem Bonita	22J	425269	7014457	993	2000	16	306
Tamandua tetradactyla	Southern tamandua	Herval do Oeste	22J	450756	6989683	546	1900	17	284
Euphractus sexcinctus	Six-banded armadillo	São Miguel Oeste	22J	254380	7036758	546	2000	18	476
Didelphis sp.	Opossum	Chapecó	22J	337652	7000646	614	2000	17	394
Didelphis sp.	Opossum	Chapecó	22J	338357	6999798	650	2000	18	395
Tupinambis merianae	Black and white tegu	Saudades	22J	291201	7031073	434	1900	18	443
Procyon cancrivorus	Crab-eating raccoon	Irani	22J	413152	7021102	1029	2000	16	318
Sapajus nigritus	Black capuchin	Campos Novos	22J	490173	6971352	918	1800	16	242
Cerdocyon thous	Crab-eating fox	Guatambu	22J	326345	6999699	550	1900	18	408
Nasua nasua	Ring-tailed coati	Modelo	22J	295987	7036662		1900	18	435
Leopardus wiedii	Margay	Campos Novos	22J	481469	6969851	893	1900	16	257
Leopardus wiedii	Margay	Seara	22J	371130	6995507	-	2000	17	363
Leopardus guttulus	Tiger cat	Chapecó	22J	326816	7014931	-	1900	18	405

3.3. Statistics

To express the mean values of $\delta^{18}O$, we have obtained the average ($\bar{x}$) and its deviation (**SD – Standard deviation-**) and the median (**Md**) and its deviation (**MAD -Median Absolute Deviation-**), which is a measure less influenced by extreme values. To characterize the distribution, we have used quartiles and the interquartile range (**Qr** and **IQR** respectively). For the identification of outliers we have used the usual criteria (±2 * **MAD** and ±1.5 * **IQR**). The first of them identifies as outliers those values above or below the mean ± 2 * MAD, while the second those values that are outside the range comprised by the interquartile range 1.5 * (Q1-Q3). To analyze how variable the values obtained are, we have used the coefficient of variation (**SD** / $\bar{x}$ * 100). To evaluate the normality of the distributions we have used the Shapiro-Wilks test. Likewise, we used **the ANOVA (Tukey) test** to evaluate the homogeneity and grouping of the data at 0.05 significance level for normal distributions.

4. RESULTS

Table 2 shows the $\delta^{18}O$ values obtained for the mammals analyzed. The mean of the 17 samples is $\delta^{18}O$ 0.2 ± 1.3 ‰, and the Md is similar (0.4 ± 1.0 ‰) because extreme values are few. In this sense, just one outlier was identified (EIL-5053; see Table 2; Figure 5) using the range provided by the Md ± 2 MAD. However, this value is not detected as an outlier using the 1.5 IQR range. Indeed, the distribution of the values is normal (Shapiro-Wilks, p = 0.36), and although carnivores seem to present lower values (Figure 5), this trend is not decisive, since the ANOVA and Tukey post-hoc test show no difference between guilds (p = 0.13). In addition, the confidence intervals are similar between the different guilds (Figure 5). Whilst all these results show a significant homogeneity, it is noteworthy that they present at the same time some dispersion (CV is = 617%). This variability includes a wide range of $\delta^{18}O$ values (~ 5 ‰) between the maximum (1.9 ‰) and minimum (-2.9 ‰), although this difference decreases (~ 2.5 ‰) when comparing the 10th and 90th percentiles (Table 2).

In environments without human intervention, where organisms naturally drink environmentally available water, ^{18}O values are expected to correlate with altitude or distance from the sea (*cf.* Dansgaard, 1964; Gonfiantini *et al.*, 2001; Poage and Chamberlain, 2001). However, there is no correlation with altitude (*Pearson r* = -0.37; p = 0.2) or distance from the sea (*Pearson r* = 0.2; p = 0.4) (Figure 6). If outlier EIL-5053 is excluded, there is also no correlation with altitude or distance to the sea. The results also did not allow verifying any relationship between the body mass and $\delta^{18}O$ values (*Pearson r* = 0.05; p = 0.8).

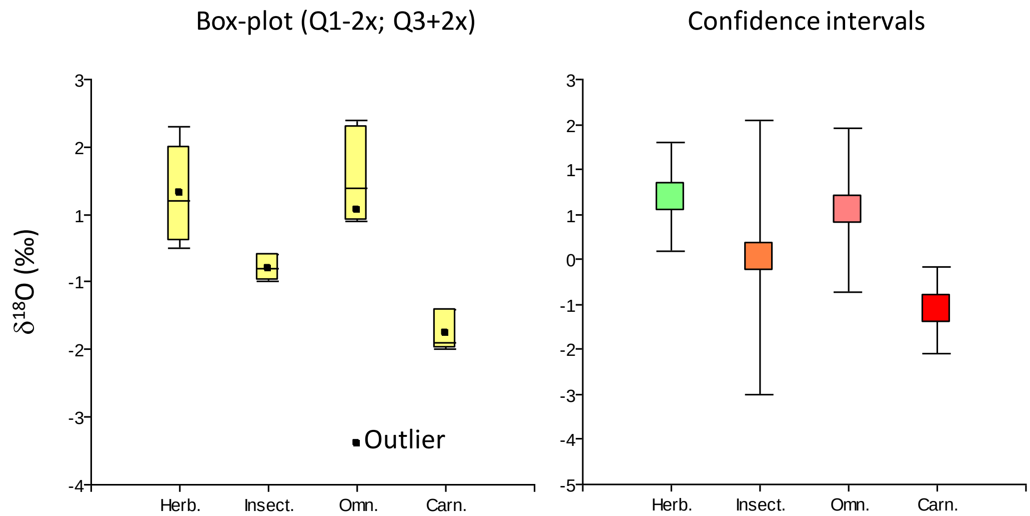

Figure 5. $\delta^{18}O$ values (Q1-2x; Q3+2x) and confidence intervals for each guild.

Table 2. Isotopic values of mammals and summary statistics.

Scientific name	Common Name	Guild	Body mass*	Code	wt%N	wt%C	C/N	$\delta^{18}O$ (‰)
Mazama gouazoubira	Gray-brocket	Herbívoro	20	EIL 5062	4,3	12,0	2,8	0,1
Mazama sp.	Brocket deer	Herbívoro	20	EIL 5063	3,9	12,7	3,3	1,5
Mazama nana	Dwarf brocket deer	Herbívoro	16	EIL 5064	4,8	14,0	2,9	1,8
Hydrochoerus hydrochaeris	Capibara	Herbívoro	50	EIL 5065	4,2	13,0	3,1	0,0
Cavia fulgida	Cavy	Herbívoro	0,3	EIL 5066	2,6	8,5	3,3	0,7
Tamandua tetradactyla	Southern tamandua	Insectivoro	7,0	EIL 5067	4,6	14,0	3,1	-0,5
Tamandua tetradactyla	Southern tamandua	Insectivoro	7,0	EIL 5068	4,2	12,9	3,1	-0,1
Euphractus sexcinctus	Six-banded armadillo	Omnívoro	5,0	EIL 5069	4,3	13,8	3,2	0,5
Didelphis sp.	Opossum	Omnívoro	2,0	EIL 5070	3,6	11,1	3,1	1,4
Didelphis sp.	Opossum	Omnívoro	2,0	EIL 5071	4,2	14,1	3,4	1,8
Procyon cancrivorus	Crab-eating raccoon	Omnívoro	6,0	EIL 5073	3,3	10,6	3,2	-2,9
Sapajus nigritus	Black capuchin	Omnívoro	3,0	EIL 5074	4,1	12,9	3,1	1,9
Cerdocyon thous	Crab-eating fox	Omnívoro	7,0	EIL 5075	3,2	10,4	3,3	0,4
Nasua nasua	Ring-tailed coati	Omnívoro	4,0	EIL 5076	4,6	14,9	3,2	0,9
Leopardus wiedii	Margay	Carnívoro	3,5	EIL 5078	4,3	13,4	3,1	-1,5
Leopardus wiedii	Margay	Carnívoro	3,5	EIL 5079	4,2	13,6	3,2	-0,9
Leopardus guttulus	Tiger cat	Carnívoro	3	EIL 5080	4,4	13,3	3,0	-1,4

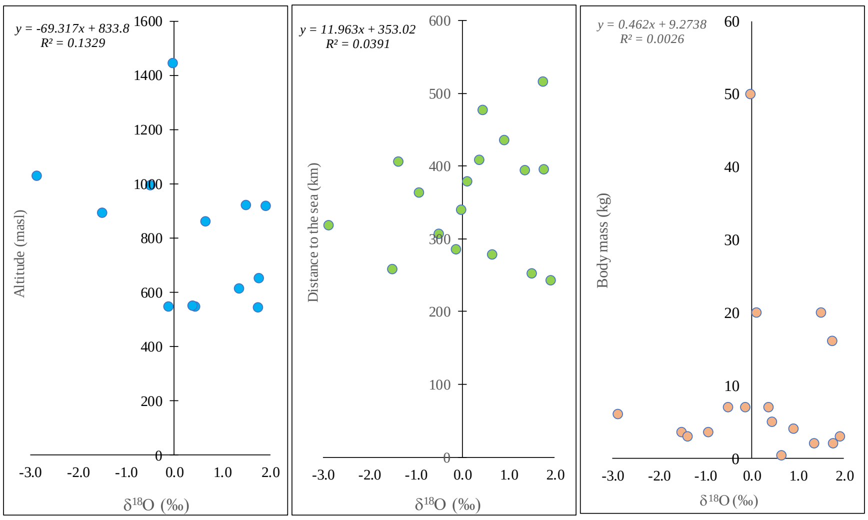

Figure 6. Distribution of $\delta^{18}O$ values related with altitude (left), distance to the sea (center), and body mass (right).

5. DISCUSSION

The mean (0.2 ± 1.3 ‰) and the median (0.4 ± 1.0 ‰) obtained in the previous section are outside the expected natural range for this oxygen-18 ecozone (-2.5 ‰ | -0.5 ‰; see Section 2.2). Indeed, the values of the samples analyzed here are higher than those obtained in three data series of pre-Columbian mammals of late Holocene Age. The ANOVA Tukey test groups these three precolumbian series and differentiates the modern one (F = 27.54; p = 0.0001) (see also Figure 7). The differences are related to the intake of water with higher $\delta^{18}O$ values for the mammals analyzed in this work, supporting the idea that they usually drink water from artificial ponds (Figure 7). Likewise, the low coefficient of variation of the samples obtained in pre-colonial contexts should be highlighted, compared to the highly variable CV of modern mammals. This can also be explained by the ingestion of the water contained in artificially manipulated reservoirs (Figure 7).

GROUP	X	n	SE		
B	-2.27	17	0.21	A	
A	-1.99	19	0.2	A	
C	-1.49	9	0.29	A	
D	0.22	17	0.21		B

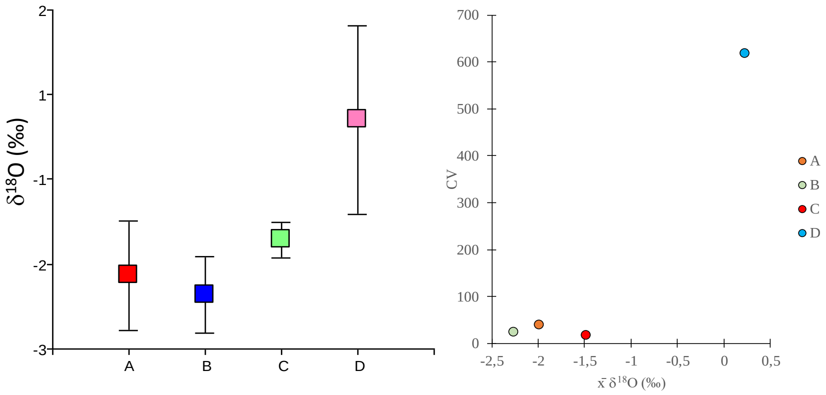

Figure 7. Left: box-plot and Tukey test (p = 0.0001; α = 0.05) of $\delta^{18}O$ values of local mammals. A, B and C were obtained in the bioapatite of pre-Columbian mammals. D: $\delta^{18}O$ values of the apatite of mammals analyzed in this study. Right: mean of $\delta^{18}O$ values plotted with the CV values. A, B and C are pre-Columbian data series. D is the data series obtained in this study. The pre-Columbian data were taken from Loponte *et al.* (2016) and Loponte and Otallagano (2022).

Within the analyzed series, carnivores (felids) and insectivores show a trend of more negative values (Figure 5). These results are similar to those obtained in other regions (Lee-Thorp, 2008; Suyt and Sealy, 2022). In fact, four values of faunivores obtained in this study fall within the natural model. This could be related to any number of reasons. First, felids have greater ranges of action and the ability to overcome obstacles. This allows for a greater chance of encountering natural water sources in the region. Second, felids seem to prefer to drink free-flowing water than stagnant water (Fritz and Handl, 2018; Pachel and Neilson, 2010; Sponheimer and Lee-Thorp, 1999; 2001; Wang, 2002). Regarding the intake of foods with more negative values for carnivores, this does not seem to be supported in this study, since the prey of felids in the region are basically those analyzed here, which have higher values. Data on $\delta^{18}O$ of insects consumed by insectivores in the region are not available. Further studies are therefore required to analyze this issue. Finally, *Procyon cancrivorus* behaves as an outlier compared to the rest of the samples, and certainly falls outside the expected values for this ecozone. For now, we don't have an explanation for this result. The absence of correlation between altitude and distance to the sea with $\delta^{18}O$ values can also be considered as further evidence of the intake of artificially manipulated water.

6. CONCLUSIONS

The values of $\delta^{18}O$ of the wild species analyzed in this study are higher and more variable than the expected in the local natural model. This probably reflects the dependence of mammals on the artificial water reservoirs built throughout the upper Uruguay River Basin. These results highlight the need for a sanitary control of these ponds to avoid the transmission of diseases from domestic livestock to native mammals, which are in a situation of population decline due to the loss and fragmentation of the natural landscape. The results obtained in this study require an expansion of the sample sizes, including in protected areas, where mammals have more options for the intake of water not manipulated by man, in such a way as to be able to increase the support of the results obtained here.

7. ACKNOWLEDGMENTS

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