Late Quaternary millennial-scale stability of the feeding strategy of the gray brocket deer [*Subulo gouazoubira* (G. Fisher, 1814); Mammalia] in Southeastern Brazil.

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Abstract

There is limited evidence, both contemporary and archaeological/paleontological, regarding the feeding ecology of the gray brocket deer (*Subulo gouazoubira*) in Brazil's Cerrado biome. Using stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope analyses of dental enamel

bioapatite from individuals recovered from Cuvieri Cave, Minas Gerais, we explore dietary and environmental changes between the Late Pleistocene and Holocene epochs in this biome. The average Pleistocene $\delta^{13}C$ was -10.9±1.7‰ and $\delta^{18}O_{dw}$ was -7.9±0.4‰, while Holocene individuals averaged a $\delta^{13}C$ of -10.5±1.2‰ and $\delta^{18}O_{dw}$ of -5.8±0.9‰. The $\delta^{13}C$ data suggest no significant shift in feeding strategy between the Late Pleistocene and Holocene, with both sets of individuals displaying $\delta^{13}C$ values consistent with browsing in environments other than closed canopy forests. However, $\delta^{18}O_{dw}$ values indicate an approximate temperature increase of 3.5°C between the two epochs and/or a reduction in precipitation. This study highlights the stability in the feeding behavior of the gray brocket deer despite biotic and abiotic changes between the epochs.

Introduction

The gray brocket deer [*Subulo gouazoubira* (G. Fisher, 1814); Mammalia; (Bernegossi et al. 2023)] is a small to medium-sized deer (weight 11–25 kg) that occurs primarily in tropical, subtropical, and semiarid regions south and east of the Amazon forest (Fig. 1). It inhabits moderately humid to dry regions in areas with some tree cover, avoiding dense forests and occasionally feeding in open habitats, if close to covered areas (Black-Décima & Vogliotti, 2016). The gray brocket deer eats a variety of vegetal parts (leaves, twigs, flowers, roots, bark, buds, seeds, and fruits) from plant species of different photosynthetic pathways (i.e., C_3, C_4, and CAM), complementing its diet with fungi, feces, invertebrates, bones, and soil (see Black-Décima and Vogliotti 2016; Black-Décima et al. 2010 and references therein). Their diet varies throughout the year and by geographic location, according to food availability, but the species is primarily considered to be a browser (Black-Décima et al., 2010; Black-Décima & Vogliotti, 2016).

Although several recent studies (Azevedo et al., 2021; Dantas et al., 2020; Tejada et al., 2020) provide Quaternary and modern comparative data for this taxon in the Brazilian Cerrado domain (Klink & Machado, 2005; Ribeiro & Walter, 2008), where gray brocket deer is considered an abundant species (Marinho-Filho et al., 2002), there is no specific study of its current and past diet. Given the dietary flexibility of the species, it is possible that its diet varied over time given the extinction of several species during the late Quaternary (Cartelle, 1999). At a minimum, a horse (*Equus neogeus* Lund 1840), two ground sloths (*Catonyx cuvieri* Lund 1839, and *Valgipes bucklandi* Lund 1844) and the saber-toothed cat (*Smilodon populator* Lund 1839) became extinct after ~20,000 cal yr BP, with the last three species

having final appearance dates younger than ~13,500 cal yr BP (Hubbe et al., 2013). Furthermore, there is evidence for vegetation and climate changes at least over the last 30,000 years (Azevedo et al., 2021; Bissa & Toledo, 2015; Cheliz et al., 2021; Novello et al., 2019; Raczka et al., 2017; Turcq et al., 1997).

For nearly fifty years, the analysis of the stable isotopes of light elements (particularly carbon and oxygen) has been an important means for reconstructing the diet and ecology of ancient animals (see reviews in Clementz, 2012; Koch, 2007). While made more difficult by the sometimes-complex physiologies of the taxa under study, preservation, and diagenetic effects, stable isotope analysis provides crucial insights into animal behavior spanning archaeological and geological time. In particular, owing to its composition and structure (Lee-Thorp, 1989; LeGeros, 1991), the carbon and oxygen isotope makeup of the carbonate fraction of dental enamel bioapatite has been demonstrated to preserve biogenic signals of feeding ecology and paleotemperature/paleohydrology stretching back hundreds of millions of years (Koch 2007:Table 5.2).

In this study, we employed stable isotope analysis ($\delta^{13}C$ and $\delta^{18}O$) on dental enamel bioapatite to explore potential variations in the feeding strategy of gray brocket deer during the late Quaternary within the Cerrado, using samples obtained from Late Pleistocene and Holocene deposits at Cuvieri Cave, Lagoa Santa, Minas Gerais, Brazil (Figure 1; Hubbe et al. 2011; Haddad-Martim et al. 2017).

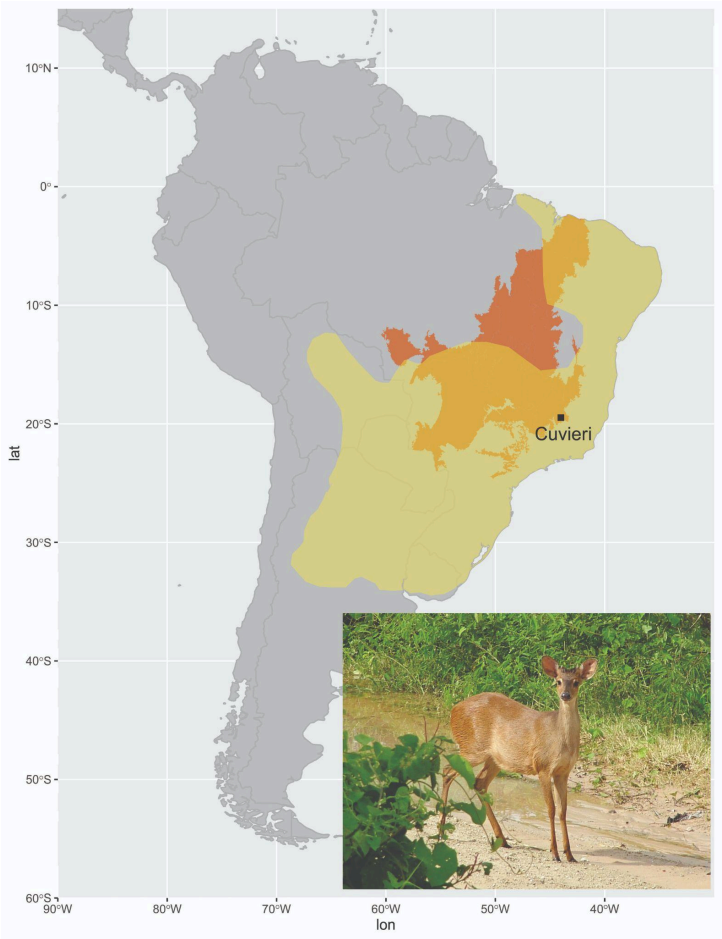

FIG 1. The current distribution range of the gray brocket deer (*Subulo gouazoubira*; yellow area) and Cerrado domain (orange area). The lower right picture depicts an adult male gray brocket deer. Black symbol represents the fossil locality Cuvieri Cave. Gray brocket deer distribution based on IUCN (2008). Cerrado distribution based on IBGE (2015). Photo by: José Maurício Barbanti Duarte.

Material and Methods

The essential information regarding analyzed samples from the Cuvieri Cave fossil site is presented here. For more information about the Cuvieri Cave fossil site, please refer to Hubbe et al. (2011, submitted) and Haddad-Martim et al. (2017).

Four lower right third molars were sampled from late Pleistocene facies and 25 lower left third molars were sampled from Holocene facies of Cuvieri Cave (Table 1). The discrepancy in sample sizes between the Late Pleistocene and the Holocene reflects the abundance of teeth available and is not the result of an intentional sampling strategy. For details regarding

sample chronology, please refer to Hubbe et al. (2011, submitted) and Haddad-Martim et al. (2017).

All third molars sampled, save two, presented some degree of wear. The isolated tooth CV-L3B-P6143 had no observable wear, but based on the position of this tooth on the tiny alveolar fragment preserved, it was presumably close to being in occlusion. The third molar preserved in the hemimandible CV-L2-12556 was still erupting, but the other molars had observable wear. By this logic, all sampled individuals were already feeding regularly by the time of their death.

The teeth selected were not identified *a priori* to the species level given the difficulties in determining species based on dental morphology alone. However, we conclude that at least the majority of them if not all belong to *S. goauzoubira* for the following reasons:

First, from a total 81 individuals identified as *Mazama*/*Subulo* for the Holocene and Pleistocene deposits from Cuvieri Cave [based on the minimum number of individuals (MNI); Hubbe et al., in prep], 86% (64 out of 74) of the individuals from the Holocene and 57% (4 out of 7) from the Late Pleistocene were positively identified as gray brocket deer. The remaining individuals (13) were not identified at the species level because available bones were too fragmented or polished for such identification. Thus, it is likely that most individuals not identified to the species level represent gray brocket deer, and that the sampled teeth are probably from this species.

Second, even if we assume that the individuals not identified at the species level are red brocket deer (*M. americana*), our samples from the Holocene and the Late Pleistocene likely include several gray brocket deer individuals. We consider the MNI to be a good estimator of the total amount of individuals in each time interval because: i) most specimens (i.e., a bone or tooth, or their fragment; (Lyman, 1994) found in Cuvieri Cave deposits are the results of pitfall entrapment; ii) around 75% and 78% of the area of the Holocene and Late Pleistocene deposits were excavated, respectively; and iii) for the Late Pleistocene deposit almost all the volume of the fossiliferous sedimentary facies were excavated, and for the Holocene deposit a volume of 3.3 m^3 was excavated (Haddad-Martim et al., 2017; Hubbe et al., 2011). Although we did not reach the bottom of this deposit, vertical stratigraphic displacement is limited (Hubbe et al., 2011, submitted) and, consequently, the MNI represents most individuals from the excavated sediments. Thus, considering that the MNI is a good approximation of the sample space of deer for each epoch, in the scenario where individuals not identified to the species level are considered red brocket deer, for the Holocene we

sampled at least 15 gray brocket deer and the chance of sampling 19 or more gray brocket deer is ~98%, while for the Late Pleistocene we sampled at least 1 gray brocket deer, and chance of sampling two or more gray brocket deer is of ~88%.

Third, our results suggest that all individuals sampled for stable isotope analysis (n=29) did not feed in closed forest environments (see below), which is in agreement with what we know about gray brocket deer biology/feeding ecology, and which stands in contrast with the current understanding of red brocket deer biology (i.e., that it inhabits and feeds in closed forest environments; Black-Décima & Vogliotti, 2016; Duarte & Vogliotti, 2016; Gallina-Tessaro et al., 2019). Palynological, environmental, and sedimentological evidence supports the existence of a non-canopy forest environment during the time of formation of the facies in Cuvieri Cave from which teeth were sampled (Hubbe et al., submitted). Even though it is possible that red gray brocket deer evolved to inhabit closed forest environments only very recently, given that we certainly sampled gray brocket deer teeth for both time intervals, it is more likely that our results reflect the fact that our sample consists largely/exclusively of gray brocket deer.

$\delta^{13}C$ and $\delta^{18}O$ Isotope Analyses

Carbon and oxygen stable isotope analysis was performed on bioapatite extracted from dental enamel. Both isotope measures are well-established in archaeology, geology, and paleontology, with decades of supporting literature, and are widely used for the reconstruction of ancient animal diet, paleoecology, and paleotemperature (see reviews in Clementz, 2012; Koch, 2007).

Knowledge of the ratio of ^{13}C to ^{12}C (expressed as $\delta^{13}C$, the ratio of heavy to light isotope in parts permil (‰) relative to an international standard, here PDB, Pee Dee Belemnite) in bone or tooth hydroxyapatite of herbivores permits estimation of the relative contribution of plants of different photosynthetic groups (C_3 vs. C_4/CAM) to an individual's diet (Bocherens et al., 1996; Cerling et al., 1997, 1998; Fox & Koch, 2004; Franz-Odendaal et al., 2002; Koch, 1998; Koch et al., 2004; Latorre et al., 1997; Macfadden & Cerling, 1996; Zazzo et al., 2000). Additionally, carbon isotope analysis also allows for the identification of taxa or individuals that habitually consumed resources from closed-canopy forests, contributing to our understanding of regional paleoecology (Tejada et al., 2020; Van der Merwe & Medina, 1991). In the analysis presented here, we applied two different diet-hydroxyapatite

fractionation values, -12‰ and -14.6‰, thus accounting for the range of proposed fractionation values for wild ungulates (Balasse, 2002; Cerling & Harris, 1999; Lee-Thorp & Van der Merwe, 1991; Passey et al., 2005; Sullivan & Krueger, 1981).

The ratio of ^{18}O to ^{16}O, or $\delta^{18}O$, in biological apatites largely reflects the oxygen isotope composition of water consumed by an organism while alive (Sponheimer & Lee-Thorp, 1999), which, in turn, varies systematically with temperature and rainfall (Dansgaard, 1964; Gat et al., 1996). As such, $\delta^{18}O$ in animal bioapatite is a widely employed "paleothermometer" allowing for proxy characterization of past climates (Koch, 1998). Observed isotopic values of hydroxyapatite carbonate require several steps of conversion before paleoclimatic determinations can be made (Pryor et al., 2014; Stevens et al., 2011). First, the observed $\delta^{18}O$ values, which were reported relative to the V-PDB standard, must be converted to the V-SMOW scale, following (Coplen et al., 1983). Next, this converted value is used to derive a value for the oxygen isotope signature of the water consumed/ingested, or $\delta^{18}O_{dw}$ (Iacumin et al., 1996; Stevens et al., 2011). Finally, $\delta^{18}O_{dw}$ is converted to surface air temperature (°C) using a formula derived from (Rozanski et al., 1992).

Extraction of hydroxyapatite followed protocols first established in (Lee-Thorp, 1989) and (Krueger, 1991) and modified by (Pestle, 2010). Approximately 150 mg (weighed to the nearest 0.1 mg) of ground enamel was placed in a 50 mL centrifuge tube. After weighing, each sample underwent 24 h of organic oxidation using 30 mL of 50% bleach (~2.5% NaOCl). Samples then were rinsed to neutral pH through a repeated process of centrifugation, decanting, and addition of distilled water. Next, labile carbonates were removed by the addition of 30 mL of 0.1 M acetic acid to each centrifuge tube for a total of four hours, with a 5 min vacuum treatment after 2 h. After the acid treatment, each sample was rinsed to neutral pH before being placed in a 50 ˚C oven overnight to dry the resulting product. Start and end weights were recorded for all hydroxyapatite samples (weighed to the nearest 0.1 mg) and used to calculate the hydroxyapatite yield (wt%).

Isotopic analysis was performed in the Marine Geology and Geophysics Stable Isotope Laboratory at the Rosenstiel School of Marine and Atmospheric Science, University of Miami. Analysis took place using a Kiel-IV Carbonate Device coupled to a Thermo Finnigan DeltaPlus IRMA, employing an OCC (optically clear calcite) standard calibrated to NBS-19. Standards were analyzed in every sample set at the beginning and end of the run, as well as in-between the analyzed samples to ensure instrumental stability. Check samples of the standard were also run as unknowns in every run to verify measurement accuracy (Szpak et

al., 2017). Long-term precision for the analysis of both $\delta^{13}C$ and $\delta^{18}O$ was better than 0.1‰. Initial (observed) results for both isotope systems are presented relative to the V-PDB standard (Koch, 2007).

Statistical Analysis

We utilized boxplots generated with ggplot2 (Wickham, 2016) to explore differences in feeding strategy between epochs. Due to the limited sample size, particularly for the Late Pleistocene, we refrained from conducting inferential statistical tests to compare central tendencies.

Results

$\delta^{13}C$ and $\delta^{18}O$ Isotope Analyses

All samples yielded sufficient material for analysis, with post-treatment hydroxyapatite yields averaging 72.2±6.8%. Observed $\delta^{13}C_{PDB}$ values ranged from -13.7 to -8.4‰ (mean -10.6±1.3‰), while $\delta^{18}O_{PDB}$ values averaged -3.3±1.1‰, with an overall range extending from -5.5 to -1.4‰ (Table 1).

Regarding $\delta^{13}C$, application of a 12‰ fractionation value yielded an average dietary carbon isotope signature of -22.6±1.3‰, whereas a 14.6‰ fractionation produced an average $\delta^{13}C_{diet}$ of -25.2±1.3‰. If one considers the latter value, the analyzed individuals would appear to have been consuming almost pure C_3 plants, which have an average $\delta^{13}C$ of 25.5±2‰, with C_4 plants (e.g., tropical grasses, which average 9.5±1.0‰) forming, on average, only 4.1±4.8% (range 0.0–15.7%) of individual diet. Application of the 12‰ fractionation value yielded higher estimates of C_4 consumption, averaging 18.2±7.9% (range 0.0–32.0%). Regardless of which fractionation value is applied, C_3 plants formed the overwhelming majority of the analyzed individuals' diets, with a small contribution coming from C_4 or CAM plants

There was no meaningful difference in $\delta^{13}C$ values between individuals of the Pleistocene and the Holocene (Fig. 2). Average Pleistocene $\delta^{13}C$ was -10.9±1.7‰, while Holocene individuals averaged a nearly identical -10.5±1.2‰. By extension, there was little difference in the consumption of C_4 plants between the two epochs (Figure C4). Both Holocene and Pleistocene individuals have $\delta^{13}C$ signatures that bound modern *Subulo gouazoubira* from the

western Amazon (Tejada et al., 2020) and a Pleistocene-age individual from Intertropical Brazil (Dantas et al., 2020), while a sole Pleistocene individual (only identified to the genus *Mazama*) from the southwestern Amazon reported in Azevedo et al. (2021) had a lower $\delta^{13}C$ (-15.5‰), perhaps indicating feeding in a more closed forest.

The overall range of $\delta^{18}O_{dw}$ (extrapolated as noted above from the measured $\delta^{18}O$ values of apatite carbonate) was -6.1±1.1‰, with a range that extends from -8.4‰ to -4.2‰. The Pleistocene individuals had an average $\delta^{18}O_{dw}$ of -7.9±0.4‰, and the Holocene individuals averaged -5.8±0.9‰ (Fig. 3). When converted to surface air temperature, this equates to an average temperature at the time of apatite formation for the Pleistocene individuals of 10.9±0.6°C versus 14.4±1.5°C for the Holocene samples .

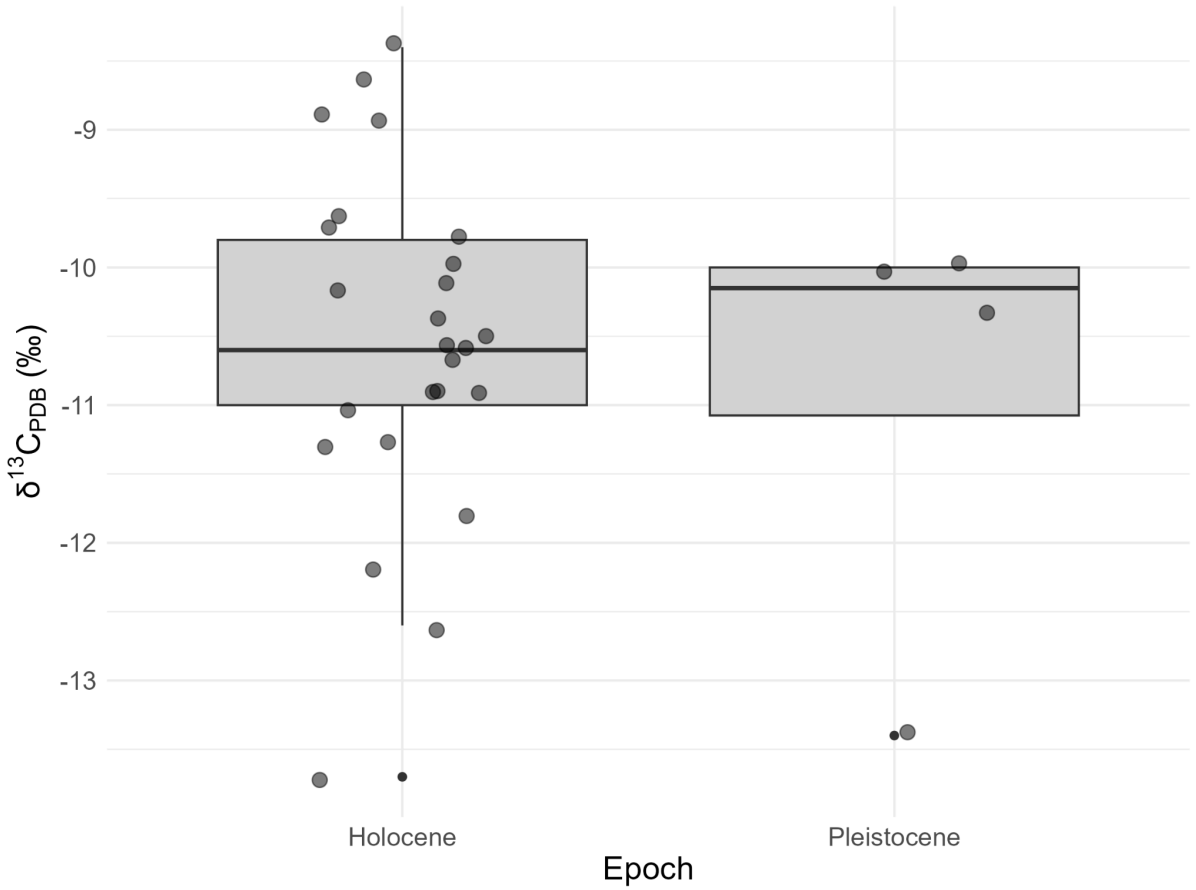

Figure 2. $\delta^{13}C_{PDB}$ (‰) values (dots) and boxplot for Cervidae teeth from Cuvieri Cave Holocene and Pleistocene deposits.

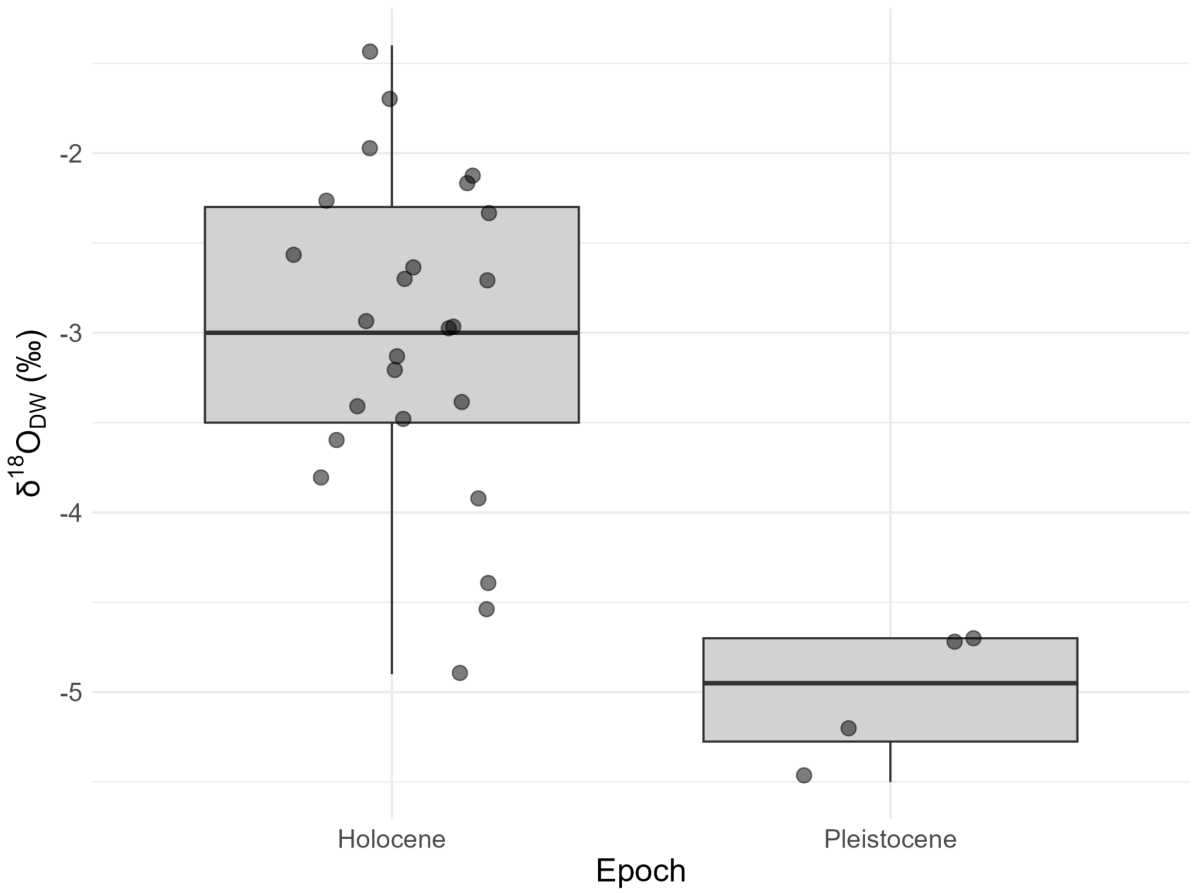

Figure 3. $\delta^{18}O_{dw}$ (‰) values (dots) and boxplot for Cervidae teeth from Cuvieri Cave Holocene and Pleistocene deposits.

Table 1. Anatomical, chronostratigraphic, and isotopic data for dental enamel samples from Cuvieri Cave's Holocene and Pleistocene deposits. The "depth" column refers to the vertical distance between the location where the sample was found during excavation and the adopted zero reference point in the excavated areas.

Excavation #	Anatomical part	Depth (cm)	Epoch	Apatite yield	δ13CPDB (‰)	Diet maximum δ13C (fractionation -12‰)	Diet minimum δ13C (fractionation -14.6‰)	Maximum % C4 plants	Minimum % C4 plants	δ18OPDB (‰)	δ18OSMOW (‰) Coplen 1983	δ18Odw (‰) Stevens, et al. 2011	Temperature (°C) Rozanski, et al. 1992
CVL3B 352	Hemimandible	-112.5	Pleistocene	71.5%	-10.0	-22.0	-24.6	21.6%	5.4%	-4.7	26.0	-7.6	11.4
CVL3B P2895	Hemimandible	-130[a]	Pleistocene	77.9%	-13.4	-25.4	-28.0	0.6%	0.0%	-5.5	25.3	-8.4	10.1
CVl3B P3961	isolated tooth	-168[a]	Pleistocene	74.8%	-10.3	-22.3	-24.9	20.2%	3.9%	-5.2	25.5	-8.1	10.6
CVL3B P6143	isolated tooth	-257[a]	Pleistocene	84.1%	-10.0	-22.0	-24.6	21.7%	5.5%	-4.7	26.0	-7.6	11.4
CVL2 687	Hemimandible	-66	Holocene	86.6%	-11.3	-23.3	-25.9	13.5%	0.0%	-1.4	29.4	-4.2	17.2
CVL2 2140	Hemimandible	-70.5	Holocene	69.9%	-10.4	-22.4	-25.0	19.3%	3.0%	-3.2	27.6	-6.0	14.1
CVL2 3361	Hemimandible	-75	Holocene	62.0%	-12.6	-24.6	-27.2	5.5%	0.0%	-3.0	27.8	-5.8	14.5
CVL2 5958	Hemimandible	-75	Holocene	66.7%	-10.6	-22.6	-25.2	18.1%	1.8%	-2.2	28.7	-5.0	15.9
CVL2 2957	Hemimandible	-75	Holocene	74.1%	-12.2	-24.2	-26.8	8.2%	0.0%	-2.7	28.1	-5.5	15.0
CVL2 4321	isolated tooth	-76	Holocene	74.9%	-10.9	-22.9	-25.5	16.2%	0.0%	-2.9	27.9	-5.7	14.6
CVL2 6730	Hemimandible	-80.5	Holocene	63.6%	-10.5	-22.5	-25.1	19.0%	2.7%	-2.6	28.3	-5.4	15.2
CVL2 6912	Hemimandible	-88	Holocene	70.4%	-11.8	-23.8	-26.4	10.9%	0.0%	-3.4	27.5	-6.2	13.8
CVL2 8210	isolated tooth	-88.5	Holocene	70.8%	-10.6	-22.6	-25.2	18.0%	1.8%	-1.7	29.2	-4.5	16.7
CVL2 10743	Hemimandible	-99	Holocene	71.3%	-13.7	-25.7	-28.3	0.0%	0.0%	-3.1	27.8	-5.9	14.3
CVL2 10801	Hemimandible	-99	Holocene	67.7%	-10.2	-22.2	-24.8	20.5%	4.2%	-2.7	28.1	-5.5	15.0
CVL2 10823	isolated tooth	-99	Holocene	68.7%	-11.3	-23.3	-25.9	14.0%	0.0%	-3.4	27.4	-6.3	13.7
CVL2 10981	Hemimandible	-100	Holocene	72.3%	-10.7	-22.7	-25.3	17.3%	1.0%	-4.4	26.3	-7.3	11.9
CVL2 10998	Hemimandible	-100	Holocene	66.2%	-9.8	-21.8	-24.4	23.4%	7.1%	-3.9	26.9	-6.7	12.9
CVL2 12417	Hemimandible	-102	Holocene	55.8%	-8.9	-20.9	-23.5	28.8%	12.5%	-2.1	28.7	-4.9	16.0
CVL2 11439	isolated tooth	-102	Holocene	79.1%	-8.6	-20.6	-23.2	30.7%	14.5%	-4.5	26.3	-7.4	11.8
CVL2 12184	isolated tooth	-104	Holocene	76.9%	-8.9	-20.9	-23.5	29.0%	12.8%	-3.5	27.3	-6.4	13.5
CVL2 12808	isolated tooth	-106	Holocene	74.7%	-8.4	-20.4	-23.0	32.0%	15.7%	-2.3	28.5	-5.1	15.6
CVL2 11942	Hemimandible	-107	Holocene	74.2%	-10.9	-22.9	-25.5	16.1%	0.0%	-2.6	28.3	-5.4	15.2
CVL2 12646	isolated tooth	-107	Holocene	79.9%	-9.6	-21.6	-24.2	24.4%	8.1%	-3.0	27.8	-5.8	14.4
CVL2 12945	isolated tooth	-108	Holocene	69.6%	-11.0	-23.0	-25.6	15.6%	0.0%	-4.9	25.9	-7.8	11.2
CVL2 13197	isolated tooth	-109	Holocene	79.8%	-10.1	-22.1	-24.7	21.3%	5.1%	-2.3	28.6	-5.1	15.7
CVL2 12556	Hemimandible	-110	Holocene	61.8%	-10.9	-22.9	-25.5	16.4%	0.2%	-2.0	28.9	-4.8	16.2
CVL2 12555	isolated tooth	-110	Holocene	74.9%	-10.0	-22.0	-24.6	21.7%	5.4%	-3.8	27.0	-6.6	13.0
CVL2 14618	Hemimandible	-114	Holocene	73.1%	-9.7	-21.7	-24.3	24.0%	7.7%	-3.6	27.2	-6.5	13.3
			Mean	72.2%	-10.6	-22.6	-25.2	18.2%	4.1%	-3.3	27.5	-6.1	13.9
			sd	6.8%	1.3	1.3	1.3	7.9%	4.8%	1.1	1.1	1.1	1.9
			Minimum	55.8%	-13.7	-25.7	-28.3	0.0%	0.0%	-5.5	25.3	/	10.1
			Maximum	86.6%	-8.4	-20.4	-23.0	32.0%	15.7%	-1.4	29.4	-4.2	17.2

[a]Since this specimen was retrieved during the sieving of sediments from the excavations, its depth was determined as the midrange depth of the corresponding sedimentary facies.

Discussion

While the sample size for the Pleistocene remains small, and thus prohibits robust statistical comparison of the diets of the two epochs, the data at hand do not indicate a diachronic change in the feeding strategy for the gray brocket deer in the Cerrado between the Late Pleistocene and the Holocene, at least based on the aspects of diet captured in carbon stable isotopes (Fig. 1). Our analysis supports gallery/open forest browsing as the main feeding strategy for all gray brocket deer individuals (i.e., maximum $\delta^{13}C$ = -8.4), consistent with the current understanding of the species' feeding strategy (Black-Décima et al., 2010; Black-Décima & Vogliotti, 2016). Our study aligns with research conducted on this taxon in other regions of Brazil (Azevedo et al., 2021; Dantas et al., 2020; Tejada et al., 2020), and with studies of browsers from elsewhere in the southern hemisphere before/after the LGM, which similarly did not observe a significant shift in $\delta^{13}C$ values between Late Pleistocene and Holocene samples (Sealy et al., 2020). This stands in contrast to studies conducted in the northern hemisphere, which reported differences between epochs (see Sealy et al. [2020] and references therein).

Given the dietary flexibility of gray brocket deer (Black-Décima et al., 2010; Black-Décima & Vogliotti, 2016), and the present and past diversity of plant species in the study region (Raczka et al., 2017), either estimate of C_4 contribution presented above (4.1±4.8% or 18.2±7.9%) seems entirely realistic, as does the variability observed among the Holocene individuals. What is more certain from our results is that, by any fractionation estimate, the plants being consumed by these individuals were not predominantly originating from closed canopy environments, aligning with other isotopic studies (Tejada et al. 2020) and observational studies of the species' biology (Black-Décima et al., 2010; Black-Décima & Vogliotti, 2016), but see (Grotta-Neto et al., 2019). Plant tissues from such environments will possess $\delta^{13}C$ values of -35‰ or lower (Van der Merwe & Medina, 1991), nearly 10‰ lower than the lowest estimated dietary value for the twenty-nine analyzed gray brocket deer individuals (-25.7‰).

Unlike the case of the carbon isotopes, comparison of the Pleistocene and Holocene individuals reveals clear differences in the oxygen isotope composition between epochs. As a consequence, one can make certain climatic inferences (Fig. 3), albeit contingent on the small sample size and uncertainties around physiological factors. The difference between these two subsets in average $\delta^{18}O_{dw}$ (2.1‰) suggests a warming of 3.5 °C between the epochs

when applying the temperature coefficient provided in (Rozanski et al., 1992), which is consistent with the understanding that the Late Pleistocene was colder than the Holocene in sub-tropical South America (Baker & Fritz, 2015; Cruz et al., 2006). However, while unlikely, we cannot exclude the possibility that the sampled teeth of the four Pleistocene individuals were formed primarily during colder winter months while those of the Holocene specimens formed primarily during hotter summer temperatures, which adds a cautionary note to the interpretation of this difference.

The increase in $\delta^{18}O$ observed in the transition from the Late Pleistocene to Holocene samples could be also a consequence of the reduction in precipitation suggested by contemporary stable isotope records observed in speleothems from the Cerrado and other nearby locales (Azevedo et al., 2021; Cruz et al., 2005; Novello et al., 2019). Indeed, an increase in herbivore enamel $\delta^{18}O$ is associated with both warmer and drier conditions (Kohn et al., 2022). Thus, the observed 2.1‰ increase in *Subulo* enamel $\delta^{18}O$ could be associated with a similar magnitude (~2.5‰) rebound in speleothem $\delta^{18}O$ seen in speleothem records as the Holocene dry period begins after the conclusion of the Younger Dryas, post 12,900-11,600 bp (Novello et al., 2017):3). Thus, multiple reasonable explanations exist for the observed shift in $\delta^{18}O$, both of which (a rise in temperature and a decrease in rainfall) are consistent with locally available paleoclimatic records.

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AUTHORS CONTRIBUTION

AH: Conceptualization, Formal analysis, Investigation, Funding acquisition, Writing - Original Draft, Writing - Review & Editing; WJP: Conceptualization, Formal analysis, Investigation, Data Curation, Funding acquisition, Supervision, Writing - Original Draft, Writing - Review & Editing; MH: Conceptualization, Writing - Review & Editing; AA: Investigation, Writing - Review & Editing; ENC: Investigation, Writing - Review & Editing; REO: Investigation, Resources, Writing - Review & Editing; EDL: Investigation, Writing - Review & Editing; WAN: Funding acquisition, Writing - Review & Editing.

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