



# Sheep and goat management practices at Perdigões (Southern Portugal, 4<sup>th</sup> millennium BC) via sequential carbon and oxygen isotopic analyses of tooth enamel

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## Abstract

The archaeological site of Perdigões (Reguengos de Monsaraz, Portugal) was a centre of social and ceremonial importance with diverse funeral traditions from the middle 4th to the 3rd millennium BC. Previous strontium isotope analysis of human remains indicates that these funerary contexts contained individuals from a wide catchment area. Together with artefactual evidence, it suggests Perdigões was a part of a large interaction network of settlements that may have spanned Iberia, Northern Africa, and the Central Mediterranean. The stable isotopic analysis of domesticated animals' bones and teeth can provide insights into human behaviour via the choices connected to animal pasture and foddering regimes. Sheep and goats were pivotal species for Neolithic and Bronze Age communities in Iberia, and the herding strategies of individual flocks would have been dependent on local vegetation and geography as well as production strategies. Here we present a detailed and comprehensive picture of husbandry practices of sheep/goat remains found at Perdigões, using stable isotopic values from suids and deer as a baseline for the local environment. The incremental stable  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopic results from sheep/goat molars provide insight into seasonal variations in pasture and foddering practices, thereby shedding light on human management strategies. Differences between individuals may suggest that sampled sheep/goats individuals did not come from a single flock. The results reinforce the argument that Perdigões was a central place of significance, attracting communities and their herds both within the vicinity of the site and also further afield.

**Keywords** Pasturing management · Seasonality · Incremental dental sampling · Neolithic · Suid (*Sus* sp.), deer (*Cervus elaphus*)

## Introduction

The Perdigões archaeological complex, located in inland Alentejo (Reguengos de Monsaraz, Évora, Southern Portugal), is a 16 ha ditched enclosure with a long continuous occupation spanning from the Late Middle Neolithic to Late Chalcolithic/Early Bronze Age (3400–2000 BC) (Valera 2017, 2023; Valera et al. 2020). The site, in its long duration, covered an historical context characterized by a demographic growth, agricultural and herding intensification (although hunting and gathering still maintained economic relevance), development of large-scale interaction networks, increasing craft specialization and significant investments in monumentality and practices of ideological display, associated to moderate levels of social inequality (Valera 2015; Blanco-González et al. 2018). Its topographical location and interaction with the surrounding landscape in the Ribeira do Alámo

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Valley, along with the handling of the dead — reflecting different treatments of bodies in association with diverse architectures and material assemblages — suggest different social statuses, identities, or provenance (Valera et al. 2014). These diverse mortuary practices, together with strontium isotope data and “exogenous” provenance of items and raw material, suggest that Perdigões was part of a broader network of exchange and social interaction. The structural features, combined with the artefactual findings and funerary practices, suggest the site was a major centre of social and ceremonial importance, drawing communities from different areas across the Iberian Peninsula and further afield to bring their dead to this final resting place (Valera et al. 2017, 2020). Animal remains are abundantly found commingled with human burials (Cabaço 2009) and cremations (Almeida and Valera 2022) but also in non-funerary contexts, such as pits and ditches (Costa 2013, 2018; Almeida and Valera 2021). Even if differences might occur concerning the structured deposition of specific animal body parts, the overall fauna spectra identified at the site roughly follow the common subsistence pattern as reflected at settlement sites (Valente and Carvalho 2014; Schirmacher et al. 2024). However, the site’s role as a major aggregation centre of potentially socially and culturally diverse people raises the possibility of identifying different herding practices. To explore this further, this study focuses on one of the most common species at the site, sheep/goat, through the application of geochemical methodologies.

Herding practices, specifically the foddering strategies, are deeply intertwined with human behaviour and intrinsically tied to landscape exploitation and the definition of settlement areas (Berthon et al. 2021). These practices are influenced by local environments and economic constraints, particularly in terms of person/time management. As a result, different communities may adopt different pasturing regimes. The application of stable isotope analysis has been vital in deciphering key aspects of prehistoric animal husbandry, encompassing temporal changes in techniques, seasonal variations in food resources, adjusting age at weaning, birth seasonality, and detection of animal movement associated with activities such as trade or grazing (Towers et al. 2011; Makarewicz 2017a; Kinaston 2023). Stable isotope analysis of the mineral fraction of enamel, bioapatite, holds significant potential for understanding herding strategies, as it allows us to directly uncover an individual animals’ diet within a fixed period. The stable isotopic ratios of this tissue are effected by dietary and climatic factors, and given the lack of secondary mineralisation preserves a chronological record of isotopic changes throughout the tooth’s growth, covering the tooth development (Balasse 2002). The carbon isotopic composition of bioapatite reflects diet and in particular the type of plants eaten based on their photosynthetic pathways  $C_3$  or  $C_4$  (Balasse et al. 2002). Oxygen isotopic

values of bioapatite are precipitated in equilibrium with body water, which is a reflection of local drinking water temperature and can thus serve as a proxy for local climates (Balasse 2002; Balasse et al. 2002). Obligate-drinking species (e.g. suid and deer) primarily derive their ingested water from meteoric sources, hence their tissue  $\delta^{18}O$  is closely aligned to the isotopic composition of environmental water (Pederzani and Britton 2019). Obligate drinkers tend to typically have lower body water  $\delta^{18}O$  values compared to non-obligate drinkers (sheep/goat), who acquire their water from the ingested plants. Leaf water tends to be  $^{18}O$ -enriched compared to local meteoric water due to evapotranspiration effects (Dongmann et al. 1974; Epstein et al. 1977). Therefore, carbon and oxygen isotopic values from sequential samples of molar enamel can provide us with an approximately monthly-scale resolution during tooth mineralization, generating a window onto herd management strategies and, by extension, human behaviour.

The concepts of mobility and social interaction play a central role in social organisations and are crucial for gaining a deeper understanding of past human behaviour (Valera et al. 2020). Previous strontium isotope studies from Perdigões exhibit a significant scaled movement of humans and animals, which further strengthens the interpretation of the site as a complex aggregation centre within a large interaction network (Valera et al. 2020). Although strontium isotopes are commonly applied to infer pastoralist mobility, oxygen isotopes can also be extremely informative, as they reflect the geospatial isotopic variation in  $\delta^{18}O$  values in the environment, enabling to explore the movement of herds and pastoralists across the landscape (Makarewicz 2017b; Miller and Makarewicz 2017). Paired sequential analyses of oxygen and carbon can shed light on the movement of animals and exploitation of different habitats on a seasonal basis (Pederzani and Britton 2019). To further explore the human behaviour and social complexity at the site, stable isotopes of carbon and oxygen in sequential samples of enamel bioapatite were employed to reconstruct and explore the diet. Together with deer and suid stable isotopic values from bioapatite, we aim to shed light on sheep/goat herding strategies and potentially discern the dietary and movement patterns of these animals. This will fill a missing gap in understanding animal-human relationships at Perdigões, as well as in Iberia as a whole during the Late Neolithic.

## The archaeological site of Perdigões

### General contextual information

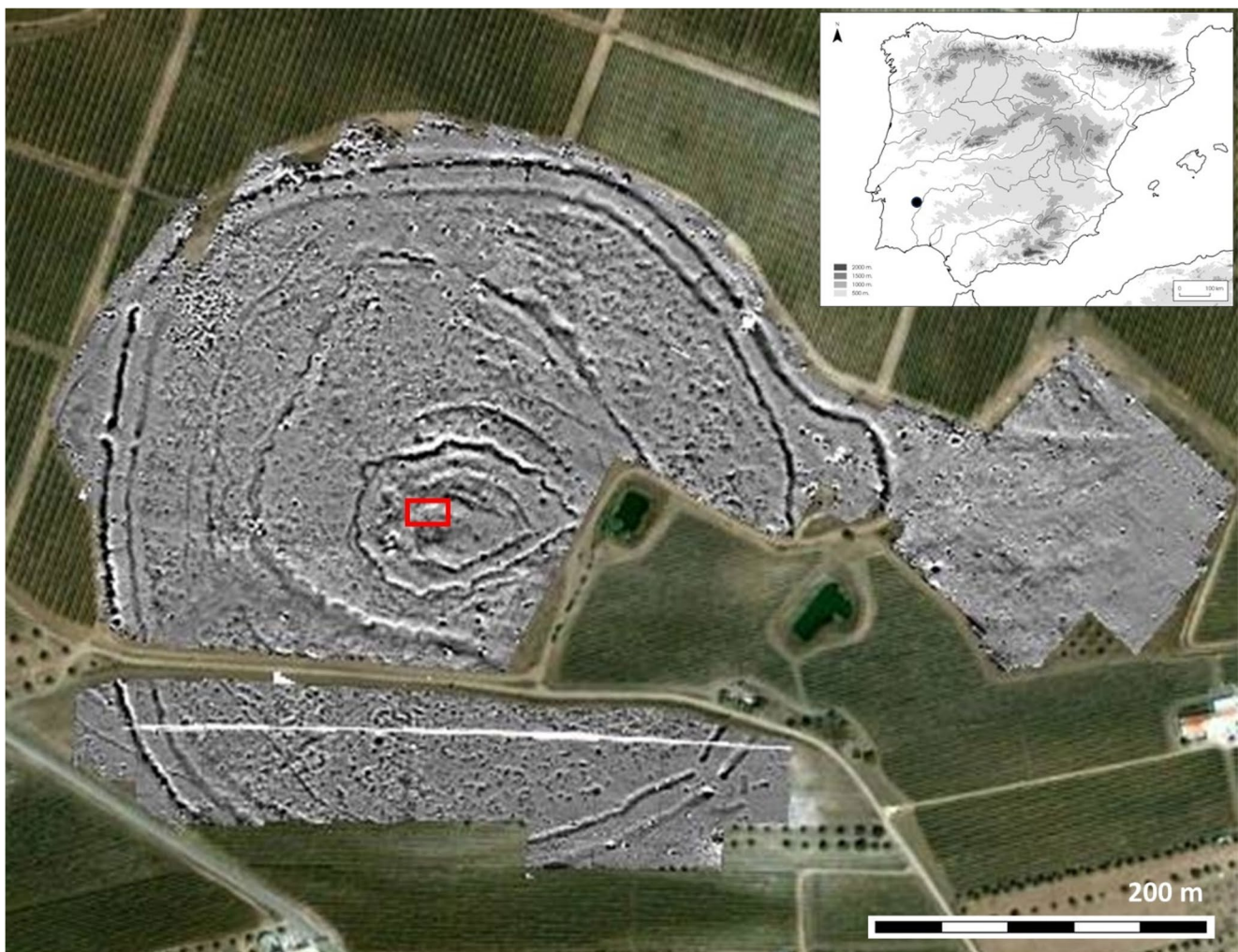
The Perdigões enclosure, an archaeological site with a complex sequence of occupation throughout Late Prehistory, has

been the target of extensive research over the past 25 years under a long-term research program led by ERA - Arqueologia, S.A. (Portugal), resulting in numerous publications (Valera 2023; Fig. 1). Situated in a natural amphitheatre open to the East towards the Ribeira do Álamo Valley, the site encompasses a significant concentration of ditched enclosures (15 roughly concentric ditches), walled enclosures, and thousands of pits (Valera et al. 2014; Valera 2017; Godinho et al. 2019). The set of enclosures presents a long and continuous chronology, dating back to the Late Middle Neolithic, with smaller enclosures positioned at the centre of the natural theatre (Žalaitė et al. 2018; Godinho et al. 2019). During the Late Neolithic, the site started to expand exponentially, reaching its peak in the Late Chalcolithic, before abruptly declining at the end of the 3rd millennium BC. Throughout this timeframe, the site underwent several architectural transformations, including the opening and

closing of the negative features. However, it consistently adhered to its astronomic and ideological relationship with the topography and landscape, with the gates aligned with the summer and winter solstices, allowing the site's horizon to function as an annual calendar (Valera et al. 2014; Valera 2015; Žalaitė et al. 2018).

### Faunal assemblages

Perdigões has one of the largest faunal assemblages (current NISP=30.000) for Iberian Late Prehistory, spanning the Late Middle Neolithic – transition to the Early Bronze Age. Remains have been recovered in different types of contexts and with diverse archaeological associations. The relevance of hunted species (deer, auroch and horse) is most notable during the Neolithic and towards the end of the 3rd millennium BC (Costa 2018; Almeida and Valera



**Fig. 1** Location of Perdigões site in Iberia and of the contexts of provenance of the animal remains (in the red box) in the magnetogram of the enclosure. Photo credits to Dr. António Valera

2021). Almeida and colleagues (2024) argued that deer were a social element during Late Prehistory, gaining symbolic value as a part of social practices of food-sharing and feasting events. Other species, most notably suids but also bovine and caprine (i.e., sheep/goat) have also been included in these events or, together with dogs, used in structured depositions. Focusing on the Neolithic data, the site has scarce evidence related to agriculture, especially cereals, but important hunting practices are registered as associated with the construction of the first ditches (Costa 2018). With the Late Neolithic, together with a rise in agricultural indicators, there was a significant growth of domesticated species and a decrease of hunted ones, although these remain relevant. Suids and caprine kill-off patterns show slaughtered mostly during the first or second year of age. Moreover, sheep/goats were slaughtered aged less than 24 months during the Late Middle Neolithic (50%) and Late Neolithic (73%: 46% have ages under 12 months, of which 43% are infants). The Neolithic age of death recorded at the site for caprine was interpreted as natural (Costa 2018) or related to social practices of food-sharing events (Valera 2018). A possible mixed meat and milk profile was suggested for some Chalcolithic samples but the influence of seasonality or a ritualized component in the slaughter patterns can not be disregarded (Almeida and Valera 2021).

From a taphonomical perspective, some major differences occur regarding bone surface preservations due to the specificities of the contexts where the remains are recovered (Costa 2013, 2018; Almeida and Valera 2021). Overall, the Neolithic assemblages show a higher preservation of bone surfaces compared to the Chalcolithic and especially the Chalcolithic-Early Bronze Age samples. Bone surface modifications related to butchering and consumption are also registered, albeit with differences regarding context and chronology (Cabaço 2012, 2017; Costa 2013, 2018; Valera et al. 2019, 2024; Almeida and Valera 2021, 2022).

### Palaeoenvironmental and archaeobotanical data

The Alentejo region, where the Perdigões site is located, exhibits a semi-arid Mediterranean climate, marked by hot and dry summers, and relatively rainy and mild winters. Paleoclimate reconstructions conducted in the Iberian Peninsula suggest a temperature increase from the Younger Dryas onwards (Liu et al. 2023). During the early Holocene in the western regions of Iberia, there is an overall trend of wetter environmental conditions and a shift towards drier conditions from approximately 7.5 cal ka BP to the present (Mauri et al. 2015; Liu et al. 2023). Historical records covering the period from 1871 to 2008 for the Alentejo region revealed an overall annual precipitation of < 1000 mm (IPMA 2023).

The archaeobotanical record in Iberia suggests the existence of a well-developed agricultural system, with evidence pointing to its presence in several areas since the Early Neolithic (Peña-Chocarro et al. 2018). The only data available from Portuguese archaeological contexts derives from two sites in the Estremadura region, Lapiás das Lameiras and São Pedro de Canaferrim (Sintra), dated to the 6th millennium cal BC (Lopez-Doriga and Simões 2015; Peña-Chocarro et al. 2018). Evidence of cultivated domesticated species includes naked barley (*Hordeum vulgare* var. *nudum*), bread/macaroni wheat (*Triticum aestivum/durum*), and pulses such as broad bean (*Vicia faba*), celtic bean (*Vicia sativa*), lentil (*Lens culinaris*), and pea (*Pisum sativum*) (Lopez-Doriga and Simões 2015).

Palaeoenvironmental analysis of archaeological deposits originating from Perdigões indicates an open park-like environment dominated by *Poaceae* (grasses) in the Late Neolithic, with scattered trees of *Pinus* (Pine) and *Quercus* sp. (Oak) (Wheeler 2010; Danielsen and Mendes 2013). The presence of *Plantaginaceae* (Plantain family) indicates natural wet meadows and pastures, while the presence of spores *Pteropsida* (Pteridophytes) and *Pteridium* (Bracken) could possibly indicate a surrounding woodland and shaded areas near the site (Wheeler 2010). Hence, an environment composed mainly of C<sub>3</sub> plants is expected for the Alentejo region during the Late Neolithic.

### Previous stable isotopic analysis

Despite its potential, stable isotopic analysis of anthropological and archaeozoological material from Portuguese contexts has only begun relatively recently with Perdigões being subject to several investigations for strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ), carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes (Žalaitė et al. 2018; Valera et al. 2020; Table 1). While strontium isotopes, as discussed earlier, are key indicators of mobility, nitrogen isotopes offer complementary insights into dietary practices, as they are influenced by protein intake and reflect trophic-level positioning in the food chain (Schoeninger and DeNiro 1984; Lee-Thorp 2008; Katzenberg and Waters-Rist 2019). Previous isotope studies from Perdigões reveal significant scales of mobility of both humans and animals, from the local settlement area to more distant surrounding regions, with evidence of varying mobility patterns, even within funerary monuments (Valera et al. 2020). Sheep/goat, suid, and deer from the Neolithic appear to have mostly moved within the local settlement area of Perdigões. However, during the Chalcolithic, suid and sheep/goat results suggest a higher degree of mobility possibly related to herding strategies (Žalaitė et al. 2018; Valera et al. 2020).

The  $\delta^{13}\text{C}$  isotopic signature is extremely useful for discriminating the consumption of different types of plants,

**Table 1** Summary of previous carbon ( $\delta^{13}C$ ), nitrogen ( $\delta^{15}N$ ) and strontium ( $^{87}Sr/^{86}Sr$ ) (bone collagen) and strontium ( $^{87}Sr/^{86}Sr$ ) (enamel) results of faunal remains from Perdigões analysed at HERCULES Laboratory (University of Évora, Portugal), Laboratory of Isotope Geology (University of Aveiro, Portugal), Memorial Applied Archaeological Sciences Laboratory (MAAS) and CREAT facilities of Memorial University of Newfoundland (St. John's, Canada). Legend: \* Isotopic values obtained in Žalaitė et al. (2018); + Isotopic values obtained in Valera et al. (2020). MN-Middle Neolithic; M-LN: Middle/Late Neolithic; LN: Late Neolithic; Chalco: Chalcolithic

Species	Sample Number	Period	N	Tissue	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			$^{87}Sr/^{86}Sr$ (‰)			Ref
					Mean	SD	Mean	SD	Mean	SD	Mean	SD		
<i>E. caballus</i>	EcN1, EcN2	MN	2	Bone	-20.7	0.2	4.9	1.0	-	-	-	-	*	
<i>B. primigenius</i>	BpN1, BpN2	M-LN	2	Bone	-20.2	0.1	6.5	0.1	-	-	-	-	*	
<i>B. taurus</i>	BtN1, BtN2, BtN3, BtN4	M-LN	3	Bone	-20.4	0.2	6.3	0.6	2	Enamel	0.71403	0.003	*	
<i>C. elaphus</i>	CeN1, CeN2	M-LN	2	Bone	-19.8	1.1	6.8	1.3	2	Enamel	0.71555	0.001	*	
<i>Ovis/capra</i>	O/cN1, O/cN2, O/cN3, O/cN4	M-LN	4	Bone	-20.2	0.4	4.7	1.0	2	Enamel	0.71451	0.0004	*	
<i>Sus</i> sp.	SN1, SN2, SN3, SN4	M-LN	4	Bone	-20.1	0.7	5.5	0.7	2	Enamel	0.71260	0.001	*	
<i>C. familiaris</i>	CfN1	LN	1	Bone	-19.3	-	9.8	-	-	-	-	-	*	
<i>Equus</i> sp.	EsC1, EC1, EC2, EC3	Chalco	1	Bone	-21.4	-	5.4	-	3	Enamel	0.71370	0.004	* +	
<i>B. taurus</i>	BtC1, BtC2	Chalco	2	Bone	-17.3	3.7	7.4	1.7	1	Enamel	0.71525	-	*	
<i>C. elaphus</i>	CeC1, CeC2, CeC3, CeC4, CeC5, CeC6, CeC7	Chalco	3	Bone	-19.8	0.2	4.5	0.2	5	Enamel	0.71440	0.002	* +	
<i>Ovis/capra</i>	O/cC1, O/cC2, O/cC3, O/cC4, O/cC5, O/cC6	Chalco	2	Bone	-20.3	0.1	5.6	1.6	5	Enamel	0.71468	0.001	*	
<i>Sus</i> sp.	SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC8	Chalco	4	Bone	-20.2	0.4	6.6	1.1	4	Enamel	0.71635	0.001	*	
<i>C. familiaris</i>	CfC2, CfC3, CfC4	Chalco	4	Bone	-19.5	0.8	8.7	1.1	2	Enamel	0.71280	0.001	*	
<i>O. cuniculus</i>	OcC1	Chalco	1	Bone	-21.9	-	3.7	-	-	-	-	-	*	

such as  $C_3$  plants which are predominant in the Iberian Neolithic contexts. Variation in  $\delta^{13}C$  values in plants can occur as they are sensitive to growing conditions (van der Merwe 1982; Tieszen 1991). Factors such as light levels and soil/atmospheric humidity can influence photosynthesis efficiency and respiration and consequently impact the isotopic fractionation of  $^{12}C/^{13}C$  (van der Merwe 1982; Farquhar et al. 1989; Tieszen 1991; Arens et al. 2000).  $C_3$  plants typically exhibit a  $\delta^{13}C$  mean of  $-26.5\text{‰}$  (range:  $-38\text{‰}$  to  $-22\text{‰}$ ), whereas  $C_4$  plants display an average of  $-12.5\text{‰}$  (range:  $-6\text{‰}$  to  $-19\text{‰}$ ) (Smith and Epstein 1971; van der Merwe 1982; O’Leary 1988; Tieszen 1991). Plants and low branches of trees growing under a dense forest canopy can exhibit depleted  $\delta^{13}C$  values, i.e., more negative values, compared to the same plant type growing in more open conditions (Broadmeadow and Griffiths 1993; Drucker et al. 2008). A minimum  $\delta^{13}C$  average of  $-33.2\text{‰}$  for tropical forests and  $-31.5\text{‰}$  for temperate canopies has been reported (Broadmeadow and Griffiths 1993), with values as negative as  $-35\text{‰}$  and  $-37\text{‰}$  recorded for subtropical monsoon forests and Amazonian forests, respectively (van der Merwe and Medina 1991). These isotopic ratios are then passed predictably on to consumers, such as ruminants. For example, in boreal and temperate forests in northern latitudes, the stable isotopic values of archaeological herbivores are found to be c.  $-22.3\text{‰}$ . However, the Iberian forests have a more open canopy and therefore, animals grazing within forests possibly have higher  $\delta^{13}C$  values than those in Central and Northern Europe. Based on previous palaeoenvironmental reconstructions from Perdigões, we would expect animals grazing in the open park-like environment to exhibit more positive  $\delta^{13}C$  values, albeit depleted  $\delta^{13}C$  values could potentially be observed in animals grazing under dense canopies or wetland meadows. Stable isotopic analysis of bone collagen revealed that all animals from Neolithic/Chalcolithic consumed a diet based on  $C_3$  plants. Furthermore, the management practices for domesticated animals were similar between phases, with slight differences possibly attributed to environmental and animal behavioural aspects, or pastoralist strategies (Žalaitė et al. 2018).

## Materials and methodology

### Materials

Sheep and goat teeth are morphologically very similar however they can be distinguished based on morphological criteria (Boessneck et al. 1964; Halstead et al. 2002; Balasse and Ambrose 2005; Gillis et al. 2011). It is important to distinguish between these two species as they have different physiology that may impact stable isotopic results for

example, see Balasse and Ambrose (2005). Five lower second molars and three lower third molars were selected for sampling with some individuals having both M2 and M3 sampled (OC1000, OC1002, OC1004M2/M3, OC1007, OC1009, OC1010 and OC1011; Table 2). These were identified by REG as sheep based on established and assessed morphological distinctions (Halstead et al. 2002; Balasse and Ambrose 2005; Gillis et al. 2011). However, morphological criteria cannot be completely relied on, and only with further proteomic analysis would be completely confident in these determinations (Buckley et al. 2010). Therefore, it is better to err on the side of caution and continue to describe these individuals as sheep/goat. The crown of second molars in Soay sheep are completed between 9 and 11 months (Witzel et al. 2018). The same study reported that the mineralisation of the crown of the third molars begins at approximately 12 months and is completed by 26 months. Crown formation timings may change due to breed, diet and local environment (Noddle 1990), and these estimates should be assumed as an approximation.

Alongside the caprine teeth, red deer (*Cervus elaphus*; 1019, 1020, 1022; Table 2) and suid (*Sus* sp.; 1015.I1, 1015.I2, 1016.I1; Table 2) teeth were also collected for incremental analysis. In line with previous stable isotope studies of the fauna material from the site, we assume that Neolithic deer and suid remains recovered were from grazing in local environments. Suid molars, unlike high crown ruminant teeth, have previously been found to not record seasonal variation in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (Frémonteau et al. 2012). Moreover given the constraints of the faunal assemblage, we were limited to sampling suid incisors (1015.I1, 1015.I2 and 1016.I1). Formation and eruption of suid incisors is limited, however in general the lower first incisor (I1) begins formation between 2 and 3 months after birth and is complete at least by 17 months. The crown of the lower second incisor (I2) begins to

form between 6 and 10 months after birth and is completed at least when the animal is 24 months-old (McCance et al. 1961; Frémonteau et al. 2012). Red deer lower M2 mineralisation begins around 4 months, coinciding with the weaning period, and the crown is fully formed at 9 months. The M3 mineralisation begins around 13 months with completion of the crown at 26 months based on a study on modern red deer from Richmond Park, London (Brown and Chapman 1991). For red deer, two lower second and one third molar were selected (1019, 1020 and 1022).

### Stable isotope sampling and analysis

For all individuals selected for this study, we sampled tooth enamel for the determination of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotope ratios. Prior to sequential enamel sampling, the teeth surfaces were cleaned using an abrasive drill bit. A sequence of enamel samples was then drilled from the apex of the crown to the enamel-root junction (ERJ) with a Komet drill bit (no. 835–104–010). The mean weight was  $6.3 \pm 1.6$  mg,  $5.1 \pm 1.4$  mg and  $6.5 \pm 1.4$  mg for sheep/goat, suid and deer, respectively. The mean number of sequential samples taken was  $10.4 \pm 3.6$ , with an average distance of  $2.2 \pm 0.6$  mm. The pre-treatment protocol followed Balasse (2002) and Tornero et al. (2013). Enamel powder samples were treated for 4 h in 0.1 M acetic acid ( $\text{CH}_3\text{COOH}$ ; 0.1 ml solution/0.1 mg of sample), rinsed five times with distilled water ( $\text{dH}_2\text{O}$ ) and freeze-dried. Dry samples were analysed on MAT253/Kiel IV carbonate device at Cardiff University (UK). The analytical precision was estimated from an internal standard (BCT63 carrara marble) used by the Cardiff laboratory for  $\delta^{13}\text{C}$  analyses of 0.02‰ and  $\delta^{18}\text{O}$  analyses of 0.03‰. The isotope ratios are expressed using the delta notion ( $\delta$ ) in parts per thousand (‰) relative to the internationally recognized measurement standards Vienna Pee Dee Belemnite (V-PDB) for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ .

**Table 2** List of sheep/goat (*Ovis/Capra*), suid (*Sus* sp.) and deer (*Cervus elaphus*) teeth selected for incremental stable isotopic analysis, archaeological (structure, context and chronology) and dental (side and grant WS (Grant 1982). Legend: W – Worn

Sample number	Species	Structure	Context	Chronology	Tooth	Side	Wear stage
1000	<i>Ovis/Capra</i>	Pit 82	[510]	Middle Neolithic	M3	L	5 A
1002	<i>Ovis/Capra</i>	Pit 80	[450]	Late Neolithic	M2	R	5 A
1004.M2	<i>Ovis/Capra</i>	Hypogeum 1	[241]	Late Neolithic	M2	R	5 A
1004.M3	<i>Ovis/Capra</i>	Hypogeum 1	[241]	Late Neolithic	M3	R	5 A
1007	<i>Ovis/Capra</i>	Pit 80	[599]	Late Neolithic	M2	L	5 A
1009	<i>Ovis/Capra</i>	Pit 82	[510]	Middle Neolithic	M3	L	5 A
1010	<i>Ovis/Capra</i>	Hypogeum 1	[182]	Late Neolithic	M2	L	5 A
1011	<i>Ovis/Capra</i>	Hypogeum 1	[183]	Late Neolithic	M2	R	4 A
1015.I1	<i>Sus</i> sp.	Hypogeum 1	[220]	Late Neolithic	I1	–	W
1015.I2	<i>Sus</i> sp.	Hypogeum 1	[220]	Late Neolithic	I2	–	W
1016.I1	<i>Sus</i> sp.	Pit 82	[509]	Middle Neolithic	I1	–	W
1019	<i>Cervus elaphus</i>	Pit 80	[556]	Late Neolithic	M2	R	W
1020	<i>Cervus elaphus</i>	–	–	Late/Middle Neolithic	M2	L	W
1022	<i>Cervus elaphus</i>	Ditch 12	[239]	Late Neolithic	M3	L	W

The isotopic composition of an animal's dietary source can be estimated through the  $\delta^{13}\text{C}$  value recorded in its tissues (DeNiro and Epstein 1978). This estimation takes into account the isotopic fractionation effects that occur during the incorporation of the dietary carbon into the consumer's tissues, commonly termed as  $\Delta$  (difference) or  $\epsilon$  (enrichment factor) consumer diet-tissue (DeNiro and Epstein 1978; Lee-Thorp 2008). Fractionation will vary among different tissues influenced by their chemistry and biosynthetic pathways (Lee-Thorp 2008). The enrichment of  $\delta^{13}\text{C}$  values between diet and bioapatite has been shown to vary between species depending on dietary composition, physiology, and body size (Cerling and Harris 1999; Balasse 2002; Passey et al. 2005; Zazzo et al. 2006, 2010). We have chosen to use two enrichment values for diet-bioapatite: 12.5‰ for suids and 14.5‰ for ruminants (Cerling et al. 2021). It is important to clarify that none of the individuals were analysed for both sequential dental enamel and bulk bone collagen. For comparison with previously published  $\delta^{13}\text{C}$  isotopes on bulk bone collagen (Žalaitė et al. 2018), we applied a 5.1‰  $^{13}\text{C}$ -enrichment between diet and collagen (Ambrose and Norr 1993). Stable isotopic results were statically tested using non-parametric tests Mann-Whitney and Kruskal-Wallis in SPSS v.28, and all standard deviation is given to  $1\sigma$ .

## Results and discussion

The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are reported in Table 3; Figs. 2 and 3. The  $\delta^{13}\text{C}$  values vary between  $-13.9\text{‰}$  (*Sus* sp.) to  $-10.2\text{‰}$  (*Ovis/Capra*) (overall average  $\delta^{13}\text{C} = -12.3\text{‰} \pm 0.8$ ; average  $\delta^{13}\text{C}$  amplitude across species =  $1.5\text{‰}$ ), while  $\delta^{18}\text{O}$  values range from  $-3.9\text{‰}$  (*Cervus elaphus*) to  $3.9\text{‰}$  (*Ovis/Capra*) (overall average  $\delta^{18}\text{O} = -0.008\text{‰} \pm 1.7$ ; average  $\delta^{18}\text{O}$  amplitude across of species =  $3.0\text{‰}$ ). The enamel bioapatite of sheep/goat displayed the most significant variation and range of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values (Table 3; Figs. 2 and 3).

### Sequential series of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in sheep/goat

The average  $\delta^{13}\text{C}$  value of enamel bioapatite in sheep molars was  $-12.1\text{‰}$ , with a range of  $-13.6\text{‰}$  to  $-10.2\text{‰}$  ( $\delta^{13}\text{C}$  amplitude =  $2.0\text{‰}$ ; Fig. 4a-h). Among the individuals,

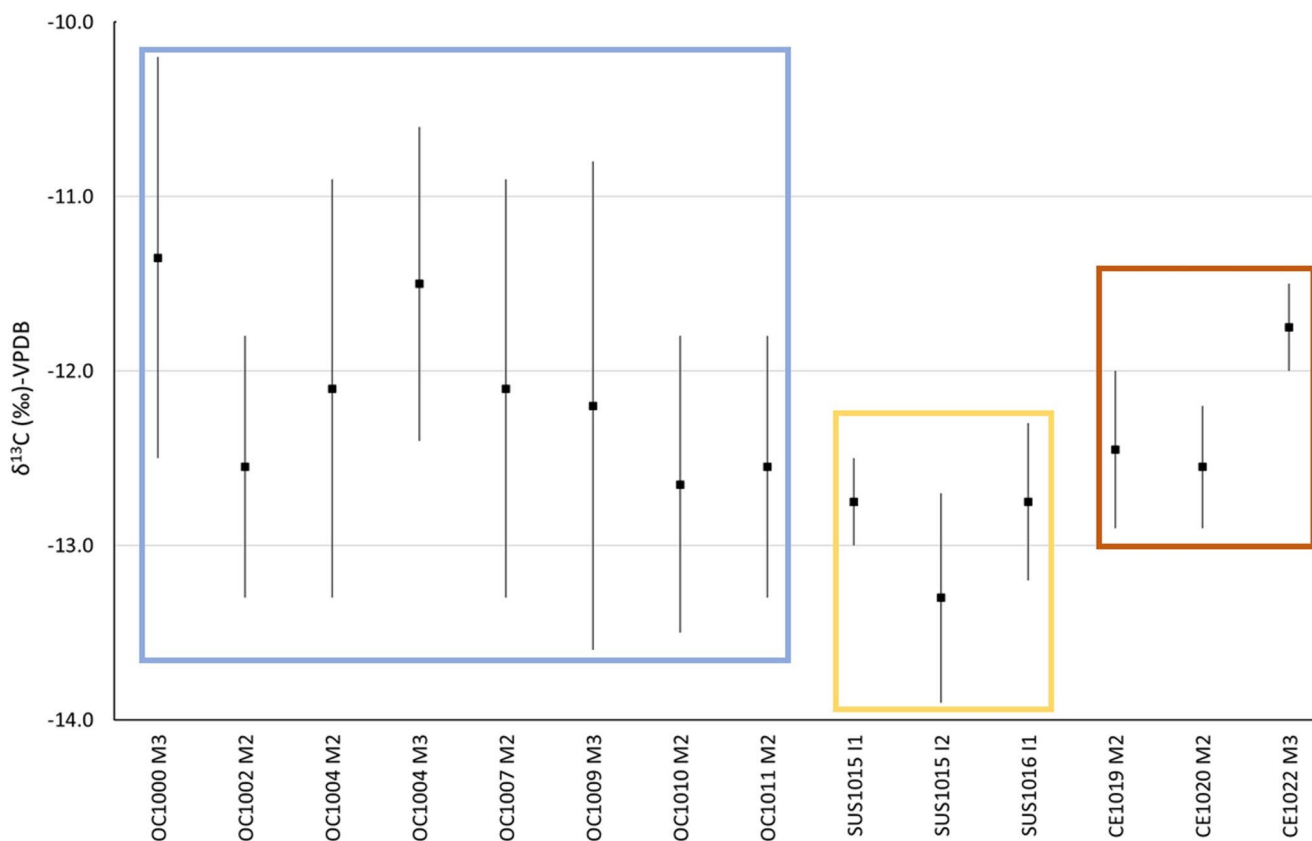
the third molar of OC1000 exhibited the highest  $\delta^{13}\text{C}$  value recorded ( $-10.2\text{‰}$ , Fig. 4a), while OC1009 M3 yielded the largest amplitude ( $\delta^{13}\text{C}$  amplitude =  $2.8\text{‰}$ , Fig. 4f). Statistical differences were observed for  $\delta^{13}\text{C}$ , particularly in the third molar of OC1004, which differed significantly from all individuals except OC1000, including the values recorded in the M2 of the same individual ( $\delta^{13}\text{C}$  Kruskal-Wallis  $p < 0.001$ ;  $\delta^{13}\text{C}$  Mann-Whitney OC.1004.M3 ~ OC.1004.M2  $p < 0.05$ ; Supplementary Material 2). OC1000 and OC1004. M3 exhibited the highest  $\delta^{13}\text{C}$  average ( $-11.4\text{‰}$ ), and  $\delta^{13}\text{C}$  values recorded ( $-10.2\text{‰}$ , and  $-10.6\text{‰}$ , respectively: Fig. 4a and d). Among sheep, OC1004.M3 displayed a different pattern of variation in  $\delta^{13}\text{C}$  values. In this specimen, the upper part of the crown showed an almost stable sequential record of  $\delta^{13}\text{C}$  values, among the highest values recorded in Perdígões, ranging from  $-10.8\text{‰}$  to  $-10.6\text{‰}$ .

Using a +14.5‰ enrichment factor to estimate the values for diet (Cerling et al. 2021), sheep/goat individuals consumed plants with an average  $\delta^{13}\text{C}$  value of  $-26.6\text{‰}$  (range:  $-28.1\text{‰}$  to  $-24.7\text{‰}$ ). Sheep/goat  $\delta^{13}\text{C}$  values indicate a diet predominantly based on  $\text{C}_3$  plants; however, a high variation was detected throughout the seasonal cycle. Seasonal variations in plant  $\delta^{13}\text{C}$  values can be explained by changes in temperature, light and water availability, salinity, and variations in  $\text{CO}_2$  concentrations (Farquhar et al. 1982, 1989; Tieszen 1991; Navarrete et al. 2019; Gillis et al. 2021). We hypothesised that the differences observed can also be attributed to different herding strategies or animal behaviour: higher  $\delta^{13}\text{C}$  values indicate grazing in open areas, whereas low  $\delta^{13}\text{C}$  values could reflect a slight canopy that may be explained by feeding in woodland or waterlogged resources. The presence of an area characterised by woodlands and shaded areas near the site has been documented in palaeoenvironmental studies at Perdígões (Wheeler 2010). Plants growing under these conditions will show a  $\delta^{13}\text{C}$ -depletion ("canopy effect") that will be passed on to herbivores grazing in these areas, thus leading to low  $\delta^{13}\text{C}$  values recorded in their tissues (Drucker et al. 2008; Bonafini et al. 2013). Waterlogged conditions may also lead to low foliar  $\delta^{13}\text{C}$  values (Fan et al. 2018).

The average  $\delta^{18}\text{O}$  value for sheep/goat was  $0.6\text{‰}$  and varied between  $-2.6\text{‰}$  to  $3.9\text{‰}$  ( $\delta^{18}\text{O}$  amplitude =  $3.8\text{‰}$ ; Figs. 3 and 4a-h). OC1010M2 exhibited the highest

**Table 3** Descriptive statistics of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of enamel bioapatite of sheep/goat (OC), suid (*Sus*) and red deer (CE) per species and type of tooth sampled (incisor (I), second (M2) and third (M3) molar). N=Number

Species/Tooth	N teeth	Average N of samples/tooth	$\delta^{13}\text{C}$ (‰)				$\delta^{18}\text{O}$ (‰)			
			Average	SD	Min	Max	Average	SD	Min	Max
OC/M2	5	12.6	-12.4	0.7	-13.5	-10.9	0.5	1.4	-2.1	3.9
OC/M3	3	14	-11.7	1	-13.6	-10.2	0.8	1.5	-2.6	3.5
Sus/I	3	8.7	-13	0.4	-13.9	-12.3	-1.2	1.1	-2.6	2.8
CE/M2	2	5	-12.6	0.3	-12.9	-12	-1.4	1.7	-3.5	1.3
CE/M3	1	5	-11.8	0.2	-12.0	-11.5	-3.2	0.5	-3.9	-2.8

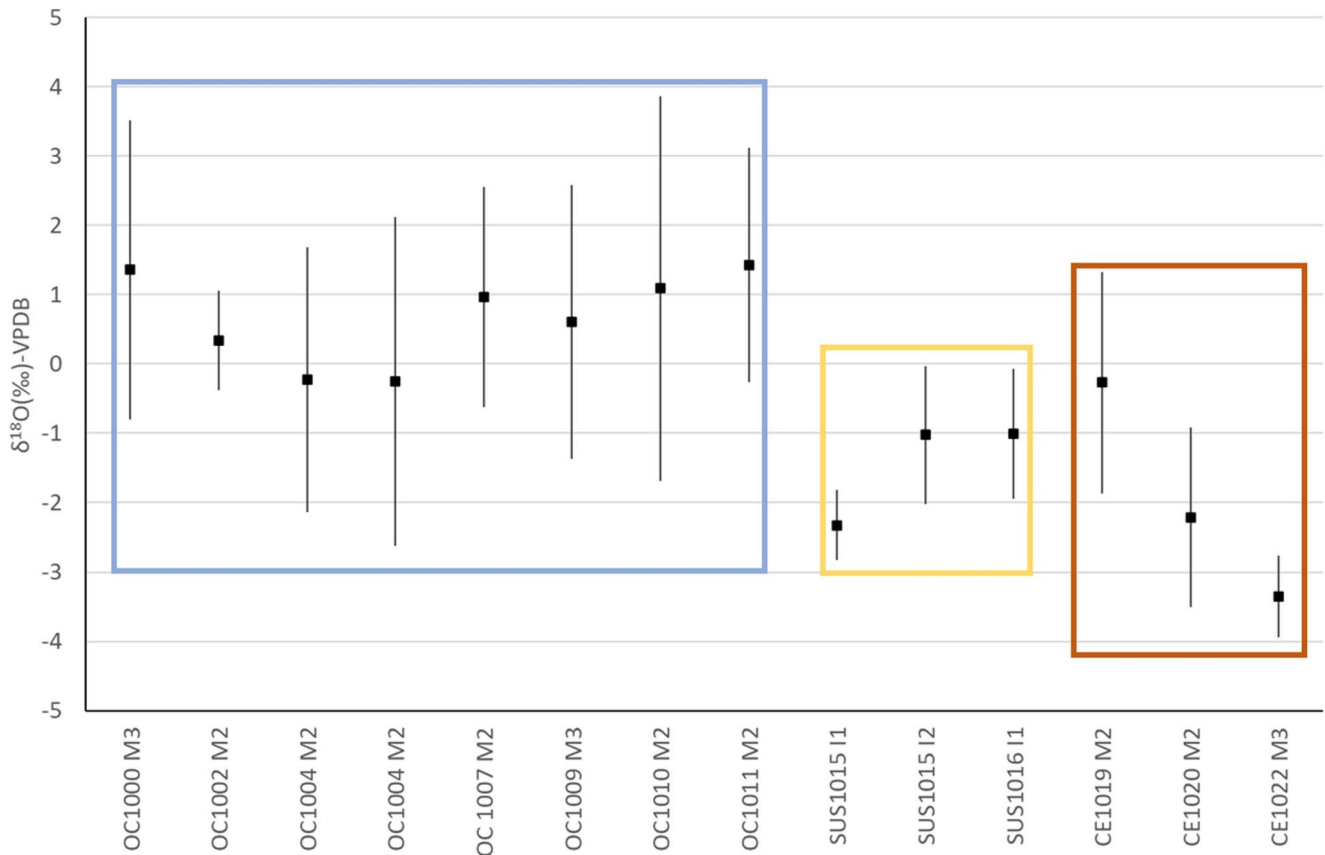


**Fig. 2** Comparative graph of  $\delta^{13}\text{C}$  values of enamel bioapatite of sheep/goat (OC - blue), suid (Sus - yellow) and deer (CE - orange) (incisor (I), second (M2) and third (M3) molar, see Supplementary Material 1)

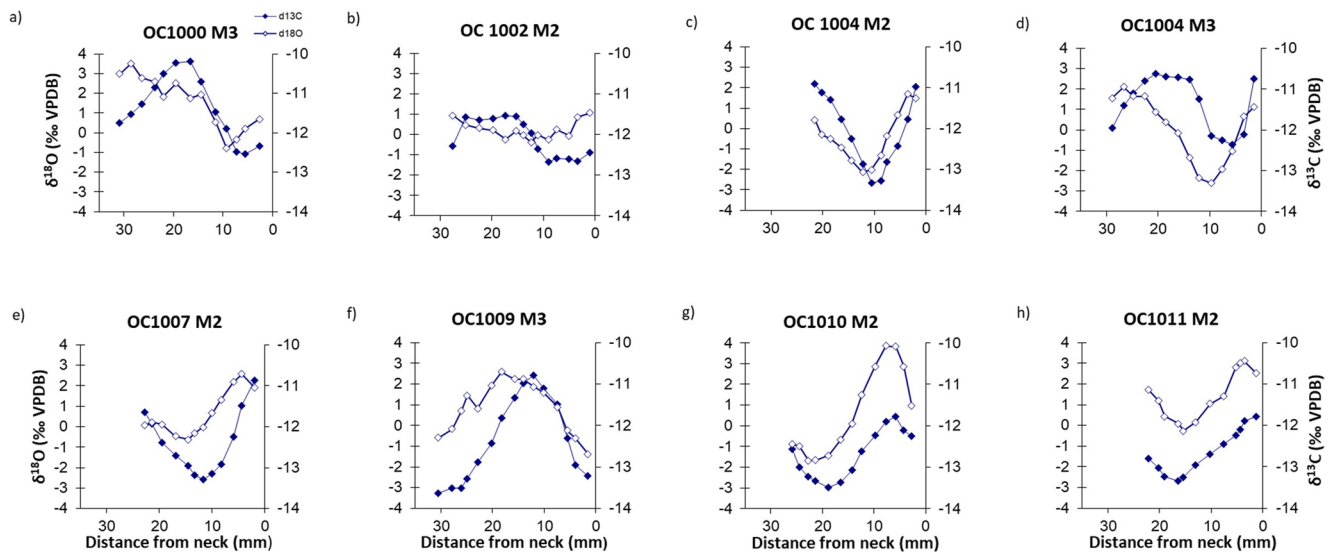
$\delta^{18}\text{O}$  value (3.9‰) and largest amplitude ( $\delta^{18}\text{O}$  amplitude = 5.5‰; Figs. 3 and 4g). The  $\delta^{18}\text{O}$  values registered in the enamel bioapatite of individuals OC1000 and OC1011 differed significantly from those of OC1004 (M2 and M3) and OC1002 ( $\delta^{18}\text{O}$  Kruskal-Wallis  $p=0.013$ ; Supplementary Material 3). Individuals OC1000 and OC1011 displayed the highest  $\delta^{18}\text{O}$  averages (1.6‰ and 1.4‰, respectively), whereas OC1004 (M2 and M3) and OC1002 exhibited the lowest values among sheep/goats (−0.4‰, 0‰ and 0.2‰, respectively). All sheep/goat specimens sampled displayed a complete sinusoidal  $\delta^{18}\text{O}$  sequence representing a complete seasonal cycle, except for individual OC1002, which also had the lowest amplitude ( $\delta^{18}\text{O}$  amplitude = 1.4‰; Fig. 4b).

The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values recorded in individuals (OC1010.M2/OC1011.M2) followed a similar pattern, where the highest or lowest  $\delta^{13}\text{C}$  values occur with the respective higher/lower  $\delta^{18}\text{O}$  values. We also observed in some individuals (OC1000.M3, OC1004.M2/M3, OC1007.M2, OC1009.M3) the following pattern: Taking OC1004.M3 as an example, at the tooth apex, the  $\delta^{18}\text{O}$  values are decreasing while the  $\delta^{13}\text{C}$  values reach their maximum (−10.6‰), and then towards the base of the tooth,  $\delta^{18}\text{O}$

values reach their minimum (−2.6‰) which is followed by that of  $\delta^{13}\text{C}$  (−12.4‰, Fig. 4d). Moreover, we observe that Individual OC1004 consumed plants depleted in  $^{13}\text{C}$  during the formation of M2 compared with that of M3 (Figs. 4c-d). It is possible that the seasonal supplementation of fodder either depleted in  $^{13}\text{C}$ , such as leafy-hay (Balasse et al. 2012) or enriched with  $^{13}\text{C}$ , such as meadow grasses (Makarewicz 2017a), would have caused this observation. The same pattern was observed in individual OC1000 (Fig. 4a). At La Draga, Navarrete et al. (2019) suggested that the high  $\delta^{13}\text{C}$  values registered in winter months could be related to animals feeding on surplus from agricultural by-products as a means to overcome seasonal deficiencies and improve reproduction and breeding. The use of supplementary fodders in Neolithic and more recent prehistoric herding contexts have been detected using stable isotopes: leafy-hay (Balasse et al. 2013; Tornero et al. 2020; Gillis et al. 2024) or enriched  $^{13}\text{C}$  sources such as  $\text{C}_4$  plants (Makarewicz and Pederzani 2017). Although  $\text{C}_4$  plants cannot be ruled out, they are present within the natural environment in very small proportions in Southwestern Europe (Laffranchi et al. 2016), making it unlikely that this type of plant was included in the sheep/goat diet.



**Fig. 3** Comparative graph of δ<sup>18</sup>O values of enamel bioapatite of sheep/goat (OC - blue), suid (Sus - yellow) and deer (CE - orange) (incisor (I), second (M2) and third (M3) molar, see Supplementary Material 1)



**Fig. 4** δ<sup>13</sup>C and δ<sup>18</sup>O values of enamel bioapatite of sheep/goat (OC) second (M2) and third (M3) molars: **a.** 1000.M3; **b.** 1002.M2; **c.** 1004.M2; **d.** 1004. M3; **e.** 1007.M3; **f.** 1009.M3; **g.** 1010.M2; **h.** 1011.M2. Dark blue diamonds and lines are δ<sup>13</sup>C and white diamonds with blue lines are δ<sup>18</sup>O values

The sequential series of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in sheep/goat from Perdigões revealed that animals experienced shifts in pasture sources during their lifetime. The  $\delta^{13}\text{C}$  values suggest the consumption of plant sources with strong seasonality in carbon isotopes, or different types of animal feed during specific times of the year, e.g., leafy hay. It is clear from the sampled individuals that the source of pasture plants during winter appears to be depleted in  $^{13}\text{C}$ . In northern European contexts, this could be attributed to the consumption of forest resources. However, given the palaeoenvironment, it is unlikely there were dense forest canopies. An alternative explanation is that the  $\delta^{13}\text{C}$  values are a reflection of cool and humid conditions during winter that impacted the uptake of  $^{13}\text{C}$ . If stable isotope values reflect husbandry practices and namely fodder choice by herders, then we propose that individuals experienced different management strategies and possibly originated from different regions. This is exemplified in the case of OC1002, which showed the lowest variation of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values throughout the annual cycle (Fig. 4b). A reduced variation in a sequence of  $\delta^{18}\text{O}$  values has been proposed as a potential signal of rapid seasonal migrations between diverse environments, thereby minimising the animal's exposure to climatic variations that impact local drinking water (Makarewicz and Pederzani 2017). According to this model, herders move the animals to cooler regions in the summer and warmer regions in the winter (Pederzani and Britton 2019). Therefore, the restricted variation observed in OC1002 could be attributed to movements associated with climatic and environmental conditions, and/or migration from another region to the site.

### Sequential series of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in suid and red deer

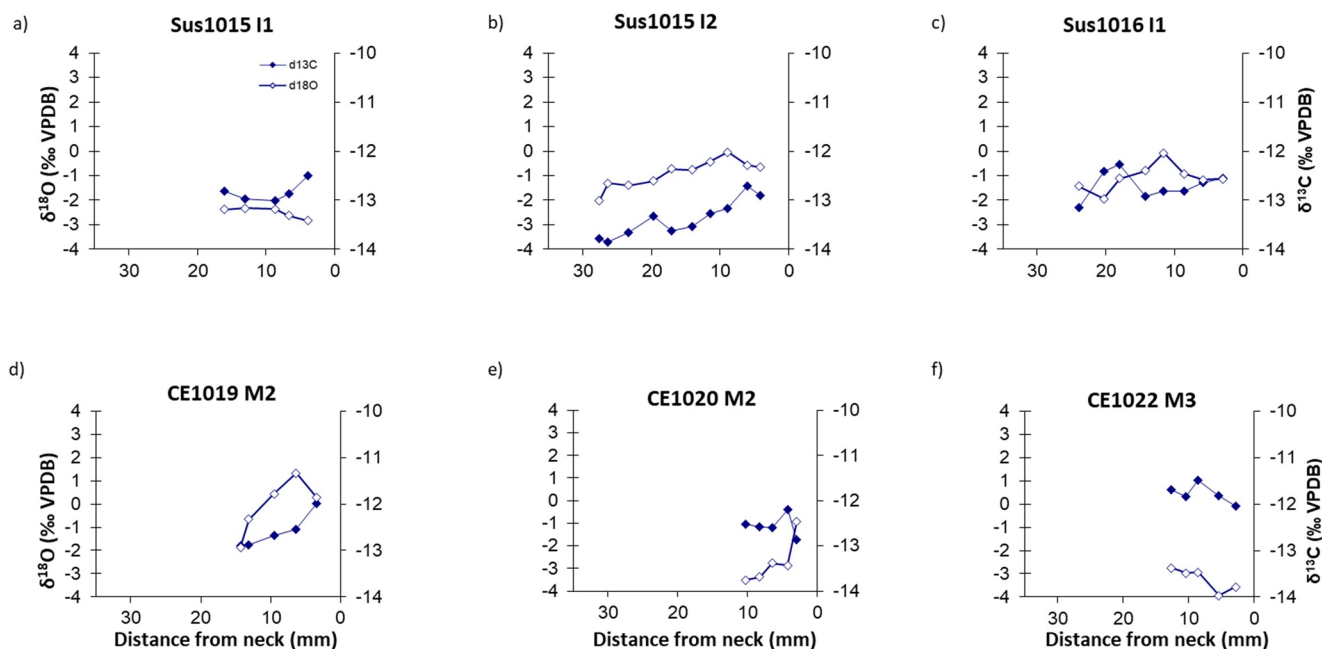
The average  $\delta^{13}\text{C}$  value of enamel bioapatite of suid incisors was  $-13\text{‰} \pm 0.4$ , with a range spanning from  $-13.9\text{‰}$  to  $-12.3\text{‰}$  ( $\delta^{13}\text{C}$  amplitude =  $0.8\text{‰}$ ; Fig. 5a-c). Among all the species examined in this study, the second incisor of Sus1015 displayed the lowest carbon value recorded ( $-13.9\text{‰}$ ). Statistically significant differences were observed between the carbon values of the incisors (I1 and I2) of suid 1015, and 1016.I1 ( $\delta^{13}\text{C}$  Kruskal-Wallis  $p=0.002$ ; Supplementary Material 2). The I1 of suid 1015 exhibited the lowest average  $\delta^{13}\text{C}$  registered ( $-13.3\text{‰}$ ) when compared to the I2 and 1016.I1 ( $-12.8\text{‰}$  and  $-12.7\text{‰}$ , respectively). However, caution should be taken when comparing these values, as the first incisor of 1015 has a short crown and therefore does not capture the same sequence as 1015.I2 (Fig. 5a). Suids consumed plants with  $\delta^{13}\text{C}$  values between  $-26.2\text{‰}$  and  $-24.8\text{‰}$  ( $\delta^{13}\text{C}$  average =  $-25.4\text{‰}$ ) using the 12.5‰ enrichment factor. Suids are typically considered omnivores; however, they are extremely adaptable and can feed on a broad

range of food items (Madgwick et al. 2012; Villalba-Mouco et al. 2018). The  $\delta^{13}\text{C}$  values of suids indicate a diet based on  $\text{C}_3$  plants and fall within the range obtained for sheep/goats. Hence, suids were foraging in environments similar to those of herbivores.

The average  $\delta^{18}\text{O}$  value for the suids samples was  $-1.4\text{‰} \pm 0.8$ , varying between  $-2.8\text{‰}$  to  $0\text{‰}$ , thus displaying the lowest amplitude observed ( $\delta^{18}\text{O}$  amplitude =  $1.6\text{‰}$ ; Fig. 5a-c). The  $\delta^{18}\text{O}$  values recorded in the first incisor of 1015 differed significantly from those of the second incisor of the same individual, and of 1016.I1 ( $\delta^{18}\text{O}$  Kruskal-Wallis  $p<0.001$ ; Supplementary Material 3). Similarly, the I1 of suid 1015 exhibited the lowest  $\delta^{18}\text{O}$  average recorded ( $-2.4\text{‰}$ ) when compared to the I2 and 1016.I1 ( $-0.9\text{‰}$  and  $-1.1\text{‰}$ , respectively). Unlike the  $\delta^{18}\text{O}$  curves of sheep, suids did not exhibit strong seasonal signals. However, 1016.I1 appeared to show some variation in  $\delta^{13}\text{C}$  values with respect to  $\delta^{18}\text{O}$ , i.e. at the apex of the tooth,  $\delta^{13}\text{C}$  values were high, while  $\delta^{18}\text{O}$  values were at their lowest (Fig. 5c) and this pattern was reversed towards the base of the tooth. Frémondeau and colleagues (2017) observed similar patterns in suids, i.e., low values in summer and high values in autumn and winter. These patterns have been interpreted as possibly indicative of foraging under forest canopy and consumption of forest fruits (Frémondeau et al. 2012, 2017). Fruits tend to be  $^{13}\text{C}$ -enriched by up to  $3\text{‰}$  in comparison to leaves in  $\text{C}_3$  plants (Cernusak et al. 2009).

The average  $\delta^{13}\text{C}$  value from deer molars was  $-12.3\text{‰} \pm 0.5$  and ranged from  $-12.9\text{‰}$  to  $-11.5\text{‰}$  ( $\delta^{13}\text{C}$  amplitude =  $0.7\text{‰}$ ; Fig. 5e-f). The  $\delta^{13}\text{C}$  values of CE1022 were statistically different from those of CE1019 and CE1020 ( $\delta^{13}\text{C}$  Kruskal-Wallis  $p=0.012$ ; Supplementary Material 2). Specimen CE1022 displayed the highest  $\delta^{13}\text{C}$  value ( $-11.8\text{‰}$ ) in comparison to CE1019 and CE1020 ( $-12.6\text{‰}$ ). The plant  $\delta^{13}\text{C}$  values for the red deer diet had a mean  $\delta^{13}\text{C}$  value of  $-26.7\text{‰}$  (range:  $-27.4\text{‰}$  to  $-26\text{‰}$ ) considering the  $^{13}\text{C}$  enrichment factor of  $+14.5\text{‰}$ . Red deer feed mainly on trees, shrubs, and grass in boreal and closed forests (Drucker et al. 2008) but have wide ecological niches. The  $\delta^{13}\text{C}$  values indicate a predominant diet of  $\text{C}_3$  plants with limited seasonal variation. The low  $\delta^{13}\text{C}$  values suggest animals browsing under a forest canopy as proposed for a few sheep/goat specimens. These animals were possibly feeding on fodder resources in the nearby woodland at Perdigões.

Turning to the oxygen values from deer, the average  $\delta^{18}\text{O}$  value was  $-2.0\text{‰} \pm 1.7$ , varying between  $-3.9\text{‰}$  to  $1.3\text{‰}$  ( $\delta^{18}\text{O}$  amplitude =  $1.6\text{‰}$ ; Fig. 5e-f). Again, CE1019 was significantly different from CE1020 and CE1022 ( $\delta^{18}\text{O}$  Kruskal-Wallis  $p=0.010$ ; Supplementary Material 2), as this individual presented the highest amplitude of oxygen values ( $\delta^{18}\text{O}$  amplitude CE1019 =  $3.2\text{‰}$ ) when compared to other samples ( $\delta^{18}\text{O}$  amplitude CE1020 =  $2.6\text{‰}$ ;  $\delta^{18}\text{O}$



**Fig. 5** Distribution of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of enamel bioapatite of suids (Sus) and red deer (CE) remains from Perdigões: **a.** Sus1015.I1; **b.** Sus1015.I2; **c.** Sus1016.I1; **d.** CE1019.M2; **e.** CE1020.M2; **f.** CE1022.M3. Dark blue diamonds and lines are  $\delta^{13}\text{C}$  and white diamonds with blue lines are  $\delta^{18}\text{O}$  values

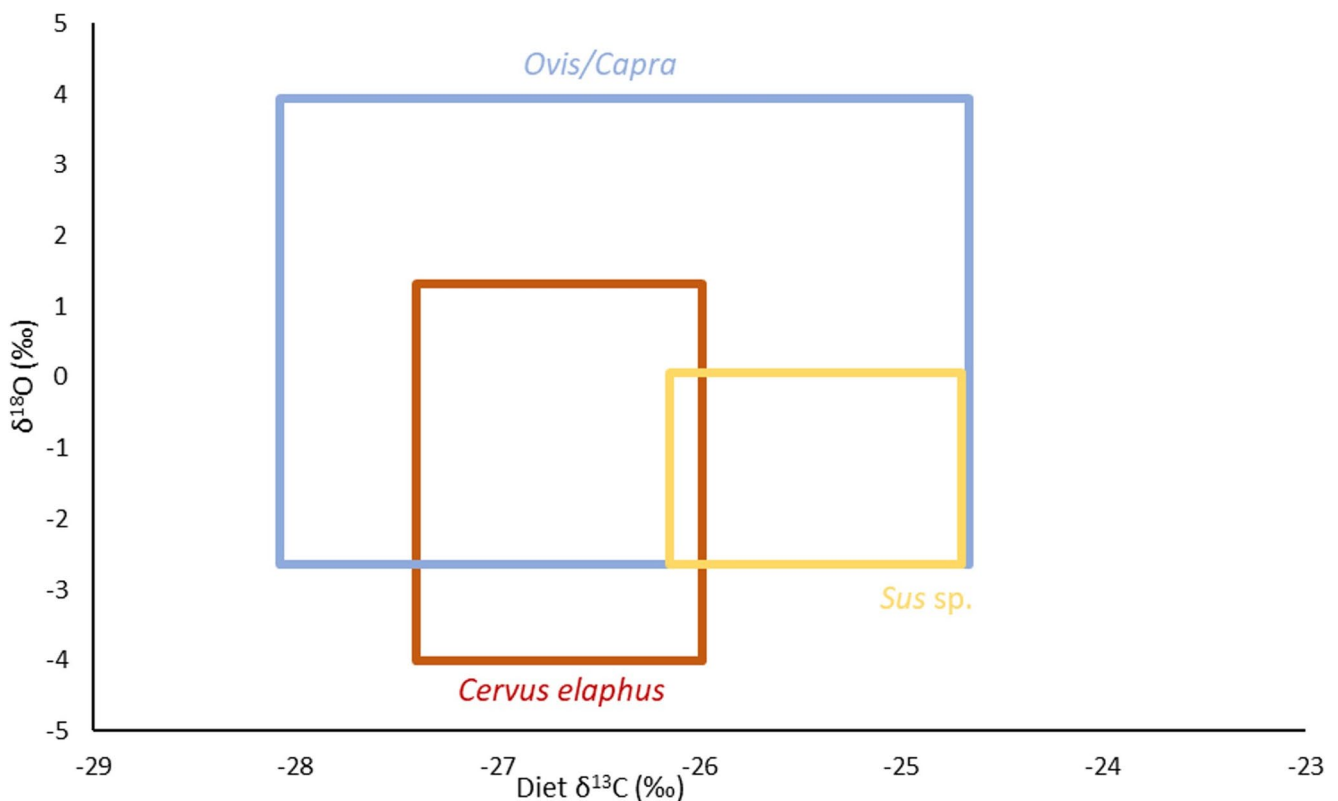
amplitude  $\text{CE1022} = 1.2\%$ ). Red deer samples were among the shortest sequential series of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  recorded. Red deer exhibited mild seasonal signals, where  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  followed the same pattern: an increase/decrease in  $\delta^{18}\text{O}$  was followed by a similar tendency in the  $\delta^{13}\text{C}$  isotopic signature. In CE1020, the pattern was inverse: in the upper part of the crown, an increase in  $\delta^{18}\text{O}$  values was followed by an opposite trend in  $\delta^{13}\text{C}$  values, followed by a slight decrease in  $\delta^{18}\text{O}$  while  $\delta^{13}\text{C}$  increases. At the base of the crown, a sharp increase in  $\delta^{18}\text{O}$  is followed by an abrupt decrease in  $\delta^{13}\text{C}$ . The tooth analysed for this individual was an M2, which begins formation at four months around weaning. The  $\delta^{18}\text{O}$  values observed might reflect milk consumption, as it leads to an increase in  $\delta^{18}\text{O}$  body water (Stevens et al. 2011).

### Reconstructing palaeoenvironment and sheep/goat diet

The  $\delta^{13}\text{C}$  values of the reconstructed diet span from  $-28.2\%$  to  $-24.7\%$ , indicating a diet predominantly based on  $\text{C}_3$  plants. Our data shows that, in general, there is an overlap of  $\delta^{13}\text{C}$  values among the different taxa analysed (Fig. 6). However, the carbon values recorded in suids differed significantly from those of sheep/goat and deer ( $\delta^{13}\text{C}$  Kruskal-Wallis  $p < 0.001$ ;  $\delta^{13}\text{C}$  Mann-Whitney  $\text{Sus} \sim \text{Ovis/capra}$   $p < 0.001$ ;  $\delta^{13}\text{C}$  Mann-Whitney  $\text{Sus} \sim \text{Cervus elaphus}$   $p < 0.001$ ). Although carbon values fall within the sheep/goat and deer range, suids displayed a higher  $\delta^{13}\text{C}_{\text{diet}}$  average.

The overall trend at Perdigões was higher  $\delta^{13}\text{C}$  values during summer and lower values during winter, likely reflecting seasonal plant variation in the environment. In the Mediterranean,  $\delta^{13}\text{C}$  plant values can fluctuate up to 4‰ over the seasonal cycle (Navarrete et al. 2019). This seasonal pattern is more evident in the sheep/goat  $\delta^{13}\text{C}$  isotopic signature than in suids and deer. Sheep/goats were grazing in open environments as well as woodlands, whereas suids appear to forage in open environments and deer primarily feed on woodlands. Plants growing in shaded environments are more depleted in carbon isotopic values, with the degree of depletion potentially linked to the level of shade, specifically the percentage of cover in the woodland (Bonafini et al. 2013). While deer and sheep appear to feed in a similar woodland environment, the lower  $\delta^{13}\text{C}$  values registered in the majority of sheep/goats indicate that the areas they were grazing are more closed than deer or are being supplied with supplementary leafy hay. Alternatively, they may have been grazing on water meadows.

Sheep/goats exhibited different ranges of  $\delta^{13}\text{C}$  values, possibly directly related to human management practices, such as herds experiencing seasonal movement of animals between grazing grounds to prevent overgrazing (Tornerio et al. 2018). Two of the individuals (OC1000.M3 and OC1004.M3) exhibit different  $\delta^{13}\text{C}$  value ranges, suggesting differences in their feeding and management regimes. Interestingly, these individuals also display slightly different patterns in  $\delta^{18}\text{O}$  values when compared to others, as discussed earlier. This could potentially reflect that these



**Fig. 6** Range of Diet  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of enamel bioapatite of sheep/goat (*Ovis/Capra* - blue), suid (*Sus* sp. - yellow), and red deer (*Cervus elaphus* - red) from Perdigões. These  $\delta^{13}\text{C}$  values were calculated using Cerling et al. (2021), 12.5‰ for suids and 14.5‰ for ruminants

animals came from a different community or region. Previous nitrogen values obtained at Perdigões suggest that ruminant species, such as sheep/goat and deer, were grazing in environments with distinct isotopic signatures ( $\delta^{15}\text{N}$  range of 3.3‰ to 9.8‰ for Neolithic animals) probably related to diversified management practices or the provenance of animals (Žalaitė et al. 2018). However, animals within the same species, fed with a similar diet, may display variations in the  $\delta^{13}\text{C}$  values recorded in their tissues, possibly reaching up to 2‰ (DeNiro and Epstein 1978). This shift may contribute to the variability observed among different specimens (Žalaitė et al. 2018). Physiological differences between diverse species should also be considered, as differentiation in bioapatite-diet carbon isotopic fractionation can occur in animals with different digestive physiologies (Passey et al. 2005; Cerling et al. 2021).

The Alentejo region is characterised by dry summers and relatively rainy winters, with a mean annual precipitation of less than 1000 mm. Water availability, particularly emphasizing the dominant role of rainfall, is described as the major factor affecting carbon isotopic composition (Hartman and Danin 2010). Previous studies (Stewart et al. 1995; Kohn 2010) have identified a negative correlation between  $\delta^{13}\text{C}$  values in  $\text{C}_3$  plants and mean annual precipitation. Thus, we would expect plants in dry environments to exhibit high

$\delta^{13}\text{C}$  values, while plants in tropical rainforests would display low  $\delta^{13}\text{C}$  values. If we consider a range of  $\delta^{13}\text{C}$  values for  $\text{C}_3$  plants spanning from  $-23\text{‰}$  to  $-31.5\text{‰}$  (mean  $\delta^{13}\text{C} = -28.5\text{‰}$ ; Kohn 2010), the environment at Perdigões inferred by  $\delta^{13}\text{C}_{\text{diet}}$  would be marked by dry seasons with plant  $\delta^{13}\text{C}$  values reaching high values ( $-24.7\text{‰}$ ), and mildly wet seasons with lower  $\delta^{13}\text{C}$  values ( $-28.2\text{‰}$ ). However, it should be noted that, even within the same environment, the isotopic composition of  $\text{C}_3$  plants can vary significantly (Kohn 2010). Moreover, the  $\delta^{13}\text{C}$  values of plants in drier environments containing areas with wetter conditions, e.g., along rivers and springs, may be depleted seasonally or during a year with higher precipitation (Kohn 2010). Therefore, the lower plant  $\delta^{13}\text{C}$  values detected at Perdigões may be related to its proximity to the Guadiana River.

### Comparison with previous studies

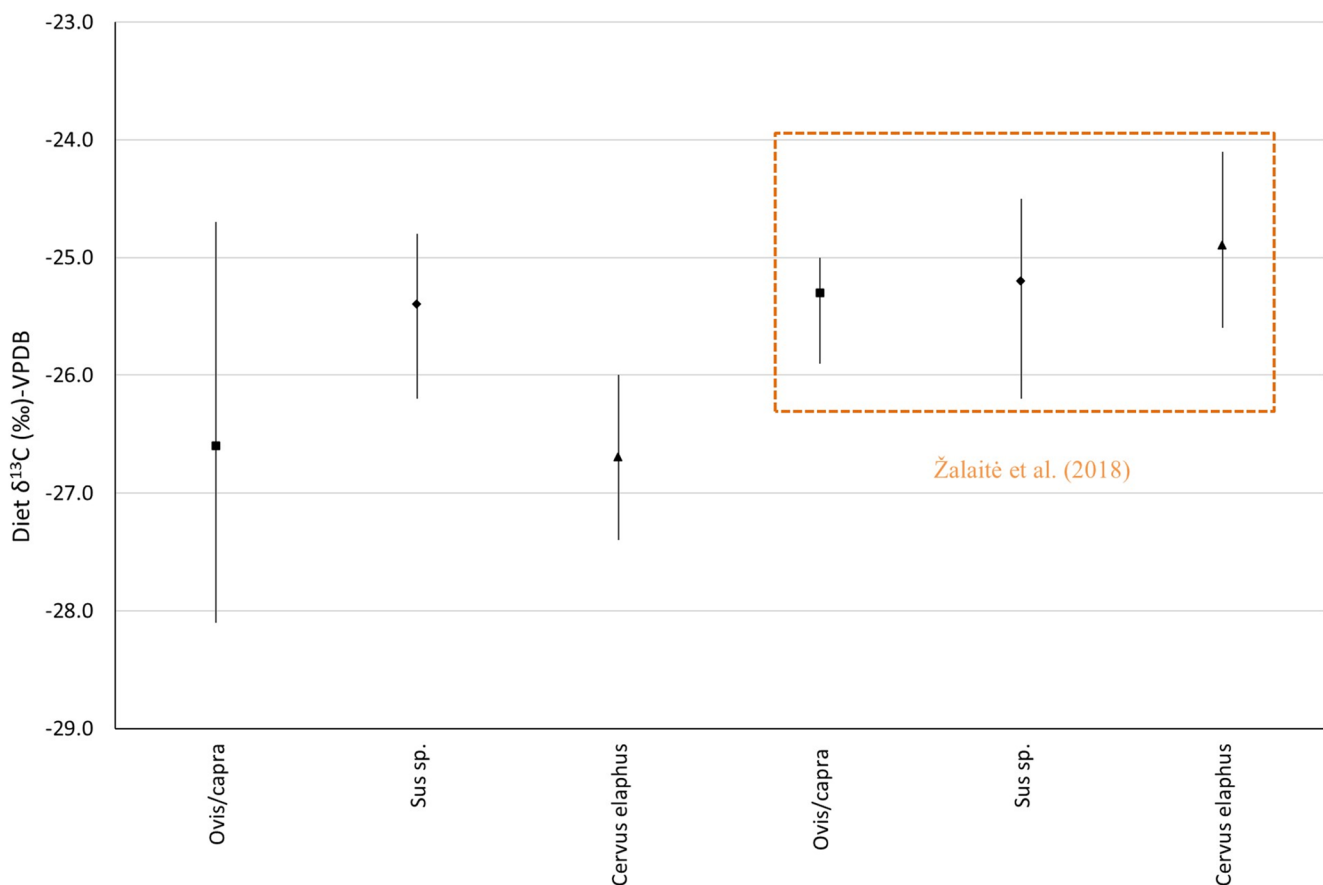
Considering a 5.1‰  $^{13}\text{C}$ -enrichment between the protein component of diet and collagen (Ambrose and Norr 1993), sheep/goat, deer and suid previously analysed (Žalaitė et al. 2018) had a diet mainly composed of  $\text{C}_3$  plants with  $\delta^{13}\text{C}$  values ranging from  $-26.2\text{‰}$  to  $-24.1\text{‰}$  ( $^{13}\text{C}$  average =  $-25.2\text{‰} \pm 0.6$ ). We compared this with our dataset assuming the +12.5‰ and +14.5‰ enrichment between

bioapatite-diet for suids, sheep/goat and deer, respectively (Fig. 7).

The striking difference between our study and that of Žalaitė et al. (2018) could be related to the following factors. Firstly, the tissue analysed in Žalaitė et al. (2018) was bone collagen, whereas in our study was enamel bioapatite. Isotopic values measured in bone collagen reflect long-term averages of diet, which are intrinsically linked to several factors, including the age of the individual (Lee-Thorp 2008). Bone turnover rates generally decrease with age, resulting in an attenuation of the recorded isotopic signature, reflecting recent and past dietary intakes (Hedges et al. 2007; Tsutaya and Yoneda 2013). In contrast, enamel bioapatite provides a high-resolution record of isotopic dietary signals throughout the tooth's growth, spanning from months to years (Balasse 2002). Hence, the time resolution accessed in our study enabled us to discern dietary changes during the first years of the animal's life at Perdigões that were obscured in the dietary signal average of bone collagen in Žalaitė et al. (2018). Besides the temporal resolution of bone collagen versus enamel bioapatite, the

dietary component preserved in these tissues we are accessing is also differentiated. The carbon isotopic values in bone collagen in Žalaitė et al. (2018) reflect the animal's dietary protein intake. On the other hand, the bioapatite carbonate analysed in our study reflects the whole diet consumed (e.g., carbohydrates, fat, and protein) (Ambrose and Norr 1993). Consequently, this contributes to the differences observed between the studies. Conducting future isotopic investigations at Perdigões, wherein both tooth enamel and bone collagen from the same individual specimens are analysed, would be extremely advantageous. This would allow for a more accurate examination of the different aspects of their diet as well as the variation between the tissues.

Secondly, the isotopic enrichment factors defined for our study may also contribute to the differences detected. For the suids, for which we applied an enrichment factor specific to this species (+12.5‰), it is possible to observe an overlap in the  $\delta^{13}\text{C}$  dietary values. However, for sheep/goat and deer, for which we calculated a diet-bioapatite  $\delta^{13}\text{C}$  enrichment defined for ruminants, it is clear that our  $\delta^{13}\text{C}$  dietary values exhibit a broader range for sheep/goat and



**Fig. 7** Distribution of Diet  $\delta^{13}\text{C}$  values of *Ovis/Capra*, *Sus* sp. and *Cervus elaphus* from Perdigões from the present study (left) and from Žalaitė et al. (2018) (orange square). The  $\delta^{13}\text{C}$  values in our study

were adjusted by Cerling et al. (2021, 12.5‰ for suids and 14.5‰ for ruminants). The  $\delta^{13}\text{C}$  values from Žalaitė et al. (2018) were adjusted by 5.1‰ according to Ambrose and Norr (1993)

lower  $\delta^{13}\text{C}$  values for deer. Applying a species-specific isotopic enrichment between diet and bioapatite is crucial as previous studies based on controlled feeding experiments have reported large variability among species spanning from 9‰ to 15‰ (DeNiro and Epstein 1978; Ambrose and Norr 1993; Cerling and Harris 1999; Balasse 2002; Passey et al. 2005; Zazzo et al. 2010). Therefore, our results emphasize the need for more studies to further understand bioapatite-diet spacing for specific-species.

### Neolithic sheep/goat herding strategies at Perdigões

The stable isotopic values provide insights into the individual life histories of the caprine herds at Perdigões. In previous strontium analyses, four suids out of six were found to have values from outside the local settlement range, while among deer remains three out of seven were outside the local range (Valera et al. 2020). Previous strontium isotopic studies at Perdigões show that most sheep/goats studied moved within the local settlement network range of the Álamo Valley (see Valera et al. 2020). The strong variation in suids and deer was suggested to be associated with large ranges or parts of the remains being brought to the site after the animals were killed (Valera et al. 2020). Žalaitė et al. (2018) observed a difference in strontium values in faunal remains from the Neolithic to the Chalcolithic, linking it to a possible change in herding sites from the northwest of Reguengos de Monsaraz in the Neolithic to the northeast during the Chalcolithic.

Based on the  $\delta^{18}\text{O}$  values, we observed that sheep/goat teeth were significantly  $^{18}\text{O}$ -enriched compared to the suid and deer teeth (Fig. 6;  $\delta^{18}\text{O}$  Kruskal-Wallis  $p < 0.001$ ;  $\delta^{18}\text{O}$  Mann-Whitney *Ovis/Capra* ~ *Sus*  $p < 0.001$ ;  $\delta^{18}\text{O}$  Mann-Whitney *Ovis/capra* ~ *Cervus elaphus*  $p < 0.001$ ). This is expected for sheep/goat to have higher values being non-obligate drinkers albeit overlap with animals raised in similar environments. Suids in general have short home range, and we found most of the sheep  $\delta^{18}\text{O}$  values overlap with the values from the latter, while some individuals only slightly overlap by approximately 0.1 to 0.8‰ (OC1000, OC1002, OC1007 and OC1011). There are two scenarios in light of the strontium results. The first is that sheep/goats overlap significantly with the suid data were raised by communities outside the local settlement area. The second is that these are all local animals. Moreover, two deer individuals (CE1019 and CE1020) fall within the suid isotopic range. Mandibles (and other body parts) from hunted animals are usually removed at the primary butchery site, often near the kill site, unless these elements hold important symbolic value to communities (Russell 2012). Previous faunal analysis at the site has highlighted the significance of deer remains (Almeida et al. 2024). Animals' parts of crania, including mandibles,

have been found associated with funerary and non-funerary contexts, with interpretations comprising food-refuse and structured depositions, with a complexity of scenarios being found at Perdigões (Cabaço 2012, 2017; Costa 2013; Valera et al. 2019; Almeida et al. 2024). Regarding hunted animals, our results may support these analyses with evidence that the mandibles come from outside the local territory. Due to their symbolic significance, they might be brought from another locality to be interred with the dead or, as seems to be the cases reported here since these are non-funerary contexts, as a by-product of food-sharing events or used in social display practices.

This difference observed in sheep/goat may be attributed to them grazing on a broader range of environments. This is supported by the wide amplitude of  $\delta^{13}\text{C}$  values detected in this study, which could arise from different husbandry practices or animal behaviour. On the other hand, another explanation for these individuals not presenting "local"  $\delta^{18}\text{O}$  values from Perdigões may be related to the fact that they were brought to Perdigões, and hence, both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values recorded in their tissues reflect "local" values from another region. However, it should also be noted that the variation observed in  $\delta^{18}\text{O}$  values between taxa may be related to a complex combination of factors that include differentiated mineralization rates, metabolic differences related to body size and feeding behaviours (Makarewicz and Pederzani 2017). Within a herd, there may be variation in  $\delta^{18}\text{O}$  values due to environmental variables (relative humidity, rainfall amount and temperature), and differentiated leaf evapotranspiration rates of plants that cannot be explained just by different animal management practices.

### Conclusions

Sheep and goats were a dominant species in Neolithic faunal assemblages in the Mediterranean and played a pivotal role in the earliest husbandry communities in the Iberian Peninsula. They were managed for meat and possible milk production, and they have been managed both extensively and intensively by communities past and present. Management strategies are often governed by the local environment and fodder availability, as well as human behaviour. We have investigated the potential herding strategies of sheep and goats at Perdigões, using incremental stable isotopic analysis of molars within a framework where suids and deer represent the local baseline. The results revealed that the sheep/goats brought to the site displayed different management practices and, thus, might have been raised as part of several herds. This chimes with the interpretation that animals were being brought to the site as Perdigões was a major social centre that attracted communities from beyond

the local region. The data suggests high variation throughout the seasonal cycle in sheep and goats, possibly explained by supplementary forage, such as leafy hay, during winter. The results also potentially highlight the transportation of deer from outside the settlement catchment, demonstrating their important symbolism to these communities.

This study demonstrates the potential of using high-resolution sequential time series in tooth enamel to examine changes in dietary intake throughout the seasonal cycle, which were not detected in previous studies at the site using bulk bone collagen. Conducting more incremental analyses of bioapatite with a larger sample size, combined with strontium isotopes, would allow for a more comprehensive assessment of the extent and complexity of the seasonal migrations of sheep and goats at Perdigões. Thus potentially evaluate similarities and differences in human management practices. Additionally, based on our comparison with previous analysis, this research emphasizes the need for more thorough studies into species-specific isotopic enrichment between diet and bioapatite. This is exemplified in the case of the suids analysed in our study, wherein the application of a species-specific enrichment allowed us to reconstruct the carbon isotope values of the diet more accurately. In contrast, for sheep/goats and deer, we applied isotopic enrichments specific to ruminants. Using non-specific enrichment factors that do not account for physiological variations between species can result in biased interpretations in paleodietary studies, potentially obscuring or amplifying differences. This highlights the importance of using appropriate enrichment factors tailored to each species to ensure accurate dietary reconstructions.

Our study provides further insights into the cultural identity and human behaviour, as well as confirming observations from archaeological and archaeozoological analysis, for an important transformative period of increasing social and cultural complexity in the Iberian Peninsula. Further isotopic analysis of animal remains from prehistoric sites in Iberia and, more specifically, local sites in the Alentejo region, would enhance our understanding of herding strategies in the Late Neolithic and the important role that domesticated and wild species played within these societies. Moreover, extending this study to other periods, e.g. Chalcolithic and Bronze Age, would provide a more nuanced picture of the evolution of herds and the communities that managed them throughout prehistory.

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**Data availability** Data is provided within the manuscript or supplementary information files.

## Declarations

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