1 **Transhumance in the Early Neolithic? Carbon and oxygen isotope insights into sheep**
2 **husbandry at Arene Candide, Northern Italy**

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16 **Abstract**

17 Vertical transhumance is historically known as an animal management practice in the Mediterranean
18 that mitigates the risk of overgrazing and unpalatable pastures. It has long been debated whether the
19 practice developed together with the spread of the Neolithic in Europe or if it was adopted later. This
20 study explores the evidence for transhumant pastoralism at Arene Candide, Northern Italy during the
21 Neolithic. We examine the carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope values of sequentially sampled
22 sheep teeth from the Early and Middle Neolithic layers at Arene Candide. The pattern of reduced $\delta^{18}O$
23 profile amplitude as well as inverse cyclical isotope variation characterised by lower $\delta^{13}C$ during the
24 summer season suggest that some of the sheep analysed were moved to higher altitude pasture areas
25 during the summer months. The dating of the analysed samples suggest that small-scale transhumance
26 occurred at Arene Candide during the Cardial phase (5400 – 5300 BC). Because the sample size in
27 this study is small, it remains difficult to project transhumant pastoralism as a widespread practice in
28 the Mediterranean region. Further studies of this kind will provide a greater understanding of the
29 development and use of vertical transhumance.

30 **Keywords:** Sheep, Neolithic, Carbon isotopes, Oxygen isotopes, Transhumance, Animal management
31 **Declaration of interests:** No conflicts of interest
32
33

1. Introduction

The origins of transhumance - a pastoral animal husbandry practice involving the periodic movement of herds of domestic animals between complementary seasonal pastures - has been hotly debated in the archaeological community. This is a traditional animal husbandry practice in the Mediterranean that remains in practice to this day. Some researchers suggest that this practice allowed farming to adapt to the climatic extremes of the lowland regions of the Mediterranean, characterized by mild winters and hot summers (Barker 1972; Halstead 1987). The movement of herds to the upland regions during cool and well-watered summers avoids unpalatable dry browse and overgrazing of the lowland pasture (Geddes 1983). This practice also relates to social and spatial organisation of sites and landscapes, wherein shepherds build pens and storage facilities or use rock shelters and caves for animals and themselves (Segui 1999).

There is no consensus in the research community about the origins of the practice but dates as early as the Neolithic have been proposed (Higgs et al. 1968; Geddes, 1983). There is a wide difference of opinion, with others placing its origins in the Chalcolithic, Iron Age and even in the Medieval Period (Walker 1983; Halstead 1987; Arnold and Greenfield 2006). At Arene Candide transhumance has been proposed to originate in the Late Neolithic (Barker et al. 1990; Maggi and Nisbet 1991). Halstead (1987) argues that most mountain pastures seem to be a product of human interference (woodland clearing or grazing), thus likely limited during prehistoric times; similarly, lowlands would have been wooded providing graze to animals even when the shallow rooted grasses dry out in the summer months. Therefore, there would not have been as much environmental pressure for transhumant practices as there is in the later historic and modern times (Halstead 1987). Furthermore, it is theorised that the scale of agriculture and animal husbandry would not have been large enough to require such a form of pastoralism, which would arrive with the intensification of agriculture in the Chalcolithic and Bronze Age (Arnold and Greenfield 2006; Greenfield 2006).

Archaeologists have previously tried to investigate potential transhumant practices in various sites based on the contents of animal coprolites and the age-at-death profiles of zooarchaeological assemblages (Akeret and Jacomet 1997; Arnold and Greenfield 2006). Plant macrofossils in sheep/goat faeces from a Neolithic site in Switzerland, for example, suggest that animals were feeding outside of the settlement during the summer months as mainly winter plant macrofossils were evident (Akeret and Jacomet, 1997). Similarly, the absence of 2-6 month old individuals in the caprines (*Ovis sp./Capra sp.*) from southeast European assemblages could indicate that the animals were moved from sites during the summer months, potentially to the highland regions where pasture is more palatable (Arnold and Greenfield 2006). However, the available evidence cannot definitively demonstrate the presence of transhumant pastoralism: it only indicates the absence of animals at sites during certain months. There is very little isotopic evidence on transhumant activities, especially in the Mediterranean with the one of the only published studies coming from Bronze Age Crete (Isaakidou

1 et al. 2019), which demonstrates an important research gap in the investigations of origins of
2 transhumant pastoralism in Europe.

3   In this study, we investigate sheep (*Ovis aries*) husbandry in the earliest Neolithic occupation
4 phases (from Impressa to the beginning of Square Mouth Pottery - SMP culture) at Arene Candide, a
5 stratified cave site in Liguria, in Northwest Italy, via sequential isotopic analyses of tooth enamel
6 carbonates. Results indicate that there is no straightforward answer with regards to transhumance in
7 the Early Neolithic of Liguria. Some caprines appear to have been moved vertically on a seasonal
8 basis, while others were not. Perhaps unsurprisingly, the observed pattern likely reflects a complex
9 system of caprine management, probably as a response to farmers and farming adapting to a new
10 agrarian landscape.

2. Methodological background

2.1 Stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes

13   Oxygen isotope values derived by sequential analysis of caprine teeth can characterize climate,
14 altitude, temperature and geographic location in which the animal was raised (Rozanski et al. 1993).
15 Given the developmental timeline of tooth enamel, this means that tooth enamel sampled in this way
16 records the fine scale chronological variation in what can be very local and variable processes, many
17 of which reflect human decision-making. Enamel bioapatite oxygen values are directly related to body
18 water, which is influenced by the oxygen isotope value of ingested water and food (Luz et al. 1984;
19 Fricke and O'Neil 1996). It is through these relationships that variations in $\delta^{18}O$ values in enamel can
20 be related to the seasonal cycle of oxygen in local waters (Balasse et al. 2002).

21   Seasonal changes in temperature and seasonally modulated evapotranspiration decrease $\delta^{18}O$
22 values of meteoric water in winter and increase them in summer (Rozanski et al. 1993). Meanwhile,
23 changes in altitude influence $\delta^{18}O$ values in meteoric water in an inverse relationship, as altitude
24 increases, $\delta^{18}O$ values decrease (Ambach et al. 1967). When sampled sequentially along the axis of
25 tooth growth, this allows for the reconstruction of past climatic conditions as well as investigation of
26 season of birth and altitudinal mobility in hypsodont animals.

a.s.l. (m)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Mean
90	-9.8	-9.6	-8.3	-6.3	-4.1	-2.9	-1.7	-2.0	-3.4	-5.3	-7.8	-9.0	-6.2
290	-10.3	-10.1	-8.7	-6.7	-4.4	-3.2	-2.0	-2.2	-3.7	-5.7	-8.2	-9.4	-6.7
490	-10.7	-10.5	-9.1	-7.1	-4.8	-3.6	-2.3	-2.5	-4.0	-6.0	-8.7	-9.8	-7.1
690	-11.1	-11.0	-9.5	-7.5	-5.2	-4.0	-2.5	-2.8	-4.3	-6.4	-9.1	-10.2	-7.6
890	-11.5	-11.5	-9.9	-7.9	-5.6	-4.4	-2.8	-3.0	-4.6	-6.8	-9.6	-10.6	-8.0
1090	-11.9	-11.9	-10.3	-8.3	-6.0	-4.7	-3.1	-3.3	-4.9	-7.1	-10.1	-11.1	-8.4

Table 1 Annual variation in $\delta^{18}O$ values in Arene Candide and the altitude effect. Arene Candide is located at 90 m a.s.l. Data sourced from Bowen (2019) at http://www.waterisotopes.org. (Bowen and Revenaugh 2003; Bowen et al. 2005; IAEA/WHO 2015).

1   At Arene Candide an altitude of around 1100 m above sea level can be reached within 10 km

2 of the site and an altitude of 2000 m within 20 km of the site (Fig 1). According to an algorithm the

3 altitude effect around Arene Candide area is estimated to be ca. -0.2‰/100m (Table 1) (Bowen 2019),

4 similar to observed values across Northern Italy (Longinelli and Selmo 2003; Longinelli et al. 2006;

5 Longinelli and Selmo 2010). Considering this, in the event of seasonal transhumant activities up to a

6 4‰ decrease in summer $\delta^{18}O$ values is expected, leading to a reduced amplitude of variation.

7   Carbon isotope values expressed in herbivore enamel carbonate represent the isotope value of

8 the ingested plants that are metabolised, with a ~13‰ offset from diet (Lee-Thorp et al. 1989; Cerling

9 and Harris 1999). Additional factors forcing $\delta^{13}C$ variation in plants include temperature, aridity,

10 photosynthetic pathway and altitude (Jim et al. 2004; Körner et al. 1988; Tieszen 1991). High

11 temperatures increase $\delta^{13}C$ values due to reduced stomatal conductance forced by moisture

12 conservation by the plants (Tieszen 1991). Meanwhile, photosynthetic pathway (e.g. C_3 Calvin-

13 Benson or C_4 Hatch-Slack) influence carbon fractionation in plants, resulting in the ranges in $\delta^{13}C$

14 values of: -33‰ to -20‰ in C_3 plants and -20‰ to -10‰ in C_4 plants (Bender 1971; Kohn 2010). In

15 the southern Mediterranean region, where the warm season is dry and hot and the cool season is mild

16 and wet, the landscape is dominated by C_3 plants (Sage et al. 1999). However, some C_4 plants are

17 present, usually emerging in mid-spring when the climate becomes warmer and more arid (Sage et al.

18 1999). Additionally, plant biodiversity changes with altitude, with the prevalence of C_4 plants

19 decreasing as altitude increases in favour of C_3 plants (Tieszen and Boutton 1989).

20 *2.2 Sequential Sampling of Tooth Enamel and Transhumance*

21   Mandibular molars in caprines start to form in utero for the first molar (M1), at approximately

22 1-3 months of age for the second molar (M2) and 9-12 months for the third molar (M3) (Weinreb and

23 Sharaw 1964; Milhaud and Nezit 1991). Crown formation continues until approximately 6-7 months

24 for first molars, 12 months for second molar and 22-24 months for third molar (Weinreb and Sharaw

25 1964; Milhaud and Nezit 1991; Zazzo et al. 2010). Studies have also observed that tooth enamel

26 mineralisation in sheep has a five to six-month delay compared to expected crown formation (Zazzo

27 et al. 2010; Balasse et al. 2012a). Because tooth enamel does not remodel once formed and is resistant

28 to diagenetic change, isotope values can be related to a specific time in an animal's life (Weinreb and

29 Sharaw 1964; Zazzo et al. 2010; Balasse et al. 2012a). This has been used to provide information

30 about the season of birth and altitudinal mobility in wild and domestic caprines (Tornero et al. 2016;

31 Balasse et al. 2017; Makarewicz 2017; Isaakidou et al. 2019).

32   Seasonal variability in the $\delta^{18}O$ value of ingested water is recorded as enamel $\delta^{18}O$ values,

33 while diet is represented by enamel $\delta^{13}C$ values. Analysis of the two in tandem permits the

34 investigation of location and diet on a seasonal scale (Jim et al. 2004). In sedentary animal husbandry

35 regimes, $\delta^{13}C$ values might be expected to covary with $\delta^{18}O$ values. $\delta^{13}C$ values of ingested plants are

influenced by temperature, dependent on moisture availability, suggesting that the highest values should be observed during the summertime, when $\delta^{18}O$ values are also highest with the converse during wintertime (Tieszen 1991). In addition, the hot and arid summer climate in the Mediterranean favoured by C_4 plants will increase $\delta^{13}C$ values in sheep dependent upon the proportion of C_4 forage consumed by the animals (Tieszen and Boutton 1989; Smedley et al. 1991; Sage 1999). In the event of transhumant pastoralism, $\delta^{18}O$ and $\delta^{13}C$ profiles are expected to have an inverse relationship and, in some cases, a reduced $\delta^{18}O$ amplitude can be expected (Knockaert et al. 2018). This is partly due to cooler temperatures in higher-altitude areas as opposed to hot and dry summers in lowlands (Rozanski et al. 1993). Furthermore, C_4 plants are less common in the higher altitudes of Liguria, and thusly their contribution to the diet is expected to be reduced in such settings (Tieszen and Boutton 1989; Sage 1999). In conjunction with lower temperatures forcing lower $\delta^{13}C$ values in plants, this would be expected to result in lower dietary $\delta^{13}C$ values in sheep transhumant in the summer to higher altitudes. Alternatively, altitude can also lead to an increase in $\delta^{13}C$ values (Körner 1988). Despite complications in these isotope systems, we expect seasonally transhumant sheep in this setting to have lower $\delta^{13}C$ values in the summer than in the winter.

3. Materials

3.1 Arene Candide

Arene Candide is a cave site located in Liguria, northern Italy (Fig. 1). The site is a coastal cave, located in terrain which rises steeply from the coast and reaches over 1000 m above sea level within only 10 km from the coastline (Rowley-Conwy 2000). The site contains occupational layers spanning from the Upper Palaeolithic to the Byzantine Period; the site is well known for its Neolithic sequence, which is considered one of the best articulated stratigraphies in the Western Mediterranean region (Binder and Maggi 2001; Arobba et al. 2017, Binder et al. 2017).
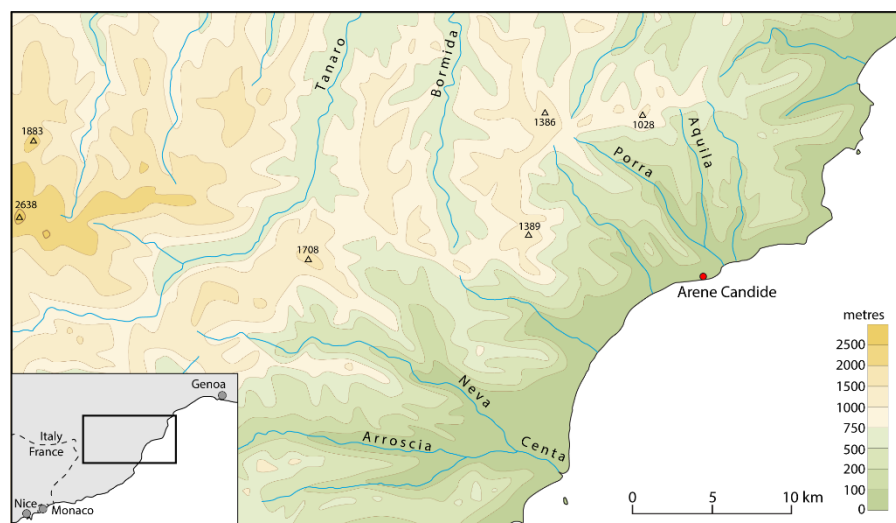

Fig. 1 Location of Arene Candide, Liguria, northern Italy (44°09′45″ N, 08°19′43″ E, 90m a.s.l.). Geographical coordinates reported in Arroba et al. (2017)

At Arene Candide during the early Neolithic 60% of the faunal assemblage consisted of caprines, which increased up to 90% during the Middle Neolithic (Rowley-Conwy, 2000). Pigs and red deer were relatively common during the Early Neolithic, but later decreased in prevalence, while cattle (*Bos Taurus*) were never common at the site (Rowley-Conwy, 2000). Age-at-death profiles of caprines at Arene Candide suggest that they were kept for both their meat and milk, which would have maximised the production as the herd size were theorised to have been small (Rowley-Conwy 1997, Rowley-Conwy 2000, Rowley-Conwy et al. 2013). This pattern changed to a more meat focused husbandry during the Middle Neolithic, which also includes the introduction of goats (*Capra hircus*) (Rowley-Conwy 1997).

3.2 Sheep and Sampling

The third molars of seven sheep (*Ovis aries*) from the Neolithic layers of the most recent excavations in Arene Candide (2011–2012) were selected for oxygen and carbon isotope analyses. The M3 teeth were loose, not embedded in mandibles. It can be difficult to distinguish loose M1s from M2s on morphological grounds, and because these teeth have different developmental timelines (Weinreb and Sharaw 1964; Milhaud and Nezit 1991) misidentification can be problematic. Therefore, we selected M3s, which are easy to identify, even if loose. The M3 teeth discussed here were identified as sheep, not goats, using the criteria developed by Halstead et al. (2002, 548-9, fig. 2d). In the overview by Zeder and Pilaar (2010, fig. 6), criteria 1, 2, 6 and 7 produced clear and consistent results while criteria 4 and 5 were more variable. In addition, goats are almost entirely absent in the Early Neolithic sequence at Arene Candide (Rowley-Conwy et al. 2020). Therefore, we conclude that all the specimens analysed in this study are sheep.

The dates of the stratigraphic layers from which teeth were recovered range from the Impressa to the Early Square Mouth Pottery phase, which represent the Early Neolithic and initial stages of Middle Neolithic at Arene Candide. To place the individual samples more securely within the sequence, each tooth was AMS dated, largely in agreement with the stratigraphic sequence (Table 2). The attritional wear stages were assigned from b to g based on Grant (1982, fig.2). Sequential enamel sampling was performed on the buccal side of the mesial lobe of lower third molars, with the teeth derived from different sides of the skeleton. Therefore, to ensure that the teeth belong to different individuals, tooth wear and gross morphological differences were used to confirm that specimens came from different individuals.

Specimen	Side	Wear	Context	Period	Lab Code	C^{14} Age	Calibrated C^{14} Age (cal. BC)
DAC-1	Dextral	d	US 311 (Ph 7)	Cardial/EN	GrM-18746	6220 ± 35	5621-5491 (95.4%)
DAC-2	Sinistral	b	US 281 (Ph 16)	Early SMP/MN	GrM-18747	6085 ± 30	5202-5176 (3.9%) 5071-4906 (91%) 4864-4857 (0.5%)
DAC-3	Dextral	d	US 308 (Ph 8)	Cardial/EN	GrM-18808	6155 ± 30	5212-5018 (95.4%)
DAC-4	Sinistral	e	US 306 (Ph 9)	Cardial/EN	GrM-18809	6207 ± 28	5291-5245 (8.3%) 5231-5056 (87.1%)
DAC-5	Dextral	g	US 306 (Ph 9)	Cardial/EN	GrM-18810	6190 ± 30	5227-5041 (95.4%)
DAC-6	Dextral	f	US 320 (Ph 5b)	Impressa/EN	GrM-18811	6705 ± 45	5711-5545 (95.4%)
DAC-8	Sinistral	e	US 232 (Ph 21)	Early SMP/MN	GrM-18812	6055 ± 30	5046-4882 (89.4%) 4871-4848 (6.0%)

Table 2 Sample information and radiocarbon age. EN – Early Neolithic; MN – Middle Neolithic; SMP – Square Mouth Pottery.

4. Methods

Sample preparation was carried out in the isotope preparation lab at the University of Durham. Tooth enamel from each specimen was sampled due to its resistance to diagenetic changes post-deposition, unlike dentine or bone (Fricke and O'Neil 1996). Tooth enamel surfaces were cleaned by abrasion using a tungsten carbide burr, that removed any obvious surface contamination. Subsequently, the enamel was sequentially sampled using a diamond-tipped dental burr starting at the crown (earliest forming part of the tooth) continuing to the cervix (latest forming portion of the tooth). Each sample consists of a groove perpendicular to the tooth growth axis drilled through the thickness of the enamel layer, but without penetrating the dentine. The sampling spanned the whole crown, including the lowest part which in some cases bore softer enamel indicative of incomplete mineralisation. The placement of each sample relative to the enamel/root junction (ERJ) was measured and later used to normalise the data. Between 18 and 24 samples were drilled from each tooth depending on the crown length, giving a total of 141 samples from 7 teeth.

No pre-treatment was applied to these samples. There is ongoing debate in the community over which pre-treatment procedures if any, should be applied to enamel carbonate samples, with no agreement. Use of H_2O_2 pre-treatment has been demonstrated to be ineffective in remove organic matter and it's corrosive nature dissolves the carbonate modifying its structure and crystallinity (Grimes and Pellegrini 2013, Pellegrini and Snoeck 2016). NaClO pre-treatment while effective in removing organic matter has been shown to induce the absorption of exogenous carbonates, forcing a significant non-linear change in isotope values of the samples (Zazzo et al. 2006, Snoeck and Pellegrini 2015). Acetic acid treatment is another pre-treatment option. It has been demonstrated to be effective in removing organics and secondary carbonates (Pellegrini and Snoeck 2016). However, it has been demonstrated that this pre-treatment procedure also forces changes in stable isotope values,

1   with even a brief immersion in the 0.1 M acetic acid dissolving a significant unquantifiable

2   percentage of material (Jeffrey et al. 2015, Snoeck and Pellegrini 2015, Pellegrini and Snoeck 2016).

3   Experiments have demonstrated that an acetic acid pre-treatment forces unquantified changes in

4   isotope values of modern samples that do not have exogenous carbonates (Skippington et al. 2019).

5   For these and other reasons no pre-treatment procedures were applied, similarly to some other

6   researchers (e.g. Pellegrini et al. 2011, Jeffrey et al. 2015) due to the significant and unquantified

7   effect of the pre-treatment on isotope values and structural carbonate. As this was consistent

8   methodology across all samples and all of them come from the same depositional environment and

9   seem to be well preserved it is unlikely that this influences the interpretation of the data.

10   Carbonate isotope analysis of the samples was carried out in the Saskatchewan Isotope

11   Laboratory, University of Saskatchewan. Carbonate samples were roasted in a vacuum oven at 200°C

12   for one hour to remove water and volatile organic contaminants that may confound stable isotope

13   values of carbonates.

14   Stable isotope values were obtained using a Finnigan Kiel-IV carbonate preparation device

15   directly coupled to the dual inlet of a Finnigan MAT 253 isotope ratio mass spectrometer. Twenty to

16   fifty micrograms of enamel were reacted at 70°C with three drops of anhydrous phosphoric acid for

17   420 seconds. Evolved CO_2 was cryogenically purified before being passed to the mass spectrometer

18   for analysis. Isotope ratios were corrected for acid fractionation and ^{17}O contribution using the Craig

19   (1957) correction and reported in per mil (‰) notation relative to the VPDB scale. Data were directly

20   calibrated against the international standard NBS-19 that has the following values: $\delta^{13}C = 1.95$‰

21   VPDB and $\delta^{18}O = -2.20$‰ VPDB. Precision/accuracy of data were monitored through routine analysis

22   of NBS-19. Precision/accuracy of $\delta^{13}C$ and $\delta^{18}O$ are 0.05‰ and 0.11‰, respectively (n = 25). Actual

23   sample errors may be greater than these due to sample heterogeneity.

24   $\delta^{18}O$ values are reported relative to Vienna Standard Mean Ocean Water (VSMOW) using the

25   equation: $\delta^{18}O_{VSMOW} = 1.03091 \times \delta^{18}O_{VPDB} + 30.91$ given in Sharp (2007). All isotopic results are

26   reported in standard delta notation.

27   **5.   Results**

28   *5.1 Variation in $\delta^{13}C$ and $\delta^{18}O$ values*

29   There is considerable variation in $\delta^{13}C$ and $\delta^{18}O$ values within each tooth (Table 3,

30   Supplementary Table 1). In all specimens, $\delta^{18}O$ values show variation along the tooth crown, and

31   therefore through time, with an amplitude between 1.0‰ and 3.7‰. As variation is expected with

32   changes in temperature, this likely represents natural seasonal variation in precipitation and

33   groundwater $\delta^{18}O$ values (Rozanski et al. 1993). When $\delta^{18}O$ profiles are compared, two distinct

34   patterns are observed, based on amplitude and shape of the curves (Fig. 2). DAC-1 has an amplitude

of 3.7‰ with pronounced peaks while other specimens have smaller amplitudes, mostly under 2‰.

Two distinct $\delta^{18}O$ patterns could indicate different husbandry practices including different seasons of

birth and/or altitudinal mobility. Specimens DAC-2 to DAC-8 fall into two distinct clusters, with

DAC-5 and DAC-6 exhibiting $\delta^{18}O$ values clustering around 26‰ whereas DAC-2, 3, 4, 8 exhibit

$\delta^{18}O$ values around 24‰ to 25‰. The 2‰ difference between the groups is likely caused by year-to-

year variation in $\delta^{18}O$ values in ingested water as the samples are not contemporaneous.
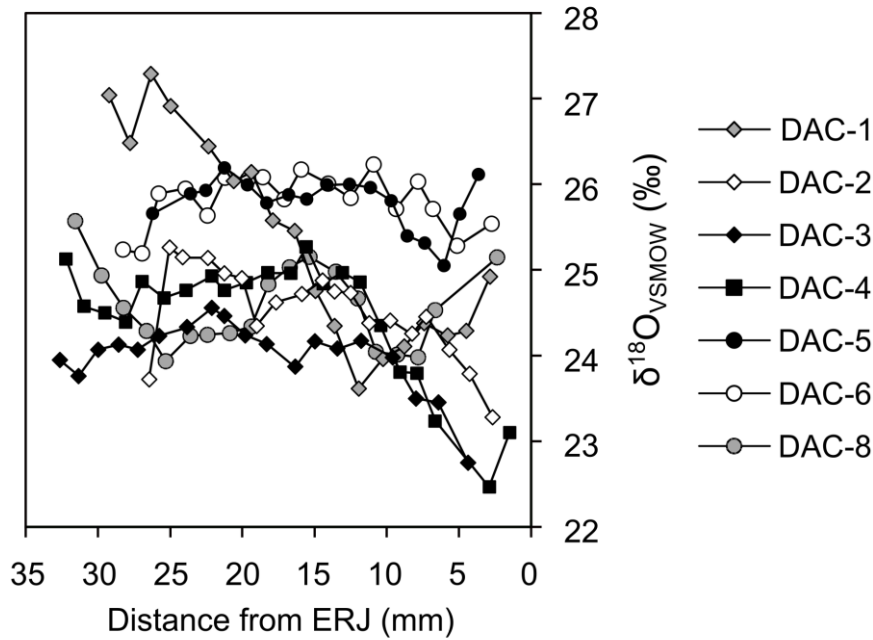
Fig. 2 Enamel sequential bioapatite oxygen isotope profiles from all specimens

Specimen	$\delta^{18}O_{min}$ (‰)	$\delta^{18}O_{max}$ (‰)	$\Delta^{18}O$ (‰)	$\delta^{13}C_{min}$ (‰)	$\delta^{13}C_{max}$ (‰)	$\Delta^{13}C$ (‰)
DAC-1	23.6	27.3	3.7	-11.8	-9.6	2.1
DAC-2	23.3	25.3	2.0	-12.0	-9.9	2.1
DAC-3	22.8	24.6	1.8	-11.0	-9.4	1.6
DAC-4	22.5	25.3	2.8	-11.7	-10.0	1.7
DAC-5	25.1	26.2	1.1	-11.0	-9.4	1.6
DAC-6	25.2	26.2	1.0	-12.3	-10.6	1.6
DAC-8	23.9	25.6	1.6	-10.4	-8.8	1.5

Table 3 Summary of $\delta^{18}O$ and $\delta^{13}C$ values of tooth enamel carbonate from Arene Candide: minimum and maximum values, amplitude of intra-tooth variation (Δ). The values are expressed relative to the VPBD standard for $\delta^{13}C$ and VSMOW for $\delta^{18}O$

Variation in $\delta^{13}C$ values likely reflects seasonal changes in diet values related to temperature

changes, moisture abundance, and proportional availability of plants that utilize different

9

1   photosynthetic pathways (Table 3, Fig. 3) (Tieszen and Boutton 1989; Tieszen 1991). This can be

2   better understood by comparing them with the $\delta^{18}O$ profiles. Visual assessment of $\delta^{18}O$ and $\delta^{13}C$

3   profiles suggest that all but sample DAC-4 covary (Fig. 3). This is confirmed by covariance and

4   Pearson correlation coefficient using the following equations (Table 4).

5
$$Cov(\delta^{18}O, \delta^{13}C) = \frac{\sum(\delta^{18}O - \overline{\delta^{18}O})(\delta^{13}C - \overline{y\delta^{13}C})}{n}$$

6
$$r(\delta^{18}O, \delta^{13}C) = \frac{Cov(\delta^{18}O, \delta^{13}C)}{\sigma(\delta^{18}O) \times \sigma(\delta^{13}C)}$$

7   Both statistics determine the degree to which the two samples covary – when one variable

8   increases the other follows, with positive values showing positive covariance and negatives denoting

9   negative covariance. Correlation coefficient also measures how strong this relationship is from r=-1 to

10   r=1, with ±1 representing a perfect negative/positive correlation. These calculations demonstrate that

11   samples DAC-4 and DAC-5 display negative covariance and correlation between $\delta^{18}O$ and $\delta^{13}C$

12   profiles. In addition, sample DAC-6 shows close to neutral correlation between $\delta^{18}O$ and $\delta^{13}C$ profiles

13   and can be grouped together with DAC-4 and DAC-5. This negative (or neutral in the case of DAC-6)

14   covariance/correlation suggests that these specimens could have been under a different husbandry

15   regime.

Specimen	Period/Date	Covariance	Correlation
DAC-1	Cardial/EN	0.678	0.880
DAC-2	Early SMP/MN	0.171	0.710
DAC-3	Cardial/EN	0.108	0.700
DAC-4	Cardial/EN	-0.026	-0.089
DAC-5	Cardial/EN	-0.004	-0.030
DAC-6	Impressa/EN	0.021	0.160
DAC-8	Early SMP/MN	0.159	0.840

Table 4 Covariance and Pearson correlation statistics between $\delta^{18}O$ and $\delta^{13}C$ datasets. The age of the specimens is presented for reference

16   *5.2 Modelling of $\delta^{18}O$ data*

17   Normalising data to the periodic cycle identified from $\delta^{18}O$ values permits elimination of inter-

18   individual variability in tooth size, that allows comparison between the samples (Balasse et al. 2012a).

19   Data sequences were modeled using the following equation mainly based on a cosine function

20   (Balasse et al. 2012b):

21
$$\delta^{18}O_m = A.\cos(2\pi\,(x - x_0)/X) + M$$

22   Where $\delta^{18}O_m$ is the modelled $\delta^{18}O$; x is the distance from enamel-root junction; X is the period (in

23   mm), corresponding to the length of the tooth crown potentially formed over a whole annual cycle; A

1 is the amplitude [= (max - min)/2] (in ‰); x_0 is the delay (in mm), which depends on the time of the
2 year when the tooth started forming - $\delta^{18}O$ reaching its maximum when $x = x_0$; M is the mean [= (max
3 + min)/2] expressed in ‰.

4   The best fit of the model was determined using an iterative method and minimisation of the
5 sum of the square of the difference between the model and the measurements (the method of least
6 squares) as described in Balasse et al. (2012b). The results from the calculation of the best fit for
7 combines variations of X, A, x_0 and M are shown in Table 5. The fitting of the model to the data is
8 estimated using Pearson's correlation coefficient, r=1 representing a perfect fit of the model to the
9 database:

10
$$r = \frac{Cov(\delta^{18}O, \delta^{18}O_m)}{\sigma(\delta^{18}O) \times \sigma(\delta^{18}O_m)}$$

11 The accuracy of the model varied between 0.72 and 0.97 (Table 5). Correlation coefficient >0.90
12 demonstrates that the model is very close to the dataset and is observed only in specimens DAC-1,
13 DAC-3 and DAC-4 (Balasse et al. 2012a, b). However, if models with correlation coefficient above
14 0.75 are accepted the interpretation of birth seasonality and vertical mobility do not change.

Specimen	Crown length (mm)	X (mm)	A (‰)	x_0 (mm)	M (‰)	x_0/X	r (Pearson)
DAC-1	29.24	34.27	1.52	26.31	25.48	0.77	0.98
DAC-2	26.46	300.64	24.88	18.79	0.00	0.06	0.79
DAC-3	32.66	325.84	24.35	21.48	0.00	0.07	0.91
DAC-4	32.22	295.66	25.01	22.05	0.00	0.07	0.89
DAC-5	26.22	9.61	0.20	21.51	25.80	2.24	0.72
DAC-6	28.26	335.87	26.07	15.43	0.00	0.05	0.78
DAC-8	25.57	15.32	0.57	31.51	24.59	2.06	0.91

Table 5 Calculation of the best fit model (method of least squared) for combined variations of X (period), A (amplitude), x_0 (delay) and M (mean). When r = 1, the series are identical

15   Period length (X), represents the length of an annual cycle corresponding to the length of the
16 tooth crown and varied between 9.6 and 335.8 mm (Balasse et al. 2012a). X values in some of the
17 samples are considerably higher than what is expected and observed in similar studies, where they are
18 close to the length of the crown (Balasse et al. 2012a, b; Balasse 2013). Considering that the third
19 molar in sheep forms over 12-14 months, one full annual cycle (or slightly more) should be
20 represented within a single tooth (Weinreb and Sharav 1964; Balasse et al. 2003). Other parameters,
21 such as amplitude and mean also deviate from the model estimates. Amplitudes of some samples
22 DAC-2, 3, 4, 6 are higher than expected as they should represent the difference between the highest
23 and lowest $\delta^{18}O$ values.

1   The DAC-1 model seems to fit what is expected the best as the X is 34.27, meaning that just

2   under one annual cycle is represented while amplitude and mean are reasonable. DAC-1 also has the

3   highest correlation coefficient, demonstrating that the model is a near perfect fit. Other samples have

4   lower Pearson correlation coefficients but still within acceptable boundaries except for DAC-5 with

5   r=0.72. High period and amplitude values as well as low mean could be explained with external

6   environmental and cultural factors affecting $\delta^{18}O$ values, thus while the model did not fully fit the data

7   can still provide important insights. However, the DAC-5 model cannot be accepted due to the low

8   correlation coefficient and abnormal values in other parameters: low period and amplitude.

9
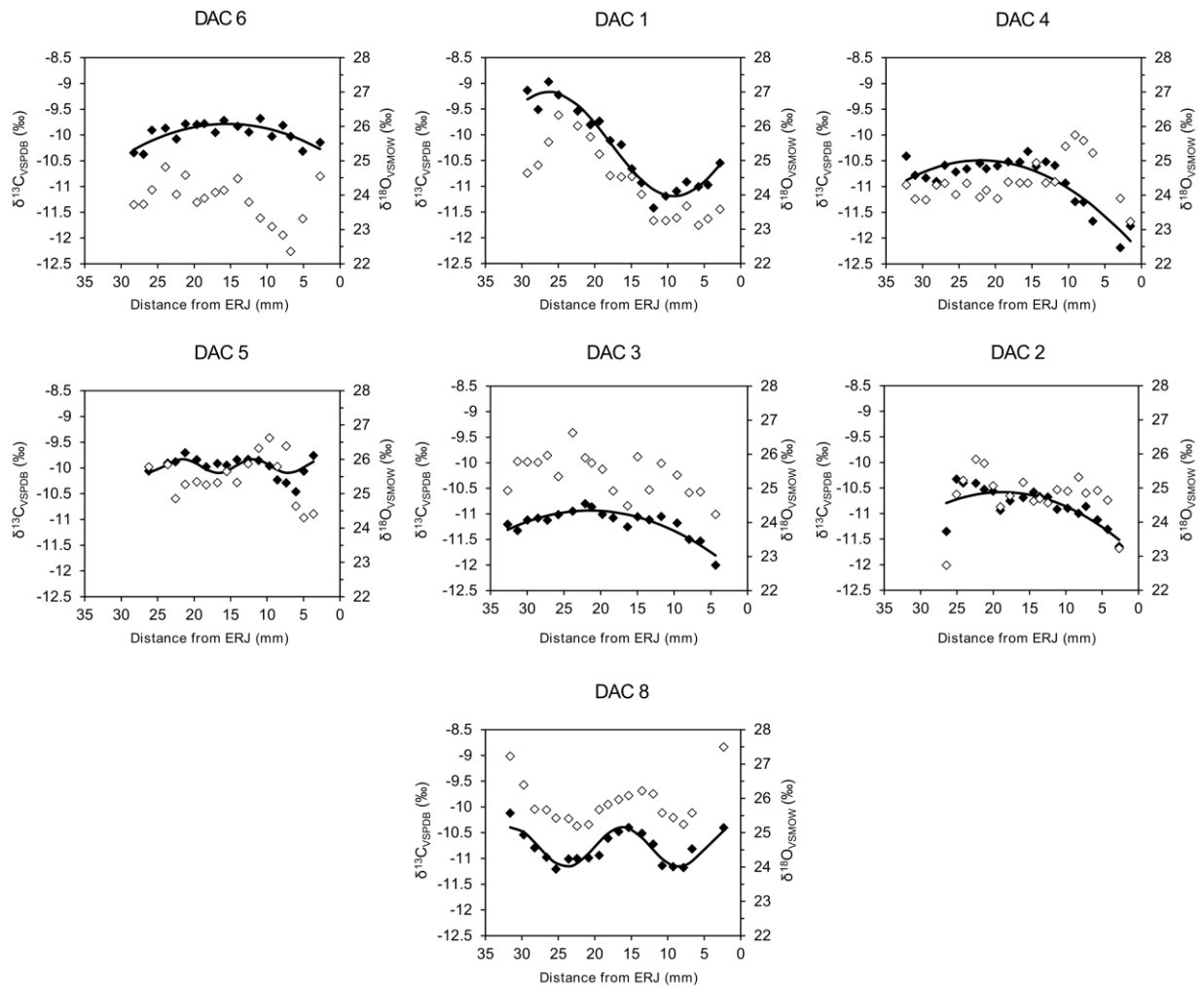
Fig. 3 $\delta^{18}O$ (black symbols) and $\delta^{13}C$ (white symbols) values in Arene Candide sheep. Samples are located along each tooth crown by their distance from the enamel-root junction (ERJ). The symbols represent the unmodeled isotope values, black line represents the modelled $\delta^{18}O$ values. The specimens are arranged in a chronological order. Raw data is provided in Supplementary Table 1

10

11

6. Discussion

6.1 Reduced seasonal $\delta^{18}O$ amplitude

Based on model estimates of modern meteoric water $\delta^{18}O$ values (Bowen and Revenaugh 2003; Bowen et al. 2005; Bowen 2019) the expected modern amplitude of annual variation in meteoric waters at Arene Candide should be around 8.1‰ (Table 1). Comparable annual $\delta^{18}O$ variations are currently reported for a Darfo-Boario pluviometer, located 300 km away from the cave in the Lombard pre-Alps (Longinelli and Selmo 2003). In comparison with the modelled and observed annual variation of meteoric water, the amplitude of variation of $\delta^{18}O$ values for the tooth enamel samples from Arene Candide is reduced to 1‰–3.7‰. However, caution is warranted when comparing modern values with prehistoric ones. Furthermore, the amplitude of variation in tooth $\delta^{18}O$ values can also differ slightly from the meteoric waters due to oxygen fractionation upon the ingestion of water and sheep feeding behaviours (Fricke and O'Neil 1996). Sheep are generally grazers and get most of their water from grasses rather than drinking (Makarewicz and Pederzani 2017). $\delta^{18}O$ values of their samples therefore record these sources. However, sometimes sheep browse - consuming leaves, shoots or fruits of woody plants, all of which could influence tooth $\delta^{18}O$ values. Plants with deeper roots generally have lower $\delta^{18}O$ values compared to short-rooted plants, such as grasses with higher $\delta^{18}O$ values related to evaporative effects taking place at the air-ground interface (Heller 1968).

All of the specimens analysed in this study had on average a 5‰–6‰ reduction in $\delta^{18}O$ amplitude that is not consistent with the expected 8‰ annual variation. Some researchers have attributed this reduction of amplitude to altitudinal mobility (Isaakidou et al. 2019). As $\delta^{18}O$ variation over the annual cycle is influenced by seasonal temperature variability, moving sheep to a cooler climate in higher altitudinal zones could force damping of summer $\delta^{18}O$ values (Rozanski et al. 1993). Evidence also demonstrates that reduction in seasonal $\delta^{18}O$ variation could be caused by time-averaging by sampling strategy (Zazzo et al. 2005). However, the reduction in amplitude at Arene Candide is too great compared to other published studies of sheep (e.g. Balasse et al. 2013; Tornero et al. 2016; Knockaert et al. 2017) to be caused by sample averaging alone, thus, we believe that altitude change had also affected the sheep. Karstic soils and subsequent use of deep soil water, which has lower $\delta^{18}O$ values compared to rainfall, by plants during the summer droughts, as has been demonstrated in grapevines by Savi et al. (2019) could also affect the $\delta^{18}O$ amplitude of sheep enamel carbonate. However, as this would generally affect longer rooted plants, such as shrubs and trees it is unlikely that it would have significantly changed sheep enamel values. Sheep have been observed to preferentially graze on herbaceous plants and grasses (up to 89% in some studies), which would not likely access the deep soil water (Bartolome et al. 1998; Castro and Fernandez-Nuñez 2016).

An altitude of around 1100 m above sea level can be reached within 10 km of Arene Candide and within 20 km of the site altitudes of 2000 m can be found (Fig 1). Considering that the altitude

13

1   effect in the area is around -0.2‰/100m, in the event of seasonal transhumant activities up to a 4‰

2   reduction in the summer δ^{18}O values could be expected in turn decreasing the amplitude of variation.

3   This is consistent with the Arene Candide dataset as the observed reduction in amplitude varied

4   between 4.3‰ and 7.0‰. The argument for altitudinal mobility is especially convincing for

5   specimens DAC-2, 3, 4, 5, 6 with amplitude ranging from 1.0‰ to 2.8‰. The models of these

6   specimens are dampened with no clear seasonal variation in comparison to the rest of the models. On

7   the other hand, some of the profiles while having reduced amplitude still have a distinct cyclical

8   variation representing seasonal changes. For example, the DAC-1 δ^{18}O profile has an amplitude of

9   3.7‰, lower than what would be expected in West Liguria, but with distinct summer and winter

10   values. Similarly, DAC-8 also has a reduced amplitude of variation (1.6‰), but the annual cycle

11   remains pronounced. If these two specimens were to be considered as representative of the seasonal

12   δ^{18}O variation at Arene Candide during the Neolithic, the reduced amplitude and flat models of

13   specimens DAC-2, 3, 4, 5, 6 could represent vertical transhumance.

14   There is no correlation between the pattern of reduced amplitude of variation and the

15   archaeological phases from which the sheep derive. The sheep that show this reduction in amplitude

16   of variation come from Impressa (DAC-6), Cardial (DAC-3 to DAC-5) and Early SMP (DAC-2)

17   periods. Meanwhile, the ones that show distinct annual variation (DAC-1 and DAC-8) come from the

18   Cardial and Early SMP phases. Thus, there is no consistent indication of *when* transhumance could

19   have been initiated. In addition, Cardial specimens display both patterns, perhaps explained by the

20   presence of two different flocks of sheep, one sedentary and one transhumant, or possibly sex

21   differences in mobility. However, herd size may not have been large enough for several flocks as

22   large herd sizes are rarely demonstrated in Prehistory (Halstead, 1987).

23   Reduced δ^{18}O amplitude as evidence for vertical transhumance is based on comparison with an

24   estimate of expected variation observed in modern data. However, it is not known if the modern data

25   is directly comparable with prehistoric δ^{18}O values and it does not account for prehistoric year by year

26   variations in precipitation and ambient temperature. Thus, due to complex sourcing of body water in

27   caprines, establishing vertical transhumance using δ^{18}O tooth data alone is problematic (Makarewicz

28   et al. 2017). Another issue is that the vertical gradient effect on water δ^{18}O values does not always

29   behave in a predictable way. Knockaert et al. (2018) found that flocks of sheep raised at different

30   altitudes in the Pyrenees did not have δ^{18}O values reflecting these known differences; sheep grazed

31   higher in the mountains did not record a decreased δ^{18}O amplitude. Researchers suggested that this

32   could have been caused by different water sources available to sheep (river *versus* seasonal ponds).

33   This implies that the pattern of decreasing δ^{18}O values with increasing altitude in meteoric water does

34   not always reflect the expected δ^{18}O altitudinal gradient (Knockaert et al. 2018). As Arene Candide is

35   surrounded by a mosaic of different environments: coastal marshes, dunes, river plains and karst

36   plateaus, this phenomenon might also affect the sheep at the site. However, it is not clear if reduced

1     $\delta^{18}O$ amplitude of variation at Arene Candide was caused by altitudinal mobility or other factors.

2   Nonetheless, our results most likely point to the observed patterns being consistent with transhumance

3   as the majority of the samples demonstrated the reduction in $\delta^{18}O$ amplitude of ~4‰–6‰, consistent

4   with the altitude that could be reached regularly near Arene Candide.

5   *6.2 Covariance in $\delta^{13}C$ and $\delta^{18}O$ profiles*

6   The majority of specimens in this study had positive covariance and correlation coefficients

7   between $\delta^{13}C$ and $\delta^{18}O$ sequences: DAC-1, r=0.88, DAC-2, r=0.71, DAC-3, r=0.70, DAC-6, r=0.16,

8   DAC-8, r=0.84. This relationship suggests that during summers, when $\delta^{18}O$ values are highest, $\delta^{13}C$

9   values are also highest (Table 3). This pattern is consistent with sedentary sheep herds as has been

10   previously observed in Iron Age Catalonian sheep (Valenzuela-Lamas et al. 2016). Higher summer

11     $\delta^{13}C$ values and lower winter values likely correspond to seasonal plant variation in the environment

12   and sheep diet as well as seasonal variation in plant $\delta^{13}C$ values (Smedley et al. 1991). Because plant

13     $\delta^{13}C$ values can be affected by temperature, low temperatures during winter months can lead to lower

14   sheep tooth $\delta^{13}C$ values (Körner et al. 1988; Tieszen 1991). Meanwhile, during summer months, heat

15   and moisture stress reduce stomatal conductance, increasing $\delta^{13}C$ values. Additionally, the hot and

16   arid summer climate found at Arene Candide, will increase the proportion of C_4/C_3 plants (Tieszen

17   1991; Sage et al. 1999). The mosaic environments in the region, such as coastal salt marshes, dunes,

18   etc. could also affect the variety of plants available for graze, however it is unlikely that they affected

19   the variation of bioavailable carbon to a large degree. All samples demonstrate a C_3 plant dominated

20   diet, with some consumption of C_4 plants during summer being supported by samples DAC-1 and

21   DAC-8 as they have some values above the expected C_3 diet range (above -10‰) (Balasse et al.

22   2013). This contribution of C_4 plants in the summer months suggests that covariant $\delta^{13}C$ and $\delta^{18}O$

23   profiles found in samples DAC-1 and DAC-8 support a sedentary herding regime.

24   Sheep DAC-4 and DAC-5 display inverse covariance between $\delta^{18}O$ and $\delta^{13}C$ values. Opposing

25   covariance has been attributed to vertical mobility (Tornero et al. 2016; Makarewicz 2017;

26   Makarewicz et al. 2017), or winter foddering (Balasse et al. 2013; Makarewicz 2017; Makarewicz et

27   al. 2017). Inverse $\delta^{13}C$ and $\delta^{18}O$ sequences may be related to temperature changes and reduced

28   availability of C_4 plants in the highlands compared to the lowland plains (Tieszen and Boutton 1989;

29   Tieszen 1991). Thus, sheep feeding on plants available in the highlands during the summer months

30   would have lower $\delta^{13}C$ values compared to those feeding in the lowlands. Tornero et al. (2016) has

31   observed this pattern in wild mouflons in Armenia during the Late Glacial Maximum and attributed it

32   to vertical mobility. Similar results have been observed in Late PPNB Jordanian and Chalcolithic

33   Anatolian sheep (Makarewicz 2017; Makarewicz et al. 2017).

34   Winter foddering, which has been hypothesised for Arene Candide, can lead to a similar effect.

35   Makarewicz (2017) has proposed that seasonal supplementation of the caprine diet with high $\delta^{13}C$

1   fodder would produce high δ^{13}C tooth values during the winter season (Maggi and Nisbet 2000;

2   Arobba et al. 2014). Similarly, Balasse et al. (2013) have also proposed a possibility of winter

3   foddering with plant food (C$_3$ and C$_4$) collected during summer as an explanation for inverse δ^{13}C and

4   δ^{18}O sequences. This is supported by a study of modern caprines fed with high δ^{13}C winter fodder as

5   the practice resulted in winter values similar to that of summer grazing (Makarewicz and Tuross

6   2006). Similar data has also been observed from domestic and wild modern sheep in Mongolia

7   (Makarewicz and Pederzani 2017). It is possible that during winter the diet of caprines at Arene

8   Candide was supplemented by fodder collected during summer, which could explain higher δ^{13}C

9   values during colder months of the year. Possible sources of fodder that have been observed at

10 comparable sites include collected grass, leaves, branches as well as stored cereals and their by-

11 products (Hejcman et al 2014; Moreno-Garcia and Pimenta 2015).

12 Therefore, it is difficult to determine whether specimens DAC-4 and DAC-5 have inverse δ^{13}C

13 and δ^{18}O sequences due to altitudinal mobility, winter foddering or a combination of both. As

14 Makarewicz (2017) explained, distinguishing transhumance and winter foddering is difficult.

15 Although, she proposes that in some cases it is possible by comparing δ^{18}O values of caprines against

16 δ^{18}O values of obligate and non-obligate-drinking herbivores in the same area (Makarewicz 2017).

17 *6.3 Were sheep in Arene Candide transhumant?*

18 Two of the samples from this study show strong evidence of transhumance. DAC-4 and DAC-5

19 have reduced amplitudes of variation in their δ^{18}O sequences as well as inverse covariance between

20 δ^{18}O and δ^{13}C profiles, which is often assigned to vertical mobility. However, interpretations of the

21 DAC-5 δ^{18}O dataset are tenuous due to low correlation coefficient, long period, low amplitude and

22 mean values. Alternatively, inverse δ^{18}O and δ^{13}C sequences in specimens DAC-4 and DAC-5 could

23 be argued to be a result of winter foddering with high δ^{13}C value plants as has been proposed in some

24 studies (Maggi and Nisbet 2000; Balasse et al. 2013; Arobba et al. 2014; Makarewicz 2017;

25 Makarewicz et al., 2017). Nonetheless, the presence of both reduced amplitude of variation *in*

26 *addition to* inverse sequences suggests transhumance in these two specimens.

27 Three other specimens (DAC-2, 3, 6) display reduced amplitudes of variation but unlike the

28 above specimens they did not display inverse covariance between their δ^{18}O and δ^{13}C profiles. Some

29 researchers argue that reduced amplitude of variation can indicate vertical mobility, but this line of

30 evidence alone is problematic (Makarewicz et al. 2017). It is therefore possible that specimens DAC-

31 2, 3, 6 were transhumant, while there is a possibility that the reduced amplitude was caused by other

32 factors, such as different drinking water sources (Knockaert et al. 2018). Lastly, specimens DAC-1

33 and DAC-8 do not show evidence for vertical mobility. Even though both specimens have reduced

34 δ^{18}O amplitude of variation, their δ^{18}O and δ^{13}C values exhibit distinct seasonal patterns and have a

strong positive correlation between $\delta^{18}O$ and $\delta^{13}C$ profiles. Therefore, it is unlikely that these specimens were transhumant.

This evidence presents a potential differentiation in herding practices among separate groups of sheep during the Early Neolithic. This could imply that small-scale transhumance was being practiced, while the rest of the sheep remained sedentary at the site. Similar practices have been previously reported for a nearby area of Pietra Ligure (Cleary and Delano Smith 1991). This pattern could also indicate differential management dependent on sex. These results are consistent with theories of the origins of transhumance in the Early Neolithic proposed by Barker (1972) and Geddes (1983). It is suggested that transhumant animal herding was developed as part of the adaptation of the Neolithic in the Mediterranean climate. There is also evidence from wild sheep that migrate seasonally between lowland and upland pastures, that could further encourage transhumant animal husbandry (Tornero et al. 2016). As two of the sheep in this study showed strong evidence of transhumance, while three others have partial evidence of vertical mobility, it is likely that transhumant sheep herding practices in Arene Candide existed on a small scale. Small-scale transhumance herding could make sense, especially if the resources were sparse in the lowland regions and the climate was arid during the summer months. Having some sheep in the lowlands near the site, would provide year-round access to the animals for consumption, while moving part of the flock to the higher altitude grazing land would reduce the strain on the lowland pasture.

7. Conclusion

This study presents evidence of transhumant pastoralism at Neolithic Arene Candide, Liguria, Italy. Strong evidence was observed in two of the specimens from Cardial levels, while three showed some evidence of vertical mobility, although this interpretation remains problematic. Alternatively, there could have been winter foddering practices on site or access to different sources of water that would have affected the $\delta^{18}O$ and $\delta^{13}C$ values in a similar way as transhumance. A future analysis of a larger sample size could help clarify this with more certain determinations of transhumant activities at the site. Nonetheless the combination of inverse relationship of $\delta^{18}O$ and $\delta^{13}C$ profiles and reduced amplitude of $\delta^{18}O$ variation observed in this study suggests that small scale transhumance could have been practiced in Arene Candide during the Early Neolithic. This supports arguments for the Neolithic origin of transhumance put forward by Geddes (1983) and Barker (1972). However, due to the small sample size of this study, it is difficult to quantify the extent of this practice, but based on the differing isotope profiles in the Arene Candide sheep it is possible that a part of the flock was transhumant, while the rest were kept close to the settlement. This would have allowed for year-round access to the flock, maximising the productivity without depleting the summer pasture.

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