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Neolithic sheep birth distribution: results from Nova Nadezhda (sixth millennium BC, Bulgaria) and a reassessment of European data with a new modern reference set including upper and lower molars.

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Abstract
During the course of the diffusion of Neolithic agropastoral societies across Europe, animal husbandry was adapted to local constraints and resources, involving changes in practices, as well as in animal physiology. As a result, the timing of animal breeding was impacted, with consequences on the organization of agro-pastoral tasks and the seasonal availability of animal productions. Past sheep birth seasonality can be investigated through the reconstruction of the seasonal cycle recorded in molars, based on the sequential analysis of stable oxygen isotope ratios ($\delta^{18}O$) in enamel. Modern sheep serve as comparative material to define the season of birth. In the present study, we provide new reference values for winter births in the sheep third molar (M3) using data from the modern Kemenez sheep herd. The dataset also includes paired upper and lower M3s in order to test the comparability of results obtained from both teeth. Results show a moderate shift in the isotopic record between upper and lower M3s. The consecutive difference in the assessment of the timing of birth is one month, on average. Additionally, we provide a new set of results for sheep from Nova Nadezhda (Bulgaria, early sixth millennium BC), combining upper and lower molars, in order to expand data relating to the earliest stages of the introduction of sheep to Europe. At Nova Nadezhda, sheep were born in late winter and spring, and the pattern of birth distribution does not indicate the control of sheep reproduction by separating males from females. When compared to previously published results at other Neolithic and Chalcolithic sites in the Balkans, corrected for the shift between upper and lower M3s, no latitudinal and chronological trend is observed between the Southern Balkans, Northern Balkans and Hungarian plains over the early sixth to the second half of the fifth millennia BC. This apparent uniformity for the length (3-4 months) and timing of the birth period could be challenged in the future by enlarged datasets.

Keywords
Sheep, birth seasonality, stable oxygen isotopes, modern reference, Nova Nadezhda.

Introduction

The Neolithic diffusion of domestic plants and animals across Europe from the seventh to the fourth millennia BC involved the reshaping of agropastoral systems in keeping with local constraints and resources, leading to the adaptation of practices as well as changes in animal and plant physiology (Balasse and Tresset, 2009; Bogaard *et al.*, 2013; Marinova and Valamoti, 2014; Orton *et al.*, 2016; Balasse *et al.*, 2017; Salavert, 2017; Ivanova *et al.*, 2018). In particular, the dispersal of plants and animals initially domesticated in south-eastern Anatolia towards higher latitudes led to selection in the photoperiod responsive biological cycles of imported domesticates. This led, for example, to the differential expansion across Europe of two variants of domestic barley with different flowering-time adaptations (Jones *et al.*, 2008). The timing of animal breeding (i.e. when, in the yearly cycle, did births occur) was also impacted. This may have been especially true for sheep, which, in temperate latitudes, show strongly photoperiodic-controlled seasonal reproductive behavior, inherited from their wild ancestor (Hafez, 1952; Karsch *et al.*, 1984; Malpaux *et al.*, 1997; Thiéry *et al.*, 2002). Currently, most European sheep are short-day breeders: they enter a period of sexual activity when the length of the day begins decreasing in late summer and up to early winter, with births occurring five months later, from winter to early summer. As an additional consequence of photoperiodic control, the breeding season is further reduced in higher latitudes, with a later onset of sexual activity (Hafez, 1952). The consecutive constraints with regard to the organization of agro-pastoral tasks and the availability of animal production on an annual scale, were probably just as strong in ancient agropastoral systems as in present-day husbandries (Balasse and Tresset, 2007; Chemineau *et al.*, 2008). Current research aims to clarify the pattern of sheep lambing in the Neolithic (i.e. the spread of births, the shape of the distribution, and the timing of births), on regional and European scales (Balasse *et al.*, 2017).

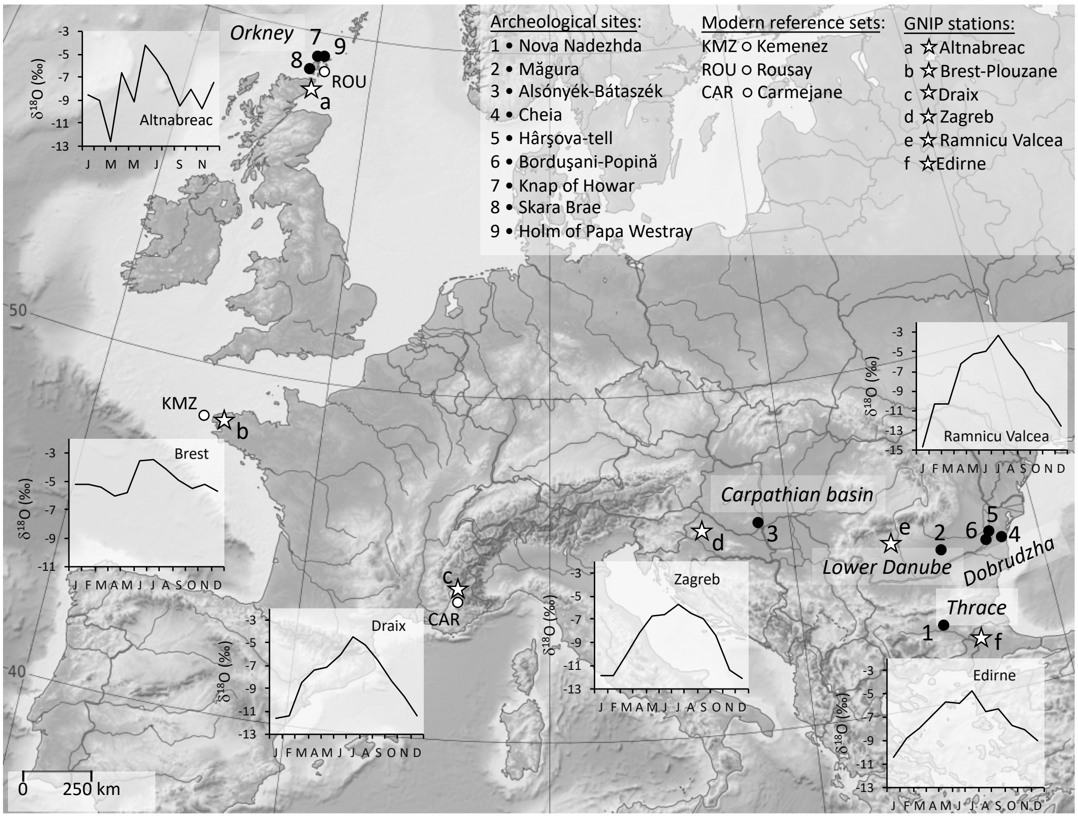
Past sheep birth seasonality can be investigated through the reconstruction of the seasonal cycle record in sheep molars, using sequential analysis of the stable oxygen isotope composition ($\delta^{18}O$) of enamel (Balasse *et al.*, 2003, 2012). A review of the data obtained from European sites dated from the sixth to the third millennia BC demonstrated a restricted breeding season for sheep, with lambing occurring over a three-to-four-month period, from late winter to early summer (Balasse *et al.*, 2017). Later lambing was observed at higher-latitude sites (Orkney), confirming a rapid adaptation of sheep breeding behavior to the photoperiodic conditions of North Atlantic Europe (Balasse *et al.*, 2017). Further work should help to refine the picture on a regional scale. Nevertheless, two methodological points were left unresolved. Firstly, some of the assemblages included in previous studies considered upper and lower third molars together (Balasse *et al.*, 2017). Although no major delay is expected in the timing of the formation of upper and lower molars in sheep (Milhaud and Nezit, 1991), the comparability of the results obtained from both molar rows still requires careful investigation, given the high resolution of $\delta^{18}O$ sequential sampling. Secondly, the great majority of $\delta^{18}O$ sequential datasets used to reconstruct sheep birth seasonality were obtained from the third molar. This choice is often dictated by the necessity to secure tooth identification in assemblages mostly composed of isolated teeth, where second and first molars may be mixed up. Yet, available modern reference sets, used to determine the season of birth, were obtained from sheep second molars (Blaise and Balasse, 2011; Balasse *et al.*, 2012). Although the lack of modern references on the third molar does not preclude an adequate description of the distribution of births (seasonality of births i.e. the spread of the lambing period and the number of peaks), the season of births (the time of the year when births occurred) cannot be directly determined (Balasse *et al.*, 2017).

The present study addresses these two methodological points by introducing a new modern reference set; the Kemenez sheep herd (Brittany). The sample includes paired upper and lower third molars in order to test for comparability between the sequences of $\delta^{18}O$ values measured in both teeth as well as to provide reference values for winter births in the third molar. This new reference set interlinks to previous ones (Blaise and Balasse, 2011 Balasse *et al*., 2012) through the second lower molar. The synthesis for Neolithic Europe published in Balasse *et al.* (2017) is then revised through a normalization of the data obtained from upper and lower M3s. Additionally, we provide a new set of results for sheep from the archaeological assemblage from Nova Nadezhda in Bulgaria (early sixth millennium BC), mixing upper and lower molars, in order to expand data relating to the earliest stages of the introduction of sheep to Europe. Previously published data for sheep birth seasonality in the Early Neolithic of Southeast Europe include the Starčevo-Criş 1 assemblages of Măgura-*Boldul lui Moş Ivănuş* in the Northern Balkans (Romania; Balasse *et al*., 2013) and Alsónyék-Bátaszék in the Hungarian plains (Balasse *et al.*, 2017). In the lower Danube and the Carpathian basin where Măgura and Alsónyék-Bátaszék lie, occupying an intermediate position between the Mediterranean and the Central European bioclimatic zones, the prevailing type of husbandry is characterized by a strong predominance of ruminants among livestock, a higher relative proportion of cattle and milk exploitation as evidenced from organic residue analysis in pottery. This contrasts with the sub-Mediterranean Southern Balkans where husbandry focused primarily on caprines and where the incidence of dairy residues in pots is low (Ethier *et al.*, 2017). Investigating sheep birth seasonality at Nova Nadezhda should therefore determine whether a disparity can also be discerned in the rhythms of husbandry systems, between this assemblage located in the Southern Balkans and previously acquired data in the temperate area of the Balkans.

Material

Kemenez is a small island (27 hectares) located in the Molene archipelago, off Brest and the western coast of Brittany (latitude 48°N; Figure 1). The region is subjected to an oceanic climate characterized by mild temperatures (around 7.5°C on average in winter and 15°C in summer) and high regular rainfalls. Monthly precipitation amounts exceed 100 mm from October to February and are the lowest in July (Lambert et al., 2018). Data related to the oxygen isotopic composition of precipitation ($\delta^{18}O_P$) at the nearest Global Network of Isotopes in Precipitation (GNIP) station at Brest Plouzane is available for the years 1998-2002. Over this time period, the monthly $\delta^{18}O_P$ values were comprised between -5‰ and –6 ‰ in the winter, and -3 ‰ and -4‰ in the summer on average; however, interannual variability is high. The yearly amplitude of variation varied from 3.6 ‰ in 2002 to 7.5 ‰ in 1998 (IAEA/WMO, 2020). The Kemenez reference set is composed of 20 sheep from a Ouessant x Landes de Bretagne cross breed, raised on the island of Kemenez (KMZ; Molène, Finistère). In this herd, sheep are kept under free-range conditions, with no extra food supply. Uncontrolled reproduction leads to winter lambing. Over a seven-year period from 2008 to 2013 and in 2015, a total of 255 referenced births occurred, mainly from mid-January to mid-April (N= 240 or 94 %), with a marked peak over a six-week period during February until mid-March (N = 187 or 73 %). A small number of out-of-season births are also observed in summer (August) and autumn (October) (Figure 2). The specimens used for stable isotope analysis were retrieved from the semi-articulated skeletons of individuals who had died of natural causes or were slaughtered due to a pathological condition, collected between 2013 and 2017. In total, the KMZ sheep reference set is composed of 31 teeth, including four lower second molars (M2) and 19 lower third molars (M3), eight of which were paired with an upper M3 (Table 1).

The archaeological site of Nova Nadezhda, in south-eastern Bulgaria (Figure 1), is situated on a low-lying alluvial terrace in the Middle Maritsa Valley floodplain. The site covers an area of more than five hectares and consists of two low tell-like formations separated by a stream. Excavations carried out in the northwest tell revealed continuous occupation from the beginning of the Early Neolithic (c. 6000 cal BC) to the Final Chalcolithic (c. 4000 cal BC), then again in the Early Iron Age. The Early Neolithic occupation spanned approximately four centuries (6000 – 5600/5500 cal BC). The archaeological features include five concentric enclosure ditches used successively from the innermost (earliest) to the outermost (latest). The latest ditch may have been combined with a palisade. One Early Neolithic building was excavated, overlapping three of the inner ditches and most probably contemporaneous with the latest ditch. The material culture is typical of the Karanovo I period (Bacvarov *et al.*, 2016).



Culture group/period	Geographic area	Dates cal BC	Sites (# on map)
Karanovo I	Thrace	ca. 6000-5600	1
Starčevo-Criş	Carpathian basin/Lower Danube/C. Balkans	ca. 6000-5600	2, 3
Hamangia	Dobrudzha	ca. 5200-4500	4
Gumelniţa	Lower Danube/Dobrudzha	ca. 4500-4000	5, 6
Orcadian Neolithic	Orkney	ca. 3500-2700	7, 8, 9

Figure 1: Archaeological cultures mentioned in the text. Location of archaeological sites, modern reference sets and Global Network of Isotopes in Precipitation (GNIP) stations. Available modern data for average monthly oxygen isotope composition of precipitation at Altnabreac (1981-1982) ; Brest Plouzane (1998-2002) ; Draix (2008-2013) ; Zagreb (1975-1995) ; Ramnicu Valcea (2012-2016) and Edirne (2008-2016) (IAEA/WMO, 2020).

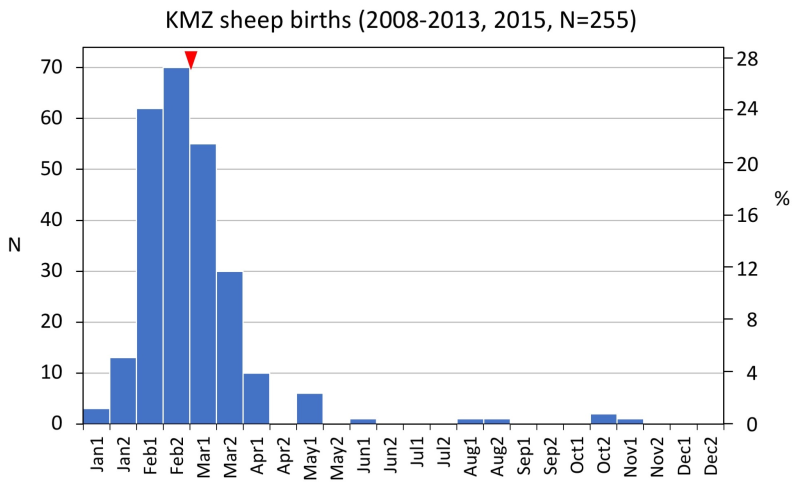


Figure 2: Distribution of births in the Kemenez sheep herd over the years 2008-2013 and 2015. Jan1: first two weeks of January; Jan2 last two weeks of January. 17 individuals are missing from this count (unknown dates of birth).

The early Neolithic faunal assemblage at Nova Nadezhda is primarily made up of bones from domesticated caprines—sheep and goat. Where distinctions between the two taxa could be made, sheep are overwhelmingly more abundant than goats. It would seem that caprines were the primary focus of husbandry, although cattle herding and pig rearing were also practiced. Hunting also seems to have been an important activity, and deer (mostly red deer and roe deer) were the second most abundant taxon after caprines. The mortality profiles of sheep mandibles suggest a mixed kill-off pattern not focusing on any single product. Mandibles from the earliest age group (0-2 months) are completely absent, with slaughtering peaks at 1-2 years and again at 4-6 years. The low sample size (n= 22 mandibles) precludes any definitive assessment of caprine husbandry, but it is more than likely that the slaughter pattern was determined by the need to ensure a stable population of animals for meat, breeding, and exchange. Organic residue analysis of pottery detected animal fats in 20 % of the corpus (total n=45), among which 89 % and 11 % were identified as ruminant and non-ruminant adipose fats respectively. No dairy fats were identified (Ethier *et al*., 2017).

The sheep remains included in this study are composed of 11 lower third molars and three upper third molars from the fill of the second earliest to the latest ditches, with a majority of teeth recovered from the second latest ditch (nine teeth out of a total of 14) (Table 2).

	Lower M2	Lower M3	Upper
KMZ Ovis 1	1**		
KMZ 10013	1**	1*	
KMZ 00018	1**	1**	
KMZ 20001	1	1	1
KMZ 20038		1	1
KMZ 20025		1	1
KMZ 10026		1	1
KMZ 10033		1	1
KMZ 40001		1	1
KMZ c123		1	1
KMZ c119		1	1
KMZ c122		1	
KMZ 00004		1	
KMZ c116		1	
KMZ c147		1	
KMZ 10046		1	
KMZ 10051		1	
KMZ c128		1	
KMZ 00017		1*	
KMZ c127		1*	
TOTAL	**4**	**19**	**8**

Table 1: The Kemenez sheep composing the modern reference set. (*) could not be modelled; (**) data from Balasse *et al.* (2017).

code	lower/upper	piece ID	Feature	Structure
NNd Ovis 1 M3	L	53.56	53	latest ditch
NNd Ovis 2 M3	L	56.4	56	second earliest ditch
NNd Ovis 3 M3	L	1005.5.8	054/2	second latest ditch
NNd Ovis 4 M3	L	54.2.89	054/2	second latest ditch
NNd Ovis 5 M3	L	54.1.27	054/1	second latest ditch
NNd Ovis 6 M3	L	54.2.95	054/2	second latest ditch
NNd Ovis 7 M3	L	54.3.55	054/3	second latest ditch
NNd Ovis 8 M3	L	54.1.79	054/1	second latest ditch
NNd Ovis 9 M3	L	55.1.7	055/1	third ditch
NNd Ovis 10 M3	L	53.9	53	latest ditch
NNd Ovis 11 M3	L	54.3.44	054/3	second latest ditch
NNd Ovis 12 M3	U	1005.9.2	054/2	second latest ditch
NNd Ovis 13 M3	U	1004.7.3	054/2	second latest ditch
NNd Ovis 14 M3	U	55.2.11	55	third ditch

Table 2: Provenance of the sheep molars from Nova Nadezhda.

Principles and methods

Stable oxygen isotope ratios ($\delta^{18}O$) from the mineral fraction (bioapatite) of vertebrate skeletons are indirectly linked to local precipitation $\delta^{18}O$ (Land *et al.*, 1980; D'Angela and Longinelli, 1990). Body water tracks changes in surface water $\delta^{18}O$ values through ingested water. Bioapatite precipitates in oxygen isotopic equilibrium with body water, offset by temperature-dependent fractionation held constant in homeothermic mammals (Longinelli, 1984). The oxygen isotopic composition of precipitation ($\delta^{18}O_P$) varies with the origin and the trajectory of the air mass. At the location where condensation occurs it is then affected by the air temperature and precipitation amount (Rozanski *et al*., 1993). In continental Europe, the $\delta^{18}O_P$ values are mainly affected by the air temperature and its seasonal variations: the highest $\delta^{18}O_P$ values are recorded when air temperature is the highest in the summer months, the lowest when air temperature is the lowest in the winter months (Rozanski *et al*., 1993). The seasonal variations in $\delta^{18}O_P$ values in the present days at Nova Nadezhda and Eastern Europe are consistent with this pattern (Figure 1). However, correlation between $\delta^{18}O_P$ values and rainfall amount have been observed in maritime temperate regions, including in the British Isles (Tyler et al., 2016) and in particular in northern Scotland (Fuller et al., 2008). This would apply the late Neolithic archaeological sites in Orkney. The data recorded at Brest Plouzane, the nearest GNIP station to Kemenez, similarly shows inverse correlation between the rainfall amount and $\delta^{18}O_P$ values. At these locations where the amount effect applies, the highest $\delta^{18}O_P$ values are recorded when the monthly precipitation is the lowest – in the summer months, the lowest $\delta^{18}O_P$ values when the monthly precipitation are the highest, in the winter months (IAEA/WMO, 2020; Figure 1). Consequently, although different controls apply to the precipitation $\delta^{18}O$ values in the different study areas (Eastern Europe /North Western Europe maritime regions), the seasonal patterns are comparable at all locations.

Sheep molar crowns grow over a one to one-and-a-half-year period, during which enamel bioapatite records changes in environmental stable isotope values, through diet. A sequential sampling procedure detects cyclical variations in $\delta^{18}O$ values, reflecting the seasonal cycle. As the timing of tooth growth is fixed within a species - notwithstanding some degree of variability (Milhaut & Nezit,1991) on which the influence of sex, breeding age and nutritional regime were shown to be statistically non-significant in sheep molars (Worley et al., 2016) - the season of birth determines the sequence of the annual cycle recorded in a given tooth (Bryant *et al.*, 1996; Fricke and O'Neil, 1996). Variability in the birth season may be described through the comparison of the position of the maximum value of the $\delta^{18}O$ cycle in the tooth crown (Balasse *et al.*, 2003). A quantitative estimation of inter-individual variability involves modelling the seasonality in $\delta^{18}O$ sequences and normalizing the distances in order to eliminate the tooth size factor (Balasse *et al.*, 2012).

The $\delta^{18}O$ values were measured in the carbonate fraction of enamel bioapatite. Enamel was sequentially sampled following the procedure described in Balasse *et al.* (2003) on the posterior (M2), middle (lower M3) or anterior lobe (upper M3). Archaeological enamel powders from Nova Nadezhda were pre-treated to eliminate diagenetic carbonates (0.1 M acetic acid for 4h at room temperature, 0.1ml/mg; Tornero et al., 2013). Oxidizing agents, aimed at removing organic matter, are not deemed necessary in enamel pretreatment and were shown to cause isotopic shift due to unwanted bioapatite dissolution or incorporation of exogenous carbonates (Pellegrini and Snoeck, 2016). For this reason they were not applied. No pretreatment was applied to enamel from modern teeth. Bioapatite samples weighing 580-620 µg were reacted with 100 % phosphoric acid at 70°C in individual vessels in an automated cryogenic distillation system (Kiel IV device), interfaced with a DeltaVAdvantage isotope ratio mass spectrometer. The analytical precision within each run, estimated from six to eight analyses of our laboratory carbonate standard (Marbre LM, expected value -1,83 ‰ calibrated to the NBS19 international standard) was lower than 0.05‰ for $\delta^{18}O$ values. Over the period of analysis of the Kemenez and Nova Nadezhda enamel samples, the analysis of 220 Marbre LM gave an average $\delta^{18}O$ value of -1.99±0.04 ‰ (Supplementary Material 1).

The $\delta^{18}O$ sequences were modelled using an equation derived from a cosine function (Balasse *et al.* 2012 and Table S3), in order to define the position (distance to enamel-root junction) of the maximum value (x_0). The period of the cycle (X, distance over which the isotopic record covers one annual cycle) was used to normalize the distances (x_0/X) in order to eliminate inter-individual variability in tooth size. Each modelled $\delta^{18}O$ sequence yields a x_0/X ratio which may be directly compared for all specimens. The distribution (spread and shape) of these ratios within a population is an expression of the distribution of births. We use a circular representation of the data to reflect the cyclical nature of seasonality: January follows December, *i.e.* when (x_0/X) reaches 1, it also reaches 0. Because of the small size of our archaeological samples, we applied Student statistics. Births occurring outside the 95 % confidence interval were considered as outliers. The main period of birth is defined by the 66 % confidence interval around the mean (excluding the outliers).

The season of birth in archaeological specimens is estimated by comparison with reference x_0/X ratios obtained from modern sheep whose birth season is known; the Kemenez sheep are used as reference for x_0/X ratios measured in the M3. This procedure assumes a similar timing of tooth development for modern and ancient sheep breeds.

Results

Results from the sequential analysis of the Kemenez sheep molars are shown in Figure 3. All $\delta^{18}O$ sequences are shown individually in Supplementary Materials 2 and 3. In the lower M3s, the lowest $\delta^{18}O$ value for each intra-tooth sequence varies from -4.91 ‰ to -3.33 ‰ (mean-4.20 ± 0.41 ‰) and the highest $\delta^{18}O$ value varies from -2.78 ‰ to -0.11 ‰ (mean -1.47 ± 0.66 ‰). The intra-tooth amplitude of variation is 2.73 ‰ on average (from 1.21 ‰ to 4.38 ‰). The M2s delivered comparable results (Supplementary Materials 2). All sheep included in this reference set were born in 2008 or after. The oxygen isotopic composition of precipitation during these sheep's lifetime is not known. A comparison with the data available for previous years (1998-2002) suggests that the $\delta^{18}O$

values measured in the sheep enamel carbonate are higher than the $\delta^{18}O_P$ values, although the extant of the shift cannot be estimated due to great interannual variability in $\delta^{18}O_P$ values (IAEA/WMO, 2020). The amplitude of intra-tooth variation in the $\delta^{18}O$ values is also muted compared to the annual amplitude in the $\delta^{18}O_P$ values (3.6 ‰ to 7.5 ‰; IAEA/WMO, 2020). This is explained by prolonged mineralization of enamel, leading to the attenuation of the amplitude of cyclical variations (Balasse, 2003).

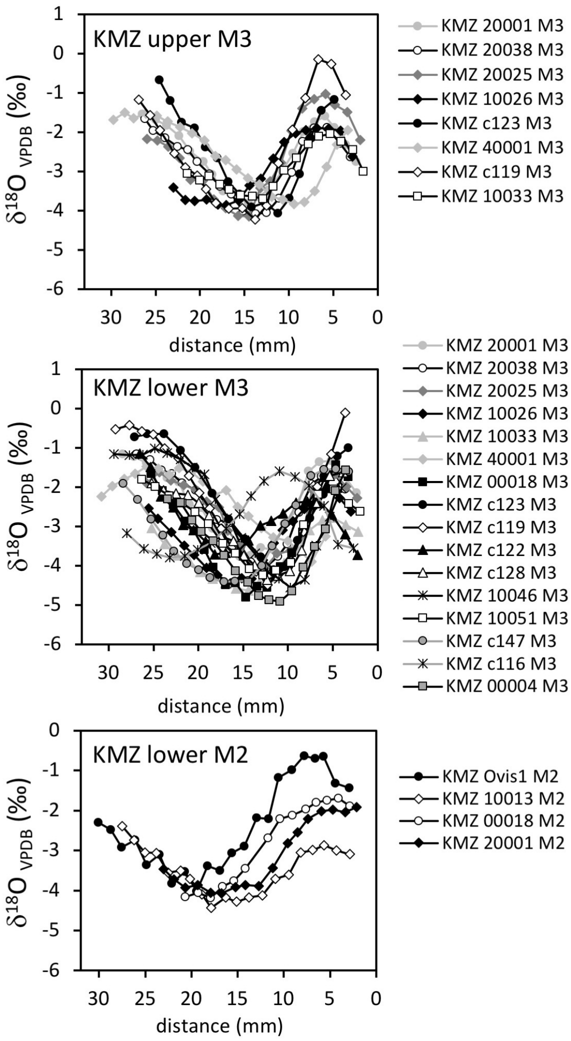


Figure 3: Results from the sequential analysis of the Kemenez (KMZ) sheep second (M2) and third molars (M3). Each sample is located in the tooth crown using its distance from the enamel-root junction.

The $\delta^{18}O$ sequences measured in upper and lower M3s are compared in Figure 4A. The difference in the $\delta^{18}O$ values measured in paired lower and upper M3s are reported in Table 3. In most cases, they exceed the analytical precision for the IRMS measurement. However, no systematic relationship may be observed between $\delta^{18}O$ values measured in the upper/lower molar. In KMZ 10033 M3, the difference is great, with a mean $\delta^{18}O$ value 0.84‰ higher in the upper molar (Figure 4 A and B). This shift is unexplained.

Results from the modelling of the $\delta^{18}O$ sequences measured in KMZ M2s and M3s are shown in Figure 5 (and Supplementary Material 4). In three of the 19 lower M3s, the results from the modelling were considered unreliable: in KMZ10013 M3 and KMZc127 M3 because the measured $\delta^{18}O$ sequence did not show a clearly identified maximum (Supplementary Material 3); in KMZ 00017M3 because it combined a rather short sequence (22 mm), a very low amplitude of variation

(1.2 ‰), and also the lowest period (X= 16.11 mm) of the data set (average 22.45±3.57 ‰ in lower M3s, N= 16; Supplementary Material 4). In the second molar, (x_0/X) ratios vary between 0.14 and 0.20. These values are compared with the previous reference data sets from Carmejane (CAR, Blaise and Balasse, 2011) and Rousay (ROU, Balasse *et al.*, 2012) on Figure 5A. In the lower M3s, (x_0/X) ratios vary between 0.001 and 0.98. Among those, eleven (x_0/X) ratios are included in the 66 % confidence interval with (x_0/X) values comprised between 0.07 and 0.26, constituting the main group of births (Supplementary Material 4 and Figure 5B). When the average (x_0/X) value is set between February and March to fit with the observed distribution of births (Figure 2), KMZ c123 and KMZ 10046, which yielded (x_0/X) ratios of 0.001 and 0.98 (or -0.02 considering the cyclicity of this parameter) respectively, reflect early winter births (late December and January). KMZ c116 and KMZ c122, with (x_0/X) ratios of 0.42 and 0.48 respectively, point to the occurrence of births in May and June. KMZ40001, with (x_0/X) values of 0.77 and 0.85 in lower and upper M3s respectively, represents an October birth (Figure 5B).

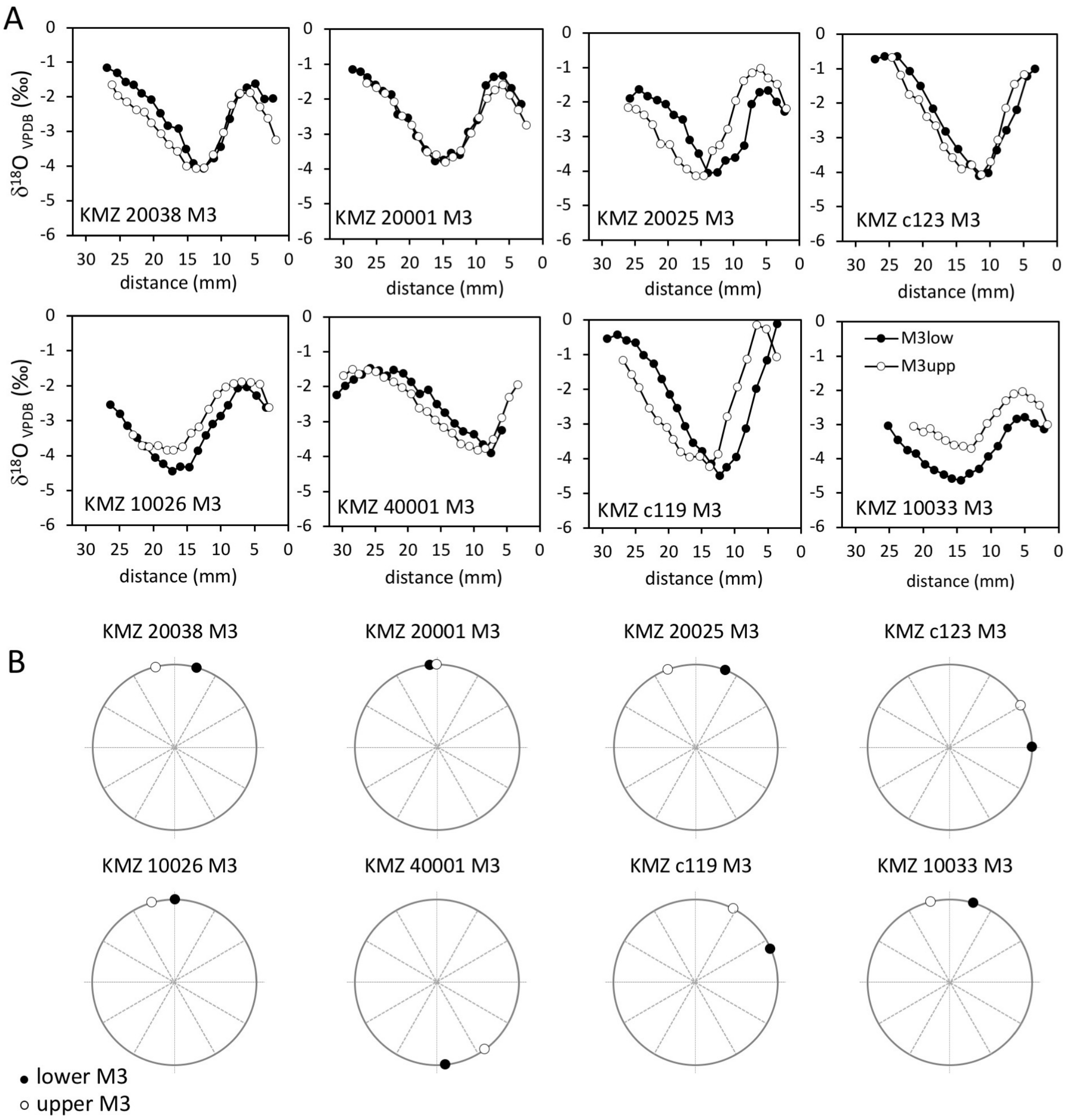


Figure 4: A- Comparison of the $\delta^{18}O$ sequences measured in upper and lower third molars (M3) from Kemenez. B – Comparison of the normalized location in tooth crown of the maximum $\delta^{18}O$ value (x_0/X) in upper and lower third molars.

	Lower M3				Upper M3				Δ (upper-lower)			
	min	max	A	mean	min	max	A	mean	min	max	A	mean
KMZ 20001 M3	-3.77	-1.16	2.61	-2.46	-3.80	-1.53	2.27	-2.67	-0.03	-0.38	-0.35	-0.20
KMZ 20038 M3	-4.07	-1.16	2.91	-2.61	-4.07	-1.65	2.42	-2.86	0.00	-0.50	-0.49	-0.25
KMZ 20025 M3	-4.06	-1.63	2.44	-2.85	-4.14	-1.03	3.11	-2.58	-0.07	0.60	0.68	0.26
KMZ 10026 M3	-4.45	-2.03	2.42	-3.24	-3.85	-1.89	1.96	-2.87	0.60	0.14	-0.46	0.37
KMZ 10033 M3	-4.63	-2.78	1.85	-3.71	-3.69	-2.04	1.66	-2.87	0.94	0.75	-0.19	0.84
KMZ 40001 M3	-3.90	-1.47	2.43	-2.69	-3.83	-1.50	2.33	-2.66	0.08	-0.03	-0.10	0.03
KMZ c119 M3	-4.49	-0.11	4.38	-2.30	-4.22	-0.14	4.08	-2.18	0.26	-0.04	-0.30	0.11
KMZ c 123 M3	-4.11	-0.64	3.47	-2.38	-4.06	-0.66	3.40	-2.36	0.05	-0.02	-0.07	0.01

Table 3: Minimum and maximum $\delta^{18}O$ values measured in each intra-tooth sequence, amplitude of intra-tooth variation (A) and mean (=(min+max)/2). Difference between upper and lower third molars (Δ = upper -lower).

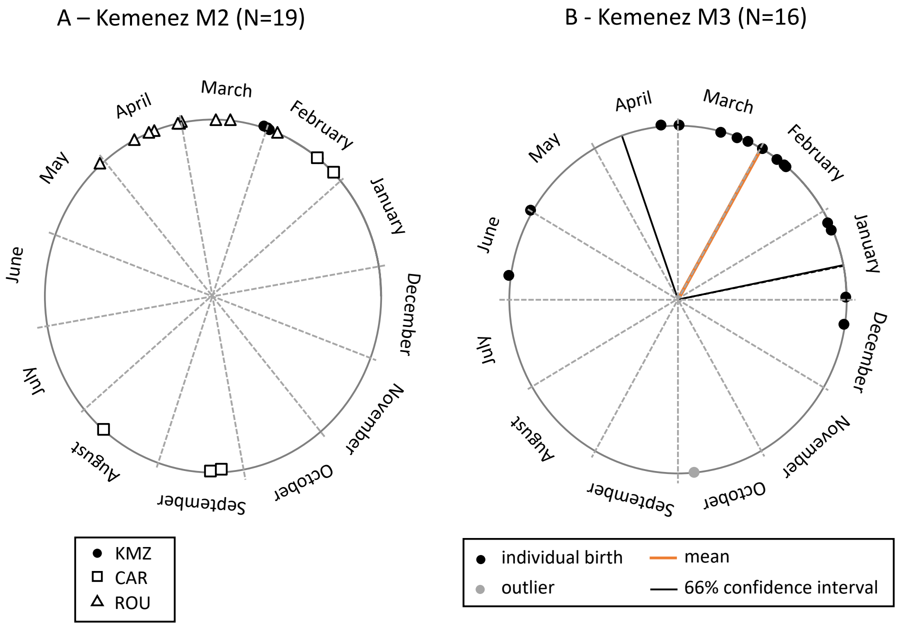


Figure 5: Kemenez (KMZ) sheep births positioned in the yearly cycle, as reflected from the normalized location in tooth crown of the maximum $\delta^{18}O$ value (x_0/X) (A) in the second (M2) and (B) in the third molar (M3). Comparison with previous reference values measured in the M2 at Carmejane (CAR; squares; January/February and September births; Blaise and Balasse, 2011) and Rousay (ROU; diamonds; April/May births, Balasse *et al*., 2012). Mean and 66% confidence interval. The outlier (grey) is outside of the 95% confidence interval.

Considering paired M3s, the upper M3 (x_0/X) value is similar to or higher than in the lower M3, with an average difference of 0.073±0.04, or approximately one month (26 ±14 days) (Supplementary Material 4 and Figure 5B). In all cases, a higher (x_0/X) ratio for the upper M3 is explained by a higher x_0 value in this tooth (+ 0.47 to +2.78) compared to the lower M3, which does not correspond to a longer period (X) in the upper M3. In some cases, the difference is actually further accentuated by a shorter period in the upper M3 (up to -2.4 mm in KMZ 10033 and KMZ 40001). This result could be indicative of a later onset of tooth growth in the upper dentition. Although acquired on a limited number of individuals (N=8) this average difference between upper and lower M3s (x_0/X) ratio will be used as a first approximation to correct the data acquired in the upper third molar of archaeological specimens.

Results from the sequential analysis of sheep M3 from Nova Nadezhda are shown in Figure 6. The $\delta^{18}O$ sequences are individually shown in Supplementary Material 5. The $\delta^{18}O$ values vary between -8.6 ‰ and +0.1 ‰. Intra-tooth variation follows a marked sinusoidal pattern with amplitude varying between 2.5 ‰ and 7.0 ‰ (mean 5.3 ‰). Results from the modelling of the $\delta^{18}O$ sequences are shown in Figure 7 (and Supplementary Material 6). The $\delta^{18}O$ sequences retrieved from the three upper molars (NND Ovis 12, 13 and 14) cannot be matched with any of the sequences measured in the lower molars (Supplementary Material 6), confirming that all teeth belong to different individuals. The position in the tooth crown of the maximum $\delta^{18}O$ value normalized to the period of the annual cycle (x_0/X) varies from 0.17 to 0.47 in lower molars; from 0.39 to 0.40 in upper molars. Considering corrected (x_0/X) values for upper molars (-0.073), the amplitude of variation for all 14 specimens from Nova Nadezhda is 0.30, or approximately 3.6 months. The main peak of births, as defined by the 66% confidence interval, occurs over 2 months. Sheep births distribution at Nova Nadezhda is compared to previous datasets from archaeological sites dated from the sixth to the third millennia BC (Balasse *et al.*, 2017) in Figure 8, where all previously published (x_0/X) ratios measured in upper M3s have been normalized to the lower M3s by correcting by -0.073. The timing of sheep births in the archaeological sites is determined by comparison with the KMZ reference value for winter births in Figure 9. The timing of sheep births at Nova Nadezhda is set within the first half of the spring.

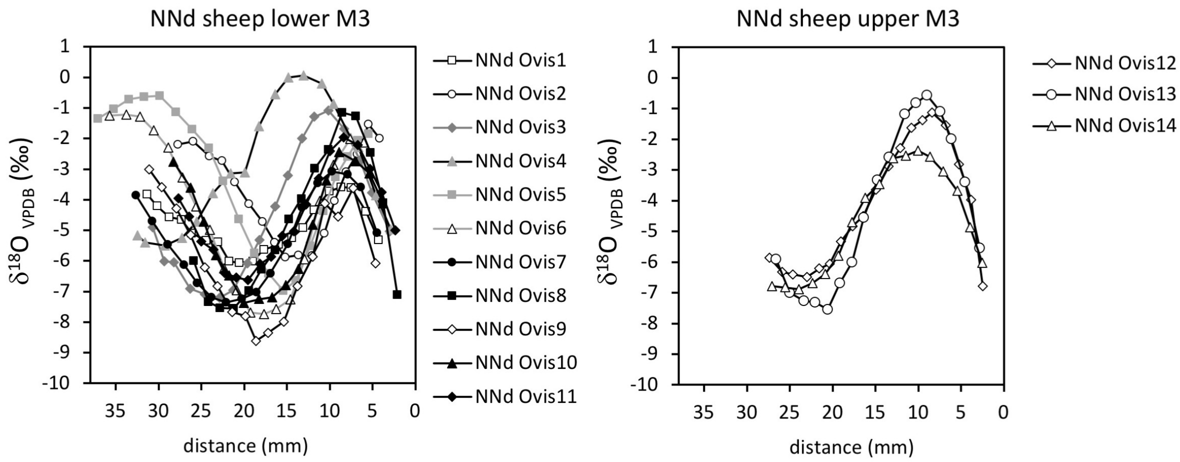


Figure 6: Results from the sequential $\delta^{18}O$ analysis of third molars (M3) of 14 sheep from Nova Nadezhda. Each sample is located in the tooth crown using its distance from the enamel-root junction.

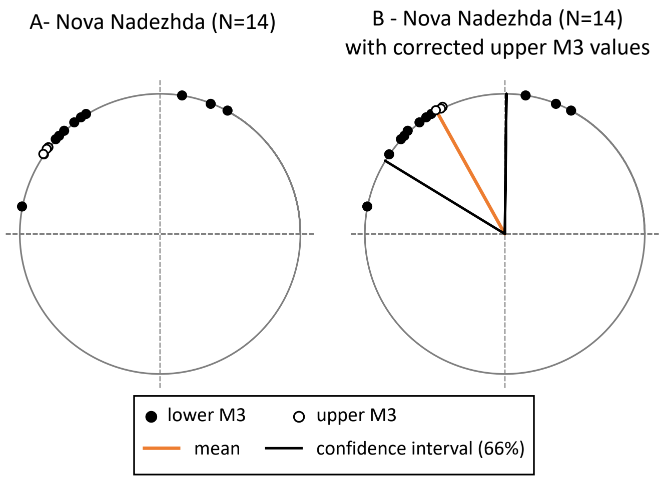


Figure 7: A - Nova Nadezhda sheep births positioned in the yearly cycle, as reflected from the normalized location in tooth crown of the maximum $\delta^{18}O$ value (x_0/X) in the third molar (M3). B- After correction of (x_0/X) ratios obtained in the upper molars.

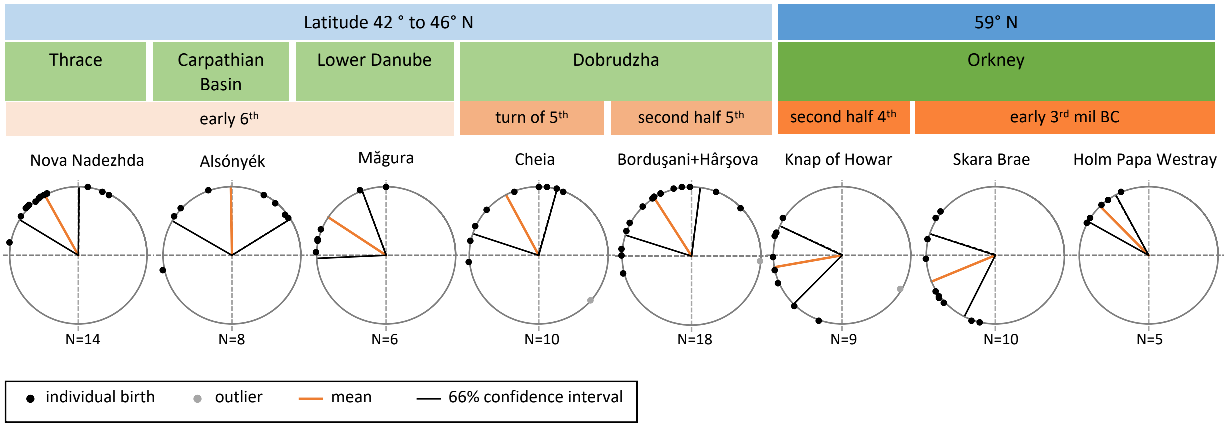


Figure 8: Distribution of sheep births at Nova Nadezhda and other archaeological sites cited in the text. Data: this study, Balasse *et al.*, 2017 and Balasse *et al.*, 2013. All (x_0/X) ratios measured in upper M3s have been normalized to the lower M3s by correcting by -0.073. In order to enlarge the size of the dataset, Hârşova-tell and Borduşani-Popină, contemporaneous tell sites located in close vicinity and showing great similarity in the orientation of the economy (Bréhard and Bălăşescu 2012), are considered together.

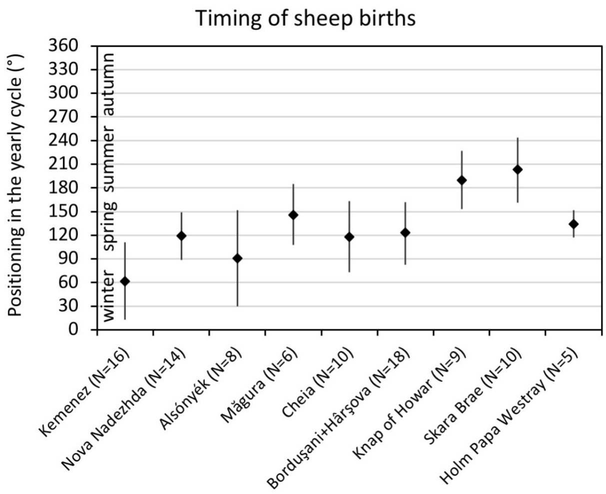


Figure 9: Timing of sheep births at Nova Nadezhda and other archaeological sites cited in the text, estimated from the comparison with the modern reference set at Kemenez, defining winter births.

Discussion

Correspondence between the new Kemenez and previous modern sheep reference sets

Considering the lower M3s at Kemenez, when the average measured (x_0/X) ratio is set between February and March to fit with the observed distribution of births (Figure 2), there is a good agreement between the expected shape of the distribution and the one reconstructed from the stable isotope analysis, except for one individual whose birth is set in late December (Figure 5B) while the earliest recorded births occurred in early January. Overall, this suggests minimal inter-individual variability in the timing of the lower M3 growth within the sampled population. Considering the M2, when comparing the reference values obtained from the Kemenez sheep and those previously obtained from Carmejane (CAR; Blaise and Balasse, 2011) and Rousay (ROU; Balasse *et al.*, 2012) respectively on a Préalpes du Sud breed and a Shetland cross, there is a good agreement between the three sets as long as their relative positioning in the yearly cycle is considered (Figure

5A): the (x_0/X) ratios measured in KMZ, reflecting mainly February and March births, are correctly ranked in between the January and February births at Carmejane and the April/May births at Rousay. When setting the sequence of births within the yearly cycle using the KMZ as a reference for February/March, the Carmejane births are correctly attributed to February and September (although one belonging to the last group is wrongly attributed to August), while the Rousay births are globally set in March and April instead of April/May. This slight discrepancy may result from interbreed variability in the timing of tooth growth, although this would need to be investigated further on a higher number of individuals and breeds in the future. This creates uncertainty in the attribution of birth to a month of the year and imposes the season as a more reliable resolution scale. The main group of births at Kemenez provides values ($0.07 < x_0/X < 0.26$) that will be used as reference values for winter births when defining the lambing period in past breeds.

Sheep birth distribution at Nova Nadezhda

The results obtained at Nova Nadezhda point to a main peak of births over a two-month period. This spread and shape of of births distribution are similar to those observed in wild ovine populations, as well as in domestic herds with no human control over reproduction. Observations in European mouflon populations report peaks of fertilization/births over periods of three to seven weeks (Hadjisterkotis & Bider, 1993; Garel *et al.*, 2005). At Kemenez, where reproduction occurs without human control and males and females are kept together all year round, the main peak of births occurs over a six-week period. However, a lower number of births directly precedes and follows this main peak, and isolated births occur out-of-season in the summer and autumn (Figure 2). In domestic herds, reproduction can be controlled by separating males and females and reintroducing males at the onset of the breeding season. This practice, aimed at synchronizing births, leads to massive lambing at the very beginning of the lambing season, and the absence of out-of-season births (Etienne *et al.*, 2002). At Nova Nadezhda, the occurrence of births before and after the main peak does not indicate this kind of control over sheep reproduction. At this site, the non-occurrence of out-of-season births – observed elsewhere in Neolithic European assemblages (Balasse *et al.*, 2017 and Figure 8) – may also be due to sampling bias given the small assemblage size. Birth synchronization is observed in modern industrialized as well as in traditional husbandry and may be driven by different factors. In the case of large herds, in view of the intense effort required to monitor lambing and take care of the youngest animals, synchronization allows farmers to concentrate on this task at a given time in order to reduce time conflicts with other tasks in the agro-pastoral calendar. For nomadism and herding systems involving mobility, synchronized births are preferable because young animals are vulnerable to long-distance moves. In modern husbandry, another objective is to be able to provide the market with large quantities of products at the most advantageous time (Barth, 1961; Digard 1981; Mace, 1993; Etienne *et al.*, 2002). The absence of this type of control of sheep reproduction at Nova Nadezhda may simply be due to the lack of need for it. Although the orientation of production cannot be clearly inferred from the slaughtering profile on account of the low number of caprine dental remains, a strong focus on seasonal production (milk, tender meat) is not evidenced either.

Sheep birth season

After correction of the data obtained from upper M3s at Nova Nadezhda and all previously published European assemblages (Figures 8 and 9), the main conclusions in Balasse *et al.* (2017) are refined here. In all instances, the main peak of sheep births occurred over a period of two to three months except at Alsónyék-Bátaszék in the Hungarian plains, where the peak is spread over four months with an earlier onset of the breeding season (Figure 9). The small number of individuals included in most assemblages included in this comparison prevents from discussing further the archaeological significance of this find. The new Kemenez reference set shows that births globally occurred in the spring at sites in the Balkan area (Figure 9). Comparatively to the Balkans (Bulgaria, Hungary and

Romania), the onset of the breeding season occurred later under higher latitudes (in late spring at Knap of Howar and Skara Brae in Orkney), possibly as a consequence of the photoperiodic control of the fertility cycle in sheep (Hafez, 1962).

Latitudinal and chronological trends in the Balkans

Figures 8 and 9 compare the distribution and timing of sheep births at Nova Nadezhda in the Southern Balkans with contemporaneous sites of the Starčevo-Criş 1 culture in more northern parts of the Balkans: Măgura-*Boldul lui Moş Ivănuş* in Romania and Alsónyék-Bátaszék in the Hungarian plains (Figure 1). In contrast to Nova Nadezhda, where husbandry focused mainly on caprines, cattle played a more important meat-supplying role at Alsónyék-Bátaszék, where they appear in similar proportions to caprines (Nyerges 2013; Nyerges & Biller 2015) and at Măgura, where the larger body mass of cattle compensates for the predominance of caprines (2/1) (Bălăşescu *et al.*, 2005). The presence of dairy fat residues in ceramic vessels also attests to milk exploitation at Alsónyék-Bátaszék, whereas no dairy residues were detected at Nova Nadezhda (Ethier *et al.*, 2017). In spite of different climatic settings and different herding strategies in terms of flock composition and the orientation of animal exploitation, the results do not suggest any major shift in the timing of sheep births between the sub-Mediterranean southern and temperate northern parts of the Balkans. With 14 specimens, the Nova Nadezhda sample is the largest ever published for the Early Neolithic in south-eastern Europe and for the European Neolithic as a whole. As a result, in addition to determining the timing of the main birthing period, the shape of sheep birth distribution could also be described. This level of description is probably less reliable for other sites available for comparison in Figure 8 with smaller sample sizes. It is reasonable to assume that this apparent uniformity may be challenged in the future with enlarged datasets.

In Romania, the early Chalcolithic assemblage at Cheia (early fifth millennium cal BC, Hamangia culture) and the Chalcolithic assemblages at Hârşova-tell and Borduşani-Popină (second half of the fifth millennium cal BC, Gumelniţa A2 culture; Figure 1) allow for the investigation of the stability of sheep birth seasonality over time. Over this time period in Romania, the composition of domestic stock was very different among cultural groups. At Cheia, as well as at other sites from the Hamangia culture, caprines were outnumbered by cattle but nonetheless played an important second role and pigs were rare (Bălăşescu and Radu, 2004; Balasse *et al.*, 2014). In the Gumelniţa, where pigs were considerably more important but cattle and caprines were nonetheless predominant, sheep were probably exploited for meat, with a focus on early culling, and cattle mortality profiles suggest milk exploitation (Bréhard and Bălăşescu 2012). At all three Chalcolithic sites, the timing of sheep births remained similar compared to birthing at Măgura and at all Early Neolithic sites in the Balkans as a whole (Figures 8 and 9). However, again, reduced sample sizes prevent more detailed comparisons of the shape of sheep birth distribution and may possibly contribute to this apparent uniformity.

Conclusions and recommendations

The Kemenez sheep provide a solid new reference set of $\delta^{18}O$ sequences for winter births and the very first reference set for the sheep third molar. It also provides the first direct comparison of $\delta^{18}O$ sequences measured in upper and lower sheep third molars. The results from this comparison on eight paired third molars showed a moderate shift in the positioning of the isotopic sequences in tooth crowns: the maximum $\delta^{18}O$ were recorded at a greater distance from the enamel-root junction i.e. at an earlier stage of tooth formation in the upper compared to the lower third molar. The consecutive difference in the final assessment of the time of year when births occurred was estimated at one month on average (26±14 days), with a later estimation when inferred from the upper molar compared to the lower molar. This discrepancy may be considered significant or not depending on the aim pursued. In temperate Europe, given the strongly seasonal reproductive behavior of sheep, the timing of the main birthing season can be defined with a limited number of sheep teeth (winter/spring *versus* autumn/winter, for example, which are currently the two main patterns). When pursuing this objective, upper and lower molars may be directly combined without introducing too much of a bias in interpretation. However, beyond this goal, a more accurate definition of the shape of birth distribution (*i.e.,* spread of the main peak of births and positioning in the yearly cycle), would lead to refined comparisons between sites and over time, but also to conclusions on the degree of control by herders of sheep reproduction. To attain this objective, it is clear that a higher number of specimens is required - the 14 sheep from Nova Nadezhda were just enough to outline birth distribution at the site. The analysis of larger datasets is recommended, and this may be attained by the combined use of upper and lower molars, and would in this case require the normalization of the results obtained from upper and lower teeth. In this regard, the correction used in the present study was obtained on a limited number of specimens (N=8) and must be refined on a larger sample size.

The KMZ reference set also provided four $\delta^{18}O$ sequences measured in the M2. A comparison with other reference sets (Carmejane, Blaise and Balasse, 2011; Rousay, Balasse et al., 2012) showed a good agreement between the three sets as long as their relative positioning in the yearly cycle was considered. Considering the absolute positioning in the yearly cycle (month of birth) and when setting the KMZ reference values at February/March, there was a slight offset in the attribution of the Rousay births (March/April instead of April/May). This is possibly due to interbreed variability in the timing of tooth growth. Alternatively, it could be related to the small sample size of all modern reference sets currently available. Either way, until this is clarified with enlarged datasets, it is recommended to use the season as the resolution scale rather than the monthly scale

When compared to the Kemenez reference set, the extended dataset at Nova Nadezhda provide evidence for a peak of births in early spring at this site, and a birth distribution pattern pointing to the absence of sheep reproduction control by herders; i.e., no separation of males and females. The spring birth periods at Nova Nadezhda are similar to previous data from other Neolithic and Chalcolithic sites in the Balkans. In this area, no latitudinal and chronological trend could be observed for six sites located in the Southern Balkans, the Northern Balkans and the Hungarian plains and dated from the early sixth millennium to the second half of the fifth millennium BC. This apparent uniformity for the spread (2-4 months) and timing (early spring) of the birthing period could be challenged in the future by enlarged datasets, potentially showing different birth distributions and different timing for the main peak of births.

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