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INVESTIGATING SEASONALITY AND SEASON OF BIRTH IN PAST HERDS: A REFERENCE SET OF SHEEP ENAMEL STABLE OXYGEN ISOTOPE RATIOS.

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ABSTRACT

Intra-tooth sequential analysis of enamel $\delta^{18}\text{O}$ is currently used to investigate birth seasonality in past animal populations, offering new insights into seasonal availability of animal resources, herd management and seasonality of site occupation. Reference datasets are still required to address two major difficulties: (1) inter-individual variability in the record of the seasonal cycle is affected by tooth size; (2) the season of birth cannot be directly estimated from the timing of tooth growth, because of a delay in enamel mineralization. We present a dataset acquired on the lower second molar of ten modern sheep from Rousay (Orkney) born within a few weeks in April / May and submitted to the same environmental conditions until death. All sheep have recorded a sinusoidal pattern of $\delta^{18}\text{O}$ variation spanning approximately a year. From the difference between expected and measured time sequence, the delay of enamel mineralization is estimated to five to six months. The data set is then described using a model mainly based on a cosine function. The period, corresponding to the length of M2 crown formed over a year, averaged 35.8 mm. A very slight variation of tooth growth rate with time and no attenuation of the isotopic signal towards the cervical margin of the crown could be detected on this dataset. The lowest $\delta^{18}\text{O}$ values, corresponding to the sheep's first winter, were tracked at a distance from the enamel-root junction varying between 23.0 and 30.3 mm (x_{\min} mean = 27.6 mm); the highest $\delta^{18}\text{O}$ values, corresponding to the sheep second summer, between 6.3 and 11.6 mm (x_{\max} mean = 9.9 mm). Most of the variability can be attributed to tooth size. When normalized on the period, x_{\min} and x_{\max} are 0.28 (± 0.05) and 0.78 (± 0.05) on average, meaning that the Rousay sheep have recorded the minimum and maximum $\delta^{18}\text{O}$ values on average at respectively 78 % and 28 % of the end of the periodic cycle recorded in the second molar.

Keywords: Sheep, seasonality of birth, $\delta^{18}\text{O}$, tooth enamel, Orkney.

INTRODUCTION

Intra-tooth sequential analysis of enamel stable oxygen isotope composition ($\delta^{18}\text{O}$) is currently used to investigate birth seasonality in past animal populations (Balasse *et al.*, 2003; Nelson, 2005; Balasse and Tresset 2007; Britton *et al.* 2009; Henton *et al.* 2010). In terrestrial mammals, the stable oxygen isotope composition of enamel mineral (hydroxylapatite, hereafter referred to as bioapatite) is closely linked to that of meteoric precipitation (Land *et al.* 1980; Longinelli 1984; Luz *et al.* 1984; Kohn *et al.*, 1996). Precipitation $\delta^{18}\text{O}$ varies seasonally with ambient temperature at high and middle latitudes (Gat 1980, Rozanski *et al.* 1993). These seasonal changes are tracked in tooth mineral during growth, resulting in variability in the $\delta^{18}\text{O}$ of bioapatite within a tooth row (Bryant *et al.* 1996a; Bryant *et al.* 1996b; Fricke and O'Neil 1996) and within a tooth (Koch *et al.* 1989; 1995; Fricke and O'Neil 1996; Stuart-Williams and Schwarcz 1997; Kohn *et al.*, 1998; Sharp and Cerling 1998; Sharma *et al.* 2004; Nelson 2005; van Dam and Reichart, 2009; Bernard *et al.*, 2009). Because the timing of tooth growth is fixed within a species, distinct patterns of variation in enamel $\delta^{18}\text{O}$ within tooth rows reflect different seasons of birth, as exposed for the first time by Bryant *et al.* (1996a; 1996b) in their studies of fossil populations of equids. When tooth growth spans several seasons, birth seasonality can be investigated with a higher time resolution at the scale of a tooth, through sequential sampling (Balasse *et al.* 2003; Nelson, 2005; Balasse and Tresset 2007; Britton *et al.* 2009; Henton *et al.* 2010). This approach is undoubtedly of great interest for archaeologists, offering new insights into seasonal availability of animal resources, herd management and seasonality of site occupation in past societies (Balasse *et al.* 2003).

Nevertheless, potential difficulties have emerged, which need to be addressed before the method can be more widely applied to archaeological assemblages. First, the assessment of seasonality of birth is done through the assessment of inter-individual variability in the time sequence - the sequence of the seasonal cycle - recorded in a given tooth. Practically, seasonality of birth is assessed from $\delta^{18}\text{O}$ sequential series by the identification of the position in tooth crown where the highest and/or lowest $\delta^{18}\text{O}$ values of the cycle are measured, allowing comparison between individuals. However, this variability, measured as a distance on tooth crown, may also be influenced by variability in the timing of tooth development, from which depends what part of the tooth is being formed at a given age and variability in tooth full size, to which the position in tooth crown where diagnostic $\delta^{18}\text{O}$ values are measured should be related. How much of the observed inter-variability can be attributed to such developmental and morphological factors and when their significance takes over differences due to birth seasonality have not been assessed. This aspect must be investigated in a reference population of animals born within a short time period and submitted to similar external conditions.

Second, the temptation is great, to use intra-tooth sequential analysis of enamel $\delta^{18}\text{O}$ to determine the *season* of birth from the timing of tooth growth (Henton *et al.* 2010). However, the timing of tooth growth, precisely known for only very few species, might not be the timing of tooth mineralization, due to the delay in the process of enamel maturation. How this might affect the timing of the record of the isotopic signal was shown in cattle (Balasse 2002), horse (Hoppe *et al.*, 2004) and sheep tooth enamel (Zazzo *et al.* 2010). Alternatively, determination of the birth season may be done by comparison with reference datasets obtained on animals whose season of birth is known.

Such reference datasets are species and tooth dependant. For sheep, one reference set is published for the record of spring birth in the sheep third lower molar. It was acquired on primitive sheep from North Ronaldsay island (Orkney) presumably born during the population birth peak in April-May (Balasse *et al.* 2005; Balasse and Tresset 2007). In the present study, series of $\delta^{18}\text{O}$ values were measured in the lower second molars of ten sheep born within a few weeks and submitted to the same environmental conditions until they were slaughtered on the same day, 18 months later. The objectives are (1) to acquire a set of $\delta^{18}\text{O}$ sequential data for spring birth, in the lower second molar of sheep; (2) to measure inter-individual variability for a sheep population with a very narrow birth period. A model is proposed to quantify this variability while erasing the influence of tooth size.

MATERIAL

The study includes ten sheep raised on the island of Rousay in the Orkney archipelago. The region is under temperate climate, heavily influenced by the Gulf Stream, with annual amplitude of variation in temperature around 9°C. The highest ambient temperatures are usually reached in July and August and the lowest in February (Vose et al. 1992). The studied animals were derived from a working farm rather than an experiment and as such more closely mirror environmental conditions that might be encountered in traditional herding systems in the past. The sheep are a Shetland cross. They were born at the end of April/beginning of May 2003 within 4 to 6 weeks and were raised in the same cohort. They were slaughtered on the 7th of October 2004. The mandibles were collected just after slaughter and each of them provided one lower second molar for the present study. The group includes castrated rams and ewes, however the mandibles were not identified to individual. The sheep grazed on terrestrial pasture. The farm water is supplied from natural and drilled wells, and the sheep also had access to streams running through the island. However, they were rarely seen drinking from these sources: most of their water came from vegetation, including plant water and meteoric water ingested with the grass. Six of these sheep (ROU 01, ROU 04, ROU 09, ROU 11, ROU 16 and ROU 17) were included in a previous study, where their enamel stable carbon isotope compositions were used as terrestrial references for comparison with seaweed eating sheep (Balasse et al. 2009). For the present study, we completed the sequences previously sampled on these six sheep (see METHODS), and we added four individuals (ROU 6, ROU 07, ROU 08 and ROU 18).

METHODS

Enamel sampling was performed on the buccal side of the anterior lobe of the lower second molar. Tooth enamel surfaces were cleaned by abrasion with a tungsten drill bit. Enamel was sequentially sampled from a few millimetres away from the apex to the cervix of the crown using a diamond burr bit. Each sample is a groove perpendicular to the tooth growth axis, drilled through the whole thickness of the enamel layer. The sampling spans the whole crown height, including the lowest part bearing softer enamel due to incomplete mineralization (Figure 1). In the six sheep included in a previous study (ROU 01, ROU 04, ROU 09, ROU 11, ROU 16 and ROU 17), the sampling was initially stopped before reaching softer enamel (Balasse et al. 2009). Because this part of the tooth, formed at the very end of the first year, represents an important milestone in the timing of tooth growth, the sampling was completed on these teeth until as close as possible to the enamel-root junction (except for ROU 01, whose tooth had served for other scientific purpose since the first sampling). The distance of each sample from the enamel-root junction is recorded from the furthest sample margin (Figure 1). From 20 to 31 enamel samples were drilled out of each tooth depending on the crown length, giving a total of 272 samples.

Enamel powder was pre-treated for bioapatite extraction as described in Balasse *et al.* (2002). Enamel was treated for 24 hours with sodium hypochlorite 2-3 % (0.1 ml solution /mg sample) to remove organic matter and then for 4 hours in 0.1 M acetic acid (0.1 ml/mg) to remove exogenous carbonate. This protocol was designed to purify archaeological bioapatite. No exogenous carbonate is supposed to be present in modern enamel samples. However, because the first six sheep from a previous study (Balasse et al. 2009) were pre-treated for their isotopic values to be directly compared to those from archaeological samples, and because the pre-treatment is known to induce changes in the $\delta^{18}\text{O}$ values of enamel (Koch et al. 1997), all samples had to be pre-treated. For three individuals (ROU 07, ROU 08 and ROU 18) the enamel samples were analyzed before and after pre-treatment to state whether the results from Koch et al. (1997), obtained on a small number of modern enamel samples, could be confirmed.

Bioapatite samples weighing 550 to 650 μg were reacted for 240 seconds with 100 % phosphoric acid at 70°C in individual vessels in an automated cryogenic distillation system (Kiel IV device) interfaced with a Delta V Advantage isotope ratio mass spectrometer. The accuracy of the data is checked

through the analysis of our internal lab carbonate standard (Marbre LM), whose $\delta^{18}\text{O}$ value, normalized to the international standard NBS 19, is -1.83‰ . Over the period of analysis of the bioapatite samples, from November 2009 to January 2010, the analytical precision calculated from 127 analyses of Marbre LM was 0.06‰ for $\delta^{18}\text{O}$. Over the period of analysis of the six previous sheep, from March to May 2007, the analytical precision for $\delta^{18}\text{O}$ was 0.04‰ , from 51 analyses of Marbre LM. The correction applied to the enamel samples, from the measured value of Marbre LM, varied from -0.26‰ to $+0.01\text{‰}$.

RESULTS

The mean percentage of weight loss induced by the pre-treatment is $47 \pm 5\%$. Results from $\delta^{18}\text{O}$ analysis of untreated enamel bioapatite and comparison with the values measured in pre-treated corresponding samples are shown in Table 1 and Figure 2. The pattern of variation of $\delta^{18}\text{O}$ values within a tooth is similar before and after pre-treatment: the minimum and maximum $\delta^{18}\text{O}$ values are recorded in the same place in the tooth crown and the amplitude of variation within each tooth differs slightly (Table 1). The difference in $\delta^{18}\text{O}$ values between untreated and pre-treated enamel ($\delta^{18}\text{O}$ pre-treated - $\delta^{18}\text{O}$ untreated) varies from -0.3‰ to 1.9‰ and the mean difference is $1.0 \pm 0.4\text{‰}$ ($N=81$). This is close to the 0.9‰ ^{18}O -enrichment reported in Koch et al. (1997), although with a different protocol in the latter study (2% NaOCl for 1 day, then 0.1 M acetic acid for 3 days, 0.04 ml solution/mg sample). This offset is lower in the last 10 mm of tooth crown toward enamel-root junction (Figure 2).

Results from the $\delta^{18}\text{O}$ analysis of pre-treated enamel are shown in Table 2 and Figure 3. All $\delta^{18}\text{O}$ values are expressed *versus* VPDB. Overall, the $\delta^{18}\text{O}$ values vary from -6.5‰ to -1.7‰ . Within a tooth, the minimum $\delta^{18}\text{O}$ values vary between -6.5‰ and -4.6‰ and the maximum $\delta^{18}\text{O}$ values are comprised between -2.8‰ and -1.7‰ . The amplitude of variation within each second molar is between 2.7‰ and 4.0‰ (Table 2). From the lowest and highest $\delta^{18}\text{O}$ values and from intra-tooth amplitude of variation, no individual diverges significantly from the remaining individuals (Student T test, 95% confidence interval).

In all sheep, the pattern of variation of $\delta^{18}\text{O}$ is sinusoidal, most likely reflecting the seasonal cycle. In the teeth where the sampling started very close to the lobe apex (ROU6M2 and ROU18M2, Figure 3), the recorded sequence spans approximately a year. In other teeth, when the sampling started a few millimetres away from the apex (Figure 1 and Figure 3) the sequence is less than a year, due to truncation of this part of the signal. The lowest $\delta^{18}\text{O}$ values, reflecting the coldest months, were tracked at a distance from the enamel-root junction varying between 22.1 mm and 33.1 mm (Table 2). Given that the sheep were born in the spring, these correspond to their first winter. The highest $\delta^{18}\text{O}$ values, reflecting the warmest months, were tracked at a distance from the enamel-root junction varying between 5.1 mm and 13.7 mm (Table 2). These values correspond to the second summer of the sheep's life.

DISCUSSION

Difference in the pre-treatment induced offset between mature and immature enamel

In ROU08M2 and ROU18M2, a difference was observed in the pre-treatment effect in the last 10 mm of tooth crown. In this part of the tooth, enamel was not fully mineralized, as witnessed from visual observation (Figure 1) and during the sampling process from a lower resistance of enamel to the drill. This was confirmed by a lower extraction yield after pre-treatment (data shown) due to a higher initial organic content. An explanation for such a difference in the pre-treatment induced isotopic offset between mature and immature enamel may be proposed, although this observation, made on a very limited number of samples, must be confirmed. As a possible and non-exclusive explanation for the

trend induced by pre-treatment, Koch et al. (1997) suggested that partial dissolution by acetic solution might change the relative proportion of carbonate in phosphate (PO_4^{3-}) and hydroxyl (OH^-) lattice sites of bioapatite, by removing preferentially the more weakly bonded sites for example. This mechanism also predicts a direct link between the isotope value of bioapatite and lattice site occupancy, which was not tested since. If this explanation is correct, the pre-treatment offset might also differ with a change in the relative proportion of adsorbed/structural carbonate and/or carbonate present at the PO_4^{3-} / OH^- lattice site. Aoba and Moreno (1990) have shown that during enamel development, incorporation of carbonate into bioapatite crystals changes quantitatively and qualitatively. A significant amount of carbonate incorporated during the secretory phase is labile, adsorbed on the crystal surface. During maturation the labile component decreases and most of the carbonate ions are incorporated into the crystal lattice. As for the sites occupied by carbonate ions in the crystal lattice, enamel crystals at early stages of mineralization include mainly carbonate substituted into the PO_4^{3-} site, while maturation sees a decrease in the number of carbonate ions substituted per atom mass of calcium and a concomitant increase in carbonate ions substituted in the OH^- sites (Aoba and Moreno, 1990; Aoba, 1996). Therefore, the isotopic shift induced by the pre-treatment may be different in mature enamel and enamel in earlier mineralization stages, although the exact mechanisms leading to a very low shift of the isotopic signal in immature enamel, in this study case, are unexplained. Alternatively, the measurement of the $\delta^{18}\text{O}$ values on untreated immature enamel might have been affected by a higher organic content in these samples.

Inter-individual variability in the $\delta^{18}\text{O}$ values

Within the studied population, inter-individual variability is 1.1 ‰ for the maximum $\delta^{18}\text{O}$ values and reaches 1.9 ‰ for the minimum $\delta^{18}\text{O}$ values recorded in the second molar. The analytical procedure - including precision in the volume of enamel sampled (corresponding to a certain amount of time, over which the isotopic signal is averaged), differential effect of pre-treatment and analytical precision - explains only a very small part of this variability. The rest must be explained by inter-individual variability in physiology (from body water balance to the process of enamel mineralization), and possible differences in the input signal, although those would be minor, including for instance different relative proportions of plant water and drink water. The experimental protocol designed for this study does not compare with carefully controlled conditions as far as environmental factors are concerned. However, the sheep included in this study were submitted throughout their lives to environmental conditions as similar as they could ever be within the framework of a real herding system: born on the same farm, they have moved in a cohort with each other over a very small territory until they were slaughtered on the same day.

Sequence of the seasonal cycle recorded in M2 and duration of enamel mineralization

In sheep, the second molar starts to form over the second month of life and its crown is completed when the sheep reaches twelve months (Weinreb and Sharav 1964, Awassi breed; Milhaud and Nézit 1991, south Pre-Alps breed; Zazzo et al. 2010, Suffolk cross). No information on the timing of tooth growth in the Shetland breed was found. Given the consistency of previous observations on different sheep breeds, it is assumed that the timing of tooth growth in Shetland is close to that of previously cited breeds.

According to this timing of tooth development, sheep born at the end of April/beginning of May would be expected to form the earliest part of the M2 crown in the end of May/beginning of June while they were one month old and the latest part of the M2 crown in the end of April/beginning of May of the following year, while they were one year old. Unfortunately, the $\delta^{18}\text{O}$ of ingested water or, more generally the environmental water, was not controlled over the sheep lifetime. Precipitation $\delta^{18}\text{O}$ is closely related to surface air temperature at high and middle latitudes (Gat 1980, Rozanski *et al.* 1993) and consequently the pattern of monthly variations in precipitation $\delta^{18}\text{O}$ may be close to the

pattern of monthly variation of ambient temperature in its trends. The expected sequence of the seasonal cycle, estimated from monthly variation of ambient temperature is reported in Figure 4. Average temperature data from Vose et al. (1992) were obtained over 959 months between 1827 and 1906, averaging years of data. They may not be the most appropriate to compare to isotopic data acquired in a specific year. However, in the absence of direct control of precipitation $\delta^{18}\text{O}$ over the time of formation the teeth included in this study, they were the unique data set that could be referred to in this purpose.

The measured pattern of $\delta^{18}\text{O}$ variation within sheep enamel is different from expectation (Figure 4). Although enamel was sampled on the posterior lobe, where tooth wear was very light and crown height still intact, the sampling started a few millimetres lower to avoid reduction of sample size due to the narrowing of tooth crown towards the apex. Maximum crown heights are comprised between 31.2 mm and 40.4 mm (Table 2). They were reported on the graphs in order to estimate what part of the signal was truncated by the sampling in each tooth (Figure 3). The isotopic signal is missing over two to three millimetres on most teeth, except in ROU 6 M2, where the sampling started at the very beginning of the crown. In this specimen, the earliest isotopic signal is close to the mean between the maximum and minimum $\delta^{18}\text{O}$ values recorded over the year. According to the pattern of annual variations in surface air temperature (Vose et al. 1992), this could be reached in the end of October (Figure 4). Compared to the expected date of end of May/beginning of June, this suggests a five months shift in the reading of the seasonal cycle recorded in tooth enamel.

Because of the reduction of the enamel layer thickness towards the cervical margin of the crown, the sampling could only be performed as far as the enamel-root junction in one specimen (ROU 04). From this specimen and from others where only a couple of millimetres are missing, the $\delta^{18}\text{O}$ values measured at the very end of the crown would correspond to early autumn (ROU 04) to autumn (ROU 18), roughly from late September to late October. Compared to the expected date of April, this indicates a five to six months shift in the record of the seasonal cycle in tooth enamel. In a controlled study of Suffolk sheep, Zazzo et al. (2010) estimated the duration of enamel maturation time to be four months in the second molar. From measurements on thin sections of developing enamel, Suga (1982) estimated that two thirds of enamel developmental time is devoted to maturation in sheep molars. A four-month maturation would therefore correspond to six months for the whole developmental process. It is therefore apparent that Zazzo et al.'s study and the present work led to similar conclusions. This important delay in enamel maturation highlights that the season of birth cannot be deduced directly from the timing of tooth growth in sheep.

Location in crown height of maximum and minimum $\delta^{18}\text{O}$ values

In archaeological assemblages, the season and seasonality of birth are assessed from $\delta^{18}\text{O}$ sequential series by the identification of the position in tooth crown where the highest and/or lowest $\delta^{18}\text{O}$ values of the cycle are measured. This allows comparison between individuals. In our data set, the lowest $\delta^{18}\text{O}$ values were measured at a distance from the enamel-root junction varying from 33.1 to 22.1 mm and the highest $\delta^{18}\text{O}$ values at a distance varying from 13.7 to 5.1 mm (Table 2, Figure 3). This distance is directly linked to the age of the individual when this part of the tooth is forming, but it is also linked to tooth size. However, within the sampled population, the full crown height of the second molar ranges between 31.2 mm and 40.4 mm, showing that tooth size varies greatly (see also Figure 1). Direct comparison between individuals could be acceptable after normalizing the distance from the enamel-root junction to each individual's full crown height. However, the full crown height is attained - and measurable - only over a very short time in sheep molars, because the crown comes into wear at about the same time as it reaches its full length. For this reason, such normalization is difficult to envisage in archaeological assemblages, where the number of specimens of sheep second molars showing full crown height (roughly between 12 and 18 month old) might not be high enough for a study of birth seasonality/season, depending on the exploitation strategy and therefore the structure of the herd mortality profile.

Another way to eliminate inter-individual variability in tooth size is to normalize the data to the periodic cycle identified from the $\delta^{18}\text{O}$ values, which should represent a whole year record in each individual whatever tooth size and growth rate. This can be done using the model described thereafter.

Description of the model

The sequence of $\delta^{18}\text{O}$ values measured in the sheep teeth can be modelled using a function mainly based on a cosine, as follows:

$$\delta^{18}\text{O}_m = A \cdot e^{\left(\frac{x-x_B}{x_A}\right)} \cdot \cos\left(2\pi \frac{x-x_0}{X+bx}\right) + M + px$$

Where:

$\delta^{18}\text{O}_m$ is the modelled $\delta^{18}\text{O}$;

x is the distance from enamel-root junction;

X is the period (in mm), corresponding to the length of tooth crown potentially formed over a whole annual cycle;

A is the amplitude (= max-min/2) (in ‰) and depends on the amplitude of the isotopic signal;

x_0 is the delay (mm), which depends on the time of the year when tooth growth started; $\delta^{18}\text{O}$ attains maximum value when $x = x_0$;

M is the mean (= (max+min)/2) expressed in ‰;

p is the slope (in ‰.mm⁻¹) of the mean: when the sequence measured spans over a year, the mean M might change from a year to another (change in the mean annual ambient temperature for example);

x_A and x_B (in mm) model the attenuation of the amplitude A : x_A is the severity of the attenuation, according to an exponential function whose lateral shift is described by x_B . Such parameters will be important, for example, if tooth growth rate slows down towards the tooth neck.

b is the gradation of the period (no unit), also related to possible changes in tooth growth rate from the apex towards the neck of the crown.

The function is close to that proposed by Bernard et al. (2009), with the addition of the last four parameters (p , x_A , x_B , and b). Figure 5 highlights how the variation of each parameter might influence the model.

Application of the model to the dataset

The best fit of the different parameters of the model to the measured data was determined using an iterative method and a minimization of the sum of the square of the difference between the model and the measurements (method of least squares). For this study, the calculations have been carried out with Microsoft Excel software but any data analysis software can be used. First, the model was applied with p , b and x_B fixed to 0, x_A fixed to 10^6 with only X , A , x_0 and M let to vary: this will be referred to hereafter as the four-parameter model. Results from the calculation of the best fit for combined variation of X , A , x_0 and M are reported in Table 3. Among ten specimens, the period X , corresponding to the length of tooth crown mineralized over a year, varies from 30.7 to 39.0 mm (mean = 35.8 mm). The distance from the enamel-root junction where the highest $\delta^{18}\text{O}$ values is recorded ($x_{\max} = x_0$) varies between 6.3 and 11.6 mm (mean = 9.9 mm). The lowest $\delta^{18}\text{O}$ values ($x_{\min} = x_0 + X/2$) were recorded at between 23.0 and 30.3 mm (mean = 27.6 mm) from the enamel-root junction (Table 3 and Figure 6a). These results are similar to but probably more accurate than the previous estimations from raw data (5.1 to 13.7 mm and 22.1 to 33.1 mm for location of highest and lowest $\delta^{18}\text{O}$ values respectively), because analytical precision could arbitrary create false maximum and minimum data points. On this population, inter-individual variability in the position in tooth crown of highest and lowest $\delta^{18}\text{O}$ values, resulting mainly from tooth size, is respectively 5.3 mm and 7.3 mm.

When adding parameter **b** to the four-parameter model, **b** was always very low (comprised between -0.3 and 0.6), suggesting that only a very slight variation of tooth growth rate with time could be detected on this dataset. Such variation is probably easier to quantify on sequential $\delta^{18}\text{O}$ values spanning over one periodic cycle. Using this five-parameter model, the modelled x values for maximum $\delta^{18}\text{O}$ ($x_{\max} = x_0$) and minimum $\delta^{18}\text{O}$ ($x_{\min} = (\mathbf{X} + 2x_0) / (2-\mathbf{b})$) were almost identical to those obtained with the four-parameter model ($\Delta x_{\max} \leq 0.6$ mm; $\Delta x_{\min} \leq 0.2$ mm, except in ROU 08 where $\Delta x_{\min} = 2.1$ mm).

When applying all eight parameters (**X**, **A**, **x₀**, **M**, **p**, **x_A**, **x_B** and **b**) to the dataset, **x_A** always remained very high (10^6) and **x_B** very low (10^{-7} to 10^{-5}). The very small influence of these parameters on the model suggests that no attenuation of the isotopic signal towards the neck of the tooth is detected on this dataset. The slope **p** was comprised between -0.1 and 0.3 ‰.mm⁻¹. Such slopes were considered unsuitable to describe the dataset. This parameter might be more adapted to longer sequences spanning over one periodic cycle. Using the eight-parameter model, calculation of x_{\min} and x_{\max} yielded different results from those obtained with the four-parameter model ($0.6 \leq \Delta x_{\max} \leq 3.7$ mm; $0 \leq \Delta x_{\min} \leq 8.7$ mm).

Using the results from the four-parameter model, x_{\min} and x_{\max} for each individual were normalized to the respective period **X**. Values for x_{\max}/X ($=x_0/X$) vary from 0.19 to 0.37 (mean = 0.28 ± 0.05). Values for x_{\min}/X vary from 0.69 to 0.87 (mean = 0.78 ± 0.05) (Table 3 and Figure 6b). From this it may be concluded that when inter-individual variability due to tooth size is erased:

- all sheep gave very similar results. Eight are within the standard deviation around the mean; they must be considered undistinguishable. ROU 04 and ROU 18 deviate slightly from the mean, suggesting that tooth size alone cannot explain this difference. Nevertheless, both stay within an interval of plus or minus two times the standard deviation from the mean, which means that the data are coherent. Considering all eight remaining sheep, the inter-individual variability observed in the location of the highest (2.7 mm) and lowest $\delta^{18}\text{O}$ values (5.5 mm) might be attributed to tooth size.
- the Rousay sheep were born over a maximum period of 6 weeks (or 0.11 year). The precision for the whole procedure described in this study (data acquisition, modelling and normalization to the periodic cycle) is lower than the dispersion of the births (Figure 6b), suggesting that the error in the attribution of a date of birth using this procedure is better than 6 weeks.
- the Rousay sheep have recorded the minimum and maximum $\delta^{18}\text{O}$ values on average at respectively 78 % and 28 % of the end of the recorded periodic cycle, the end being defined when $x = 0$ (when crown growth reaches the enamel-root junction). These may be considered as references for births occurring at the end of April/beginning of May.

CONCLUSIONS

This dataset offers a new reference for the record of April/May birth in the sheep lower second molar. The five to six month-delay for completion of enamel mineralization re-affirms the usefulness of such reference datasets to determine the season of birth, which must not be estimated directly from the timing of tooth growth.

A model mainly based on a cosine function and involving eight parameters (**X** = period, **A** = amplitude, **x₀** = delay, **M** = mean, **p** = slope of the mean, **x_A** and **x_B** = attenuation of the amplitude and **b** = gradation of the period) was proposed to analyze the data. The last four parameters, **p**, **x_A**, **x_B** and **b**, were not found very useful in describing the dataset in this case: only a very slight variation of tooth growth rate with time and no attenuation of the isotopic signal towards the cervical margin of the crown could be detected on this dataset. However, these parameters might be important to describe cyclic stable isotope variations in teeth whose growth spans several years.

The four-parameter (period, amplitude, delay and mean) model was found very useful to determine more accurately the period and the actual location in tooth crown where the maximum and minimum

$\delta^{18}\text{O}$ values occur. The period was found to be on average 35.8 mm, suggesting that a minimum crown height of 17.9 mm (X/2) might be required to record the position of both maximum and minimum $\delta^{18}\text{O}$ values over a natural annual seasonal cycle. Tooth remains with a lower crown height might not be well suited to the determination of seasonality and season of birth. Maximum and minimum $\delta^{18}\text{O}$ values occurred on average respectively at 9.9 mm and 27.6 mm from tooth enamel-root junction. In this regard, maximum inter-individual variability due to tooth size was 5.5 mm in this population, suggesting that shifts over this distance in the record of the seasonal cycle must not be interpreted in terms of season of birth. The Rousay sheep have recorded the minimum and maximum $\delta^{18}\text{O}$ values on average at respectively 78 % and 28 % of the end of the recorded periodic cycle. These may be considered as references for births occurring at the end of April/beginning of May.

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Table 1: Minimum, maximum and mean differences in the stable oxygen isotope ratios ($\delta^{18}\text{O}$) of untreated (utr) and pre-treated (ptr) enamel carbonate samples from three specimens. Amplitude of variation of enamel carbonate $\delta^{18}\text{O}$ within each tooth.

Specimen	N	$\Delta \delta^{18}\text{O}(\text{prt-utr})$			Amplitude of variation	
		Min (‰)	Max (‰)	Mean $\pm 1\sigma$ (‰)	utr (‰)	ptr (‰)
ROU 07 M2	24	0.6	1.4	1.1 \pm 0.2	2.4	2.9
ROU 08 M2	27	-0.2	1.1	0.7 \pm 0.3	2.5	2.7
ROU 18 M2	30	-0.3	1.9	1.2 \pm 0.5	3.2	2.7

Table 2: Stable oxygen isotope ratios ($\delta^{18}\text{O}$) of enamel carbonate from the Rousay sheep lower second molars (M2). Samples are located within tooth crown by their distance from the enamel-root junction (mm). The minimum and maximum $\delta^{18}\text{O}$ values within each tooth are also indicated with location in tooth crown, together with the amplitude of variation within the tooth (Δ). The maximum crown height (Max crown H) is the distance between the apex and enamel-root junction on the posterior lobe of the tooth.

sample	mm	$\delta^{18}\text{O}$												
Max crown H	38.8		Max crown H	38.4		Max crown H	40.4		Max crown H	37.9		Max crown H	38.2	
ROU 09 M2-1	35.6	-5.6	ROU 11 M2-1	34.5	-5.7	ROU 16 M2-1	37.4	-5.2	ROU 17 M2-1	34.3	-5.4	ROU 18 M2-1	36.3	-3.3
ROU 09 M2-2	34.4	-5.5	ROU 11 M2-2	33.4	-6.0	ROU 16 M2-2	36.2	-5.4	ROU 17 M2-2	33.2	-5.4	ROU 18 M2-2	35.2	-3.6
ROU 09 M2-3	33.2	-5.7	ROU 11 M2-3	32.2	-6.1	ROU 16 M2-3	35.2	-5.7	ROU 17 M2-3	32.0	-5.6	ROU 18 M2-3	33.9	-3.6
ROU 09 M2-4	32.1	-5.6	ROU 11 M2-4	31.1	-6.2	ROU 16 M2-4	34.0	-5.8	ROU 17 M2-4	30.9	-5.7	ROU 18 M2-4	32.9	-3.9
ROU 09 M2-5	31.0	-5.5	ROU 11 M2-5	30.0	-6.4	ROU 16 M2-5	32.8	-6.1	ROU 17 M2-5	29.7	-5.7	ROU 18 M2-5	31.4	-4.2
ROU 09 M2-6	29.8	-6.0	ROU 11 M2-6	28.9	-6.2	ROU 16 M2-6	31.7	-6.2	ROU 17 M2-6	28.6	-5.7	ROU 18 M2-6	30.2	-4.6
ROU 09 M2-7	28.5	-6.2	ROU 11 M2-7	27.9	-6.4	ROU 16 M2-7	30.6	-6.2	ROU 17 M2-7	27.4	-5.9	ROU 18 M2-7	29.1	-4.8
ROU 09 M2-8	27.2	-6.0	ROU 11 M2-8	26.8	-5.8	ROU 16 M2-8	29.4	-6.4	ROU 17 M2-8	26.3	-5.7	ROU 18 M2-8	28.0	-5.0
ROU 09 M2-9	26.1	-6.2	ROU 11 M2-9	25.7	-6.0	ROU 16 M2-9	28.1	-6.1	ROU 17 M2-9	25.0	-5.5	ROU 18 M2-9	27.0	-5.0
ROU 09 M2-10	24.8	-6.0	ROU 11 M2-10	24.5	-5.8	ROU 16 M2-10	26.9	-5.9	ROU 17 M2-10	24.0	-5.3	ROU 18 M2-10	25.7	-4.9
ROU 09 M2-11	23.6	-5.8	ROU 11 M2-11	23.4	-5.4	ROU 16 M2-11	25.7	-5.9	ROU 17 M2-11	22.8	-5.1	ROU 18 M2-11	24.5	-5.0
ROU 09 M2-12	22.2	-5.6	ROU 11 M2-12	22.4	-5.2	ROU 16 M2-12	24.5	-5.7	ROU 17 M2-12	21.5	-4.8	ROU 18 M2-12	23.4	-4.9
ROU 09 M2-13	21.3	-5.1	ROU 11 M2-13	21.2	-4.5	ROU 16 M2-13	23.0	-5.2	ROU 17 M2-13	20.3	-4.4	ROU 18 M2-13	22.3	-4.5
ROU 09 M2-14	20.0	-4.7	ROU 11 M2-14	20.2	-4.3	ROU 16 M2-14	21.8	-4.9	ROU 17 M2-14	19.3	-4.1	ROU 18 M2-14	21.1	-4.3
ROU 09 M2-15	19.0	-4.5	ROU 11 M2-15	19.1	-3.8	ROU 16 M2-15	20.6	-4.3	ROU 17 M2-15	18.1	-3.8	ROU 18 M2-15	20.0	-4.0
ROU 09 M2-16	17.6	-4.1	ROU 11 M2-16	18.0	-3.3	ROU 16 M2-16	19.4	-3.8	ROU 17 M2-16	17.0	-3.4	ROU 18 M2-16	18.8	-3.5
ROU 09 M2-17	16.6	-3.7	ROU 11 M2-17	16.8	-3.4	ROU 16 M2-17	18.3	-3.7	ROU 17 M2-17	15.7	-3.1	ROU 18 M2-17	17.7	-3.3
ROU 09 M2-18	15.6	-3.4	ROU 11 M2-18	15.8	-2.8	ROU 16 M2-18	17.1	-3.4	ROU 17 M2-18	14.7	-2.9	ROU 18 M2-18	16.6	-2.9
ROU 09 M2-19	14.5	-3.1	ROU 11 M2-19	14.7	-2.8	ROU 16 M2-19	15.8	-3.2	ROU 17 M2-19	13.4	-2.6	ROU 18 M2-19	15.5	-2.8
ROU 09 M2-20	13.5	-2.9	ROU 11 M2-20	13.6	-2.6	ROU 16 M2-20	14.7	-3.0	ROU 17 M2-20	12.2	-2.3	ROU 18 M2-20	14.2	-2.4
ROU 09 M2-21	12.4	-2.7	ROU 11 M2-21	12.6	-2.9	ROU 16 M2-21	13.7	-2.8	ROU 17 M2-21	11.1	-2.4	ROU 18 M2-21	13.2	-2.5
ROU 09 M2-22	11.3	-2.6	ROU 11 M2-22	11.4	-2.6	ROU 16 M2-22	12.5	-2.8	ROU 17 M2-22	9.9	-2.3	ROU 18 M2-22	11.9	-2.3
ROU 09 M2-23	10.1	-2.8	ROU 11 M2-23	10.3	-2.6	ROU 16 M2-23	11.3	-2.8	ROU 17 M2-23	8.8	-2.2	ROU 18 M2-23	10.9	-2.5
ROU 09 M2-24	8.9	-2.7	ROU 11 M2-24	9.2	-2.5	ROU 16 M2-24	10.1	-3.0	ROU 17 M2-24	7.7	-2.2	ROU 18 M2-24	9.7	-2.4
ROU 09 M2-25	7.8	-3.0	ROU 11 M2-25	8.1	-2.4	ROU 16 M2-25	8.8	-3.1	ROU 17 M2-25	6.7	-2.6	ROU 18 M2-25	8.5	-2.7
ROU 09 M2-26	6.7	-2.8	ROU 11 M2-26	7.1	-2.6	ROU 16 M2-26	7.4	-3.0	ROU 17 M2-26	5.7	-2.5	ROU 18 M2-26	7.2	-2.9
ROU 09 M2-27	5.7	-2.8	ROU 11 M2-27	6.1	-3.0	ROU 16 M2-27	6.4	-3.1	ROU 17 M2-27	4.6	-3.0	ROU 18 M2-27	6.3	-2.8
ROU 09 M2-28	4.5	-2.8	ROU 11 M2-28	4.9	-3.1	ROU 16 M2-28	5.3	-3.1	Min	27.4	-5.9	ROU 18 M2-28	5.2	-2.9
ROU 09 M2-29	3.4	-3.4	Min	30.0	-6.4	ROU 16 M2-29	4.2	-3.5	Max	8.8	-2.2	ROU 18 M2-29	4.3	-3.3
Min	28.5	-6.2	Min	27.9	-6.4	Min	29.4	-6.4	Max	7.7	-2.2	ROU 18 M2-30	3.0	-4.1
Min	26.1	-6.2	Max	8.1	-2.4	Max	13.7	-2.8	Δ	3.7		Min	28.0	-5.0
Max	11.3	-2.6	Δ	4.0		Max	12.5	-2.8				Min	27.0	-5.0
Δ	3.6					Max	11.3	-2.8				Min	24.5	-5.0
						Δ	3.6					Max	11.9	-2.3
												Δ	2.7	

sample	mm	$\delta^{18}\text{O}$												
Max crown H	38.8		Max crown H	38.4		Max crown H	40.4		Max crown H	37.9		Max crown H	38.2	
ROU 09 M2-1	35.6	-5.6	ROU 11 M2-1	34.5	-5.7	ROU 16 M2-1	37.4	-5.2	ROU 17 M2-1	34.3	-5.4	ROU 18 M2-1	36.3	-3.3
ROU 09 M2-2	34.4	-5.5	ROU 11 M2-2	33.4	-6.0	ROU 16 M2-2	36.2	-5.4	ROU 17 M2-2	33.2	-5.4	ROU 18 M2-2	35.2	-3.6
ROU 09 M2-3	33.2	-5.7	ROU 11 M2-3	32.2	-6.1	ROU 16 M2-3	35.2	-5.7	ROU 17 M2-3	32.0	-5.6	ROU 18 M2-3	33.9	-3.6
ROU 09 M2-4	32.1	-5.6	ROU 11 M2-4	31.1	-6.2	ROU 16 M2-4	34.0	-5.8	ROU 17 M2-4	30.9	-5.7	ROU 18 M2-4	32.9	-3.9
ROU 09 M2-5	31.0	-5.5	ROU 11 M2-5	30.0	-6.4	ROU 16 M2-5	32.8	-6.1	ROU 17 M2-5	29.7	-5.7	ROU 18 M2-5	31.4	-4.2
ROU 09 M2-6	29.8	-6.0	ROU 11 M2-6	28.9	-6.2	ROU 16 M2-6	31.7	-6.2	ROU 17 M2-6	28.6	-5.7	ROU 18 M2-6	30.2	-4.6
ROU 09 M2-7	28.5	-6.2	ROU 11 M2-7	27.9	-6.4	ROU 16 M2-7	30.6	-6.2	ROU 17 M2-7	27.4	-5.9	ROU 18 M2-7	29.1	-4.8
ROU 09 M2-8	27.2	-6.0	ROU 11 M2-8	26.8	-5.8	ROU 16 M2-8	29.4	-6.4	ROU 17 M2-8	26.3	-5.7	ROU 18 M2-8	28.0	-5.0
ROU 09 M2-9	26.1	-6.2	ROU 11 M2-9	25.7	-6.0	ROU 16 M2-9	28.1	-6.1	ROU 17 M2-9	25.0	-5.5	ROU 18 M2-9	27.0	-5.0
ROU 09 M2-10	24.8	-6.0	ROU 11 M2-10	24.5	-5.8	ROU 16 M2-10	26.9	-5.9	ROU 17 M2-10	24.0	-5.3	ROU 18 M2-10	25.7	-4.9
ROU 09 M2-11	23.6	-5.8	ROU 11 M2-11	23.4	-5.4	ROU 16 M2-11	25.7	-5.9	ROU 17 M2-11	22.8	-5.1	ROU 18 M2-11	24.5	-5.0
ROU 09 M2-12	22.2	-5.6	ROU 11 M2-12	22.4	-5.2	ROU 16 M2-12	24.5	-5.7	ROU 17 M2-12	21.5	-4.8	ROU 18 M2-12	23.4	-4.9
ROU 09 M2-13	21.3	-5.1	ROU 11 M2-13	21.2	-4.5	ROU 16 M2-13	23.0	-5.2	ROU 17 M2-13	20.3	-4.4	ROU 18 M2-13	22.3	-4.5
ROU 09 M2-14	20.0	-4.7	ROU 11 M2-14	20.2	-4.3	ROU 16 M2-14	21.8	-4.9	ROU 17 M2-14	19.3	-4.1	ROU 18 M2-14	21.1	-4.3
ROU 09 M2-15	19.0	-4.5	ROU 11 M2-15	19.1	-3.8	ROU 16 M2-15	20.6	-4.3	ROU 17 M2-15	18.1	-3.8	ROU 18 M2-15	20.0	-4.0
ROU 09 M2-16	17.6	-4.1	ROU 11 M2-16	18.0	-3.3	ROU 16 M2-16	19.4	-3.8	ROU 17 M2-16	17.0	-3.4	ROU 18 M2-16	18.8	-3.5
ROU 09 M2-17	16.6	-3.7	ROU 11 M2-17	16.8	-3.4	ROU 16 M2-17	18.3	-3.7	ROU 17 M2-17	15.7	-3.1	ROU 18 M2-17	17.7	-3.3
ROU 09 M2-18	15.6	-3.4	ROU 11 M2-18	15.8	-2.8	ROU 16 M2-18	17.1	-3.4	ROU 17 M2-18	14.7	-2.9	ROU 18 M2-18	16.6	-2.9
ROU 09 M2-19	14.5	-3.1	ROU 11 M2-19	14.7	-2.8	ROU 16 M2-19	15.8	-3.2	ROU 17 M2-19	13.4	-2.6	ROU 18 M2-19	15.5	-2.8
ROU 09 M2-20	13.5	-2.9	ROU 11 M2-20	13.6	-2.6	ROU 16 M2-20	14.7	-3.0	ROU 17 M2-20	12.2	-2.3	ROU 18 M2-20	14.2	-2.4
ROU 09 M2-21	12.4	-2.7	ROU 11 M2-21	12.6	-2.9	ROU 16 M2-21	13.7	-2.8	ROU 17 M2-21	11.1	-2.4	ROU 18 M2-21	13.2	-2.5
ROU 09 M2-22	11.3	-2.6	ROU 11 M2-22	11.4	-2.6	ROU 16 M2-22	12.5	-2.8	ROU 17 M2-22	9.9	-2.3	ROU 18 M2-22	11.9	-2.3
ROU 09 M2-23	10.1	-2.8	ROU 11 M2-23	10.3	-2.6	ROU 16 M2-23	11.3	-2.8	ROU 17 M2-23	8.8	-2.2	ROU 18 M2-23	10.9	-2.5
ROU 09 M2-24	8.9	-2.7	ROU 11 M2-24	9.2	-2.5	ROU 16 M2-24	10.1	-3.0	ROU 17 M2-24	7.7	-2.2	ROU 18 M2-24	9.7	-2.4
ROU 09 M2-25	7.8	-3.0	ROU 11 M2-25	8.1	-2.4	ROU 16 M2-25	8.8	-3.1	ROU 17 M2-25	6.7	-2.6	ROU 18 M2-25	8.5	-2.7
ROU 09 M2-26	6.7	-2.8	ROU 11 M2-26	7.1	-2.6	ROU 16 M2-26	7.4	-3.0	ROU 17 M2-26	5.7	-2.5	ROU 18 M2-26	7.2	-2.9
ROU 09 M2-27	5.7	-2.8	ROU 11 M2-27	6.1	-3.0	ROU 16 M2-27	6.4	-3.1	ROU 17 M2-27	4.6	-3.0	ROU 18 M2-27	6.3	-2.8
ROU 09 M2-28	4.5	-2.8	ROU 11 M2-28	4.9	-3.1	ROU 16 M2-28	5.3	-3.1	Min	27.4	-5.9	ROU 18 M2-28	5.2	-2.9
ROU 09 M2-29	3.4	-3.4	Min	30.0	-6.4	ROU 16 M2-29	4.2	-3.5	Max	8.8	-2.2	ROU 18 M2-29	4.3	-3.3
Min	28.5	-6.2	Min	27.9	-6.4	Min	29.4	-6.4	Max	7.7	-2.2	ROU 18 M2-30	3.0	-4.1
Min	26.1	-6.2	Max	8.1	-2.4	Max	13.7	-2.8	Δ		3.7	Min	28.0	-5.0
Max	11.3	-2.6	Δ		4.0	Max	12.5	-2.8				Min	27.0	-5.0
Δ		3.6				Max	11.3	-2.8				Min	24.5	-5.0
						Δ		3.6				Max	11.9	-2.3
												Δ		2.7

Table 3: Results from the calculation of the best fit (method of least squares) for combined variation of X (period), A (amplitude), x_0 (delay) and M (mean) when the four-parameters model is applied to the sheep M2 $\delta^{18}\text{O}$ data sets.

Parameter	ROU 01	ROU 04	ROU 6	ROU 07	ROU 08	ROU 09	ROU 11	ROU 16	ROU 17	ROU 18
X (mm)	31.7	33.5	34.6	31.7	37.3	39.0	38.3	38.7	38.6	30.7
A (‰)	1.9	2.0	1.5	1.3	1.2	1.8	1.9	1.8	1.8	1.3
x_0 (mm)	8.9	6.3	10.6	10.4	11.6	9.0	10.7	10.9	9.5	11.2
M (‰)	-4.3	-4.4	-4.2	-3.2	-3.4	-4.4	-4.4	-4.5	-4.1	-3.7
p (‰/mm)	0	0	0	0	0	0	0	0	0	0
xA (mm)	1.00E+06									
xB (mm)	0	0	0	0	0	0	0	0	0	0
b	0	0	0	0	0	0	0	0	0	0
x_{\min} (= $x_0+X/2$)	24.8	23.0	27.9	26.2	30.2	28.5	29.9	30.3	28.8	26.6
x_{\max}/X	0.28	0.19	0.31	0.33	0.31	0.23	0.28	0.28	0.25	0.37
x_{\min}/X	0.78	0.69	0.81	0.83	0.81	0.73	0.78	0.78	0.75	0.87

Figure 1: Sampled sheep teeth from Rousay. Scale in mm. d: distance of sample from enamel-root junction.

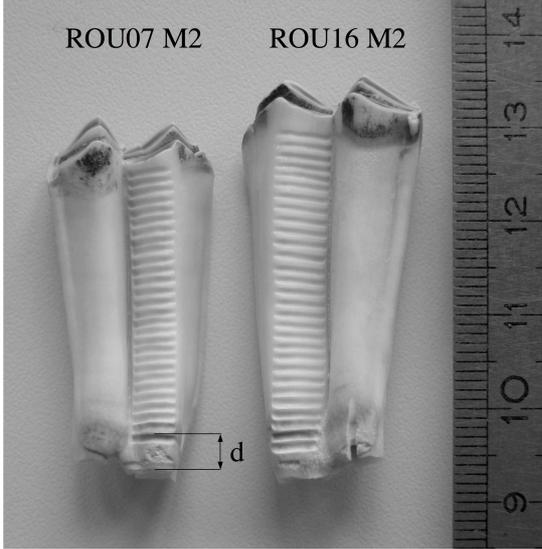


Figure 2: Results from stable oxygen isotope analysis ($\delta^{18}\text{O}$) of untreated (utr) and pre-treated (ptr) samples of enamel bioapatite carbonate from ROU 07, ROU 08 and ROU 18 second molars (M2). Sample location within the tooth is expressed as the distance from enamel-root junction (erj). In ROU 07 M2 the three last samples from were not analyzed after pre-treatment because of insufficient remaining amount.

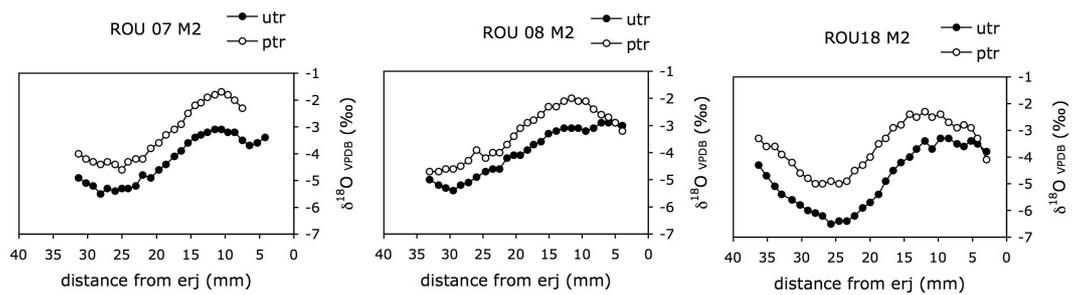


Figure 3: Results from sequential sampling and stable oxygen isotope analysis ($\delta^{18}\text{O}$) of tooth enamel from the Rousay sheep second molars. Sample location within the tooth is expressed as the distance from enamel-root junction (erj). The dash line locates the maximum crown height.

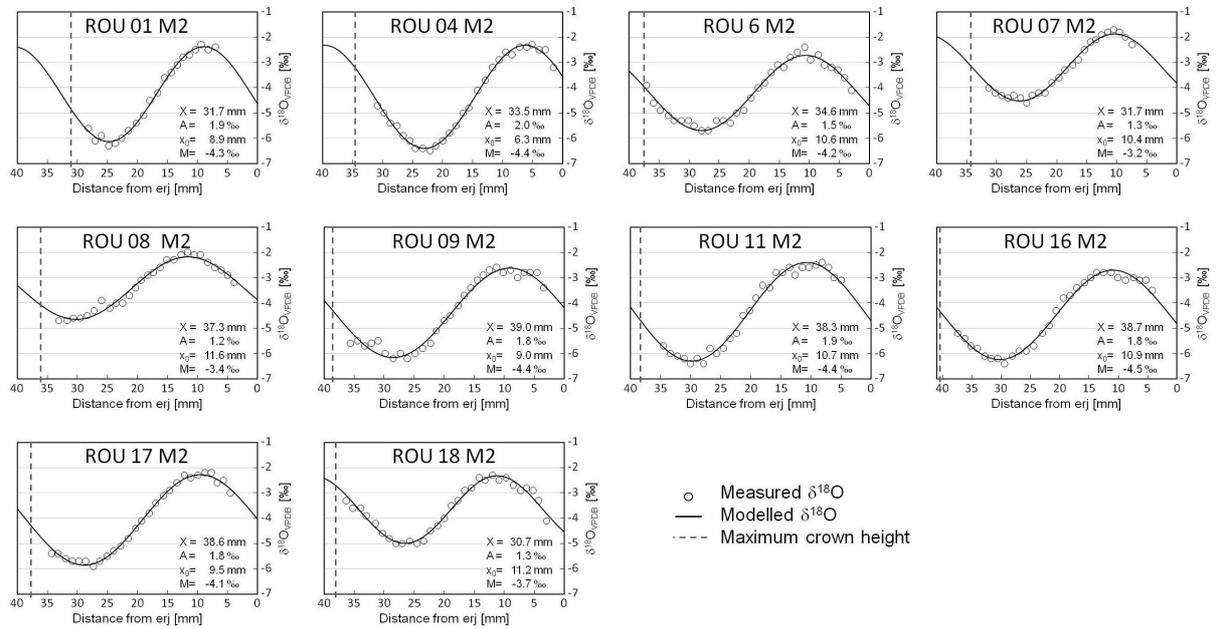


Figure 4: Average ambient temperature in South Orkney islands (Vose et al. 1992) and expected sequence of the seasonal cycle recorded in the sheep second molar, from the timing of tooth growth. The sequence measured in the sheep second molar is shifted.

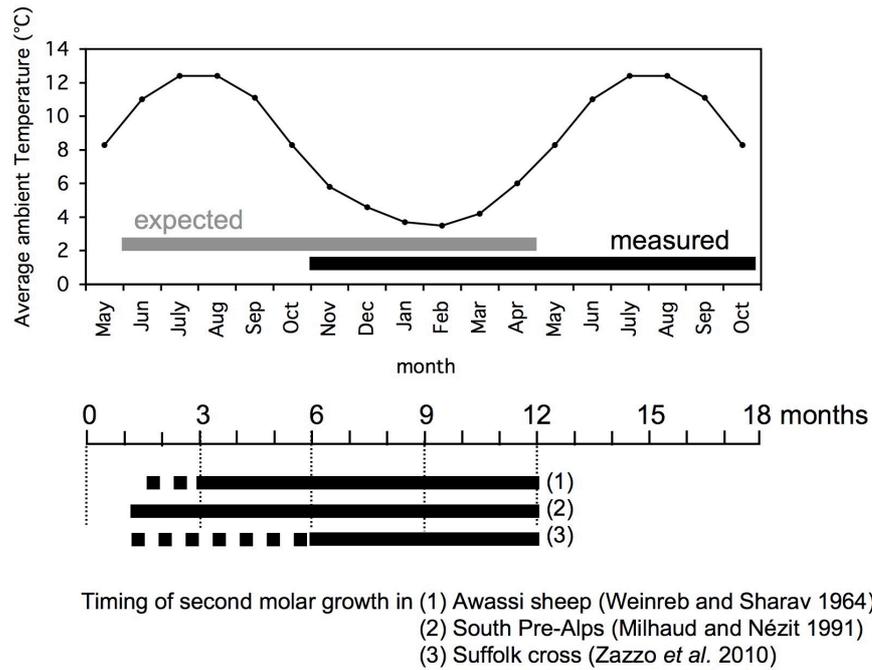


Figure 5: Definition of the eight parameters included in the model and influence of each of them on the shape of the cosine function. (a): parameters included in the four-parameter model; (b) additional parameters.

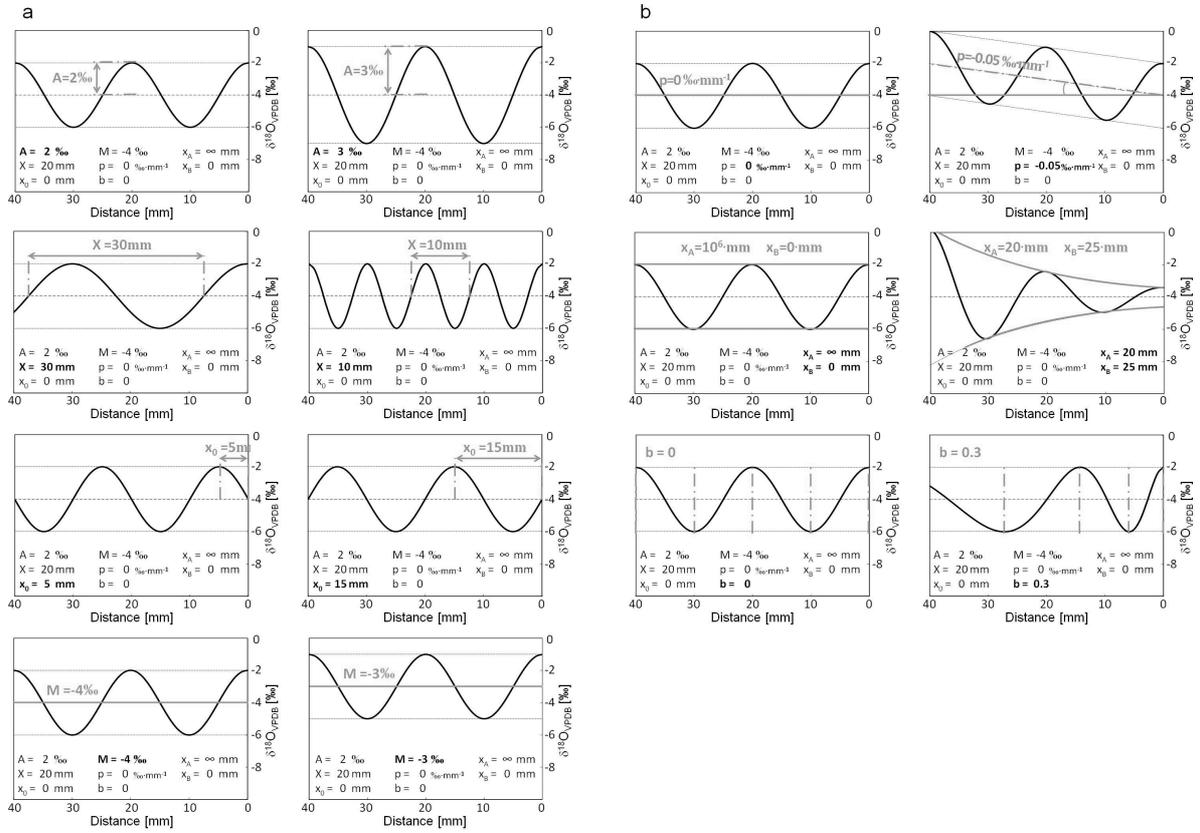


Figure 6: a - Location in tooth crown of the highest (x_{max}) and lowest (x_{min}) values in the ten specimens; b – normalisation of these distances to the period X.

