A calf for all seasons? The potential of stable isotope analysis to investigate prehistoric husbandry practices

Authors

Jacqueline Towers (corresponding author: j.r.tower1@Bradford.ac.uk)

Division of Archaeological, Geographical and Environmental Sciences, University of Bradford, Bradford BD7 1DP, UK

Mandy Jay

Department of Archaeology, Durham University, South Road, Durham DH1 3LE, UK

Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

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Ingrid Mainland

Division of Archaeological, Geographical and Environmental Sciences, University of Bradford, Bradford BD7 1DP, UK

Department of Archaeology, Orkney College UHI, Kirkwall, Orkney KW15 1LX, UK

Olaf Nehlich

Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

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Janet Montgomery

Division of Archaeological, Geographical and Environmental Sciences, University of Bradford, Bradford BD7 1DP, UK

Abstract

The Early Bronze Age barrows at Irthlingborough and Gayhurst in central England are notable for the large number of cattle (*Bos taurus*) remains associated with their human Beaker burials. Previous work using strontium isotope analysis has indicated that most of the cattle analysed, and one aurochs (*Bos primigenius*), were of local origin (Towers et al. 2010). In this study, stable isotope analysis of enamel and bone was carried out to investigate whether the mature cattle had experienced similar husbandry practices, climate and environment. Bulk carbon, nitrogen and sulphur isotope analysis of collagen suggested most were consuming similar sources of plant protein from environments probably local to the sites and this was supported by high resolution intra-enamel carbon isotope profiles. Oxygen isotope profiles indicated the aurochs and most of the cattle experienced similar climatic regimes: the only exception being an animal with a non-local strontium isotope ratio. However, a comparison of seasonality profiles of the local animals using estimated tooth formation times showed that there was no consistency in season of birth: the animals appeared to have been born throughout the year. Cattle can breed throughout the year but it requires considerable human effort and intervention to successfully overwinter young stock; it is therefore unlikely to have been carried out without good reason and benefit if winters were harsh. One reason is to ensure a continuous supply of milk. Measuring oxygen isotope profiles to identify year-round calving may thus be a potential indicator of dairying economies.

Introduction

Insight into past animal husbandry practices is essential to gain an understanding of the economic basis of prehistoric communities (Charles and Halstead, 2001). This would include aspects such as selection of species, herd/flock size, production goals (i.e. meat, wool, milk, traction) and strategies for grazing or foddering. The role of palaeodietary techniques in elucidating past husbandry practices is becoming increasingly apparent: for example, compound-specific stable isotope analysis of lipids in pottery residues is used to identify dairying (e.g. Copley et al., 2005; Dudd et al., 1999); strontium isotope analysis of tooth enamel enables the detection of animal movement (e.g. Balter, 2008; Bendrey et al., 2009; Montgomery et al., 2007; Towers et al., 2010); microwear analysis can indicate foddering and grazing strategies, and practices such as stalling or penning (Mainland, 2006; Vanpoucke et al., 2009). An approach which offers much potential is intra- and inter-tooth analysis of stable isotopes as this enables identification of how animals are husbanded in different seasons or at different stages of their life, up until tooth formation is complete (e.g. Balasse et al., 2002; Bentley and Knipper, 2005). In this paper we explore the potential of seasonal variation in oxygen and carbon isotope analysis of tooth enamel carbonate together with nitrogen, carbon and sulphur isotope analysis of bone collagen as indicators of cattle husbandry practices through the analysis of cattle remains from Irthlingborough Barrow 1 and Gayhurst Barrow 2.

The assemblages are from two of Britain's most remarkable Early Bronze Age archaeological sites, dated to around 2000 BC. They were discovered on floodplains at Irthlingborough (Northamptonshire) and Gayhurst (Buckinghamshire) (Figure 1) during gravel extraction in the 1980s and 1990s (Chapman, 2007; Dix, 1987; Halpin, 1987).

Both were round barrows notable for the unusually large quantities of cattle (*Bos taurus*) remains associated with their central human Beaker burials. The associated cattle bone assemblage at Irthlingborough Barrow 1 includes skulls from 185 animals, mandibles and scapulae from between 35 and 40 animals, and pelves from 15 animals (estimated minimum values) (Davis, 2009). There were also several aurochs (*Bos primigenius*) remains: five teeth, a fragment of horn core and two possible scapulae (Davis and Payne, 1993). The cattle bones were found mixed with limestone blocks, suggesting the presence of a cairn, originally located above a central, wooden, burial chamber (Davis and Payne, 1993). As at Irthlingborough, the burial of a single adult male in an oak-lined chamber was discovered at the centre of Gayhurst Barrow 2. The cattle remains, consisting principally of limb bones but including skulls and mandibles, were found in a ring-ditch surrounding the barrow. A minimum number of 300 animals has been estimated (Deighton and Halstead, 2007).

Potential information derived from light stable isotope analysis

Carbon

Light stable isotope ratios are usually expressed in the δ notation and units are per mil (‰). For herbivores, the $\delta^{13}C$ value of tooth enamel and bone collagen depends on the plants eaten, with a difference in $\delta^{13}C$ between diet and body tissue resulting from fractionation during the formation of body tissues from plant material components (Lee-Thorpe, 2008).

The $\delta^{13}C$ values of collagen mostly reflects the consumption of protein, whilst those of enamel carbonate are expected to reflect the chemistry of the whole diet, which would include lipid and carbohydrate components (Ambrose and Norr, 1993; Jim et al., 2004).

Bone collagen molecules are slowly replaced during life and represent an averaged dietary picture over an animal's existence (Hedges et al., 2007), whereas enamel does not turn over, forms over a short period of time and represents a set picture for that period (according to Brown et al. (1960), second molar crowns take ~12 months to form, during the first year of an animal's life, whilst third molar crowns take ~14 months to form, starting at around nine months of age).

The δ^{13}C values of plants following the two basic photosynthetic pathways (C_3 and C_4) are different, but plants from temperate Northern Europe are predominantly C_3 with no significant levels of C_4 plants present in prehistoric Britain. δ^{13}C values of C_3 plants can be highly variable both between and within species, and also according to environmental conditions (Leavitt and Long, 1982; Senbayram et al., 2008; Winkler et al., 1978). For a single plant species, several different environmental factors can alter the δ^{13}C values, such as recycled CO_2 at ground level in dense woodland (the canopy effect), sunlight levels, water availability, temperature, salinity and altitude (Heaton, 1999). δ^{13}C values of C_3 plants are therefore highly variable from sites and environments worldwide, with a modal value of –27‰ (O'Leary, 1988). A mean value of –29.4‰ has been obtained for herbs and grasses from a British meadow (Dungait et al., 2008), although it should be appreciated that contemporary values are affected by industrialization, which leads to more negative values than would be seen in prehistory (Hoefs, 1997; O'Leary, 1988). For herbivores browsing a particular location, the consumption of different species and plant parts will tend to an average for a particular time and place, but different locations and seasonal inputs may be identifiable.

The bulk $\delta^{13}C$ value of bone collagen, reflecting a lifetime of protein input, will generally be enriched over the averaged dietary value by around 5‰ after fractionation (van der Merwe and Vogel, 1978). For enamel mineral, which reflects different biosynthetic pathways, the enrichment is around 12‰ on average, but can vary with body mass and dietary physiology (Krueger and Sullivan, 1984). Values of around 14‰ have been measured for large ruminants including cattle (Cerling and Harris, 1999; Passey et al., 2005). There are many variables to be considered which cannot be covered here, but a good synthesis can be found in Lee-Thorp (2008).

Overall, $\delta^{13}C$ values of herbivore enamel and collagen depend on many factors relating to diet and environment. Of interest in this study is whether the results of intra-tooth carbon isotope analysis of enamel carbonate will show seasonal variation in $\delta^{13}C$ and whether any husbandry-related information can be inferred. Of further interest is whether domestic cattle can be distinguished from aurochs, in terms of the $\delta^{13}C$ values of their tooth enamel and bone collagen. Several isotopic studies of bone collagen have suggested that $\delta^{13}C$ values differ between the species and that this may be due to feeding habits, such as aurochs being more likely to feed under forest cover or in more watery environments (Balasse et al., 2000; Lynch et al., 2008; Noe-Nygaard et al., 2005). It has also been hypothesized, based on the location of aurochs remains in Britain, that their preferred habitat was low-lying floodplains (Hall, 2008) and that such areas were relatively open because of grazing by large herbivores (Svenning, 2002). Unless forced away from open areas by human activity, aurochs may have lived in a similar environment to domestic cattle, producing indistinguishable $\delta^{13}C$ values.

Nitrogen

$\delta^{15}N$ values from bone collagen also reflect the consumption of protein over a lifetime. Nitrogen isotope ratios are generally used in foodwebs to interpret trophic level and marine resource consumption, but for herbivorous cattle at these sites variation in $\delta^{15}N$ values is likely to be entirely related to spatial differences in local soils, plants and environments, both natural and anthropogenic (Bogaard et al., 2007; Hedges and Reynard, 2007; Stevens et al., 2008). $\delta^{15}N$ values in plants can range from −5 to +20‰, but the more positive values are from extreme arid and saline environments, whilst the very low ratios are from leguminous plants and environments of moist forest and montane (Ambrose, 1991; Heaton, 1987; Virginia and Delwiche, 1982). The $\delta^{15}N$ value of bone collagen will be enriched by between 2 and 6‰ over that of the diet, but this can be variable across a number of factors, such as species, age, trophic level, dietary protein levels and physiological stress (Hedges and Reynard, 2007; Sponheimer et al., 2003). For these cattle, a spacing of around 3‰ might be expected.

Sulphur

There are currently few publications with significant $\delta^{34}S$ data-sets from archaeological skeletal material, due to technical difficulties of the analysis which are now largely being overcome (Nehlich and Richards, 2009; Privat et al., 2007; Tanz and Schmidt, in press). Our understanding of the ways in which these can be interpreted are therefore at an early stage, although it is clear that the data can reflect mobility related to the geology of the region of plant growth at the base of the food chain and to the proximity of the coastline, where the 'sea spray' effect of marine sulphates can be reflected in the dietary resources (Richards et al., 2001; Richards et al., 2003). $\delta^{34}S$ values can also

distinguish dietary consumption of aquatic resources, although this is not likely to be relevant in this study (Nehlich et al., 2010).

There is little fractionation in the sulphur isotope system (probably $\leq$ 1‰, this being less than current analytical error for these values) (Richards et al., 2003), so that bone collagen values are expected to be similar to diet. Ranges of $\delta^{34}S$ values for terrestrial European archaeological bone collagen go from –18 up to around +20‰ (Jay and Nehlich, unpublished data; Nehlich et al., 2010; Privat et al., 2007), although fully terrestrial herbivore diets well away from the coast might be expected to be centrally placed in that range (Jay, unpublished data).

Oxygen

$\delta^{18}O$ values of both structural carbonate and phosphate in mammalian tooth enamel are linked to that of body water at the time of tooth formation (Bryant et al., 1996; Longinelli, 1984), and have been shown to be a proxy of climatic and geographic variables, such as air temperature, altitude, latitude and distance from the sea, because the $\delta^{18}O$ of body water is principally controlled by that of precipitation, ingested from surface reservoirs such as streams and plants (Dansgaard, 1964; Longinelli, 1984; White et al., 1998).

Seasonal variation of $\delta^{18}O$ can be revealed through the use of intra-tooth enamel sampling (Balasse et al., 2003). Of interest in this study is whether this method of analysis applied to Irthlingborough and Gayhurst cattle teeth will provide information on cattle birth seasonality. Examples from similar studies are sheep from the Late Stone Age site of Kasteelberg, South Africa, which show two birth seasons (Balasse et al.,

2003), and Neolithic cattle from the Knap of Howar, Orkney, which suggest a strong seasonality of birth, in contrast to cattle from Neolithic Er Yoh, Brittany (Balasse and Tresset, 2007). The Neolithic cattle data-sets were small and the authors were unable to identify which of several factors may have been responsible (e.g. climate, husbandry and genetics) for this difference.

Cattle molar intra-tooth data

Cattle have hypsodont (high-crowned) teeth, which form sequentially from the cusp of the crown to the cervix (Hillson, 2005). Thus, intra-tooth enamel sampling from a single molar, where enamel is extracted at a number of positions between the cusp and cervix, may produce time-related isotope data (Fricke and O'Neil, 1996). However, it is hypothesized that herbivore enamel mineralization is somewhat more complicated, consisting of a matrix formation stage and a maturation stage (Suga, 1982). The maturation stage, when most of the mineralization occurs (Robinson et al., 1995), has been shown to be complex, both temporally and spatially (Hoppe et al., 2004; Tafforeau et al., 2007). By measuring the carbon isotope ratios of intra-tooth enamel samples from the molars of cattle that had changed diets between plant sources with different photosynthetic pathways (C_3 and C_4), Balasse (2002) has concluded that, at any position on the molar, mineralization takes ~6-7 months.

Samples and methods

Skeletal samples

Carbon, nitrogen and sulphur stable isotope data have been obtained from extracted bone collagen for 12 domestic cattle from Gayhurst and ten from Irthlingborough, alongside an aurochs from the latter site. In addition, oxygen and carbon isotope ratios

have been obtained from the carbonate component of molar enamel for at least four domestic cattle from Gayhurst and four domestic cattle and an aurochs from Irthlingborough. Although oxygen and carbon isotope ratios may be obtained from the mineral component of bone, dentine or tooth enamel (biological apatite), the mineral component of enamel tends to be more resistant to diagenesis than that of bone and dentine, being "non-porous, and more highly crystalline, with larger crystals" (Koch et al., 1997), and has become the biological apatite of choice for isotope ratio analysis (Lee-Thorp and van der Merwe, 1991). Eight second molars and ten third molars were sampled. For the Irthlingborough domestic cattle, adjacent second and third left maxillary molars were utilised in all but one case to maximise the likelihood that different individuals were being sampled. In addition, a single third molar from an aurochs (IRTH B) and a pair of adjacent right mandibular molars from a domestic animal (IRTH 8) were also analysed. Oxygen, carbon and strontium isotope results suggest that IRTH 8 was a distinct animal (see below; Towers et al., 2010). Although pairs of second and third molars were also obtained from the Gayhurst remains, consistency in sampling with respect to tooth position (mandibular or maxillary, left or right) was not possible. However, sampling was carried out across different archaeological contexts, and oxygen, carbon and strontium isotope results do suggest different animals apart from two, designated GAY 2 and GAY 4, for which all three isotope results are very similar (see below; Towers et al., 2010); i.e. it is possible that GAY 2 and GAY 4 were the same animal.

The animals from Gayhurst for which collagen was extracted and analysed were not those for which tooth enamel was analysed. For Irthlingborough, bone collagen was extracted and analysed from the aurochs (IRTH B) and three of the domestic cattle

(IRTH 3, 7 and 8) for which tooth enamel was analysed, whilst the other seven cattle collagen samples were from the same context.

Collagen sample preparation and analysis

Collagen extraction was based on Longin's method, modified by a two-step filtering process (Brown et al., 1988; Longin, 1971). Whole bone samples were demineralized in 0.5 M HCl at 4°C. The remaining collagen was denatured in pH 3 aqueous solution at 70°C for 48 hours. The solution was filtered using Ezee filters®, followed by centrifugal filtering using Millipore ultrafilters which separated molecules smaller than 30 kD. The larger, less degraded collagen molecules were then freeze-dried. The resultant collagen product was weighed to tin capsules and the samples combusted to N_2, CO_2, SO and SO_2, and analysed using either a Thermo Finnigan DELTA Plus XL continuous helium flow gas isotope ratio mass spectrometer coupled with a Flash EA elemental analyser or a Thermo Finnigan DELTA V Plus coupled to a Eurovector elemental analyser, both at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig. The analytical standard deviation, averaged from laboratory working standards run with the samples (methionine for carbon and nitrogen, casein for sulphur), amounted to ± 0.1‰ for $\delta^{13}C$, less than ±0.1‰ for $\delta^{15}N$ and ± 0.3‰ for $\delta^{34}S$. Replicated collagen included in the sulphur runs give reproducibility at ± 0.6‰. Two replicates were run for each sample, analysed in separate batches, and the results averaged. Averaged replicates were used where possible for sulphur, although large collagen sample requirements mean that this occurred for only 6 of 21 samples analysed for this element.

The widely accepted quality tests for collagen $\delta^{13}C$ and $\delta^{15}N$ data in terms of atomic

C:N ratios of 2.9 to 3.6 and appropriate elemental percentages (approximately 30 to

47% for carbon and 10 to 18% for nitrogen) (Ambrose, 1990; DeNiro, 1985; Nehlich

and Richards, 2009; van Klinken, 1999) were met for all samples referred to in this

paper. The quality tests for sulphur suggested by Nehlich and Richards (2009) were

also met (C:S ratios of 600 ± 300; N:S ratios of 200 ± 100 and S% of 0.15 to 0.3% for

mammals).

Enamel sample preparation and analysis

Sample preparation of cattle molars from Irthlingborough and Gayhurst was carried out

at the Stable Light Isotope Facility at the University of Bradford. Using a diamond

dental burr, the cementum was removed from each tooth, the enamel surface cleaned

and between five and fourteen intra-tooth samples of powdered enamel obtained.

Sample weights of ~15 mg were sufficient to provide sufficient material for repeat

analyses if required. Further treatment of the samples followed a protocol modified

after Sponheimer (1999). To summarise, they were treated initially with 1.7% NaOCl

solution for 30 minutes to remove organic matter, then rinsed with distilled water. 0.1M

acetic acid was added for ≤ 10 minutes to remove exogenous carbonate. After further

rinsing and freeze-drying the samples were weighed into septa-capped vials and loaded

into a Finnigan Gasbench II, an automatic carbonate preparation device connected

directly to a Thermo Delta V Advantage continuous flow isotope ratio mass

spectrometer. The enamel carbonate of each sample reacted with phosphoric acid

(103%) at 70 °C to release CO_2, which was analysed by the mass spectrometer together

with CO_2 from a reference supply. Values of $\delta^{18}O_{VSMOW}$ and $\delta^{13}C_{VPDB}$ for the enamel

samples were obtained from the mass spectrometer and were calibrated to the measured

and accepted values of two internal standards and one international standard. Analytical precision was ± 0.2‰ for both $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$ (1σ).

Results and discussion

Collagen results

The collagen results are presented in Table 1. The average $\delta^{13}C$ and $\delta^{15}N$ values for Gayhurst bone collagen are –23.1 ± 0.3 and 6.0 ± 0.3‰ respectively ($n = 12$) and for Irthlingborough they are –22.9 ± 0.4 and 6.0 ± 0.4‰ respectively ($n = 10$), with the aurochs values being –22.4 and 6.8‰. These data form a relatively tight group, such as might be expected for the same general location (e.g., see Jay and Richards, 2007 for the variation in groups of Iron Age herbivores, where $\delta^{13}C$ values showed ranges from 0.9 to 2.0‰ and $\delta^{15}N$ from 1.9 to 5.0‰ at different locations). There are no extreme outliers, although the aurochs from Irthlingborough and one of the Irthlingborough domestic cattle (IRTH 12) show slightly higher $\delta^{15}N$ values than the rest of the group (Figure 2). If they are excluded from the entire group, the average $\delta^{15}N$ value is 5.9 ± 0.3‰, and these two animals then have nitrogen isotope ratios which are either equal to or outside 3 standard deviations from the mean. The domestic cattle sample with the highest $\delta^{15}N$ value is not one for which enamel $\delta^{13}C$ and $\delta^{18}O$ values are available. The collagen sample was taken from a scapula fragment, which means that it is not impossible that the original identification as *Bos taurus* rather than aurochs may be flawed (see Lynch et al., 2008 for identification issues). The other data for the identified aurochs are presented below.

The $\delta^{34}S$ values are shown in Figure 3. For the domestic cattle, they average 0.9‰ ($n = 10$) for Gayhurst and −1.1‰ ($n = 10$) for Irthlingborough, with the aurochs at −5.5‰.

As for the carbon and nitrogen isotope data, there is no statistically significant difference between the two locations if all of the cattle values are compared. However, one of the Irthlingborough animals (IRTH 6) has a higher δ^{34}S value than the other Irthlingborough cattle (5.0‰) and if this is removed from the comparison, there is a statistical difference between the Gayhurst and Irthlingborough cattle at 95%. The strontium isotope ratio obtained from animal IRTH 6 indicates it originated from outside the study area (Towers et al., 2010). It is possible, therefore, that the δ^{34}S value for this animal is also reflecting mobility, although it must be noted that the bone collagen value is averaged over a lifetime of dietary input, whilst the strontium value from the enamel is fixed early in life. Given that the strontium isotope ratios of the enamel indicate that the animal was brought into the area well before death (Towers et al., 2010), the δ^{34}S value will reflect partial equilibration with local δ^{34}S, thus bringing it closer to that of cattle raised in the Irthlingborough area. It is possible that the source location would have provided a higher δ^{34}S value (closer to that seen at Gayhurst, although the strontium would not support that origin) and this would be consistent with movement from a location closer to the coast, perhaps in the west of Britain as suggested by the strontium isotope data. In general, these δ^{34}S values are low and consistent with the central England location of the two study sites, both in terms of being away from the coast and reflecting local geology. They compare, for instance, with an average of 12.0‰ for Early Bronze Age herbivores from the East Yorkshire chalk Wolds (Jay, unpublished data). Again, the aurochs is at the extreme of the range, although not an outlier.

In general, therefore, these cattle collagen data are consistent with the animals having lived locally, as are the previously published strontium data, although there are

individuals for which this is not true. Overall this provides a context for considering

the intra-tooth enamel data.

Intra-tooth enamel carbonate results

Oxygen and carbon results for enamel carbonate are shown in Table 2. Values range

between 22.2 and 26.3‰ for δ^{18}O and between −15.5 and −13.1‰ for δ^{13}C (Table 3).

The mid-range enamel carbonate δ^{13}C value for Gayhurst is −14.3‰ and for

Irthlingborough it is −14.5‰ (Table 3). When compared to the mean bone collagen

values the resulting enamel-collagen difference of 8.8‰ and 8.4‰ for Gayhurst and

Irthlingborough respectively is broadly consistent with values obtained for herbivores

in other worldwide studies, although those available are mainly comparisons of bone

collagen with bone carbonate, rather than with enamel carbonate (Gröcke, 1997).

Investigation of birth seasonality using intra-tooth δ^{18}O results

In order to obtain seasonally related information from intra-tooth δ^{18}O values and aid

comparison between different animals, sequential intra-tooth data from the second and

third molars of each animal are displayed on a single time-related x-axis (Figures 4 and

5). This has been achieved using the chronology of cattle molar development given by

Brown et al. (1960), together with measured crown heights and predicted unworn

crown heights (Towers, 2008), and assumes a uniform rate of crown formation, which

may not be the case (Hoppe et al., 2004). Wear stage and crown height data for 221

Irthlingborough cattle second and third molars, compiled by Davis (2009), were used to

calculate unworn crown heights. The isotope ratios are plotted against the time of initial

matrix formation (as opposed to completion of maturation), relative to that of the

second molar cervical enamel, which is designated 0 months and corresponds to ~12½

months after birth (Brown et al., 1960). The isotope ratio for each intra-enamel sample represents an average of perhaps six or seven months of mineralization (Balasse, 2002).

It is apparent from Figures 4 and 5 that the enamel data for each animal generally follow a sinusoidal pattern, which is likely to reflect the seasonal variation of $\delta^{18}O$ of local precipitation in a temperate, lowland region, with highest and lowest $\delta^{18}O$ values corresponding to summer and winter temperatures respectively. However, there is variation between the profiles of different animals, in terms of absolute $\delta^{18}O$ values and profile amplitudes, possibly due to the animals being born in different years or living in different locations. The high $\delta^{18}O$ values for animal GAY 1 in the early part of its life may reflect its probable birth in western Britain, as indicated by strontium isotope ratio analysis (Towers et al., 2010). The aurochs (IRTH B) also shows relatively high $\delta^{18}O$ values. However, strontium data suggest that the aurochs and the other domestic cattle for which $\delta^{18}O$ data were obtained were local animals, or at least grazed similar geological terrain (Towers et al., 2010). The $\delta^{18}O$ profiles of animals GAY 2 and GAY 4 are remarkably similar and may indicate that GAY 2 and GAY 4 were the same individual.

Figures 4 and 5 show that the second molar cervices of these animals (0 months) were formed at different times of year, e.g. the summer $\delta^{18}O$ peaks of IRTH 9 coincide with the winter troughs of IRTH 7. Profile peaks from all the animals appear to be evenly distributed between six months before and three months after the formation of their second molar cervical enamel (Figure 6). Assuming that the second molar cervix forms at a comparable time after birth in all cattle, it follows that the animals' births must also have had a similar, multiple season distribution. In the light of known analytical errors

and unknown uncertainties in tooth wear stages and enamel mineralization, it would be premature to predict the actual season of birth for each animal. However, the multiple season distribution of Figure 6 is unlikely to have been produced by the combined magnitude of such uncertainties.

Increasing the dataset should allow a more accurate assessment of birth seasonality. It is certainly the case that cattle can mate and breed throughout the year (King, 1978). However, it has been observed that primitive breeds, living under feral conditions, tend to breed seasonally (Balasse and Tresset, 2007), their breeding behaviour being influenced by climate and the seasonal availability of food (e.g. Hall and Moore, 1986). If breeding at Irthlingborough and Gayhurst occurred across several seasons, perhaps even year-round, there must have been an adequate supply of food throughout the year, either naturally through favourable climatic conditions, or through the active provision of additional feed in winter and management of grazing land. If the climate was not sufficiently benign, considerable effort would have been required to encourage non-seasonal calving (Balasse and Tresset, 2007). Hence there must have been a significant benefit in doing so. A possible impetus might have been the continuous supply of unprocessed fresh milk, providing nutritious food even in winter. Today and in the recent past, farmers will manipulate the timing of calving in a herd to ensure a year-round supply of milk. Future work will investigate whether non-seasonal calving is a strong indicator of dairying in the past. If that proves to be the case, then oxygen isotope analysis of cattle tooth enamel would provide additional ammunition for a multi-proxy approach, together with examination of faunal remains and lipid analysis, with which to identify dairying in prehistoric communities.

Investigation of diet and environment using intra-tooth $\delta^{13}C$ results

The diet of the Irthlingborough and Gayhurst cattle and the environment in which they were feeding should be reflected in the carbon isotope composition of their enamel. Figures 7 and 8 show $\delta^{13}C$ profiles generated from a combination of second and third molar intra-tooth data. It is evident that these profiles do not all define a sinusoidal or seasonal profile like the $\delta^{18}O$ profiles (Figures 4 and 5). Of note are the $\delta^{13}C$ profiles of GAY 2 and GAY 4, which like their $\delta^{18}O$ profiles, are very similar and tend to strengthen the suggestion that GAY 2 and GAY 4 were the same animal.

Of interest from Figure 7 is the range of $\delta^{13}C$ values from animal IRTH B, the aurochs, which lies within the total range of values from all the Irthlingborough domestic animals. If the aurochs had been feeding in dense woodland or in a wetland habitat and the domestic cattle in open areas or in a drier habitat, then a distinction between the two species might have been observed, with more negative $\delta^{13}C$ values expected for the aurochs. In this respect, it appears that this particular aurochs did not feed in a significantly different environment to the domestic cattle, although the $\delta^{15}N$ values for the bone collagen may indicate that there was some differentiation which may relate to the source location of the plants being eaten, or to a difference in the types of plants consumed.

When enamel $\delta^{13}C$ and $\delta^{18}O$ values are plotted together on the same time-related x-axis, several of the $\delta^{13}C$ profiles show possible seasonal variation. Figure 9 shows $\delta^{13}C$ and $\delta^{18}O$ profiles for animal GAY 2. The peak in the $\delta^{13}C$ profile is almost concurrent with

a trough in the $\delta^{18}O$ profile, and vice versa. In comparison, the peak in the $\delta^{13}C$ profile

for animal IRTH 7 corresponds to a peak in the $\delta^{18}O$ profile (Figure 10). These plots

suggest seasonally related changes in the $\delta^{13}C$ values of the plants eaten by the cattle.

If cattle were grazing in the same type of open environment throughout the year, the

$\delta^{13}C$ values of their food might be expected to be more positive in the summer than in

the winter because of environmental factors such as decreased water availability (Mole

et al., 1994; Schnyder et al., 2006; Smedley et al., 1991). Such a seasonal variation has

been measured for grasses and herbs sampled from grazed meadowland in Somerset,

UK (Dungait et al., 2010). This scenario might explain the $\delta^{13}C$ profile of animal IRTH

7, where the peak in $\delta^{13}C$ values coincides with the summer peak of the $\delta^{18}O$ profile,

but is contradictory to that of GAY 2. However, movement in the summer into shaded

woodland from open grassland might account for the profile of GAY 2. In addition,

different plant species and different plant parts produce different values of $\delta^{13}C$

(Heaton, 1999). Therefore, a seasonal variation in $\delta^{13}C$ can also result from a

seasonally varied diet, which might involve the consumption of different species or

different plant parts available at different times of the year. Clearly, if the cattle could

neither range freely nor select their own winter fodder, their $\delta^{13}C$ values will reflect a

managed diet rather than a natural seasonal variation or movement to a different habitat.

The interpretation of $\delta^{13}C$ profiles from C_3-only diets and isolating the cause or causes

of variation is complex and there are currently very few comparative data for British

cattle from any period. Consequently, for this small study, it is not possible to draw any

firm conclusions from these data. However, the very similar profiles observed from

GAY 2 and GAY 4 and the seasonal variation observed in all cattle are unlikely to

derive from purely random biological variation and thus show the potential for increased understanding of husbandry practices in the future. As the data-set of $\delta^{13}C$ and $\delta^{18}O$ profiles increases from different geographic localities and time periods, recurring seasonal and geographic patterns are likely to be found. Clearly, the construction of seasonal and dietary profiles from animals with known dietary histories and residence, will enable the elucidation of prehistoric husbandry practices to be made with greater confidence.

Conclusions

The intra-tooth oxygen isotope ratios can provide a clear seasonal framework with which to evaluate the temporal variation of intra-tooth data for different isotope ratios such as strontium and carbon. $\delta^{18}O$ profiles also have the potential to determine the seasonality of birth, provided there is a sufficiently large sample size. In this study, the data suggest that cattle from Irthlingborough and Gayhurst were being born throughout the year. An impetus for this might have been the continuous supply of fresh milk for human consumption. The link between year-round calving and dairying is the subject of ongoing research.

Combining sequential $\delta^{18}O$ and $\delta^{13}C$ values from enamel carbonate may help in identifying differences in herd management practices, in terms of environment and fodder provision. In this study, differences between individuals and between the sites can be seen in terms of seasonal data, such that further work on both archaeological material and modern analogues may provide valuable data-sets for future interpretation. However, $\delta^{18}O$ and $\delta^{13}C$ values of enamel could not distinguish between the aurochs and domestic cattle at Irthlingborough, although the $\delta^{15}N$ and $\delta^{34}S$ values from the bone

collagen may indicate a difference in environment or food resources. If the δ^{15}N and δ^{34}S values from bone collagen indicate a different environment, whilst the δ^{18}O and δ^{13}C values from the enamel do not, it is possible that this arises from a difference in the time of life represented by these two tissues. Equally, the animal could have been mobile, moving between locations of similar geology and climate. Such mobility would not necessarily involve long-distance movement since δ^{15}N values can differ over very short distances and the low δ^{34}S value is consistent with a central region of southern Britain (Beaker People Project, unpublished data).

Acknowledgements

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Tables

Table 1. Bone collagen $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data.

Animal	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	C:N	C:S	N:S	C (%)	N (%)	S (%)	Collagen yield (%)
GAY 4877	-23.3	5.8	-0.5	3.3	538	167	42.9	15.5	0.213	1.6
GAY 4878	-23.0	6.3	0.6	3.3	597	184	42.1	15.2	0.188	1.3
GAY 4879	-23.2	5.8	No data	3.3	No data	No data	39.9	14.1	N/A	0.8
GAY 4880	-23.2	6.4	-3.3	3.3	496	153	42.4	15.1	0.228	1.5
GAY 4881	-22.8	6.2	0.6	3.3	501	156	42.7	15.5	0.227	2.2
GAY 4882	-23.5	6.2	-2.0	3.3	604	188	42.2	15.4	0.187	1.5
GAY 4883	-22.4	5.5	*5.2	3.2	669	210	43.9	16.0	0.178	3.8
GAY 4885	-23.2	5.9	3.3	3.3	679	208	41.5	14.8	0.163	1.6
GAY 4886	-23.4	5.7	-0.9	3.3	596	181	43.1	15.3	0.193	1.3
GAY 4887	-23.3	5.8	2.2	3.2	607	192	41.8	15.5	0.184	1.6
GAY 4888	-22.6	6.2	4.0	3.3	664	206	43.0	15.6	0.173	1.4
IRTH 1	-23.0	6.1	0.9	3.2	647	203	44.1	16.1	0.182	2.6
IRTH 2	-22.8	6.2	0.2	3.2	617	192	43.9	16.0	0.190	1.6
IRTH 3	-22.4	5.7	*-3.4	3.2	637	201	44.5	16.3	0.187	3.6
IRTH 4	-23.6	5.7	*-3.6	3.2	594	184	44.6	16.1	0.201	4.0
IRTH 6	-23.1	5.8	*5.0	3.2	641	202	44.2	16.2	0.184	4.1
IRTH 7	-22.6	5.4	0.4	3.2	616	192	43.8	15.9	0.190	1.1
IRTH 8	-22.6	6.0	-5.0	3.2	623	194	44.0	16.0	0.188	1.8
IRTH 10	-22.4	6.0	-1.3	3.2	682	214	42.9	15.7	0.168	1.7
IRTH 11	-23.5	5.9	*-1.2	3.2	573	178	44.0	16.0	0.206	3.2
IRTH 12	-23.1	7.0	-3.3	3.2	623	197	43.9	16.2	0.189	2.8
IRTH B (aurochs)	-22.4	6.8	*-4.9	3.2	615	192	44.1	16.0	0.192	2.7

Notes:

1. Collagen extraction utilized ultrafilters (see text) and the collagen yields should be interpreted in this context.

2. All carbon and nitrogen data are replicated and averaged data shown. Sulphur has been replicated where enough collagen was available, for one Gayhurst individual and five from Irthlingborough (these are indicated by * in $\delta^{34}S$ column).

3. The elemental ratios shown in the table are atomic and calculated using percentages weighted for relative atomic masses.

Table 2. Oxygen and carbon isotope composition values from Gayhurst and Irthlingborough cattle tooth enamel. The sample ID contains the following information: GAY/IRTH = site, 1st digit = animal number, 2nd digit = molar number and 3rd digit = position on tooth lobe (cusp = 1). Mandibular 2nd and 3rd molars are designated M_2 and M_3, maxillary 2nd and 3rd molars are designated M^2 and M^3. L = left, R = right.

Sample ID	Distance from cervix (mm)	$\delta^{18}O_{VSMOW}$ (‰) normalised	$\delta^{13}C_{VPDB}$ (‰) normalised	Sample ID	Distance from cervix (mm)	$\delta^{18}O_{VSMOW}$ (‰) normalised	$\delta^{13}C_{VPDB}$ (‰) normalised
Animal IRTH 3 (M²L)				**Animal IRTH 3 (M³L)**			
IRTH 321	32.0	24.6	-14.2	IRTH331	36.0	23.5	-14.3
IRTH 322	28.5	24.7	-13.9	IRTH332	33.5	23.4	-14.4
IRTH 324	22.5	24.9	-14.7	IRTH333	30.5	23.7	-14.2
IRTH 325	20.0	24.9	-14.9	IRTH334	28.5	24.0	-14.3
IRTH 326	17.0	24.4	-14.9	IRTH335	26.0	24.0	-14.4
IRTH 327	14.0	23.8	-14.7	IRTH336	23.5	24.2	-14.4
IRTH 328	11.0	23.3	-14.7	IRTH337	20.5	24.4	-14.4
IRTH 329	8.5	22.9	-14.7	IRTH338	18.0	24.6	-14.5
				IRTH339	15.5	24.5	-14.6
				IRTH3310	12.5	23.8	-14.3
Animal IRTH 7 (M²L)				**Animal IRTH 7 (M³L)**			
IRTH 721	39.0	23.7	-15.4	IRTH731	41.5	23.6	-13.5
IRTH 722	36.0	23.0	-15.4	IRTH732	38.5	23.6	-13.5
IRTH 723	33.5	23.2	-15.2	IRTH733	35.0	23.7	-13.5
IRTH 724	30.5	22.2	-15.1	IRTH735	29.0	23.2	-13.7
IRTH 725	27.0	22.3	-15.0	IRTH736	25.5	23.2	-13.4
IRTH 726	23.5	22.6	-14.5	IRTH737	22.5	22.8	-13.7
IRTH 727	20.0	22.7	-14.6	IRTH738	19.5	22.5	-13.9
IRTH 728	17.0	22.5	-14.7	IRTH7310	14.0	22.3	-14.2
IRTH 729	13.5	22.7	-14.4	IRTH7311	11.0	22.4	-14.2
IRTH 7210	10.5	22.7	-14.0	IRTH 7312	8.0	23.0	-14.2

IRTH 7212	7.0	23.4	-13.6				

Animal IRTH 8 (M$_2$R)				Animal IRTH 8 (M$_3$R)			
IRTH821	21.0	24.5	-15.3	IRTH831	28.5	22.6	-15.4
IRTH822	18.5	24.7	-15.2	IRTH832	25.5	22.7	-15.2
IRTH823	16.0	25.0	-15.3	IRTH833	22.0	22.4	-15.1
IRTH824	13.5	25.2	-15.3	IRTH834	19.0	22.4	-14.9
IRTH825	11.0	24.6	-15.5	IRTH835	16.5	22.4	-14.9
IRTH826	8.5	24.4	-15.4	IRTH836	14.0	22.6	-14.7
IRTH827	6.0	24.1	-15.5	IRTH837	11.5	23.0	-14.6
IRTH828	3.5	23.8	-15.5	IRTH838	8.5	23.6	-14.5
				IRTH839	6.0	23.7	-14.5
				IRTH8310	3.5	23.9	-14.6

Animal IRTH 9 (M^{2}L)				Animal IRTH 9 (M^{3}L)			
IRTH921	41.5	23.4	-14.7	IRTH931	48.0	23.0	-14.8
IRTH922	39.0	23.7	-14.4	IRTH932	44.5	22.7	-14.5
IRTH923	36.0	23.6	-14.7	IRTH933	41.5	22.9	-14.3
IRTH924	33.0	24.2	-14.8	IRTH934	38.5	23.3	-14.4
IRTH925	29.5	24.4	-15.0	IRTH935	35.5	23.3	-14.4
IRTH926	26.0	24.6	-15.0	IRTH936	32.5	23.7	-14.4
IRTH927	22.5	24.6	-15.0	IRTH937	29.5	24.1	-14.3
IRTH928	19.0	24.5	-15.1	IRTH938	25.0	24.6	-14.1
IRTH929	15.0	23.7	-15.0	IRTH939	21.5	24.9	-14.1
IRTH9210	12.0	23.3	-15.0	IRTH9310	18.0	24.5	-14.1
IRTH9211	8.5	23.0	-14.8	IRTH9311	14.5	24.6	-13.5
IRTH9212	5.5	22.9	-14.3	IRTH9312	11.0	24.6	-13.5
				IRTH9313	8.0	23.4	-13.9

Animal IRTH B (M^{3}R)			
IRTHB31	35.5	25.8	-13.9
IRTHB32	33.0	25.7	-14.1
IRTHB33	30.5	25.5	-14.0
IRTHB34	28.0	25.3	-14.0
IRTHB35	25.5	25.4	-14.0
IRTHB36	23.0	25.3	-14.0

				IRTHB38	18.5	24.4	-13.6
				IRTHB39	16.0	24.0	-13.5
				IRTHB310	13.5	23.7	-13.6
				IRTHB311	10.5	23.7	-13.7
				IRTHB312	6.5	23.6	-13.8

Animal GAY 1 (M$_2$R)				**Animal GAY 1 (M$_3$R)**			
GAY 121	30.0	26.3	-13.5	GAY 131	39.5	23.9	-13.8
GAY 122	27.5	26.1	-13.9	GAY 132	37.5	24.1	-13.9
GAY 123	25.0	25.9	-13.7	GAY 133	35.0	24.4	-13.9
GAY 124	23.0	25.2	-13.7	GAY 134	32.5	24.5	-13.9
GAY 125	20.0	25.0	-13.6	GAY 135	29.5	24.6	-13.9
GAY 126	17.0	24.8	-13.6	GAY 136	27.0	24.6	-14.1
GAY 127	14.5	24.2	-13.5	GAY 137	24.5	24.5	-14.0
GAY 128	11.5	23.8	-13.5	GAY 138	19.0	23.6	-13.8
GAY 129	9.0	23.8	-13.2	GAY 139	17.0	22.8	-13.6
GAY 1210	6.5	23.4	-13.1	GAY 1310	14.0	22.9	-13.6
GAY 1211	4.0	23.3	-13.3	GAY 1311	11.5	22.6	-13.6
				GAY 1312	9.0	22.9	-13.2
				GAY 1313	6.5	22.9	-13.1

Animal GAY 2 (M$_2$L)				**Animal GAY 2 (M$_3$L)**			
GAY 221	22.0	25.0	-14.2	GAY 231	33.0	23.0	-14.8
GAY 222	19.0	24.8	-14.7	GAY 232	30.0	23.0	-14.4
GAY 223	16.5	25.3	-14.9	GAY 233	27.0	22.8	-14.5
GAY 224	14.0	25.5	-15.2	GAY 234	24.5	23.0	-14.4
GAY 225	11.0	25.1	-15.2	GAY 235	21.5	23.2	-14.4
GAY 226	8.5	24.6	-15.5	GAY 236	18.5	23.5	-14.6
GAY 227	6.0	24.1	-15.4	GAY 237	16.0	23.7	-14.3
GAY 228	3.5	24.2	-15.2	GAY 238	13.0	24.8	-14.5
				GAY 2310	7.5	25.8	-15.1
				GAY 2311	5.0	25.5	-15.2
				GAY 2312	2.5	25.5	-14.8

Animal GAY 4 (M$_2$R)				**Animal GAY 4 (M$_3$R)**			
GAY 421+422	23.25	24.8	-14.5	GAY 431	35.0	23.0	-15.1

GAY 424+425	16.0	24.9	-15.1
GAY 426	12.5	25.3	-15.3
GAY 427	10.0	25.1	-15.4
GAY 428+429	7.0	24.5	-15.3

GAY 433	29.0	22.7	-14.5
GAY 434	26.0	22.9	-14.5
GAY 435	23.0	22.6	-14.5
GAY 436	20.5	23.2	-14.5
GAY 437	18.0	23.7	-14.3
GAY 438	15.0	24.3	-14.3
GAY 439	12.0	24.7	-14.7
GAY 4310	9.0	25.3	-15.0
GAY 4311	6.5	25.3	-15.1
GAY 4312	3.5	24.8	-14.9

Animal GAY 7 (M_2R)

GAY 721	36.0	23.6	-15.0
GAY 722	32.5	23.3	-14.7
GAY 723	29.0	23.0	-14.5
GAY 725	21.0	23.2	-14.2
GAY 726	17.5	23.1	-14.2
GAY 727	14.5	24.1	-14.2
GAY 728	11.5	24.3	-14.2
GAY 729	8.5	25.3	-14.3
GAY 7210	5.0	25.7	-14.4

Animal GAY 7 (M_3R)

GAY 731	46.0	25.4	-14.2
GAY 732	43.0	25.5	-14.3
GAY 733	39.5	25.1	-14.4
GAY 734	36.0	23.9	-14.6
GAY 735	33.0	23.8	-14.7
GAY 736	29.5	24.0	-14.8
GAY 737	25.5	24.2	-14.5
GAY 738	21.5	23.8	-14.8
GAY 739	18.0	23.6	-14.7
GAY 7310	14.5	23.5	-14.6
GAY 7311	10.5	24.1	-14.8
GAY 7312	7.5	24.4	-14.6
GAY 7313	4.0	24.9	-14.5

Animal GAY 8 (M_3R)

GAY 831	33.5	24.8	-14.8
GAY 832	31.5	24.7	-14.7
GAY 833	29.5	24.1	-14.8
GAY 834	27.5	24.2	-14.5
GAY 835	25.0	24.2	-14.5
GAY 836	22.5	24.0	-14.6
GAY 837	20.5	23.4	-14.4
GAY 838	18.5	23.3	-14.2
GAY 839	16.0	23.4	-14.0

| GAY 8310 | 13.5 | 23.3 | -14.0 |
GAY 8311	11.0	23.2	-14.1
GAY 8312	8.5	23.1	-14.2
GAY 8313	6.0	23.8	-13.8
GAY 8314	4.0	24.0	-14.1

Table 3. Minimum, maximum and mid range values of δ^{18}O and δ^{13}C for Irthlingborough and Gayhurst enamel.

Site	$\delta^{18}O_{VSMOW}$ (⁰/₀₀)			$\delta^{13}C_{VPDB}$ (⁰/₀₀)		
	min. value	max. value	mid range	min. value	max. value	mid range
Irthlingborough (*n* = 93 enamel samples; 5 animals)	22.2	25.8	24.0	-15.5	-13.4	-14.5
Gayhurst (*n* = 95 enamel samples; 5 animals)	22.6	26.3	24.5	-15.5	-13.1	-14.3

Figures

Figure 1. Outline map of Britain showing the locations of Irthlingborough and Gayhurst.
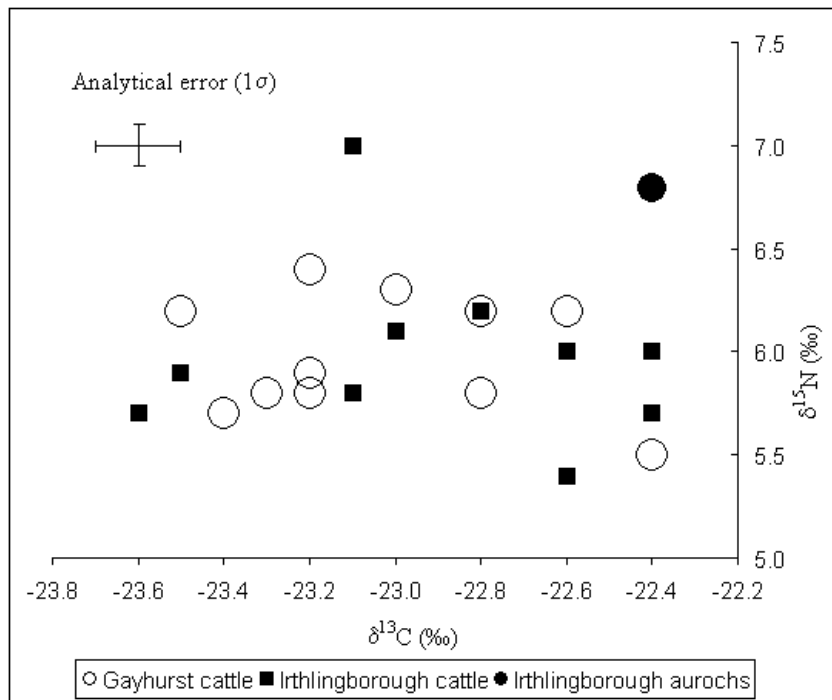
Figure 2. $\delta^{13}C$ and $\delta^{15}N$ values for Gayhurst and Irthlingborough cattle and aurochs bone collagen.
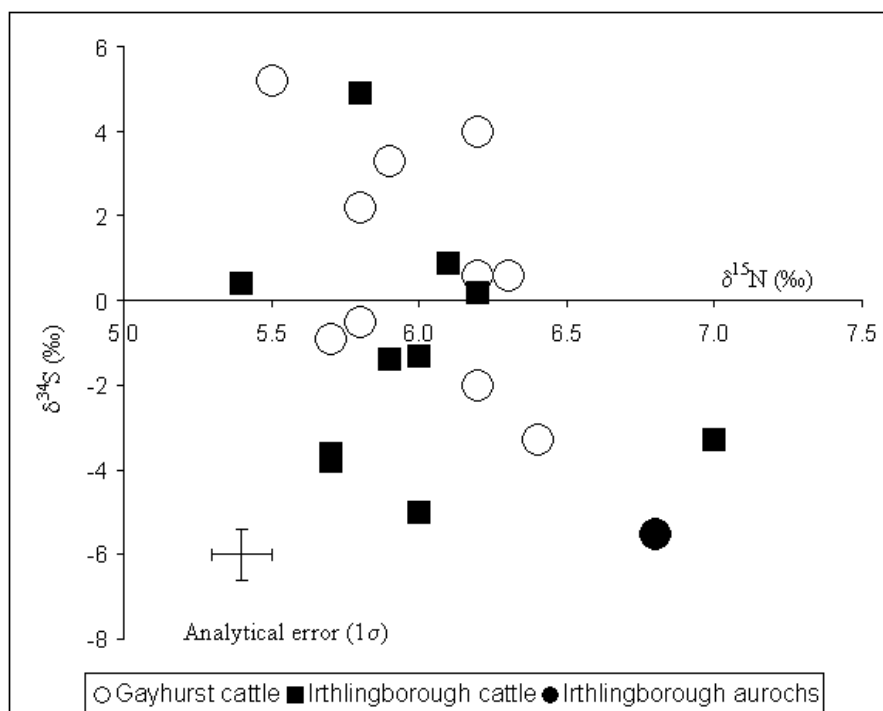
Figure 3. $\delta^{34}S$ and $\delta^{15}N$ values for Gayhurst and Irthlingborough cattle and aurochs bone collagen.
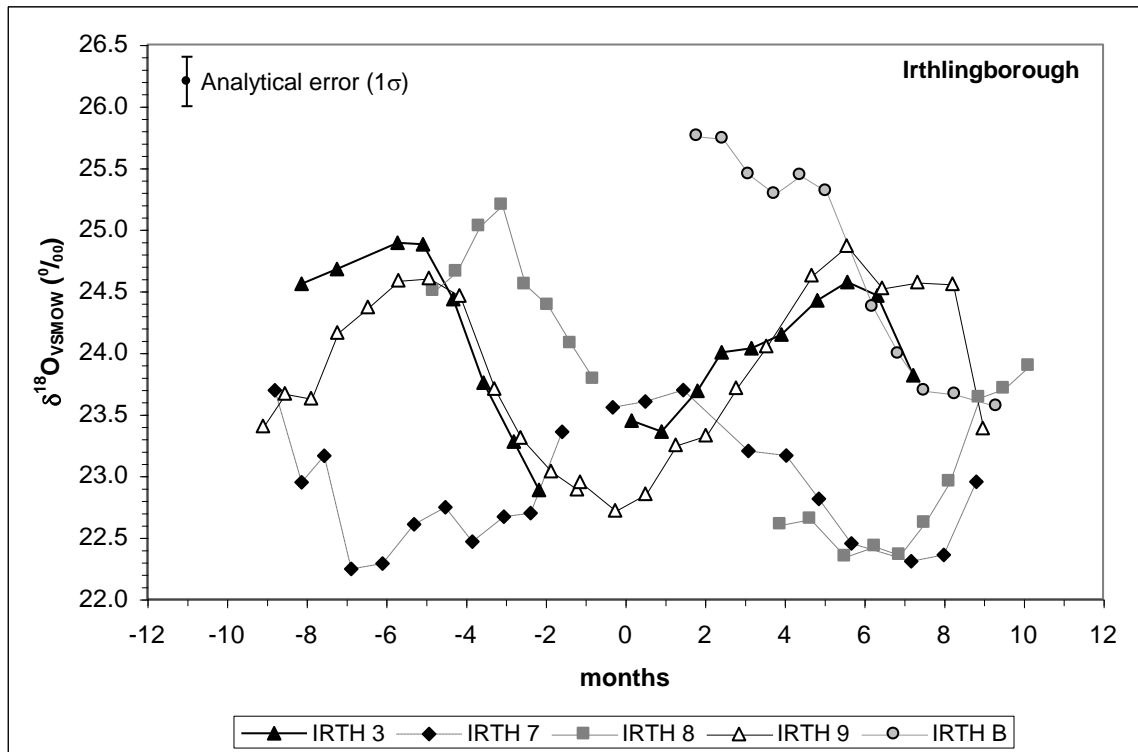
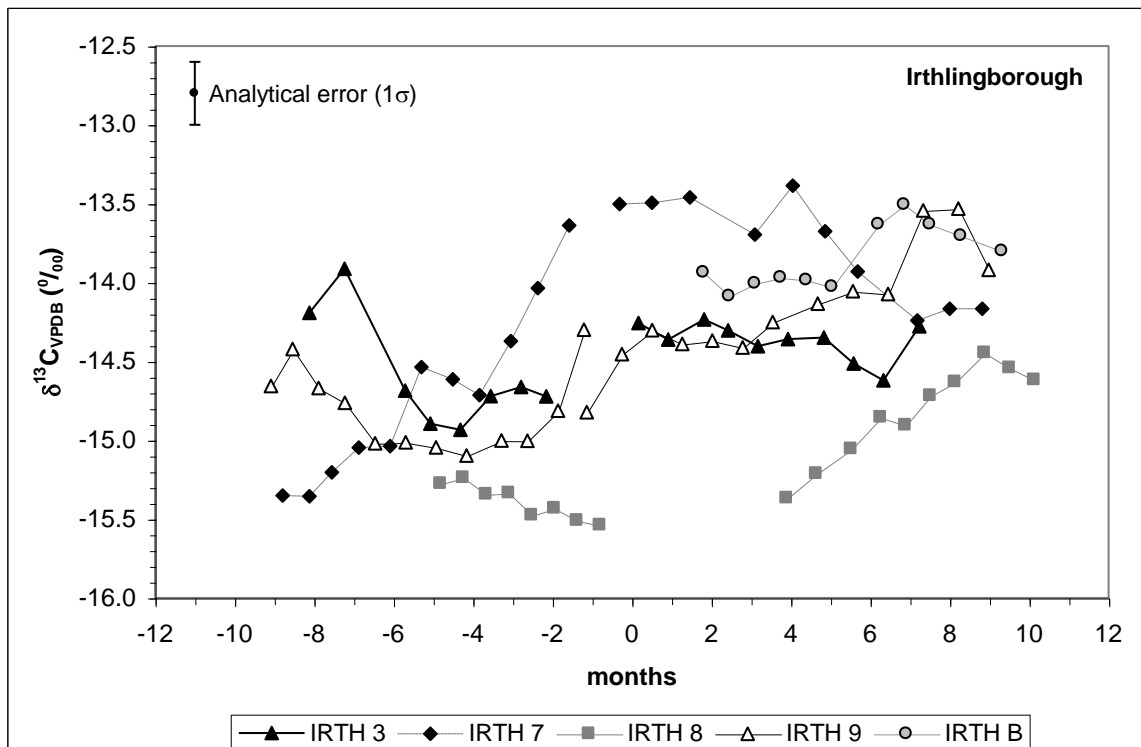**Figure 4.** Combined plot of $\delta^{18}O_{VSMOW}$ versus time of matrix formation for Irthlingborough second and third cattle molar enamel. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix.
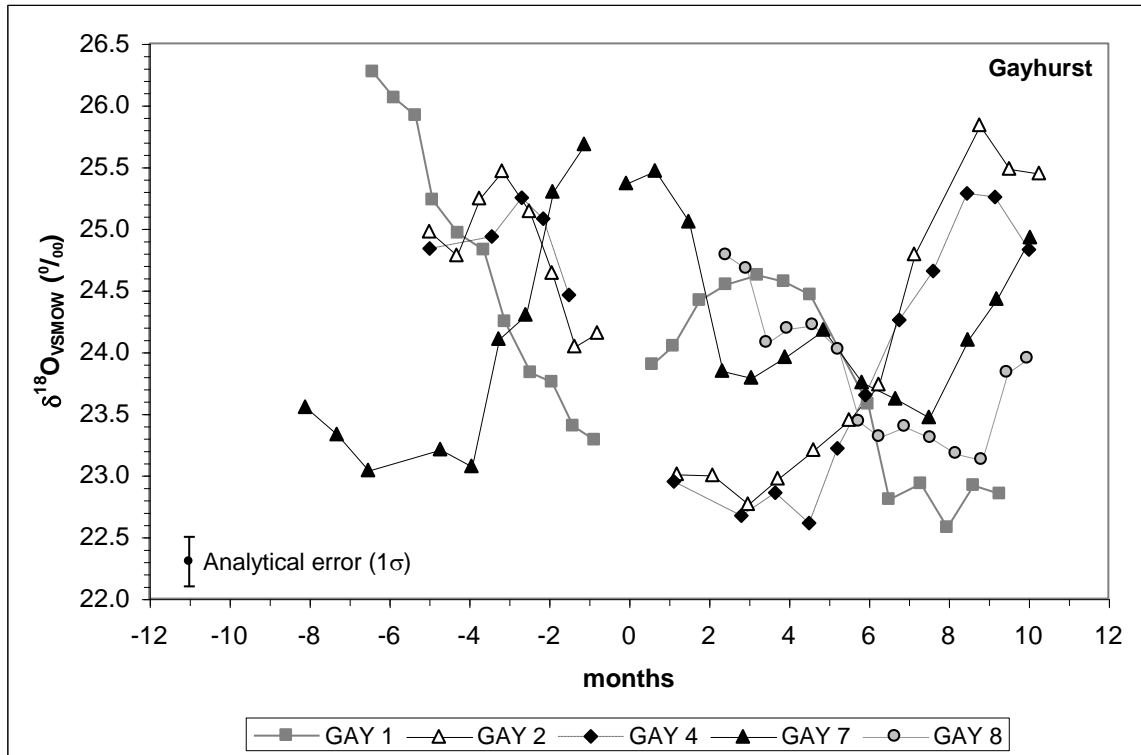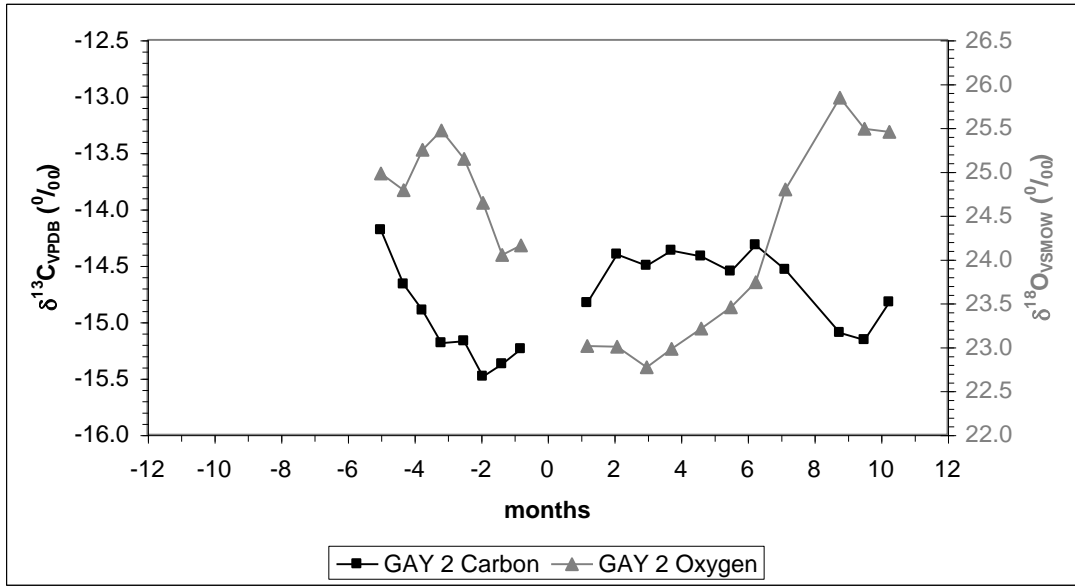
Figure 5. Combined plot of $\delta^{18}O_{VSMOW}$ versus time of matrix formation for Gayhurst second and third cattle molar enamel. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix.
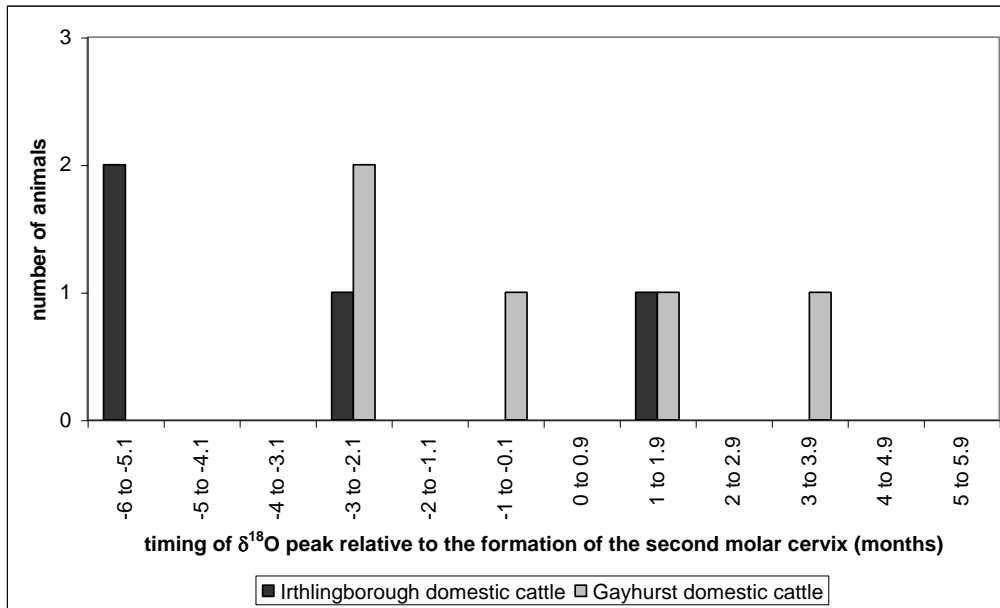
Figure 6. Histogram showing the distribution of $\delta^{18}O$ peaks relative to the formation of the second molar cervical enamel.


Figure 7. Combined plot of $\delta^{13}C_{VPDB}$ versus time of matrix formation for Irthlingborough second and third cattle molar enamel. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix.
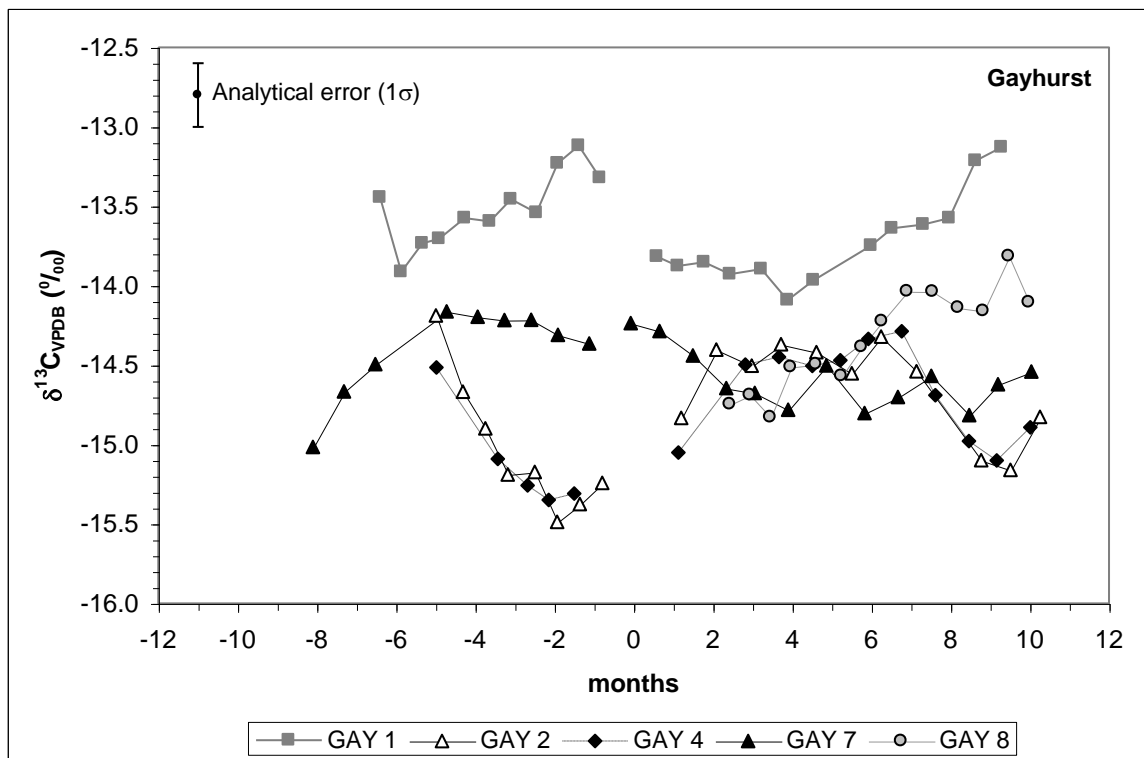
Figure 8. Combined plot of $\delta^{13}C_{VPDB}$ versus time of matrix formation for Gayhurst second and third cattle molar enamel. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix.

Figure 9. $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$ versus time of matrix formation for animal GAY 2. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix. Analytical error is $\pm$ 0.2‰ for both $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$.
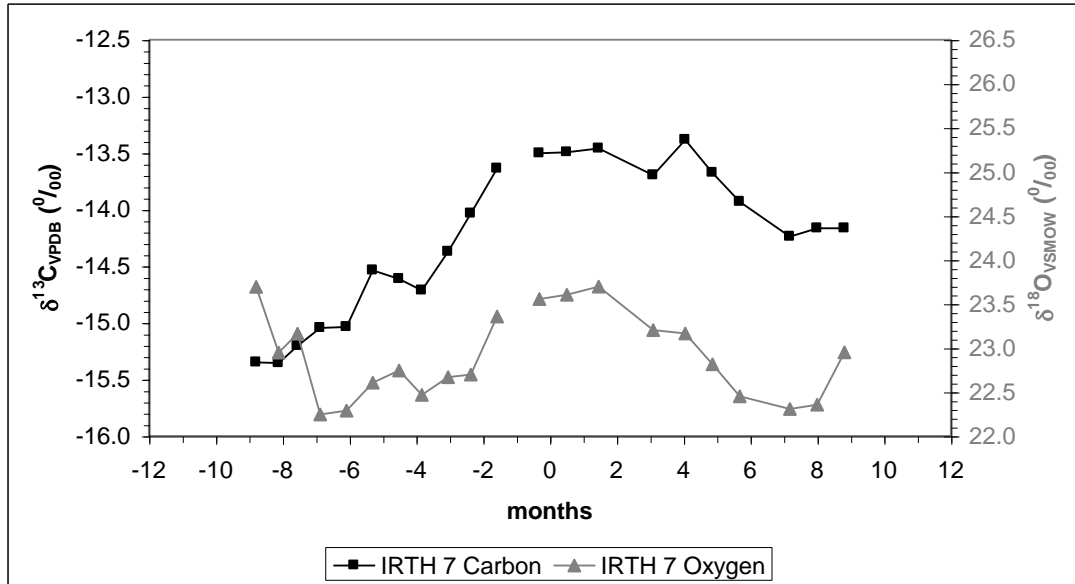
Figure 10. $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$ versus time of matrix formation for animal IRTH 7. Time of matrix formation is months before (-ve) or after (+ve) matrix formation of the second molar cervix. Analytical error is $\pm$ 0.2‰ for both $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$.