Journal of Archaeological Science 180 (2025) 106269

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: www.elsevier.com/locate/jas

Sequential multi-isotope sampling through a *Bos taurus* tooth from Stonehenge, to assess comparative sources and incorporation times of strontium and lead

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ARTICLE INFO

Keywords:
Enamel
Pb isotopes
Sr isotopes
C & O isotopes Neolithic
Stonehenge
Provenance
Movement

ABSTRACT

The aim of this paper is to use the sequential nature of enamel deposition in hypsodont teeth to study the relationship, in time and source, of strontium and lead isotopes to better understand the use of this pairing of elements for studies of movement and migration. Carbon and oxygen isotope analysis were included to place the data in their seasonal and dietary context. The study was undertaken on an M3 from a Neolithic cattle tooth excavated from Stonehenge. The animal was female based on peptide analysis. The tooth records *c.* six months of enamel deposition from winter to summer, based on $\delta^{18}O_{carbVSMOW}$ compositions, and changes in $\delta^{13}C_{carbVSMOW}$ that reflect a shift from forest to grassland food sources. $^{87}Sr/^{86}Sr$ varies from a winter value of 0.7144 to 0.7110 in summer. Lead concentrations and isotope composition shows peaks and troughs which contrast with the unidirectional change in the $^{87}Sr/^{86}Sr$. We suggest that whereas the Sr is wholly derived from dietary sources the Pb represents a balance between diet and skeletal reservoirs, the latter being scavenged during a time of metabolic stress attributed to calving and lactation. It is thus important to consider skeletal reservoirs as a source of Pb when using this element to track movement and migration. This study demonstrates the value of using the complementary isotope systems of lead and strontium in tandem, but also highlights that their integration must be undertaken with caution and with full consideration of alternative drivers of variation.

1. Introduction

Sr isotope composition is a well establish method of assessing human and animal migration across terrains (Montgomery et al., 2009; Viner et al., 2010; Towers et al., 2017a; Lazzerini et al., 2021; Barakat et al., 2023; Madgwick et al., 2019) and there are an increased number of reference maps, displaying variation in bioavailable strontium, and other isotopes, such as oxygen and sulfur across landscapes. Most recently, lead isotopes have been added to this suite of methods for tracing movement and migration.

With this layering of multiple isotopes comes the danger that not all the elements being used are deposited coevally, or from the sample source, for example, the analysis of sulfur is based on collagen analysis and so when used with isotope compositions derived from tooth enamel it is important to consider whether all the data represent the sample point in time when applied to migration analysis.

The aim of this paper is to make use of the sequential nature of enamel deposition in hypsodont teeth to assess how lead and strontium isotopes co vary, and to reflect the geogenic source of these elements, such that they can be used in tandem for migration studies. Carbon and oxygen isotopes are used to place the radiogenic data within a seasonal and dietary context.

1.1. Sample description

A tooth was needed that was free of the possible influence from

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https://doi.org/10.1016/j.jas.2025.106269
Received 13 December 2024; Received in revised form 22 April 2025; Accepted 17 May 2025
Available online 17 June 2025

anthropogenic Pb so that the geogenic uptake of strontium and lead could be studied. Therefore a Neolithic, and hence, pre anthropogenic Pb pollution, tooth was selected. The sample chosen was known, from a previous study, to have a wide range of strontium isotope variation down the cusp. The hope being that this would maximize the opportunity to look at coeval lead uptake. It was excavated from a Chalk-based site which provided a well characterized isotope burial environment.

Tooth sample, SH-3929 is a cattle molar tooth (M3), from the right mandible of an elderly *Bos taurus*, dating to 3350-2920 BC (4460 ± 45 BP), from the bottom of the ditch enclosing Stonehenge Stage 1, constructed in 2995-2900 BC (Marshall et al., 2020). It was excavated in 1924 from the east terminal of the south entrance through the ditch; a right mandible (SH-2480) of similar date but from a smaller adult *Bos taurus* was found at the bottom of the west terminal (Cleal et al., 1995). The two cattle mandibles were probably 55–270 years old (at 68 % probability) when placed on the bottom of the ditch and may have been curated (Allen and Bayliss, 1995). However, the imprecision of their measurements (±45 and ± 40) is more than twice that for high-precision-dated antlers from the bottom of the ditch (Allen and Bayliss, 1995). Quite possibly the mandibles could date to as late as the antlers' Bayesian modelled date of 2995-2900 BC but the balance of probability nevertheless places them as old when deposited. Loss of premolars in both sets of teeth supports this evidence for deposition sometime after death. On the other hand, the mandibles' well-preserved surfaces would suggest that, if they were curated, they had to have been kept in a protected environment before being placed in the ditch (Serjeantson, 1995). Ultimately the evidence for curation is equivocal although it remains the most probable interpretation (Parker Pearson et al., 2022).

1.2. Possible causes of isotope variation in a Neolithic herbivore tooth

1.2.1. Controls on oxygen isotope composition

The principal source of oxygen in herbivore teeth is from the water they drink. Rainwater in Britain has seasonal variability (Darling and Talbot, 2003) so drinking from seasonally charged sources, such as rivers and small lakes, will reflect seasonal changes and this is fixed in herbivore teeth (Towers, 2013; Morandi et al., 2021). There are some moderating conditions which could affect this basic uptake pattern 1) Ground water in Britain retains the long term average of rainfall and this value generally decreases eastward (Darling et al., 2003). Therefore, if an animal consumes water from well-mixed, equilibrated, water sources, its isotope composition will reflect the broad geographic variation of the ground water composition. 2) Evaporation, particularly from shallow lakes, can cause heavy isotope enrichment leading to higher $\delta^{18}O$ values composition (Fronval et al., 1995). 3) Alternative water sources such as moisture derived from plant consumption (Luz et al., 1990; Levin et al., 2006) and 4) Human intervention in the form of transhumance will also effect the oxygen isotope record in herbivore teeth as the change in altitude of water sources tends to suppress the isotope range recorded in the teeth (Varkuleviciute et al., 2021) but, to date, most archaeological studies of British hypsodont teeth reflect seasonal variations typical of rainfall (Towers et al. 2014, 2017a, 2017b; Evans et al., 2019).

1.2.2. Causes of variation in carbon isotope composition of herbivore tooth enamel

The $\delta^{13}C$ values measured in animal tooth enamel, provide valuable insights into diet, management practices, and the environments in which they lived. Understanding these variations helps archaeologists and researchers reconstruct past agricultural and environmental conditions. The animals ingest and fractionate carbon from plants which vary according to plant type and environment (Cerling et al., 1997). Different plants use different photosynthetic pathways (the main types are C3, C4), which affect their $\delta^{13}C$ values. (Ehleringer and Cerling, 2002). C3 plants have lower $\delta^{13}C$ values compared to C4 plants because of differences in their carbon fixation processes. Beyond this primary subdivision other processes can affect the ratios and include light Intensity, water availability, temperature, soil composition and altitude (Heaton, 1999).

Herbivores, by nature, have restricted diets (DeNiro and Epstein, 1978) and, in Neolithic Britian, these would have been dominated by the predominantly C3 plants (Treasure et al., 2019). The main drivers of seasonal variation in a British Neolithic herbivore would be changes in pastures/food sources and include 1) the 'canopy effect' (Merwe and Medina, 1991; Bonafini et al., 2013; Drucker et al., 2008) for animal grazing in, or making use of, forest resource where the $\delta^{13}C$ values of plants growing at ground level under dense tree cover tend to be lower by 2–5 ‰ and 2) marine produces for those grazing close to the coast (Blanz et al., 2020).

1.2.3. The application of Sr isotopes to movement in herbivores

Strontium isotope composition is a well-established method for studying movement and in the past (Price et al., 2002; Perry et al., 2017; Neil et al., 2020). It is based on the principle that animals ingest Sr from their food and that strontium is transferred from the erosion of underlying rocks into soils and from there enters the food chain via plants. These values pass up through the food chain unfractionated. Reference data sets of plants, and other sources provide spatial distribution maps of the Sr ratios against which a sample can be compared (Bataille et al., 2018; Evans et al., 2022a; James et al., 2022; Holt et al., 2021). By excluding areas that do not match the measured reference values, the locations in which the animals grazed can be restricted.

1.2.4. Pb isotope composition in skeletal material

Lead, like strontium, originates from geological sources. It is found in soil in low concentrations and is ingested accidently though hand to mouth transmission, in humans (Calabrese et al., 1997) and through grazing in animals. Once humans exploited Pb as a resource they exposed themselves to much higher Pb levels with associated health risks. As this study is set in Neolithic times, which predates the onset of mining in Britain in the Bronze Age (Williams and Le Carlier de Veslud, 2019), the possible effects of Pb pollution can be excluded. The variation in Pb isotopes across Britain is based on lead mineralization and the isotope composition is related to the timing of major tectonic events. This provides a broad-brush subdivision of Britain into three major Pb tectonic zones with the addition of Chalk/Limestone as a fourth zone (Evans et al., 2022b). The applicability of these rock and mineral zones to biological tracking is in its infancy and factors such as seawater/rainwater contributions are not yet assessed.

1.3. Contrasts in the way the body reacts to strontium and lead

The main aim of this study is to determine whether strontium and lead follow the same path and timescale to deposition in the tooth and thus provide comparable evidence of spatial mobility for a given point in time.

Strontium is non-toxic and non-essential, but it is may help strengthen tooth enamel and bones (Li et al., 2013) and Wang et al. (2019) showed that the presence of Sr in enamel can decrease demineralization. These observations are based on human studies and the transfer of these benefits to animal teeth is not documented, but it would seem reasonable to argue that Sr is not toxic and may be beneficial to the body in strengthening enamel, given its similar properties to calcium. This contrasts with lead.

Lead is a poison to both human and animals (Levin et al., 2021). It enters the blood stream, after ingestion and some is excreted via the kidneys. However, 94 % of the Pb burden in humans is stored in bone and tooth enamel and this can be mobilized back into the blood during events which include advanced age, broken bones, lactation, and pregnancy (ATSDR, 2024). So, while both strontium and lead ultimately have geological sources, the manner in which the body uses/excludes and stores these elements may affect the timing and sourcing of

deposition into the tooth enamel (Müller et al., 2019).

1.4. Peptide analysis to provide the sex of an animal

The use of peptide analysis as a means of determining the sex of an individual was developed for modern forensic studies (Roffey et al., 2000), and subsequently utilized in archaeological studies (Parker et al., 2019; Wasinger et al., 2019). Most recently this has been developed and applied to animals, both modern and ancient (Kotli et al., 2024). The method is based on the differing composition of Amelogenin which has two forms: AMELX (found on the X chromosome) and AMELY (found on the Y chromosome). Males typically have both AMELX and AMELY, while females only have AMELX. Analysis if the peptide chains are determined by mass spectrometry and the spectral data are interpreted and identified using a software package. While this is a useful tool for determining sex it should be noted that processes such as diagenesis can lead to protein degradation and anomalous/erroneous interpretations.

2. Sample preparation

A full cusp face of enamel was cut from an M3 tooth, using a flexible diamond rotary blade. The enamel sample was cleaned by abrasion, using a diamond bur to a depth of about 100 μm to remove surface contamination on the outer surface of the enamel, and residual dentine from the inner surface (Montgomery, 2002). It was then leached for 5 min in 5 %HNO3, to further ensure clean surfaces. The sample, measuring 35 mm in length, was then horizontally cut into nine slices so that each slice represented a stage in the growth of the tooth. The samples were numbered from the top slice 1) of the tooth (worn surface) down to slice 9 at the enamel dentine junction (EDJ). These enamel slices were then rough crushed, using an impact mortar and split into three subsamples*: c.*3 mg for O isotope analysis, *c.*5 mg for Pb concentration analysis and the remaining material (50–70 mg) for Sr and Pb isotope analysis. The samples for oxygen isotope analysis were further reduced to a powder in an agate mortar and pestle.

2.1. The preparation and isotope analysis of Pb and Sr in tooth enamel

The enamel samples were transferred to a class 100 clean suite where they were further cleaned in Teflon© distilled 2M HNO_3 acid, for ~5 min and then rinsed twice in de-ionised water, and left to soak in water for an hour on a hot plate set at 60 °C. Following this, the sample was again rinsed twice in de-ionised water, and then dried and weighed into a pre-cleaned Teflon© beaker.

The samples were then dissolved in Teflon© distilled 8M HNO_3. After evaporation to dryness, the sample was converted to bromide form by addition of 0.5M Ultrapur© HBr. The Pb fraction was collected using Eichrom© AG1X8 anion resin (Dickin, 1995). The residue from this separation was evaporated to dryness and converted to chloride form by addition of Teflon© distilled 6MHCl. Sr was collected from this fraction using Eichrom© AG50 X8 resin (Dickin, 1995).

2.2. Geochemical analysis of the tooth enamel

The *c.*5 mg samples of enamel for geochemical analysis were weighted into a beaker and dissolved in Teflon© distilled 8M HNO_3 and evaporated to dryness. These were then transferred to BGS Analytical Geochemistry for analysis.

The digested and dried down tooth sample was dissolved in a mixture of 1 % HNO_3 and 0.5 % HCl. The volume of solution added was varied to produce a solution with between 50 and 100 mg/L calcium, based on the original mass of tooth material. This concentration range is optimal for best detection limits, while minimising ICP-MS matrix effects.

These solutions were analysed on an Agilent 8900 ICP-MS/MS system using a He collision cell gas for the elements of interest. The system was calibrated using a multi-element solution prepared in a range of dilutions to encompass the expected Sr and Pb concentrations within the sample. In addition to the regular analysis of the QC solutions, during the analytical run, a series of reagent blanks were also analysed. Any matrix effects were normalised out using added internal standards including Sc, Ge, Rh, In, Te and Ir. Reproducibility of low concentration Pb in solution is ±3 % RSD (n = 76).

2.3. Strontium isotope analysis

Strontium was loaded onto a single Re Filament following the method of (Birck, 1986) and both the isotope composition and strontium concentrations were determined by Thermal Ionisation Mass Spectrometry (TIMS) using a Thermo Triton multi-collector mass spectrometer. The international standard for $^{87}Sr/^{86}Sr$, NBS987, gave a value of 0.710262 ± 0.000020 (2SD, n = 8) during the analysis of these samples and the data are normalised to the accepted value of 0.710250 (Li et al., 2021).

2.4. Lead isotope analysis

Pb isotope analysis of the samples was conducted using a Thermo Fisher Neptune Plus MC-ICP-MS (multi-collector inductively coupled plasma mass spectrometer). This mass spectrometer is fitted with the Jet interface, in which enhanced sensitivity is achieved through the use of a large volume interface pump (Pfeiffer On-Tool Booster 150) in combination with the Jet sampler and X skimmer cones. Prior to analysis, each sample was appropriately diluted (using Teflon distilled 2 % HNO_3) and spiked with a solution of thallium (Tl), which is added (in a ratio of ~1_Tl:10_Pb) to allow for the correction of instrument induced mass bias. Samples were then introduced into the instrument via an ESI 50 μl/min PFA micro-concentric nebuliser attached to a desolvating unit, (Cetac Aridus II). All isotopes of interest were simultaneously measured using the Faraday cup configuration detailed in Table 1. The acquisition consisted of 50 ratios, collected at 8.4-s integrations, following a 60 s defocused baseline measurement made at the beginning of each analytical session.

The precision and accuracy of the method was assessed through repeat analysis of NBS 981 Pb reference solution, (also spiked with Tl). Based on the estimate that the tooth would contain c 0.1 ppm of Pb this should yield 5 ng Pb for analysis. Data are corrected (normalised) relative to the known values for this reference, (taken from Thirlwall (2002): $^{206}Pb/^{204}P = 16.9417$, $^{207}Pb/^{204}Pb = 15.4996$, $^{208}Pb/^{204}Pb = 36.724$, $^{207}Pb/^{206}Pb = 0.91488$, $^{208}Pb/^{206}Pb = 2.1677$. The analytical errors, reported for each of the sample ratios, are propagated relative to the reproducibility of the session NBS 981, to take into account the errors associated with the normalization process. A secondary standard, (NBS 982), with a defined value of $^{206}Pb/^{204}Pb = 36.7432$, $^{207}Pb/^{206}Pb = 0.467084$, $^{208}Pb/^{206}Pb = 1.00016$ (Baker et al., 2004) gave $^{206}Pb/^{204}Pb = 36.7462$, $^{207}Pb/^{206}Pb = 0.46715$, $^{208}Pb/^{206}Pb = 1.00043$ after normalization to NBS981.

2.5. The preparation and isotope analysis of O in tooth enamel

The approximately 3 mg of powdered enamel were loaded into a glass vial and sealed with a septa. The sample vials were transferred to a hot block at 90 °C on the GV Multiprep system. The vials were evacuated and 4 drops of anhydrous phosphoric acid added. The resultant CO_2 was collected cryogenically for 15 min and transferred to a GV IsoPrime dual inlet mass spectrometer for isotope measurement. $\delta^{18}O$ is reported as per mil (‰)($^{18}O/^{16}O$) normalised to the PDB scale using a within-run calcite laboratory standard (KCM) calibrated against NBS-19 IAEA reference material and were converted to the SMOW scale using the published conversion equation of (Coplen, 1988): $SMOW=(1.03091 \times \delta^{18}O_{VPDB}) +30.91$. Analytical reproducibility for this run of laboratory standard calcite (KCM) for $\delta^{18}O$ $(‰)_{VSMOW}$ is±0.05 ‰ (1 σ, n = 39) and $\delta^{13}C_{VPDB}$

Table 1
)[1] Measured to allow for the correction of the isobaric interference of ^{204}Hg on ^{204}Pb*[2] Measured to allow for the correction of instrumental mass bias.

High 4	High 3	High 2	High 1	Axial	Low 1	Low 2	Low 3	Low 4
	^{208}Pb	^{207}Pb	^{206}Pb	^{205}Tl *[2]	^{204}Pb ^{204}Hg	^{203}Tl *2	^{202}Hg *[1]	

is ±0.04 ‰ (1s, n = 39). External reproducibility of the enamel data based on the analysis of duplicate sample pairs, gives $\delta^{18}O_{carbSMOW}$ ± 0.10 (1SD, n = 8) and $\delta^{13}C_{carbVPDB}$ = 0.04 (n = 8)

All isotope data are presented in Table 2.

2.6. Peptide analysis

A piece of enamel weighing approximately ~5 mg was incubated in 0.5 mL of 0.5 M ethyldiamine tetraacetic acid (EDTA; pH 8) overnight (~18 h) at room temperature. After centrifugation at 12,400 rpm, peptides from 100 μL of this were then purified using a Vivaspin C18 solid phase extraction cartridge into 50 % acetonitrile/0.1 % trifluoroacetic acid and subsequently dried to completion. This dried fraction was then digested using sequencing grade trypsin (Promega, UK) and left overnight at 37 °C. This was then applied to a prepared filter laced with 10 μL POROS R3 beads for peptide capture. Preparation of the filter plates involved washing the beads once with 200 μL 50 % acetonitrile (ACN) and twice with 200 μL 0.1 % formic acid (FA), each followed with centrifugation (1 min, 2000 rpm). The sample was incubated with the beads for 5 min at room temperature while shaking at 500 rpm, and the liquid removed by centrifugation as above, followed by two further washes with 0.1 % FA. Elution was then carried out with two 50 μL volumes of 30 % ACN/0.1 % FA each centrifuged as above and then dried down to completion by centrifugal evaporator. After resuspension with 10 μL 5 % ACN/0.1 % FA, 2 μL of the sample was subject to LC-MS/MS analysis using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a QE HF (Thermo Fisher Scientific, Waltham, MA) mass spectrometer. The mobile phase A was 0.1 % FA and mobile phase B was 0.1 % FA/ACN and the column used was a 75 mm × 250 μm i.d. 1.7 mM CSH C18, analytical column (Waters). A 1 μl aliquot of the sample was transferred to a 5 μl loop and loaded on to the column at a flow of 300 nl/min for 5 min at 5 % B. The loop was then taken out of line and the flow was reduced from 300 nl/min to 200 nl/min in 0.5 min. Peptides were separated using a gradient that went from 5 % to 18 % B in 63.5 min, then from 18 % to 27 % B in 8 min and finally from 27 % B to 60 % B in 1 min. The column was washed at 60 % B for 3 min before re-equilibration to 5 % B in 1 min. At 85 min the flow was increased to 300 nl/min until the end of the run at 90 min. Mass spectrometry data were acquired in a data-directed manner for 60 min in positive mode. Peptides were selected for fragmentation automatically by data-dependent analysis on a basis of the top 12 peptides with *m/z* between 300 and 1750 Th and a charge state of 2, 3 or 4 with a dynamic exclusion set at 15 s. The MS resolution was set at 120,000 with an AGC target of 3e6 and a maximum fill time set at 20 ms. The MS2 Resolution was set to 30,000, with an AGC target of 2e5, a maximum fill time of 45 ms, isolation window of 1.3 Th and a collision energy of 28. The amalgamated MS/MS spectra (4491 spectra, as a.MGF file) were then searched against the SwissProt database, specifiying *Bos taurus* (5999 sequences) with the variable modifications of deamidation (N/Q), oxidation (M), and phosphorylation (S/T), a peptide mass tolerance of 5 ppm, and a fragment mass tolerance of 20 ppm, with a no enzyme search.

3. Results

3.1. Oxygen isotopes

The oxygen isotope composition shows a range of values with a minimum value in slice 2 of $\delta^{18}O_{carbVSMOW}$ +23.6 ‰ and maxima $\delta^{18}O_{carbVSMOW}$ in slice 6 of 25.6 ‰ (Fig. 1). The range of 1.9 ‰ is consistent with British archaeological M3 cattle teeth ranges (Towers et al., 2014). Using the curve fitting of (Balasse et al., 2012a) we conclude that the data represent about half a year of enamel growth from just before the seasonal minimum of rainwater (winter/January) to just past the seasonal maximum rainwater (summer/July) (Darling and Talbot, 2003). As this is an M3, which develops between 10 and 24 months, the preserved enamel represents 6 months of mineralization during the second year of the animals life. (Brown et al., 1960). This provides a time frame within which to interpret the other isotope systems.

3.2. Carbon isotopes

The $\delta^{13}C_{carbVPDB}$ values form a sinusoidal curve, similar to that of the oxygen isotopes rising from a winter minimum of −14.6 ‰ to a summer peak value of −12.8 ‰ (Fig. 2) giving a composition range of 1.8 ‰.

3.3. Strontium isotopes

The Sr isotope ratios seen in this enamel define a curve from a winter value that peaks at $^{87}Sr/^{86}Sr = 0.7144$ from the top of the tooth (slice 35 mm from EDJ) to a summer value of $^{87}Sr/^{86}Sr = 0.7114$ (slice 9) (Fig. 3a). The two oldest, winter slices, have similar values giving a plateau for this part of curve and this could be taken to indicate that such values represent, or are close to, an end member composition. The last, youngest, enamel (slice 9) does not show any sign of plateau and therefore it can only be stated that the endmember is lower than the last measured value of 0.7114.

A plot of $^{87}Sr/^{86}Sr$ isotope composition vs Sr concentration displays the potential mixing line more clearly (Fig. 3b). It is modelled against two theoretical end members using an upper end member composition of Sr ppm 165 and $^{87}Sr/^{86}Sr = 0.7144$ and a lower member of Sr ppm 210 ppm and $^{87}Sr/^{86}Sr$ 0.7110. There is long term retention of Sr in the skeleton and body pool, or long-term maturation of the enamel at a microscopic scale (Montgomery et al., 2009). It is not possible, therefore, to be certain that the Sr ratios we measure reflect end-member biosphere sources, or whether they are part of a mixing profile with more extreme terminal compositions (Moots et al., 2024).

Using the isotope biosphere map (Evans et al., 2022a), the theoretical higher winter value of 0.7144 excludes most of SE England and southern Scotland. The lower summer theoretical value of 0.7110 has a similar, but higher, spatial coverage to the sample regions at the winter value (i.e. excluding most of SE England). These distributions are based on using the central 90 % model option recommended for herbivores (Evans et al., 2022a) (Fig. 4).

3.4. Lead isotopes

The Pb data do not record the smooth changes of composition observed in the oxygen, carbon and strontium isotope data. The $^{207}Pb/^{206}$ Pb and $^{208}Pb/^{206}Pb$ ratios start at 0.8511 and 2.0785 respectively. They peak in slice 4 with ratios of 0.8622 and 2.1035 respectively, and then drop back down to 0.8481 and 2.0755 before rising again to an incomplete peak in slice 9. The Pb concentrations peak in slice 2, at 0.91 ppm, but are below 0.15 ppm in all other slices (Fig. 5a–c). The isotope ratios that represent the baseline (slices 1, 2 and 6) have Pb isotope model ages consistent with Mesozoic deposits which dominate England, but the composition peak in slice 4 has a Palaeozoic

Table 2
Isotope and concentration data for the samples. *T*, μ and κ, calculated using Albarede and Juteau (1984).

slice	distance (mm) from EDJ	$\delta^{13}C$ $(‰)_{carb}$ VPDB	$\delta^{18}O$ $(‰)_{carb}$ VSMOW	Sr ppm	$^{87}Sr/^{86}Sr$	Pb ppm	$^{206}Pb/^{204}Pb$	2s %	$^{207}Pb/^{204}Pb$	2s %	$^{207}Pb/^{206}Pb$	2s %	$^{208}Pb/^{204}Pb$	2s %	$^{208}Pb/^{206}Pb$	2s %	Pb Model Age	mu	kappa
1	35.0	−14.6	23.7	166	0.71434	0.15	18.3418	0.06	15.6105	0.05	0.8511	0.01	38.1227	0.05	2.0785	0.020	270	9.67	3.77
2	31.1	−14.2	23.6	162	0.71444	0.91	18.3392	0.07	15.6062	0.06	0.8506	0.02	38.1380	0.07	2.0793	0.019	263	9.65	3.78
3	27.2	−14.5	23.8	163	0.71415	0.05	18.2839	0.07	15.6016	0.07	0.8533	0.02	38.0769	0.07	2.0827	0.016	296	9.64	3.78
4	23.3	−14.4	23.8	159	0.71381	0.08	18.0984	0.05	15.6029	0.07	0.8622	0.02	38.0659	0.09	2.1035	0.043	437	9.70	3.90
5	19.4	−14.2	24.4	167	0.71341	0.11	18.2478	0.08	15.6102	0.09	0.8555	0.01	38.0873	0.09	2.0871	0.025	340	9.69	3.82
6	15.6	−13.8	24.7	173	0.71293	0.10	18.4164	0.05	15.6182	0.06	0.8481	0.01	38.2179	0.06	2.0755	0.023	229	9.68	3.78
7	11.7	−13.3	25.5	186	0.71226	0.08	18.3642	0.04	15.6237	0.04	0.8507	0.02	38.1762	0.05	2.0788	0.025	278	9.71	3.79
8	7.8	−13.0	25.4	198	0.71174	0.10													
9	3.9	−12.8	25.4	203	0.71137	0.06	18.1205	0.03	15.6057	0.03	0.8612	0.01	37.9106	0.04	2.0920	0.020	426	9.70	3.80

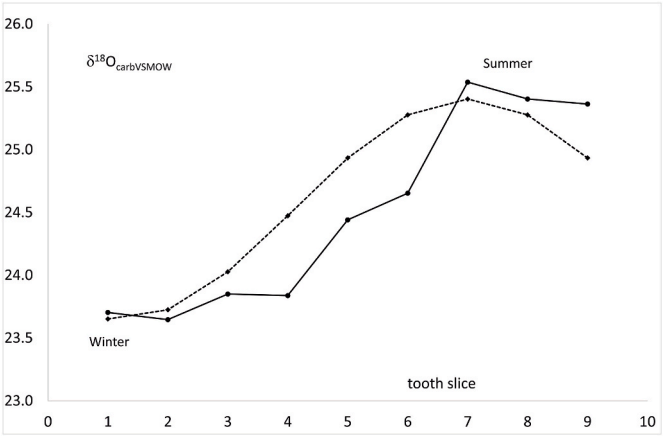

Fig. 1. $\delta^{18}O_{carbVSMOW}$ variations from the oldest (slice 1) top of the crown to the youngest (slice 9) Enamel Dentine Junction (EDJ) with theoretical curve fitted using (Balasse et al., 2012a).

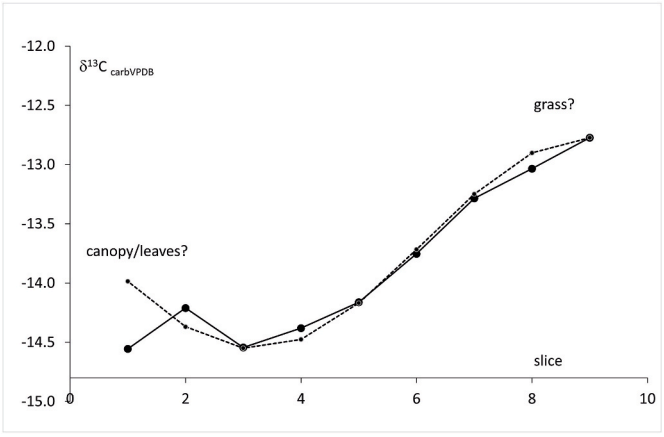

Fig. 2. $\delta^{13}C_{carbVPDB}$ variations from the oldest (slice 1) top of the crown to the youngest (slice 9) Enamel Dentine Junction (EDJ) with theoretical curve fitted using (Balasse et al., 2012a).

model age. A discussion of this pattern is provided below and undertaken in the knowledge that the interpretation of Pb isotope data in fauna, through reference to geological ore data, is a substantial interpretative step.

3.5. Peptide sexing

An initial Mascot search of the amalgamated MS/MS spectra (as.MGF file) revealed that, of the two forms of amelogenin, AMELX, was the most abundant protein present (102 peptide matches). Although the traces of two Y-specific forms were matched (WYQNMLR and YPYPSYGYEPVG/YPYPSYGYEPVGGW), these yielded particularly poor fragmentation spectra, and all are far below the ion score of cut-off of 36 recommended by Mascot for this data. Therefore, from this data alone the specimen could be interpreted as female, though noting the caveats of sexing via proteomics (i.e., false negative males are feasible due to diagenesis). Other proteins also identified as present within the sample included the enamel-specific protein amelobastin, as well as smaller amounts of serum albumin and type 1 collagen.

4. Discussion

The oxygen isotope composition of the M3 tooth slices provides a temporal framework in which the other isotopes can be contextualized.

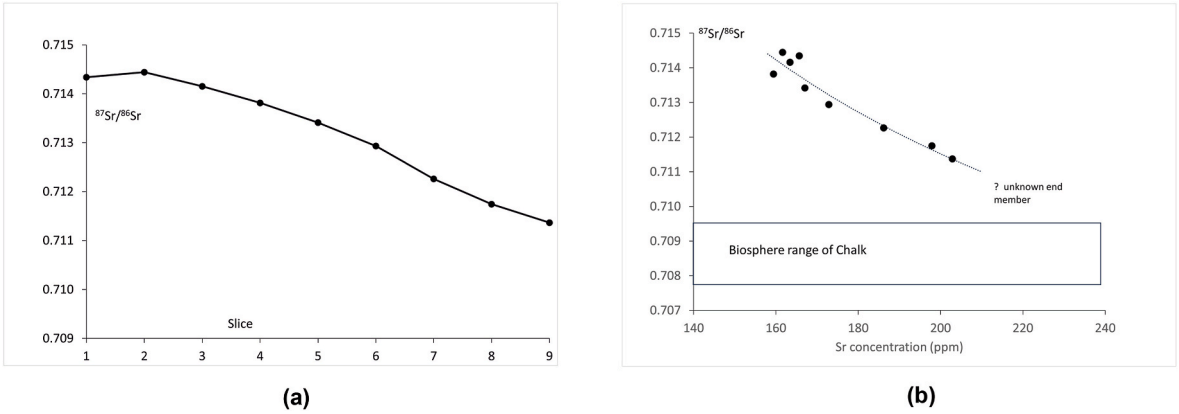

Fig. 3. a&b a) $^{87}Sr/^{86}Sr$ variation from oldest (slice 1) top of crown to youngest (slice 9) at EDJ and b) A mixing curve best fit to the $^{87}Sr/^{86}Sr$ vs Sr concentration (Sr ppm) using mixing equations from (Faure, 1986).

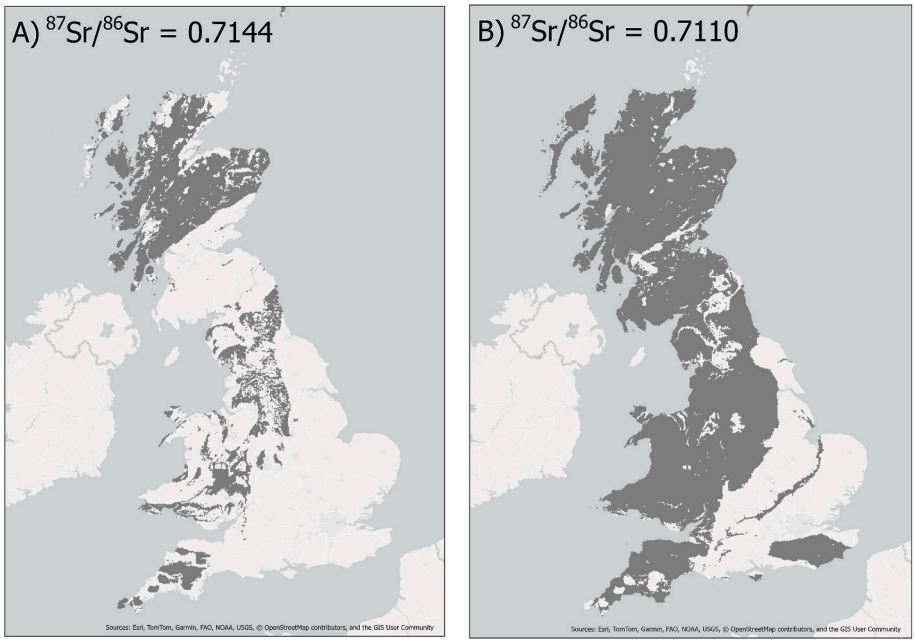

Fig. 4. Plots of the distributions of the $^{87}Sr/^{86}Sr$ ratios in the British biosphere using 90 % of the reference data for a value of a) 0.7144 and b) 0.7110 as an indication of areas that cannot be excluded as a source of food for the winter (slice 1) and summer (slice 9) possible end members.

The oxygen isotope profile shows that the tooth section preserves enamel from an approximate six-month period from winter to summer, of the animals second year of life, and the carbon isotope profile reveals a change from forest resources to open grassland grazing over this time. This provides two interpretations for the change in the Sr isotope ratios over time: 1) the animal moved from forest to grassland, a move that might be local or a more extensive distance or 2) the animal's winter forest food sources came in the form of previously harvested and stored winter fodder produce such as leaves, nuts, and twigs (Rasmussen, 1989; Balasse et al., 2012b).

The migration model simply requires that the animal move from the winter terrain to a substantially less radiogenic terrain over six months, whereas the static model of differing foddering in a farmable area requires the proximity of two different strontium isotope terrains. It is possible for very different Sr isotope domains to be abutted if the underlying geology is sufficiently different, but such a domain change can also be recorded on a single lithology and is caused by the effect of forestation (Johnson et al., 2022). As a deciduous forest will provide little forest foraging during winter, it is, perhaps, more likely that the winter signal is caused by the animal being fed on stored resources such as leaf/tree hay (Halstead, 1998), consistent with the better known more modern practice of storage and feeding grass hay in winter.

The Pb data points to a more complex picture than the Sr over the 6 months of tooth formation. The majority of the Pb isotope compositions are below $^{208}Pb/^{206}Pb = 2.085$, and $^{207}Pb/^{206}Pb = 0.854$. However, there is a clear spike in composition in slice 4 to $^{208}Pb/^{206}Pb = 2.104$ and $^{207}Pb/^{206}Pb = 0.862$ with a further upturn towards similar values on slice 9 (EDJ). The animal accessed a substantially different Pb isotope source in spring (slice 4). It is difficult to reconcile this pattern of change with the Sr data that indicates a progressive change in environmental Sr source over the history of the tooth. The lack of correlation between the Pb and Sr argues against the Sr and Pb reflecting coeval and consanguineous uptake from an external source. A number of possible explanations exist for this. 1) Diagenetic alteration. The low concentration of Pb in the tooth enamel (0.05–0.9 ppm) means the enamel is more susceptible to Pb diagenetic alteration from an external source than Sr where the enamel concentrations are higher (159–203 ppm). Could this explain the different patterns seen between the Sr and Pb isotope patterns? This explanation seems unlikely as the tooth was excavated from a Chalk substrate, the Pb isotope composition of which would drive the

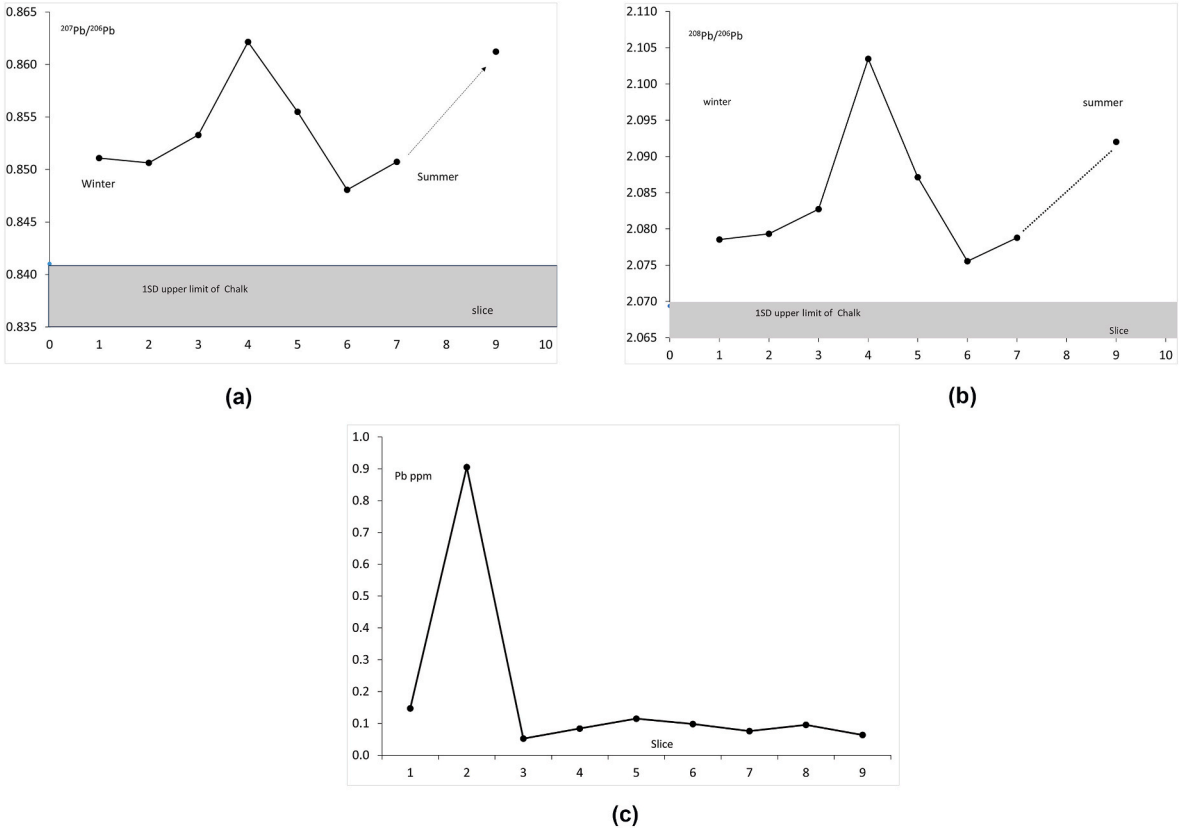

Fig. 5. a–cPlots of the variation in a) $^{207}Pb/^{206}$ Pb b) $^{208}Pb/^{206}Pb$ and c) Pb concentrations (Pb ppm) down the tooth.

$^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios to be lower, rather than higher (Fig. 5a and b). 2) The discrepancy could be related to different deposition times for Sr and Pb within the tooth structure. Müller et al. (2019) suggest that the affinity Pb has for organic material means that Pb is locked into the enamel structure during secretion when the ameloblasts form and before full mineralization is complete, whereas Sr is associated with the inorganic mineralization process. This model requires a disconnect between the geological sources of Sr and Pb such that the Sr can be modelled as a two-end member transition down the tooth whereas the Pb source switches twice between Mesozoic and Palaeozoic sources without disrupting the Sr curve. While this model is not discarded it is difficult to model geologically (i.e. it is difficult to identify source zones that could explain this pattern). 3) The disconnect could be caused by the release of previously deposited Pb due to physiological stress.

The primary cause of Pb re-mobilization in humans and animals is stress induced scavenging of elements from the skeleton and is commonly associated with pregnancy and lactation. This takes the form of bone dissolution, with the aim of releasing Ca into the blood for embryo bone growth, and/or or milk production (Liesegang et al., 2006). However, other elements, stored in the Ca sites of the bone apatite, such as Pb, will also be released and are available for deposition in tooth enamel mineralization (Gulson et al., 1998; Spencer, 1979). Such a model could be applied to this study as the peak in Pb release, which has a distinctive isotope composition, occurs in spring, a likely time for a calf to be born. Fig. 6 provides a graphical method for relating the Pb isotope composition of a sample to geological and, by association, geographic regions. The model age (*T*) is indicative of the time at which Pb was separated from its source rock and the μ value is related to the geochemical composition of the source lithology (Albarede et al., 2012). Three reference fields provide the framework for interpretation, and these are constructed as the 1SD range of μ and *T* from published papers and reports (Rohl (1996, Rohl and Needham, 1998, Blaxland et al., 1979, Barreiro, 1995, and Fletcher et al., 1993) after a 10 % exclusion was applied to outliers. The Welsh and English ore fields both have μ values above 9.8 but differ in age. The field of Southern Uplands ores shows the characteristic lower μ values of Scottish ores. The Pb isotope ratios measured in the tooth suggest that the skeletal lead could originate from a Pb source of similar age to Welsh ores whereas the dietary Pb is more consistent with English ore Pb. The μ values would seem to exclude a more northerly association. While this is currently a tentative conclusion it opens up the possibility of using Pb concentration spikes, and changes in isotope composition, to trace physiological stress, particularly in relation to pregnancy.

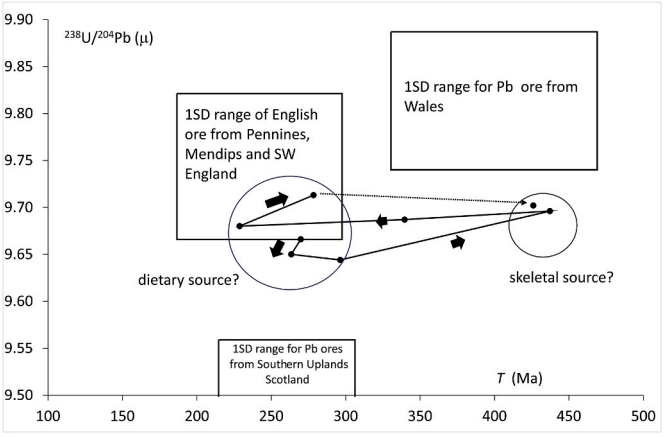

Fig. 6. The Pb isotope data are presented in the form of μ ($^{238}U/^{204}Pb$) vs *T* (Ma) (Westner et al., 2012; Albarede and Juteau, 1984). The figure shows the relationship between the composition of each enamel slice relative to the 1SD data ranges of British Pb ore data sets.

5. Conclusions

The tooth enamel from this Neolithic cow, excavated from the Stonehenge enclosure ditch, provides an insight into foddering (carbon), seasonality (oxygen), geographic origin (strontium), sex (peptides) (lactation- Pb release) and deeper skeletal history (lead). The tooth data record approximately the last six months of enamel formation in the animal's second year of life. At some time, before the mineralization of this M3, this cow had access to a Palaeozoic Pb source with a composition $^{208}Pb/^{206}Pb \geq 2.104$ and $^{207}Pb/^{206}Pb \geq 0.862$, which was locked in the animal's bones. The section of the M3 that is preserved shows that in winter the animal was fed on forest derived products with an $^{87}Sr/^{86}Sr$ isotope composition around 0.7144. As the seasons progressed into summer the animal was grazing on more open grassland with an unknown $^{87}Sr/^{86}Sr$ composition below 0.7110. During this time there were fluctuations in the Pb concentration and Pb isotope composition down the tooth into summer. As these fluctuations do not correlate with $^{87}Sr/^{86}Sr$ variation and are not attributed to diagenetic effects, we suggest they were due skeletal scavenging of Pb, during calving and lactation, which released Pb with a different isotope composition from its dietary intake.

The remains of this elderly animal were found buried at Stonehenge. It is not known if it travelled to Stonehenge alive, or its remains were, curated and deposited there. However, it is possible that the animal held some significance to the population as the cow probably died 55–270 years (at 68 % probability) before being placed on the bottom of the ditch and may have been be curated (Serjeantson, 1995; Allen and Bayliss, 1995)

This study has revealed some important considerations when looking at multi-isotope variation in tooth enamel. By linking the changes in carbon and oxygen with those in strontium we can see that the Sr isotope terrain changes are related to vegetation type and seasonality and while the shift in Sr from *c.* 0.7144 to <0.711 might previously have been see as indication a significant migratory event, new studies show that this could be either localized movement or simply movement of feed rather that the animal itself. The discrepancies between strontium (unidirectional composition change down the tooth) and Pb isotope composition (peaks and troughs) highlights the different metabolic pathways of Sr and Pb and show that care must be taken, if the two isotopes are used together as a migration tracker, to ensure that both isotopes are monitoring coeval and consanguineous isotope sources. Finally, fluctuations in Pb concentration and isotope composition may be indicative of birth induced skeletal stress in female animals.

CRediT authorship contribution statement

J. Evans: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **R. Madgwick:** Writing – review & editing, Validation. **V. Pashley:** Methodology, Formal analysis. **D. Wagner:** Methodology, Formal analysis. **K. Savickaite:** Methodology, Formal analysis. **M. Buckley:** Writing – review & editing, Methodology, Formal analysis. **M. Parker Pearson:** Writing – review & editing, Funding acquisition.

Ethical approval

We have no ethical concerns regarding this paper.

Declaration of generative AI statement

No AI or and AI-assisted technologies were used in the in the writing of this paper.

Declaration of competing interest

None.

Acknowledgements

We thank Tom Barlow of the Analytical Geochemistry section at British Geological Survey for the lead concentration analysis and Katy Mee for help drafting figures. We are grateful to Luca Bondioli, Wolfgang Müller and Robert Anczkiewicz for insightful discussions surrounding Pb, and to two anonymous reviewers. We thank Umberto Albarella for the zooarchaeological work. Funding source: AHRC grant no AH/H000879/1. Feeding Stonehenge: provisioning henges and households in southern Britain in the 3rd millennium BC.

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