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TRACKS: An isotopic investigation of the seasonality of Middle and Upper Palaeolithic humans and fauna in Estremadura, Portugal

Bethan Linscott

2020

Thesis for the Degree of Doctor of Philosophy

Dedicated to the eternally loving memory of

Dr Angelo Niko Grubišić

“Exploration is in our nature. We began as wanderers, and we are wanderers still.
We have lingered long enough on the shores of the cosmic ocean.
We are ready, at last, to set sail for the stars.”

Carl Sagan (1980)

University of Southampton

ABSTRACT

Faculty of Humanities
Archaeology
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Bethan Linscott

The karstic limestone caves of Portuguese Estremadura provide some of the richest Middle and Upper Palaeolithic archaeological assemblages in Iberia. The well-preserved skeletal remains of Neandertals and anatomically modern humans (AMH) from cave sites such as Gruta da Oliviera, Galeria da Cisterna and Gruta do Caldeirão are invaluable sources of biogeochemical information, and they offer a rare opportunity to address questions about mobility patterns and subsistence strategies in multiple human species diachronically, through direct isotopic investigation.

This research represents the first large-scale isotopic study of human and animal mobility during the Middle and Upper Palaeolithic in Europe. Using laser ablation multi-collector inductively-coupled plasma mass spectrometry (LA-MC-ICP-MS), highly spatially resolved sequential strontium isotope analyses of Pleistocene tooth enamel from sites in Portuguese Estremadura are carried out alongside sequential oxygen isotope analysis, in order to reconstruct the mobility of terrestrial fauna across the local landscape. The extreme variation in the geology (and hence $^{87}Sr/^{86}Sr$) of the study area over distances of just tens of kilometres permits the detection of relatively short-distance mobility in the region over annual scales. Sequential strontium isotope data from the tooth enamel of two Neanderthals (Pike *et al.,* unpublished), a Magdalenian human (Pike *et al.,* unpublished), and two Solutrean humans (this study) are considered in the context of strontium and oxygen isotope data from contemporaneous fauna, in order to investigate the relationships between human mobility, animal mobility and seasonality across the Middle and Upper Palaeolithic. These data are further synthesized with existing archaeological evidence from the study sites and surrounding region, in order to address wider questions about range size and changes in subsistence behaviour in response to climatic and environmental change.

Results suggest that red deer, ibex, horse and extinct rhinoceros would have been present within a 30km radius of the study sites, and were likely available as resources throughout the year. Based on strontium isotope data and studies of lithic raw material sources, the mobility of both Neanderthals and AMHs appears to be consistent with ranges of between 20-30km, though both isotopic and archaeological evidence suggest that the pattern of landscape changed over time. Settlement patterns appear to have shifted from short term, possibly seasonal residential mobility during the Middle Palaeolithic, to more logistical mobility patterns during the Upper Palaeolithic; perhaps as a response to changing arboreal cover during the climatic oscillations of the Late Pleistocene.

This study further considers our current collective understanding of the complex nature of enamel formation, and its implications for the interpretation of highly spatially resolved isotopic analyses in both archaeological and modern contexts. The potential for the strontium isotope analysis of biological apatites via LA-MC-ICP-MS in seasonality studies is reflected in the data presented here, and suggestions for further refinement of the method (particularly with regard to our understanding of isotope incorporation) are put forward.

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Academic Thesis: Declaration of Authorship

I, Bethan Linscott, declare that this thesis (TRACKS: An isotopic investigation of the seasonality of Middle and Upper Palaeolithic humans and fauna in Estremadura, Portugal) and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

5. I have acknowledged all main sources of help;

6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

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Signed: Bethan Linscott

Date: 25/08/2020

1. Introduction

Understanding the subsistence strategies and mobility patterns of hominin groups is central to the study of human evolution. The importance of palaeodietary studies is self-evident in the sense that the success of any given terrestrial species is, at the most basic level, dependent on its ability to secure nutritionally adequate, reliable food and water sources; and for tool users, the necessary raw materials. The availability of these essential resources dictates the geographical space that can be occupied permanently by that species, as well as population size (Mellars, 1995). Where hominins are concerned, the decisions that are made regarding food procurement have a subsequent effect upon settlement patterns, social organisation and mobility (ibid). Thus, if we are to form a comprehensive understanding of hominin behaviour, it is imperative that we first seek an understanding of the ways in which these groups procured resources across time and geographical space.

The goal of palaeodietary studies is not simply to identify the resources exploited by hominins, but rather to characterise the range of resources available in the landscape and understand the role of hominin decision-making in the development of their subsistence strategies. It is argued that the essential aim of a food procurement strategy is to mitigate risk; that is, to ensure that a food source is *a*) secure and reliable; and *b*) retrievable within acceptable limits of energy expenditure (Gamble, 1984; Jochim, 1976). Knowledge of the seasonal availability of prey and plant foods, anticipation (planning depth) and food storage are key tactics in risk reduction, and can contribute to long-term food security (Gamble, 1999). Mobility, defined as "the strategic displacement of residential sites from one location to another in search of resources necessary for the subsistence of a group" (Sánchez-Hernández *et al.*, 2014: 317), is intrinsically linked to these subsistence behaviours, and as such, reconstructing the mobility patterns of hominins can contribute greatly to our collective understanding of hominin subsistence in the Middle and Upper Palaeolithic periods.

Rapid methodological advancements in isotopic analysis have made it possible to reconstruct the dietary ecology, mobility and seasonality of individuals with increasing temporal resolution through the direct analysis of skeletal material. Sequential ^{87}Sr/^{86}Sr isotope data measured sequentially along the growth axis of tooth enamel permits the reconstruction of movement across varying geologies during the time of enamel formation, whilst sequential δ^{18}O measurements taken parallel to ^{87}Sr/^{86}Sr samples enables said movement to be anchored to seasons. Previously, such work could only be carried out on large animal teeth due to large sample size requirements. With the development of novel laser ablation methodologies and a general reduction in sample size requirements due to instrumental advances, these analyses can now be performed on much smaller hominin teeth. When combined with archaeological, zoological and palaeoclimatic information, such data can permit the detailed reconstruction of human subsistence behaviours and wider ecological settings during the

Pleistocene. In regions and site complexes that played host to more or less continuous human occupation throughout the Middle and Late Pleistocene, there arises the possibility of examining changes in subsistence behaviour in response to environmental and climatic pressures, as well as comparisons between different human species.

It has been proposed by some that the subsistence strategies of European Neanderthals may have contributed to their disappearance. Based on zooarchaeological and stable isotope data, their dietary habits have been described as narrow, with a primary focus on the hunting of large game and little input from small game or plant foods (Richards and Trinkaus, 2009b). The anatomically modern humans who superseded them, on the other hand, are argued to have exploited a much broader range of resources including aquatic foods, small game and plants, alongside large terrestrial game. Such evidence has led researchers to suggest that both human groups were in direct competition for large game, and that the wider dietary breadth of anatomically modern humans gave them the 'upper hand' over Neanderthals and their more narrow, specialised subsistence strategies during the climatic deterioration of MIS 3 (O'Connell, 2006). This view, however, has been contested. Recent dental microwear analysis (El Zaatari *et al.*, 2011; El Zaatari *et al.*, 2016; Henry, Brooks and Piperno, 2014) and compound specific stable isotope analysis (Naito *et al.*, 2016b) has provided evidence that Neanderthal diet was in fact broader than previously suspected, suggesting that the factors contributing to Neanderthal displacement by anatomically modern humans may be more complex than the inability of the former to adapt their subsistence strategies according to climatic conditions. A thorough comparison of Neanderthal and anatomically modern human subsistence behaviours through the synthesis of newly obtained strontium, oxygen, carbon and nitrogen isotope data, new seasonality data and existing zooarchaeological data for sites across Europe may help us to detangle the complex causes of the displacement of the former.

The Pleistocene cave deposits of Portuguese Estremadura offer an invaluable opportunity to carry out such a study. The Almonda karstic system (Torres Novas) provides what is perhaps the richest collection of Middle and Late Pleistocene human and animal remains in western Iberia, characterised by human specimens dating to the Lower, Middle and Upper Palaeolithic (Daura *et al.*, 2017; Trinkaus, Maki and Zilhão, 2007; Trinkaus *et al.*, 2011). Human remains of these ages are relatively rare in Iberia south of the Pyrenees, and as such, those recovered from the Almonda karstic system and nearby sites are invaluable sources of biogeochemical information. Seasonality data for fauna in the region is virtually non-existent, except for one short study carried out at the cave site of Lapa do Picareiro by Bicho *et al.* (2000). This lack of data severely limits the extent of our understanding of Middle and Late Pleistocene hominin subsistence, in an area which otherwise offers the rare opportunity to investigate temporal change and inter-species differences in subsistence strategies. This project therefore aims to advance our understanding of Middle and Late Pleistocene hominin mobility and subsistence through the isotopic investigation of human remains from sites in Portuguese Estremadura, as well as the mobility and seasonality of associated prey species from sites in the region. Isotopic data will be contextualised through the synthesis of existing artefactual, zooarchaeological and palaeoclimatic data in order to reconstruct wider ecologies. This may permit us to address the question of whether differences in subsistence strategies between modern humans and Neanderthals contributed to the apparent disappearance of the latter, at least in Iberia.

1.1 Aims and objectives

The Middle and Upper Palaeolithic faunal remains recovered from the Almonda karstic system and Gruta do Caldeirão in Estremadura provide an excellent record of the prey species exploited by Middle and Upper Palaeolithic human groups in the region. As such, they offer the opportunity to explore the movement of faunal species across the local landscape, which consequently is likely to have influenced human mobility patterns and subsistence strategies. The overarching aim of this research project is to investigate (both directly and indirectly) the subsistence patterns of Middle and Upper Palaeolithic human groups in Estremadura through the isotopic analysis of faunal and human skeletal remains. The overarching archaeological aims of this project are therefore:

a) To investigate whether the mobility of major prey species such as red deer, ibex and horse in central Portugal is seasonal in nature, and establish whether their mobility patterns change over time during the Pleistocene;

b) To investigate the mobility patterns of anatomically modern humans and Neanderthals in central Portugal, and determine whether range size and seasonal behaviour can be observed through the sequential strontium isotope analysis of tooth enamel;

c) To examine whether the mobility patterns of these two human groups are the result of the availability of local fauna, and potentially establish links between certain geographical regions and the exploitation of specific resources at certain times of the year;

d) To investigate whether the mobility patterns and subsistence strategies of Neanderthals and anatomically modern humans differ between the Middle and Upper Palaeolithic of Portuguese Estremadura - this has wider implications for our understanding of the cognition and ecology of these populations.

Each of these aims will be addressed with the following objectives:

a) To investigate the patterns of seasonal movement of Middle and Upper Palaeolithic fauna through the sequential strontium $^{87}Sr/^{86}Sr$ analysis of Pleistocene tooth enamel via laser ablation multi-collector inductively-coupled plasma mass spectrometry (LA-MC-ICP-MS) and sequential oxygen isotope analysis (EA-IRMS) of the same tooth enamel;

b) To investigate the mobility of anatomically modern humans and Neanderthals in central Portugal through sequential strontium isotope analysis of the enamel of permanent dentition via laser ablation multi-collector inductively-coupled plasma mass spectrometry (LA-MC-ICP-MS)

c) To investigate the relationship between animal and human mobility by comparing faunal isotope data with the human $^{87}Sr/^{86}Sr$ profiles gathered in this study, as well as those previously obtained by Pike et al. (unpublished);

d) To examine diachronic change in mobility between the Middle and Upper Palaeolithic by bringing together archaeological and isotopic evidence from the study region, in order to reconstruct the subsistence strategies of both human groups.

2. Human subsistence in the Middle and Upper Palaeolithic

2.1 *No such thing as competition?* Neanderthal and AMH subsistence in Europe

Knowledge of the resources consumed by a species, whether extinct or extant, is essential if its ecological niche is to be understood. Diet can have a profound effect upon the geographical distribution, mobility patterns, social organisation and behaviour of a species, and as such, the reconstruction of hominin subsistence strategies over the course of the Pleistocene is of considerable value in human evolutionary studies. In particular, the subsistence strategies of Neanderthals and anatomically modern humans (AMH) has taken centre stage over the last several decades, amidst growing evidence for behavioural similarities and genetic introgression between the two species (Hoffmann *et al.*, 2018; Fu *et al.*, 2015). The apparent disappearance of Neanderthals and simultaneous spread of anatomically modern humans in Europe approximately 45,000-40,000 years ago has raised questions about dietary competition between the two populations (Stewart, 2004). Based primarily on zooarchaeological evidence and heavily influenced by bulk collagen stable isotope data, many have argued that the diversification of anatomically modern human diets to include, fish, plants and small game alongside large terrestrial herbivores gave them a selective advantage over the Neanderthal population, who by contrast are argued to have obtained the majority of their calories from the meat of large game such as mammoth, horse, deer and ibex (for example, see Hockett and Haws, 2009a; Hockett and Haws, 2005; Hoffecker, 2009; Richards *et al.*, 2001; Richards and Trinkaus, 2009a). Growing evidence, however, contests the notion that the Neanderthal dietary range was limited in comparison to the incoming anatomically human population, and highlights the need for a more comprehensive understanding of *how* resources were obtained by these two human groups – if indeed they can be distinguished, and evidence to the contrary is certainly building (Peeters and Zwart, 2020). The following chapter is a brief review of the existing evidence for human subsistence strategies in Europe.

Information derived from archaeological faunal assemblages and stone tool evidence has, over the years, contributed to the enduring concept of the narrow Neanderthal diet. Comprehensive reviews of the faunal assemblages at European Middle Palaeolithic sites compiled by Boyle (2000), Conard and Prindiville (2000), Patou-Mathis (2000) and Tortosa *et al.* (2002) illustrate an emphasis on large game hunting, with assemblages often (though not always) characterised by the dominance of one or two large herbivorous species such as horse, reindeer or ibex; the taxon varying depending on geographical location. Faunal assemblages at a number of AMH sites, in contrast, are argued to exhibit evidence for the increased exploitation of aquatic resources, small game such as leporids and birds, and plant foods alongside large game (Fa *et al.*, 2013; Hockett and Haws, 2009a; Hockett and Haws, 2005). The recovery of specialised tools such as fishhooks (for example, see O'Connor, Ono and Clarkson, 2011) from Upper Palaeolithic contexts and the comparative lack of specialised technology in Middle Palaeolithic deposits has led some to suggest that Neanderthals simply lacked the technological innovations and/or the cognitive abilities required to exploit small, fast-moving terrestrial prey or aquatic foods (Stiner and Kuhn, 2009).

Brown (2010) however, argues that much of the zooarchaeological research to date has been carried out in regions of relatively poor resource diversity, and as such, contrasts in the range of fauna exploited by Neanderthals and anatomically modern humans is likely to be a result of differences in overall availability rather than cognitive or technological abilities. Indeed, there is zooarchaeological evidence that in certain areas, Neanderthals were exploiting a variety of aquatic resources originally thought to be targeted exclusively by anatomically modern humans. Marine mollusc shells have been recovered from Mousterian contexts at sites such as Gorham's Cave and Vanguard Cave on the island of Gibraltar (Finlayson, Barton and Stringer, 2001), Grotta del Moscerini on the west coast of Italy (Stiner, 1994) Figueira Brava on the coast of central Portugal (Antunes, 2000) and Los Aviones on the east coast of Spain (Zilhão *et al.*, 2010b); many of which exhibit fire damage argued to be indicative of human activity. The remains of marine reptiles and mammals have also been recovered from Mousterian deposits, including tortoise at Gorham's Cave in Gibraltar (Finlayson et al. 2001), monk seal at Grotta di Sant'Agostino in Italy (Tozzi, 1970) and Vanguard Cave in Gibraltar (Álvarez-Fernández, 2015) and dolphin at Vanguard Cave and Figueira Brava (Antunes, 2000). Rabbit remains were recovered from Mousterian deposits at Gruta Nova da Columbeira, Portugal (Carvalho, Peireira and Manso, 2018), Les Canalettes Aveyron, France (Cochard *et al.*, 2012) and Bolomor Cave, Spain (Blasco and Peris, 2009) amongst others. At many of the above sites these faunal remains were cut-marked, reinforcing the fact that flesh was removed from the bones (presumably for consumption) by humans. In certain regions, then, evidence suggests that Neanderthals were equally capable of exploiting small game and aquatic resources as were anatomically modern humans; despite the theory that they were technologically or cognitively unable to do so.

Bulk collagen $\delta^{13}C$ and $\delta^{15}N$ data in particular have given support to the concept of a 'narrow' Neanderthal diet in comparison to a more diverse AMH diet, in which the meat of large herbivores provided the majority of the calorific intake. To date, bulk collagen $\delta^{13}C$ and $\delta^{15}N$ data obtained from adult Neanderthal remains at eight sites: Scladina (Bocherens *et al.*, 1991) and Spy Cave (Bocherens *et al.*, 2001; Semal *et al.*, 2009) in Belgium; Les Pradelles (Bocherens *et al.*, 2005; Fizet *et al.*, 1995), St. Césaire (Bocherens et al. 2005), Les Rochers-de-Villeneuve (Beauval *et al.*, 2006) and Jonzac (Richards *et al.*, 2008b) in France; Vindija in Croatia (Higham *et al.*, 2006; Richards *et al.*, 2001) and Feldhofer in Germany (Richards and Schmitz, 2008; Schmitz *et al.*, 2002). In each case, the authors conclude that the similarity of Neanderthal δ^{13}C and δ^{15}N values to coeval carnivores (such as wolves and hyenas) points to Neanderthals as top-level carnivores in their respective ecosystems, with a clear emphasis on the high proportion of animal protein in the diet derived from large terrestrial herbivores (Figure 1).

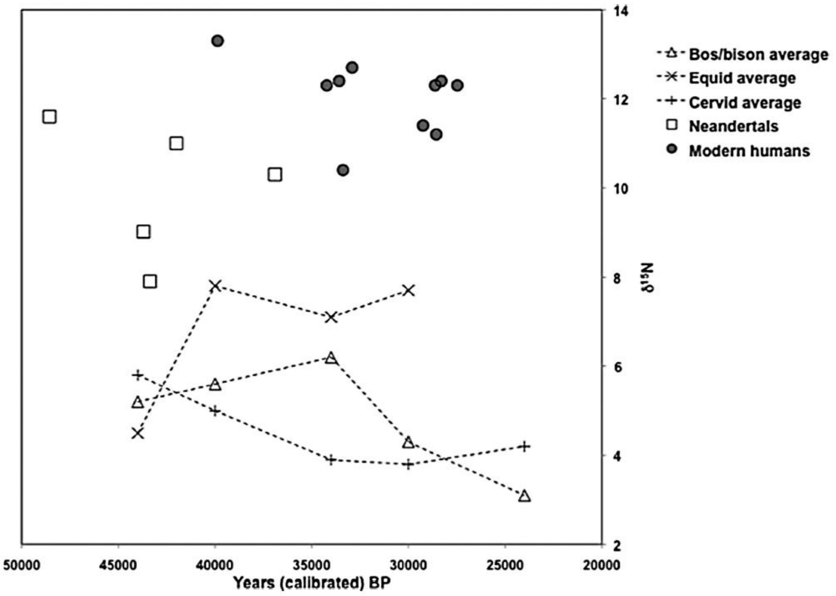


Figure 1. "δ^{15}N bone collagen values of directly radiocarbon-dated Neanderthals and early modern humans compared to the average δ^{15}N values of directly radiocarbon dated herbivores from Northern Europe over the period 50,000 to 20,000 cal BP" (Richards and Trinkhaus 2009: 16037).

δ^{13}C and δ^{15}N data obtained for collagen from Upper Palaeolithic sites including Oase (Trinkaus *et al.*, 2003), Cioclovina and Muerii (Trinkaus *et al.*, 2009; Soficaru, Doboş and Trinkaus, 2006) in Romania, Paviland (Jacobi and Higham, 2008) and Eel Point (Schulting *et al.*, 2005) in the UK, Brno-Francouzská (Richards *et al.* 2001; Pettitt and Trinkaus, 2000) and Dolní Věsetonice (Richards *et al.* 2001) in the Czech Republic, and Arene Candide (Pettitt *et al.*, 2003) in Italy, on the other hand, are argued to reflect more diverse diets than observed among Neanderthals. The particularly high δ^{15}N and variable δ^{13}C values of a number of these individuals with respect to those of local herbivores are argued by (Richards and Trinkaus, 2009a) to reflect the consumption of aquatic resources such as freshwater fish. These authors suggest that the stable isotope evidence may reflect the failure of Neanderthals to alter their subsistence strategies in response to changing climatic conditions, whilst the flexibility of modern human diets allowed them to adapt and spread throughout Europe around 40, 000 years ago.

Bulk collagen δ^{13}C and δ^{15}N values in adult human bone collagen, however, primarily reflect only the protein intake (Hedges and Reynard, 2007), and as such they do not indicate the calorific proportions of every food source in the diet. Indeed, contributions from low protein, high carbohydrate foods such as underground storage organs cannot be detected; even if they constituted a considerable portion of the calorific intake. Equally, the varying nitrogen content in animal and plant foods (approximately 14% and 1% on average, respectively (Phillips and Koch, 2002) can have a significant effect on the resultant δ^{15}N value of the consumer. A small contribution of animal-based foods can increase the consumer tissue δ^{15}N considerably, whilst a comparatively large contribution of plant-based foods to the diet may only slightly alter the δ^{15}N value of the consumer tissue (Bocherens, 2009). As such, bulk collagen δ^{13}C and δ^{15}N values alone are likely to overemphasize the contribution of animal protein to the diet; a phenomenon that has lent support to the long-standing theory that Neanderthals subsisted almost exclusively on the meat of large terrestrial herbivores. Furthermore, the interpretation of Palaeolithic human bulk collagen stable isotope values (particularly if mixing models are used) is also entirely dependent on comparative faunal values which, ideally, should be contemporaneous and should represent all possible dietary protein components (Bocherens, 2009; Phillips, 2001). Factors such as poor collagen preservation at any given site, post-depositional mixing (particularly in cave sites) and financial restraints may result in the production of a relatively limited set of comparative faunal isotope data (or may even lead analysts to use comparative faunal data that is not contemporaneous, or is from geographically farther afield). In such situations, human bulk collagen stable isotope values may be interpreted as reflecting considerably different subsistence strategies than they represent in reality.

Novel biomolecular methods are further challenging the theory that Neanderthal diet was not as varied as that of anatomically modern humans. New lines of evidence such as compound specific carbon and nitrogen isotopes, dental calculus and aDNA compliment the information provided by faunal assemblages, and have offered new insights into the range of resources exploited by Neanderthals. Most notably, all of these approaches provide evidence for the consumption of plant foods. Analysis of the dental calculus of three adult Neanderthals (Shanidar III; Iraq, and Spy I and II; Belgium) revealed the presence of a multitude of starch grains in the oral environment of these individuals. In the dental calculus of Shanidar I, grass seed starch grains characteristic of the tribe *Triticae* were identified; several of which bore likeness to barley. One starch grain was also tentatively identified as a legume, but many were too damaged to be confidently identified. Nevertheless, almost half of the starch assemblage exhibited evidence of cooking; more specifically, boiling or baking. Phytoliths were also identified; 80% of which were attributed to date palm (Henry, Brooks and Piperno, 2011). These findings are consistent with results of analyses of macro- and microbotanical analyses of Mousterian levels at cave sites in Isreal – at Kebara, the charred remains of edible grasses and legumes were recovered (Lev, Kislev and Bar-Yosef, 2005), and at Amud, phytoliths associated with date palm leaves were identified (Madella *et al.*, 2002). At Spy Cave, the dental calculus of the two individuals also produced a myriad of starch grains; almost half of which are attributed to underground storage organs (USOs); potentially water lily. Other plant starches were identified, with some tentatively attributed to the *Andropogoneae* tribe (to which sorghum belongs). In a more recent study (Power *et al.*, 2018) plant microremains were also identified in the dental calculus of Neanderthals from Vindija Cave (Croatia) and Guattari and Fossellone Caves (Italy). *Triticae* starches were recovered from dental calculus at all three sites, though the former exclusively exhibited starches attributed to legumes, USOs and potentially nuts or sedge seeds. The analyses further revealed possible evidence for the consumption of fungi at the sites of Guattari and Fossellone, in the form of boletoid spores.

As well as preserving physical microfossil remains, dental calculus also has the potential to provide genetic evidence for the range of resources exploited by Neanderthals. Ancient DNA analysis of the dental calculus of five Neanderthal individuals from El Sidrón (Spain) and Spy Cave revealed the presence of a variety of plant, animal and fungal resources in the oral environment (Weyrich *et al.*, 2017). In the El Sidrón individuals, plant resources including pine nuts and moss were identified, along with edible eukaryotic foods such as fungi. Wheat pathogens were identified in the El Sidrón 1 calculus, and although this is interpreted as evidence of self-medication for a dental abscess, the presence of such pathogens suggests that edible grains were available to these individuals and may have been purposely collected as a food source. The Spy II calculus also exhibited genetic material attributed to edible fungi, though in contrast to El Sidrón, wild sheep and woolly rhinoceros DNA was also recovered. It should be noted, however, that the presence of genetic material in the oral environment does not necessarily indicate consumption; indeed, certain types of resource processing may involve the use of the mouth (Clement, Hillson and Aiello, 2012). Furthermore, it is argued by some (Dickson, Oeggl and Stanton, 2017) that although the dental calculus of the El Sidrón individuals exhibits genetic evidence for the presence of moss, it is unlikely to be a major constituent (if at all) to the diet due to its low nutritional value and poor palatability.

Dental calculus preserves microfossil and genetic evidence for taxa that were present in the oral environment, but whether those taxa were there as a result of consumption or accidentally (perhaps through the use of the mouth as a tool) is difficult to discern. Compound specific carbon and nitrogen isotope data, on the other hand, offer direct evidence for the foods consumed – and, through modelling, may offer insights into the proportions of plant- and animal- derived proteins in the diet.

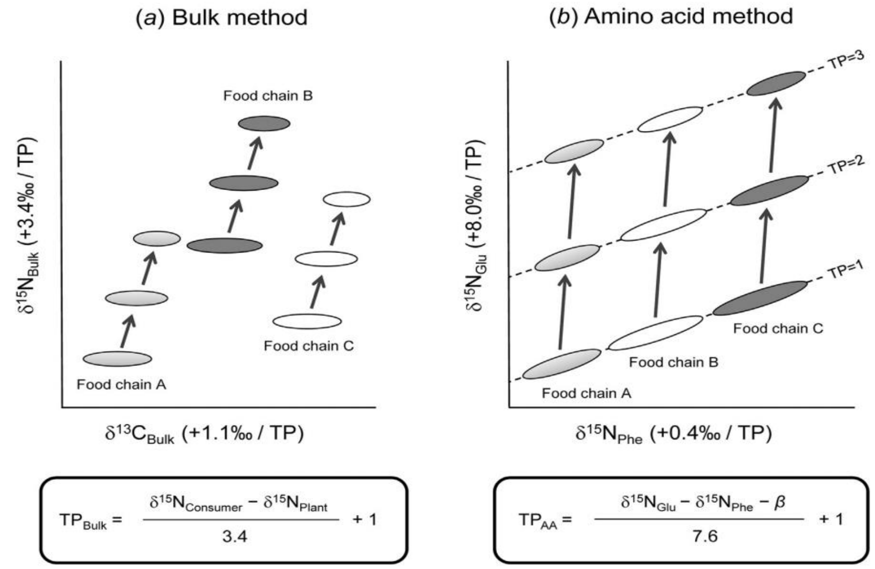


Figure 2. Comparison of trophic position estimations based a) bulk collagen and b) single amino acid (AA) stable isotope values. *β* is the $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ of the primary producer. Using the bulk method, individuals at the same trophic position may have different collagen $\delta^{15}N$ values, whereas in the AA method, individuals at the same trophic position will exhibit $\delta^{15}N$ values on the same trophic line. From Naito et al. (2016a: 84).

Unlike bulk collagen $\delta^{13}C$ and $\delta^{15}N$ analyses, compound-specific isotopic analysis reconstructs trophic position by measuring the $\delta^{15}N$ spacing between essential and non-essential amino acids in bone collagen (usually phenylalanine and glutamic acid, respectively). Phenylalanine undergoes a negligible ^{15}N enrichment of approximately 0.4 ± 0.4‰ from prey to consumer, whilst glutamic acid undergoes a more significant ^{15}N enrichment of 8.0 ± 1.1‰ from prey to consumer (Figure 2; Naito *et al.*, 2016a). Phenylalanine therefore acts as an indicator of the $\delta^{15}N$ value of the primary producers in a given food chain, and as such the isotopic spacing between phenylalanine and glutamic acid can be used to “offset the background fluctuation in $\delta^{15}N$ of ecosystems” (Naito *et al.* 2016a: 83). Single AA analysis of Neanderthal and animal bone collagen from the cave sites at Spy and Scladina, Belgium, suggests that rather than being heavily dependent on mammoth as a source of dietary protein (as suggested by the bulk collagen $\delta^{15}N$ values), prey with lower $\delta^{15}N_{Phe}$ such as bovids and equids were probably preferred by the Spy Neanderthals (Figure 3). Whilst conventional bulk collagen stable isotope values suggest that the Spy Neanderthals occupied a similar dietary niche to hyenas, the AA method implies that these Neanderthals occupied a trophic position similar to wolves. The application of a mixing model to the data also suggests that up approximately 20% of the protein in the diet of these Neanderthals could have been obtained from plant foods (Naito *et al.*, 2016a), suggesting that their diets were likely more omnivorous than had previously been thought.

The single amino acid method for carbon and nitrogen stable isotope analysis is still in its infancy, and as such there are caveats that must be borne in mind when considering trophic position estimations based on δ^{15}N data obtained for phenylalanine and glutamic acid. One such caveat is that at the current time, the value of β ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ of the primary producer) used in the trophic position calculation is based upon a limited dataset of published plant AA δ^{15}N values (Chikaraishi *et al.*, 2011). (O'Connell and Collins, 2018) demonstrate that by using different β values based on other published C_3 plant δ^{15}N AA values, a range of trophic positions can be estimated for the Spy Cave Neanderthals. A better understanding of the variability in plant $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ depending on species and photosynthetic pathway would therefore aid in the refinement of the method.

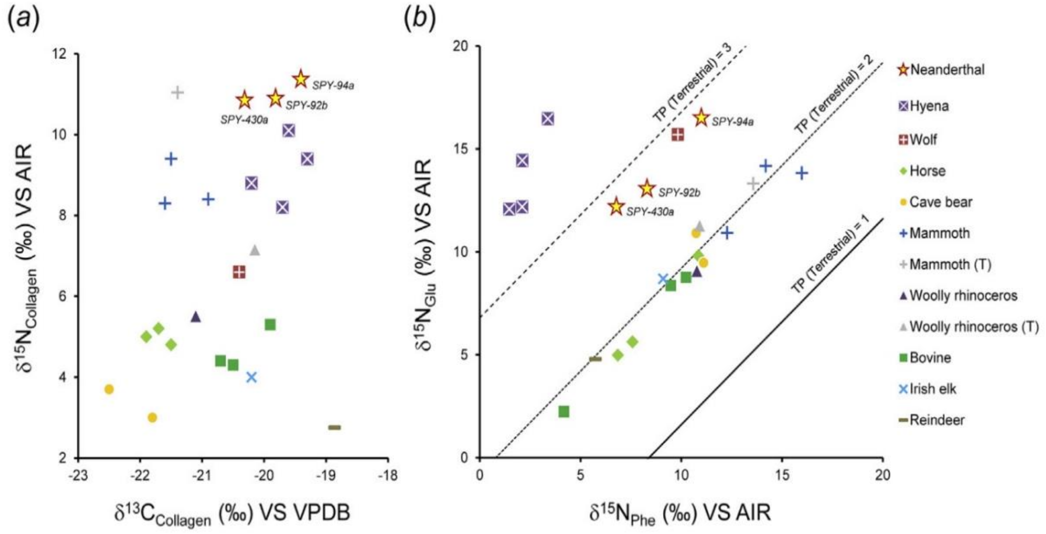


Figure 3. Comparison of a) bulk collagen stable isotope data and b) AA stable isotope data for the Spy Cave Neanderthals and associated fauna. From Naito *et al.* (2016a: 86).

Nevertheless, as it stands, targeting essential and non-essential amino acids in bone collagen for stable isotope analysis has the potential to provide a greater insight into trophic relationships than conventional bulk collagen stable isotope analyses, and in the case of Spy Cave, such data has already contributed to the growing body of evidence against Neanderthals as purely top-level carnivores.

Until recently, it was thought that the range of foods exploited by Neanderthals was much narrower than anatomically modern humans, with the latter targeting a wider range of low-return resources including plants, fish and small mammals alongside larger prey. This theory was primarily based on the dominance of large herbivore remains and the comparative lack of evidence for the exploitation of low-return resources at Neanderthal sites, which when combined with bulk collagen stable isotope values pointed to a diet high in protein derived from large terrestrial herbivores. Zooarchaeological evidence across Europe, however, illustrates the fact that in regions where such resources were geographically available, Neanderthals exploited aquatic resources and small game. New lines of evidence are also adding weight to the argument that in fact, the range of resources exploited by Neanderthals was comparable to that of anatomically modern humans. Microfossils and ancient DNA preserved in dental calculus provides evidence for the presence of both plant and animal foods in the oral environment of Neanderthals, and compound specific stable isotope data contests the conclusions traditionally drawn from bulk collagen carbon and nitrogen isotope data. Taken together, this evidence and the fact that species representation varies significantly depending on site location and time period serves to reinforce the theory put forward by (Brown, 2010) – that differences in the range of fauna exploited by anatomically modern humans and Neanderthals is likely to be a result of geographical and temporal availability rather than the supposed comparatively lesser cognitive and technological abilities of the latter. Perhaps in light of the ongoing evolution of our understanding of the complexity of Neanderthal behaviour (see Finlayson, 2019), it seems somewhat impertinent to consider 'Neanderthal' and 'AMH' diet at all, since the apparent behavioural and physiological gap between the two is increasingly smaller - for example, recent work suggests that both groups expressed themselves symbolically, where previously only the latter was deemed capable (Hoffmann *et al*. 2018). Considering that the range of resources exploited by human groups appears to have been heavily dependent on geographically available resources, it is increasingly important that their subsistence strategies should not be generalised in the way that they have over the last few decades – rather, human diet should be contextualised geographically and climatically, regardless of species.

2.2 Hunting strategies and food procurement

If Middle and Upper Palaeolithic diets were relatively similar in the range of foods exploited, then the key question becomes: *how did food procurement strategies differ geographically and temporally, if it all*? In the late 20th Century, it was argued by some that obligate scavenging was the primary mode of calorie procurement by archaic human populations - including Neanderthals - chiefly as a result of cognitive and behavioural limitations. Such conclusions were based on faunal assemblages recovered from Middle Stone Age contexts at a number of sites; namely Klasies River Mouth in South Africa (Binford, 1981; Binford, 1984; Binford, 1985), Grotte de Combe-Grenal and Grotte Vaufrey in the Dordogne region in France (Binford, 1981; 1985), Grotta dei Moscerini and Grotta Guattari in Italy (Stiner, 1991b; Stiner, 1991a; Stiner, 1994). The presence of carnivore tooth marks on faunal remains recovered from Middle Palaeolithic occupation layers, the high abundance of low energy return body parts such as heads and feet at certain sites, and a relative lack of cut marks – which, when present, were often found on the bones of less calorifically valuable body parts rather than the shafts of long bones – led Binford and Stiner to argue that the remains were the product of obligatory scavenging by the respective hominins. These arguments have been countered on the basis that the recovery methods and analytical procedures employed for these sites were biased; for example, at Grotte Vaufrey, long bone shafts that could not be assigned to a species or genus were excluded from analysis, and at Grotte de Combe-Genal, bone fragments that could not be identified were discarded entirely (Marean, 1998). The discovery of Middle Palaeolithic fire-hardened spears at the site of Schöningen in Germany (Thieme, 1998), however, essentially ended the debate as to whether Neanderthals were able to hunt or were obligate scavengers (White, Pettitt and Schreve, 2016). Evidence as to how such weapons were deployed has also been brought to light. Perforations in fallow deer skeletons were identified in Middle Palaeolithic contexts at Neumark-Nord in Germany, and based on ballistic experiments, have been interpreted as wounds from short-range spears (Gaudzinski-Windheuser *et al.*, 2018). The central issue, then, is "…not *whether* Middle Palaeolithic people could hunt, but rather *when* and *how* they chose to hunt" (Burke, 2000, p. 281).

Faunal assemblages at Mousterian sites are frequently monospecific; that is, the abundance of one prey species is twice as great as the next most common species (Patou-Mathis, 2000). Such sites are particularly common after MIS 7 (Patou-Mathis, 2000). The dominating species often varies depending on geographical location (White et al., 2016 and references therein); for example, more than 90% of the faunal remains in Late Middle Palaeolithic (LMP) levels at the Ortvale Klde rockshelter in Georgia are identified as *Capra caucasica* (Adler *et al.*, 2006), whilst *Cervus elaphus* comprise more than 90% of the faunal assemblage in Late Mousterian levels at the site of San Francesco in Italy (Valensi and Psathi, 2004). The prevalence of faunal assemblages dominated by just one or two large prey species suggests that Neanderthals were selectively targeting specific species and, in cases where mortality profiles exhibit biases towards certain age groups or sex, may have been selectively hunting specific individuals within local populations in order to maximise gain (White, Pettitt and Schreve, 2016). It is argued that such strategies, particularly with regards to seasonally available prey species, likely involved a considerable degree of forward planning, organisation, co-operation and a comprehensive understanding of the behaviour of the prey and the surrounding landscape (Daujeard *et al.*, 2017). These are abilities that, in the past, were associated exclusively with behavioural modernity.

2.3 Neanderthal and AMH mobility

Human mobility strategies are intrinsically linked to the procurement of resources. If resources such as food, water and raw materials are restricted geographically and seasonally, then hunter-gatherer groups must engage in a degree of mobility if a range of resources are to be exploited (Lieberman and Shea, 1994). Binford (1980) describes two models of resource acquisition (Figure 4) based on observations of modern hunter gatherer groups, which he refers to as *residential* and *logistical* mobility. In the residential system, groups occupy a residential base camp and gather food daily, ranging out from the central residential base in search of resources and gathering them as they are encountered. Food is rarely stored, and the residential base (which acts as the primary centre of subsistence activities and resource processing) may be moved multiple times over an annual cycle as local resources are depleted. In the logistical system, on the other hand, populations deploy specialised task groups such as hunting parties in order to obtain specific resources. These task groups may leave the residential base for varying lengths of time, occupying short-term 'field camps' selected specifically for the exploitation of specific resources, which are often geographically and temporally constrained.

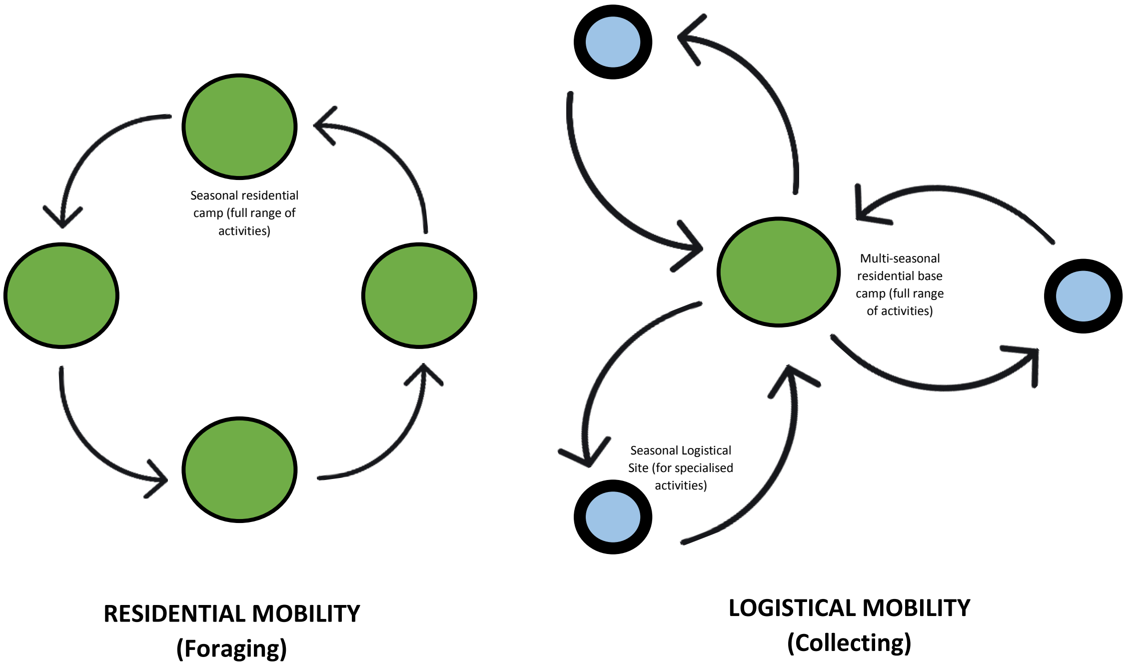


Figure 4. Schematic diagram of the differences between residential and logistical mobility strategies. After Lieberman and Shea (1994: 317).

The residential base camps act as the hub from which the task force operates, and might for example be positioned along the routes of migratory species such as caribou. If the task force successfully obtains the desired resource, the field camp may also be host to processing activities, facilitating the transport of the obtained goods back to the primary residential base. Populations following a logistical-type mobility often engage in the temporary storage of food in caches, permitting the large-scale procurement of food by specialised task groups and subsequent distribution to consumers without the need for large-scale immediate transport. To summarise both strategies: "Foragers [residential] move consumers to goods with frequent residential moves, while collectors [logistical] move goods to consumers with generally fewer residential moves" (Binford 1980: 15). These models have been applied in several isotopic investigations of mobility in recent years (for example, see

Standen *et al.*, 2018; Lugli *et al.*, 2019), but in this study they act solely as a conceptual framework to aid in examination of the ways in which hunter-gatherer systems are related organisationally. This is primarily because the archaeological analyses of many sites in Portuguese Estremadura are still under way, and the incomplete nature of the record renders more rigid, statistical approaches to the reconstruction of mobility and settlement patterns such as those employed by Clark and Barton (2017) and Cortell-Nicolau *et al*. (2019) unsuitable at this time. Since so many sites in central Portugal have been classified as 'temporary camps' akin to those described by Binford based on existing archaeological evidence (see Chapter 3), the Binford (1980) models offer an appropriate and useful framework with which to explore the conditions under which resource procurement patterns change over time in the study area.

According to these models, whether a group adopts a residential or logistical strategy appears to depend not on resource abundance, but primarily upon resource *distribution*. In an environment where all resources are distributed evenly within the locality of the residential base, foraging for food on an 'encounter' basis will suffice. However, in environments where food resources are distributed incongruously as a result of terrain or climatic conditions, logistical collecting strategies may offer the most effective risk mitigation. Naturally, environments that experience considerable temperature extremes are more likely to play host to uneven resource distribution, prompting a greater reliance on logistical strategies as opposed to residential strategies.

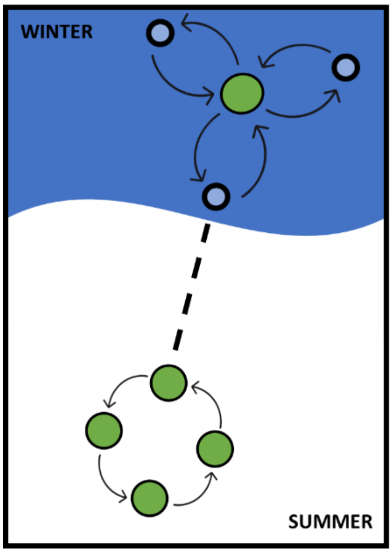


Figure 5. Model of MIS 3 Neanderthal landscape use, with residential mobility during the summer (fauna distributed evenly) and logistical mobility during the winter (fauna distributed unevenly). Adapted from Richter (2006: 28).

Which strategies, then, were likely to have been employed by different human groups in Europe? Are these models too simplistic? The answer to the first question is likely to depend entirely upon geographical location. Neanderthals and anatomically modern humans appear to have adapted their subsistence behaviours based on their situation and habitat (Brown, 2010); indeed, evidence suggests that the dietary ranges of both groups varied geographically depending upon the local availability and distribution of resources (see Section 2.1). Since the distribution of food sources is a key factor in determining the mobility strategies of human groups, it is logical to propose that mobility is likely to vary both geographically and temporally. Studies attempting to reconstruct Neanderthal mobility at sites across Europe have encountered varying patterns - for example, at a number of MIS 5-7 sites in southern France such as Payre (Daujeard *et al.*, 2012), and Taubach and Weimar (MIS 5) in Germany

(Moncel and Rivals, 2011), faunal dental micro-wear and lithic evidence is argued to be reflective of highly seasonal, residential mobility among Neanderthal groups. On the other hand, studies of lithic and faunal assemblages recovered from Mousterian layers at sites such as Kebara and Tabun led Lieberman and Shea (1994) to the conclusion that the Neanderthal occupants of those sites engaged in a mobility pattern that more closely follows the logistical model. As such, any attempts to generalise are likely to be futile – rather, the mobility patterns of both Neanderthals and modern humans are highly likely to be a function of the landscape and resource distribution.

With regard to the question of whether these models are too simplistic, it seems unlikely that mobility strategies are as clear cut as those described by Binford (1980) in reality; rather, they are the "two extremes of a continuum" (Wallace and Shea, 2006: 1295), and aspects of both strategies may be employed depending on the landscape and climate. At the Bavarian site of Sesselfelsgrotte, lithic evidence recovered from the MIS 3 layer hints at variable land use and mobility patterns over the course of each annual cycle (Richter, 2006). Lithic provenancing suggests that at the beginning of each annual cycle, a wide range of raw material sources were utilised, whilst the end of the cycle exhibited decreased raw material variability. Richter (2006) argues that such a shift in the degree of variability is indicative of a change in the mode of mobility, with Neanderthal groups engaging in a higher degree of group mobility in the first part of the cycle, and decreased mobility towards the end of cycle. Archaeologically, this is interpreted as a shift from circulating land use during spring and summer, during which time groups moved between ephemeral camps in order to hunt dispersed fauna (collecting raw materials along the way or from within a short radius of the site), to a radiating system suited for the exploitation of migrating herds during the autumn and winter months, at which time a small number of raw material sources were repeatedly exploited through the deployment of specialised task groups (Richter 2006). In essence, then Sesselfelsgrotte acted as one of several temporary camps visited during the spring and summer months, and subsequently as a more permanent residential basecamp during the autumn and winter months (Figure 5). Further evidence for variable mobility patterns that combine certain aspects of both circulating and radiating models has also been observed at sites mentioned previously, such as Payre in France and Taubach in Germany (Moncel and Rivals, 2011).

If Neanderthal and anatomically modern human mobility patterns are to be compared, it is necessary to focus on a location occupied by both species (though not necessarily at the same time) so as to negate the potential effects of contrasts in terrain. One region that has attracted considerable research attention for this reason is the Levant; both groups subsisted in the area during the Late Pleistocene and observed broadly the same climatic and environmental conditions. The Levant, then, offers the opportunity to investigate the behavioural differences between Neanderthal and AMH groups diachronically. The Levantine Mousterian, according to (Lieberman and Shea, 1994), spans from 150,000 years ago to approximately 45,000 years ago, and is represented archaeologically at sites such as Tabun, Kebara, Qafzeh and Amud. Archaic human remains were recovered from Mousterian deposits at Tabun, Kebara and Amud, whilst anatomically modern human remains were recovered from earlier deposits (tentatively dated to between 80,000 and 120,000 ka) at Skhul and Qafzeh. A selection of the fauna associated with these hominin remains were analysed by Lieberman and Shea (1994). Cementum annuli analysis of gazelle teeth from Mousterian levels at Kebara cave revealed that 52% of the sample (n=30) had been killed during the dry season, and 48% had been killed during the wet season, implying a multiseasonal occupation of the site and year-round hunting of the taxon. In contrast, fauna associated with early modern human fossils at Qafzeh exhibit cementum annuli consistent with single-season deaths – all were killed during the dry season. At Tabun, Mousterian gazelle reflected the same multi-season killing pattern as was observed in Mousterian specimens from Kebara cave, whilst those from Tabun layers associated with early modern human remains again exhibited cementum annuli consistent with solely dry-season deaths.

Leiberman and Shea (1994) argue that the results of the cementum annuli analysis provide insights into differences in hunting strategies between archaic and early modern human populations. They state that the fact that the gazelle were brought into the Kebara and Tabun caves year-round during the Mousterian period suggests that these archaic human groups may have practiced a radiating mobility strategy, utilising a primary multi-seasonal base camp and a series of nearby seasonal logistical sites. Based on the cementum annuli of the fauna associated with early modern humans, however, it seems likely that these human groups engaged in a circulating mobility strategy, whereby Tabun and Qafzeh were only occupied briefly during the dry season and the spring/summer respectively, in order to exploit seasonal game. Hunter-gatherer bands utilising this circulating mobility strategy generally move from one seasonal residential camp to another in an annual cycle. The reasons for the inferred differences in mobility and subsistence strategies between archaic and modern human populations at these Levantine sites remain to be seen.

Portuguese Estremadura offers an invaluable opportunity to examine the differences (or lack thereof) in the mobility patterns of Neanderthal and anatomically modern human groups diachronically, since the archaeological record of the region documents near-continuous occupation through the Middle and Upper Palaeolithic periods. The concept of residential and logistical models of mobility (Binford, 1980) will be used henceforth as frameworks to aid in the exploration of the existing archaeological data in the region and the isotopic data collected here, and although they may offer one possible reconstruction they should not be deemed an accurate reflection of the true patterns of mobility engaged in by human groups in the study area.

3. Site background

The study area, Portuguese Estremadura, essentially encompasses Central Portugal. Its northern and southern boundaries are marked by the Mondego and Tagus river basins respectively, and to the east and west it is edged by Precambrian mountains and the Atlantic Ocean respectively (Marks *et al.*, 1994). Before addressing the cave sites upon which this work is based, it is first useful to examine the geology of Portugal, and of the study region.

3.1 Geological setting

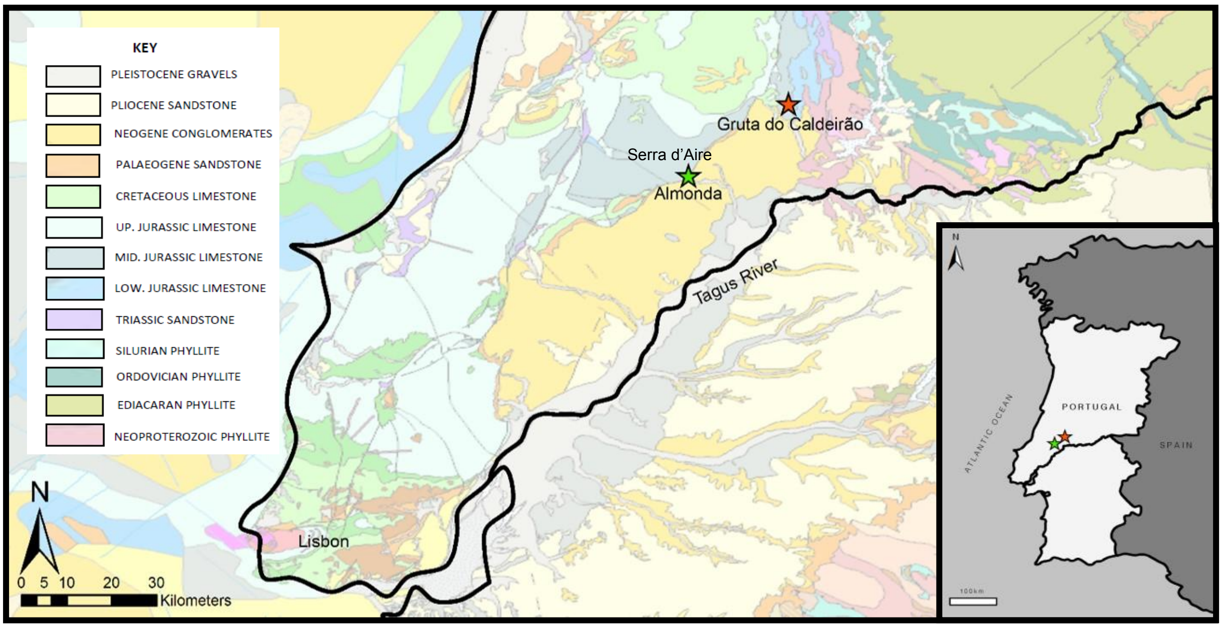


Figure 6. Geological map of Portuguese Estremadura. Produced using LNEG Geological Map of Portugal 1:50 000.

Three main structural units comprise the geological setting of Portugal: the Iberian Massif to the northeast and to the south, the Meso-Cenozoic basins of central Portugal, and the Meso-Cenozoic basin of the Algarve. Estremadura is located along the central Atlantic coast of Portugal, and occupies part of the Mesozoic Lusitanian Basin and the Cenozoic Lower Tagus Basin. The region is geologically heterogeneous, but is dominated by the Jurassic and Cretaceous deposits of the Lusitanian Basin. The basin stretches approximately 300km from the north to the south, and 180km from the east to the west; offshore included. It is divided by faults into three sectors; the Northern Lusitanian Basin (NLB), the Central Lusitanian Basin (CLB) and the Southern Lusitanian Basin (SLB). The study area lies within the CLB; a sector characterised in the northeast by Jurassic limestone massifs of marine origin. Cretaceous conglomerate and sandstone deposits are located to the west and the south of the basin, whilst pockets of Triassic sandstone can be found to the southeast. Basalts are present in the volcanic complexes in the vicinity of Lisbon (Waterman *et al.*, 2014). In the east, the basin is bordered by the Late Palaeozoic Hercynian bedrock of the Iberian Meseta (Schneider, Fürsich and Werner, 2009).

The study sites are in the direct vicinity of the Central Limestone Massif; more specifically the Serra d'Aire, a Middle Jurassic limestone relief which overthrusts the Lower Tagus Tertiary Basin (Angelucci and Zilhão, 2009). Karstic cave systems permeate the limestone, and modern springs (including the Almonda River) discharge water from the base of the Arrife, cutting through the Miocene sediments to the southeast (Waterman *et al.,* 2014). The geology in this region is particularly diverse; varying over just tens of kilometres (see Figure 6). This heterogeneity naturally produces bioavailable ^{87}Sr/^{86}Sr values that vary significantly over short distances, making it an ideal region within which to investigate the mobility patterns of human groups and the fauna they exploited.

3.2 The Almonda karstic system

Figure 7. Drone view above the escarpment showing entrances to caves within the Almonda karstic system mentioned in the text. Adapted from Deschamps and Zilhão (2018: 4).

Middle and Upper Palaeolithic sequences in western Iberia are perhaps best known from cave and open-air sites in Portuguese Estremadura, in the locality of the Mesozoic-Cenozoic Central Limestone Massif and the adjacent sedimentary basin of the Tagus River. The cave complex at the core of this work, the Almonda karstic system, consists of around 12 kilometres of mapped subterranean galleries (Trinkaus *et al.*, 2011) and is located 30km from the west coast of Portugal in association with the spring of the Almonda River (Hoffmann *et al.*, 2013). Recent work within the Almonda karstic system has sought to investigate the effects of climate change on human evolution and settlement in western Iberia, primarily through uranium-series dating of speleothems and correlation with palaeoclimatic data from marine cores (Hoffmann *et al.* 2013). Excavations of Gruta da Aroeira, Gruta da Oliviera and Galeira da Cisterna (see Figure 7) during the Late 20th Century yielded human and animal remains from the Lower Palaeolithic (Daura *et al.*, 2017), Middle Palaeolithic (Trinkaus, Maki and Zilhão, 2007) and Upper Palaeolithic (Trinkaus *et al.*, 2011) respectively. Based on zooarchaeological and material evidence, many of these sites have been interpreted as temporary settlements, occupied in short, repeated intervals in order to allow efficient exploitation of seasonal resources (Gameiro, Aubry and Almeida, 2008).

3.2.1 *Gruta da Oliviera*

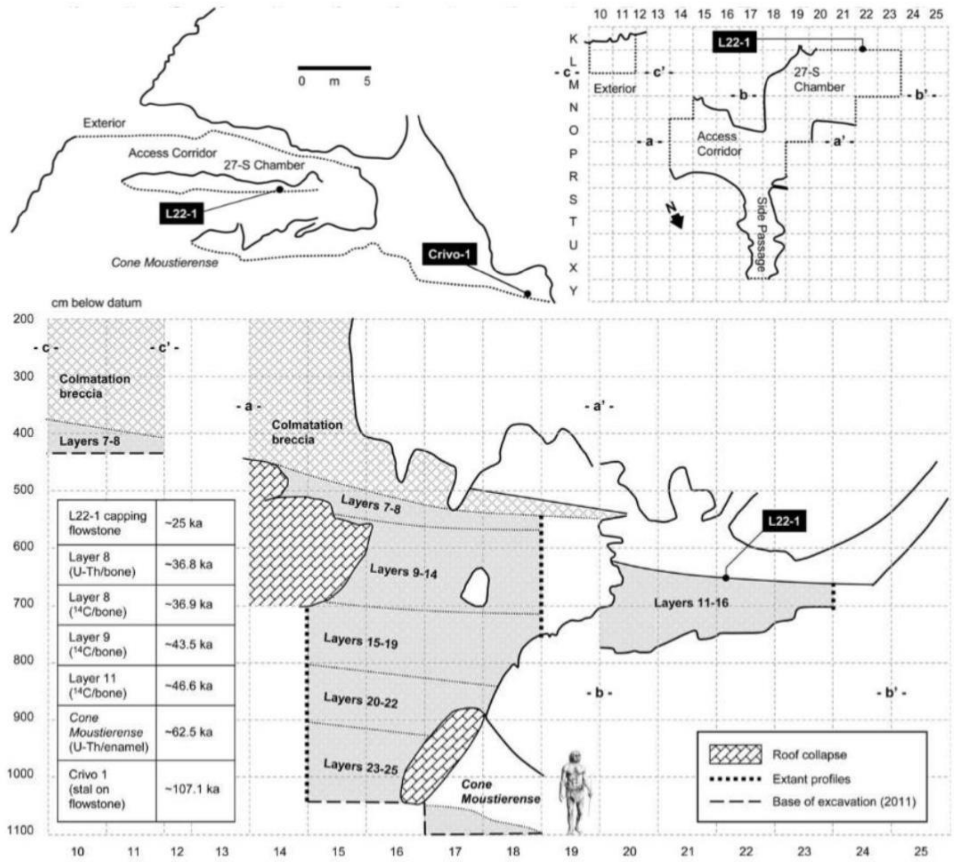


Figure 8. Schematic topographic profile of Gruta da Oliviera (top); stratigraphic profile and available dates (bottom). From Willman *et al.* (2012: 40).

The Gruta da Oliviera (39° 8′ 30′′ N, 88° 36′ 49′′W) is a collapsed entrance to the Almonda karstic system located half way up the escarpment at 115m (Figure 8). The site contains a seven-metre-thick Middle Palaeolithic sequence and has yielded a total of nine Neanderthal remains, two of which are teeth. These were recovered from Levels 9, 10, 11, 17, 18, 19 and 22; dates for which are provided in Figure 8 (discussed in depth by (Trinkaus, Maki and Zilhão, 2007) and (Willman *et al.*, 2012). The proximity of the Gruta da Oliviera to essential natural resources such as fresh water (provided by the spring of the Almonda River), hunting grounds and lithic raw materials would have made the site particularly attractive during the Mousterian. The presence of characteristic Levallois stone tool technology, an abundance of hearth material and tens of thousands of faunal remains (many of them burnt) suggest that the site may have been used for the processing and consumption of locally hunted fauna including red deer, ibex and tortoise (Zilhão *et al.* 2010). Whether such hunting behaviour was seasonal in its nature remains to be seen, but the isotopic data obtained in the current study may help to address this question.

Layer 7 is archaeologically sterile, containing no lithics but yielding a faunal assemblage that was likely to have been accumulated naturally by carnivores. Layer 8 represents the last human occupation of Gruta da Oliviera after the main portion of the cave had collapsed, and based on two radiocarbon dates obtained from pieces of burnt bone, dates to around 31,900 ± 200 BP to 32,740 ± 420 BP (Trinkaus, Maki and Zilhão, 2007). Layers 9 to 14 yielded clear evidence of human occupation of the side passage and the '27-S' chamber (see Figure 8). Layer 14 in particular provides a wealth of material, including the remains of a hearth and an abundance of associated stone tools and débitage, burnt animal bone, charcoal and a considerable faunal assemblage. Importantly, a manual phalanx and the proximal end of a right ulna were discovered in Layers 9 and 10 respectively, and are assigned to the species *Homo neanderthalensis.* Trinkaus *et al.* (2007) suggest that these Neanderthal bones may represent the same individual, but this is difficult to investigate further with such fragmentary remains. A radiocarbon date obtained for an animal bone recovered from Layer 9 provides a date of 43.5 ka cal BP.

Figure 9. (Top) Oliviera 8; M$_2$ or M$_3$. The specimen was broken during excavation. (Bottom) Oliviera 9; P$_3$. From Willman *et al.* (2012: 45-46).

Layers 15 to 19 also yielded strong evidence for human occupation of the surviving 'porch' of the cave (known as the 'Exterior'), through material redeposited in the excavation area as a result of slope run-off. High concentrations of cremated tortoise shells were excavated from these layers, implying the processing and cooking of foodstuffs by human occupants. In the 1997 and 1998 field seasons, the diaphysis of a tibia (Oliviera 4) and the distal end of a right humerus (Oliviera 3) were recovered from Layers 18 and 19 respectively, and were morphologically assigned to the Neanderthal species. These remains are described in detail in Trinkaus et al. (2007). Interestingly, the tibia fragment exhibits evidence of carnivore gnawing in the form of a puncture (likely from the canine of a canid). Again, it is suggested that these two Neanderthal bones may be derived from the same individual, but such a hypothesis is difficult to prove or disprove. In excavations during the 2008 season, a further three Neanderthal remains were recovered from Layers 17 and 18. A proximal manual phalanx (Oliviera 5) and a postcanine tooth fragment (Oliviera 6) were identified in Layer 17, and the distal diaphysis of a right humerus (Oliviera 7) was recovered from Layer 18. Oliviera 3 and

7 are both distal diaphyses of right humerii, and as such represent two individuals. Oliviera 5 and 6, then, could derive from either of these individuals – either separately or together.

During the deposition of Layers 20 to 22, the interior of the cave was once again occupied by humans, evidenced by the presence of a large hearth and associated flint and bones scatters in Layer 21. The hearth itself is approximately 1.5m in diameter, and rather dominates Layer 21. In 2008, Layer 22 produced two of the Neanderthal dental specimens (Figure 9) at the heart of the current study – Oliviera 8; a lower molar suspected to be an M_3 based on the amount of cuspal wear (Willman *et al.*, 2012), and Oliviera 9; a lower first premolar (P_3). Coming from the same layer, it is suggested that these two teeth may have originated from the same individual, if Oliviera 8 is indeed an M_3. The lowermost layers, however, represent a roof collapse, and as such they contain few artefacts and a small faunal assemblage scattered throughout Layers 23 to 25.

Fauna

The study of the faunal remains from the lower part of the Gruta da Oliviera sequence is yet to be completed, but an overview of the faunal assemblage from the upper part of the sequence (Levels 7 to 13) is available in Zilhão *et al.* (2010). Throughout these layers, red deer is always the most abundant species. Ibex remains are present in Layers 9 to 13, but they are most abundant in Level 13 and become increasingly sparse in the overlying deposits. The same is true of horse and rhinoceros remains, which are present in Layers 8 to 13 but are better represented in the lower levels than the upper levels. Tortoise remains were recovered in every level, and based on the NISP is the third most abundant species in Layers 7 to 13. Other large terrestrial herbivores including boar (Layers 8 and 13) and a bovid (Layer 11) are present, but are ephemeral.

Carnivore remains primarily including those of bear, fox and lynx were recovered in most levels, but they represent only 4% of the total assemblage NISP. This, combined with the fact that the age distribution of the red deer and horse is similar to the Mousterian human-accumulated faunal assemblage of the Gruta do Caldeirão, is argued to be evidence that the faunal assemblage in the Mousterian layers at Gruta da Oliviera is not the result of carnivore activity, but rather is the product of human activity. The presence of a considerable number of burned animal bones in the Mousterian layers, particularly in Layer 14 in which they were discovered in association with a hearth, lends support to the notion that the majority of the large terrestrial herbivore remains were accumulated by humans. Further down the sequence in Layers 15-19, excavators recovered the cremated and highly clustered remains of tortoise shell, argued to be evidence of the roasting of this species whole over hot coals.

Lithics

The Gruta da Oliviera stone tool assemblage is still undergoing analysis, but a preliminary report of the lithic assemblage from Levels 8 and 9 is provided by (Marks, Monigal and Zilhão, 2001). In the former, quartzite is the most commonly exploited raw material, though flint and quartz are also present. All three of these raw materials would have been readily available within approximately 30km of the site. 95 tools and débitage were recovered in Layer 8, including characteristically Mousterian Levallois flakes. 112 pieces including retouched tools and débitage were recovered from Level 9, reflecting a similar proportion of raw material usage. In both layers, the tools are generally rare. They include denticulates, notched tools and retouched flakes. According to Marks *et al.* (2001), the assemblage is characteristic of other Late Mousterian stone tool assemblages in Portugal (Angelucci and Zilhão, 2009). In the lower levels, which remain under study (10-14), a range of lithic artefacts including backed microliths, bladelet cores and Levallois flakes were recovered. Handaxes and flake blanks were excavated from Levels 15-18, and an increase in the use of flint is observed Marks *et al.* (2001).

3.2.2 *Galeria da Cisterna*

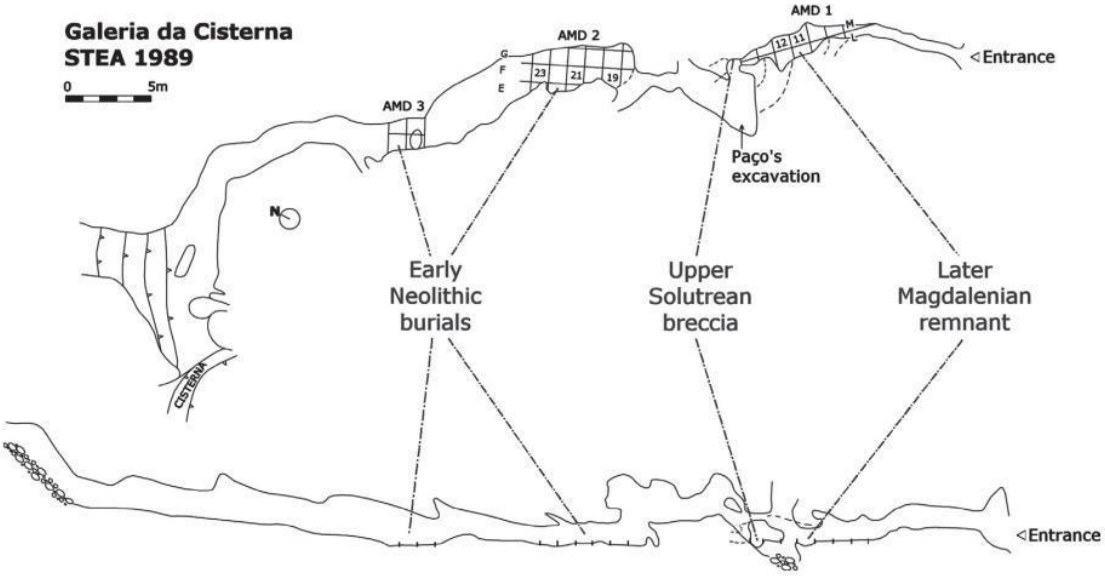


Figure 10. Topographical plan and profile of the Galeria da Cisterna. The areas preserving archaeological deposits of different periods are indicated. From Trinkaus et al. (2011: 398).

Galeria da Cisterna is a long, meandering fossil spring measuring approximately 100 metres in length and contains sediments dating to the Late Pleistocene and the Holocene (Trinkaus *et al.*, 2011). Figure 10 provides a topographical plan and profile of the cave, and pinpoints the locations of three excavation zones (AMD1, AMD2 and AMD3). These zones were excavated between 1987 and 1989, and were previously untouched. Five deposition levels are described in AMD1 by Trinkhaus *et al.* (2011) – Level 1, a Holocene 'dark-earth' deposit; Levels 2 and 3, remnant Pleistocene sediments dating to the Allerød (13, 580 – 13, 740 cal BP) and Younger Dryas (12, 730 – 12, 830 cal BP); Level 4, dating to the Older Dryas and Level 5, a sterile fluviatile deposit. Two animal bones from Level 3 of AMD1 provided direct radiocarbon dates of 12, 680 – 12, 880 cal BP and 13, 500 – 13, 820 cal BP (2σ), placing the deposit within the later Magdalenian (ibid). Level 4 lies directly below Level 3, and is therefore inferred to be >14, 500 cal BP.

Level 3 and Sublevel 3a of Zone AMD1 produced fifteen human bone and dental specimens, described in full by (Trinkaus *et al.*, 2011). Based on the characteristics of the dental remains, it is suggested by the aforementioned authors that three individuals of varying ages (young child – young adult) are represented in Level 3 of the AMD1 zone. One of the human remains recovered from Level 3, the left premolar (P_3) of Cisterna 2, underwent sequential strontium isotope analysis in 2014. The results of this analysis are integral to the current study.

Fauna

A full report on the Galeria da Cisterna fauna is yet to be published, but a preliminary description of the somewhat small assemblage is available in (Trinkaus *et al.*, 2011). The Younger Dryas deposit, Level 3, yielded large terrestrial species such as red deer, ibex and wild boar, but is dominated by the remains of rabbit. Bats, birds and amphibians are also represented, and one wildcat bone was recovered. The Older Dryas deposit, Level 4, yielded the remains of ibex, rabbit, hare, wildcat, fox and birds. It seems likely that the bird, bat and amphibian remains were accumulated naturally or by carnivores, whilst the large terrestrial herbivores such as red deer and ibex were brought into the cave by its human occupants. The diversity of fauna present in Levels 3 and 4 at Galeria da Cisterna is similar to that observed at contemporaneous sites such as Gruta do Caldeirão (see Section 3.3), although unlike Gruta do Caldeirão, no horse remains were recovered. The small size of the assemblage may explain the lack of equid remains, though a gradual decrease in the abundance of equid remains from the middle to Upper Palaeolithic is observed at Gruta do Caldeirão.

Lithics

A total of 52 lithic artefacts were recovered from Levels 3-3a, whilst 44 were recovered from Level 4. Only four retouched tools were identified in Levels 3 and 4; a pointed blade and a backed bladelet in the former, and a denticulate blade and a dihedral burin in the latter. In Level 3-3a, the most abundant artefact type is the flake, whilst in Level 4, chips are the most abundant form of débitage. In level 3, the lithic assemblage is comprised of 31% flint, 31% quartz, 36% quartzite and 2% basalt, whilst Level 4 is overwhelmingly dominated by quartzite (71%), with flint and quartz comprising 20% and 9% of the assemblage respectively. Although no detailed investigation of the assemblages has been published to date, it seems likely that many of these raw materials were collected locally, as flint, quartz and quartzite would have been available in the vicinity of the site (approximately 30km).

3.2.3 *Lapa dos Coelhos*

Lapa dos Coelhos is a small limestone cavity situated approximately 10 metres above Galeria da Cisterna (Bicho, Haws and Almeida, 2011). Eight stratigraphic layers were identified (Figure 11) during excavations between 1997 and 2010, spanning the Mousterian through to the Final Magdalenian. Level 10, the lowermost layer, is ascribed to the Mousterian based on the ephemeral presence of characteristic Levallois technology. As a result of the recovery of a fragmented leaf point, Levels 6 – 8 are tentatively attributed to the Solutrean, though other tools and débitage uncharacteristic of the Solutrean are also present in these layers. Levels 3 and 4 belong to the Upper and Final Magdalenian respectively, based on radiocarbon dating of animal bones. These layers are characterised by the presence of bone projectiles and hooks, as well as fish remains. The geological characteristics of each of the eight stratigraphic levels are discussed at length in (Almeida *et al.*, 2004). Lapa dos Coelhos is a particularly small cavity, with a usable space of less than 10m^2. In line with Binford's descriptions of hunter-gatherer site function, Gameiro *et al*. (2011) argue with some certainty that Lapa dos Coelhos was not a residential base camp, but rather a temporary camp with a specific short-term function.

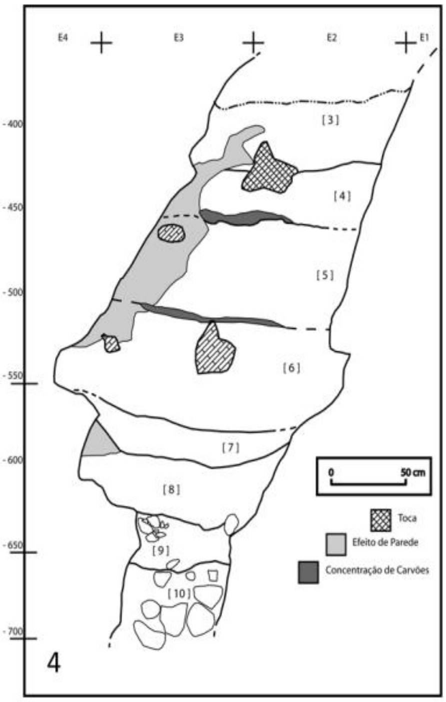


Figure 11. Stratigraphic cross section of Lapa dos Coelhos. From Gameiro et al. (2017: 367).

Fauna

A full zooarchaeological report on Lapa dos Coelhos is yet to be published, but preliminary results are available and can be found in (Gameiro, Davis and Almeida, 2017). A variety of taxa are present in the Lapa dos Coelhos faunal assemblage, including large terrestrial herbivores, carnivores and fish. As implied by the name of the cave itself, however, the zooarchaeological assemblage is dominated by the remains of rabbit and hare. In the Solutrean levels (6 – 8), deer, ibex, aurochs, horse, wild boar, hare, rabbit, bear, hyena and wolf are represented. In the overlying Magdalenian levels (3 and 4), a decrease in the variety of herbivorous species can be observed, with only deer, ibex, boar, hare and rabbit present. In these Magdalenian deposits, carnivores are virtually non-existent, with only three carnivore remains recovered (identified as fox and wild cat). Fish bones and aquatic mollusc shells, however, are present in Levels 3 and 4. Approximately 89% of the fish remains were excavated from Level 4, where the bones of salmonids, shad, carp and barbell are present in association with a combustion structure. Three bone tools interpreted as fishing hooks were identified in Level 4. Two saltwater periwinkles (*Littorina obtusata*) plus more than 30 shells from the freshwater mollusc *Theodoxus fluviatilis* were also recovered from this level; of which 15 of the latter are pierced.

The proportion of lagomorph remains increases significantly from the Solutrean to the Upper and Final Magdalenian levels at Lapa dos Coelhos, with rabbit and hare comprising 42% in the former and 94% and 98% in the latter respectively. This is comparable to temporal shifts in lagomorph abundance at Gruta do Caldeirão, where a considerable increase can be observed between Solutrean and Magdalenian assemblages (Davis, 2002; Lloveras *et al.*, 2011).

Lithics

In the Mousterian level (10), only seven stone artefacts were excavated; six of which are made from quartzite. The presence of a Levallois core and a Levallois flake prompted the assignment of Level 10 to the Mousterian, though it is argued that the presence of these stone artefacts is likely due to natural accumulation of run-offs from other occupied cavities within the karstic system, rather than physical human occupation of the site (Zilhão *et al.*, 2013).

The Solutrean levels (6 – 8) yielded a much greater abundance of lithic artefacts, with a total of 508 lithic pieces recovered including 26 retouched tools. 61% of these artefacts are made from quartz, which is local and can be collected nearby, whilst flint accounts for 22% of the lithic assemblage. 76% of this flint was identified as being locally sourced, with approximately 5% suspected to be Oxfordian and could be gathered more than 30km away from the site. A perforated bear canine and a bone artefact described by Gameiro *et al.* (2017) as a 'tip' were also recovered from the Solutrean layers. The presence of a broken but typically Solutrean leaf point prompted the attribution of Layer 8 to the Solutrean, but the majority of the artefacts are somewhat uncharacteristic of the Solutrean technocomplex seen elsewhere. Gameiro *et al.* (2017) put forward two possible explanations for this: first, that these layers may represent the 'Solutro-Gravettian' phase described by (Zilhão, 1997), or alternatively, that these layers represent the sedimentary mixing of Solutrean and Magdalenian deposits. They argue that the latter explanation is more plausible.

The highest abundance of stone artefacts was excavated from the Final Magdalenian level (3), from which 6379 pieces were recovered. Of these, a total of 303 retouched artefacts were identified. The most common raw material represented in this layer is flint (62%), followed by quartzite (19%), quartz (16%) and other sources (2%). Approximately 80% of this flint can be collected within 10km of the site, whilst 12% was identified as Oxfordian and could have been collected approximately 30km away. The remainder of the flint was not identifiable to source. Level 3 exhibits a particularly high percentage of blades (60%), though very few of the pieces could be refitted. The Upper Magdalenian level (4) also contained an abundance of stone artefacts; 2055 pieces in total. 96 of these artefacts are retouched, and 47% of the total assemblage is made from flint. Quartz pieces comprise 36% of the assemblage, whilst quartzite represents 10%. 99% of the flint is from local sources, with only four pieces of Oxfordian flint present. As is the case for Level 3, blades are abundant (52% of the assemblage), but no refitting pieces were identified. As well as stone artefacts, five bone implements were also recovered – three of which have been interpreted as fish hooks, and two of which are likely to represent projectile weapons.

3.3 Gruta do Caldeirão

Gruta do Caldeirão is a south-facing cave site (39° 38′ 54″; 8° 24′ 54″ W) which lies close to the Nabão River, approximately 7 kilometres north of Tomar and 140 kilometres northeast of Lisbon within the Tagus valley, Estremadura. It is the largest Late Pleistocene collection in Portugal (Davis, Robert and Zilhão, 2007), and was excavated between 1979 and 1988. It consists of a 6-metre-thick sequence spanning the Mousterian, Early Upper Palaeolithic, Solutrean, Magdalenian and Neolithic periods (Trinkaus, Bailey and Zilhão, 2001).

Human remains have been recovered from Solutrean (H-Fa), Magdalenian (Eb) and Neolithic (Ea) levels at Gruta do Caldeirão. To date, 11 human specimens (assigned Caldeirão 1-11) have been published; 9 of which were recovered from Solutrean levels and 2 of which were recovered from Magdalenian contexts. These are described by Trinkaus *et al.* (2001) in detail. These 11 specimens are suspected to represent 8 individuals, all aged between childhood and early adulthood. During 2015, the Gruta do Caldeirão collection was re-examined, and a further 26 human remains were identified in levels Eb and ABC-D. These are currently undergoing direct radiometric dating and are yet to be published.

Fauna

Gruta do Caldeirao provides us with the largest Late Pleistocene faunal collection in Portugal, and as such, has attracted a great deal of research interest over the last several decades. A broad overview of the faunal assemblage is provided by Davis (2002), but several publications explore aspects of the collection in greater detail. Rodent remains from the site are discussed at length by Povoas *et al.* (1992), and mollusc shells are reported by (Callapez, 2003). A detailed analysis of the lagomorph remains (in particular, their mode of accumulation) was carried out by Lloveras *et al.* (2011), and the function of the cave through time was discussed in depth by Davis *et al.* (2007).

The ungulate portion of the Gruta do Caldeirao faunal assemblage is typical of the Late Pleistocene in Iberia, and is dominated by the remains of red deer, ibex and horse. Wild boar, aurochs chamois and goat are also represented. Vast quantities of rabbit and hare remains were recovered throughout the sequence, and a variety of smaller species including fish (a single salmonid bone), birds (partridges, songbirds, and corvids), rodents and shellfish. A plethora of carnivores are also represented, including species such as hyena, lynx, bear, lion, leopard, wildcat, wolf and fox. The proportions of these species (both herbivore and carnivore) vary through time from the Mousterian to the Magdalenian, providing insights into both carnivore and human behaviour at the site and the function of the cave itself. Of the large terrestrial herbivores, red deer is the most abundant species in every stratigraphic layer.

However, their overall contribution in each layer changes through time; increasing from 39% in the Mousterian layers (K, L M, N) to 52% in the Solutrean layers (Fa, Fb, Fc, H and I; Davis, 2002). The proportion of red deer falls again in the Magdalenian layer (Eb), amounting to just 32%. The proportion of horse remains drops steadily from the Mousterian to the Magdalenian, contributing 19% of the Mousterian faunal assemblage and just 3% of the Magdalenian collection. Goat remains also decrease in percentage over time, from 18% in the Early Upper Palaeolithic layers (Ja, Jb) to just 1% in the Magdalenian. Where carnivores are concerned, some species can be observed to decrease in abundance with time; others increase. Hyena and wolf, for example, decrease in abundance from the Mousterian to the Magdalenian level, from which no remains were recovered at all. The proportion of lynx, on the other hand, increases from 9% in the Mousterian to 15% in the Magdalenian, and badger remains (which are non-existent in the Mousterian layers) account for 9% in the Magdalenian deposit.

Rabbits are the most abundant remains in every cultural unit except for the Neolithic. The number of rabbit remains increases over time, with 806 recovered from Mousterian levels, 1551 recovered from Early Upper Palaeolithic layers, 9406 recovered from Solutrean layers, and a final slight decrease to 5248 recovered from the Magdalenian layer. The question of how such vast quantities of remains were accumulated (and by whom) is addressed by Lloveras *et al.* (2011). By comparing taphonomic data from the Mousterian and Solutrean lagomorph assemblages, Lloveras *et al.* (2011) argue that the former was accumulated primarily by Eagle Owls (*Bubo bubo*) and the latter a product of human activity. This conclusion is drawn based on the presence of damage to the Mousterian bones indicative of carnivore activity, such as digestion corrosion and puncture marks. The comparative lack of such evidence in the Solutrean assemblage, combined with the presence of anthropogenic cut marks, burn marks and bone breakage patterns consistent with marrow extraction points to humans as the primary accumulators of the Solutrean lagomorph assemblage, though a small proportion of the remains may also have been the result of carnivore activity.

The gradual increase in rabbit remains observed over time is considered to be significant in terms of human behaviour. Human occupation of the cave during the Mousterian period is evidenced by the presence of stone tools and accompanying cut marks on ungulate bones, but it seems that lagomorphs such as hare and rabbit did not play as great a role in the subsistence strategies of Neanderthals as they did in the subsistence strategies of the Upper Palaeolithic humans who occupied Gruta do Caldeirão during the Solutrean period. This is readily apparent at other Portuguese sites such as Lapa do Picareiro and Vale Boi, and it is suggested that this may be the result of ecological changes which forced human groups to shift from large terrestrial herbivores to smaller game, and/or the development of new hunting technologies such as traps and nets (Lloveras *et al.* 2011).

Lithics

A detailed study of the lithic assemblage at Gruta do Caldeirão was carried out by Zilhão (1997), but a brief overview is provided here. The stone tool assemblages recovered from the Mousterian and Early Upper Palaeolithic layers are relatively small and the bone:tool ratios are low, suggesting that the site was not used intensively by humans during those periods. However, the presence of a high concentration of points and barbs in the lithic assemblages of layers Ja and I (which make up more than 50% of the total retouched tools in those layers) leads *Davis et al.* (2007) to suggest that specialised hunting may have taken place in the latest early Upper Palaeolithic and the earliest Solutrean. Furthermore, a greater number of stages of the Chaîne opératoire are represented in the lithic assemblages of the Solutrean and Magdalenian layers, suggesting that the site was likely to have been occupied for a greater length of time – possibly in seasonal, residential occupations.

3.4 Climatic context

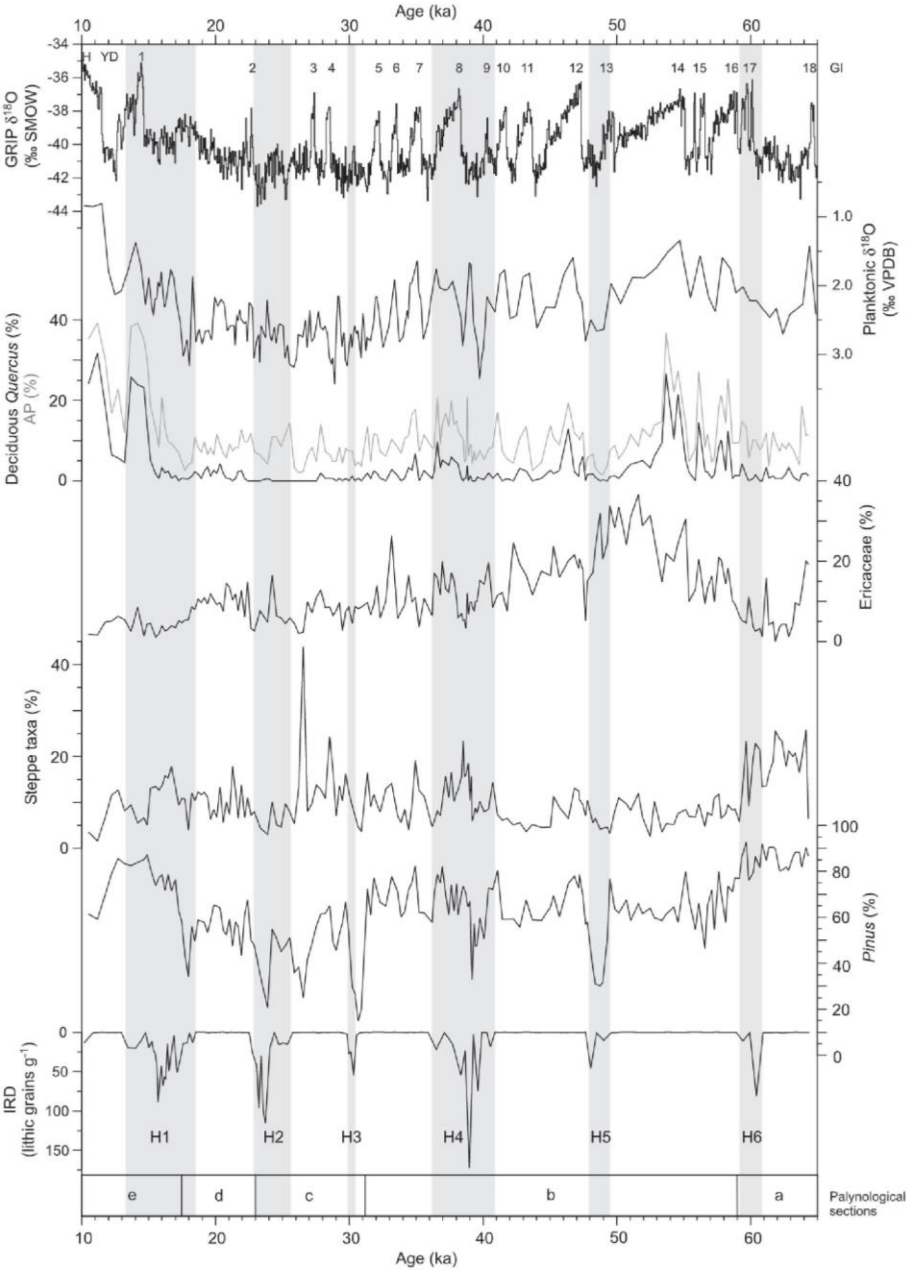


Figure 12. Key pollen taxa and marine proxy data from the MD95-2039 core plotted on the GRIP-derived time scale. From Roucoux *et al.* (2005: 1644).

The subsistence strategies, mobility patterns and geographical distribution of any given animal is intrinsically linked to the availability and distribution of the resources upon which it survives. The distribution and availability of these resources may change in response to fluctuations in temperature; not only on a seasonal basis at higher and lower latitudes, but also on a larger scale as global climatic conditions change over time. If changes (or lack thereof) in human and animal subsistence strategies and mobility patterns in Central Portugal are to be investigated, then an understanding of the oscillating nature of the climate over the course of the Middle and Upper Palaeolithic is essential.

A series of climatic cycles known as Dansgaard-Oeschger (D-O) events occurred during the Middle and Upper Palaeolithic periods in Europe, characterised by very abrupt warming episods (often occurring in less than 60-100 years) and followed by slow climatic deterioration (Rasmussen, Thomsen and Moros, 2016). Brief but extreme stadial episodes known as Heinrich Events (HE) occasionally punctuate these slow periods of cooling, and can often be correlated with the larger stadial periods recorded in the Greenland ice cores (Rasmussen *et al.* 2016). D-O events and HE events can be clearly seen in North Atlantic marine sediment cores within the Ruddiman belt (stretching from Newfoundland in North America to Portugal on the western European coast) in the form of ice-rafted debris (IRD) released from the Laurentide ice sheet (Rasmussen *et al.,* 2016).

Our current understanding of climate during MIS 3 to MIS1 in Central Portugal is derived primarily from marine and terrestrial sediment cores (though the latter are relatively limited), along with environmental evidence from archaeological deposits such as those at the Gruta da Oliveira. Analysis of marine core MD95-2039 (Figure 12), located in the sedimentary catchment basin of the Douro River, illustrates a relatively dry, cold and open Estremaduran landscape between 65 and 59ka, with small tree and shrub patches dominated by typical steppe taxa and *Juniperus* scrub (Roucoux *et al.*, 2005). These conditions were likely brought about by the H6 event; a period of increased climatic severity. Between 59ka and 31ka, arboreal pollen is observed to fluctuate on a millennial scale, with *Quercus, Pinus* and Ericaceae gradually declining with each D-O stadial. Two very significant decreases in *Pinus* are evident in this section of the MD95-2039 core, corresponding with HE events 5 and 4 respectively, which produced colder, drier and more severe conditions.

Between 31ka and 23ka, cold and dry conditions prevailed, with herbaceous and steppe-like vegetation dominating the landscape. During this phase, the H2 event (26.5 – 24.3 ka cal BP; (Sánchez Goñi and Harrison, 2010) evident in Galician marine sediment cores MD99-2331 and MD03-2697 coincided with another significant reduction in *Pinus* and the expansion of herbaceous taxa, implying a sharp increase in aridity (Naughton *et al.*, 2007). In cores MD95-2042 and MD95-2039, this event appears to have prompted an abrupt drop in sea surface temperature, accompanied by a significant decline of warm-adapted vegetation, though small patches appear to have survived precariously in refugia along the coast (Naughton *et al.*, 2009; Bicho *et al.*, 2015). In southwestern Iberian marine sediment cores such as SU81-18, ODP-976 and SO76-6KL, an expansion of semi-desert vegetation is evident, but the reduction in *Pinus* is less severe (Naughton *et al.,* 2007). Contemporaneous Level H at Gruta do Caldeirão has yielded microfauna consistent with these inferences; indeed the presence of the common vole (*Microtus arvalis*) implies a dry and open environment (Povoas *et al.*, 1992).

The H2 event was succeeded by the Last Glacial Maximum (LGM; 24.3ka cal BP), which heralded a return to full glacial conditions in northern Europe (Álvarez-Solas *et al.*, 2011). Ice cover reached its maximum extent during this period and the Gulf Stream was displaced southwards; running from Cape Hatteras (NC, North America), directly to Portugal (Straus, 1991; Straus, 1992). In northwestern Europe, the severe cold and aridity brought about by this event appears to have resulted in a contraction in human range, and an apparent increase in the number of sites across the Iberian Peninsula and Italy (Straus, 1992). Conditions in Portugal appear to have been, however, warmer, moister and more stable than preceding periods (Roucoux *et al.*, 2005). Marine sediment cores (MD99-2331 and MD03-2697) document the expansion of *Pinus* and the continued presence of herbaceous communities, and the presence of pollen from temperate trees suggests that patches of temperate vegetation survived in scattered refugia. This seems consistent with microfauna recovered from Solutrean levels Fb and Fa at Gruta do Caldeirão; the latter dated to just after the LGM (Trinkaus *et al.,* 2001; Zilhão, 2013). A strong percentage of wood mouse (*Apodemus sylvaticus),* Mediterranean pine vole (*Microtus duodecimcostatus*) and Lusitanian pine vole (*Microtus lusitanicu*s) in these levels points to the presence of forests and damp meadows (Povoas *et al.,* 1992). Towards the end of this phase, however, the contraction of tree populations is evident in the MD95-2039 core, which coincides with the onset of the H1 cooling event (Roucoux et al., 2005).

The H1 event (dated to between 18ka and 15.6ka cal BP; Sánchez Goñi and Harrison, 2010) is characterised in the Galician marine sediment cores by a cold and humid phase, with moist conditions reflected by the presence of *Isotes* fern (Naughton *et al.,* 2007). Conversely in the southwest, trees such as *Pinus* persisted whilst markers for steppe conditions increased (Turon, Lézine and Denèfle, 2003). Subsequently, a period of warming known as the Bölling-Allerød (D-O 1) occurred between 14.6ka and 12.8ka cal BP (Rasmussen *et al.*, 2014), after which an extremely abrupt and severe cold period known as the Younger Dryas persisted in Europe until approximately 11.5ka cal BP (Blockley *et al.*, 2006). In Portugal, pollen data from the south-western marine sediment core SU81-18 documents the expanse of temperate plant species and the establishment of open woodland during this period; as do north-western cores such as MD95-2039 and MD99-2331 (Fletcher *et al.*, 2010). Similarly, in Magdalenian level Eb of Gruta do Caldeirão, an overall decrease in the percentage of field vole (*Microtus agrestis*) and high percentages of wood mouse and garden dormouse (*Eliomys quernicus*) imply a decrease in humidity, and the onset of more Mediterranean conditions (Povoas *et al.,* 1992). Large species such as Spanish ibex and chamois essentially disappear in Eb, whilst wild boar (*Sus scrofa*) numbers escalate (Davis, 2002; Povoas, 1992).

To conclude, pollen and marine isotope data from marine sediment cores off the coast of Portugal appear to be relatively consistent with Greenland ice core records for the last 70,000 years. The Estremaduran landscape was characterised by fluctuations in arboreal cover, which appear to be correlated with HE events. Nevertheless, the persistence of temperate vegetation during cold phases suggests that certain Portuguese regions acted as refugia (Figueiral and Terral, 2002), providing favourable and reliable habitats for humans, animal and vegetation. The Gruta do Caldeirão microfauna documents a roughly similar trend in terms of the changes observed in the pollen records of the cores, with evidence for colder, steppe-like conditions during the Solutrean and gradual amelioration of conditions and expansion of Mediterranean woodland throughout the Magdalenian. The microfaunal evidence is further supported by the apparent decline of cold-adapted species such as Spanish ibex and chamois in Magdalenian levels; again illustrating the gradual onset of warmer conditions after the LGM.

3.5 Evidence for Middle and Upper Palaeolithic subsistence and mobility strategies in Portuguese Estremadura

Portuguese Estremadura (and in particular, the Almonda karstic system) offers us the rare opportunity to consider both Neanderthal and anatomically modern human subsistence strategies and mobility patterns diachronically. Although few seasonality studies have been carried out to date, the region provides a rich zooarchaeological record of the species exploited by human groups throughout the Middle and Upper Palaeolithic, and has the potential to inform our understanding of changes (or lack thereof) in subsistence strategies over time. The following section is a brief overview of the zooarchaeological and lithic evidence for Neanderthal and AMH subsistence and mobility strategies in Portuguese Estremadura, bringing together evidence from the sites sampled in this study and others in the region.

3.5.1 Middle Palaeolithic subsistence and mobility

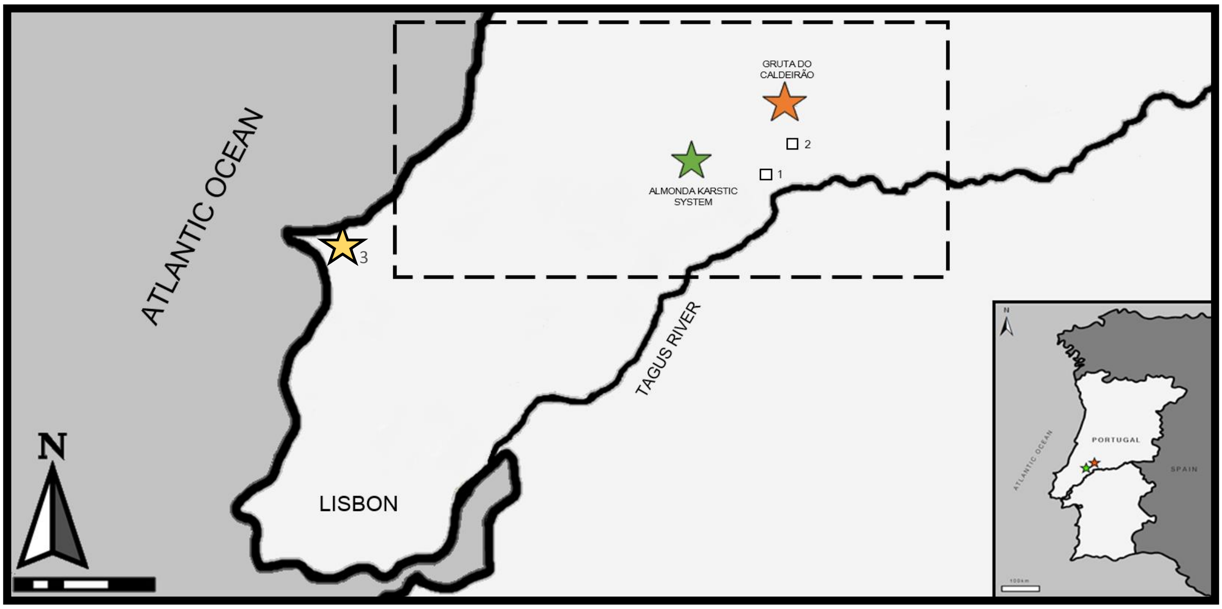


Figure 13. Map of Central Portugal showing the study area (dashed box) and key Mousterian sites. White squares indicated open air sites (1. Ponte de Pedra and Fonte da Moita; 2. Santa Cita). Stars indicate cave sites (3. Gruta Nova da Columbeira). The latter falls outside of the study area, but is briefly discussed in the text.

The Middle Palaeolithic is represented in a number of open air and cave sites in Portuguese Estremadura (Figure 13), though the latter are the primary source of the faunal assemblages from which our current understanding of Middle Palaeolithic subsistence is derived – perhaps due to more suitable conditions for preservation. Open air sites within the study area such as Santa Cita (Bicho and Ferring, 2001), Ponte da Pedra (Rosina and Grimaldi, 2001) and Fonte da Moita (Rosina, Boton and Grimaldi, 2000) offer valuable insights into mobility patterns, and are discussed in depth in Section

10.2 in the context of the isotope data gathered in the current study. The following is a brief overview of three key Middle Palaeolithic cave sites in Central Portugal, which provide both faunal and lithic assemblages.

Rich deposits associated with Neanderthal occupation were identified at Gruta do Aroeira (Galeria Pesada) in the Almonda karstic system (Figure 13), and preliminary reports paint a picture of the site as a temporary camp occupied seasonally in a circulating mobility system (Marks *et al.*, 2002). Cut-marked cervid remains (including those of fallow deer and red deer) dominate the faunal assemblage, comprising 81% of the total NISP. Horse, rhinoceros, ibex and tortoise are also present, and the cut marks and fracture patterns identified on these faunal remains are consistent with intensive butchering and marrow extraction by humans, suggesting that butchering activities took place at the site. Analysis of the relative abundances of skeletal elements present amongst the large herbivore taxa suggest that cervid carcasses were brought into the cave whole, whilst equids were likely to have been brought back in parts. The presence of juvenile red deer in Level C suggests a spring season of death, though a dedicated investigation of red deer seasonality is yet to be carried out. Rabbit and small bird remains are abundant, but a lack of anthropogenic cut marks and a high proportion of hind limb elements imply that they were accumulated as a result of raptor activity. It is deemed likely, therefore, that the cave was occupied by humans during the spring and summer months, and primarily by raptors and other carnivores such as bear during winter. Marks *et al.* (2002) conclude that Gruta do Aroeira was not an ephemeral campsite, but rather a temporary base camp revisited on a seasonal basis, at which a range of activities took place. These conclusions are drawn based on the fact that a) the remains of young bear, raptors and raptor-accumulated mesofauna were recovered, suggesting that the cave was occupied by carnivores during the winter; b) the remains of anthropogenically accumulated foetal and infant Cervid remains were recovered, implying that humans occupied the cave during the spring and summer months, and c) discrete lenses of artefacts can be observed within levels, suggesting that the site was repeatedly visited. Gruta do Aroeira appears to be more than an ephemeral camp, however, since evidence of both primary and secondary butchering activities is abundant. Tool rejuvenation, but a comparative lack of cores, is further argued to point to the site as a temporary camp at which a range of activities took place, likely on a seasonal basis.

Gruta da Oliviera has also yielded a considerable faunal assemblage since excavations began in 1989. Mousterian occupation levels are dominated by red deer remains, but ibex, horse, rhinoceros and rabbit are also present. Interestingly in layers 15-19 (dating to 70, 000 – 44, 000 years calBP), excavators identified the clustered, burnt remains of tortoise shells, along with a multitude of bones of the same taxon. Cut and burn marks on the bones suggest beyond reasonable doubt that these remains were accumulated primarily by the human occupants of the cave, although the presence of several carnivore coprolites and hyena teeth implies that Gruta da Oliviera was, at times, occupied by carnivores as well (Zilhão *et al.*, 2010a). The Mousterian deposits are suggested to represent a palimpsest, produced as a result of alternating human and carnivore occupation. What becomes more difficult to establish is the timescale of the alternation; in short, for how long did Neanderthal groups utilise the cave before the its primary occupants switched to carnivores, and vice versa? Zilhao *et al.* (2010) discuss the results of the analysis of charcoal recovered from Mousterian levels, whereby tree species indicative of cold phases such as *Pinus sylvestris* and *Erica arborea* were identified. In contrast, palynological analysis of the hyena coprolites provides evidence for more temperate species such as *Quercus* and *Betula.* They tentatively suggest that such evidence may reflect the use of the Gruta da Oliviera alternately by humans and carnivores depending on environmental conditions; humans exploiting the shelter of the cave during the harsh, cold stadial periods, and carnivores inheriting the site during the more amenable interstadial periods.

Middle Palaeolithic levels at Gruta Nova da Columbeira, a limestone cave located on the west coast of Portugal near Bombarral, provide a detailed insight into shifts in subsistence and mobility patterns over the period. Uranium-thorium dating of levels 7-9 imply that the Middle Palaeolithic deposits date to between 54 and 61 ka. The faunal assemblage recovered from layers 6-8 is dominated by red deer, comprising more than 50% of the identifiable pieces, but ibex, horse, aurochs, rhino and tortoise are also present (Cardoso, Raposo and Ferreira, 2002). Carnivores including hyena, lynx, wolf and bear are also present at the site, with hyena comprising 9% of assemblage from Layers 6-8. Analysis of the lithic assemblages across the Mousterian levels points to changing patterns of mobility, with the site alternating between use as a permanent residential base and a temporary camp (ibid). These inferences are based on the following observations: high occurrences of retouched tools in levels 9, 6 and 4 suggest that the site was occupied intermittently, with humans and carnivores occupying the cave alternately, whilst levels 7 and 8, on the other hand, exhibit a high density of combustion residues and lithic artefacts, combined with a low occurrence of stone tool retouch implies that the site was occupied for longer durations. The presence of such a range of prey species in the Mousterian layers is argued by Cardoso, Raposo and Ferreira (2002) to reflect the nature of Neanderthals as 'non-specialised' hunters, exploiting the abundant red deer in the sheltered valleys and lowlands, as well as ibex along the exposed limestone crags in the vicinity of the site and aurochs grazing along the coastal grasses.

3.5.2 Upper Palaeolithic subsistence and mobility

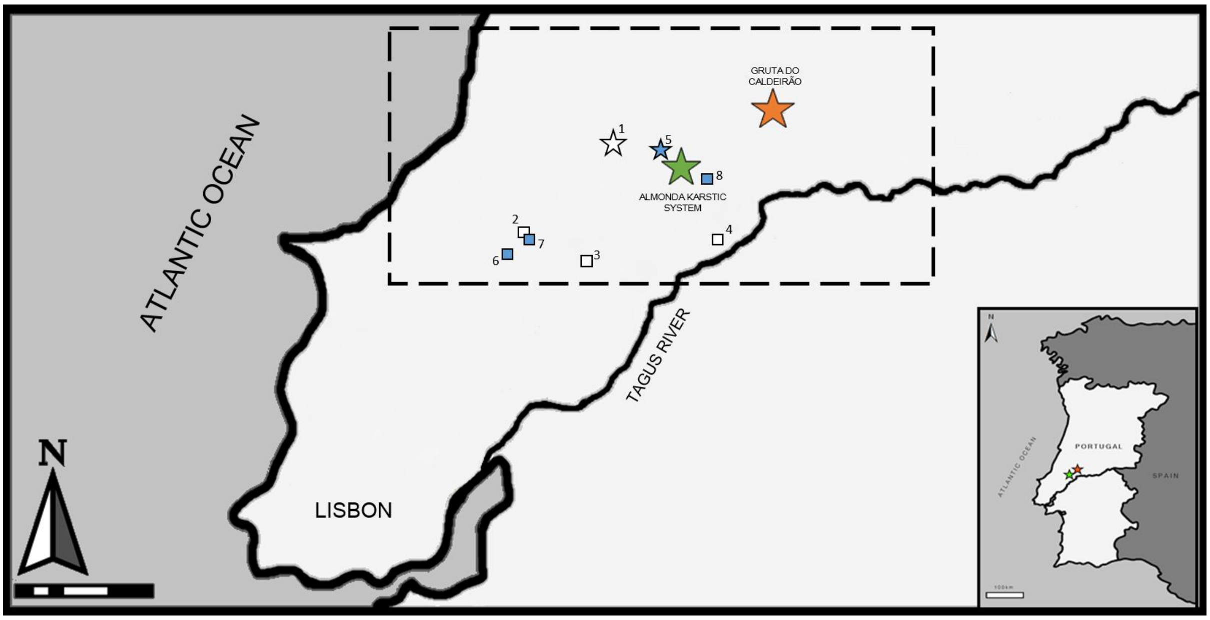


Figure 14. Map of Central Portugal showing the study area (dashed box) and key Solutrean and Magdalenian sites. The white star and squares indicate Solutrean cave and open air sites respectively (1. Lapa do Anecrial; 2. Olival de Carneira; 3. Passal and Olival de Arneiro; 4. Casal do Cepo). The blue star and squares indicate Magdalenian cave and open air sites respectively (5. Lapa do Picareiro; 6. Bocas; 7. Cabeço de Porto Marinho; 8. Bairrada).

A number of open air sites, caves and rockshelters in Portuguese Estremadura have produced Upper Palaeolithic material that has the potential to inform our understanding of the subsistence strategies and mobility patterns of human groups during the Late Pleistocene (Figure 14). The range of fauna exploited by anatomically modern humans appears not to differ significantly from that of Neanderthals; indeed, both hunter-gatherer groups appear to have exploited a range of large terrestrial herbivores; primarily red deer, though the contribution of species such as wild boar and ibex appears to have fluctuated over time. During the Upper Palaeolithic, small game appears to have become a more extensively exploited resource; in particular the remains of rabbit are found in abundance in Solutrean deposits at Gruta do Caldeirão, and in Magdalenian deposits at Lapa do Picareiro and Lapa dos Coelhos. Combined lines of archaeological evidence suggest that Upper Palaeolithic humans living in Portuguese Estremadura may have engaged in varying patterns of mobility over time. Solutrean open air sites located within the study area such as Casal do Cepo (Zilhão, 1997), Olival de Carneira, Olival de Arneiro and Passal (Zilhâo *et al.*, 1995), and Magdalenian sites such as Bairrada (Zilhão, 1997) and Cabeço de Porto Marinho (Bicho, 2002) are discussed in detail in terms of the inferences to be made from their assemblages with regard to mobility in Section 10.2. The following section focuses on the evidence for the range of animal resources exploited at three major sites during the Solutrean and Magdalenian periods in Portuguese Estremadura – Gruta do Caldeirão, Lapa dos Coelhos and Lapa do Picareiro respectively.

Faunal remains recovered from Solutrean deposits at Gruta do Caldeirao are discussed in length by Davis *et al.* (2007), Davis (2002), and Lloveras *et al.* (2011). The ungulate portion of the collection is dominated by *Cervus elaphus, Capra and Equus;* typical for the Late Pleistocene in Iberia (Davis *et al.,* 2007). Aurochs, wild boar, chamois and goat were also recovered. A number of these large herbivore remains exhibit clear evidence of human activity, including cut marks, burn marks and signs of marrow extraction. Like many Upper Palaeolithic sites in Estremadura, lagomorph remains were recovered in vast quantities. Detailed taphonomical studies of leporids from Middle and Upper Palaeolithic levels were carried out by Lloveras *et al.* (2011). Based on the increase in the ratio of rabbits to ungulates over time, Davis (2002) proposes that Gruta do Caldeirão provides evidence of resource intensification by humans; giving weight to the theory that human exploitation of small mammals such as rabbits was not significant until the Upper Palaeolithic. Preliminary evidence suggests that the site was not an ephemeral campsite used briefly for hunting; rather, it was occupied for longer periods of time. This tentative conclusion is based primarily on the fact that all stages of lithic reduction are present, and the presence of skeletal and dental remains of humans of a variety of ages (including adults, adolescents and infants), suggesting that family groups frequented the site, not just teams of specialised hunters (Zilhão, 1997).

A range of prey species were also identified in the faunal assemblages of Magdalenian deposits at Lapa dos Coelhos. Rabbit remains are abundant, though red deer and caprids are also well represented. Along with terrestrial mammals, the remains of both freshwater and anadromous fish (including trout, salmon and barbell) were recovered. The lithic assemblages of Levels 3 and 4 attest to the use of the site as something more short-term than a residential base camp; indeed, very little refitting could be achieved, suggesting that much of the Chaîne opératoire was carried out elsewhere. The absence of cores and the high proportion of incomplete tools such as flakes implies that semi-prepared pieces were brought to the site, in order to be retouched for functions as required. Taken together, Gameiro *et al.* (2011) argue that this is evidence for short stays at the site. They further suggest that the high quantity of lithic artefacts recovered from the Upper Palaeolithic levels, particularly Level 3, reflect repeated short-term visits to the cave. The typological nature of the lithic assemblage hints at the use of the site. The presence of fish vertebrae and worked-bone 'fish hooks' in Level 4 paint a clear picture of fishing activities, and the high proportion of lithic points (many of which are broken) potentially attests to the repair of hafted hunting weapons used to target larger, terrestrial prey. Puncture marks associated with impact fractures were identified in three animal bones in Level 3, supporting the notion that such tools were used as projectiles. The technological and typological variety of the tools associated with their frequency in both Upper and Final Magdalenian levels suggest a palimpsest context, resulting from successive occupations of hunter-gatherer groups. As such, Lapa dos Coelhos is considered to be a site of short-term occupation; a shelter used repeatedly (perhaps seasonally) during the Upper Palaeolithic for carcass butchery and specialised tasks such as the hunting of red deer, caprids and rabbit (Bicho and Haws, 2012; Hockett and Haws, 2009b).

Another major site of particular significance is Lapa do Picareiro, a cave with well-preserved Magdalenian deposits located on the west face of the Serra d'Aire. Red deer comprise c.70% of the identifiable macrofauna, making this species the most abundant large herbivore in the Tardiglacial assemblage. Wild boar are the second most abundant species of macrofauna, comprising approximately 29% of the collection. Chamois, aurochs and roe deer are also present, though the remains are relatively ephemeral. Analysis of the red deer and wild boar remains (in particular, the representation of different skeletal elements) revealed two contrasting processing patterns. The wild boar remains are dominated by cranial elements, followed by metapodials and phalanges. Limb bones, vertebrae and ribs are highly underrepresented, suggesting that the heads were removed (in order to reduce transport costs) and the remaining carcass was relocated to another site or area. Bicho, Haws and Hockett (2006) describe this as a classic reverse utility pattern, whereby the least useful elements are consumed or abandoned, and the most useful parts are transported elsewhere. Red deer, on the other hand, exhibit a much more equal overall skeletal representation, suggesting that they were processed differently. It is suggested that fillets of meat may have been smoked or dried at the site to be transported; perhaps to larger residential sites. Around 9000 leporid bones were also recovered from Tardiglacial levels at Lapa do Picareiro; approximately 80% of which (c.146 individuals) were located in or around two hearth features. Burn marks were frequently identified on foot bones and the ends of long bones, indicating that the animals were likely roasted whole over hot coals and consumed at the site (Bicho *et al.*, 2000). Several hundred fish bones from both anadromous shad and marine sardine species were also recovered from the Tardiglacial deposits, most of which are vertebrae.

Much of the faunal and lithic evidence points to the use of Lapa do Picareiro as a temporary seasonal camp, occupied briefly in order to hunt and process red deer and wild boar. Several red deer molars from Stratum F (11 700 – 12 300 BP) were selected for cementum annuli analysis, and both exhibited late autumn/early winter deaths. Two wild boar phalanges were also examined (one from Stratum F and one from Stratum E; 10 000 – 11 700 BP), and based on epiphyseal fusion rates, the individuals both died during autumn/winter. The lithic assemblage is dominated by chips produced through the sharpening of tools, and in contrast, very few cores are present, suggesting that function-specific tools or blanks were brought to site ready-made. Rabbits appear to have been hunted or trapped in the vicinity of the Lapa do Picareiro, and subsequently consumed at the site in order to sustain the hunters. Extensive bone breakages imply that marrow extraction and grease rendering also took place.

3.5.2 Isotopic evidence

Very little isotopic work has been carried out in Portuguese Estremadura to date, but the data that do exist provides the foundations for the current study. Palaeodietary isotope studies of Upper Palaeolithic humans and associated fauna from Galeria da Cisterna and Gruta do Caldeirão were carried out by Linscott (2015, 2016; unpublished, Figures 15 and 16), and sequential strontium isotope analysis of Neanderthals and anatomically modern humans from Gruta da Oliviera and Galeria da Cisterna (see Section 10.2) were carried out by Pike *et al.* (unpublished).

Upper Palaeolithic

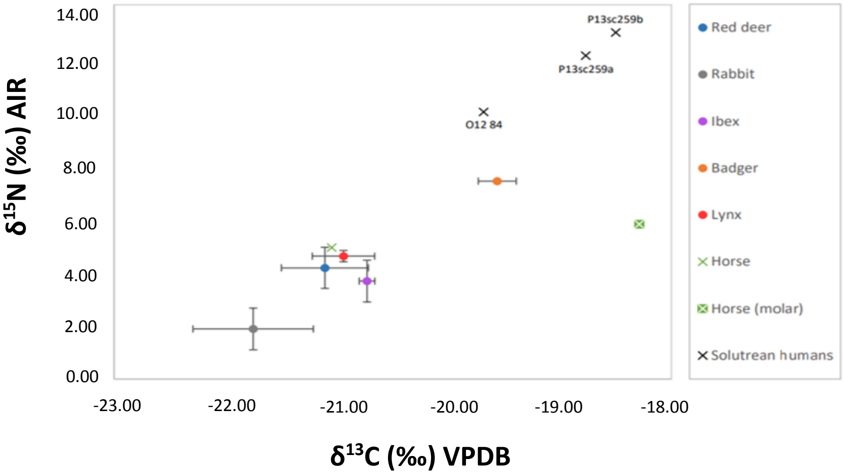


Figure 15. Stable C and N isotope data for bone collagen recovered from Solutrean humans and fauna from Gruta do Caldeirão. P13sc259a (Caldeirão 6) is the apical portion of an I2 tooth root, P13sc259b is the crown dentine of the same tooth, and O12 84 is derived from the bone collagen of a mandible. From Linscott (2016: 49).

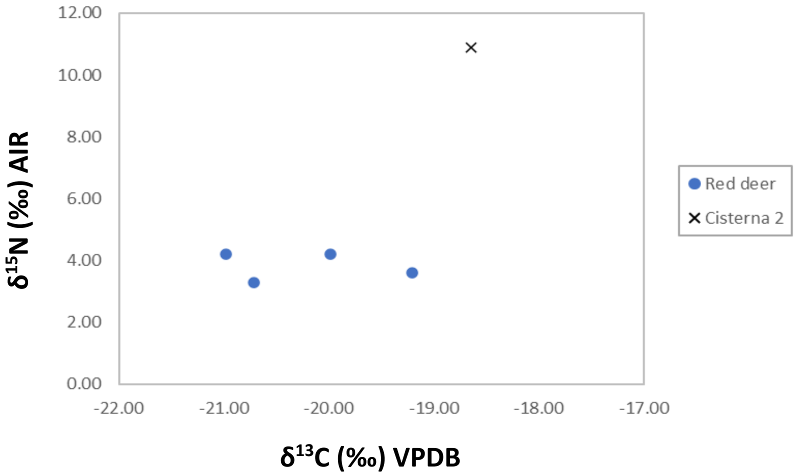


Figure 16. Stable C and N isotope data for bone collagen recovered from a Magdalenian human (Cisterna 2) and red deer from Galeria da Cisterna and Lapa dos Coelhos respectively. Data from Linscott (2015).

The isotopic data for the two Solutrean humans (O12-84 and P13sc259), the Magdalenian human (Cisterna 2) and the associated fauna indicates broadly terrestrial diets. The poor level of organic preservation at the Magdalenian sites prevented the production of a large faunal dataset with which to interpret the human isotope data, but nevertheless, the consumption of an amount of aquatic protein by the Magdalenian individual is implied by the relatively high δ^{15}N. This is consistent with archaeological evidence recovered from contemporaneous deposits at Lapa dos Coelhos, where the remains of fish were found alongside bone implements assumed to be fishing hooks (Gameiro *et al.,* 2011). The high δ^{15}N values of the Gruta do Caldeirão human bone collagen also implies a potential aquatic protein input to the diets of these individuals, despite the scarcity of zooarchaeological evidence for fish at the site. This may be due to the consumption of freshwater or marine resources at other sites in the region, prior to the deposition of the human remains at Gruta do Caldeirão. Such an explanation would support existing archaeological evidence, suggesting that human subsistence in Estremadura during the Upper Palaeolithic was characterised by the temporary use of cave sites as strategic camps for the specialised hunting of seasonal prey.

4. Strontium and Oxygen isotope analysis in archaeology

4.1 Geochemical principles of strontium isotope analysis

Strontium is an alkali earth metal and exists as four naturally occurring stable isotopes – ^{88}Sr, ^{87}Sr, ^{86}Sr and ^{84}Sr. Whilst ^{88}Sr, ^{86}Sr and ^{84}Sr are formed solely though nucleosynthesis, ^{87}Sr is also produced through the decay of radioactive rubidium, ^{87}Rb, which has a half-life of approximately 4.96×10^{10} years (Villa *et al.*, 2015). Therefore, the ^{87}Sr/^{86}Sr ratio of a geological unit is a function of the amount of the initial ^{87}Sr/^{86}Sr and ^{87}Rb /^{86}Sr ratios initially present at the time of its formation, and the amount time elapsed since its formation (Bentley, 2006).

4.1.1 From the geosphere to the biosphere

It has been proven experimentally that ^{87}Sr/^{86}Sr ratios are not significantly affected by mass fractionation in nature; in other words, strontium isotope ratios pass from the geosphere into the biosphere without major alteration (Flockhart *et al.*, 2015). Soils reflect the ^{87}Sr/^{86}Sr ratios of the bedrock from which they are derived, and groundwater will echo the strontium isotope signal of deposits through which it passes (Montgomery, 2010). These ^{87}Sr/^{86}Sr ratios are subsequently adopted by the tissues of plants growing within the sediment or water as a result of strontium uptake (ibid.).

Strontium readily substitutes for calcium in calcium-bearing minerals due to both cations sharing the same valency (Blaschko *et al.*, 2013). The calcium component of the primary mineral phase present in animal bones and teeth, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], is therefore commonly replaced by strontium during biomineralisation (Hillson, 1986). This substitution appears be benign; indeed, to date there is no evidence for the regulation of strontium through homeostatic mechanisms (Montgomery, 2010). Strontium and calcium are metabolised by animals in the same way, and as such, teeth and bones will reflect the ^{87}Sr/^{86}Sr ratios of food and water consumed during the formation and remodelling of these respective tissues (Blaschko *et al.*, 2013; Ericson, 1985). As such, the ^{87}Sr/^{86}Sr ratios observed in the biomineralised tissues of a given organism should primarily echo the isotopic signal of the underlying geology of the area in which said tissue was formed or remodelled (Flockhart *et al.,* 2015), though there are several major factors that can affect the strontium isotope variation in the biosphere.

4.1.1.1 Strontium isotope variability in the biosphere

The deposition of aeolian sediments and foreign wind-transported dust particles can significantly alter the ^{87}Sr/^{86}Sr composition of soils. Loess can contribute substantially to the silicate fraction of native soils, and can travel thousands of kilometres before deposition (for example, dust particles originating in Asia have been identified over 6,000km away in Hawai'i (Chadwick *et al.,* 1999). Soils with considerable contributions from aeolian sediments may therefore have bulk $^{87}Sr/^{86}Sr$ compositions that result from the mixing of local geology and loess. The depth at which a sediment sample is taken for $^{87}Sr/^{86}Sr$ measurement is relevant in light of this, since the relative contribution of wind-transported strontium will be greater towards the surface in comparison to lower levels, where the weathering of bedrock is greater (Bentley, 2006). The contribution of loess may be a more considerable problem in arid or volcanic regions in comparison to more temperate biomes, but should nevertheless be borne in mind here.

In areas of high rainfall, plants may also adopt a significant proportion of their overall strontium from rainwater, resulting in a value that lies somewhere on a mixing curve between the soil and rain $^{87}Sr/^{86}Sr$ values. Indeed, (Chadwick *et al.*, 1999) established that in the highly weathered geological environments of the oldest Hawaiian mountain chains, the amount of strontium delivered to plants by rainwater is comparable to that delivered by the weathering of local rocks; essentially producing an overall $^{87}Sr/^{86}Sr$ composition that is a mixture of both sources of strontium. It follows, then, that on a swathe of homogenous underlying geology, two sedentary populations may exhibit different tissue $^{87}Sr/^{86}Sr$ values depending on the amount of rainfall received by their locality (Montgomery, 2010). The tissue $^{87}Sr/^{86}Sr$ values of coastal communities gathering and consuming resources along the seaboard may also be affected by sea spray, since the concentration of strontium in saline fluids is much greater than in freshwater. Plants growing within the reach of coastal sea splash and sea spray may therefore be subject to $^{87}Sr/^{86}Sr$ values that lean away from the underlying geology and towards the mean ocean seawater value (*ibid*).

As well as environmental factors, the sampling methods used when generating data for bioavailable strontium maps can have considerable effects upon the $^{87}Sr/^{86}Sr$ variation in a given area. In a study of strontium bioavailability, Britton *et al.* (2020) found that even when sampled upon the same underlying geology, different plant species exhibited inconsistent $^{87}Sr/^{86}Sr$ compositions due to varying root depth. As such, specialisation in the plant consumption of grazing species (and indeed humans) could theoretically result in isotope variation even when gathered upon isotopically homogenous underlying geology. Whilst sampling plants with different root depths in order to capture the bulk soil variability of an area may mitigate this issue, it is perhaps unlikely to resolve the potential issue of anthropogenic $^{87}Sr/^{86}Sr$ input. Agricultural processes such as liming can alter the $^{87}Sr/^{86}Sr$ of surface waters, thereby affecting the bioavailable strontium isotope composition of nearby areas regardless of underlying geology. In their study of the effects of lime treatment upon non-calcareous soils in Denmark, Thomsen and Andreasen (2019) observed that samples taken along 1.5km of a stream running through lime-treated farmland decreased from 0.7131 to 0.7099, potentially altering the strontium isotope composition of plants growing in the locality. As such, it is important to establish (where possible) to what degree any given study area has experienced agricultural activity such as liming, since samples taken from anthropogenically altered areas may not necessarily reflect the bioavailable strontium isotope values of the time period in question.

4.1.1.2 Dietary sources of strontium

Another major factor affecting the strontium isotope value of animal tissues (in particular, omnivores) is the composition of the diet. Strontium concentration varies between sources, and as such the overall body plasma $^{87}Sr/^{86}Sr$ value of an individual will be a mixture weighted towards the dietary source(s) with the highest strontium concentrations. Compared with fruits, vegetables and cereals, meat and fat have particularly low strontium isotope concentrations, and as such the body fluid strontium isotope signature in an omnivore will lean more towards plant-based dietary sources than animal-based sources (Montgomery, 2010; Millour *et al.*, 2012). Drinking water can also be an important source of strontium in the diet (Weber *et al.,* 2020). Since an abundance of cut-marked faunal remains were recovered from Palaeolithic deposits in the Almonda karstic system and Gruta do Caldeirão, we can be relatively confident that the meat of large terrestrial fauna contributed to the diets of Middle and Upper Paleolithic humans, but preservation bias makes it impossible to establish through archaeological means the contribution of plant foods. It should be borne in mind, then, that an unknown proportion of the individuals' measured enamel strontium isotope composition is likely to be derived from plant foods, and therefore weighted isotopically towards the substrates upon which they were gathered. Shellfish, fish and salt also bear high concentrations of strontium (Millour *et al.* 2012), and can contribute significantly to the body plasma $^{87}Sr/^{86}Sr$ if consumed (Fenner and Wright, 2014). The presence of marine shells at the study sites combined with the potential isotopic evidence for freshwater fish consumption by the Cisterna 2 individual (Section 3.5.2) suggests that riverine and marine resources were indeed consumed, and as such the comparatively high strontium contribution from these foods should be borne in mind when examining the measured enamel strontium isotope data.

4.2 Laser ablation MC-ICPMS

Despite its relative youth as a sampling technique, laser ablation has become routine within a number of fields including geology, biology and materials sciences over the past several decades (Durrant and Ward, 2005). Coupled to a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS), laser ablation systems offer high-throughput, spatially resolved sampling, often with accuracy and precision comparable to that of conventional solution based methods such as TIMS. The following is a brief introduction to the methodology.

Laser ablation (MC)-ICP-MS involves the in-situ single-spot or raster sampling of solid matrices within a closed sample cell. Laser pulses volatilise and mobilise material from the selected sample region, which is then swept out of the chamber by the carrier gas(es) and onwards to the ICP for ionisation. The wavelength of the laser chosen for ablation dictates its interaction with the sample matrix - lower wavelengths are generally considered to provide more clearly defined ablation pits, and as a result, higher spatial resolution (Durrant and Ward, 2005).

At the ICP torch, argon gas is passed through a concentric quartz-tube structure wrapped within a radio frequency induction coil at one end. Plasma production is initiated by a spark generated by a Telsa unit, which ionises a portion of these argon atoms. Oscillating radio frequencies are generated to produce an intense electromagnetic field (Bazilio and Weinrich, 2012), which causes charged particles to move through the plasma in a circular motion, generating temperatures of several thousand degrees centigrade (Pollard *et al.*, 2007). Cations and electrons accelerating through the existing plasma then ionise other argon molecules, hence producing a constant flow of plasma (Bazilio and Weinrich, 2012). The plasma will reach equilibrium and a constant temperature of between 6,000 - 10,000°C, providing that sufficient argon gas is supplied. Sample molecules flowing into the torch as an aerosol are dried to a solid state and subsequently atomized, at which point collision with charged argon atoms prompts sample ionisation (ibid). These ions then reach the interface, at which point a pressure reduction brings the sample gas to the correct pressure and temperature for entrance to the mass spectrometer. Following exit from the interface, ions are focused into a narrow beam and selected based on their mass to charge (*m/z*) ratios. In multicollector systems, a series of faraday cups (often up to twelve) or ion counters are employed to collect masses of interest. The use of multiple faraday cups eliminates the difficulty in implementing jump routines for time resolved analyses (Woodhead *et al.*, 2005a).

4.3 Why laser ablation?

A number of recent strontium isotope studies of incrementally formed samples have illustrated the merits of LA-MC-ICP-MS (Haak *et al.*, 2008; Pryor *et al.*, 2016; Woodhead *et al.*, 2005b). Indeed, the ability to produce sample spots with diameters of <200µm opens up the possibility of generating in-situ climatic, dietary and migratory data recorded in microscopic, temporal growth increments such as those found in tooth enamel, speleothems and otoliths at a greater resolution than is possible through mechanical micro-sampling (Lewis *et al.,* 2016; Woodhead *et al.,* 2005). Laser ablation also offers a greatly reduced sample preparation time and fast sample throughput in comparison to conventional solution-based analyses (Simonetti, Buzon and Creaser, 2008). Despite this, conventional methods are still routinely used in archaeological strontium isotope analysis, perhaps primarily as a result of the ongoing difficulty of overcoming isobaric interferences (Lewis *et al.,* 2014). Whilst LA-MC-ICP-MS offers a variety of unique qualities that make it well-suited for the investigation of palaeomobility, its merits should not serve to mask its limitations – rather, its limitations should be carefully considered with respect to those of conventional analyses.

Conventional methods such as thermal ionisation mass spectrometry (TIMS) and inductively coupled plasma mass spectrometry (ICP-MS) are generally considered to be the benchmark in strontium isotope analyses in terms of their high degree of precision and accuracy (Jackson and Günther, 2003). The ion exchange chemistries associated with sample preparation permit the removal of many of the chemical species that present isobaric interferences in laser ablation MC-ICP-MS, offering greater precision and accuracy than can be achieved via LA-MC-ICP-MS analyses of bioapatites (Pollard *et al.,* 2007). Recent work, however, has demonstrated that LA-MC-ICP-MS can generate isotopic data of a quality comparable to that offered by TIMS (Simonetti *et al.,* 2008; and references therein).

Laser ablation MC-ICP-MS also offers the opportunity to sample with incredibly high spatial resolution. Geological and biological materials such as zoned crystals, speleothems, otoliths and tooth enamel develop incrementally, recording fluctuations in isotopic ratios and chemical composition as they form. As such, they harbour a wealth of temporally resolved information on a microscopic scale, which can be accessed if sufficiently highly resolved, accurate and precise sampling methods are available. Davidson *et al.* (2001) carried out one of the earliest isotopic studies using laser ablation MC-ICP-MS, in which isotopically zoned feldspar crystals were analysed with sample radii of 100-300µm. The ability of the laser ablation system to carry out both single-spot sampling and rastering enabled not only the discrete sampling of distinct zones within the feldspar crystal, but also the continuous sampling of the crystal from the rim to the core. The latter also enabled the integration of isotopic data with topographical surface maps of the feldspars, which provided a wealth of information about changes in the magmatic environment during crystal formation (Davidson et al., 2001). Laser ablation sampling methods are now used widely in geological research, not only in mineralogical provenancing studies but also in single crystal dating applications (Guillong *et al.*, 2016; Paton *et al.*, 2007). The spatially resolved sampling of biological samples is also a growing topic of research. Using an excimer laser, Woodhead *et al.* (2005) were able to sequentially sample fish otoliths with diameters of 500µm, ablating rectangular craters with lengths of 147µm and widths of just 3µm. This produced approximately 2000 data points across a single otolith, demonstrating a clear shift in $^{87}Sr/^{86}Sr$ values between the otolith core and the rest of the sample. The high resolution sequential sampling of tooth enamel is also possible, as demonstrated by Pryor *et al.* (2016). Using a laser spot size of 150µm, strontium isotope data were collected down the growth axis of reindeer enamel samples at $10\mu m^{-1}$, producing a far greater number of temporally resolved data points than have been collected in similar studies employing conventional mechanical sampling. Horizontal line scanning (raster analysis) is not the only sampling option for the generation of spatially resolved isotopic data.

Intrinsically linked to spatially resolved laser ablative sampling is reduced sample destruction. Ablation pits are invisible to the naked eye, and as such, LA-MC-ICP-MS considered to be an ideal methodology for the analysis of rare remains such as hominin teeth, where mechanically destructive sampling is often unfeasible. However, in order to generate high spatially and temporally resolved strontium isotope data from tooth enamel, the sectioning of teeth is desirable. This, as Nowell and Horstwood (2009: 1338) put it, "…is about as sample destructive as one can get." Understandably, the sectioning of hominin teeth is unlikely to be an option unless extant breakages are present, as was the case in the study of Neanderthal mobility carried out by Pike *et al.* (unpublished). Alternatives to sectioning have been explored; indeed, the sampling of external enamel surfaces was investigated by Roux *et al.* (2014). The suitability of this method, however, is a subject of debate. Nowell and Horstwood (2009) suggest that micromilling combined with TIMS offers the least destructive analytical methodology, arguing that with appropriate micromill hardware and software, sampling resolution comparable to laser ablation can be achieved. It has also been suggested that laser drilling, which involves the off-line ablation of a sample and subsequent collection of ablated material for preparative ion exchange chemistries and TIMS analysis, may be a viable alternative to LA-MC-ICP-MS (Willmes *et al.*, 2013). As of yet, however, no published examples of the use of this technique exist. Whilst such a method has the potential for greater accuracy, minimal sample destruction and no flat sample surface requirements, the process would undoubtedly be significantly slower than LA-MC-ICP-MS.

TIMS analysis, whilst boasting excellent within-run precision and accuracy, is undoubtedly a more time consuming process. Ion exchange chemistries must first be employed in order to separate the desired element from the rest of the sample matrix in order to reduce isobaric interference (Pollard *et al.,* 2007), and refined samples must then be mounted in a solid state onto a filament for subsequent introduction, ionisation and analysis (Walczyk, 2004). Laser ablation MC-ICP-MS, on the other hand, does not require time-consuming chemical sample preparation. Though some chemical pretreatment may be carried out for archaeological enamel samples suspected to have been affected by diagenetic processes, the only preparative measure required is the mounting of the sample within the ablation cell; whether that be through the use of a resin block (see Woodhead *et al.,* 2005) or through other means. Where the analysis of an individual TIMS samples may take up to two hours to complete (not counting the several days required to complete preparative chemistry), a single laser ablation analysis can be carried out in approximately two minutes (Davidson et al., 2001; Simonetti *et al.,* 2008).

4.4 Considerations

Whilst LA-MC-ICP-MS offers a number of advantages for spatially resolved strontium isotope analyses, a series of inherent caveats must be taken into account. A number of isobaric interferences on key masses can cause considerable offsets in strontium isotope ratios, which must be appropriately dealt with if precise and accurate measurements comparable to conventional solution analyses are to be obtained (Woodhead *et al.,* 2005). A lack of appropriate matrix-matched international standards also presents difficulties in regards to instrument calibration (Lewis, Coath and Pike, 2014; Horstwood, Evans and Montgomery, 2008a). Furthermore, difficulties in maintaining laser focus across uneven surfaces and variation in the degree of instrumental isotopic fraction must be carefully considered (Vroon *et al.*, 2008). All of these factors, if not appropriately taken into account and addressed, may contribute to a reduction in the precision and accuracy of strontium isotope analysis via LA-MC-ICP-MS.

4.4.1 Isobaric interferences

Rubidium

^{87}Rb produces an isobaric interference on mass 87, directly affecting ^{87}Sr/^{86}Sr measurements (Woodhead *et al.,* 2005). In conventional solution analyses, the application of ion exchange chemistries enables the removal of rubidium from the sample prior to analysis, effectively eliminating the risk of mass interference (Copeland *et al.*, 2008). However, the absence of preparative chemistries in laser ablation MC-ICP-MS results in the need to correct for the isobaric interference of ^{87}Rb on ^{87}Sr. As rubidium has no interference-free stable isotopes, the correction is often carried out by monitoring ^{85}Rb, which must first be stripped of interferences from doubly-charged rare earth elements (Yb^{2+} and Er^{2+}) as well as calcium dimers (^{42}Ca^{43}Ca+) (Woodhead *et al.,* 2005; Lewis *et al.,* 2014). ^{87}Rb can then be stripped from the ^{87}Sr peak based on an assumed natural ^{87}Rb/^{85}Rb ratio of 0.3856 (Copeland *et al.,* 2008) after appropriate mass bias corrections have been carried out (Woodhead *et al.,* 2005).

Krypton

Krypton exists as an unavoidable impurity within helium and argon carrier gases (Paton *et al.*, 2007). Stable krypton isotopes interfere with masses 86 and 84, and as such, they can alter the ^{84}Sr/^{86}Sr ratio used in mass bias corrections (ibid). Woodhead *et al.* (2005) advocate the use of on-peak gas blanks, during which the instrument parameters are kept constant apart from the closure of the laser shutter to prevent sample ablation. This is generally the preferred method in both geological and archaeological studies (Davidson *et al.*, 2001; Copeland *et al.*, 2010; Horstwood, Evans and

Montgomery, 2008b; Lewis *et al.*, 2014; Pryor *et al.*, 2016). Other analysts consider peak-stripping via mass 83 to be a preferable correction method (eg. Christensen *et al.*, 1995) though interference from doubly-charged rare earth elements and diatomic molecules must also be considered if a peak-stripping approach is to be employed for the analysis of calcium-bearing matrices.

Diatomic molecules

Isobaric interference on masses of interest from calcium dimers and argides have been recognised in strontium isotope analyses of both geological and archaeological materials via LA-MC-ICP-MS. Diatomic molecules such as ^{48}Ca^{40}Ca[Ar], ^{46}Ca^{40}Ca[Ar] and ^{44}Ca^{40}Ca [Ar] have the potential to interfere at masses 87, 86 and 84 respectively, and as such their presence can have measurable effects on the ^{84}Sr/^{86}Sr ratio; a constant which is often used as a quality control (Woodhead *et al.,* 2005). The influence of Ca dimers and argides is often monitored via mass 82 (^{42}Ca^{40}Ca/^{42}Ca^{40}Ar) and subsequently stripped from the 86 and 84 peaks based on natural abundance ratios (see Copeland *et al.,* 2010; Horstwood *et al.,* 2008; Woodhead *et al.,* 2005).

Calcium phosphate (^{40}Ca^{31}P^{16}O)

The chemical nature of bioapatite presents the analyst with one molecular interference in particular that is seldom encountered in geological strontium isotope studies. The inherent presence of phosphates and calcium in tooth enamel permits the formation of calcium phosphates within the plasma, producing an isobaric interference of ^{40}Ca^{31}P^{16}O on mass 87 (Horstwood *et al.,* 2008). The degree of ^{40}Ca^{31}P^{16}O interference is related to the strontium concentration of the sample; the greater the concentration of strontium, the lower the level of interference (Lewis *et al.,* 2014). ^{40}Ca^{31}P^{16}O interference therefore becomes increasingly problematic when dealing with samples that exhibit low strontium concentrations, which are not uncommon in archaeological enamel (Nowell and Horstwood, 2009). Despite this, ^{40}Ca^{31}P^{16}O interference has been overlooked entirely in some publications. In a study of Neanderthal mobility at the site of Lakonis, Greece, Richards *et al.* (2008) carried out strontium isotope analysis of a histological tooth section via LA-PIMMS. The data were interpreted as the first evidence for Neanderthal mobility over a range of approximately 20km, but a failure to appropriately address the effects of isobaric interferences calls this into question. Whilst the strontium concentration was not reported by Richards *et al.* (2008), Nowell and Horstwood (2009) argue that the strong correlation between the inverse of the analysis length and the ^{87}Sr/^{86}Sr ratio measured during each spot analysis is indicative of variable strontium concentration, hence the ^{87}Sr/^{86}Sr values considered to reflect three distinct geologies are likely, in fact, to be nothing more than analytical artefacts partially caused by varying ^{40}Ca^{31}P^{16}O interference.

Although $^{40}Ca^{31}P^{16}O$ interference has the potential to significantly offset $^{87}Sr/^{86}Sr$ ratios in LA-MC-ICP-MS analyses of bioapatites, very few studies to date have directly addressed the problem. Attempts have recently been made to directly minimise the effects of $^{40}Ca^{31}P^{16}O$ formation within the plasma through dedicated tuning protocols (de Jong, 2013), customised plasma interfaces (Lewis *et al.,* 2014), and most recently, 'conventional' interference corrections based on strontium concentration and interference/signal ratios (Irrgeher, Galler and Prohaska, 2016). The efficiency of oxide formation within the plasma is dependent on a number of factors, including sampler and skimmer cone orifice size (Vaughan and Horlick, 1990), the flow rate of sample gas, and the addition of nitrogen to the carrier gas flow (Foster and Vance, 2006; Lewis *et al.*, 2014). By measuring the $^{238}U^{16}O^+/^{238}U^+$ ratio in NIST SRM 610 and treating this as a proxy for $^{40}Ca^{31}P^{16}O$ production efficiency, de Jong (2013) was able to tune the instrument (a Thermo-Finnigan Neptune MC-ICP-MS) for significantly reduced oxide formation. Repositioning the plasma torch, increasing the flow rate of helium and increasing the operating power to 1500W enabled the $^{238}U^{16}O^+/^{238}U^+$ratio to be reduced to 0.035%, hence the accuracy of apatite standard $^{87}Sr/^{86}Sr$ measurements could be brought within 100ppm at 2σ. Building on this, Lewis *et al.* (2014) attempted to directly reduce oxide formation through the development of a unique plasma interface, which utilised a flow of helium gas directly behind a customised skimmer cone, akin to a collision cell. Through the deployment of an inert stationary gas phase after plasma ionisation, it was hoped that $^{40}Ca^{31}P^{16}O$ molecules would dissociate prior to mass selection, hence reducing the effects of $^{40}Ca^{31}P^{16}O$ interference. This new interface was tested through the analysis of two bone and enamel reference materials, as well as two Pleistocene rhinoceros molars. An optimum helium flow of 0.0475l/min brought LA-MC-ICP-MS $^{87}Sr/^{86}Sr$ values to within 90ppm of the TIMS values (Lewis *et al.,* 2014). Combining this custom plasma interface with low oxide tuning as outlined by de Jong (2013), it was possible to achieve LA-MC-ICP-MS accuracy to within analytical uncertainty for samples with strontium concentrations of >90ppm.

Doubly-charged rare earth elements (REEs)

Elements with low ionisation potentials may form doubly charged ions (Taylor, 2001). The presence of doubly charged rare earth elements (REEs) such as erbium (Er) and ytterbium (Yb) in sample matrices is therefore a concern in strontium isotope analysis via LA-MC-ICP-MS, as ions such as these will exhibit an apparent *m/z* value half of that observed for the corresponding singly charged isotopes (Taylor, 2001; Paton *et al.*, 2007). $^{168}Yb^{2+}$ and $^{168}Er^{2+}$ will interfere with ^{84}Sr, whilst $^{172}Yb^{2+}$, $^{174}Yb^{2+}$ and $^{176}Yb^{2+}$ will interfere with strontium at masses 86, 87 and 88 respectively. Furthermore, $^{170}Yb^{2+}$ and $^{170}Er^{2+}$ have the potential to interfere with ^{85}Rb, a measurement essential for the correction of ^{87}Rb on ^{87}Sr. Indeed, the greater the interference of these two doubly charged ions on mass 85, the greater the amount of ^{87}Rb will be stripped from the ion current of mass 87 due to an overestimation of ^{85}Rb;

hence the production of a lower $^{87}Sr/^{86}Sr$ ratio than is present in reality (Ramos, Wolff and Tollstrup, 2004; Vroon *et al.*, 2008). As $^{84}Sr/^{86}Sr$ and $^{86}Sr/^{88}Sr$ ratios are used in quality control and mass bias corrections (Woodhead *et al.,* 2005), and because rubidium has no interference-free stable isotopes (Paton *et al.,* 2007), doubly charged rare earth element interferences can therefore present a considerable obstacle where strontium isotope analysis via LA-MC-ICP-MS is concerned.

REE interference has been approached in a number of ways in multiple fields of study. In their strontium isotope study of perovskite (ABO_3) derived from kimberlites, Paton *et al*. (2007) indirectly monitored the impact of doubly charged Er and Yb ions by observing $^{166}Er^{2+}$ and $^{173}Yb^{2+}$ at masses 83 and 86.5 respectively, subsequently subtracting associated contributions from the aforementioned masses based on assumed isotopic ratios. However, calcium argides and dimers at mass 83 ($^{43}Ca^{40}Ar$ and $^{43}Ca^{40}Ca$) were not taken into account, potentially corrupting the corrections made for doubly charged REEs (though, as the authors argue, perhaps minimally considering that $^{43}Ca[^{40}Ca^{40}Ar]$ represents just 0.13% of Ca ions). Copeland *et al.* 2010 utilised this method during their study of Pliocene-Pleistocene rodent tooth enamel, but upon finding that the REE signals obtained were low enough to be insignificant within the measured external precision, the authors chose to use the non-REE-corrected $^{87}Sr/^{86}Sr$ values in order to reduce data scatter. Woodhead *et al.* (2005) suggest that REE interference is generally not a major concern in the strontium isotope analysis of modern carbonates, as they tend to contain very low abundances of such elements. Ancient carbonates (and bioapatites), on the other hand, are flagged as being more prone to REE incorporation and subsequent interference; primarily due to diagenetic processes (Woodhead *et al.,* 2005; Lewis *et al.,* 2014). Woodhead *et al.* (2005) suggest that monitoring yttrium (Y) at mass 89 can aid in alerting the analyst to the presence of doubly charged rare earth elements in the sample matrix (Woodhead *et al.,* 2005), and indeed, this method is commonly used in archaeological studies (see De Jong, 2013; Pryor *et al.*, 2016). As diagenetic alteration is a matter of concern in archaeological strontium isotope studies, it is not uncommon for samples with high REE concentrations to simply be excluded from further analysis due to the risk of diagenetic Sr (De Jong *et al.*, 2010; Lewis *et al.*, 2014).

4.4.2 Instrumental isotopic mass fractionation

Isotopic mass fractionation is inevitable during mass spectrometry, occurring to different extents depending on the system in question (Le Roux *et al.*, 2014). The reasons for this fractionation are varied, and can occur during ionisation, mass separation and detection (Walczyk, 2004). In LA-MC-ICP-MS, two primary causes are identified by Jackson and Gunther (2003): laser-induced fractionation and ICP-induced fractionation. The former is considered to be a result of the preferential volatilisation of lighter isotopes under low-energy ablation conditions, and the simultaneous preferential condensation of the heavier isotope within the ablation crater (Jackson and Günther, 2003). Furthermore, undulating sample surfaces can result in areas of reduced laser focus, which can affect the efficiency of the ablation and cause variations in the degree of isotopic mass fractionation across a sample (Balter *et al.*, 2012). ICP-induced fractionation, on the other hand, is thought to occur as a result of incomplete volatilisation and ionisation (Jackson and Günther, 2003). Both of these fractionation sources, amongst others, can result in artificially varied isotope ratios (Balter *et al.,* 2012).

Instrumental isotopic fractionation is commonly addressed through application of an exponential mass bias law, which utilises the deviation of the measured ratio of two internal stable isotopes from their natural ratio (Paton *et al.,* 2007). In strontium isotope analyses via LA-MC-ICP-MS, an exponential mass bias law and the natural ^{86}Sr/^{88}Sr ratio (0.1194) is routinely used as a correction for instrumental isotopic mass fractionation (Le Roux *et al.,* 2014).

4.4.3 Matrix matched standards

Ideally, an in-house analytical standard for the strontium isotope analysis of archaeological human and animal teeth should have a strontium concentration comparable to archaeological specimens, a homogenous ^{87}Sr/^{86}Sr ratio, and a matrix identical or highly similar to tooth enamel. However, modern tooth enamel is generally unsuitable in that strontium concentrations are often far greater than those in archaeological specimens, and isotope ratios it exhibits are likely to be inhomogeneous due to the inherently incremental nature of its formation. The latter of these problems, however, can be rectified through controlled feeding studies. A study involving the feeding of isotopically homogenous foodstuffs to pigs from birth until death was carried out at the University of Bristol (Lewis *et al.,* 2017) resulting in the formation of pig tooth enamel with homogenous ^{87}Sr/^{86}Sr values. These teeth were analysed via TIMS to determine ^{87}Sr/^{86}Sr values, and were subsequently sectioned and mounted in epoxy for analysis via LA-MC-ICP-MS. These teeth are used within the current study as an in-house standard.

4.5 Principles of oxygen isotope analysis

The oxygen isotope ratios ($^{18}O/^{16}O$) in mammalian tooth enamel carbonate and phosphate can be used as a proxy for local temperature at the time of tooth formation. The oxygen isotope composition of precipitation differs as a result of variables including altitude, latitude, distance from the coast and air temperature (Gourcy, Groening and Aggarwal, 2005).The latter of these parameters, air temperature, varies with the seasons in mid and high latitudes. The highest $\delta^{18}O$ values correlate with the warmest seasonal temperatures, and the lowest $\delta^{18}O$ values are associated with the coldest annual temperatures (Balasse *et al.*, 2003). Body water reflects the oxygen isotope composition of the meteoric water ingested by the individual, and as such, those $\delta^{18}O$ values are incorporated into the carbonate and phosphate fractions of tooth enamel *(ibid).* Seasonal temperature variations in rainfall $\delta^{18}O$ are therefore recorded as sinusoidal profiles in teeth whose enamel form over the course of a year or longer, and those data can be recovered through sequential oxygen isotope analysis. Conversely, if an individual engages in migratory behaviour, the measured $\delta^{18}O$ profile may appear flat or dampened since the act of avoiding climatic extremes ensures a degree of stability in environmental conditions experienced by the individual (Britton *et al.,* 2009).

Certain physiological factors can complicate the interpretation of oxygen isotope signals from tooth enamel. Of particular importance is the nature of water intake, which can vary between terrestrial animal species – whilst most are obligate drinkers and require the intake of liquid water to survive, some (non-obligate) are able to survive purely on the water stored within the vegetation they consume (Pederzani and Britton, 2019). Because non-obligate drinkers obtain all or most of their water from vegetation, their body water $\delta^{18}O$ values are often lower than obligate drinkers (Sun *et al.,* 2019). This is because leaf water is often enriched in ^{18}O relative to meteoric water as a result of evapotranspiration (*ibid*). The amount of enrichment can even vary spatially within a single plant (Wang and Yakir, 1995). Different plant species, depending on the depth of their root systems, may also exhibit $\delta^{18}O$ values that differ from the local meteoric water – shallow-rooted plants sample water near to the soil surface, which is liable to undergo a degree of enrichment due to evaporation. Deep-rooted plants, on the other hand, sample water that is less susceptible to evaporative processes, and therefore less likely to become enriched through these means (Pederzani and Britton, 2019). Since evapotranspiration and source water enrichment can result in variations in the $\delta^{18}O$ values of plants in the same locale, it should be borne in mind that preferences for certain browse or graze in non-obligate drinkers may lead to inhomogenous oxygen isotope profiles in tooth enamel, regardless of whether seasonal temperature change occurs.

Time-resolved oxygen isotope data in tooth enamel can be recovered through several analytical techniques. The most widely employed method involves the mechanical sampling of teeth along the growth axis of the enamel, either by drilling sequential samples down external enamel surface, or by removing a full slice of enamel and dividing it into samples (with any associated dentine removed) along the growth axis. These samples are then powdered and treated with acetic acid in order to reduce the impact of secondary mineralisation upon the measured isotope ratio. Once pre-treated, the samples are then subjected to analysis via isotope ratio mass spectrometry. This usually involves digestion in phosphoric acid, in order to obtain $\delta^{13}C$ and $\delta^{18}O$ values for CO_2 derived from the carbonate phase of the enamel. The number of sequential samples that can be taken depends heavily on the size of the tooth, the sampling method and the limit of detection of the instrument used, and can vary from just two or three to somewhere in the region of fifteen to twenty where large animal teeth are concerned.

In order to achieve greater spatial resolution for small samples containing time-resolved oxygen isotope data, such as human tooth enamel and fish otoliths, several micron-scale methods exist. The first of these, laser ablation GC-IRMS, has been under development for some time at the University of Utah (Cerling and Sharp, 1996; Passey and Cerling, 2006). The method involves the spot analysis of polished cross sections or outer enamel surface using a CO_2 laser. Very few facilities in the world are capable of this type of analysis, however. The alternative method, secondary ion mass spectrometry (SIMS), involves the use of a focussed ion beam in order to eject ions from the sample surface. The collision causes the ejection of atoms and molecules from the sample surface, which are then ionised. These 'secondary ions' are collected, and the stable isotope ratios are measured using an on-line mass spectrometer (for example, see Kita *et al.,* 2009). Spot sizes generally range from 1-25µm using conventional ion micro-probe SIMS, but as a result of recent advances in Nano-SIMS technology, some instruments can now achieve spot sizes at the nanometre scale.

5. Teeth

An understanding of oral physiology is essential if isotope measurements from archaeological and modern dental tissues are to be interpreted, and regardless of species, most mammalian dentition is comprised of the same basic units discussed in this chapter. Teeth are comprised of two primary elements, evident in Figure 17 – the crown, which is exposed within the mouth and provides the working surfaces of the tooth, and the root, which anchors the tooth within the bone of the mandible or maxilla. The crown is coated in enamel, a highly crystalline and almost entirely inorganic substance. The bulk of the crown and root is composed of a mineral and organic composite known as dentine, through which runs a central pulp cavity containing blood vessels and nerves. Layers of cementum sheathe the root, acting as a connective tissue between the root and the alveolar bone.

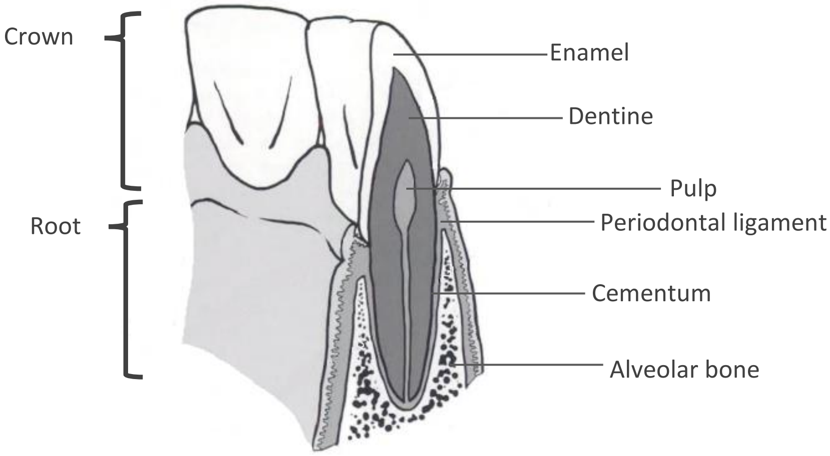


Figure 17. Cross section of an incisor depicting primary elements of tooth. Adapted from Hillson (1986: 10).

Tooth formation and eruption age varies between species. Many species develop two sets of teeth – the first, a set of deciduous dentition which is utilised during childhood before replacement by a set of permanent, mature dentition. Crown formation begins first, followed by the gradual elongation of the root within the mandible or maxilla. Often, teeth erupt upon completion of the crown and before the completion of the apex of the root (AlQahtani, Hector and Liversidge, 2010).

5.1 Enamel structure and formation

Enamel formation is a complex process, but can be considered to occur in two major stages – matrix secretion (or 'apposition') and the maturation stage, during which the enamel attains its full mineral content. These two phases can be broken down into five smaller, distinct stages – the presecretory stage, during which ameloblasts prepare for initial matrix deposition; the secretory stage, during which the enamel matrix is deposited and approximately 14% of the total mineral content is established (Humphrey *et al.,* 2008); the transition phase, in which the ameloblasts undergo cytological changes in preparation for maturation; the maturation stage, during which the matrix proteins are removed and mineralisation is completed, and finally the protective stage, at which point the ameloblasts sheath the enamel until eruption (Hand and Frank, 2014). The complex nature of the mineralisation stage has direct implications for time-resolved isotopic analyses of enamel, and as such will be discussed at length in Section 5.1.2 with respect to the rate of strontium and oxygen isotope incorporation. A brief overview of the matrix production and mineralisation processes is provided in this section.

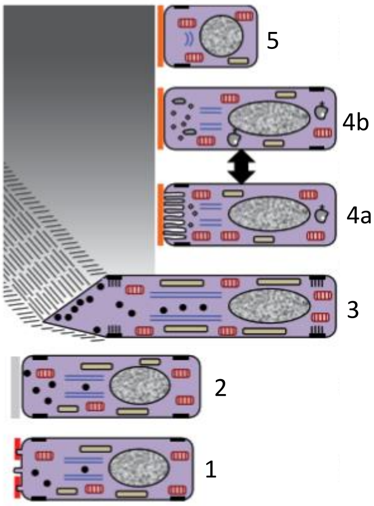


Figure 18. Ameloblasts during the enamel formation process. 1) presecretory stage; 2) early secretory stage; 3) secretory stage; 4a) ruffle-ended ameloblasts during maturation; 4b) smooth-ended ameloblasts during maturation; 5) protective stage. Adapted from Hand and Frank (2014: 70).

Enamel formation is carried out by specialised functional cells known as ameloblasts (Figure 18). The presecretory stage of enamel formation involves the differentiation of epithelial and peripheral cells into ameloblasts; a process during which the cells elongate and the nucleus relocates to the proximal end of the cell. Small projections form at the distal end of the cell in preparation for matrix secretion through exocytosis (Hand and Frank, 2014). At the early secretory stage, a protein-rich, mineral-poor matrix containing vital structural proteins such as amelogenins, enamelins and ameloblastins, as well as long, thin crystals of carbonated hydroxyapatite is synthesised and secreted by the ameloblasts (Gallon *et al.*, 2013; Passey and Cerling, 2002; Smith, 1998). As the matrix is deposited, the ameloblasts move increasingly further away from the dentin-enamel junction and each develops a Tomes' process at its distal end (Hand and Frank, 2014). It is at this stage that rod and interrod structures are formed. Matrix secreted at either side of the tip of the Tomes' process produces interrod enamel, and as the ameloblast retreats, the Tomes' process leaves a narrow cavity which is subsequently infilled to form enamel rods (ibid). Matrix secretion progresses from the apex of the crown to the cervix. During the transition phase, ameloblasts undergo cytological changes and their function switches to mineralisation (He and Swain, 2008). During this stage, ameloblasts cease to secrete matrix proteins and initiate the production of proteinases, which later degrade the organic phase of the matrix (Simmer and Hu, 2001). During maturation, two morphologically distinct types of ameloblasts are active: smooth-ended ameloblasts, which undertake the removal of matrix proteins and water, and ruffle-ended ameloblasts, which aid in the mineralisation process (Hand and Frank, 2014). The matrix degradation products are replaced by enamel fluid, allowing the crystallites continue to grow in width and thickness (Robinson, 2014). Ameloblasts form incremental layers in enamel, initially creating successive dome-like structures at the cusp before forming what are described by Hillson (1986: 121) as consecutive 'sleeve'-shaped layers, as growth proceeds towards the cervix (Figure 19). Once enamel has completely mineralised, it is considered to be a 'dead' tissue – it does not remodel during life, as bone does (ibid.). As such, isotopic and chemical signatures preserved within enamel reflect conditions at the time of its formation, which is either *in utero* or during childhood/adolescence (Bentley, 2006).

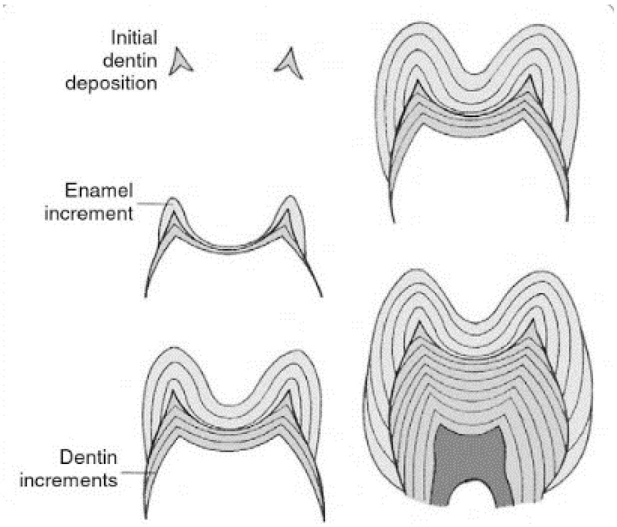


Figure 19. Simple diagram illustrating the incremental pattern of enamel formation, with growth proceeding from the cusp to the cervix. From Chiego (2013: 70).

5.1.1 Apposition

5.1.1.1 Incremental structures

Much research regarding the incremental nature of the growth of enamel has been carried out over recent years. Several types of incremental bands are observable within enamel, and have been used as a basis for the calculation of crown extension rates (Guatelli-Steinberg *et al.*, 2005). Two incremental markers in particular are readily visible in histological sections of enamel viewed under transmitted light microscopy: cross striations, which record daily growth increments, and striae of Retzius (often known simply as striae), which reflect longer periods of growth (Hillson, 1986; Reid and Ferrell, 2006). Cross striations (see Figure 20) are a product of the circadian nature of ameloblast activity, and appear as alternating dark and light bands approximately 3-4µm apart in humans; the former exhibiting a reduced crystal concentration (Antoine and Hillson, 2015; Li and Risnes, 2004; Reid and Ferrell, 2006). Direct experimentation using chemical labelling in the early 20th Century

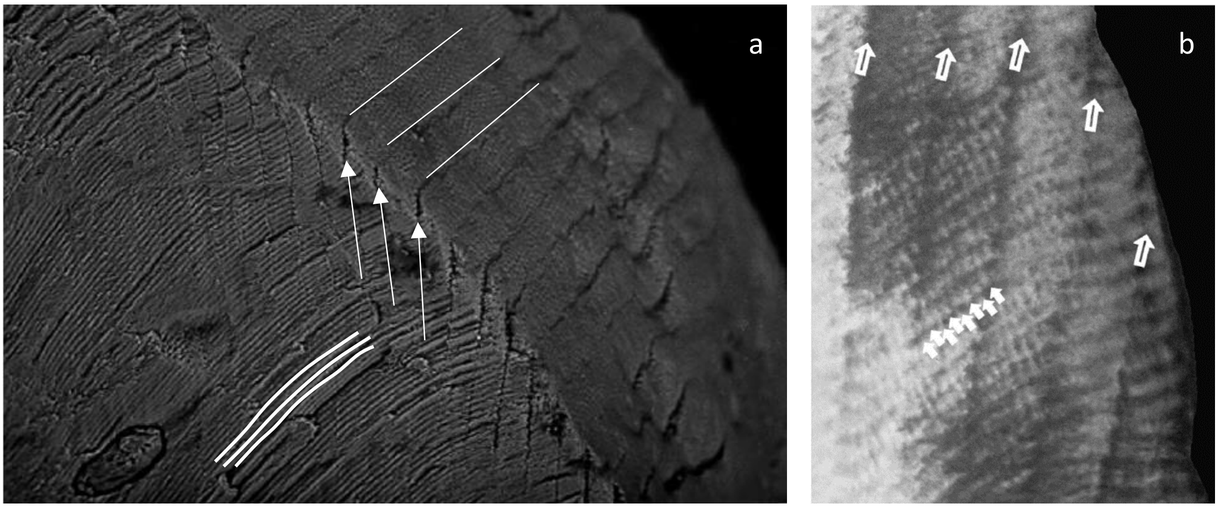


Figure 20. a) SEM image of the tooth enamel of a Miocene hominid illustrating striae of Retzius (white arrows), perikymata (straight white lines) and enamel prisms (curved white lines), and **b)** polarised light micrograph of the same specimen depicting daily cross striations (small white arrows) and striae of Retzius (large white arrows). Note that the number of daily cross striations between the striae of Retzius is regular with a periodicity of eight. Adapted from (Kelley and Smith, 2003): 312, 316).

demonstrated that cross striations in humans and some animals form over a period of approximately 24 hours (Bromage, 1989; Bromage, 1991; Massler and Schour, 1946; Schour and Poncher, 1937). The underlying mechanical cause for the alternating dark and light bands is suggested to be the CO_2 capacity of blood, which varies between night and day as a result of sleep (Fitzgerald, 1998). Despite rejections of cross-striation regularity in the past (Warshawsky, 1989), it is now widely accepted that these bands reflect daily growth, and this regularity forms the basis of calculations of enamel extension rates.

It is suggested that striae of Retzius (Figure 20) are also a product of physiological rhythm, formed repeatedly over a longer period of around 6-11 days in apes (Li and Risnes, 2004). The Striae of Retzius outcrop at the surface of the tooth enamel as perikymata, visible as concentric ridges (Guatelli-Steinberg *et al.*, 2005). The exact cause of this rhythm remains unknown, though (Dean and Scandrett, 1996) suggest that the underlying cause may be related to the analogous striations observed in dentine (known as 'Andresen lines'). Striae of Retzius appear as brown bands when viewed in transmitted light microscopy, and occur with constant periodicity (i.e, the number of daily cross-striations present between each band) within an individual (Fitzgerald, 1998). As such, their frequency (the number of striations identified at the junction with the surface of the enamel) can be multiplied by the periodicity in order to calculate the formation time of the imbricational crown (Reid and Ferrell, 2006). An estimated formation time for the appositional enamel at the cusp of the crown can then be added to this in order to estimate the time required for the full thickness of the crown to be secreted (ibid). It is essential to bear in mind, however, that the amount of time represented by the appositional structures discussed here does not necessarily reflect the *total* crown formation time, as full mineralisation may occur a considerable time after the appositional enamel has been laid down.

5.1.1.2 Rate of apposition

The rate at which the mineral poor, protein-rich matrix forms during the appositional stage of enamel formation (otherwise known as the enamel extension rate) varies between species, and can also vary spatially within individual tooth crowns. Knowledge of the spatial differences in extension rates in tooth crowns of various species is vital if time resolved isotope profiles are to be interpreted with reference to biological timeframes.

Of particular relevance to the current research is the body of published work on the extension rate of Neanderthal and anatomically modern human enamel. As dental development is intrinsically linked to the duration of somatic growth periods, this topic receives great interest with regards to its implications for rate at which Neanderthals reached sexual maturity. A number of researchers argue that the enamel extension rate of Neanderthals is comparable to that of modern humans (Guatelli-Steinberg *et al.*, 2005; Reid, Guatelli-Steinberg and Walton, 2008). Others oppose this view, arguing instead that Neanderthal dental development occurred much more rapidly (Rozzi and de Castro, 2004; Smith *et al.*, 2009; Smith *et al.*, 2010; Smith *et al.*, 2007). The debate undoubtedly stems from the difficulties in obtaining histological samples for analysis; understandably, the sectioning of hominin teeth is not usually feasible. Nevertheless, histological sections of Neanderthal molars from Lakonis, Greece, and Scladinia, Belgium, were examined by Smith *et al.* (2007; 2009), enabling periodicities to be examined directly. Periodicities of seven and eight days were recorded, respectively, and data gathered through the synchrotron imaging of fossil hominin teeth is consistent with this (Macchiarelli *et al.*, 2006; Smith *et al.*, 2010). Indeed, in a study of ontogenetic differences between Neanderthals and modern humans, Smith *et al.* (2010) applied synchrotron imaging to Neanderthal and modern human teeth, enabling enamel cross-striations to be counted virtually. Neanderthal specimens exhibited an average periodicity of 7.4 days; significantly lower than modern humans (who exhibited an average periodicity of 8.3) but not significantly different to that of fossil *Homo sapiens* (Smith *et al.,* 2010).

Varying extension rates within individual tooth crowns have been observed in both humans and fauna. Recent evidence gathered by (Smith *et al.*, 2018) suggests that there is a gradient in the extension rate along the growth axis of human enamel. According to their study, the extension rate of human enamel is rapid near the cusp of the crown and decreases towards the enamel-root junction. Data were obtained through the production of micro-CT scans and histological sections of an anatomically modern human molar and two Neanderthal molars from the site of Payre, France (Figure 21). Extension rate was calculated by dividing the distance along the DEJ by the duration of enamel formation (based on appositional structures). Interestingly, the disparity in enamel extension rate along the growth axis of the crown seems evident in the varying maxima and minima of summer and winter peaks in seasonal $\delta^{18}O$ data obtained from the innermost enamel closest to the DEJ. A similar gradient in enamel extension rate was observed in soay sheep third molars by (Kierdorf *et al.*, 2012), who noted a decrease in enamel extension rate from the cusp of the crown to the cervix - with rates of between 180µm and 217µm per day at the upper regions of the crown and a marked decrease towards the cervical portion.

5.1.2 Maturation

Whilst the incremental structures of enamel clearly illustrate the geometry of the appositional stage, the direction and timing of the onset of enamel maturation has remained poorly understood for decades. The following is a brief summary of key works that have attempted to characterise maturation in various mammalian species over the last century.

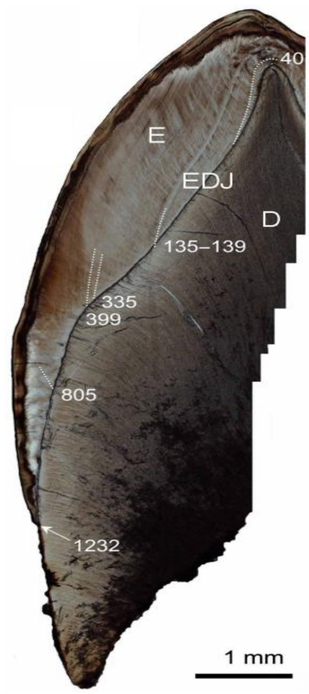


Figure 21. Histological section of the Payre 1 anatomically modern human molar. Number-labelled dotted lines mark the number of days represented by appositional structures. From Smith *et al*. (2018: 2).

5.1.2.1 Timing of onset

In the past, it was argued by some that total mineralisation of the tooth enamel could not occur until the matrix had been deposited to the full extent of the thickness of the crown (von Beust, 1928; Mellanby, 1929; Diamond and Weinmann, 1940). Later work led researchers to the conclusion that matrix production and maturation occur simultaneously during crown formation (Crabb and Darling, 1960; Crabb, 1959; Avery, Visser and Knapp, 1961; Avery, 1962; Engfeldt and Hammarlund-Essler, 1956; Allan, 1959). There is now a large body of evidence that the latter theory is the correct one, and it is widely agreed that in mammals, enamel maturation begins before the full thickness of the crown has been secreted.

The timing of the onset of maturation (i.e, the length of time between matrix secretion and the start of maturation in a given area of enamel) can, however, vary spatially within individual tooth crowns; a phenomenon that has implications for the interpretation of sequential isotope profiles due to isotopic mixing. A number of studies report that the onset of maturation in the innermost band of enamel (closest to the dentine-enamel junction; DEJ) is much faster than elsewhere in the crown. This has been observed in a variety of taxa, including humans (Crabb, 1959; Engfeldt and Hammarlund-Essler, 1956), rodents (Suga *et al.*, 1987), rhinoceros (Tafforeau *et al.*, 2007) and Rhesus monkeys

(Avery, Visser and Knapp, 1961). Green *et al.* (2017) also observed that the timing of the onset of maturation varies spatially across the crown in sheep, with a greater pause between apposition and maturation at the cusps of the crown in comparison to cervical enamel.

5.1.2.2 Geometry

It is also widely agreed that in mammals, enamel maturation occurs in a broadly sequential manner from the cusp to the cervix of the crown, echoing the direction in which the appositional front proceeds. Early evidence for this stems from microradiographic work and microhardness tests involving the sectioning of partially mineralised teeth. Engfeldt and Hammarlund-Essler (1956), Crabb (1959) and Avery, Visser and Knapp (1961) carried out radiographic analyses of sectioned human and primate teeth, observing highly radiopaque zones (which, by implication, had reached an advanced level of mineralisation) that spread longitudinally from the enamels cusps down to the cervical portion of the crown. No distinct boundaries were observed between the high and low mineralised areas; that is, areas of increased mineralisation ‘diffused’ from areas of older matrix to younger matrix rather than following a set number of increments behind the matrix production front (Avery et al. 1961: 1009). This suggests that although maturation geometry roughly follows the geometry of apposition, regularly spaced incremental structures such as the striae of Retzius do not necessarily reflect the pattern of maturation. Microhardness tests carried out by Avery *et al.* (1961) upon developing human molars seem to support this observation, with the highest Knoop Hardness numbers observed in the molar cusps and along the DEJ, decreasing towards the enamel-root boundary (see Figure 22).

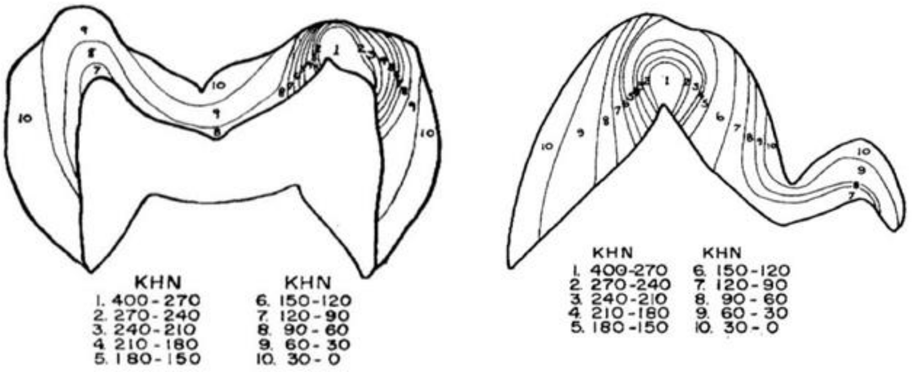


Figure 22. Left: Projection diagram of a buccolingual section of a human maxillary permanent first molar. Zones 1 and 2 are in the range of adult hardness. Right: Projection diagram of a buccolingual section of a human permanent premolar. Note that these are not discrete increments; only ranges of microhardness and not demonstrable morphologic divisions of enamel matrix like Retzius lines. From Avery et al. (1961: 1011).

Later histological investigations involving chemical labelling and microradiography of rodent (Suga *et al.*, 1987), cattle (Suga *et al.*, 1979), caprid (Suga, 1982) and dog (Suga *et al.*, 1977) dentition over the course of the latter 20th Century lead Suga to argue that there are in fact four progressive stages of mineralisation, each of which progresses in a specific direction. In the Suga (Suga, 1982) system, the appositional stage of enamel formation is considered to be the first stage of mineralisation whereby the primary hydroxyapatite is laid down and enamel rods are seeded. This stage is followed by secondary mineralisation, which “starts abruptly and extends toward the deeper layers…” (Suga, 1989: 191). As this wave of mineralisation nears the DEJ, a tertiary wave proceeds from the edge of the inner enamel to the outer surface. This tertiary period of mineralisation is slower and takes longer than secondary mineralisation, and proceeds at a steeper gradient than earlier stages (see Figure 23). The outer layer continues to mineralise when the middle and innermost enamel is beginning to cease mineralisation. This heavy mineralisation of the outer enamel layer is referred to as quaternary mineralisation. Suga’s maturation stages are commonly cited in recent archaeological works concerned with sequential isotopic analysis (for example, see Balasse, 2002; Makarewicz and Pederzani, 2017; Tafforeau *et al.*, 2007; Towers *et al.*, 2017; Montgomery, Evans and Horstwood, 2010) These works reference the complex mineralisation geometries described by Suga and their implications (including signal averaging) where sequential isotope analyses are concerned.

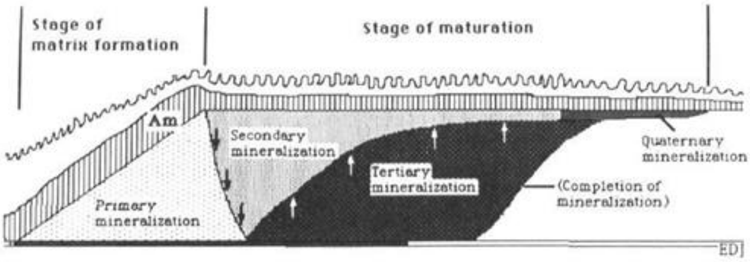


Figure 23. Schematic drawing illustrating the four stages of progressive mineralisation in developing tooth enamel, as revealed by microradiography. From Suga (1989: 196).

More recent works, however, contest the notion that maturation is characterised by multiple waves of mineralisation. In a study of modern sheep dentition via synchrotron X-ray microtomographic imaging (µCT) and Markov Chain Monte Carlo (MCMC) simulation, Green *et al.* (2017) were able to produce the first ever dynamic model of enamel mineralisation patterns. In their approach, single-energy monochromatic X-ray beams are used to calculate the density and distribution of hydroxyapatite in developing sheep tooth crowns, and MCMC methods are used to estimate increases in hydroxyapatite in discrete locations across the enamel. Their results show clear apposition and maturation fronts, with the latter proceeding in a single, primary maturation wave from the cusp towards the cervical portion of the crown, rather than multiple maturation waves as described by Suga. The data suggest that although matrix secretion and maturation proceed in the same direction relative to the DEJ, both phases are discontinuous and exhibit distinct geometries. As evidenced by Figure 24a, the appositional stage occurs at a steep angle to the DEJ whilst the maturation stage exhibits a more variable, diffusive front that is nevertheless largely perpendicular to the DEJ.

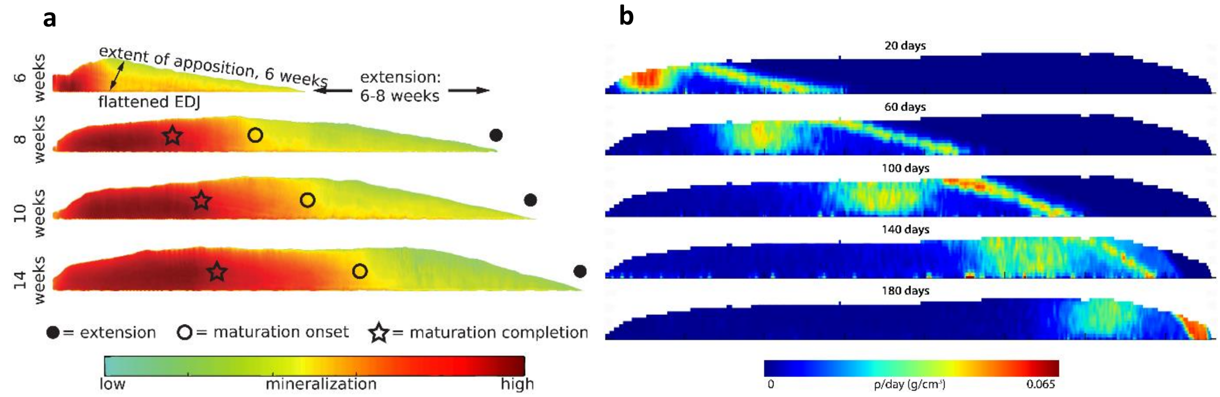


Figure 24. a Mineral densities of sheep teeth at different developmental stages. Solid black circles denote the progress of extension, open circles denote the onset of maturation, and open stars show the completion of maturation. **b** Snapshots of the completed dynamic model showing the progression of apposition and maturation over time. The crown apex is to the left, and the cervix is to the right. From Green et al. (2017: 6, 9).

Similar maturation geometries were recently observed in a histological study of enamel carried out by (Trayler and Kohn, 2017), which sought to determine maturation geometry in a selection of modern fauna including guanaco, sheep, mule deer, horse and cow. Five teeth with partially formed enamel were sectioned parallel to their growth axes and examined through electron microscopy. Calcium X-ray maps were collected for each specimen and used as a proxy for mineral content, with low X-ray intensity indicative of poorly mineralised matrix and high X-ray intensity representative of heavily mineralised mature enamel (Figure 25). The maturation geometry is deduced based on the transition between low and high X-ray intensities. What is strikingly evident is that maturation angles vary between species, but all appear to exhibit a high angle between the dentine-enamel junction and the maturation front. Again, the data suggest that maturation geometry is independent of the geometry of apposition, and this must be borne in mind when designing sampling strategies for sequential stable isotope analysis.

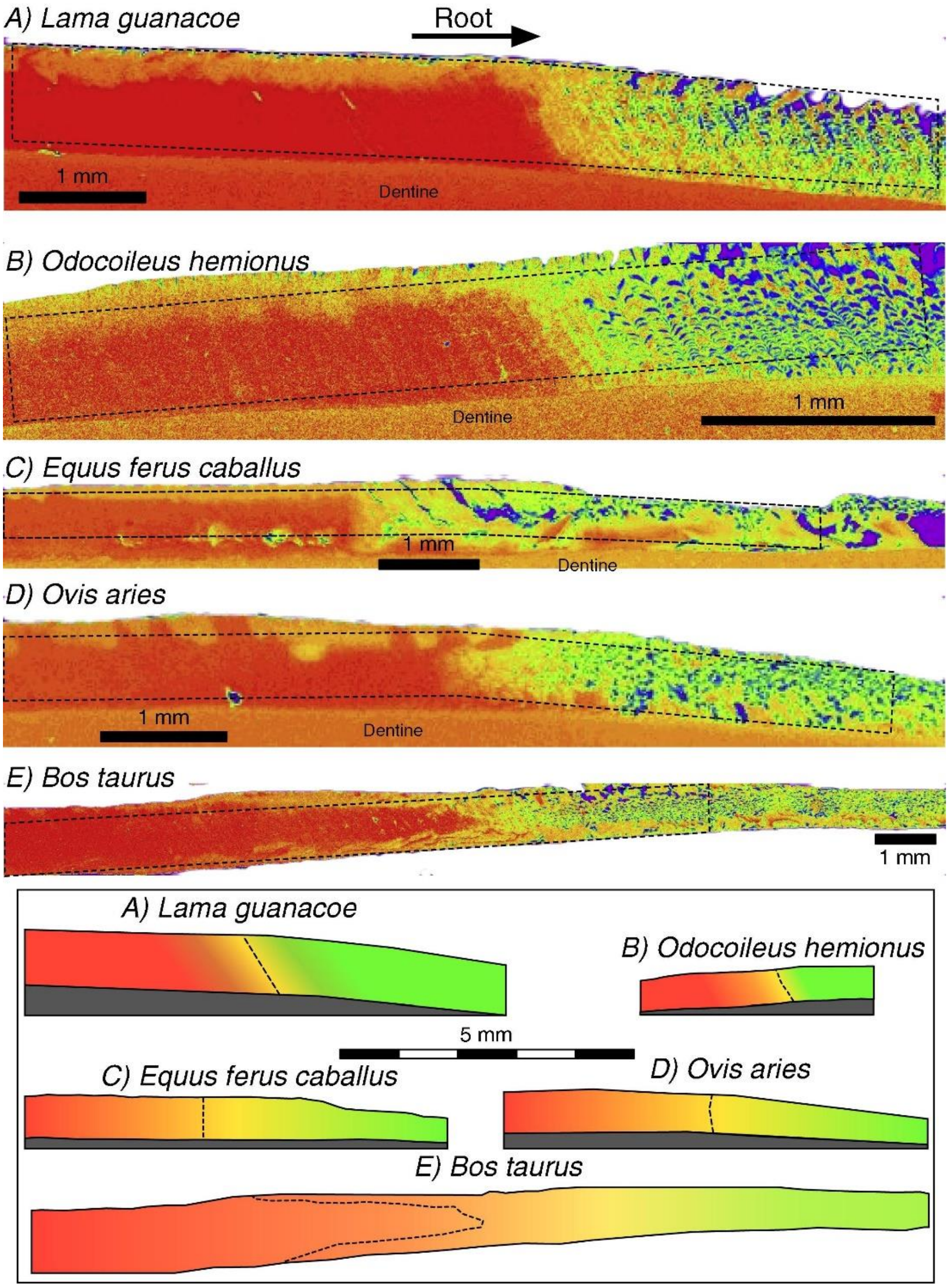


Figure 25. X-ray maps of calcium zoning for teeth with both mature and immature enamel. Warm colours indicate higher relative concentrations; blue/purple = pore space. From Trayler and Kohn (2017: 40).

Enamel maturation geometry, then, was poorly characterized until recently. This has limited palaeoclimatic, archaeological and palaeontological sequential isotope studies of tooth enamel for decades, as uncertainties surrounding the directions in which maturation proceeds have shed doubt on whether sequentially recovered isotope values can be considered temporally meaningful. However, recent works utilising novel methods including synchrotron X-ray imaging, calcium X-ray mapping and modelling have offered new insights into maturation geometry, and the data allow us to discuss a number of key maturation characteristics with confidence. Firstly, maturation proceeds from the apex of the crown to its cervix, though it is not necessarily directly related to appositional structures such as circadian cross striations and Retzius lines. As such, extreme care must be taken if incremental structures are used to constrain isotope series temporally, as sequential isotopic data recovered from fully mineralised teeth may not necessarily reflect the isotopic composition of enamel during the appositional stage. Secondly, maturation appears to begin before the completion of the appositional stage; in other words, the two stages occur concurrently and it is possible for a high degree of mineralisation to be attained in the apical portion of the crown before the full matrix volume has been reached at the cervix of the crown (Engfeldt and Hammarlund-Essler, 1956). Thirdly, based on recent synchrotron imaging (Green *et al.*, 2017), maturation appears to occur in a single wave that follows behind the appositional front, rather than in multiple waves as (Suga, 1989) argues. Finally, the enamel adjacent to the EDJ appears to mineralise ahead of the rest of the volume of the crown (Crabb, 1959; Engfeldt and Hammarlund-Essler, 1956; Trayler and Kohn, 2017) - this spatial difference in the rate of mineralisation is discussed in more depth in the following section.

5.2 Isotope incorporation

The fact that tooth enamel formation involves multiple stages (primarily apposition and maturation) has implications for time-resolved isotopic analyses. The spatial distribution of isotopes depends primarily upon the direction (or geometry) of the appositional and maturational phases of enamel, and the amount of time represented by spatially resolved isotope profiles in tooth enamel will depend on ***a)*** the proportion of strontium deposited during the appositional stage versus the maturational stage, ***b)*** the amount of time required for a given area of enamel to be fully mineralised (termed 'the maturation length'), and ***c)*** the residence time of the isotope in body pools before its incorporation into the enamel (termed the 'reservoir effect'). A detailed understanding of these factors is therefore necessary if sequential isotope data are to be interpreted appropriately. The following is a review of the impact upon sequential isotope measurements of four key factors – maturation geometry, maturation rate, maturation length and the reservoir effect.

5.2.1 Isotopic averaging

Despite abundant evidence that the pattern of enamel mineralisation is broadly sequential, the question of whether meaningful time-resolved isotopic data can be recovered at high spatial resolution still stands. There are two potential causes of isotopic mixing in tooth enamel, which may dampen or attenuate dietary input signals - the first relates to the metabolism and residence time of isotopes in the body before incorporation within the enamel, and the second relates to the averaging of isotopic signals incorporated during the appositional and maturational stages. These effects may vary between species, as well as between different isotope systems.

5.2.1.1 Oxygen isotope residence time

Although few attempts have been made to directly establish oxygen residence times in the body, work carried out by Podlesak *et al.* (2008) offers some insights into the turnover rates of both oxygen and hydrogen. In their study of experimentally-fed woodrats, the oxygen and hydrogen isotopes in body water underwent complete turnover within approximately 14 days, and tooth enamel recorded a switch from isotopically enriched drinking water to depleted drinking water within 27 days (Podlesak *et al.*, 2008). It has been suggested based on carbon, nitrogen and sulphur isotope studies that half-life is related to body mass (Vander Zanden *et al.*, 2016), and if this is the case, one might expect the residence time of oxygen isotopes in large mammals (including humans) to be considerably longer than in that observed in the rats studied by Podlesak *et al.* (2008). In a study of an experimentally-fed sheep, Smith *et al.* (2018) measured clear responses in forming enamel to drinking water with artificially increased $\delta^{18}O$ (administered at 202 days) and lowered $\delta^{18}O$ (administered at 262 days); suggesting that oxygen isotope ratios are rapidly incorporated in tooth enamel.

A body of biomedical studies have produced data that provide insights into the human turnover rate of water. These works utilise doubly labelled water (DLW); water in which hydrogen and ^{16}O are replaced ('labelled') with deuterium and ^{18}O. Such studies involve the administration of DLW to a subject either by ingestion or intravenous injection, and the subsequent monitoring of the isotopic composition of tissues such as hair, and/or body fluids such as plasma, blood and urine over time. The isotopic composition of the body water is measured prior to administration of the DLW in order to establish a baseline, with which the DLW 'elimination time' can be established; that is, the amount of time required for the isotopic dose to be excreted from the body and replaced with new, unlabelled water inputs to the body pools. The rate of labelled water dilution then provides a measure of the water turnover per unit of time (Raman *et al.*, 2004). The primary aim of the majority of these studies is to determine metabolic rate (energy expenditure; EE), and as such, the turnover rates used to calculate EE are often not published. Those that do publish DLW elimination rates, however, have the potential to inform our understanding of oxygen isotope turnover in the human body (and hence oxygen isotope incorporation into the enamel). A relatively recent study of 16 endurance athletes and sedentary men between the ages of 18 and 25 determined that the biological half-life of D_2O is approximately 6 days for the former and 8 days for the latter (Shimamoto and Komiya, 2003); consistent with the results of a study of 458 men and women between the ages of 40-80 (Raman *et al.* 2004) as well as measurements and estimates in earlier studies (Schloerb *et al.*, 1950; Schoeller, 1988; Westerterp, 1999). The rate of total body water turnover will vary depending on factors such as physical activity (slower in sedentary individuals), age (slower in older individuals), sex (slower in females) and water intake (slower in individuals with reduced water intake), but on the whole, data suggest that body water in adults will undergo a complete isotopic turnover in 3-5 weeks. In infants, the water turnover rate is considerably faster, with individuals aged 1-3 months exhibiting water turnover rate per body weight four fold greater than children aged 13-15 years (Fusch *et al.*, 1993).

5.2.1.2 Strontium residence time

Some workers argue that the residence time of strontium in the body before its incorporation into enamel may render sequential, time-resolved analyses futile (Montgomery, 2010; Montgomery, Evans and Horstwood, 2010). Studies using radioactively labelled Ca and P demonstrate that the transport of these elements from the blood into the enamel during its formation is very fast, occurring in a matter of seconds to minutes (Muller *et al.,* 2019; Smith, 1998), but this does not necessarily indicate that strontium absorbed from the diet is instantaneously incorporated. The skeleton plays a major role in calcium homeostasis; that is, the storage of calcium ions until they are released in order to meet metabolic requirements (Balasse *et al.*, 2002). Because strontium readily substitutes for calcium, the skeleton usually contains approximately 99% of all strontium in the body (Schroeder *et al.* 1972; Sugihira and Suzuki, 1991), and it is likely that it behaves in the same way as calcium metabolically. The exchange of calcium (and hence strontium) between the skeleton and the body fluid is heavily dependent on factors such as age, health, sex, bone turnover rates and diet (Skulan and DePaolo, 1999). If dietary strontium is incorporated into the skeleton of an individual and steadily released as a result of bone turnover, or in 'bursts' as a response to dietary deficiencies, then the strontium isotope ratios in that individual's tooth enamel may actually be an average of the ingested strontium over a period of weeks, months or even years. This was recently suggested to be the underlying reason for ^{87}Sr/^{86}Sr 'spikes' observed in the incisor strontium isotope profiles of rats in controlled feeding studies (Weber *et al.,* 2020), though overall results suggest that despite these spikes, tooth enamel in rats and guinea pigs is fully equilibrated to diet within 2 months.

Montgomery *et al.* (2010) argue that evidence exists for long-term metabolic averaging of strontium isotopes in the human body, in the form of data collected in several published works in non-archaeological fields (namely Gulson *et al.*, 1999; Dahl *et al.*, 2001; Degteva and Kozheurov., 1994; Tolstykh *et al.*, 1997). By monitoring the lead isotope composition of the urine and blood of pregnant and lactating women who had moved to Australia prior to conception, Gulson *et al*. (1999) were able to determine the extent of lead remobilisation from the skeleton. Lead that had been deposited in skeletal stores prior to the move to Australia (thereby exhibiting lead isotope ratios different to local Australian values) was shown to be circulating in the bloodstream during late pregnancy and lactation, months after entering Australia. The extent of remobilisation was observed to differ between individuals by almost an order of magnitude; likely as a result of differences in factors such as diet and body mass. Although strontium is unlikely to be entirely analogous to lead, Montgomery *et al.* (2010) imply that as both are bone-seeking isotopes, the residence time of the former may be similar to that of the latter. However, these studies focus primarily on the retention time of strontium in adults, and therefore the data may not be representative of its retention time in infants and children. Permanent dental enamel forms up to the age of approximately 15 years, during which time the skeleton is highly vascular and active. Bone turnover in infants and children can be around twice the rate of turnover in adults (Bilezikian, Raisz and Martin, 2008), and as such, the residence time of strontium may be considerably shorter during the time at which enamel is mineralising.

One might suppose that the rate of strontium incorporation into continuously forming tissues such as hair and nails could be used to approximate the residence time of strontium in the body, and indeed, several studies involving sequential strontium analyses of human hair and nails have been carried out. Mancuso and Ehleringer (2018) carried out a strontium isotope study of human fingernail keratin in a group of individuals who had recently moved into Salt Lake City (SLC) from other regions. $^{87}Sr/^{86}Sr$ values in the fingernails of these individuals were observed to equilibrate rapidly to the local $^{87}Sr/^{86}Sr$ value upon arrival in SLC (within 4-5 weeks). However, endogenous sources of strontium are thought to be the primary constituent of the $^{87}Sr/^{86}Sr$ value in hair and nails (Hu *et al.,* 2020), with only 0.2µg of dietary strontium entering keratin tissues per day. If the $^{87}Sr/^{86}Sr$ value of the endogenous strontium contribution to hair and nails remains constant (as observed in the SLC residents used as a control group) but a significant shift in dietary $^{87}Sr/^{87}Sr$ occurs at a known point in time, then it may be possible to determine the length of time required for hair and nails to equilibrate to the new diet and hence determine strontium residence time in humans. If such a study is carried out, it should be borne in mind that a residence time calculated through these means still may not be reflective of the speed at which strontium is incorporated into tooth enamel and dentine, as calcium (and hence strontium) is actively transported across the dental epithelium as a primary constituent of the mineral phase, unlike in keratin.

5.2.1.3 Maturation length

The second potential cause of isotopic mixing is the temporal spacing between the appositional and maturational stages of tooth enamel formation. Studies of the mineralisation of rat incisor enamel suggest that approximately 14% of the total dry weight of mineral is deposited during the secretory stage, with ~65% deposited in the subsequent maturation stage and a further 30-35% deposited after enamel proteins have been removed in their entirety (Smith, 1998). The amount of mineral deposited at each of these stages and the amount of time between apposition, the onset of mineralisation and complete maturation (referred to from here onwards as 'maturation length') is likely to vary between species, and has been observed to vary even within individual tooth crowns depending on the sampling location (Green *et al.,* 2017). This poses a potentially considerable problem for sequential analyses, as the isotopic composition of any discrete spot sample may in reality be a time-averaged signal produced by two or more successive mineral deposition events (Humphrey *et al.*, 2008).

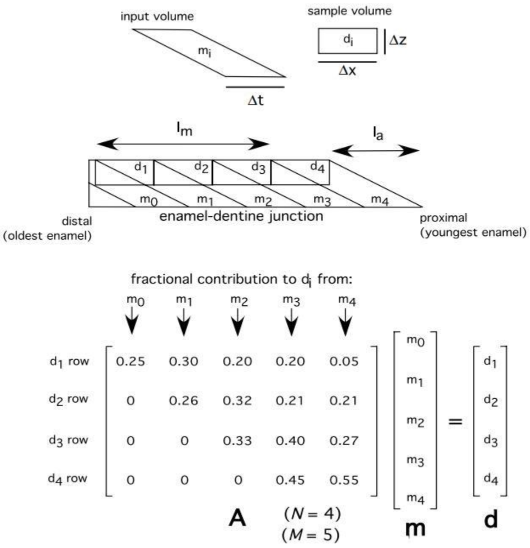


Figure 26. An illustration of the relationship between **A** (the averaging matrix), **m** (the input vector) and **d** in the Passey and Cerling (2002) forward model. $\mathbf{l_m}$ and $\mathbf{l_a}$ are maturation length and length of apposition respectively, whilst each **m** represents the isotopic composition of the body fluid during matrix deposition. **d** is the final isotopic value of the mature enamel. From Passey *et al.* (2005: 4113).

Few attempts to tackle this issue have been made to date. (Passey and Cerling, 2002) produced a forward model enabling the prediction of the appearance of primary input signals (the isotopic composition of the body fluid at a given time) in measured sequential tooth enamel signals, taking into account varying sampling strategies and different maturation parameters. The forward model is based on a system of equations, illustrated in Figure 26 ad 27:

$$\mathbf{Am} = \mathbf{d}$$

Where **A** is the averaging matrix (which deals with sampling and maturation geometries), **m** is the isotope values of the input signals, and **d** is the final measured enamel isotope value. Assuming that a tooth has a constant appositional length (l_a) and a constant length of maturation (l_m), a constant overall growth rate and a linear increase in hydroxyapatite content across the maturation length, the model is expressed as follows:

$$\delta_{ei} = (f_i * \delta_{mi}) + (1 - f_i) * \frac{\sum\limits_{n=i+1}^{i+1+l_m} \delta_{m_n}}{l_m}$$

Where **δ_{ei}** represents the final isotope value of the enamel, **δ_{mi}** is the isotopic composition of body fluids during apposition, **f_i** is the original mineral content, and **$1\text{-}f_i$** represents the remaining fraction of mineral deposited linearly across the maturation length. **i** is a volume of enamel parallel to the apposition surface. Unless the sampling strategy involves milling of samples parallel to the surface of apposition (in which case the above equation can be used), the following model is suggested:

$$\delta_{ci} = \frac{1}{l_a} \sum_{n=i-l_a}^{i} \delta_{e_n}$$

Where **δ_{ci}** represents the isotope value of a column of enamel sampling multiple **i** volumes. The isotope value of columns of varying depths can be modelled by altering the value of **l_a** (Figure 26).

To test the model, sequential carbon and oxygen isotope samples were taken along the growth axis of a modern hippopotamus (*H. amphibus*) canine. **l_a** and **l_m** were estimated for the tooth, and a hypothetical input series was generated based on expected carbon and oxygen for the region. The input series was manipulated by trial and error until the predicted isotope profile successfully matched the measured data. A controlled feeding study was later carried out, during which the diet of a rabbit was switched from pure C_3 to pure C_4 and the $\delta^{13}C$ value of the individual's breath was measured regularly (Passey *et al.*, 2005). With the $\delta^{13}C$ breath profile treated as the input profile, the Passey and Cerling (2002) forward model predicts the enamel profile relatively accurately (see Figure 28).

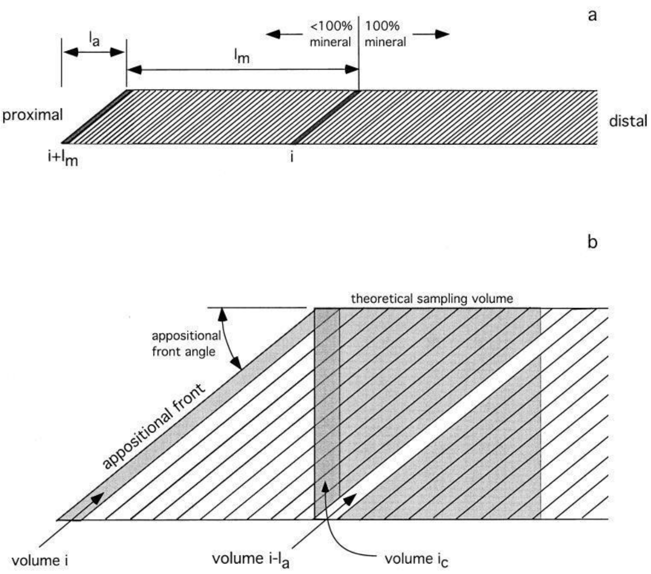


Figure 27. Illustration of enamel cross sections parallel to the growth axis of the tooth. Volume **i** is receiving its last 'dose' of mineralisation in (**a**), whilst in (**b**), **i** is newly forming at the appositional front. From Passey and Cerling (2002: 3328).

In a later publication, Passey *et al.* (2005) developed the model further, using inverse methods to quantitatively predict the original input signal **m** based on observed sequential isotope profiles from tooth enamel. There are infinite solutions for **m** that perfectly predict the measured isotope data **d**, but these solutions can be narrowed down based on expected isotope values for ecological systems. The method is tested through the generation of enamel isotope profiles derived from hypothetical input signals, and the subsequent inversion of these enamel profiles to estimate the original hypothetical input values. Comparison of the predicted input profile with the true (hypothetical) profile enabled the accuracy of the inverse method to be evaluated, and indeed, the accuracy appeared to vary depending on the isotope profile considered. The inversion method produces a series of possible solutions, some of which predict the stepped dietary change well whilst others exhibit deviations as artefacts of the model. Because both accurate and inaccurate solutions for **m** can be produced, the authors highlight the need for model conditions to be reported alongside solutions and ensure that the prediction error is similar to the measurement error.

Whilst the solution produced by the forward model in the Passey and Cerling (2005) study is promising in its accuracy, it must be born in mind that both rabbit and hippopotamus incisors and canines (respectively) are continuously growing, and as such the forward model may not be appropriate for monophyodont dentition. Both the forward and inverse models rely on the assumption that teeth grow and mineralise at a constant rate; an assumption that may be appropriate for polyphyodont dentition but is perhaps inappropriate for teeth with finite growth; especially considering the evidence for changing growth rates in monophyodont teeth (Bendrey *et al.*, 2015; Green *et al.*, 2017). The model is also rather simplistic in its view of the mineralisation process, assuming that the angle of apposition and the angle of maturation are the same and remain constant, which may not be the case (Trayler and Kohn, 2017; Green *et al.*, 2017). The model further assumes that apposition and maturation lengths are constant, and requires that both are known despite the fact that in most cases, both $\mathbf{l}_a$ and $\mathbf{l}_m$ are unknown and must be estimated. Finally, the model assumes that isotope values from both the original matrix and the subsequent mineralisation stage contribute to the final isotope value; in other words, that no isotopic exchange or recrystallisation takes place. When taken together, these assumptions are likely to make both the forward and inverse models unsuitable for use in the interpretation of isotope profiles in monophyodont dentition, without significant alteration.

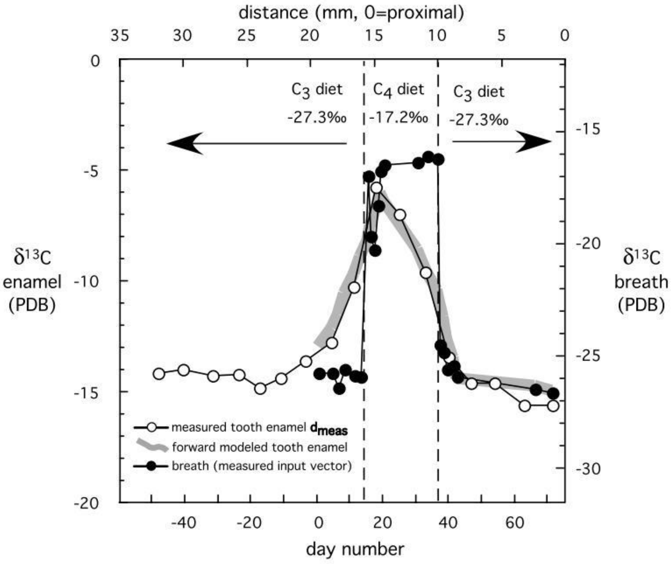


Figure 28. Rabbit tooth enamel isotope profile (grey line) forward modelled using the breath profile (black circles) as the input values. The true, measured isotope profile is denoted by white circles and the timing of the dietary change is marked by dashed lines. From Passey *et al.* (2005: 4108).

5.2.1.4 *Reequilibration*

A recent study undertaken by Trayler and Kohn, (2017) suggests that the isotopic mixing anticipated by the aforementioned researchers as a result of two-phase enamel formation may not, at least for oxygen isotopes, be as considerable a problem as implied elsewhere. Appositional bioapatite contains half as much CO_3 as mature enamel (here termed ‘maturational bioapatite’), and as such the former should contribute approximately 25% of the total mineral content and approximately 50% of the total CO_3. It is hypothesised by the authors that if isotopic signals from both the appositional and maturational bioapatite contribute to the isotopic profile of the completed enamel, and if an individual experiences seasonally varying $\delta^{18}O$ signals during enamel formation, then there should be a spatial separation between the carbonate and phosphate profiles – specifically, the carbonate $\delta^{18}O$ profile should be shifted seasonally earlier than the phosphate $\delta^{18}O$ profile, because the carbonate component is weighted towards an earlier phase of enamel formation tan the phosphate phase (Trayler and Kohn, 2017). Five teeth (three fully mineralised and two immature) from a variety of fauna including horse, goat, bison and red deer were subsampled perpendicular to their growth axes, and carbonate and phosphate $\delta^{18}O$ profiles were collected. No detectable lag between $\delta^{18}O_c$ and $\delta^{18}O_p$ was observed in any of the teeth analysed (Figure 29). Several explanations for this are offered – firstly, that the maturation length of enamel is simply fast enough to eliminate lag, or secondly, isotopic signals in appositional enamel are overwritten or ‘reset’ during the subsequent maturation phase, potentially as a result of recrystallisation of appositional bioapatite, or through the diffusion and exchange of oxygen isotopes between appositional enamel and body fluids (which, by nature, are predominantly comprised of water). If reequilibration takes place, it must occur in a process which enables mature enamel to retain appositional structures such as circadian cross striations and striae of Retzius. The authors speculate that pseudomorphic replacement reactions may enable the survival of appositional structures, as appositional crystals are replaced and isotopically reequilibrated.

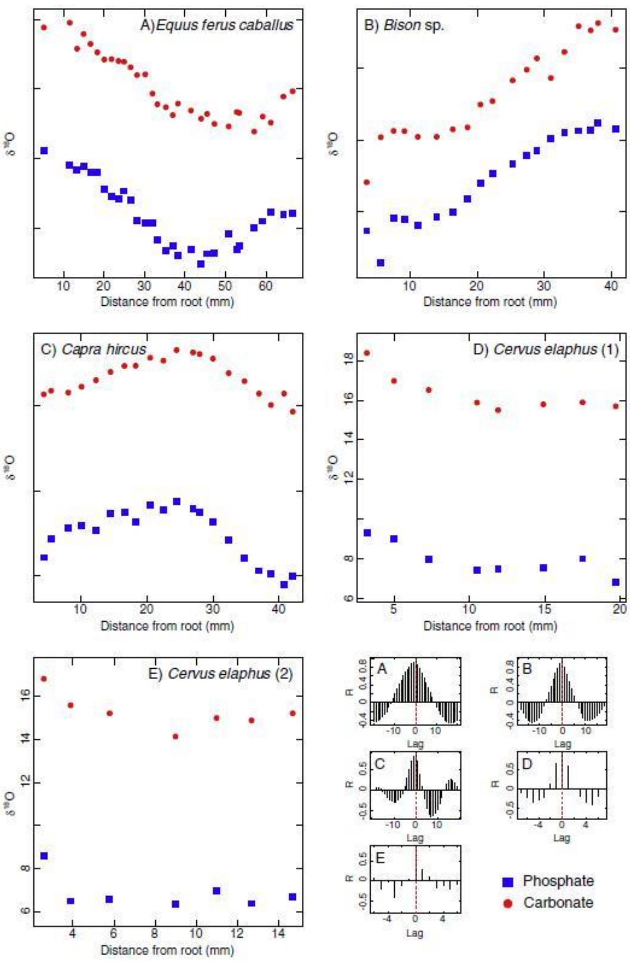


Figure 29. Plots of paired CO_3 and PO_4 $\delta^{18}O$ values and their corresponding cross correlations. From Trayler and Kohn (2017: 38).

To date, no studies have attempted to establish whether strontium isotopes in enamel are reequilibrated during maturation. It is suggested by Trayler and Kohn (2017) that there may be only very little reequilibration of regularly analysed trace elements (such as strontium) if at all, evidenced by the fact that calcium-bound fluorescent labels formed during matrix secretion are relatively well preserved in mature enamel. Indeed, in a study of Soay sheep enamel formation, fluorescent labels produced by calcein and oxytetracycline doses administered during enamel apposition were shown to be preserved in fully mature enamel (Kierdorf *et al.*, 2013). If strontium isotopes are *not* reequilibrated during maturation, then it is likely that strontium isotopes in tooth enamel are an average of values laid down during both apposition and maturation, and as such, a modified version of the Passey and Cerling (2002) may be necessary in order to interpret strontium isotope profiles. In order to determine whether strontium isotopes are reequilibrated during maturation, and in order to develop a strontium-specific model if the answer to the former is that no reequilibration takes place, a knowledge of the proportions of strontium deposited at each enamel formation stage is required.

As strontium is metabolised by the body in the same way as calcium, it is perhaps first necessary to examine the routing of calcium during apposition and maturation. Calcium is actively transported across the enamel epithelium by Ca^{2+}-ATPases; during apposition the deposition of mineral is limited to the very tip of growing crystals to enable lengthwise extension but not growth in width. For this to occur, calcium deposition must be steady and highly regulated, and indeed the presence of Ca-ATPases at the proximal ends of appositional ameloblasts is argued to allow the removal of excess calcium from enamel fluid during matrix secretion (Humphrey *et al.*, 2008). At this stage, calcium levels in enamel are argued to be lower than body fluid as a result of regulation, whilst during maturation the rate of calcium transport is heightened 4-fold (Hubbard, 2000), with approximately 86% of the total calcium deposited during this stage based on a study of developing rat incisor enamel (Smith, 1998). Is strontium actively transported, or does it undergo passive transport across the enamel epithelium? Humphrey et al (2008) argue that strontium transport is not actively regulated during apposition nor maturation, as a variety of other tissues known to actively transport calcium (for example, the mammary gland and placenta) exhibit passively transported strontium. They argue that if strontium reflects physiological levels when incorporated into enamel, then 39.4% of the strontium isotope signal is laid down during apposition (in contrast to just 14% of calcium) with the remainder laid down during maturation. If this is the case, and if no recrystallisation or isotopic exchange of strontium occurs during maturation, then strontium isotopes in any discrete enamel sample may be highly time-averaged.

(Hubbard, 2000) argues that the concept of 'one-way traffic', that is, the one-way transport of calcium from interstitial fluid to enamel hydroxyapatite is too simplistic, and that equilibration via isotopic exchange with loosely bound calcium may take place. Three stages following the entry of calcium into the enamel compartment are discussed by Hubbard (2000): firstly, diffusion through the enamel fluid; secondly, interaction with enamel fluid solutes; and thirdly, association with developing and existing mineral phases. During the first two stages, isotopic exchange between proteins and hydroxyapatite constituents (phosphate and bicarbonate) is likely to occur rapidly until equilibration is complete. In the final stage, it is suggested that whilst some calcium is deposited in addition to the existing mineral volume (allowing growth in width of the existing crystallites), a proportion of the incoming calcium is instead exchanged with existing calcium at the crystallite surface without increasing the overall mineral volume (Hubbard, 2000). Indeed, in a study of ^{45}Ca uptake in rat incisors, Moran, Deaton and Bawden (1995) found that between 50 to 90% of the radioisotope was incorporated into the enamel via isotopic exchange rather than net calcium uptake. If this is the case, and if strontium is transported into the enamel compartment in an identical way to calcium, then one might tentatively suggest that strontium is also partially equilibrated during the maturation stage, as oxygen is (Trayler and Kohn, 2017).

If reequilibration of strontium isotopes does not occur, then the impact of a time-averaged signal upon mobility studies will depend heavily on the speed at which enamel maturation occurs. If, as Crabb (1959) argues, both stages occur simultaneously within a given crown and the maturation front follows very closely behind the secretion front (a matter of days, for example), then the effects of isotopic mixing are likely to be minimal. If on the other hand the maturation front follows slowly behind the appositional front, the implications for sequential analyses are significant. For strontium isotope profiles in faunal enamel, data pertaining to the timing of apposition and maturation collected by Green *et al.* (2017) for sheep tooth crowns may be used as a rough guide for the degree of time-averaging at a given position. For human teeth, however, more work is required in order to establish whether strontium isotopes are reequilibrated during maturation, and if not, further research will be required in order to gauge the degree of time averaging across the crown.

5.2.1.5 The way forward?

This section illustrates the complexity of tooth enamel formation, particularly where isotope incorporation is concerned. Maturation is the stage of enamel formation during which the majority of the total hydroxyapatite content of tooth enamel is lain down, and for more than half a century, researchers have struggled to define its geometry and rate both in humans and animals. Early microradiographic work established that maturation proceeds from the apex to the cervix of the crown, and that it does not necessarily occur in a regimented, incremental fashion as matrix production does (Avery, Visser and Knapp, 1961). Suga argued that enamel mineralisation is a four-stage process, involving successive waves of maturation that proceed independently with their own distinct rates and geometries. Recent synchrotron imaging has established that this is not the case; rather, maturation proceeds from the apex to the cervix of the crown in one continuous wave following apposition (Green *et al.*, 2017). The rate of maturation cannot be assumed to be constant; rather, the rate at which a given portion of enamel reaches full mineralisation depends on its location within the crown. The innermost enamel adjacent to the DEJ, for example, appears to mineralise faster and ahead of the rest of the crown, whilst the outermost enamel mineralises later and more slowly (Trayler and Kohn, 2017). Sampling along the DEJ to target the region with the least time lag between apposition and maturation (and therefore mitigate the effects of isotopic mixing) is recommended by Müller et al. (2020).

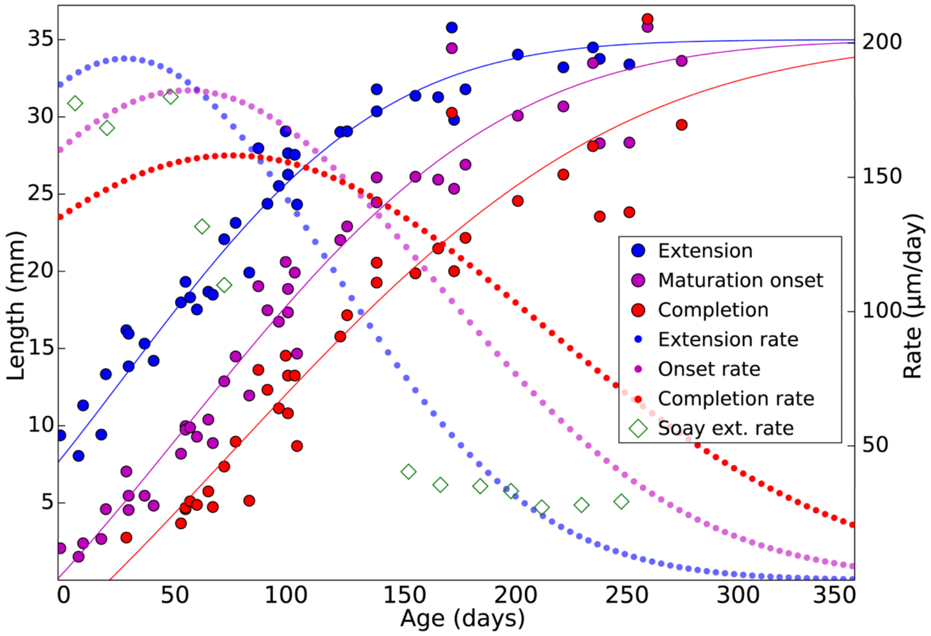


Figure 30. Measurements of enamel extension rate (blue circles), maturation onset (purple circles) and maturation completion (red circles), starting at the dentine horn tip and proceeding along the DEJ until crown completion. Solid lines are integrated as Gaussian functions. From Green *et al.* (2017: 42).

Ecological interest in the isotopic archives of tooth crowns in the later 20th and early 21st Centuries has prompted concerns regarding time averaging; that is, the averaging of isotopic signals incorporated into tooth enamel during apposition and maturation, which are chronologically discrete. New evidence from modern animal teeth suggests that the time gap between the onset of apposition and the completion of maturation can vary within an individual tooth crown depending on location within the enamel (Green *et al.* 2017), and attempts have been made to untangle potentially averaged signals using forward and inverse modelling techniques (Passey and Cerling 2002; Passey *et al.* 2005). Nevertheless, measurement of the timing of the onset and completion of enamel maturation in Dorset sheep (Green *et al.,* 2017) suggests that the maximum spacing between apposition and the onset of maturation (for this species, at least) is approximately 50 days (see Figure 30). This suggests that whilst the timing of the mobility inferred from measured strontium isotope profiles cannot be precise, it can still be attributed to season, providing that accompanying oxygen isotope data are present. This is sufficient for the scope of the current work, as the primary focus is the broad investigation of seasonality, but may not be suitable if a higher degree of temporal resolution is required. Where oxygen isotope measurements are concerned, Trayler and Kohn (2017) suggest that, based on a lack of detectable lag between oxygen isotope profiles in enamel phosphate and carbonate from a range of animal species, isotopic reequilibration (or ‘overprinting’) during maturation negates the need for complex mixing models. If this is the case, then sequential isotope profiles recovered from tooth crowns are in fact representative solely of the timespan of the maturation stage, rather than a mixture of signals from both the apposition and maturation stages.

As of yet, no work has focussed solely on mature human tooth enamel, and as such, the implications of time averaging and varying maturation rates must be borne in mind when considering sequential isotope data from anatomically modern human and Neanderthal enamel. Further research is required in order to a) determine the rate of mineralisation across human tooth crowns; particularly molars; b) establish whether strontium and oxygen isotopes are reequilibrated during the maturation stage; c) if isotopic reequilibration does not take place, develop a numerical model in order to estimate input signals from time-averaged isotopic profiles. There are several possible ways in which these areas could feasibly be examined. Sequential carbon isotope analysis of enamel structural carbonate via SIMS or laser ablation may allow maturation rates to be calculated in individuals with known seasonal switches from C_3 to C_4 based diets, such as the Sudanese mummies from Wadi Halfa (White, 1993). Sequential carbon isotope analysis of the hair of these individuals indicated a seasonal shift in diet from C_4-based foods in the summer to C_3-based foods in the winter (Schwarcz and White, 2004). The number of years taken for a molar tooth crown to mineralise could be established based on the number of C_4 to C_3 cycles observed in a sequential carbon isotope profile of a molar belonging to one of these individuals. Alternatively, or in addition, synchrotron imaging coupled with a dynamic MCMC model (much like the approach of Green *et al.* [2017]) may allow rates of maturation in human tooth enamel to be established with a higher spatial resolution. Establishing whether strontium and oxygen isotopes are reequilibrated during maturation is perhaps a more difficult task, though for the latter, the same technique used by Trayler and Kohn (2017) could feasibly be used providing that the teeth are derived from individuals who experience seasonal extremes. Methods for obtaining highly spatially resolved oxygen isotope data have been explored by (Aubert *et al.*, 2012; Krzemińska *et al.* 2017), indicating the potential for novel approaches such as SHRIMP II in the elucidation of oxygen isotope incorporation. To investigate whether strontium isotopes are reequilibrated during the maturation stage, an ideal study would involve the analysis of enamel from an individual who moved between two very distinct geologies (*A* and *B*) during the time of formation of said enamel. It can be hypothesised that if reequilibration does not take place during apposition, the strontium isotope signals *A* and *B* will appear damped due to averaging with appositional signal(s). If such a study were to be carried out and the results lead to the conclusion that isotopic reequilibration does *not* occur in human tooth enamel, then model such as those outlined by Passey and Cerling (Passey and Cerling 2002; Passey *et al.* 2005) would need to be adjusted to take into account potential variation in maturation rates. This would enable the original isotopic input signal to be estimated.

Such experiments are not possible within the scope of the timescale of this study, but may be possible in the future – in which case, the sequential oxygen and strontium isotope data produced in this study can be revisited. In the current work, strontium isotope data are interpreted (for both fauna and humans) on the basis of the work of Green *et al.* (2017), whereby the approximate maximum time elapsed between apposition and the onset of maturation is around 50 days. Whilst the precise timing of movement cannot be determined, it can feasibly be attributed to season, if oxygen isotope data for the same specimen are available.

5.3 Enamel diagenesis

Tooth enamel is widely thought to be less vulnerable to chemical alteration via diagenesis than dentine or bone, and as such it is usually the primary target for strontium isotope analysis (Balasse, 2002; Lee-Thorp and Van der Merwe, 1991). Its crystalline, virtually non-porous nature makes it more resistant to leaching than dentine or bone (Hillson, 1986), but it is by no means immune to diagenetic alteration.

Like bone, it has been demonstrated that biogenic strontium signatures in enamel have the potential to be affected by diagenesis in the form of secondary mineralisation. Hoppe *et al.* (2003) measured the $^{87}Sr/^{86}Sr$ ratios of sequential 0.1N acetic acid leachates from the skeletal remains of Neogene and

Quaternary mammals preserved at a number of midden sites across the Northern Hemisphere, identifying strong evidence for the diagenetic alteration of ^{87}Sr/^{86}Sr ratios in tooth enamel. In some samples, initial enamel leachates exhibited ^{87}Sr/^{86}Sr ratios similar to local terrestrial values; demonstrating a clear deviation from the homogenous ^{87}Sr/^{86}Sr ratio of seawater (0.7092) expected for marine hydroxyapatites (ibid.). However, after successive 0.1N acetic acid treatments, the ^{87}Sr/^{86}Sr ratios of the samples were returned to values close to the expected seawater ratio (0.7092), suggesting that the procedure had removed approximately 95% of the diagenetic strontium (Hoppe et al., 2003). This implies that the vast majority of strontium contamination is a result of secondary mineralisation, and can be effectively removed using pre-treatment procedures. Sequential 0.1N acetic acid rinses, or related variations, are now a commonly used pre-treatment procedure in strontium isotope analyses (for example, see Hoppe *et al.,* 1999; Copeland *et al.,* 2010; Pellegrini *et al.,* 2008).

5.4 Dentine

5.4.1 Structure and formation

Dentine is a hard, dense tissue that makes up the bulk of the crown and the root of any given tooth. Like bone and enamel, the primary inorganic component of dentine is hydroxyapatite, though it is also comprised of a significant proportion (c.25%) of organic material (Hillson, 1986). The majority of this organic phase is type 1 collagen, though small quantities of non-collagenous proteins, lipids and water are also present (ibid). Dentine is therefore a living tissue, though unlike bone, it does not undergo remodelling throughout life (Bentley, 2006). Providing that no significant diagenetic alteration has taken place, dentine is ideal for the retrieval of childhood carbon and nitrogen isotope signals preserved within collagen, and as such has been targeted for analysis in a variety of fields including archaeology, zoology and climate science.

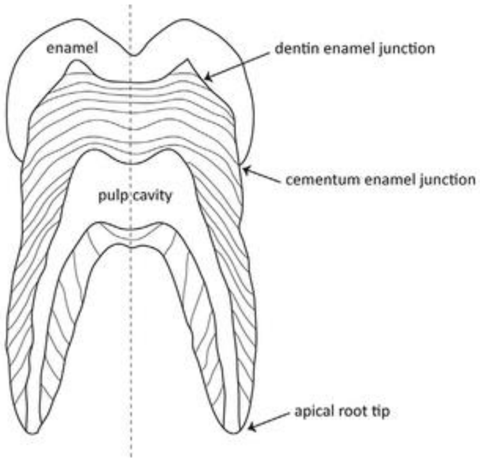


Figure 31. Simplified cross section of a human molar depicting incremental dentine formation. Adapted from Greenwald *et al.* (2016: 149).

Dentine formation begins just prior to the formation of the corresponding enamel, and continues until the complete mineralisation of the root apex. Like enamel, dentine formation begins with the production of an organic matrix by protein-synthesising cells known as odontoblasts. This matrix is comprised primarily of collagen fibrils, which are randomly orientated but build up layer by layer in what have been described as ‘felted mats’ (Hillson, 1986: 151). These collagen fibrils are embedded within a glycosaminoglycan and phosphoprotein matrix (Stevens and Lowe, 2005). Initially, predentine forms in concentric layers beneath the enamel cusps, filling the entire volume of the crown before the predentine front proceeds downwards to produce a series of sloping layers (see Figure 31). The centre of the structure remains hollow, later becoming the pulp cavity through which blood vessels and nerves run. Dentine tubules form the dominant structure within the dentine (Figure 32), produced by the retreat of odontoblasts and subsequent creation of cytoplasmic extensions known as odontoblastic processes (Young *et al.*, 2006). These tubules radiate outwards from the pulp cavity to the enamel-dentine junction, generally following a subtle sinusoidal curve (Stevens and Lowe, 2005). The mineralisation front follows shortly behind the predentine formation front. During mineralisation, hydroxyapatite crystallites are seeded in matrix vesicles and proceed to grow outwards radially within collagen fibrils. This creates interlocking spherical structures known as calcospherites (Hillson, 1986). Tubules may also become infilled through the seeding of hydroxyapatite crystallites from the odontoblast processes, producing a particularly highly mineralised material known as peritubular dentine (ibid). Three major types of dentine can be identified in human dentition – primary dentine, which makes up the majority of the dentine within the root and crown and does not undergo remodelling after formation; secondary dentine, which forms around the pulp cavity and is remodelled throughout life; and tertiary dentine, which is produced by odontoblasts as a response to damage (Arana-Chavez and Massa, 2004; Hillson, 1986).

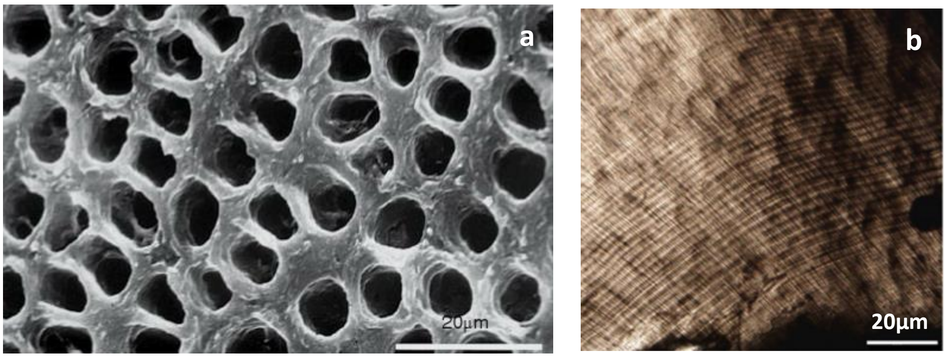


Figure 32. a) Scanning electron microscopy of human dentinal tubules after acid etching. From Hand and Frank (2014: 108); **b)** long-period Andresen lines in dentine (curving from left to right of image). From Smith *et al.,* 2013).

Like enamel, incremental structures are visible in histological thin sections of dentine. Much like the circadian cross striations observed in tooth enamel, dark and light bands of daily predentine deposition known as von Ebner's lines are evident in mineralised dentine (Hillson, 1986). These increments have been successfully used to age certain species (Dean and Scandrett, 1996). Contours in dentinal tubules known as 'contour lines of Owen' have also been observed, but these lines do not appear to be regularly spaced or incremental; indeed, they are likely to be related to the occurrence of physiological disruptions during dentine formation (ibid). Andresen lines (Figure 32b), however, do appear to follow a regular rhythm. The periodicity of these lines varies between species, but they have been observed to occur with the same periodicity of Retzius lines in any given individual, suggesting that the underlying causes of these long-period increments may be interconnected (Dean and

Scandrett, 1996). The regular layering of dentine from beneath the cusp of the enamel to the apex of the root provides the basis for the sequential sampling of tooth roots for time-resolved isotopic analyses (Balasse *et al.*, 2001; Fuller, Richards and Mays, 2003).

5.4.2 Dentine diagenesis

Chemically, dentine bears many similarities to cortical bone (Montgomery, 2010) with around 75% of its weight comprised of inorganic hydroxyapatite, and 25% comprised of an organic phase (Hillson, 1986). The crystallites comprising dentine are shorter than those present in enamel, and its structure is therefore considerably more porous (Hillson, 1986). Its porosity renders the mineral phase of dentine similarly vulnerable to the types of diagenetic alteration that threaten biogenic chemical and isotopic signatures in bone (Tütken and Vennemann, 2011). However, dentine collagen may be targeted for sequential carbon and nitrogen isotope analysis, as the collagen present in dentine is the same type as is present in bone (see Section 2.1.3.2).

Whilst the incremental nature of its growth may make dentine a tantalising target for time-resolved isotopic analyses, it is widely recognised to be unsuitable for strontium isotope analysis due to the susceptibility of the mineral phase to diagenesis. Compared with enamel, the porosity of dentine is greatly increased by the presence of dentine tubules, which exhibit diameters of approximately 1µm (Kohn, Schoeninger and Barker, 1999). Not only does the porosity of dentine put it at risk of the formation of a secondary mineral phase, it also creates the potential for partial or complete turnover of the original strontium isotope signal via ion exchange (Budd *et al.*, 2000). The degree of diagenesis is often variable, and as is the case with bone, highly dependent on burial conditions and site hydrology. The susceptibility of dentine to diagenesis is often used to the advantage of the analyst in strontium isotope studies, whereby the $^{87}Sr/^{86}Sr$ value of the dentine is considered to be reflective of the local burial environment and used (± two standard deviations) as an indication of the 'local' isotopic range (Bentley, Price and Stephan, 2004). Depending on the burial conditions, complete isotopic equilibrium of the dentine with the burial environment may not always occur (see Bentley *et al.,* 2004), rendering such values less reliable for local range determinations.

6. Reconstructing Palaeolithic subsistence and mobility using strontium and oxygen isotopes

6.1 Humans

Whilst strontium and oxygen isotope analyses have been performed widely in archaeology over the past several decades, very few studies have attempted to reconstruct human mobility through the sequential $^{87}Sr/^{86}Sr$ analysis of tooth enamel via LA-MC-ICP-MS (de Jong *et al.* 2010; Lugli et al. 2017; Richards *et al.* 2008;). Strontium isotope analysis has traditionally been used within archaeology as a way of identifying migrant individuals in past societies, with the aim of providing the archaeologist with insights into kinship networks, the spread of cultures and technologies and the movement of peoples (for example, Haak *et al.,* 2008). These studies usually involve the comparison of a bulk enamel $^{87}Sr/^{86}Sr$ value with the 'local' strontium isotope signal for the area of interest, often derived from diagenetic bone (Bentley, Price and Stephan, 2004), the tooth enamel of short-ranging faunal species (Copeland *et al.*, 2011), sediment leachates (Haak *et al.*, 2008), modern plants, or a combination of these. In this way, individuals buried within a given locality whose enamel $^{87}Sr/^{86}Sr$ values fall outside of those expected for the area are considered 'non-local', and are assumed to have moved into the burial area sometime after childhood (Bentley, 2006). However, new analytical innovations have led to novel approaches in the study of human mobility, and what follows is a brief review of Palaeolithic mobility studies which utilise LA-MC-ICP-MS.

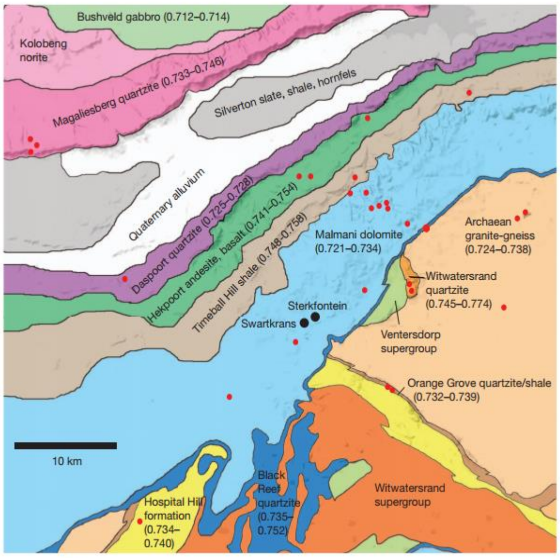


Figure 33. Map of Sterkfontein Valley showing the locations of Sterkfontein and Swartkrans, geological zones and sampling areas. From Copeland *et al.* (2011: 77).

One of the first applications of strontium isotope analysis to early hominin tooth enamel was carried out by Copeland *et al.* (2011). Prior to biogeochemical work, attempts to reconstruct range and residence patterns of Pliocene-Pleistocene hominins were based primarily upon observations of osteological morphology, raw material sourcing, phylogenetic models, and observations of extant hominoid groups. These methods, however, were insufficient to inform about broader ecology, group size and social structures. As such, eight *Australopithecus africanus* and eleven *Paranthropus robustus* specimens from Sterkfontein and Swartkrans respectively were selected by Copeland *et al.* (2011) to undergo strontium isotope analysis via minimally destructive laser ablation MC-ICP-MS. The diverse geological setting of the fossil-bearing caves rendered the region ideal for such analysis (Figure 33), with the "closest non-local geologies 2-3km to the southeast, 5-6km to the northwest and >30km in roughly northeast and southwest directions from the site" (Copeland *et al.* 2011: 76). The local range was determined through the strontium isotope analysis of 38 mammalian teeth recovered from contemporary layers, along with modern plant and 'short-ranging' fauna within a 50km range of the sites, sampling eleven geological substrates. The hominin tooth samples were swabbed with acetone and 0.1M acetic acid in order to remove contaminants, and the surface of the enamel was sampled with several linear laser scans.

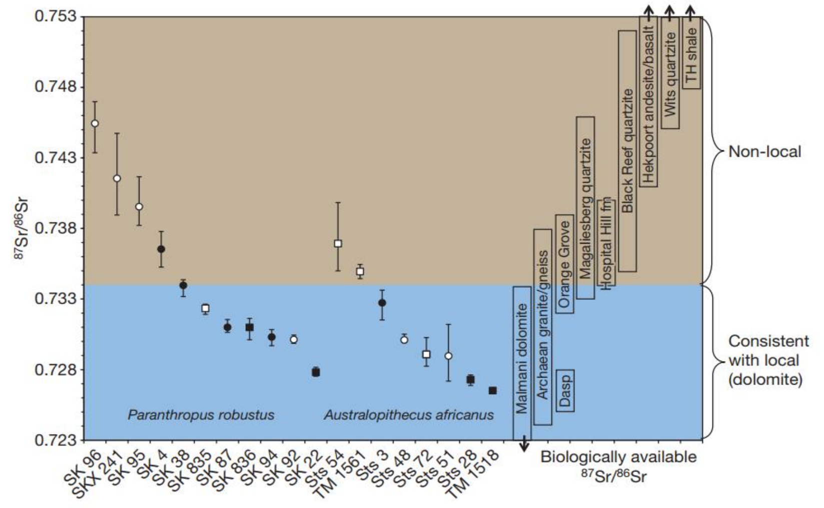


Figure 34. Strontium isotope ratios of australopith tooth enamel and biologically available $^{87}Sr/^{86}Sr$ ratios across the Sterkfontein Valley. Whiskers show the intra-tooth range of laser scans. From Copeland *et al*. (2011: 77).

No statistically significant difference in $^{87}Sr/^{86}Sr$ values was observed between *A. africanus* and *P. robustus*, but a significant difference was observed between small hominins (whose teeth are below the size mean for that species) and large hominins (whose teeth are above the size mean for their species). Based on the 'local range' established through the strontium isotope analysis of archaeological and modern fauna and flora, more than 50% of small hominins were non-local (Figure 34). Significantly fewer of the large hominins were non-local (11%). The authors tentatively suggest that the difference in size may be a result of sexual dimorphism, and discuss the implications of the strontium isotope results if the small and large teeth do in fact represent females and males respectively. Assuming this is the case, the authors speculate that the strontium isotope result may reflect female exogamy; a pattern shared by some human groups and several extant primates.

Sillen and Balter (2018) discuss key issues that should be borne in mind when considering the results of this study. Firstly, there is the inherent difficulty in distinguishing males from females in the genus *Paranthropus*; particularly as hominins appear to exhibit low dental sexual dimorphism when compared to extant apes (Plavcan, 2012). Sillen and Balter (2018) argue that, as a consequence, the only conclusion that can be drawn is that some individuals died on geologies different to those upon which they were born, and until sex assignments can be made with greater confidence, conclusions about male vs. female mobility cannot be made. Interestingly, Balter *et al.* (2012) point out that the higher degree of intra-tooth variability in the smaller teeth may in fact be a result of the curved surfaces of the enamel, which may have caused the laser to fall out of focus – thereby producing variation in the size of the ablated particles and inducing fractionation during ionisation.

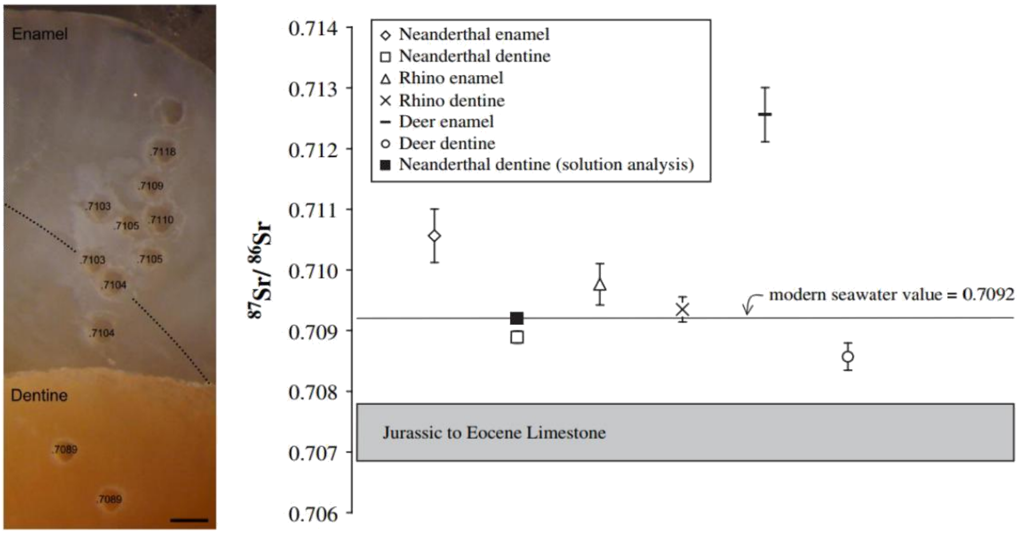


Figure 35. (*Left*) Image of the sampled enamel area showing the strontium isotope values of individual laser-ablation pits. (*Right*) Strontium isotope ratios of Neanderthal, rhino and deer enamel and dentine obtained by LA-PIMMS. (Richards *et al.* 2008: 1253, 1255).

The only published study involving the $^{87}Sr/^{86}Sr$ analysis of Neanderthal tooth enamel via LA-MC-ICP-MS was carried out by (Richards *et al.*, 2008a), and involved sequential strontium isotope analysis of a c. 40,000 year old Neanderthal third molar from the Palaeolithic site of Lakonis, Greece. The nature of Neanderthal mobility has generated long-standing debates, and has traditionally been investigated through the study of raw material sources, associated faunal assemblages and Neanderthal biology. Richards *et al.* (2008) sectioned a Neanderthal third molar and carried out discrete $^{87}Sr/^{86}Sr$ spot analyses via laser ablation, using a NewWave UP213 laser coupled with a Thermo Neptune MC-ICP-MS. Tooth enamel from a deer and a rhino (also recovered from the site) were analysed alongside the Neanderthal molar. The sample spots were spaced in order to follow visible incremental structures, and the mean strontium isotope ratios recovered from these discrete ablation pits were plotted (Figure 35). Based on the data, the authors argue that the strontium isotope ratios in the Neanderthal tooth enamel are not consistent with habitation along the coast, nor the local limestone – and as such, conclude that the individual must have lived in a more geologically radiogenic region during the formation of the third molar; subsequently moving to the area around Lakonis sometime before death. They infer from this that the Neanderthal must have travelled at least 20km in order to reach the Lakonis site from the nearest geologies with matching, more radiogenic strontium isotope values.

This study, however, suffers from a number of pitfalls. Isobaric interferences can occur on key masses during LA-MC-ICP-MS analyses of biological apatites and have the potential to produce highly inaccurate data. Richards *et al.* (2008a) make no attempt to identify, monitor and address the isobaric interferences present during their analyses; instead, they apply a constant 'correction' of -0.001 based on the average of the offsets (LA-TIMS) of their two in-house standards. In their response to Richards *et al.* (2008), (Nowell and Horstwood, 2009) assert that by applying a blanket correction in this way and neglecting to make appropriate corrections for isobaric interferences, any variation observed in the $^{87}Sr/^{86}Sr$ data may simply be an artefact of the analysis rather than genuine biogenic variation. This is due to the fact that such a correction assumes that the magnitude of any given interference does not vary across the geometry of the enamel; an assumption that is decidedly unfounded. The Sr/(CaP) has been observed to vary spatially across enamel, and the magnitude of the $CaPO^+$ interference increases with lower strontium concentrations. As such, the $^{87}Sr/^{86}Sr$ profile in an isotopically homogenous tooth that has spatially varying strontium concentrations can appear to vary, leading to erroneous geological interpretations. In this case, Richards *et al.* (2008) identify three regions across the crown which exhibit 'distinct' strontium isotope values. For the aforementioned reasons, and because no within-run uncertainties were reported (and values can only be 'distinct' if they fall outside the uncertainties of others), this interpretation is perhaps tenuous.

Richards *et al.* (2008a) further conclude that the strontium isotope values observed in the Neanderthal enamel are not indicative of habitation along the coast, nor on the limestone in the immediate vicinity of the Lakonis site. The 'non-local origin' of the Neanderthal is based on this observation. However, the average $^{87}Sr/^{86}Sr$ value of the discrete spot data collected from the Lakonis rhino is concluded to reflect local inhabitation in the vicinity of the site, despite being within the uncertainty of the average Neanderthal values. By applying a variable correction to the $^{87}Sr/^{86}Sr$ data based on estimates of strontium concentration, Nowell and Horstwood (2009) eliminate much of the isotopic heterogeneity in the data reported by Richards *et al.* (2008). The subsequent average of the Neanderthal $^{87}Sr/^{86}Sr$ data falls within the uncertainty for modern sea water. Whilst the Nowell and Horstwood (2009) corrected data cannot be considered to be entirely accurate, it does serve to illustrate the ways in which interference corrections (or lack thereof) can have a profound affect upon $^{87}Sr/^{86}Sr$ data from archaeological enamel and the interpretations subsequently drawn from such results. The Richards *et al.* (2008) study, though pioneering in its attempt to directly reconstruct Neanderthal mobility, is a prime example of the ways in which laser ablation can produce poor data if necessary considerations are not made with regard to isobaric interferences.

In 2017, strontium isotope analysis via laser ablation MC-ICP-MS was carried out upon a human tooth from Middle Pleistocene deposits at the site of Isernia La Pineta, Italy. Lugli *et al.* (2017a) selected a deciduous human incisor and several rodent, bison and rhino from the site for LA-MC-ICP-MS analysis. Discrete samples were ablated from the enamel surface of the incisor following the growth axis (Figure 36), and the local range for the site was determined based on the average $^{87}Sr/^{86}Sr$ value of the rodent teeth. Based on the fact that the average $^{86}Sr/^{87}Sr$ value of the human enamel falls within the local range of the site, Lugli *et al.* (2017) argue that the mother of the individual was not mobile; rather, she stayed around the vicinity of the site during pregnancy.

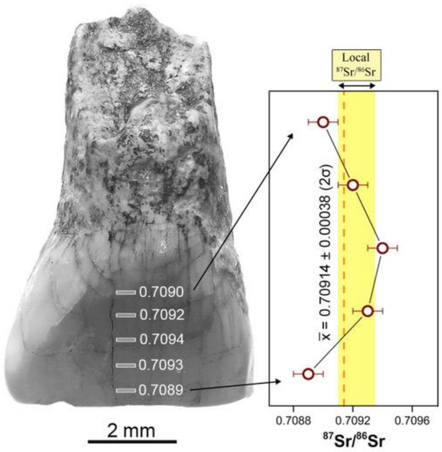


Figure 36. Human deciduous incisor IS42 from Isernia La Pineta and in situ Sr isotope results. From Lugli *et al.* (2017: 3).

The enamel of deciduous human incisors forms before birth in the uterus of the mother (Al Qhatani *et al.* 2010). The authors propose that the sequential $^{87}Sr/^{86}Sr$ values of the incisor enamel are therefore reflective of the mother's mobility during pregnancy; an assumption that is based on little to no evidence. In pregnant women, there is a greatly increased demand for calcium from the growing foetus; particularly during the third trimester (Kent *et al.*, 1993). Markers for increased bone turnover are observed in pregnant women during this time; likely as a result of the mobilisation of skeletal calcium (which comprises 99% of the total body store of calcium) in order to meet the increasing calcium demands of the foetus (Kovacs and Kronenberg, 1997). As strontium substitutes for calcium and behaves in the same way metabolically, it seems reasonable to argue that a portion of the strontium incorporated into the tooth enamel of the growing foetus is in fact routed from the mother's skeletal stores, and likely does not reflect the immediate diet of the mother (and hence the geological substrate upon which the mother subsists during pregnancy). The $^{87}Sr/^{86}Sr$ values in deciduous teeth are therefore likely to be an average of dietary values and homogenised skeletal values.

Even if one were to assume that the $^{87}Sr/^{86}Sr$ values in deciduous enamel are entirely reflective of the mobility of the mother, the results remain inconclusive. The authors argue that the fact that the average $^{87}Sr/^{86}Sr$ value of the deciduous enamel falls within the local range is evidence of limited mobility of the mother during pregnancy. However, at no point in their report do they consider the possibility of diagenetic alteration. Although tooth enamel is generally more resistant to diagenesis than dentine or bone, it is by no means immune. In Figure 36, it is evident that the surface of the tooth enamel is riddled with hairline cracks, which has likely allowed the percolation of ground water through the outermost layers of enamel. Depending on site hydrology, this may have permitted isotopic exchange and partial isotopic equilibration of the outermost enamel layers with the burial environment. This would also produce a series of homogenous tooth enamel $^{87}Sr/^{86}Sr$ values that are similar to that of the surrounding geology. No steps were taken by the authors to monitor the impact of diagenesis, and as such it cannot be ruled out that the $^{87}Sr/^{86}Sr$ values of the tooth enamel are not biogenic, but are in fact diagenetic.

Whilst it is beyond the scope of this study to provide a full review of the numerous methods employed in the reconstruction of human mobility in prehistory, it is useful to note that innovative recent studies are pushing to combine multiple lines of evidence and tap into previously overlooked archives of biogeochemical information in order to investigate human mobility in the Pleistocene. In 2019, Moncel *et al.* set out to synthesize TIMS strontium isotope data (derived from micro-drilled enamel samples) alongside lithic procurement data in order to reconstruct the mobility patterns and subsistence strategies of the Neanderthals who occupied Payre, southeast France. The range distances implied by the results of both types of analyses were consistent, suggesting that the Payre Neanderthals were mobile across the Rhone valley and nearby higher-elevation plateaus (*ibid*). In contrast, as an alternative to strontium isotope analysis, Weißing *et al.* (2019) employed a multi-isotope approach using sulphur, carbon and nitrogen systems in their study of Neanderthal and anatomically modern human mobility and subsistence in Belgium. Sulphur isotope ratios ($^{34}S/^{32}S$) in the biomass vary geographically, affected by factors such as local geology, hydrology, proximity to the coast and modern anthropogenic activity (Nehlich, 2015). These isotopic signatures are passed from plants into consumers with very little fractionation, and since sulphur is present in the essential amino acid methionine, the $^{34}S/^{32}S$ values of an individual's collagen directly reflect those of the protein sources in the diet (*ibid*). Since the $\delta^{34}S$ values of collagen from two different populations of Belgian Neanderthals (those of Goyet Cave, and those of Spy Cave) differ, the authors suggest variation in the mobility and land use. Based on their comparison with isotopic baselines, the $^{34}S/^{32}S$ values of the Spy Neanderthals' collagen reflect local signals, suggesting a local geographical range, whilst the sulphur isotope signatures of the Goyet individuals indicate non-local signatures, which is suggested either to *a)* reflect a different geographical range, or *b)* that the individuals were killed elsewhere and brought into the study area. Both of these studies, along with those that employ LA-MC-ICP-MS discussed here, illustrate the promising ability of novel techniques to shed light upon the mobility patterns and land use of Palaeolithic human groups, as well as the importance of combining multiple lines of evidence in order to elucidate such behaviour.

6.2 Fauna

Isotopic methods have been applied in studies of both modern and prehistoric fauna, using a multitude of isotopic systems including H, C, N, O, S and Sr (Rubenstein and Hobson, 2004). Archaeological studies of seasonality and animal mobility are often concerned primarily with the relationship between human groups and the wild fauna and flora upon which they survive. The subsistence strategies of hominin groups during the Pleistocene are intrinsically linked to the seasonal availability of plant and animal resources (Birch *et al.*, 2016), and as such, interpretation of the former requires an understanding of the latter. Prey mobility patterns are likely to be affected by temporal changes in climate, forage and landscape, and as such, reconstruction of animal mobility patterns across the span of the Pleistocene offers the potential for a more comprehensive understanding of temporal changes in human subsistence strategies. Although this is a key area of research, relatively few studies to date have attempted to untangle the relationship between animal and human mobility during the Pleistocene through isotopic means.

Archaeological faunal mobility studies utilising biogenic strontium isotope values in tooth enamel have, to date, focussed primarily on the difference in bulk enamel strontium isotope ratios compared to 'local' strontium isotope ratios (Hoppe *et al.*, 1999; Hoppe and Koch, 2007; Pérez-Crespo *et al.*, 2012). The assumption underlying faunal migration studies is that the bulk $^{87}Sr/^{86}Sr$ ratio of an individual's enamel is biogenic, and records the bioavailable strontium signal characteristic of the geographical region in which the individual dwelled during the mineralisation of the tooth. If this signal differs to the strontium isotope ratio expected for the area in which the individual died and was buried, it is usually concluded that movement between distinct geological areas must have taken place (Bentley, 2006). A number of studies of the migratory behaviours of both modern and archaeological fauna have been carried out following this principle. In 1999, Hoppe *et al.* carried out the first study of extinct proboscidean migratory behaviour

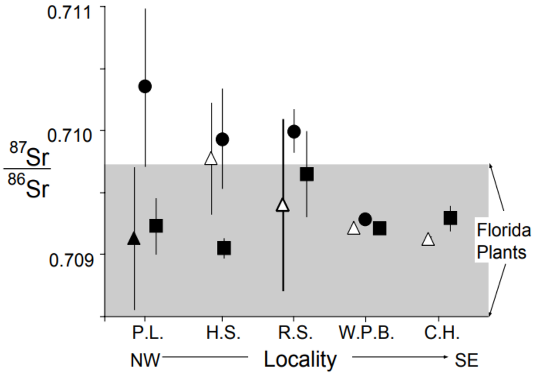


Figure 37. Average Sr isotope ratios of bulk samples of mastodons (circles), mammoths (squares), and deer and/or tapirs (triangles). P.L. is Page-Ladson–Aucilla River, H.S. is Hornsby Springs, R.S. is Rock Springs, W.P.B. is West Palm Beach, and C.H. is Cutler Hammock. The grey box represents the local Sr range. From Hoppe *et al.* (1999: 441).

using strontium isotope ratios in late Pleistocene mammoth and mastodon teeth from Florida, North America. The study compared bulk enamel strontium isotope ratios of proboscideans from five sites, as determined through TIMS, with those of contemporary deer, rabbit and tapir, as well as modern plant and water samples from several locales in the study area (Figure 37). In general, faunal and environmental values were similar; leading the authors to conclude that for the most part, proboscidean ranges were short and localised. Mastodons from the northernmost site, Page Ladson, exhibited significantly higher $^{87}Sr/^{86}Sr$ ratios than the local flora and fauna, suggesting that they may have undertaken migrations of 120-300 km at the very least (Hoppe *et al.*, 1999). It was concluded that in late Pleistocene Florida, mammoths ranged locally whilst mastodons undertook significantly larger migrations, moving across distinct geologies before their deaths in the north of the state. The apparently sedentary behaviour of the mammoths in question is argued to be evidence against existing theories advocating long-range mammoth migration, as proposed by (Churcher, 1980). However, it is critical to note that movement across homogenous geologies may not be detectable isotopically, and as Hoppe *et al.* (1999) acknowledge, the coastline of Florida is dominated by homogenous Pleistocene sediments. Therefore, if mammoths were moving primarily along the coast, they may have traversed as much as 700 km before encountering another distinct geological region, leaving little or no isotopic evidence for movement (*ibid).*

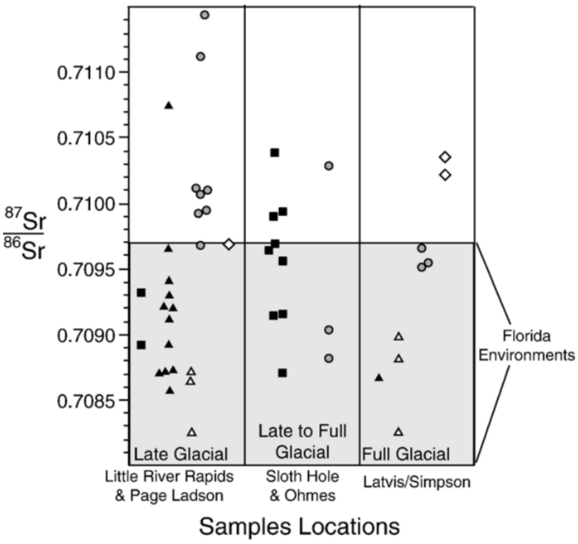


Figure 38. The $^{87}Sr/^{86}Sr$ ratios of tooth enamel from the Aucilla River. Black squares =mammoths; grey circles =mastodons; black triangles = deer; white triangles =tapir; white diamonds= equids. Shaded grey area represents the range of $^{87}Sr/^{86}Sr$ ratios expressed by Florida environmental samples. From Hoppe and Koch (2007: 350)

Diachronic changes in animal migration patterns are also a topic of great interest, as an understanding of the extent to which certain fauna respond to environmental changes and climatic fluctuations can offer insights into causes of extinction (Hoppe and Koch, 2007). In a continuation of their previous work on the Pleistocene fauna of Northern Florida, Hoppe and Koch (2007) set out to investigate potential changes in the migration patterns of Aucilla River mammoths and mastodons before and after the LGM, with the intention of exploring the validity of existing theories for the extinction of North American megafauna. The decline of these proboscideans has been attributed to a variety of factors, including ecological shifts and resulting nutritional stress (Haynes, Martin and Klein, 1984), intensive hunting by humans (Churcher, 1980), and the onset of increasingly arid conditions, which may have prompted both of these (Haynes, 1991). In line with climate-based extinction theories, variations in pollen assemblages suggest that the study region had experienced significant climatic fluctuations between 50,000-11,200BP, primarily in the form of wet-dry cycles (Hoppe and Koch, 2007, and references therein). Mastodon bulk enamel samples dated to before the LGM generally exhibited low ^{87}Sr/^{86}Sr ratios, implying short ranges within less radiogenic, local Floridian environments. Late Glacial mastodons, on the other hand, exhibited comparatively high ^{87}Sr/^{86}Sr ratios, suggesting possible migrations of over 150km into more radiogenic Georgian environments to the north (Figure 38). It seems apparent, then, that mastodons in Florida altered the extent of their migrations after the LGM, perhaps as a response to ecological pressures.

6.2.1 Sequential micro-sampling

Understanding the mobility and subsistence behaviours of animals is a key objective in palaeoecology, and indeed, is a major topic in studies of hominin subsistence. A number of faunal migration studies have therefore attempted to achieve greater temporal resolution through the sequential sampling of tooth enamel (Britton *et al.*, 2009; Henton *et al.*, 2017; Julien *et al.*, 2012; Hoppe *et al.*, 1999; Pellegrini *et al.*, 2008; Price *et al.*, 2015; Pryor *et al.*, 2016; Pryor, Stevens and Pike, 2016). As enamel forms incrementally from the cusp of the crown to the cervix, ^{87}Sr/^{86}Sr ratios of sequential micro-samples taken along the growth axis will reflect the ^{87}Sr/^{86}Sr ratios of food and water consumed during the formation period of that enamel layer (Bentley, 2006). Hence, temporally-resolved isotopic profiles can be reconstructed (Britton *et al.* 2009). Two micro-sampling methods have been employed: mechanical drilling, and more recently, laser ablation. The former generally involves the sectioning of the tooth (see Pellegrini *et al.* 2008) or the sampling of buccal or lingual enamel surfaces (see Britton et al. 2009), and the subsequent mechanical removal of samples at regular intervals using dental burrs or cutting discs. The latter is a relatively new development, involving the use of a laser sampling system to ablate microgram samples from enamel surfaces or sections.

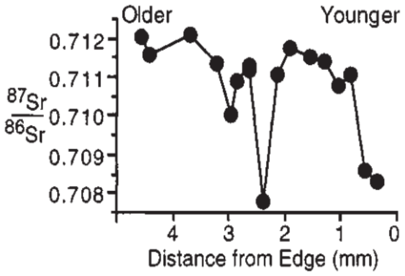


Figure 39. ^{87}Sr/^{86}Sr ratios of microsamples from a Page-Ladson mastodon. From Hoppe *et al.* (1999: 441).

Acknowledging the intrinsic limitations of using bulk enamel samples to investigate seasonal mobility, Hoppe *et al.* (1999) utilised a computerised microsampler in order to remove a series of enamel samples from a thin section of a mastodon molar in an effort to study Pleistocene proboscidean migration. In order to reconstruct a temporally-resolved sample series, 16 samples with a depth of 0.15mm were milled parallel to enamel growth increments. Large variations in ^{87}Sr/^{86}Sr ratios were observed over a period of growth estimated to be approximately two years (Figure 39), suggesting repeated movement between two geologically distinct areas (Hoppe *et al.*, 1999). Whilst the analysis of bulk enamel samples permitted the identification of individuals that had moved away from their geological area of birth, the micro-sampling of a molar section enabled the reconstruction of not only of the migratory patterns of a single individual, but also the specific timing of these movements.

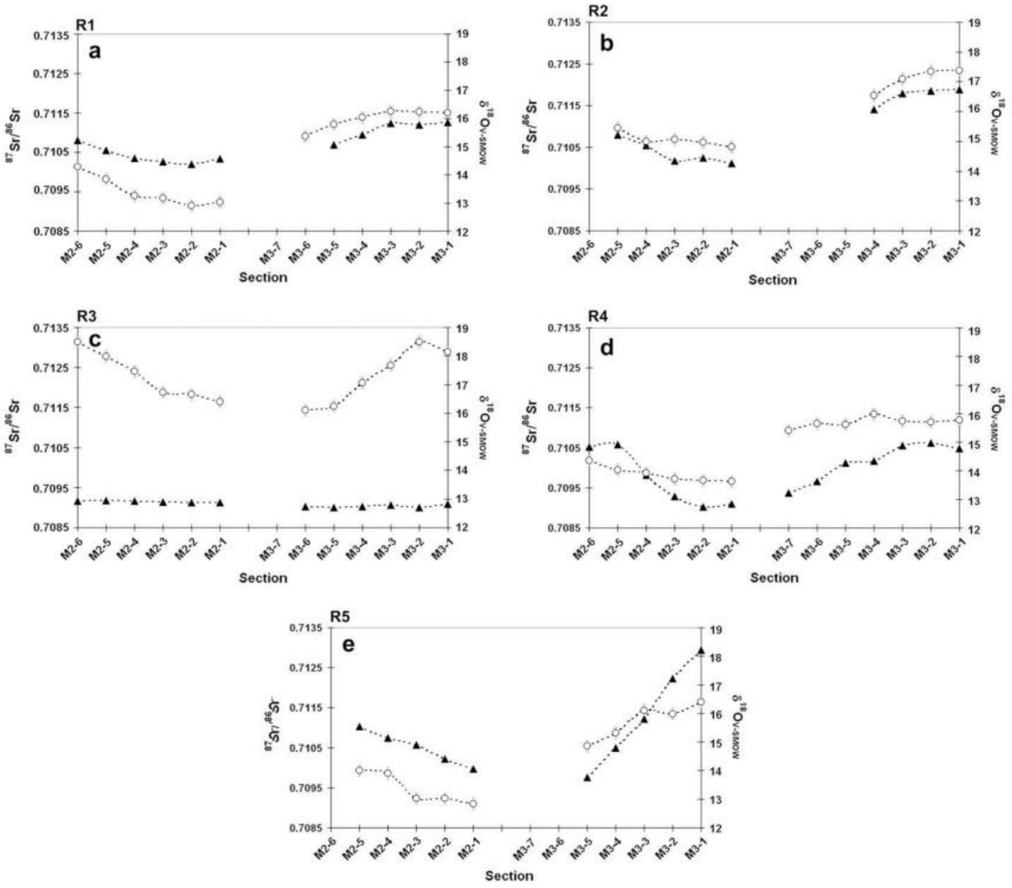


Figure 40. Plots **a–e** demonstrate intra-tooth isotope values of $^{87}Sr/^{86}Sr$ (solid symbols) and $\delta^{18}O$ (open symbols) for M2 and M3s of each of the five animals analysed. From Britton *et al.* (2009: 1169).

The application of oxygen isotope analysis to mechanically removed enamel micro-samples can enable the reconstruction of annual climatic cycles, hence allowing $^{87}Sr/^{86}Sr$ ratios to be anchored to seasons. Britton *et al.* (2009) carried out a preliminary study of the suitability of the method, utilising sequential strontium and oxygen isotope analyses to investigate the seasonal migration patterns of modern caribou (*Rangifer tarandus granti*) in Alaska. The intra-tooth $\delta^{18}O$ values of these migratory animals were expected to be smoothed due to their avoidance of temperature extremes, as opposed to the sinusoidal trend expected to be exhibited by non-migratory species (ibid). Of the five individuals analysed, four (R1, R2, R4, R5; Figure 40) demonstrated greater inter-tooth variation than intra-tooth variation in $\delta^{18}O$ profiles, suggesting that they had indeed engaged in migratory behaviour. The remaining individual (R3) demonstrated a sinusoidal $\delta^{18}O$ profile indicative of exposure to an uninterrupted seasonal cycle, heavily supported by the consistent $^{87}Sr/^{86}Sr$ ratios across intra- and inter-tooth samples. The authors conclude that, in all likelihood, the anomalous individual (R3) belongs to the species *R. tarandus tarandus* (domestic reindeer) rather than *Rangifer tarandus granti;* a distinction that is difficult if not impossible to make on osteological grounds.

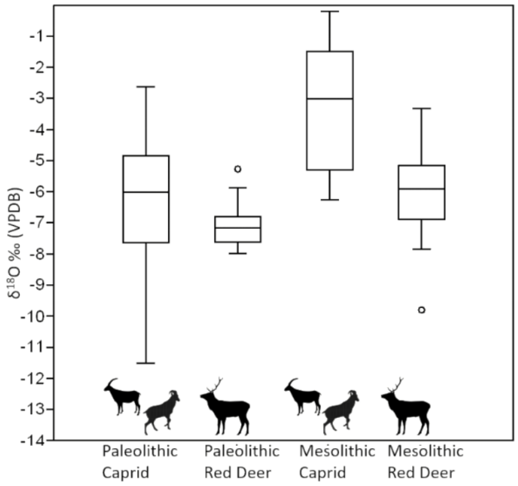


Figure 41. Boxplot of $\delta^{18}O$ values in caprids and red deer through time, where box = 50% and whiskers are 95%. From Birch *et al.* (2016: 11).

Combined strontium and oxygen isotope analyses have also been applied to a number of Pleistocene faunal assemblages (Britton *et al.*, 2011; Julien *et al.*, 2012; Pellegrini *et al.*, 2008; Price *et al.*, 2015), several of which have offered somewhat unexpected new insights into the ecologies of extinct and extant species. For example, in a study of European steppe bison from Eastern Ukraine, Julien *et al.* (2012) employed sequential strontium, oxygen and carbon isotope analyses in order to investigate migratory behaviours during the Late Pleistocene. In samples where the length of enamel was sufficient for annual cycles to be recorded, $\delta^{18}O$ values exhibited clear sinusoidal fluctuations, indicative of the animal's exposure to climatic extremes (Britton *et al.*, 2009). Homogenous $^{87}Sr/^{86}Sr$ ratios across the time series represented by the enamel attest to the apparent sedentism implied by $\delta^{18}O$ values, suggesting that very little movement across distinct geological regions took place. Interestingly, slight increases in $\delta^{13}C$ values correlate with decreases in $\delta^{18}O$ values, implying that bison may have shifted to the consumption of lichen during the cold seasons - during which time grasses became scarce (Julien *et al.* 2012). The lack of evidence for seasonal movement opposes the theory that European steppe bison engaged with the landscape in a similar way to their extant North American relatives, and offers an explanation for the ubiquity of their remains at Middle and Upper Palaeolithic sites in Europe.

Birch *et al.* (2016) utilised also employed sequential $\delta^{18}O$ analysis of tooth enamel in order to investigate the migratory behaviour of ungulates across the Late Pleistocene and Early Holocene in the northeastern Adriatic region, and the potential of those behaviours to affect the subsistence strategies of local hunter-gatherer groups. Ten red deer and fourteen caprid (ibex and chamois) molars from three sites (Pupićina, Nugljanska and Vela Špilja Lošinj) were selected, ensuring that each stratigraphic horizon was represented by at least two teeth from each taxon where possible. Samples of 3-4mg were drilled from the enamel surface sequentially from the crown to the cusp, and were pre-treated to any remove organic matter and exogenous carbonate. Intra-tooth $\delta^{18}O$ and $\delta^{13}C$ values in caprids were more varied than in red deer, illustrating species-specific foraging behaviours and mobility. Caprids exhibited little to no change in $\delta^{18}O$ or $\delta^{13}C$ variability across time, suggesting that their mobility did not change considerably between the Late Pleistocene and Early Holocene. Red deer, however, exhibited significantly different intra-individual $\delta^{18}O$ ranges between the Late Pleistocene and Holocene (Figure 41). It is argued that this may be evidence for a shift in mobility over time, with individuals migrating seasonally during the Pleistocene and ranging across smaller distances during the Holocene. Interestingly, this switch in red deer mobility coincides with a shift in the archaeological record to considerably more heterogeneous faunal assemblages at the study sites. Zooarchaeological evidence suggests that diets became broader during the Holocene, incorporating more sedentary, predictable fauna – perhaps as a response to the unpredictable nature of red deer mobility at this time (Birch *et al.* 2016). Although the study is somewhat limited by the small sample size, it illustrates the potential for ecological knowledge to inform our interpretation of changes in human subsistence over time.

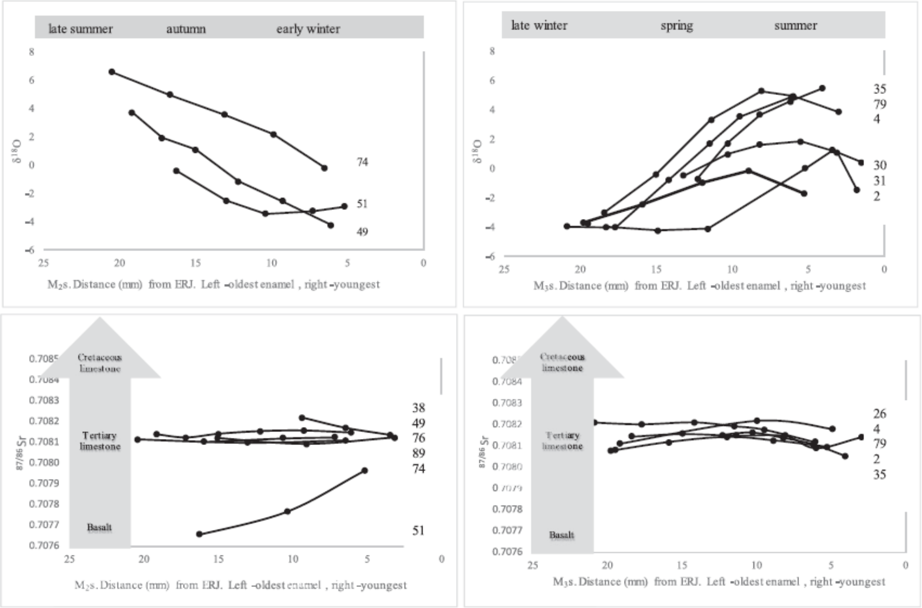


Figure 42. Charts showing $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ data for all gazelle molar specimens. M2 data are on the left, M3 data are on the right. From Henton *et al.* (2017: 155).

A key area of interest to researchers is the transition from mobile hunter-gatherer subsistence strategies to more sedentary lifeways – indeed, the study of the origins of sedentism is crucial in the pursuit of an understanding of the origins of civilisation. Much energy has been poured into the study of the Near East during the Epipalaeolithic and Natufian periods in particular, as some of the earliest evidence for sedentism can be found as early as 12, 500BP in the Levant (Jones, 2012). Two atypical Epipalaeolithic 'mega-sites' in modern-day Jordan have garnered continued research interest with regards to the question of sedentism, due to their sheer size and evidence for continuous occupation - Kharaneh IV and Wadi Jilat VI; both of which are found in the Azraq Basin, Jordan. Evidence for structures, occupation floors and burials survive at both sites; a stark contrast to the contemporary yet more ephemeral, single-occupation campsites common in their vicinity (ibid). There are two key theories concerning the primary reason for their existence – the first postulating that the 'mega-sites' represent aggregations of people exploiting the seasonal migration of gazelle (Goring-Morris, 1998). Several early studies based on zooarchaeological assemblages and ethno-historical accounts suggested that gazelle - now extinct in the Levant - migrated over long distances, resulting in the seasonal occupation of the 'mega-sites' along migration routes in order to exploit their seasonal movements. The second theory contests the first, suggesting instead that the sites were occupied throughout the year (Byrd and Garrard, 1990). Central to this debate is the mobility of the fauna, primarily gazelle, upon which the inhabitants of these sites depended. Several workers have attempted to address the question through dedicated study of the seasonality and mobility of Epipalaeolithic Levantine gazelle (Henton *et al.*, 2017, Figure 42; Jones, 2012). In the latter study, dental cementum analysis was carried out in order to determine the season of death of seventeen gazelle from Kharaneh IV and five gazelle from Wadi Jilat VI. Results suggested that groups of individuals from both sites had been killed throughout the year. Whilst spring and summer deaths tended to be the most common, autumn and winter deaths were nevertheless evident, suggesting that both Kharaneh IV and Wadi Jilat VI may have been occupied during multiple seasons (Jones, 2012). However, dental cementum analysis alone is not capable of providing a clear-cut answer to the question of which of the theories (if either) is correct, because it cannot provide direct insights into the mobility of the gazelle population. Henton *et al.* (2017) therefore set out to investigate Epipalaeolithic gazelle mobility through sequential multi-isotope analysis of gazelle molars recovered from the sites.

In order to investigate the mobility of Epipalaeolithic gazelle in the Azraq Basin, Henton *et al.* (2017) selected fourteen M3 and eight M2 teeth from a total of twenty Epipalaeolithic gazelles from Kharaneh IV for multi-isotope analysis. Six sequentially drilled samples of >20mg, taken along the buccal surface from the crown to the enamel-root junction for each tooth, were subjected to strontium, oxygen and carbon isotope analysis. Dental microwear analysis was also carried out for each tooth, on the basis that microwear represents the types of food consumed just a few weeks before death, and spring/summer foods can often be distinguished from autumn/winter foods due to the variations in the types of wear they create. The sequential strontium isotope data suggest that all of the individuals stayed exclusively on the Tertiary limestone during the formation of their second and/or third molars, with the singular exception of one individual who moved from the less radiogenic basalt onto the Tertiary limestone during the formation of the M2. The sinusoidal nature of the $\delta^{18}O$ data further implies that the gazelle did not range long distances; rather they remained in a region where both cold and warm extremes were experienced throughout the year. The carbon isotope results are consisted with the $\delta^{18}O$ data, illustrating a seasonal change in forage that likely occurred as a result of local seasonal availability. Henton *et al.* (2017) concluded that the gazelle were therefore available to inhabitants of Kharaneh IV all year round during the Epipalaeolithic, and indeed, the dental microwear further supported their conclusions. Some gazelle had died with dental microwear wear attributed to consumption of fibrous spring/summer grasses, whilst other had others died with microwear attributable to the consumption of autumn/winter browse. No trends were observed, suggesting that they all died at different times - hence, it was likely that they had been hunted throughout the year.

Although the sample size is relatively small, the results gathered by Henton *et al.* (2017) highlight the value and potential of isotopic analysis in the investigation of human and animal seasonality in prehistory. Prior to dedicated isotopic analysis of the material, it was believed that large Epipalaeolithic sites in the Near East such as Kharaneh IV and Wadi Jilat VI were positioned along gazelle migration routes, and were occupied seasonally by human groups in order to exploit the annual movement of gazelle herds. These theories were based on zooarchaeological evidence and ethno-historical accounts of gazelle mobility. Combined isotopic, dental wear and cementum annuli evidence (Henton *et al.*, 2017; Jones, 2012), however, has shed new insights into the palaeoecology of Epipalaeolithic gazelle in the region, and strongly suggests that the gazelle did not migrate over long distances. This revelation changes our understanding of the settlement patterns and subsistence behaviours of the human groups at these mega-sites – if gazelle were available in the Azraq basin throughout the year, then the sites themselves may also have been occupied year-round. Not only does this provide us with new insights into the sites as discrete entities, but also has wider implications for our understanding of the wider origins of sedentism in the Epipalaeolithic.

6.2.2. LA-ICP-MS

Very few studies to date have attempted to explore animal mobility and seasonality in Pleistocene fauna via LA-MC-ICP-MS; primarily as a result of the past difficulties in obtaining reliable data from biological apatites. Nevertheless, (Pryor, Stevens and Pike, 2016) set out to investigate the mobility and seasonal behaviour of an adult horse recovered from a Magdalenian context at the site of

Klementowice in the southeast of Poland, utilising a novel laser ablative sampling for strontium isotope analysis. The site, and many like it, has been interpreted as a temporary camp occupied at specific times of the year in order to exploit the seasonal availability of certain prey species. Sequential strontium, oxygen and carbon isotope analyses were carried out on singular M2, with the former collected via laser ablation MC-ICP-MS. Two adjacent strips of enamel spanning the entire length of the enamel from the crown to the enamel-root junction were removed; one mounted as a longitudinal section in preparation for laser ablative sampling, the other divided into ten sequential aliquots taken perpendicular to the axis of enamel extension. Carbon and oxygen isotope samples were pre-treated with acetic acid to remove exogenous carbonates, and the enamel slice intended for strontium isotope analysis was pre-ablated to remove any surface contaminants. 2027 individual ^{87}Sr/^{86}Sr measurements were made over the entire length of the enamel, with a 20-point moving average (Figure 43) illustrating a trend from more radiogenic values towards the crown to less radiogenic values towards the enamel-root junction. A gradual trend can also be seen in the δ^{18}O data, moving from lower values at the crown to higher values at the enamel-dentine junction. The carbon isotope values are relatively stable along the entire length of the enamel.

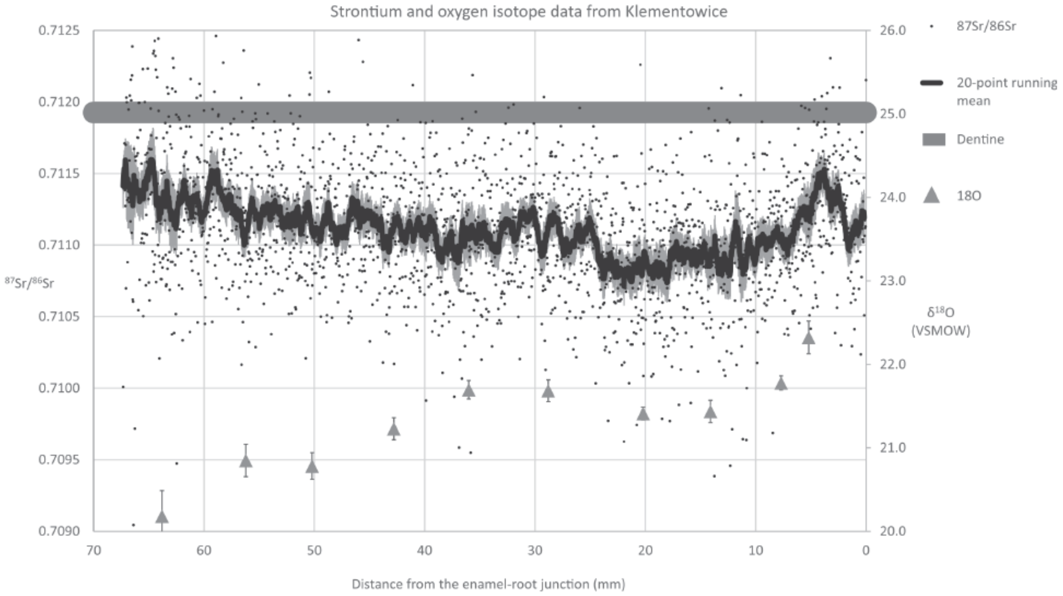


Figure 43. ^{87}Sr/^{86}Sr and d^{18}O data collected for the horse M2 recovered from a Magdalenian context at the site of Klementowice, Poland. Small black dots are individual ^{87}Sr/^{86}Sr measurements, and the black line over grey shading is a 20-point standard error envelope. From Pryor (2016).

In horses, the M2 begins to form between five and six months of age and therefore may overlap with the nursing period. (Pryor, Stevens and Pike, 2016) argue that there is little evidence for the interference of a nursing signal upon the $\delta^{18}O$ values, as nursing generally produces elevated $\delta^{18}O$ values and the $\delta^{18}O$ values observed near to the crown are, in fact, the lowest seen across the entire length of the enamel. As such, the data are interpreted as being purely reflective of seasonal temperature, leading the authors to conclude that the first season visible in the M2 enamel carbonate is winter/spring – evident in the comparatively low $\delta^{18}O$ values. The M2 mineralises over a period of approximately two years, with the upper 40-50mm of enamel reflecting one climatic year. A summer peak in $\delta^{18}O$ can be observed at approximately 35mm from the enamel-root junction, followed by a second winter minimum at approximately 15mm from the ERJ. Interestingly, a sudden drop and subsequent increase in $^{87}Sr/^{86}Sr$ values can be seen coinciding with the autumn and spring seasons respectively, as represented by the $\delta^{18}O$ data (see Figure 44). If Magdalenian horses in the study region moved between geologically distinct regions during the autumn months, support might be lent to the theory that sites such as Klementowice were indeed temporary hunting camps, occupied repeatedly at certain times of the year in order to exploit the seasonal availability of mobile fauna. Cementum annuli data from other horses at Klementowice suggests that they were hunted and killed during the autumn season (ibid), offering further support to this theory.

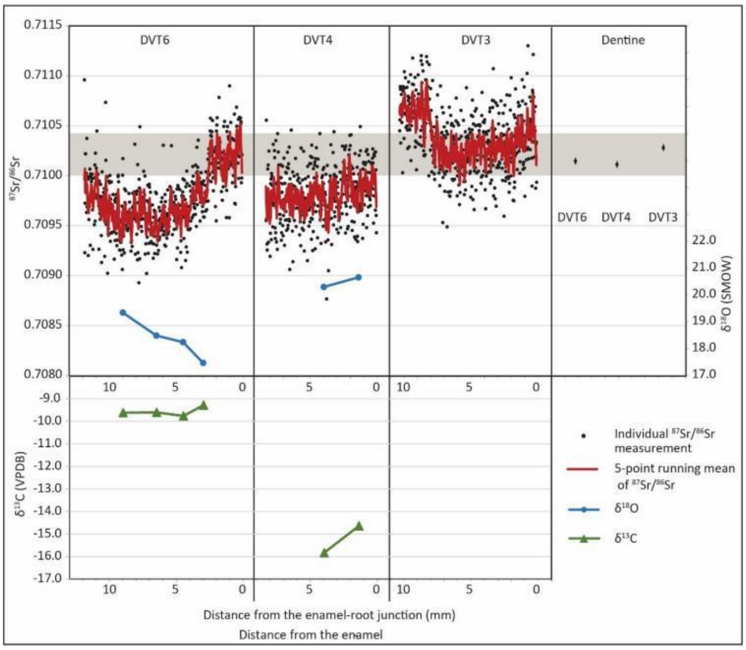


Figure 44. Sr, O and C isotope data for the three reindeer molars/premolars from Dolní Věstonice II. Black dots = individual strontium measurements, with the 5-point moving mean shown in red. The solid grey bar indicates the mean and 2-standard deviation distribution of dentine strontium isotopic measurements from a large sample of teeth from Dolní Věstonice II. From Pryor *et al.* (2016b).

A similar approach was later employed by Pryor *et al.* (2016) in a study of three Gravettian reindeer individuals recovered from the Upper Palaeolithic site of Dolní Věstonice II in the Czech Republic, famed for the discovery of early clay figurines. The site has been interpreted as a seasonal camp, occupied during the winter in order to exploit fur-bearing fauna such as wolves and foxes (*ibid*). Mammoth steppe environments during the last glacial period experienced highly seasonal changes in climate, which likely affected the availability of certain food resources over a yearly basis. This seasonal availability of resources likely had an impact upon the food procurement behaviour and mobility of human groups living in the region, and as such, understanding the subsistence strategies of these societies may offer insights into the ways in which they were able to flourish in such harsh environments. One molar and two premolars from three reindeer recovered from Gravettian deposits at the site were subjected to strontium, oxygen and carbon isotope analyses, along with dental cementum analysis, in order to reconstruct mobility patterns of the individuals. 5mm-wide strips of enamel spanning the crown to the ERJ were cut from the teeth and mounted for strontium isotope analysis, whilst discrete samples were drilled adjacent to the strips for carbon and oxygen isotope analyses. $^{87}Sr/^{86}Sr$ values from the dentine were measured and used to determine the local range for the site.

Two of the teeth are argued to exhibit clear mobility patterns – DVT6 and DVT3. The strontium and oxygen isotope data from the upper left 4th premolar DVT6 (Figure 44) suggest that the individual spent time in an area that was less geologically radiogenic than the Pavlov Hills during the period of enamel formation, before transitioning to a region isotopically identical to the archaeological sites during autumn or winter. The abrupt drop in $^{87}Sr/^{87}Sr$ from more radiogenic values to values identical to those of the site in the upper 3rd premolar (DVT3) is also argued to be indicative of a migratory event, though the season during which this occurred is not known, as oxygen data are not yet available. Both individuals appear to have ranged between areas that are more and less radiogenic than Dolní Věstonice II, but in order to establish the location of these areas, further environmental sampling is required in order to build a wider $^{87}Sr/^{86}Sr$ map. The upper left 1st molar (DVT4) on the other hand exhibits comparatively homogenous $^{87}Sr/^{86}Sr$ values; the majority of which reflect less radiogenic geologies than the site itself. It is likely that both the homogenous $^{87}Sr/^{87}Sr$ profile and elevated $\delta^{18}O$ data reflect the fact that the M1 forms partially *in utero*, and as such, the authors tentatively suggest that the strontium isotope values reflect the location of the region in which the calf was born. Results of the dental cementum analysis suggests that all three individuals were killed some time during the winter, suggesting that they were present around the Pavlov hills during winter months. This adds to a growing body of evidence for the seasonal use of Dolní Věstonice II by human groups to exploit winter resources; namely reindeer prey, fur-bearing species such as fox and wolf, and firewood.

The data presented in this work will contribute to a small but growing European dataset. Work of this kind is sparse, particularly as a result of the difficulties associated with the use of laser ablation systems in sequential $^{87}Sr/^{86}Sr$ analysis. This is unfortunate, as seasonality data for the Middle and Upper Palaeolithic is vital for a comprehensive understanding of the subsistence strategies of human groups and the effects of long-term climatic and short-term seasonal pressures upon those strategies. This project will provide new data in a region within which minimal work of its kind has been carried out previously, and may offer insights not only into Pleistocene ecology, but also the causes of changes in human subsistence patterns over a vast span of time.

6.2.3. Animal seasonality and mobility in archaeology and beyond

Whilst the reconstruction of prehistoric animal mobility has intrinsic value as an insight into the effects of climate change and human impact upon the behaviour and ecology of both extinct and extant species through time, it also has the potential to directly inform our understanding of the hunting strategies and landscape use of contemporaneous human groups. This notion is central to the current study, and was explored previously by Britton *et al.* 2011 in their study of Pleistocene reindeer mobility at the Neanderthal rock shelter site of Jonzac, France. The Mousterian deposit is characterised by a cut-marked bonebed, dominated by prime age reindeer individuals – the remains of which are argued to point, based on zooarchaeological evidence, to season-specific hunting episodes by Neanderthals (see Jaubert *et al.* 2008; Steele *et al.* 2009). Upon sequential strontium isotope analysis of reindeer tooth rows, it was established that these individuals had likely taken part in the same seasonal migratory events, lending support to the idea that the rock shelter itself was selected as a tactical location along the migratory route of reindeer herds (Britton *et al.* 2011).

Similar work was carried out in 2008 by Pellegrini *et al.,* who sought to establish whether the settlement patterns and hunting strategies of human groups in late-glacial central Italy could be attributed to the seasonal altitudinal migration of prey species such as red deer and horse. Sequential strontium and oxygen isotope data from the tooth enamel of these species, however, did not provide evidence for altitudinal mobility between the uplands and lowlands of the study area, suggesting that the movements of hunter-gatherers through the landscape were unlikely to be in response to altitudinal prey mobility. This study in particular illustrates the importance of combining multiple lines of evidence in such an investigation, since the possibility of animal mobility across homogenous geologies (which would not necessarily result in $^{87}Sr/^{86}Sr$ variation) could not be ruled out. Indeed, if isotopic reconstructions of animal mobility are to inform our understanding of the landscape use and hunting strategies of human groups, then we must consider them in the context of as much archaeological data as possible. Zooarchaeological evidence such as season of death (via *cementum annuli*) and skeletal element representation, the characteristics of lithic and artefactual assemblages, and even comparative human $^{87}Sr/^{86}Sr$ data (this study; Pike *et al.* unpublished) are invaluable sources of information that, when combined with isotope data such as those discussed in this chapter, can offer detailed new insights into human resource procurement and landscape use.

The sequential strontium and oxygen isotope analyses described in this section are also directly applicable to other fields of research, and are increasingly used for modern ecological and conservatory applications. For example, understanding the migratory routes of endangered species (particularly those that are elusive and prove difficult to track through more conventional GPS tracking) can aid in their protection; particularly when migration routes are encroached upon by human activity. Members of the small migratory bat species *Pipistrellus nathusii* are regularly killed by wind turbines along the coast of Germany, and as such an understanding of the migratory route of this species (and many others) would be beneficial in preventing future bat fatalities. A study carried out by Kruszynski *et al.* (2021) utilised a combination of stable hydrogen isotope (δ^2H) and $^{87}Sr/^{86}Sr$ analyses of *Pipistrellus nathusii* fur keratin in order to reconstruct migration corridors, which aided in the identification of the provenance of individuals captured along suspected routes. However, the possibility of exogenous strontium in hair keratin (Mancuso and Ehleringer, 2018) potentially reduces the reliability of these assignments. $^{87}Sr/^{86}Sr$ analysis via LA-MC-ICP-MS analysis of incredibly small tooth enamel samples recovered from fatally injured bats along migration routes may help mitigate this problem, and also offer a more detailed, time-resolved reconstruction of migratory routes providing that tooth formation is synonymous with migrations. A similar conservation application for highly spatially resolved strontium isotope analyses via LA-MC-ICP-MS is the reconstruction of fish migrations through analysis of otoliths ('ear bones'), numerous examples of which exist in the literature (Brennan *et al.*, 2015; Hegg, Giarrizzo and Kennedy, 2015) and are informing our understanding of the effects of human activity upon the migratory routes of fish. $^{87}Sr/^{86}Sr$ analyses of illegal wildlife materials such as rhinoceros horn and elephant ivory also offer potential for conservation-based provenance studies (Cerling *et al.,* 2018), allowing tissues to be traced back to their origin - which may subsequently serve as a basis for heightening protection in such areas, and provide evidence for prosecution in the case of illegal trade.

The isotopic reconstruction of the mobility of prehistoric animal groups not only offers profound opportunities for the scientific community to explore changes in natural ecology and human behaviour through time, but also illustrates a great and growing potential for the application of these methods to extant species whose migratory routes are threatened by human activity. Further research, such as that of the current study, will aid in the continual refinement of methodologies and beneficial application of such techniques to a growing range of fields.

7. Materials

7.1 Fauna

A range of animal teeth from different stratigraphic layers of cave deposits in Portuguese Estremadura were selected for analysis. As molar crowns are the last to form in both the mandible and maxilla, these were preferentially targeted. Faunal specimens with a sequence of molars available (usually fragments of mandible or maxilla with in-situ dentition) were selected where possible. The sampled fauna include red deer, horse, ibex and extinct rhinoceros. Red deer are consistently abundant across this span of time, providing an excellent opportunity to investigate temporal changes (or lack thereof) in the mobility patterns of this species.

Tooth formation times vary depending on species, and although we do not always have data for the exact formation and eruption times of Pleistocene fauna (particularly for extinct species), modern proxies based on analyses of the dentition of analogous extant fauna can be used with caution to provide a rough framework for the interpretation of isotope data. As such, tabulated formation times for the dentition of each species are given in Chapter 8.

Table 1. Mousterian faunal material from Gruta da Oliviera.

ID	Element	Spit	Layer	Period	Taxon
O16-1829	Molar fragment	A70	24	Mousterian	*Equus sp.*
P16-787	Mandible with two molars	A66	20	Mousterian	*Capra pyrenaica*
N15-417	Mandible fragment with molar	A68	22	Mousterian	*Equus sp.*
P16-916	Molar fragment	A74	26	Mousterian	*cf. Stephanorhinus sp.*
O17-1229	Maxillary fragment (left) with P2-P3-P4	A66	20	Mousterian	*Cervus elaphus*
N15-379	Mandible fragment with P2-P3-P4	A68	22	Mousterian	*Equus caballus*
P15-403	Molar	A66	20	Mousterian	*Capra pyrenaica*
O15-667	Molar fragment	A67	21	Mousterian	*cf. Stephanorhinus sp.*

Table 2. Upper Palaeolithic faunal material from Lapa dos Coelhos.

ID	Element	Spit	Layer	Period	Taxon
F3-88	Maxilla +teeth	16	4	Upper Magdalenian	*Capra*
F3-72	Mandible P2 P3 P4 M1 M2 M3	15	4	Upper Magdalenian	*Cervus elaphus*
H3SE-s/n	Row of upper molars	35	8	Solutrean	*Capra pyrenaica*

Table 3. Upper Palaeolithic faunal material from Gruta do Caldeirão.

ID	Element	Spit	Layer	Period	Taxon
M14sc351	Loose upper molar	F3 toca	Fa	Solutrean	Capra pyrenaica
M14sc359	Loose upper molar	F3 toca	Fa	Solutrean	Capra pyrenaica
N14-265	Upper molar row	I1	I	Solutrean	Capra pyrenaica
P11-643	Maxilla with molars	J5E	Jb	Gravettian	Capra pyrenaica
P11-650	Loose molar, part of P11-643 (?)	J6E	Jb	Gravettian	Capra pyrenaica
N14-241	Mandible with tooth row (M1 to M3)	H1	H	Solutrean	Cervus elaphus
N14-269	Mandible with P4 to M3 row of teeth	I2	I	Solutrean	Cervus elaphus
O13-164	Upper molar (M3?)	Am3/Col.A	Fa	Solutrean	Equus sp.
O13-241	Lower molar (M1 or M2)	F6	Fa	Solutrean	Equus caballus

7.2 Humans

Two Neanderthal individuals from a cave within the Almonda karstic system, Gruta da Oliveira, were subjected to sequential $^{87}Sr/^{86}Sr$ analysis via LA-MC-ICP-MS by Pike *et al.* (unpublished). An anatomically modern human from a Magdalenian deposit at Galeria da Cisterna (also within the Almonda karstic system) also underwent $^{87}Sr/^{86}Sr$ analysis during that study. The existing $^{87}Sr/^{86}Sr$ profiles of these individuals will be re-visited alongside the Solutrean human and Middle/Upper Palaeolithic faunal data collected in the current project, in order to consider diachronic changes in mobility and subsistence behaviours.

As discussed in depth in Chapter 5, the mineralisation rates of human tooth enamel are not precisely known. However, in the absence of such information, the rough formation times for each tooth can be tentatively used as a framework when interpreting isotope data. Table 5 gives tooth formation data after AlQahtani *et al.* (2010).

Table 4. Neanderthal and AMH specimens discussed and/or analysed in this study.

ID	Element	Layer	Period	Taxon	Analysed by:
Oliviera 8	Lower molar (M_3)	22	Mousterian	*Homo neanderthalensis*	Pike *et al.*
Oliviera 9	Lower first premolar (P_3)	22	Mousterian	*Homo neanderthalensis*	Pike *et al.*
Cisterna 2	Lower left premolar (P_3)	3	Magdalenian	*Homo sapiens*	Pike *et al.*
Caldeirão 6	Right I_2	Fa	Solutrean	*Homo sapiens*	This study
Caldeirão 5	Right I_2	Fa	Solutrean	*Homo sapiens*	This study

Table 5. Timing of human tooth formation and eruption. After AlQahtani et al. (2010).

Tooth	Initial cusp formation	Crown completed	Root completed	Full eruption
i1	<30 weeks in utero	1.5 months	2.5 years	10.5 months
i2	<30 weeks in utero	4.5 months	2.5 years	1.5 years
c	30 weeks in utero	7.5 months	3.5 years	2.5 years
m1	<30 weeks in utero	10.5 months	3.5 years	1.5 years
m2	30 weeks in utero	10.5 months	3.5 years	2.5 years
i1	<30 weeks in utero	Birth	2.5 years	10.5 months
i2	<30 weeks in utero	1.5 months	2.5 years	1.5 years
c	30 weeks in utero	10.5 months	3.5 years	2.5 years
m1	<30 weeks in utero	7.5 months	3.5 years	1.5 years
m2	30 weeks in utero	10.5 months	3.5 years	2.5 years
I1	4.5 months	4.5 years	11.5 years	7.5 years
I2	1.5 years	5.5 years	11.5 years	9.5 years
C	7.5 months	5.5 years	15.5 years	12.5 years
P1	2.5 years	6.5 years	14.5 years	11.5 years
P2	3.5 years	6.5 years	14.5 years	12.5 years
M1	4.5 months	3.5 years	9.5 years	6.5 years
M2	2.5 years	8.5 years	16.5 years	13.5 years
M3	8.5 years	14.5 years	23.5 years	20.5 years
I1	7.5 months	3.5 years	8.5 years	7.5 years
I2	<1.5 years	4.5 years	10.5 years	7.5 years
C	10.5 months	5.5 years	14.5 years	11.5 years
P1	2.5 years	6.5 years	14.5 years	11.5 years
P2	2.5 years	6.5 years	14.5 years	12.5 years
M1	4.5 months	3.5 years	10.5 years	6.5 years
M2	2.5 years	8.5 years	16.5 years	12.5 years
M3	8.5 years	14.5 years	23.5 years	20.5 years

8. Methods

8.1 Strontium isotope analysis

8.1.1 Sample preparation

Human and animal teeth were sampled in the stable isotope laboratory at the Department of Archaeology, University of Southampton. Enamel slices retaining dentine strips approximately 1-2mm thick were removed from human and animal tooth specimens parallel to the growth axis of the tooth using a diamond-tipped cutting disc and a Dremel tool, subsequently ultrasonicated in Milli-Q water, and finally dried in a vacuum oven. Because of their archaeological value and the need to retrieve the specimens post-analysis, human tooth samples were mounted in a blu-tack block for ablation. Slices of enamel were removed from animal tooth crowns in the same manner, but were instead mounted in Epoxy resin and polished using 1200, 1000 and 800 grit papers on a Buehler Metaserv Grinder-Polisher.

8.1.2 Analysis

Table 6. Faraday cup configuration for ^{87}Sr/^{86}Sr isotope analysis using the Thermo Neptune MC-ICP-MS system. The isotopes of interest and the main isobaric/molecular interferences are given.

Cup		**L3**	**L2**	**L1**	**C**	**H1**	**H2**	**H3**	**H4**
Mass		82	83	84	85	86	87	88	89
Isotope of interest		^{82}Kr	^{83}Kr	^{84}Sr	^{85}Rb	^{86}Sr	^{87}Sr	^{88}Sr	^{89}Y
Isobaric interference	**Rb**						^{87}Rb		
	Kr			^{84}Kr		^{86}Kr			
	Yb			^{168}Yb^{2+}	^{170}Yb^{2+}	^{172}Yb^{2+}	^{174}Yb^{2+}	^{176}Yb^{2+}	
	Er		^{166}Er^{2+}	^{168}Er^{2+}	^{170}Er^{2+}				
Molecular interference	**Ca dimers**		^{40}Ca^{43}Ca	^{40}Ca^{44}Ca	^{42}Ca^{43}Ca	^{40}Ca^{46}Ca		^{40}Ca^{48}Ca	
						^{42}Ca^{44}Ca		^{42}Ca^{46}Ca	
						^{43}Ca$_2$	^{43}Ca^{44}Ca	^{44}Ca$_2$	
	Ca argides		^{43}Ca^{40}Ar	^{48}Ca^{36}Ar					
				^{46}Ca^{38}Ar		^{48}Ca^{38}Ar			
				^{44}Ca^{40}Ar		^{46}Ca^{40}Ar		^{48}Ca^{40}Ar	
	Ca-P						^{40}Ca^{31}P^{16}O		

Tooth enamel slices were analysed using a New Wave Research 213nm solid state homogenised-beam excimer laser (NWR213) coupled to a Thermo Finnegan Neptune MC-ICP-MS. Helium was swept through the laser ablation chamber for 10 minutes prior to analysis (overnight if geological samples were previously run), and mixed with argon and nitrogen prior to introduction to the plasma. Oxide production was monitored via ^{254}(UO)$^{+}$/^{238}U^{+} following the method of de Jong (2013), and ^{87}Sr/^{86}Sr data were collected with an integration time of 1.14 seconds per measurement cycle. Krypton introduced as an impurity present in the helium gas source was corrected for by carrying out on on-peak gas blank, and the $^{87}Sr/^{86}Sr$ value was corrected for the ^{87}Rb interference using the natural $^{87}Rb/^{85}Rb$ ratio of 0.385617. Instrumental mass fractionation was corrected for using an exponential fractionation law (Russell, Papanastassiou and Tombrello, 1978) and the natural $^{86}Sr/^{88}Sr$ ratio of 0.1194 (Nier, 1938). Samples that exhibited high concentrations of yttrium either in the form of discrete spikes or in specific regions of the enamel were examined post-analysis, and data removed as per Section 8.1.4.

Prior to isotopic analysis, human enamel samples were surface cleaned by rapidly ablating the plotted laser path to remove any surface contaminants. For this purpose, a spot size of 150µm, a repetition rate of 10Hz and a 50µm/s^{-1} translation rate was utilised for human teeth. For surface cleaning the animal teeth, which are generally larger, the translation rate was increased to 100µm/s^{-1}. For human enamel samples, the subsequent analysis along the pre-ablated laser track involved the use of a 150µm spot size, a repetition rate of 20Hz and a translation rate of 2µm/s^{-1}, whilst for fauna the translation rate was increased, depending on the specimen, to between 30µm/s^{-1} and 50µm/s^{-1} to account for the greater length of the samples. Gas blank data were collected for 60 seconds prior to each analysis with the same parameters, except with the laser shutter closed.

8.1.3 Standards

In order to monitor oxide production in the plasma, samples of NIST 610 glass were ablated along continuous 100µm lines with a translation rate of 5µm/s^{-1} and a repetition rate of 10Hz. The instrument was tuned through the alteration of a number of parameters including gas flow rates and the Z position of the plasma torch, until the $^{254}(UO)^+/^{238}U^+$ was minimised. This is treated as a proxy for the production efficiency of calcium phosphates in the plasma, with conditions producing a ratio of less than 0.01% deemed to be suitable for the subsequent analysis of bioapatites. Slight environmental variations on a day to day basis meant that the instrument often required re-tuning for minimal oxide production each day prior to analysis.

As discussed in Section 4.4.3, there are difficulties in establishing an appropriate matrix-matched standard for archaeological tooth enamel via laser ablation MC-ICP-MS. Finding an enamel specimen that exhibits homogenous $^{87}sr/^{86}Sr$ values across the entire tooth, a stable Sr concentration, and a sufficient size for repeated analysis over the course of the study period is a significant challenge. Over the course of these analyses, two known-value bioapatite standards were repeatedly analysed. An ashed and pressed bovine bone pellet known as BP (produced at the University of Bristol as part of a previous study; de Jong 2013) was utilised, along with several isotopically homogenous pig teeth produced during a previous feeding study at the University of Bristol. Across analysis days, repeated measurement of BP (n=29) gave a mean offset and standard error of 124 ± 95 ppm over the TIMS value, whilst the repeat analysis of the enamel from PT 232 (n=32) and PT 238 (n=78) gave a mean offset and standard error of 65 ± 60 and 99 ± 31 ppm over the TIMS values respectively.

8.1.4 Diagenesis and the use of ^{89}Y as a proxy for diagenetic alteration

In strontium isotope studies, the susceptibility of dentine to diagenetic alteration is often used to the advantage of the analyst, since in situations where the ^{87}Sr/^{86}Sr value of the dentine has partially or fully equilibrated with the burial environment, comparison of the enamel and dentine strontium isotope values offers an insight into the extent of diagenesis of the former. Deposits within the Almonda karstic system appear to have yielded exceptionally well-preserved enamel and dentine, evident in the fact that both the enamel and dentine of the Gruta da Oliveira 9 Neanderthal appear to exhibit near-identical strontium isotope profiles when normalised to length, despite the susceptibility of the latter to diagenesis (Figure 45).

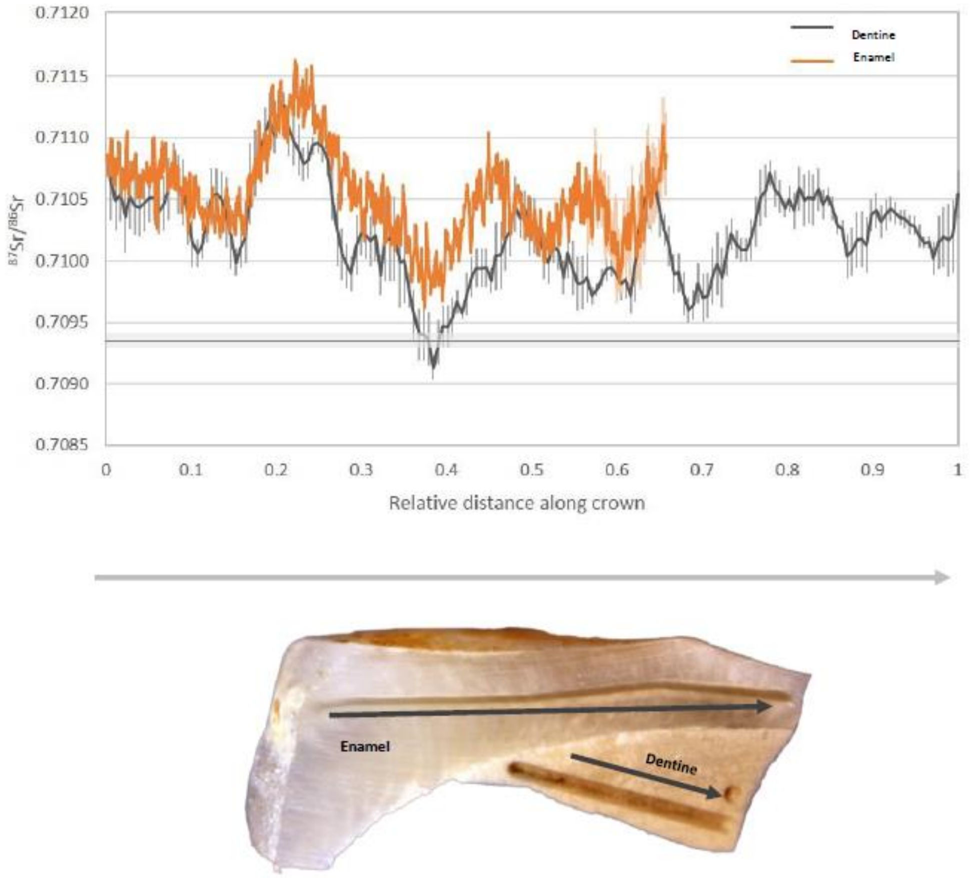


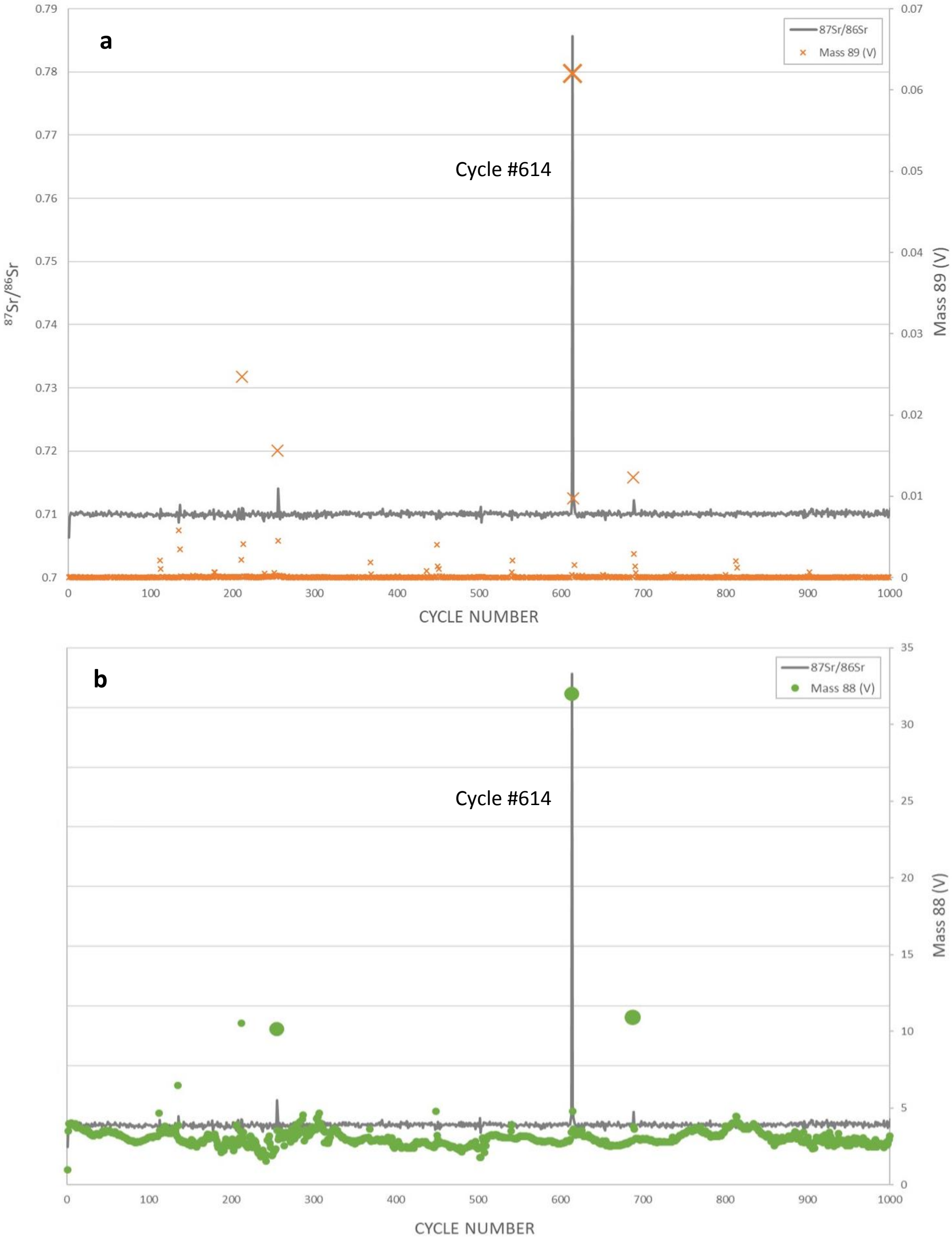
Figure 45. Top: Strontium isotope profiles of enamel and dentine from ^{87}Sr/^{86}Sr LA-MC-ICP-MS analysis of the Oliviera 9 Neanderthal, carried out by Pike *et al.* (unpublished) in 2014. When normalised to length to mitigate differences in enamel and dentine mineralisation rate, the two profiles are remarkably similar, suggesting that the dentine did not undergo a large degree of diagenetic alteration. The grey line denotes the Almonda karstic system sediment Sr value; Sr-15 (0.709314). Bottom: Laser ablation tracks in the dentine and enamel of Oliviera 9 (photograph taken by AWP and JZ). The grey arrows denote the direction of analysis.

Nevertheless, diagenesis is monitored throughout this study through the measurement of the rare earth element (REE) yttrium (89Y).In laser-ablation MC-ICP-MS studies of archaeological tooth enamel, the rare earth element (REE) yttrium (^{89}Y) is routinely monitored as a proxy for diagenetic alteration. REEs have been found to occur in relatively high abundances in chemically altered biological apatites (Trueman and Tuross, 2002; Trueman *et al.*, 2011), but occur in extremely low abundances in modern teeth (Driessens and Verbeeck, 1990; Kohn, Schoeninger and Barker, 1999). In other studies, enamel samples with consistently large REE beams across the entire profile are rejected, as it is possible that strontium uptake from the burial environment has taken place (de Jong 2013; Lewis *et al.* 2014). Of all the archaeological enamel samples analysed in this study, none were found to have consistently high yttrium contents throughout the entire isotopic profile. Nevertheless, high yttrium beams are sometimes observed in one of two forms – as a discrete 'spike', whereby one or two cycles exhibit anomalously large signal intensity at mass 89, or alternatively as gradual increases and decreases in beam size across a larger number of cycles; often occurring at the apical or cervical edges of the crown.

It should be noted that ^{40}Ar^{31}P^{18}O is likely to be produced in the plasma, and may act as a polyatomic interference on mass 89 (Willmes *et al.*, 2016). Since the method used in the current study involves careful monitoring of plasma conditions and diligent tuning in order to reduce the production of oxides within the plasma, it is assumed herein that signals at mass 89 are a result primarily of ^{89}Y.

Spikes

Large spikes in beam size at mass 89 often occur concurrently with large beams on mass 88 (for example, see Figure 46b), implying that the concentration of strontium is greater at these discrete locations. This in turn impacts the mass bias corrected ^{87}Sr/^{86}Sr ratio, often producing considerably more radiogenic values than would be expected for the study area. The voltages of these mass 88 and mass 89 spikes vary among different enamel samples, preventing a blanket cut-off from being applied. As such, in cases like the one observed in Figure 48, the anomalous cycle(s) are omitted from the ^{87}Sr/^{86}Sr profile.



Measured (V)											Measured	Rb correction		Sr MBC		
Cycle	81.5	82Kr	83Kr	84Sr	85Rb	86Sr	87Sr	88Sr	89Y		88/86	87/85Rb MBC	87Sr peak strip	87/86Sr	Moving ave (30)	Error
612	1.33E-05	0.000178	2.52E-05	0.019184	0.000166	0.33984	0.245207	2.935615	-7.6E-07		8.638224541	0.397876419	0.245140745	0.710196118	0.71274532	0.002517
613	1.41E-05	0.00026	3.71E-05	0.020514	0.011328	0.400064	0.292569	3.429847	0.000317		8.573252219	0.394847624	0.288096301	0.711700119	0.712742826	0.002517
614	2.48E-05	0.003667	0.001421	0.224407	0.761994	4.415629	3.479848	**32.01188**	**0.062027**		7.249675556	0.333210654	3.225942886	**0.785647755**	0.712681798	0.002519
615	5.52E-06	0.001115	0.001249	0.050737	0.123211	0.558412	0.453057	4.814146	0.009751		8.621138749	0.397079909	0.404132349	0.713245959	0.71016006	0.000123
616	2.73E-06	0.000587	0.000316	0.025303	0.078256	0.419294	0.333943	3.619363	0.001534		8.632036543	0.397587943	0.30282933	0.71133321	0.710069771	6.29E-05
617	2.33E-05	0.000242	7.45E-05	0.022338	0.002	0.394697	0.285473	3.408107	0.000227		8.634748849	0.397714386	0.284678074	0.710257504	0.710019331	4.59E-05

Figure 46. a) Example data (O13 241) showing $^{87}Sr/^{86}Sr$ versus ytrrium, mass 89 (V). Cycle #614 exhibits an extremely radiogenic $^{87}Sr/^{86}Sr$ value of 0.7865, which coincides with a 0.062V signal at mass 89. Several other smaller spikes in the 89 voltage coincide with less radiogenic $^{87}Sr/^{86}Sr$ values. **b)** Example data from the same sample (O13 241), showing $^{87}Sr/^{86}Sr$ versus the mass 88 voltage. Cycle #614 exhibits a large spike in voltage (32V) at mass 88, coinciding with the spikes in $^{87}Sr/^{86}Sr$ and mass 89 voltage.

There are several possible explanations for the anomalous spikes in ^{89}Y, ^{88}Sr and mass bias corrected ^{87}Sr/^{86}Sr observed in certain samples. One possibility is the ionisation and subsequent measurement of exogenous particles derived from unrelated geological samples analysed in the days or weeks prior to this study, which may have coated the internal surface of the ablation chamber capillaries and occasionally become dislodged during the ablation of the archaeological enamel samples. This may explain the comparatively very large voltages observed at mass 88 and 89; particularly when they occur in a solitary cycle. Alternatively, based on observations made in previous studies, it is possible that the ^{87}Sr/^{86}Sr value that co-occurs with such spikes in voltage is a measure of diagenetic strontium introduced to the enamel in the form of exogenous particles or secondary mineralisation from the burial environment. Tooth enamel samples destined for strontium isotope analysis via laser ablation MC-ICP-MS are ultrasonicated in deionised water in order to remove as many exogenous particles as possible, but it is likely that not all are removed. Likewise, since tooth enamel samples are not pre-treated with acetic acid prior to laser ablation, they are susceptible to the diagenetic effects of secondary mineralisation, whereby rare earth elements from the burial environment are introduced to the enamel and dentine by pore waters and are able to recrystallize. In particular, cracks and hairline fractures in the enamel likely provide routes for water flow and subsequent precipitation of secondary minerals, as was noted in a study of fossil tooth enamel carried out by Kohn *et al.* (1999).

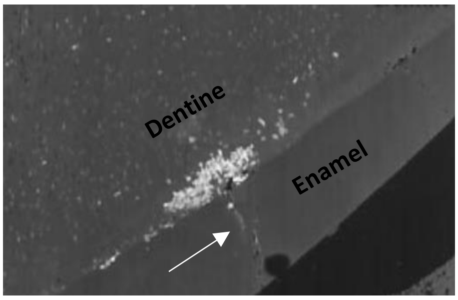


Figure 47. Oxyhydroxide precipitation (brighter patches) at the DEJ and within a hairline crack (white arrow) in a fossil pig tooth. Adapted from Kohn *et al.* (1999: 2742).

The recrystallisation of oxyhydroxides in the locality of cracks in the enamel and dentine may not necessarily, however, be entirely responsible for the presence of exogenous strontium. Kohn *et al.* (1999; and references therein) argue that the high concentrations of uranium and REEs (up to thousands of ppm) observed in fossil teeth in some cases cannot be purely the result of the admixture of biological apatite and secondary minerals; rather, it is likely that a degree of chemical alteration of the original enamel apatite crystals takes place. It may be that discrete cracks in the enamel and dentine not only facilitate the precipitation of secondary minerals, but also provide sites at which chemical alteration can take place (eg, Figure 47). Both theoretical scenarios have the potential to introduce exogenous strontium and REEs in quantities that are larger than unaltered enamel, and would produce spikes in beam voltage consistent with observations made in this study.

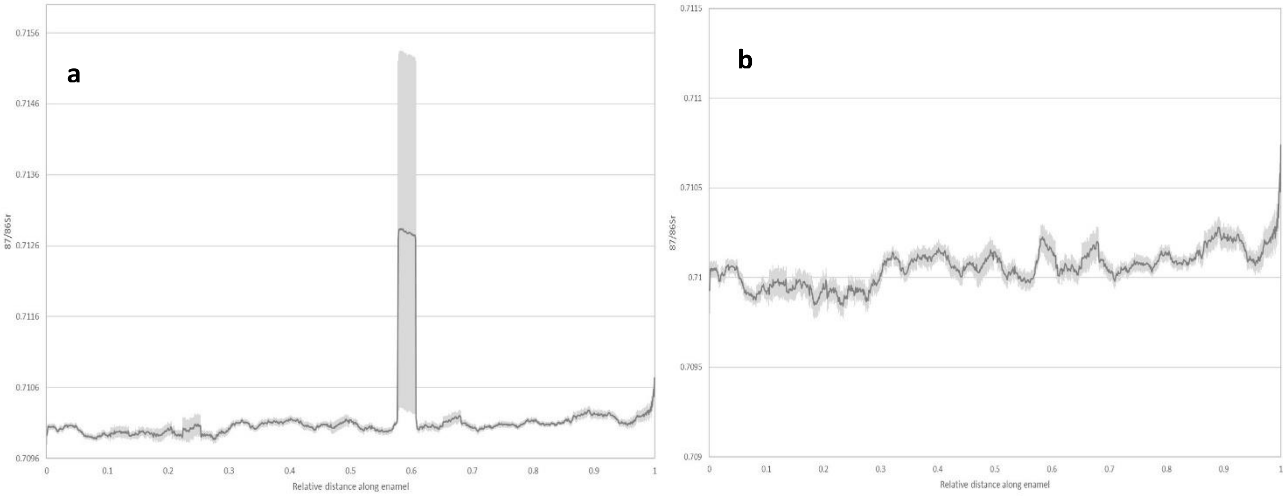


Figure 48. a) Example data (O13 241) showing a 30-point moving average of the mass bias corrected $^{87}Sr/^{86}Sr$ data prior to clipping. **b)** Example data from the same sample (O13 241) after cycles with concurrently high ^{89}Y and ^{88}Sr beams and anomalously high $^{87}Sr/^{86}Sr$ values have been removed.

Continuous

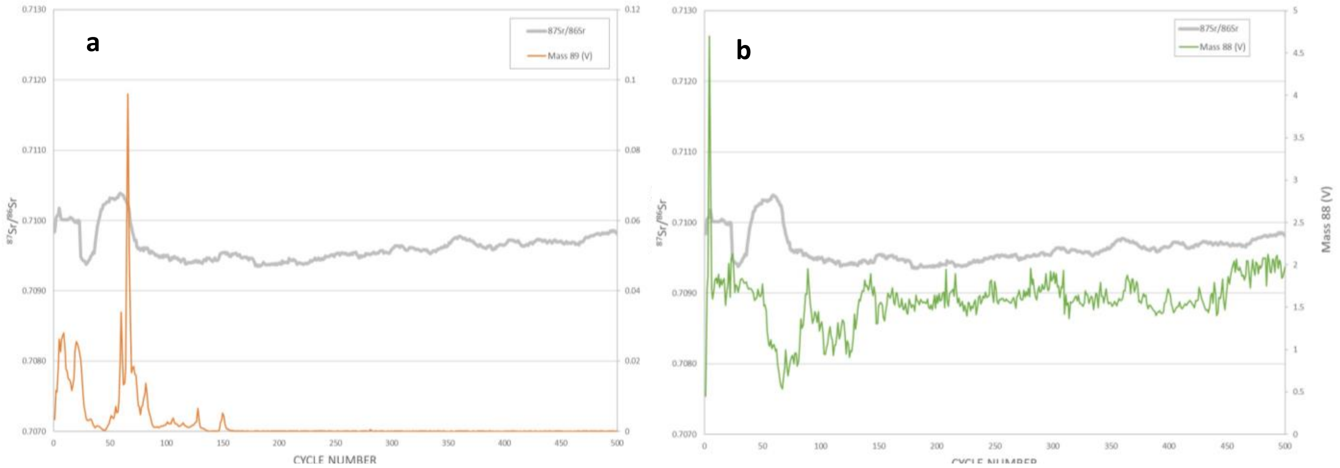


Figure 49. a) Example data (N15 417) showing $^{87}Sr/^{86}Sr$ versus ytrrium, mass 89 (V). **b)** Example data from the same sample (N15 417), showing $^{87}Sr/^{86}Sr$ versus the mass 88 voltage.

Another type of ^{89}Y occurrence involves abnormally large beams observed across a greater number of cycles, usually occurring at the apical or cervical edges of the crown. As is the case with spikes in ^{89}Y, these gradually increasing peaks in ^{89}Y appear to occur concurrently with peaks in the mass bias corrected $^{87}Sr/^{86}Sr$ value. Because they generally occur over a greater number of cycles, it seems less likely that these heightened ^{89}Y are the result of interference from exogenous particles from the burial environment or a previous unrelated analyte dislodged during ablation.

Because the sampling method involves plotting an ablation track down through the middle of the enamel from the apex of the crown to the cervix of the crown (maintaining an equal distance between the DEJ and the outer enamel surface), it seems likely that the heightened ^{89}Y and ^{88}Sr signals observed towards at the start and end of a number of ^{87}Sr/^{86}Sr profiles are in fact reflective of differential diagenetic alteration across the enamel section. Unlike the innermost enamel, the outermost enamel surfaces are exposed to the burial environment and may be subjected to a greater degree of secondary mineralisation and/or alteration of the original apatite (Schoeninger *et al.*, 2003; Willmes *et al.*, 2016). Data consistent with this explanation is presented by Kohn *et al.* (1999), who through electron microprobe, ion microprobe and cathodoluminescence methods were able to demonstrate a greater concentration of elements including manganese, iron and REEs at the outermost enamel surfaces compared with the enamel interiors of fossil tooth crowns (*Schoeninger et al.* 2003).

Figure 49 shows an example of this type of ^{89}Y signal in the apical portion of the crown of a Mousterian horse tooth from Gruta da Oliviera. Large ^{89}Y beams can be observed between cycle nos. 1 and 150, appearing to be concurrent with variable ^{87}Sr/^{86}Sr values in what is otherwise a stable ^{87}Sr/^{86}Sr profile. Interestingly in this case, the beams on mass 88 are not consistently large across the enamel section in question, and in fact are relatively small compared to those observed concurrently with ^{89}Y 'spikes' in other samples. It may be that where external enamel surfaces are concerned, the most common form of diagenetic alteration is not secondary mineralisation but direct alteration of the existing apatite, and as such the strontium measured in this region of the enamel is not additional strontium added through the precipitation of secondary minerals, but rather exogenous strontium that has replaced the original strontium through exchange. In this study, where considerable increases in ^{89}Y beam size are present across a number of cycles and appear to be concurrent with changes in the mass bias corrected ^{87}Sr/^{86}Sr, those cycles are omitted from the final mass bias corrected ^{87}Sr/^{86}Sr profile.

8.1.5 Interpretation of strontium isotope data

The interpretation of the strontium isotope profiles measured in this study can be described as follows.

If we were to assume that tooth enamel mineralisation took place in a single-stage process (i.e, not in secretory and maturational stages, but in one single process), and that the incorporation of heavy isotopes into tooth enamel occurs instantaneously (i.e, on the day of the ingestion of food and water sources, involving no incorporation of isotopes resorbed from skeletal stores), then we might expect the strontium isotope profile of an individual moving from one geological region to another over the course of six months to look something like the model in Figure 50 (though this assumes nearinstantaneous movement from Geology A to Geology B for the purpose of examining metabolic affects only). Whilst factors such as body pool strontium and maturation length may have an effect on the final measured strontium isotope profile, resulting in the peaks and troughs often observed in LA-MC-ICP-MS data, it is difficult to reverse-model without accurate information regarding the tooth enamel mineralisation rates and isotope residence time of each species. Since it is beyond the scope of this work to obtain such information through physiological study of the species represented here, the strontium isotope data will not be interpreted with reference to the specific timing of the mobility based on the position of peaks and troughs across the crown (due to the possibility of time lag). Until a time at which the extent of damping can be fully investigated based on species-specific physiological data, the strontium isotope values at the peaks and troughs of the profiles measured here will not be considered to directly reflect the absolute value of the geological location, but rather will be considered to tentatively reflect possible regions of habitation. A hypothetical strontium isotope profile and its interpretation in terms of animal mobility is presented in Figure 51.

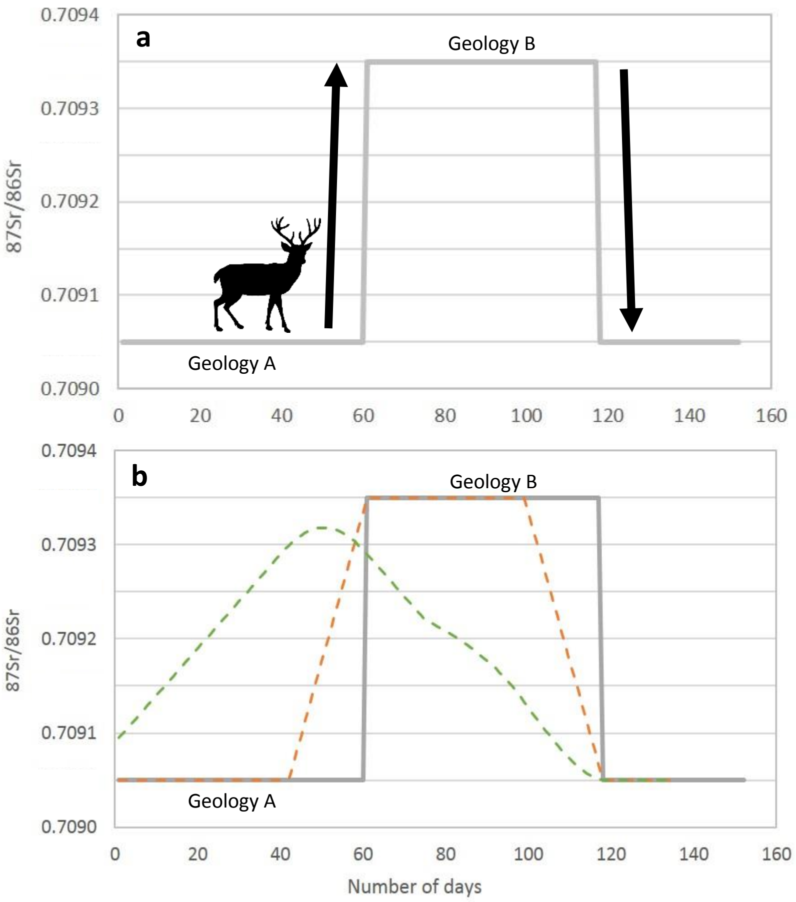


Figure 50. **a)** Simple, hypothetical $^{87}Sr/^{86}Sr$ profile for an individual moving instantaneously between two distinct geologies, assuming that strontium isotope values are incorporated immediately from dietary sources, and resorbed skeletal strontium is not incorporated. **b)** Simple model illustrating the isotopic averaging, potential lag and dampening effects of the isotopic averaging associated with strontium residence time (orange) and maturation length (green).

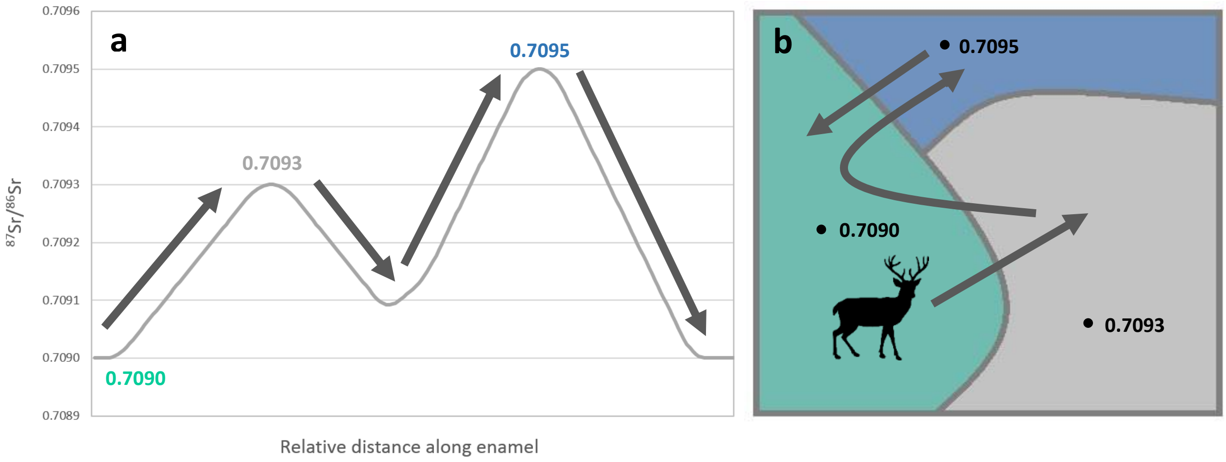


Figure 51. a) Hypothetical strontium isotope profile for the molar tooth crown of an ungulate individual mobile between three distinct geologies. **b)** Interpretation of the mobility pattern reflected in the strontium isotope profile, across a hypothetical geological map with three isotopically distinct geologies.

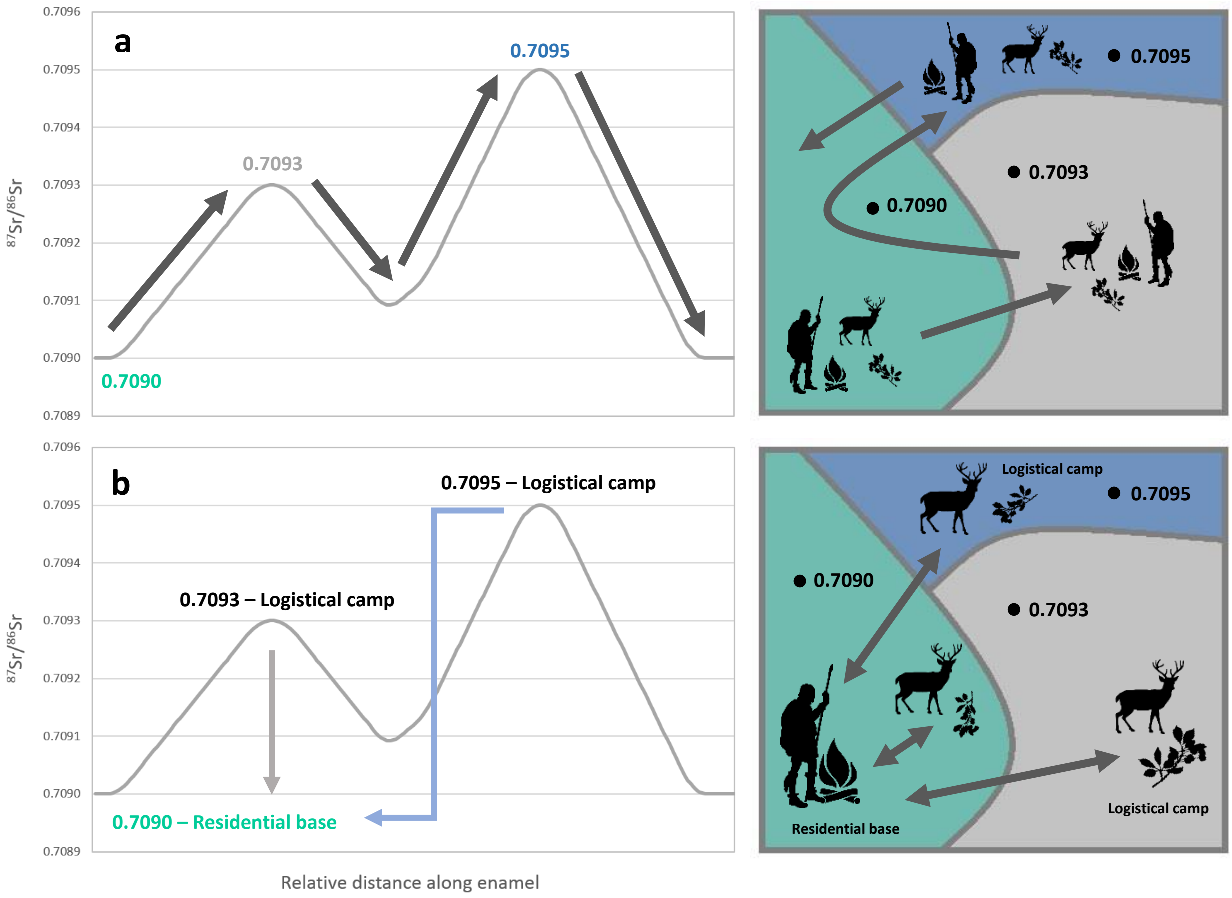


Figure 52. a) Hypothetical strontium isotope profile and corresponding geological map for the molar tooth crown of a human individual consuming resources across three distinct geologies, in a circular mobility pattern. **b)** The same hypothetical strontium isotope profile and geological map for a human individual utilising the same resources in a logistical mobility pattern; exploiting resources from different geologies on a seasonal basis.

Interpretation of human strontium isotope profiles is more complex, since different mobility patterns could in theory result in the same $^{87}Sr/^{86}Sr$ variation across the growth axis of the tooth enamel. For example, if an individual were to engage in exactly the same mobility pattern as the ungulate in Figure 51, consuming local resources as they progress (essentially following a residential mobility pattern), the resultant strontium isotope profile would be the same as the ungulate (assuming no physiological differences). However, the same profile could be generated if the human individual were to permanently occupy a base camp at 0.7090 and consume seasonal plant and animal resources from different geological substrates (brought back to the base camp from logistical hunting sites) in succession (Figure 51b). In contrast, if the individual were to exploit all resources as in Figure 52b year round, or preserved and stored seasonal resources for consumption at a later date, one might expect to see a relatively homogenous $^{87}Sr/^{86}Sr$ profile since the strontium isotope composition of the body plasma would represent a mixture of foods from different geological zones. As such, where human strontium isotope profiles are concerned, interpretation also depends on zooarchaeological and artefactual evidence from contemporaneous archaeological sites in the study area.

8.1.6 Strontium isotope map

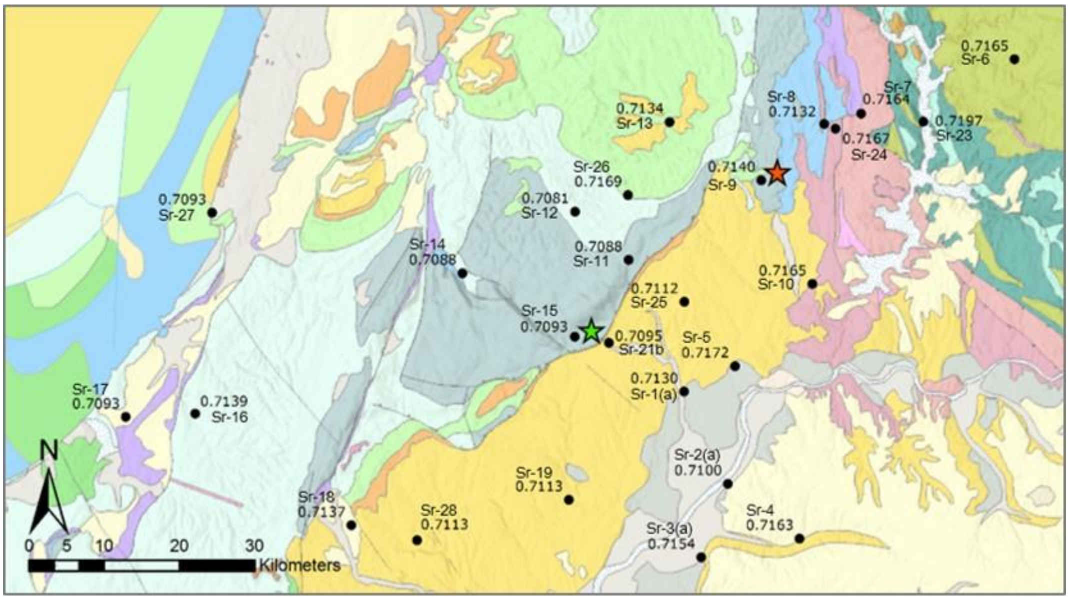


Figure 53. Strontium isotope map for the study area. Strontium isotope data were produced by Pike et al. (unpublished). The green star denotes the location of the Almonda karstic system, and the orange star represents Gruta do Caldeirão.

The strontium isotope baseline map used in this study for the interpretation of the strontium isotope profiles of the humans and fauna was produced by Pike et al. (unpublished). Sediment samples were collected at locations representative of the different geological and sedimentary units within a c. 50km radius of the sites. Subsoil samples were taken away from locations of obvious agriculture. Soils and sediments were leached for two weeks in RO 18Ω water at room temperature. The leach solution was divided into aliquots for the measurement of strontium concentration using a ThermoFisher Scientific XSeries2 ICP-MS, and for isotopic analysis using a ThermoFisher Scientific Triton Plus TIMS. Both aliquots were processed on ~50ml Sr-Spec resin (Triskem, France) columns using 2ml of 3M sub boiled nitric acid to elute and the Sr collected in 1.5ml of MQ water. The samples were dried and loaded in 1.5ml of sub boiled 1M hydrochloric acid onto an outgassed Ta filament using a Ta activator solution for subsequent TIMS analysis, using a multi dynamic procedure with a 88Sr beam of 2V. Fractionation was corrected using an exponential correction normalized to $^{86}Sr/^{88}Sr = 0.1194$. NIST 987 was run as a reference standard, and its long-term average on this instrument is 0.710243 ± 0.000021 (2σ) on 472 analyses.

8.2 Oxygen isotope analysis

8.2.1 Sample preparation

Molar dental enamel from fauna were prepared for sequential oxygen isotope analysis in the stable isotope laboratory at the Department of Archaeology, University of Southampton. Enamel slices approximately 1-2mm thick were removed from human and animal tooth specimens parallel to the growth axis of the tooth using a diamond-tipped cutting disc and a Dremel tool, and any dentine was removed using a dental burr. The enamel slices were subsequently ultrasonicated in Milli-Q water and dried in a vacuum oven. The slices were then subdivided into sequential samples, which measured between 2-5mm long depending on the length of the tooth. Each of these sub-samples was ground into a powder using an agate pestle and mortar, and subjected to a 0.1M acetic acid wash for 15 minutes in order to remove exogenous carbonates. Each sample was then washed five times in ultrapure Milli-Q water and freeze-dried to return them to a powdered state.

8.2.2 Analysis

Analysis was carried out at the Stable Isotope Mass Spectrometry Laboratory at the National Oceanography Centre, Southampton. Approximately 500μg of each sample was transferred to a Thermo KEIL IV carbonate device and automatically reacted with 106.7% phosphoric acid at 90°C in order to evolve CO_2. The CO_2 was then dried and cryogenically transferred to a Thermo Finnegan MAT 253 isotope ratio mass spectrometer. The oxygen isotope data are presented as δ-values in permil (‰) relative to the international standard Vienna Standard Mean Ocean Water (VSMOW). The typical measurement uncertainty, based on the repeated analysis of international and in-house standards, is 0.2‰.

9. Results

This chapter presents strontium and oxygen isotope data gathered from each specimen according to taxon and stratigraphic association. All samples were analysed in the manner outlined in Chapter 8. All sequential strontium and oxygen isotope data are plotted left to right, from the top of the crown to the DEJ. Given the number of uncertainties around enamel mineralisation rates and isotopic body pools for each species (and therefore the relationship between a given length of enamel and the amount of time it represents isotopically), the results are presented here against their relative distance along the growth axis of the tooth. As discussed in Chapter 6, these issues may be possible to resolve with future in-depth studies of enamel physiology, in which case the results presented here could be modelled in order to elucidate primary isotopic signals.

9.1 Fauna

9.1.1 *Red deer*

		Ages (in months)					
		Molars			**Premolars**		
Stages		**1st**	**2nd**	**3rd**	**2nd**	**3rd**	**4th**
Crypt formation	First present	*in utero*	1	5	5	6	11
	Consistent	*in utero*	< 4	10	11	11	11
Mineralisation	First present	*in utero*	< 3	9	< 14	< 13	13
	Consistent	*in utero*	4	13	< 14	14	14
Crown complete	First present	< 4	< 9	18	< 18	< 18	< 18
	Consistent	4	9	26	18	18	18

Table 7. Occurrence (in months) of crown formation stages in the molars and premolars of *Cervus elaphus* as determined through radiographs of forming dentition. Adapted from (Brown and Chapman, 1991).

Red deer *(Cervus elaphus)* are a ruminant species widespread across Europe and North America, and their mobility is variable depending on the location. In certain modern groups, red deer populations have been observed to travel between discrete summer and winter home ranges (Carranza *et al.*, 1991; Kropil, Smolko and Garaj, 2015), likely as a response to seasonal changes in forage quality. For example, in northwestern Wyoming, red deer populations are observed to migrate up to 100km between high elevation summer ranges and lower elevation winter ranges (Boyce, 1991). In a study of a modern Mediterranean red deer population, individuals were seen to occupy areas of high vegetation cover during the summer and open areas during winter/spring, in effect following high quality forage (Carranza *et al.* 1991). The following section presents the results for red deer samples taken from Mousterian, Gravettian, Solutrean and Magdalenian deposits at the study sites. Brief overviews of the results for each individual are provided.

9.1.1.1 Mousterian

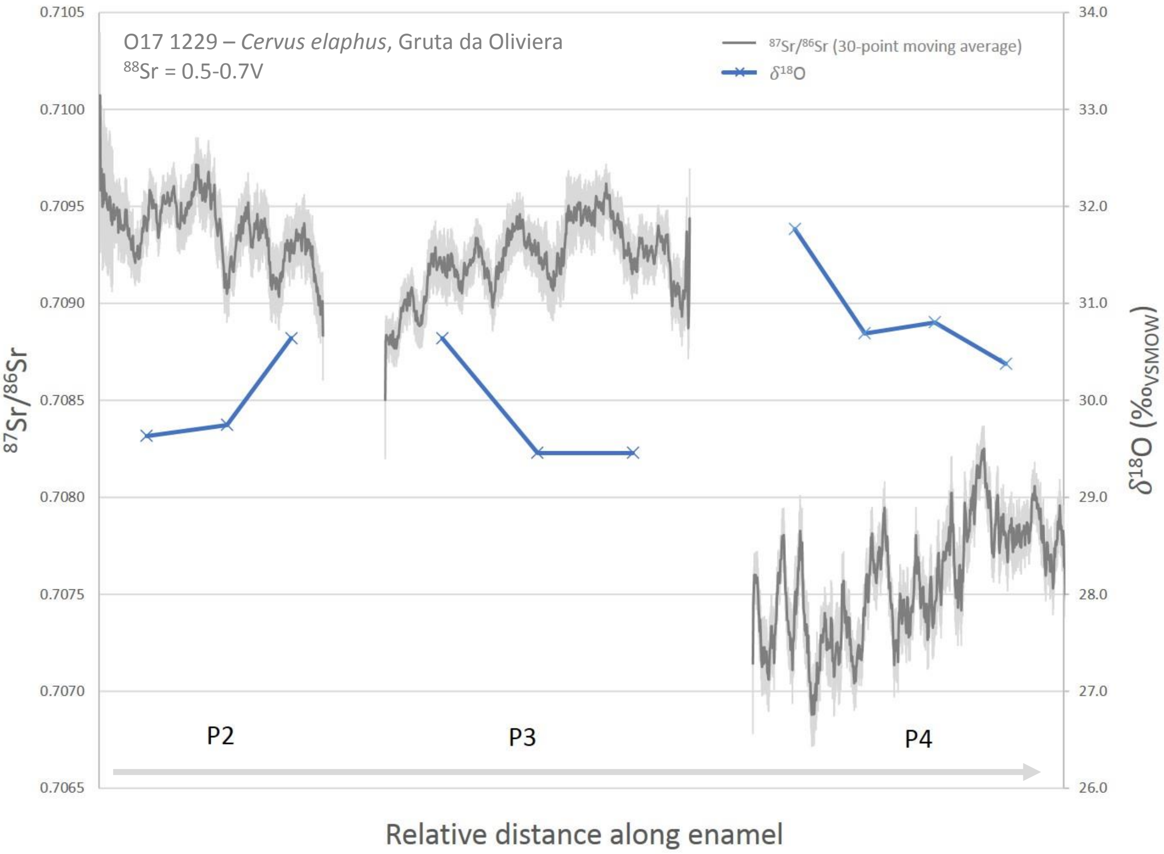


Figure 54. Top: Sequential strontium isotope profile (30-point moving average) and oxygen isotope data for O17 1229 (P2, P3 and P4; red deer). The arrow denotes the direction of analysis from the apex to the cervix of the crown.

To date, only one Mousterian red deer specimen from Gruta da Oliviera has successfully yielded isotope data. The sample, O17 1229, consists of second, third and fourth premolars. The strontium isotope profiles of the P2 and P3 appear to reflect a shift in $^{87}Sr/^{86}Sr$ between 0.7088 and 0.7095. The 0.7088 value is consistent with geological samples taken along the Middle Jurassic limestone massif, Serra d'Aire (Sr-11, Sr-14). The latter value, 0.7095, is consistent with geological samples taken in the direct vicinity of the Almonda karstic system (Sr-21b). Because the summer $\delta^{18}O$ peaks in both tooth crowns coincide with the point at which the strontium isotope profiles reach approximately 0.7088, it could be argued that this individual consumed vegetation on the higher altitude slopes of the Central Limestone Massif during the summer months, and conversely spent the winter months consuming vegetation growing at lower altitudes in the vicinity of the Almonda karstic system. This behaviour seems feasible considering that modern *Cervus elaphus* groups have been observed to migrate small distances altitudinally as a response to the availability of certain seasonal resources (Boyce, 1991). The strontium isotope values exhibited by the fourth premolar crown, however, deviate from the values observed in the P2 and P3 crowns, ranging instead between approximately 0.7073 and 0.7078. The current bioavailable strontium map of the study region does not as of yet exhibit any values consistent with this strontium isotope profile, but the oxygen isotope profile appears to show a trend from high, summer $\delta^{18}O$ values towards the apex of the crown to lower, winter $\delta^{18}O$ values at the apex of the crown. If these low $^{87}Sr/^{86}Sr$ values are the result of the individual travelling farther afield than the study area, we can at least argue that this distance cannot have been far enough geographically as to prevent seasonal $\delta^{18}O$ extremes from being recorded. Further expansion of the bioavailable strontium map may enable more detailed interpretation of the P4 strontium isotope profile.

9.1.1.2 Solutrean

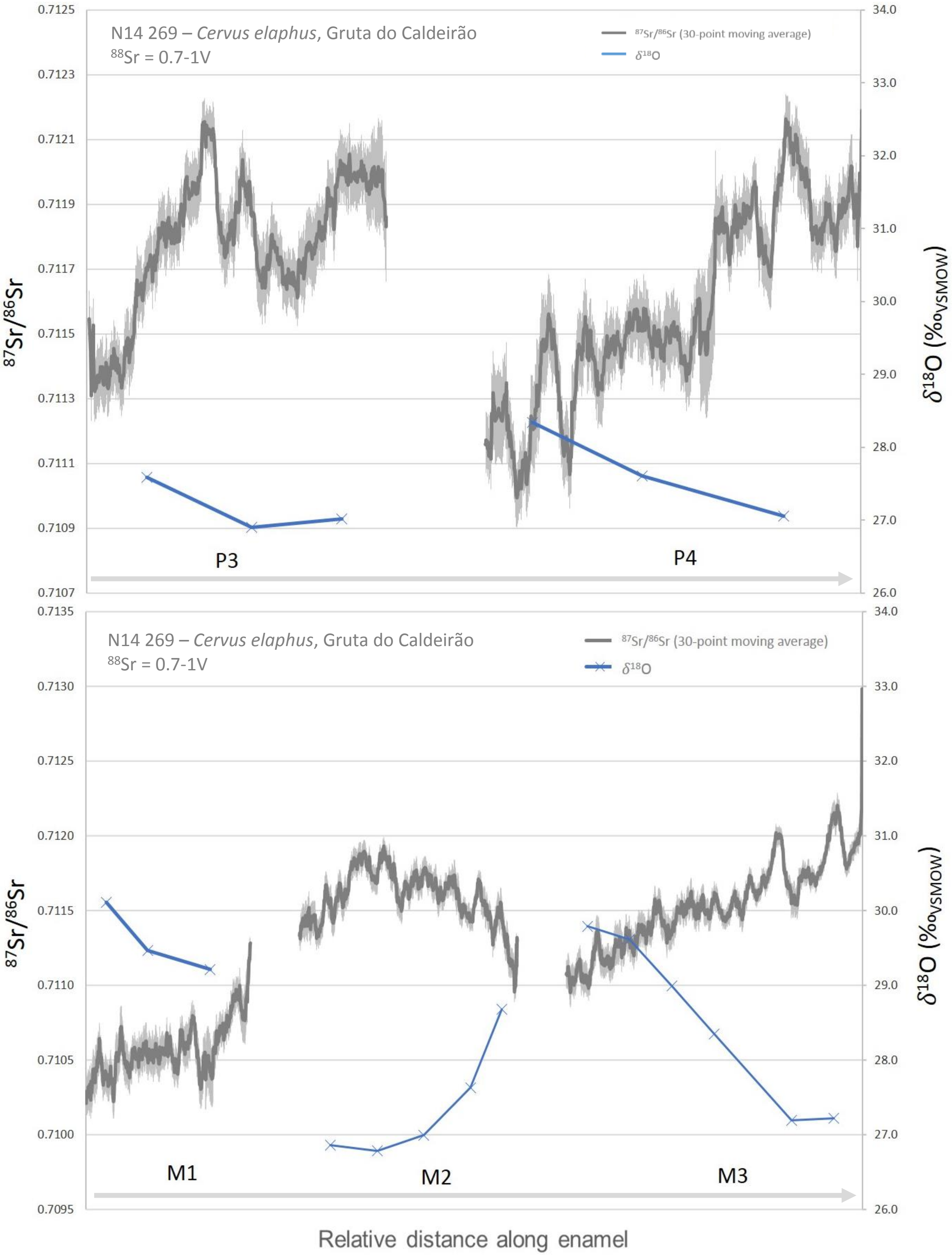


Figure 55. Top: Sequential strontium (30-point moving average) and oxygen isotope data for N14 269 (P3, P4; red deer). Bottom: Sequential strontium isotope data (30-point moving average) and oxygen isotope data for the M1, M2 and M3 of the same individual.

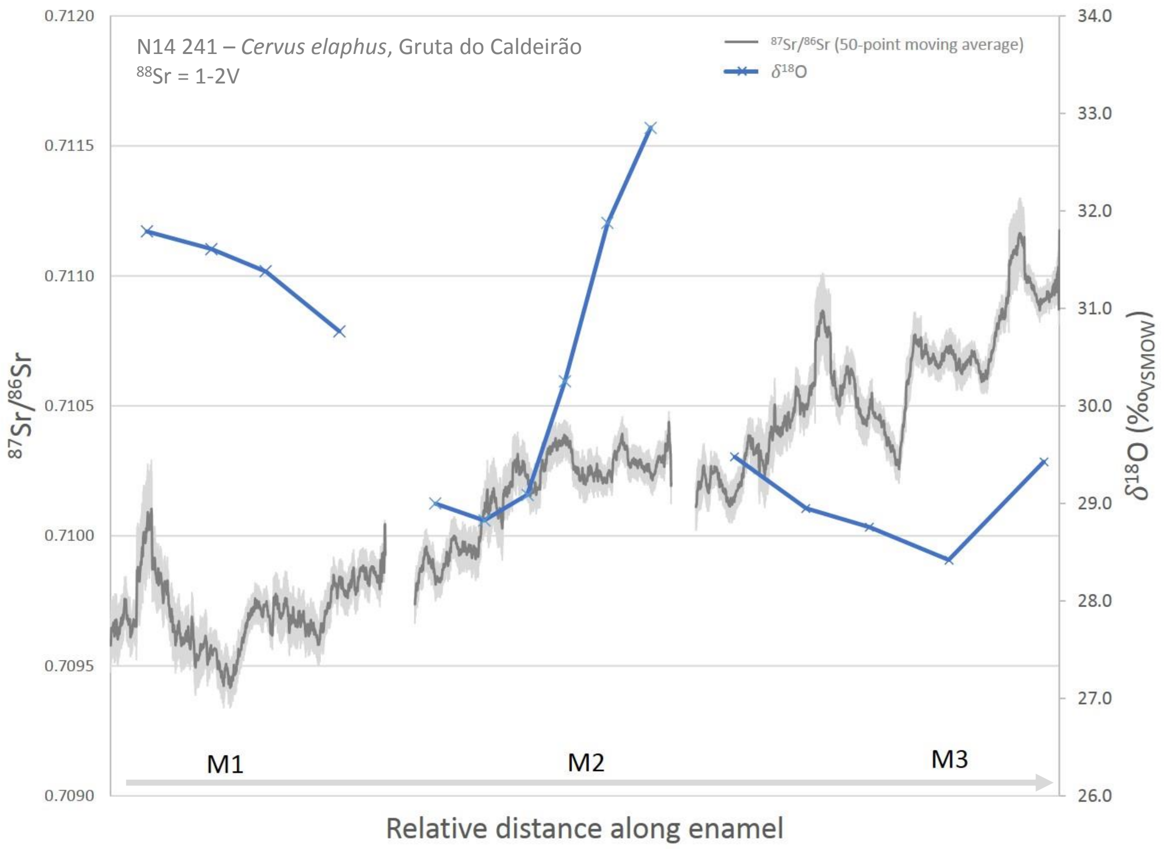


Figure 56. Sequential strontium (50-point moving average) isotope profile for N14 241 (M1, M2, M3 red deer).

Two Solutrean red deer individuals from Gruta do Caldeirão were successfully analysed. Both exhibit strontium isotope profiles indicative of mobility, though both vary in the pattern of mobility. The strontium isotope data for the third and fourth premolars of N14 269 illustrate a shift from an $^{87}Sr/^{86}Sr$ value of approximately 0.7120 to 0.7112, and returning to 0.7120. The fact that both teeth exhibit similar strontium isotope profiles is consistent with what might be expected based on crown formation times (Table 6), as both the P3 and P4 are reported to mineralise between 11 and 18 months (Brown and Chapman, 1991). Combined oxygen isotope data from both premolars appear to show half an annual cycle of temperature extremes, summer to winter, with a range of approximately 2‰. The summer $\delta^{18}O$ peak occurs in the same position in the P4 crown as the $^{87}Sr/^{86}Sr$ value of 0.7112, implying that the N14 269 individual may have consumed vegetation growing in the Neogene sedimentary basin between the Central Limestone Massif and the Tagus River (Sr-19, Sr-25 and Sr-28) during the summer months. As of yet, no sediment or plant samples in the study area match the $^{87}Sr/^{86}Sr$ value of 0.7120, but the fact that the winter $\delta^{18}O$ values in the P4 molar coincide with this strontium isotope ratio suggests that the individual may have occupied geologically distinct winter feeding grounds.

A similar pattern of mobility can be observed in the M2 and M3 of the same individual, N14 269 – again, the strontium isotope profiles exhibit a shift from 0.7120, to 0.7112, to 0.7120. As with the P3 and P4, the summer $\delta^{18}O$ peaks in the M2 and M3 occur at the same positions in the crowns as the $^{87}Sr/^{86}Sr$ value of 0.7112. According to the crown mineralisation times reported by Brown and Chapman (1991), P4 and M3 mineralisation times overlap considerably, and as such the strontium and oxygen isotope profiles of these teeth likely represent the same period of mobility in N14 269. The overlap between the mineralisation periods of the M2 (3 to 9 months) and the M3 (9 to 26 months), however, is much shorter, and as such the isotope profiles likely represent consecutive periods of mobility. Since the M2 and M3 exhibit two instances of coinciding winter $\delta^{18}O$ values and $^{87}Sr/^{86}Sr$ values of 0.7120, it could be argued that the individual engaged in a degree of seasonal mobility, whereby geologically distinct summer and winter feeding grounds were visited repeatedly. The M1 deviates slightly from this pattern, with a relatively consistent $^{87/86}Sr$ value of around 0.7105. The closest geographical match for this is sample Sr-2a, taken along the bank of the River Tagus (0.7100). Since the M1 begins mineralising *in utero* and is completed at 4 months of age, this may represent the calving grounds. In modern red deer populations, calving usually occurs in May and June, during which time the hinds separate from their social groups and locate areas with reduced population density (Clutton-Brock and Guinness, 1975). Both the strontium and oxygen isotope profiles of the N14 269 M1 are consistent with this behaviour, though the latter may be elevated to an extent due to the consumption of the mother's $\delta^{18}O$-enriched milk prior to weaning (Jay, 2009).

The other Solutrean red deer specimen from Gruta do Caldeirão, N14 241, exhibits a slightly different set of strontium isotope profiles across the M1, M2 and M3. In the M1, an increase in $^{87}Sr/^{86}Sr$ from 0.7095 to approximately 0.7099 can be observed, followed in the M2 by an increase from 0.7099 to approximately 0.7103. The M3 is further characterised by a gradual shift to more radiogenic strontium isotope values, with $^{87}Sr/^{86}Sr$ increasing from 0.7103 to 0.7110. The oxygen isotope data exhibit a range of approximately 4.5‰, showing what appears to be clear winter $\delta^{18}O$ troughs in both the M2 and the M3, and a summer $\delta^{18}O$ peak in the M2. This implies that the individual experienced seasonal temperature extremes similar to those of the N14 269 individual, but it could be argued that the N14 241 consumed vegetation from a less radiogenic area during the winter months. Whilst the $^{87}Sr/^{86}Sr$ values are within the range of geological values observed within the study area (in particular, the Neogene sedimentary basin and the banks of the Tagus river), the overall gradual shift to more radiogenic strontium isotope values across all three molars is difficult to interpret behaviourally. It is possible that age-related wear on the tooth crowns has shortened the strontium and oxygen isotope sequences, but at this point in time perhaps the most that can be inferred is that the individual was mobile across the study area - whether or not this mobility was seasonal is not clear.

9.1.1.3 Magdalenian

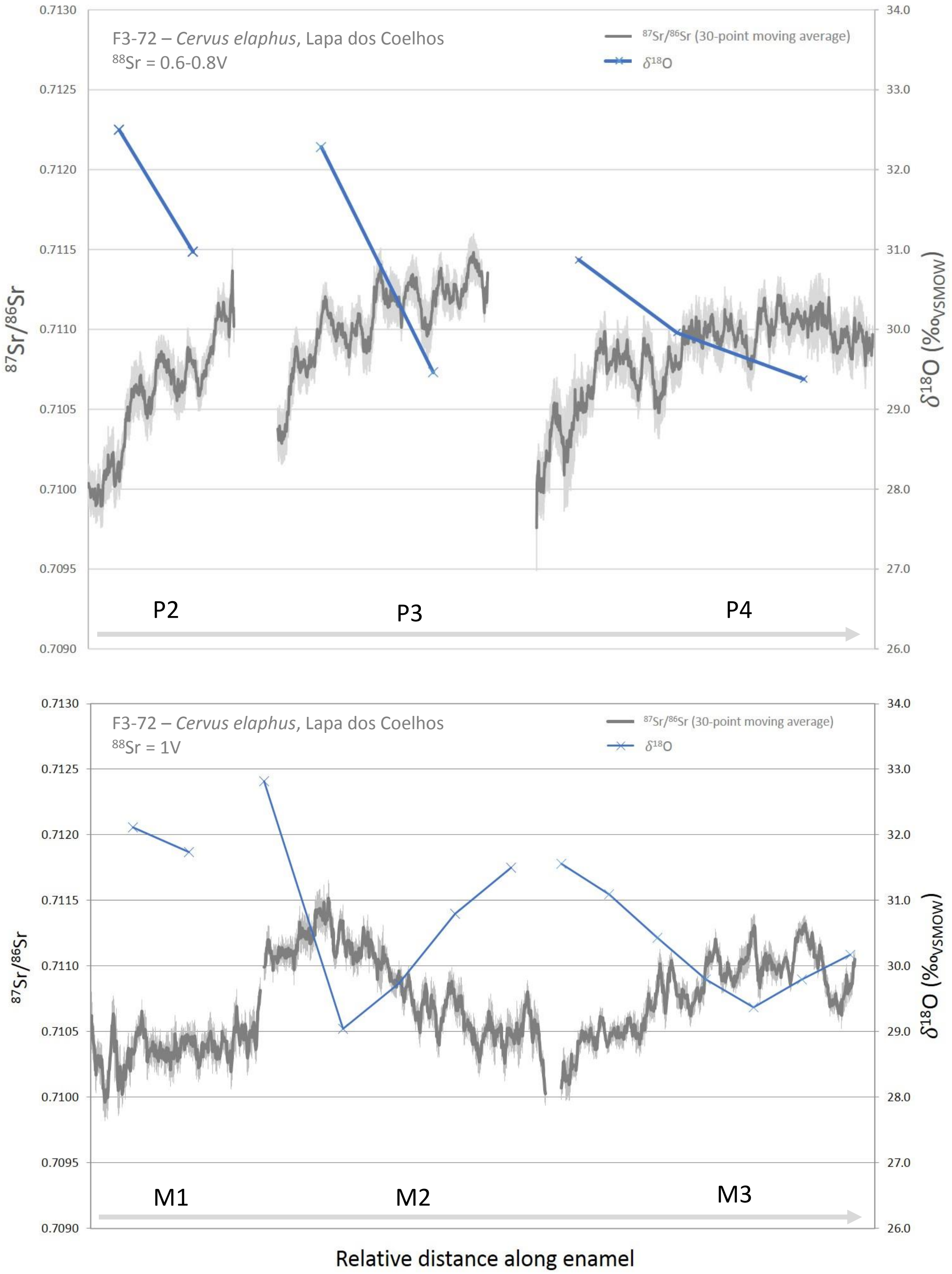


Figure 57. Top: Sequential strontium (30-point moving average) isotope profile for F3-72 (P2, P3, P4; red deer). Bottom: Sequential strontium (30-point moving average) and oxygen isotope profiles for the same individual (M1, M2, M3).

One Magdalenian *Cervus elaphus* specimen from Lapa dos Coelhos, F3-72, was analysed. The strontium isotope profiles across all teeth (P2, P3, P4, M1, M2, M3) exhibit variation, suggesting that the individual likely engaged in a degree of mobility across the local landscape during the time of dental development. In the premolars, shifts from $^{87}Sr/^{86}Sr$ values of approximately 0.7100 to 0.7111/0.7112 are evident; consistent with bioavailable Sr values from samples Sr-2a (on the banks of the Tagus River) and Sr-25 (a few kilometres northwest of the Almonda karstic system, within the Neogene sedimentary basin) respectively. Given that the strontium isotope profiles and accompanying oxygen isotope data in each tooth crown are very similar, it seems likely that the P2 P3, and P4 crowns have recorded the same movement event, due to the overlapping formation times of the teeth (see Table 7). The 1st, 2nd and 3rd molars also exhibit variation in $^{87}Sr/^{86}Sr$ values between 0.7100 and approximately 0.7112. Sequential oxygen isotope values obtained for the M2 and M3 compliment the strontium isotope profiles, with the lowest $\delta^{18}O$ values occurring at the same position in the crown as the most radiogenic $^{87}Sr/^{86}Sr$ values, and the highest $\delta^{18}O$ coinciding with the least radiogenic $^{87}Sr/^{86}Sr$. Assuming that the strontium and oxygen isotope values were incorporated into the enamel at the same time, we can infer that the individual may subsisted on resources located near to the banks of the Tagus River (Sr-2a) during the summer months, and occupied the Neogene sedimentary basin to the southeast of the Central Limestone Massif during the winter. Because the M1 begins to mineralise *in utero* and is only complete at the age of four months, the strontium isotope profile exhibited by the crown may reflect the calving ground selected by the hind.

9.1.2 Ibex

		Ages (in months)		
		Molars		
Stages		**1st**	**2nd**	**3rd**
Mineralisation	Start	*In utero*	2	9
	Completion	6	12	22

Table 8. Occurrence (in months) of crown formation stages in the molars and premolars of modern sheep. Data from Zazzo *et al.* (2010).

Capra pyrenica is a mountainous species endemic to the Iberian Peninsula, highly adapted to life amongst steep, rocky slopes. The species exhibits a high degree of sexual dimorphism; males are larger in weight and size than females, and have considerably larger horns. A faster overall growth rate is observed in females, indeed, female skeletal systems reach full ossification two years faster than males (Acevedo and Cassinello, 2009). Male and female groups are separated for most of the year, amalgamating into herds of around 100 individuals only during the rut (Straus, 1987). Iberian ibex generally engage in seasonal altitudinal mobility, occupying higher elevations during summer and moving down to lower, more amenable conditions at lower altitudes during the winter (Straus, 1987). According to Straus (1987), they usually occupy the lowest altitudes during spring, at which point they are at their physical weakest. Birth usually occurs at this time.

9.1.2.1 Mousterian

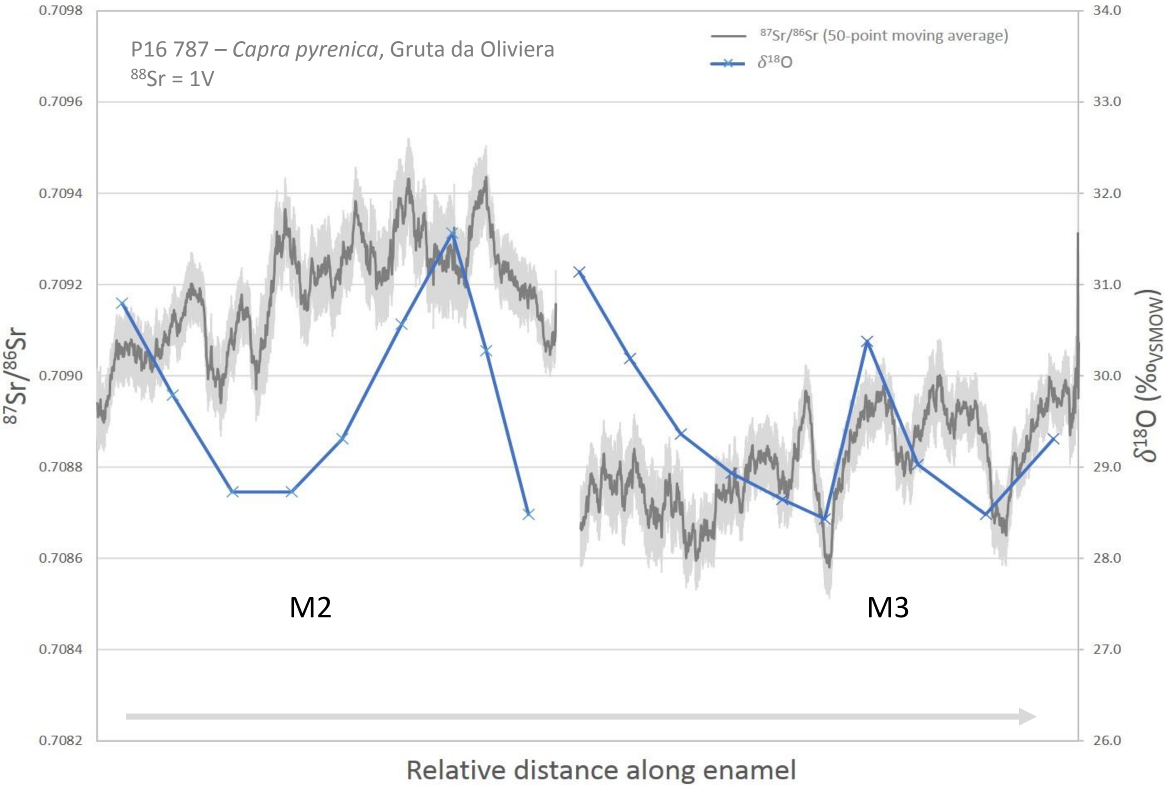


Figure 58. Sequential strontium isotope data (50-point moving average) for P16 787.

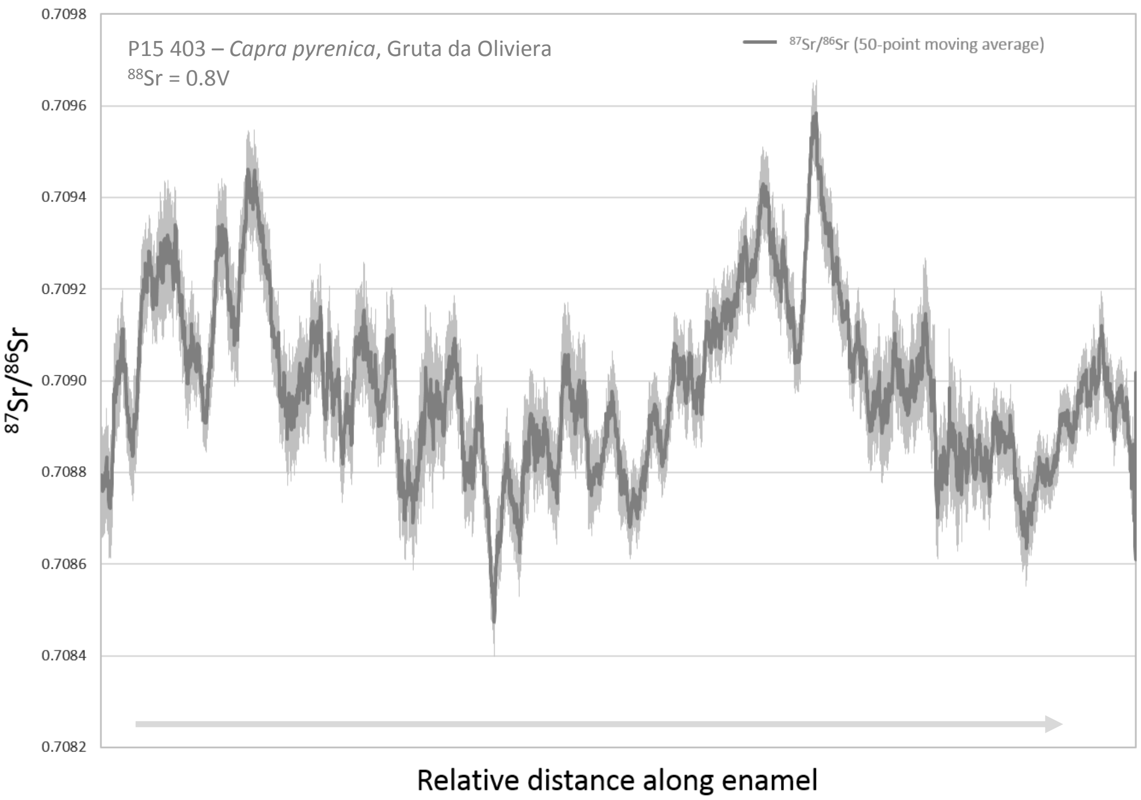
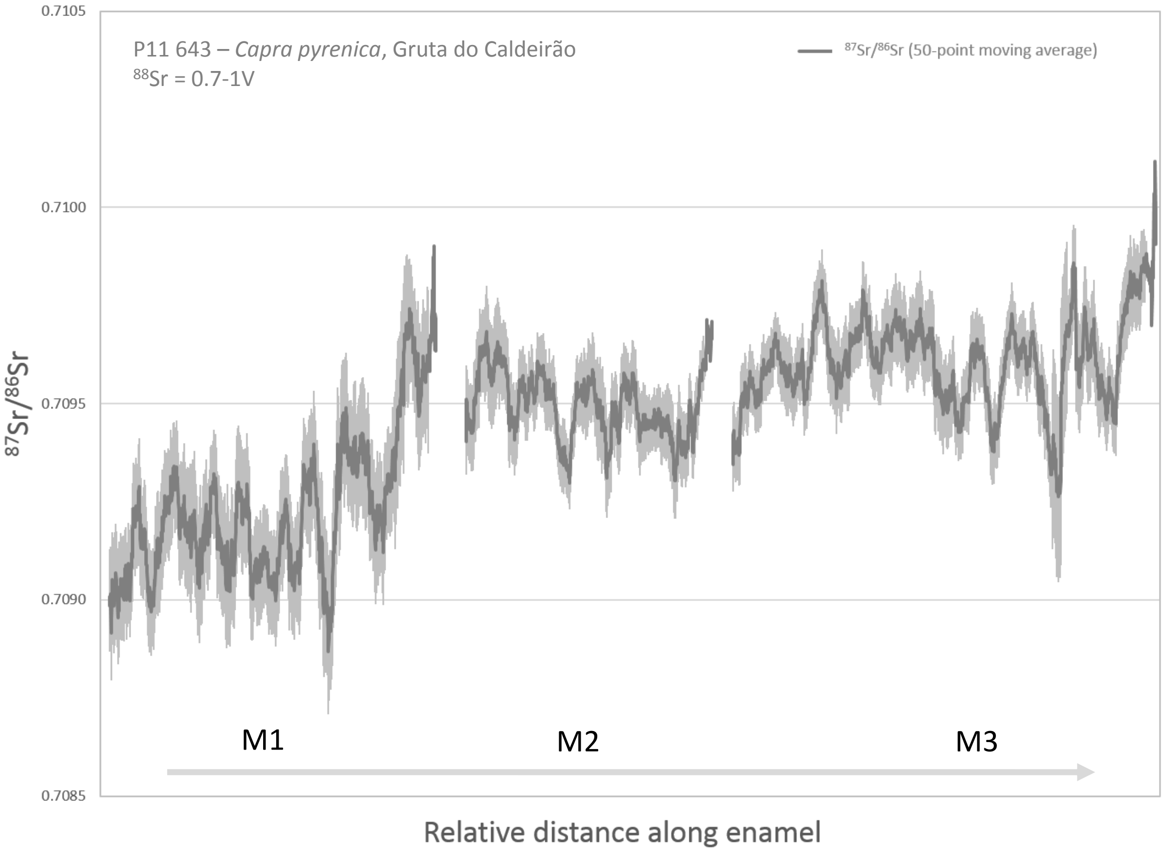


Figure 59. Sequential strontium isotope data (50-point moving average) for P15 403 (indet. molar).

According to Buchan *et al.* (2016), the M2 begins forming at the age of approximately two months and is completed at approximately 12 months, whilst the M3 begins to form at approximately 9 months and continues until the age of approximately 22 months at completion (Zazzo *et al.*, 2010). Based on the oxygen isotope profile exhibited by the M2 of the P16 787 individual, two annual temperature cycles appear to be recorded. The same is true of the M3 oxygen isotope profile, which also exhibits two summer-winter temperature cycles. Similar oxygen isotope profiles have been observed (Makarewicz and Pederzani, 2017) in the molars of modern, wild Mongolian sheep and ibex, suggesting that in certain caprine and ovine species, enamel maturation takes longer. Assuming that the oxygen isotope data provide an approximate timeline for the mineralisation time of the M2 and M3, it appears that the P16 787 individual subsisted on vegetation exhibiting ^{87}Sr/^{86}Sr values of between 0.7090 and 0.7094; consistent with the Middle Jurassic limestone in the vicinity of the Almonda karstic system (Sr-15, Sr-21b) as well as the modern-day coastline (Sr-17, Sr-27), during the first winter-summer cycle of its life. The strontium isotope profile of the M3, on the other hand, exhibits values between approximately 0.7086 and 0.7088, consistent with geological samples taken at higher high altitudes along the Serra d’Aire (Sr-14, Sr-11). Taken alone, the strontium isotope profiles of the M2 and M3 might be argued to reflect altitudinal mobility in response to the seasonal availability of nutritional resources, as observed in modern ibex populations, who are often observed to occupy higher altitude environments during summer, and lower altitudes during winter when conditions at higher altitudes become too hostile. However, when the P16 787 strontium isotope data are considered alongside the oxygen isotope data, it seems that occupation of high altitudes and lower altitudes are not concurrent with seasonal temperature changes. It may be the case that this individual did not engage in any seasonal mobility, but it should perhaps be borne in mind that if mineralisation geometry is complex, differences in sampling strategies (i.e, laser ablation vs. manual drilling of larger enamel chunks for strontium and oxygen isotope analyses respectively) has the potential to disguise isotopic variation.

The second Mousterian ibex specimen, P15 403, was also recovered at Gruta da Oliviera. Due to time restraints, it was not possible to obtain oxygen isotope data for this individual, but Figure 59 provides the strontium isotope data for the solitary P15 403 molar. The $^{87}Sr/^{86}Sr$ values exhibit by the molar range from approximately 0.7088 to 0.7093 at both extremes, and are therefore consistent with the $^{87}Sr/^{86}Sr$ values of the study area as well as those observed in the P16 787 molar crowns. In particular, these troughs (0.7088) and peaks (0.7093) in $^{87}Sr/^{86}Sr$ values are consistent with geological samples taken at higher altitudes along the Central Limestone Massif (Sr-14, Sr-11) and in close proximity to the Almonda karstic system (Sr-15, Sr-21b) and the modern day coastline (Sr-17, Sr-27) respectively. The profile exhibits a clear, repeating sinusoidal pattern, implying that the individual may have subsisted on vegetation growing within a geologically distinct area within the landscape, moved to another, and subsequently returned to the original region after a period of time. If the oxygen isotope profile of the P16 787 individual accurately reflects the amount of time taken for the M2 and M3 crowns to mineralise (between 18-24 months each), then the P15 403 $^{87}Sr/^{86}Sr$ profile may in fact reflect two annual cycles of mobility between high altitude and lower altitude feeding grounds during summer and winter respectively; consistent with observations of modern ibex populations. However, without oxygen isotope data, it is not possible to determine whether this truly is the case. Future $\delta^{18}O$ analysis of the P15 403 tooth crown will enable this hypothesis to be tested.

9.1.2.2 Gravettian



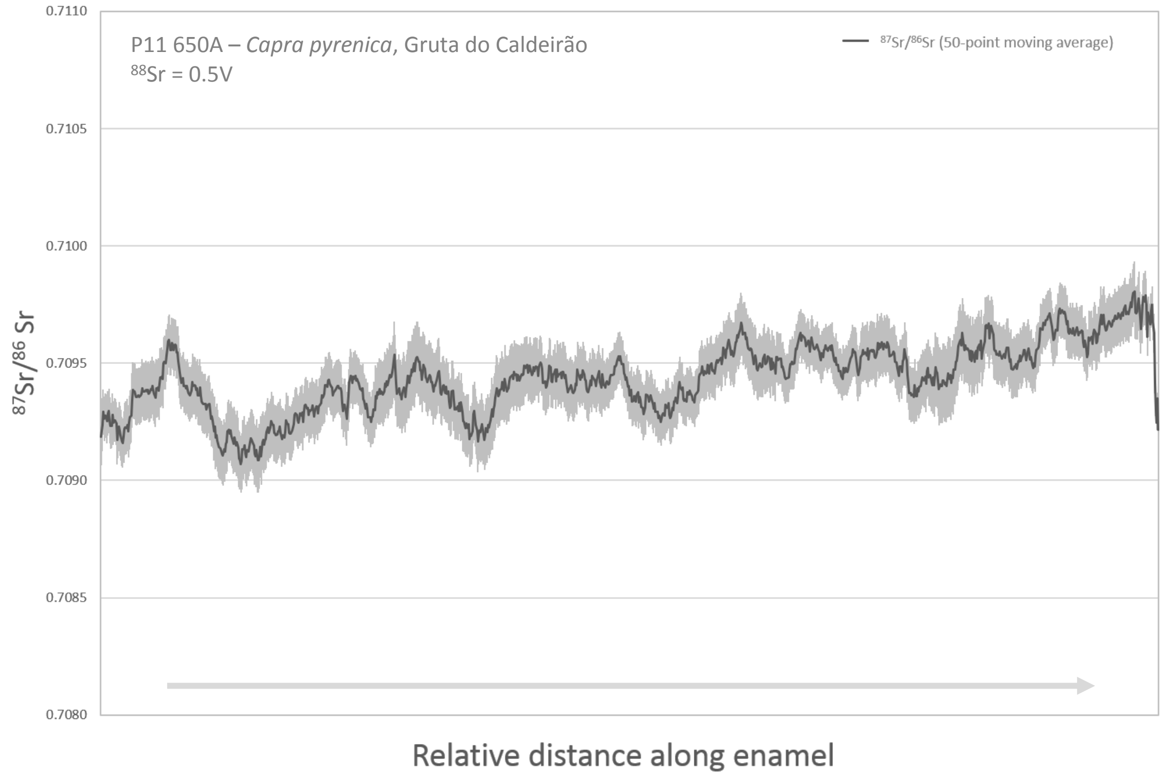


Figure 60. Top: Sequential strontium isotope data (50-point moving average) for P11 643 (M1, M2 and M3). Bottom: Sequential strontium isotope data (50-point moving average) for P11 650 A (indet. molar).

Two Gravettian *Capra sp.* samples from the Gruta do Caldeirão were analysed. There is a possibility that the molar fragment, P11 650A, belongs to the same individual as P11 643, but this is difficult to ascertain with certainty. Little variation can be seen in the strontium isotope data gathered from the M1, M2 and M3 of P11 643. The M1 data is somewhat noisy, but the average $^{87}Sr/^{86}Sr$ value is approximately 0.7093. Since the M1 began mineralising *in utero*, the likelihood is that the strontium isotope profile of this particular molar is reflective of the birthing grounds. The M2 and M3 strontium isotope profiles exhibit relatively consistent values of approximately 0.7095, and the single loose molar, P11 650A, also exhibits a consistent strontium isotope profile with an average value of approximately 0.7095. Cautiously assuming that all four molars belong to the same individual, the strontium isotope data here are rather unlike the profiles observed in the Mousterian caprids from Gruta da Oliviera, which exhibit sinusoidal variation in $^{87}Sr/^{86}Sr$ between approximately 0.7088 and 0.7093. The strontium isotope values observed in P11 650A and all three of the P11 643 molars exhibit considerably less variation, but are consistent with sediment samples from the vicinity of the Almonda karstic system, overlying the Middle Jurassic limestone (Sr-15; Sr-21b). Multiple behavioural and biological explanations could be put forward for this. It may simply be that the cusps of these molars are considerably worn, and as such do not reflect full seasonal cycles of mobility unlike the Mousterian caprids – though this is difficult to ascertain without oxygen isotope data, which due to time restraints could not be gathered for these specimens. Alternatively, if this individual occupied territory closer to the vicinity of the Gruta do Caldeirão (for example, around the Middle Jurassic limestone to the north of the cave), it may be that local bioavailable strontium isotope values do not vary altitudinally to the same extent as they do in the direct vicinity of the Almonda karstic system; hence such altitudinal movement is not detectable in the strontium isotope profiles of the molars. Equally, it may be that ibex mobility patterns changed at some time between the Mousterian and Gravettian, perhaps as a response to a change in the availability of certain resources.

9.1.2.3 Solutrean

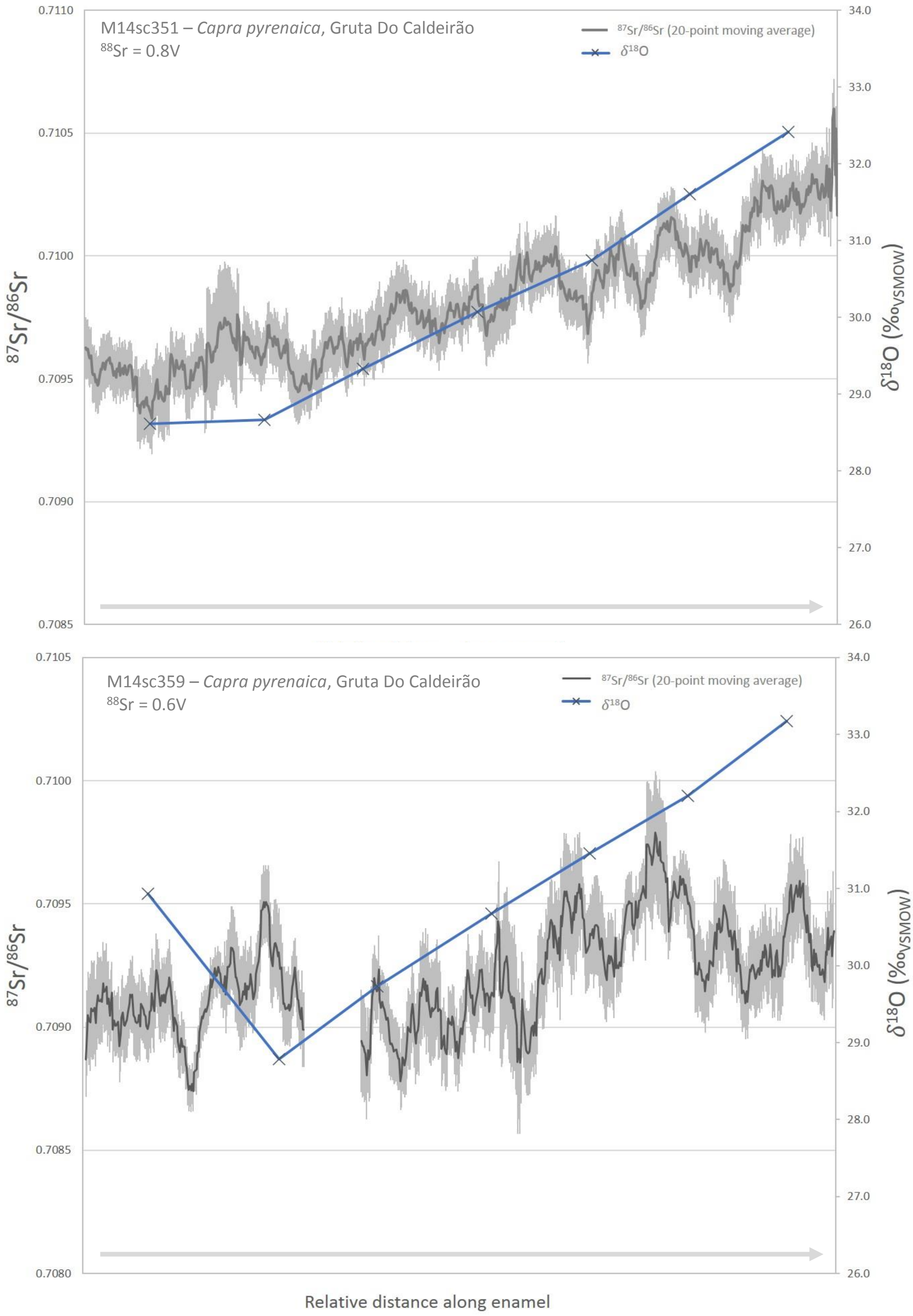


Figure 61. Top: Sequential strontium isotope data (30-point moving average) for M14sc351 (indet. molar). Bottom: Sequential strontium isotope data (30-point moving average) for M14sc359 (indet. molar). A large yttrium spike associated with a crack in the enamel of M14sc359 and anomalously high voltages on mass 88 prompted the removal of the ^{87}Sr/^{86}Sr at that point in the profile.

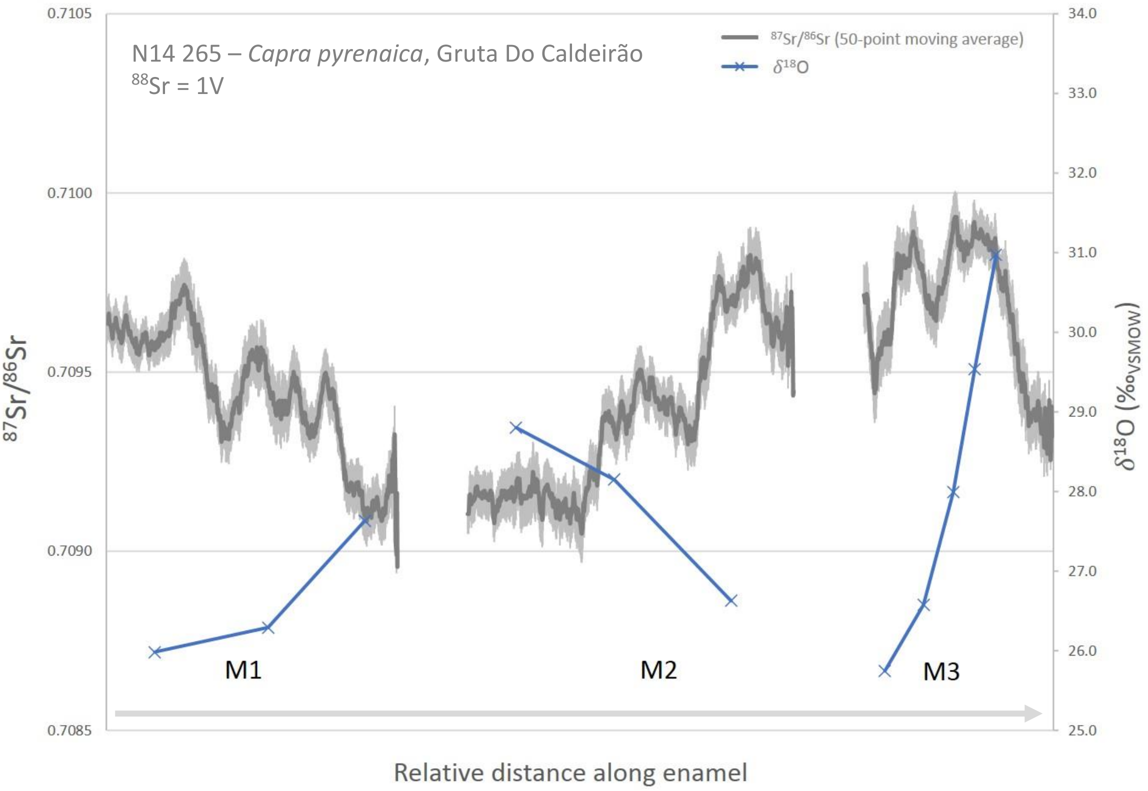


Figure 62. Top: Sequential strontium isotope data (30-point moving average) for N14 265 (M1, M2 M3).

Three Solutrean caprid specimens from Gruta do Caldeirão were analysed. Two of the individuals, M14sc351 and M14sc359, exhibit sequential oxygen isotope profiles with δ^{18}O ranges between the seasonal extremes observed in the Mousterian ibex individual, P16 787. Both enamel crowns therefore appear to be reflective of approximately six months of mineralisation, with winter δ^{18}O troughs towards the apex of the crown and summer δ^{18}O peaks towards the cervix. The shortness of both of these profiles (based on the oxygen isotope profiles) is likely the result of truncation due to enamel wear. In the M14sc351 crown, there appears to be a shift from less radiogenic ^{87}Sr/^{86}Sr values of approximately 0.7095 at the apex of the crown, to more radiogenic values of approximately 0.7105 at the cervix. The gradual shift in ^{87}Sr/^{86}Sr values along the growth axis of the tooth appears to be concurrent with the gradual increase in δ^{18}O values, and it could perhaps be argued that this offers tentative evidence for a seasonal change in feeding grounds exploited by the individual. However, such short ^{87}Sr/^{86}Sr and δ^{18}O profiles make it difficult to ascertain this.

The N14 265 individual provides a more complete isotopic record across three molars. The tooth crowns of the M1, M2 and M3 of N14 265 exhibit variation in ^{87}Sr/^{86}Sr values between approximately 0.7091 and 0.7098. In the M1, a shift in ^{87}Sr/^{86}Sr values from more radiogenic to les radiogenic values (approximately 0.7096 to 0.7091) can be observed, coinciding with an increase in δ^{18}O values of approximately 1.5‰ down the growth axis of the tooth enamel. The M1 begins to mineralise in utero and, assuming that Pleistocene Iberian ibex crown formation times are comparable to modern goat tooth mineralisation times, continues to mineralise during the first few months after birth (Zazzo *et al.*, 2010). Based on observations that the consumption of milk from the mother tends to produce enriched $\delta^{18}O$ in the tissues of suckling individuals (Tsutaya and Yoneda, 2015), it should be borne in mind that the $\delta^{18}O$ profile of this M1 may not reflect temperature changes in the local environment. The M2 strontium isotope profile exhibits a shift from values of approximately 0.7091 to more radiogenic values of around 0.7097, falling within the observed range of bioavailable $^{87}Sr/^{86}Sr$ values of the study area – in particular, between the values exhibited by the Serra d'Aire (Sr-11, Sr-14, Sr-15) and the Neogene Tagus Basin (Sr-19, Sr-21b, Sr-25, Sr-28). The oxygen isotope profile is characterised by a small 2‰ shift from more ^{18}O-enriched to less enriched values, suggesting that the shift in $^{87}Sr/^{86}Sr$ to more radiogenic values occurred during a transition from warmer to cooler conditions. The M3 crown appears to follow the opposite trends in both the $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ profiles, with the former shifting from values of around 0.7097 at the apex to less radiogenic values of approximately 0.7094 at the cervix, and the latter increasing by approximately 4.5‰ along the growth axis. This suggests that during the formation of the M3, the individual moved from one geological zone to another and consumed vegetation reflective of those $^{87}Sr/^{86}Sr$ values, and that this movement coincided with a shift from colder to warmer conditions. The extremes in $^{87}Sr/^{86}Sr$ values observed in the M3 crown are consistent with those observed in the M2 crown, and although they cannot be attributed directly to specifically to locations within the study area, they are certainly within the range of the Serra d'Aire and the Tagus Basin respectively. Because the former exhibits less radiogenic values and the latter produces slightly more radiogenic values, it could tentatively be argued that the N14 265 individual elected to move between high altitudes and low altitudes during warmer and colder months respectively.

Interestingly, the $\delta^{13}C$ profiles of M14sc351, M14sc359 and the M3 of N14 265 show a trend from more ^{13}C- depleted values to more ^{13}C-enriched values along the growth axis of the enamel, mirroring the enrichment of the $\delta^{18}O$ values along both tooth crowns. This may be a result of a phenomenon known as the 'canopy effect,' which can result in ^{13}C depleted carbon stable isotope values in the tissues of individuals subsisting in forest ecosysems (Bonafini *et al.*, 2013). This is thought to be a result of the decomposition of organic material such as leaves on the forest floor, which promotes the recycling of depleted carbon. It has been observed that modern Iberian ibex populations in southeastern Spain consume the greatest proportion of holm-oak (*Quercus ilex*) in Mediterranean forests during the autumn and winter months, and the least proportion in the summer (Martínez, 2005). If Solutrean ibex populations engaged in similar seasonal subsistence behaviours, then the trend from more ^{13}C- depleted values to more ^{13}C-enriched values along the growth axis of the enamel in the M14sc351 and M14sc359 molars and the N14 265 M3 may be a result of these individuals occupying more open, high altitude landscapes in the summer and more forested, lower altitude environments during the autumn and winter months.

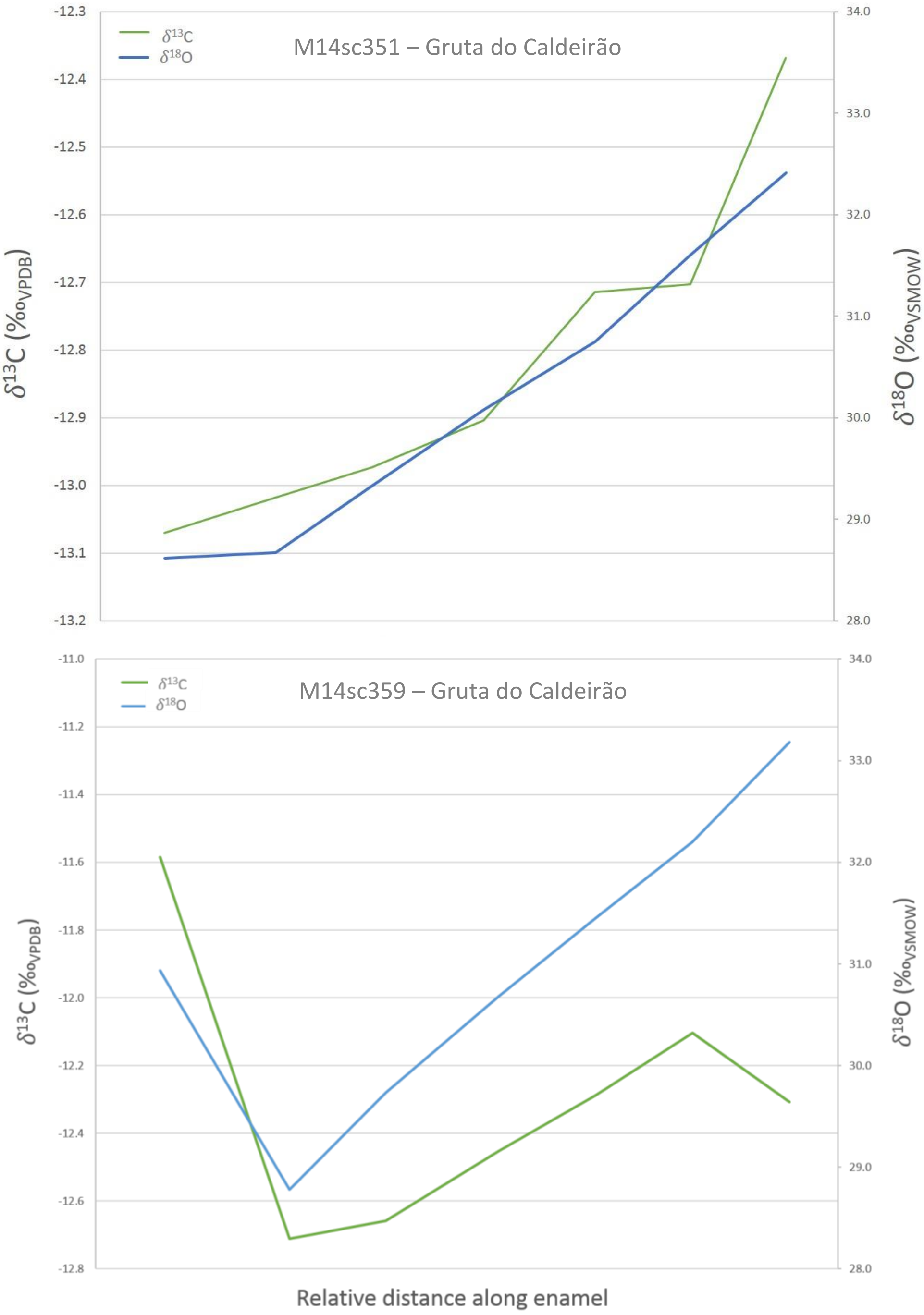


Figure 63. Top: Sequential oxygen and carbon isotope data for M14sc351 (indet. molar). Bottom: Sequential oxygen and carbon isotope data for M14sc359 (indet. molar).

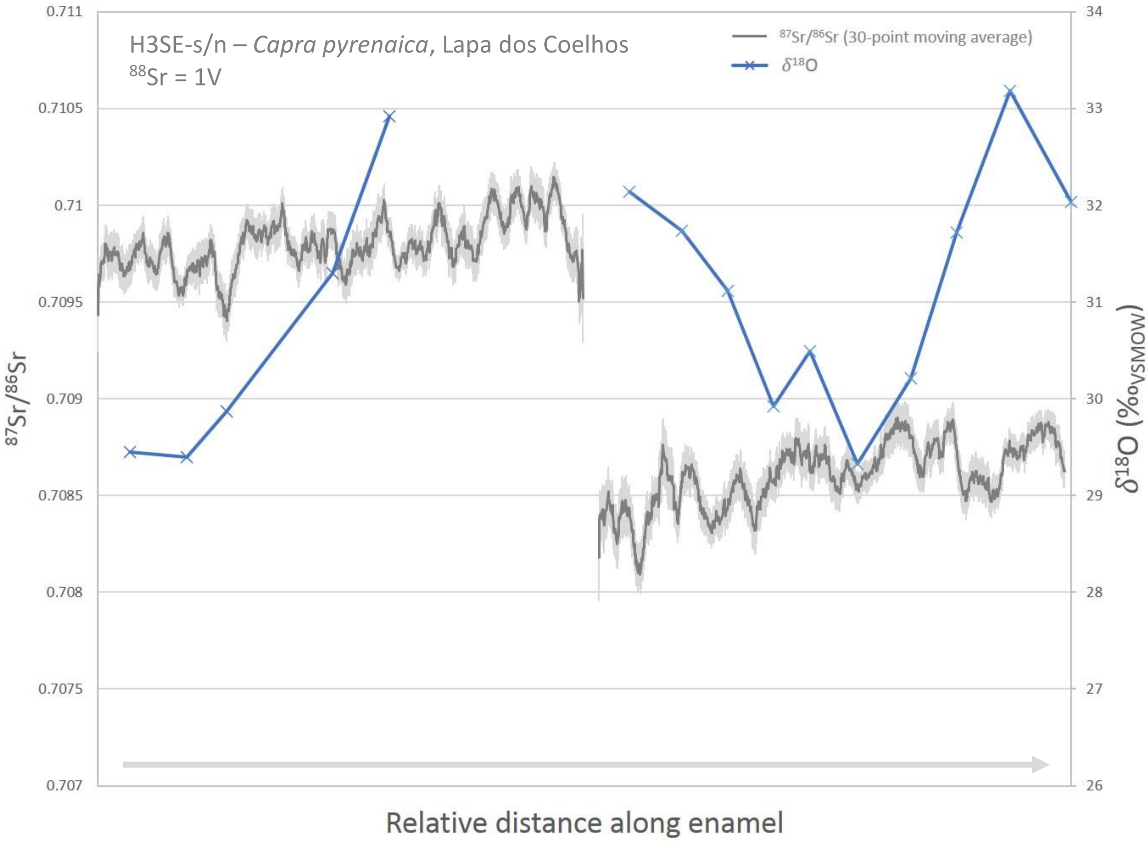


Figure 64. Sequential strontium isotope data (30-point moving average) for H3SE-s/n (M2, M3 - ibex).

One Iberian ibex individual from the Solutrean level at Lapa dos Coelhos was analysed. The molars belonging to this individuals, H3SE-s/n, are not *in situ* within maxillary or mandibular bone, and have not been positively identified as specific molars. They are tentatively assumed to be M2 and M3. Both molars exhibit relatively homogenous strontium isotope profiles, with a range of $^{87}Sr/^{86}Sr$ values between approximately 0.7095 and 0.7099 observed in the M2, and less radiogenic values ranging between approximately 0.7085 and 0.7088 in the M3. The M2 profile is relatively consistent with geological samples in the direct vicinity of the Almonda karstic system (Sr-21b), whilst values observed in the M3 are consistent with strontium isotope values obtained from samples taken along the Central Limestone Massif (Sr-11, Sr-12, Sr-14). Taken together, the strontium isotope profiles of the M2 and M3 are consistent with mobility between higher and lower altitudes across the Serra d'Aire and the Tagus Basin. Based on observations of modern ibex behaviour, one might argue that these data reflect seasonal occupation of higher altitudes during summer, and lower altitudes during winter. The oxygen isotope profiles of both molars, however, do not appear to support such a hypothesis. The sequential oxygen isotope data of M2 seems to reflect a single winter-to-summer cycle in the apical portion of the crown, representing approximately six months of formation, during which the time the individual appears to have consistently subsisted on vegetation with $^{87}Sr/^{86}Sr$ values of approximately 0.7097. In the M3, a full annual cycle appears to be represented by the oxygen isotope data, during which time the individual appears to have subsisted on vegetation within a less radiogenic region.

9.1.2.4 Magdalenian

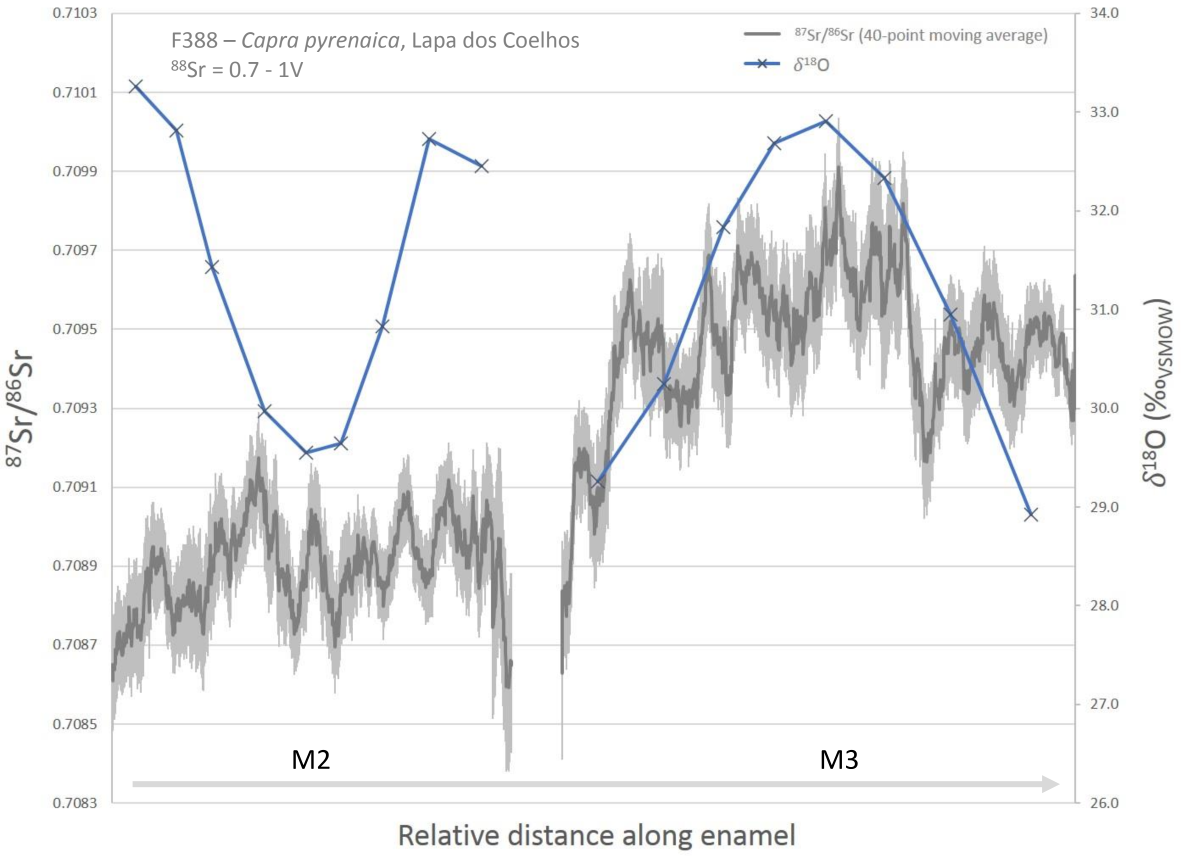


Figure 65. Top: Sequential strontium isotope data (40-point moving average) for F388 (M2, M3 - ibex).

Like the Mousterian ibex specimens from Gruta da Oliviera, the isotope data suggest that the Magdalenian ibex individual from Lapa dos Coelhos (F388) was mobile across two distinct geologies during the period of M2 and M3 formation. The ^{87}Sr/^{86}Sr profile of the M2 exhibits a value of approximately 0.7088 across the growth axis of the tooth, which rises relatively sharply to consistent values of approximately 0.7095-0.7096 in the M3. These values are consistent with the geographical samples taken at high altitudes on the Serra d'Aire (Sr-14, Sr-11) and in the vicinity of the Almonda karstic system itself (Sr-15; Sr-21b) respectively. Much like the Mousterian and Solutrean ibex specimens, the oxygen isotope data seem to contradict the idea that this shift from higher altitude regions to lower altitude regions (and vice versa) coincides with seasonal changes in temperature.

9.1.3 Horse

		Ages (in months)					
		Molars			Premolars		
Stages		**1st**	**2nd**	**3rd**	**2nd**	**3rd**	**4th**
Mineralisation	Start	0.5	7	21	13	14	19
	Completion	23	37	55	31	36	51

Table 9. Occurrence (in months) of crown mineralisation in the molars and premolars of *Equus* as determined through radiographs of forming dentition. Adapted from Hoppe *et al.* (2004).

The Mongolian wild horse (or Przewalski's horses; *Equus ferus przewalskii*) and feral horses (*Equus caballus*) are perhaps the closest extant analogues for wild horse physiology and behaviour in the Palaeolithic. Modern feral horses are preferential grazers and obligate drinkers, and generally occupy a permanent home range which can be occupied for many generations (Goodwin, 2007). Feral mares in the New Forest have been observed, for example, to occupy home ranges of up to 10km^2; each of which contained a source of water, suitable areas for grazing and natural shelter from the elements (Tyler, 1972). Horses form year-round stable social groups, usually consisting a band of mares and one or more stallions (Cameron *et al.*, 2003). The gestation length for Przewalski mares is approximately 11 months, with foals most commonly birthed in late spring or early summer (Boyd, 1991). The foals are generally nursed up until the birth of the next foal, and if the mare fails to conceive in the following breeding season, she may continue to nurse her existing offspring for an extended period of time. Indeed, foals have been observed to continue nursing until the age of 26 months (Boyd, 1991).

9.1.3.1 Mousterian

The three horse teeth selected for analysis from Gruta da Oliviera were recovered from Levels 24 and 22. Only one of those specimens could be identified at the species level.

According to studies of modern horse teeth carried out by Hoppe *et al.* (2004), the P3 and P4 mineralise between 14-36 months and 19-51 months respectively. As such, in unworn teeth, the strontium and oxygen isotope profiles in the cervical portion of the P3 should match those of the apical portion of the P4 due to the overlap in mineralisation times. The homogenous ^{87}Sr/^{86}Sr data (approximately 0.7097) recovered from the P3 and P4 of N15 379 suggests consistent occupation of just one geological region. Although this value cannot yet be matched to a specific geology within the study area, it is within the range of observed values in the vicinity of the site and the Tagus river basin. The intra-tooth variation is only in the region of approximately 1‰; a contrast to the much greater degree of intra-tooth oxygen isotope variation observed in the caprids and cervids from the same region analysed in this study. This may be a result of physiological differences between the species.

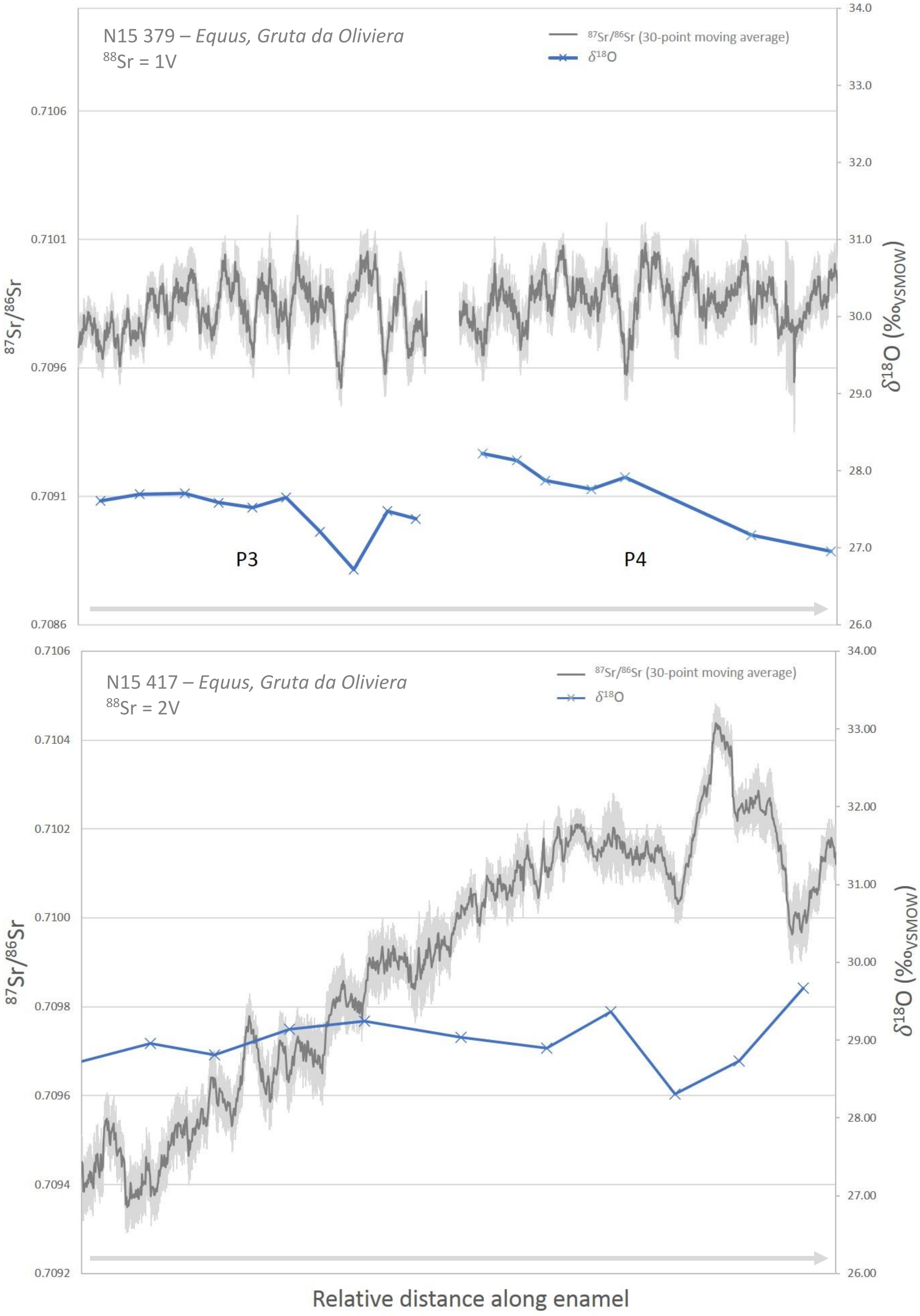


Figure 66. Top: Sequential strontium isotope data for N15 379 (P3 and P4). Bottom: Sequential strontium isotope data for N15 417 (indet. molar). Strontium and oxygen isotope data from the apical 20% of the tooth crown is omitted due to high concentrations of yttrium.

The strontium isotope profile of N15 417 contrasts with those of N15 379 in that a shift from approximately 0.7095 to 0.7101 can be observed along the growth access of the tooth. These values are still consistent with the ^{87}Sr/^{86}Sr values observed in the vicinity of the Almonda karstic system and the Tagus river basin, but a greater degree of mobility than N15 379 is implied by the variation in ^{87}Sr/^{86}Sr values across the N15 417 molar. Intra-tooth δ^{18}O variation of approximately 1‰ across the growth axis can be observed, though no obvious seasonal pattern is evident.

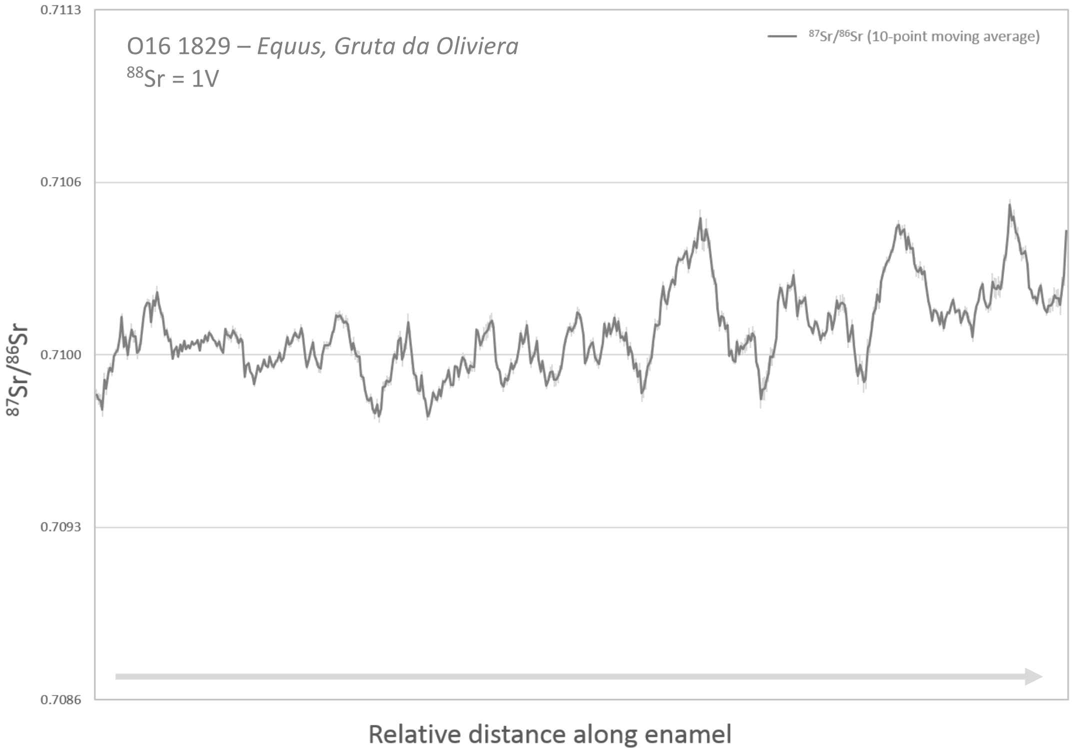


Figure 67. Sequential strontium isotope data for O16 1829 (indet. Molar fragment; analysed by AWP and JZ).

O16 1829 was analysed in 2012 by AWP and JZ. The ^{87}Sr/^{86}Sr profile observed in the apical portion of the crown is consistent with occupation of the banks of the Tagus River, with a relatively consistent value of approximately 0.7100 (Sr-2a). The cervical portion of the molar exhibits more radiogenic values of >0.7105, but elevated yttrium coincident with high voltages on mass 88 in this region of the tooth suggests that these more radiogenic ^{87}Sr/^{86}Sr values may be the result of diagenesis. If diagenesis is indeed responsible, then the fact that the strontium isotope values are considerably more radiogenic than the sediment values at the cave itself suggests that doubly-charged rare earth elements may be the cause (producing a signal on mass 87), or alternatively, secondary mineralisation of a substance with a high strontium and yttrium concentrations and more radiogenic ^{87}Sr/^{86}Sr values may have taken place.

9.1.3.2 Solutrean

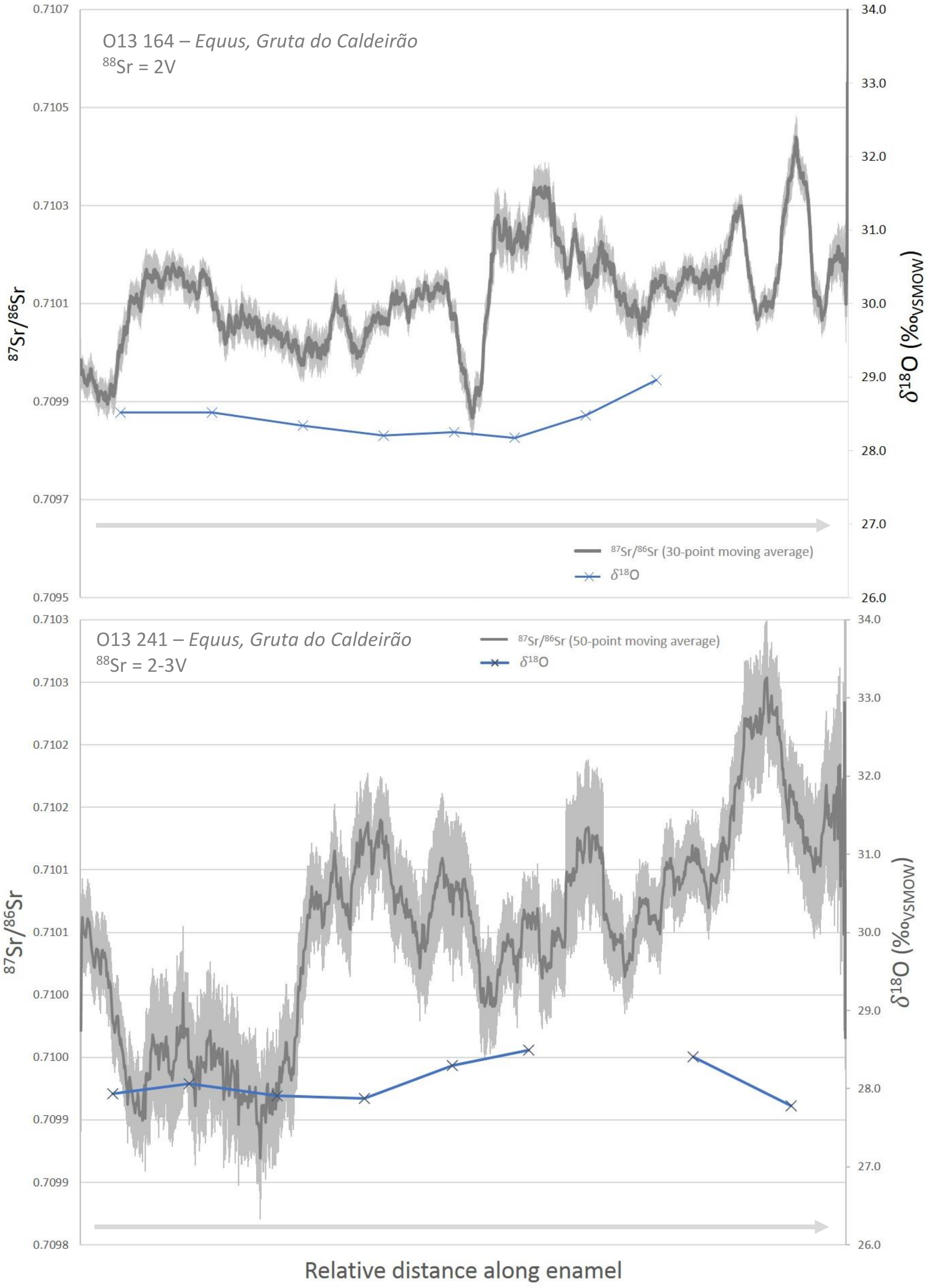


Figure 68. Top: Sequential strontium (30-point moving average) and oxygen isotope data for O13 164 (indet. molar, horse). Bottom: Sequential strontium (30-point moving average) and oxygen isotope data for O13 241 (indet. Molar, horse).

Two Solutrean horse specimens from Gruta do Caldeirão were successfully analysed; both of which are loose molars. The strontium isotope profile of O13 164 is relatively homogenous, with values ranging from 0.7100 to approximately 0.7103. Oxygen isotope data for the same tooth crown exhibits approximately 1‰ of variation, again much less than observed in red deer and ibex individuals. Nevertheless, for the period of formation time represented by the O13 164 molar fragment, the individual appears to have subsisted along the banks of the Tagus River (Sr-2a) or open similar geologies elsewhere.

The other Solutrean horse molar from Gruta do Caldeirão, O13 241, exhibits a similar strontium isotope profile with values ranging from 0.7099 to 0.7103. The average $^{87}Sr/^{86}Sr$ value is approximately 0.7100; again, consistent with occupation of the region near to the banks of the Tagus River (Sr-2a). The oxygen isotope profile of this crown exhibits a similar degree of variation to that of O13 241, and again does not seem to reflect a seasonal cycle. This may be a result of physiological differences in the way that horses metabolise oxygen in comparison to other contemporaneous herbivores in the study area. During the formation of this molar, the individual subsisted in a region with $^{87}Sr/^{86}Sr$ values consistent with a range between 0.7099 and 0.7103; perhaps reflecting occupation of the Tagus river basin and the banks of the Tagus itself.

Because both molars are loose and could not be identified as M1, M2 or M3, and as a result the oxygen isotope data are difficult to interpret, it is not possible to say with certainty that these individuals were present year-round in the area reflected by their strontium isotope profiles.

9.1.4 Stephanorhinus

9.1.4.1 Mousterian

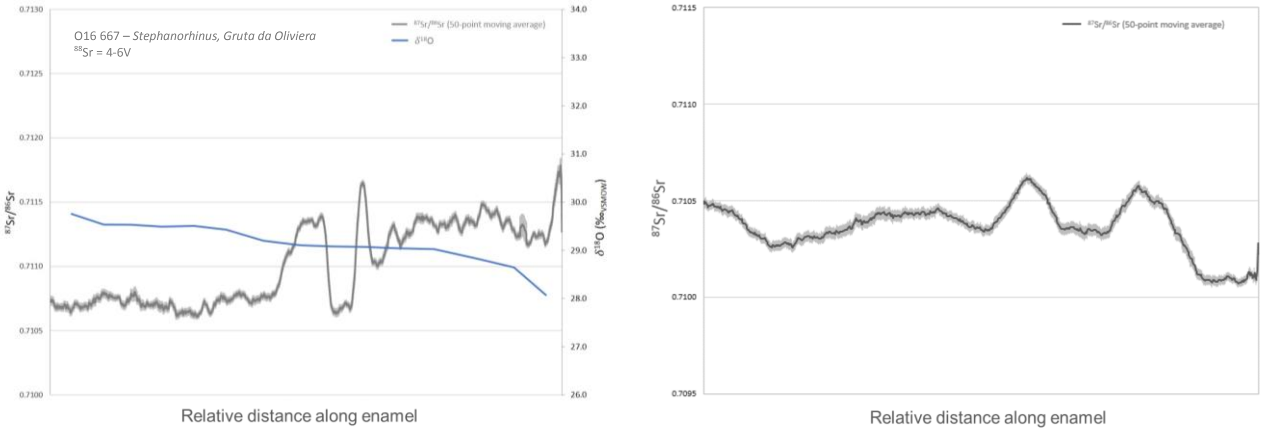


Figure 69. Left: Sequential strontium (30-point moving average) and oxygen isotope data for O16 667 (indet. molar, *Stephanorhinus sp*.). Right: Sequential strontium (20-point moving average) isotope data for P16 916 (indet. molar, *Stephanorhinus sp*.).

Two *Stephanorhinus sp*. molars were recovered from Layers 21 and 26 at Gruta da Oliviera. Their stratigraphic separation implies that they are likely to derive from two different individuals. Although they cannot be identified at the species level, assumptions can be made about their likely ecological position in the local landscape, as well as their role in Neanderthal subsistence strategies, based on information derived from studies of *Stephanorhinus kirchbergensis* and *Stephanorhinus hemitoechtus;* two Middle-Late Pleistocene species found in Europe. These species replaced the indigenous *Stephanorhinus hundsheimensis* in Europe between approximately 0.7-0.5 MA, and whilst they developed dietary specialisations in comparison to their more generalist predecessor, tooth wear data suggests that they maintained a degree of adaptability and flexibility (van Asperen and Kahlke, 2015). *S. kirchbergensis* preferred a greater proportion of browse and in the past has been referred to as the 'forest rhinoceros', whilst the diet of *S. hemitoechtus* consisted of a greater proportion of grass where available and hence has been considered to be more of a 'steppe rhinoceros'. Both species, however, essentially ate whatever else was available when their preferred diets were not possible to maintain due to environmental conditions. As such, neither species was limited to the forest or steppe, rather they were adaptable enough to subsist on varying proportions of browse and grass depending on what was readily available. No dedicated studies of modern rhino tooth crown mineralisation rates have been published to-date, but general estimations of crown formation times range between 1.5-3 years (Lugli *et al.*, 2017b).

The strontium isotope profiles obtained for the two Gruta da Oliviera *Stephanorhinus sp.* molars are generally consistent with occupation of the geological region to south-west of the Almonda karstic system, between the Serra d'Aire and the Tagus River. The sequential oxygen isotope data for O16 667 exhibits variation of approximately 1‰, but shows a trend from higher to lower values across the growth axis of the tooth. If the two end points are reflective of seasonal temperature extremes, then it could be argued that the molar fragment represents approximately six months of mineralisation from summer through to winter. This is inconsistent with observations of modern rhino crown formation times (Lugli *et al.*, 2017b), and so it is possible that either the tooth crown is considerably worn at the surface (truncating the data), nursing/weaning may have dampened the seasonal temperature signals, or there may be physiological differences in the way that this species metabolises oxygen. There appears to be a shift in $^{87}Sr/^{86}Sr$ values from 0.7107 in the apical portion of the crown to 0.7113 in the cervical half of the enamel, and may reflect occupation of the region in the locality of samples Sr-19 and Sr-25 directly to the south of the Almonda karstic system. No geographical samples currently match the $^{87}Sr/^{86}Sr$ value of 0.7107 observed in the uppermost portion of the crown, but the variation in values may reflect the consumption of plants with varying root depths or perhaps a the seasonally changing availability of browse and good quality graze.

P19 916 also exhibits potential evidence for mobility. The apical portion of the crown is dominated by an $^{87}Sr/^{86}Sr$ signal of between 0.7103 and 0.7105, although several short deviations to more radiogenic values can be observed in the cervical half of the enamel. Towards the enamel-root junction, the $^{87}Sr/^{86}Sr$ value drops to 0.7100; consistent with occupation of the banks of the Tagus River (Sr-2a). Again, this may be the result of seasonal movement to new grazing or browsing areas in response to the changing availability of resources. However, since oxygen isotope data is not available for this individual and because no studies have published detailed enamel mineralisation timings for modern rhinos, it is difficult to draw any conclusions about seasonal mobility for this individual.

10. Discussion

10.1 Animal mobility: temporal comparisons

One of the primary questions posed by this study is: *do the mobility patterns of fauna from the study area change over the duration of the Middle and Upper Palaeolithic?* This question is best explored by directly comparing sequential $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ profiles measured along the same dental array across the same species, using individuals recovered from the same sites and consecutive time periods in the archaeological record. The nature of the archaeological record means that this is not always possible, due to limits in the size of the faunal assemblages (and hence the number of individuals available to the study), the range and representation of species within a given assemblage, the availability of dental elements for analysis, the chronological resolution of the site, and the degree of preservation of the geochemical information within the sample. As such, the following section is only able to compare the strontium and oxygen isotope profiles of the study species across the span of the Palaeolithic where possible.

In order to maintain consistency, only M1, M2 and/or M3 profiles are selected, and the lengths of the strontium and oxygen isotope profiles for each tooth crown are normalised (tip of crown =0, EDJ = 1) for ease of comparison. In the case of *Equus sp.,* where only premolars and indeterminable molar fragments were available for study, all crown lengths are normalised. Because *Stephanorhinus sp.* molars were only available from Mousterian deposits at Gruta da Oliviera, the taxon is omitted from this section.

10.1.1 Red deer

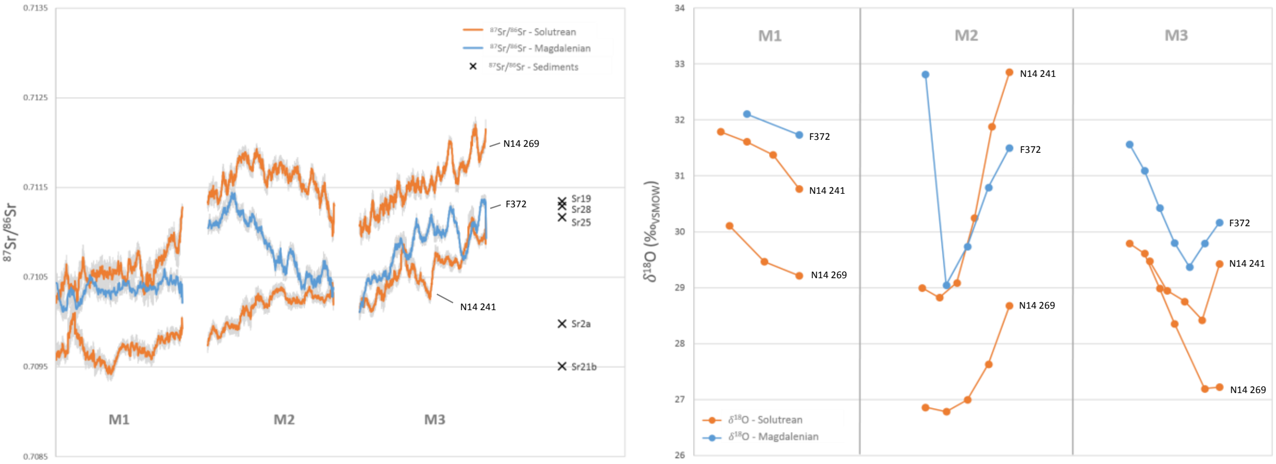


Figure 70. LEFT: Combined strontium isotope data for the M1, M2 and M3 of Solutrean and Magdalenian red deer from Gruta do Caldeirão and Lapa dos Coelhos. RIGHT: Combined oxygen isotope data for the M1, M2 and M3 of the same *Cervus elaphus* individuals.

Because of the limited availability of 1st, 2nd and 3rd molars in the red deer sample from the Palaeolithic deposits of the Almonda karstic system and the Gruta do Caldeirão, only three individuals spanning the Solutrean and the Magdalenian are available for temporal comparison. The $^{87}Sr/^{86}Sr$ profiles of all three individuals vary slightly in their absolute values, but all fall between the $^{87}Sr/^{86}Sr$ values of sediment samples Sr-21b and Sr-1b. All molars are generally consistent in strontium isotope profiles, with M1 crowns exhibiting relative homogenous values, M2 crowns showing a shift from more radiogenic to less radiogenic values along the growth axis, and M3 crowns shifting from less radiogenic to more radiogenic values. This implies that a degree of mobility was undertaken by all three *Cervus elaphus*, with individuals exiting and then revisiting (and thereby subsisting upon) specific geological substrates within the study area. The outlier in this case is the M2 crown of the Solutrean specimen N14 241, which exhibits the opposite trend in $^{87}Sr/^{86}Sr$ to the M2 crowns of the other individuals, but it is possible that this individual simply occupied different calving ground before occupying the same strontium isotope catchment as the other individuals.

The oxygen isotope profiles of the molars of all three individuals are also consistent in their overall trends, with M1 crowns characterised by a decrease in $\delta^{18}O$ value along the growth axis. M2 crowns of all three individuals follow a general increase in $\delta^{18}O$ values along the growth axis, and M3 crowns exhibit 'troughs' whereby values towards the apex of the crown are more enriched in ^{18}O, moving towards lower $\delta^{18}O$ further along the growth axis before beginning to rise again at the dentine-enamel junction. Seasonal cycles are evident across the $\delta^{18}O$ profiles, suggesting that all three individuals experienced seasonal temperature extremes and did not migrate far enough, if at all, to escape said extremes. As with the inter-individual $^{87}Sr/^{86}Sr$ data, the absolute $\delta^{18}O$ values of the individuals are not identical, with a variability of 2.7‰ in the maximum $\delta^{18}O$ values, and a variability in the lowest $\delta^{18}O$ values of 2.3‰. Inter-individual variability in the highest and lowest $\delta^{18}O$ values measured in seasonally mobile modern sheep teeth from the same population have been observed to be between 1.1‰ and 1.9‰ respectively (Balasse *et al.*, 2012), suggesting that even when environmental conditions are identical, factors such as physiology may result in variation within populations. In the case of these *Cervus elaphus* individuals, variation may also reflect factors such as differing proportions of input signals between individuals, such as plant water and drinking water.

The very limited sample size makes it difficult to make conclusive statements about differences or similarities in the mobility patterns and seasonality of the wider red deer population from the earlier to later Upper Palaeolithic, but the data tentatively suggest that these individuals, recovered from archaeological deposits dating to Solutrean and Magdalenian periods, ranged across the same landscape and engaged in similar seasonal mobility behaviours, and experienced the same or similar seasonal extremes in temperature. If these individuals are representative of the wider red deer population, it could be argued that this species was present within the study area throughout the year, perhaps moving seasonally across tens of kilometres at most due to the changing availability of suitable forage. Considering the fact that red deer are very well represented in the Palaeolithic cave deposits of the study area, it seems reasonable to suggest that the omnipresence of this species may have made them ideal targets for the Solutrean and Magdalenian human occupants of the region. The lack of comparable Mousterian *Cervus elaphus* molars in the current sample makes it impossible to investigate at this stage whether Middle Palaeolithic red deer engaged in similar mobility patterns and behaviours, but future analysis of unpublished red deer dental specimens from Gruta da Oliviera may shed light on this.

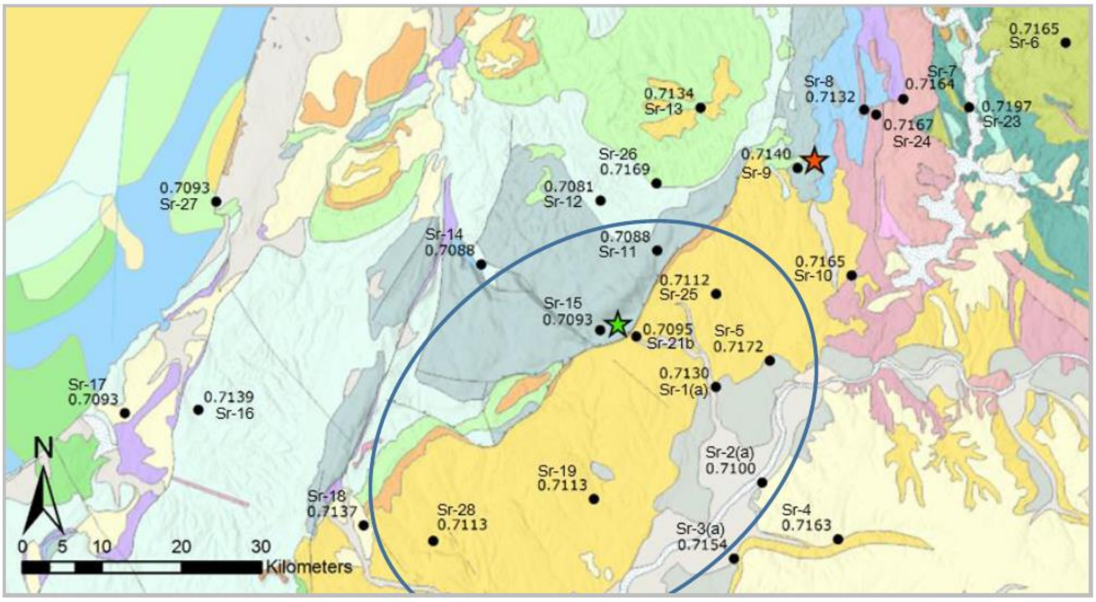


Figure 71. Strontium isotope map of the study area showing the range (blue circle) of *Cervus elaphus* individuals over the course of the Mousterian, Solutrean and Magdalenian based on their strontium isotope profiles. The green star denotes the location of the Almonda karstic system, and the orange star denotes the location of Gruta do Caldeirão.

10.1.2 Ibex

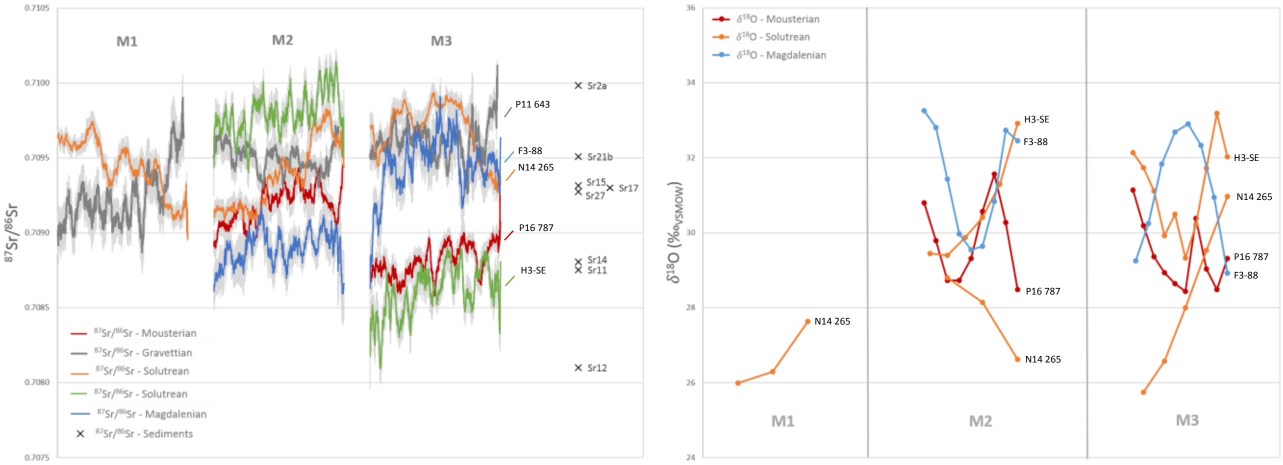


Figure 72. LEFT: Combined strontium isotope data for the M1, M2 and M3 of Mousterian, Gravettian, Solutrean and Magdalenian ibex from Gruta da Oliviera, Gruta do Caldeirão and Lapa dos Coelhos. RIGHT: Combined oxygen isotope data for the M1, M2 and M3 of the same ibex individuals.

A greater availability of *Capra sp.* molars across time periods renders it possible to compare strontium and oxygen isotope profiles from ibex individuals recovered from Mousterian, Gravettian, Solutrean and Magdalenian deposits across the study sites, though the number of individuals available for each period is still limited to two at the most due to the small size of individual faunal assemblages. As Figure 72 illustrates, the ^{87}Sr/^{86}Sr profiles of all individuals fall within the expected bioavailable values of the study area, ranging between observed ^{87}Sr/^{86}Sr values obtained for sediment samples Sr-12 and Sr-2a taken from the Central Limestone Massif and the Tagus River basin, respectively. The *Capra sp.* ^{87}Sr/^{86}Sr values are generally less radiogenic than those of the red deer, likely reflecting increased occupation of the higher altitude terrain along the Serra d'Aire. Unlike the red deer, however, there appear to be no consistent inter-individual trends in the strontium isotope profiles across the ibex molars. This is somewhat unexpected based on studies of the behaviour of extant ibex populations, who are often observed to engage in regular, altitudinal seasonal mobility between higher and lower altitude environments according to season. Although it appears that Mousterian, Solutrean and Magdalenian individuals (P16 787, H3-SE and F3-88 respectively) all spent time subsisting upon geological substrates consistent with the sediment values obtained for higher altitudes along the Central Limestone Massif (Sr-11, Sr-14), as expected, there does not appear to be much consistency in the position at which these values occur within the crowns themselves. Likewise, although the oxygen isotope values of all individuals appear to reflect both seasonal temperature extremes across their dental arcades, there does not appear to be inter-individual consistency in the occurrence of these extremes (for example, the M3 of the Solutrean individual H3-SE appears to exhibit a summer-winter-summer cycle, whilst the M3 of Magdalenian individual F3-88 exhibits the reverse – a winter-summer-winter cycle). Temperature extremes also do not appear to correlate with shifts in strontium isotope values.

There are several possible explanations for the inter-individual variation in strontium and oxygen isotope profiles observed in these ibex molar crowns, relating to animal behaviour, to physiology, and to bias resulting from differential crown wear and sampling methods. The simplest explanation is that ibex mobility changed over the course of the middle and Upper Palaeolithic, perhaps in response to changing environmental conditions. A greater sample size for each time period would enable firmer temporal comparisons to be made, but the fact that both Solutrean ibex individuals (N15 265 and H3-SE) exhibit contrasting strontium and oxygen isotope profiles, whilst conversely the Mousterian (P16 787) strontium and oxygen profiles align closely with the latter tentatively suggests that this may not be the correct explanation. It may be that the ibex sample analysed here represents a mixture of male and female individuals, which have been observed to engage in different mobility patterns depending on sex. If this is the case, physiological factors may also be at work, as Iberian ibex males and females are known to differ in their growth rates with females reaching full skeletal ossification two years faster than males (Acevedo and Cassinello, 2009). It would follow that enamel mineralisation times may also vary, resulting in different timescales presented by male and female molar crowns – this was observed in a study of modern wild sheep and ibex in Mongolia, where differences in the time taken for maxillary and mandibular crowns to mineralise resulted in the molars of some species reflecting one seasonal cycle, and others reflecting two (see Makarewicz and Pederzani, 2017). Along this line, it is also possible that individual differences in physiology regardless of sex may have an effect upon the measured isotope profiles, as observed in studies of extant sheep (Balasse *et al.*, 2012).

Alternatively, it may be the case that all ibex individuals under analysis did in fact engage in similar mobility behaviour, but the resulting trends in strontium and oxygen isotope profiles are masked by variances in the extent of enamel wear. All molar crowns were somewhat worn at the occlusal surface, and as such amount of time represented by the ^{87}Sr/^{86}Sr profiles measured along the growth axis of each crown will depend upon the amount of enamel worn away through mastication (i.e., the sequential isotope profiles may be truncated to different extents depending on the amount of enamel wear).

Whilst the sample size is relatively small and the data are difficult to interpret temporally, it is still possible to derive some broad, conclusive information from these data. Firstly, it is apparent that these ibex molar specimens, recovered from Mousterian, Gravettian, Solutrean and Magdalenian periods, all exhibit strontium isotope profiles with ^{87}Sr/^{86}Sr values consistent with those observed in the study area; in particular the Jurassic Central limestone Massif and the Tagus Basin. This implies that throughout the Middle and Upper Palaeolithic periods, this species was present within a 30-40km radius of the Almonda karstic system and the Gruta do Caldeirão respectively. Secondly, all individuals appear to have experienced seasonal temperature extremes, suggesting that they did not engage in migratory behaviours in order to escape said extremes. As such, it is likely that they were occupants of the study area year-round.

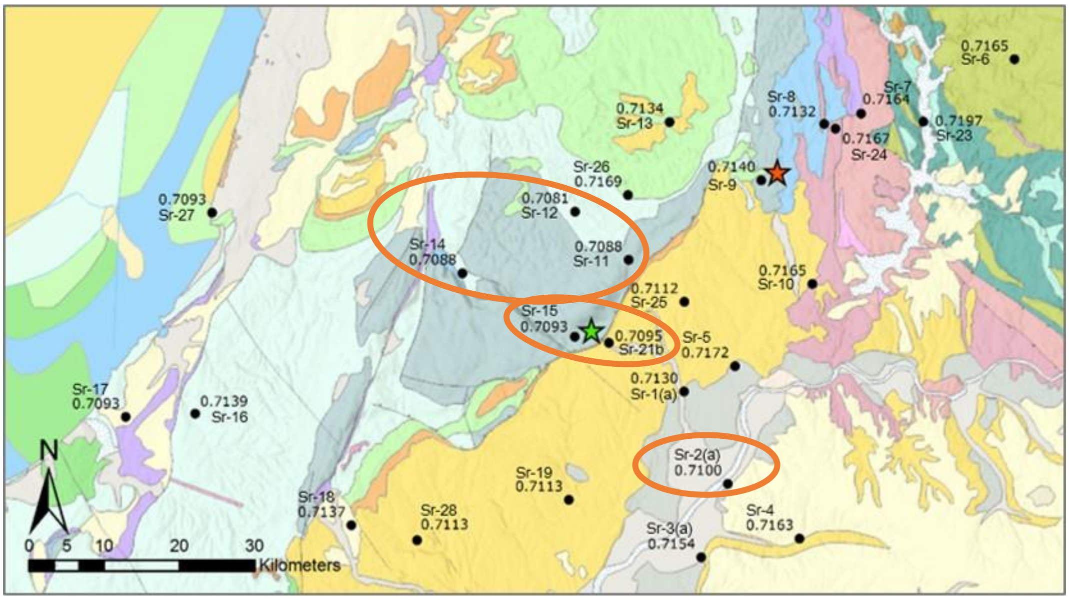


Figure 73. Strontium isotope map of the study area showing the regions (orange circles) likely occupied by *Capra* individuals over the course of the Mousterian, Solutrean and Magdalenian based on their strontium isotope profiles. The green star denotes the location of the Almonda karstic system, and the orange star denotes the location of the Gruta do Caldeirão.

10.1.3 Horse

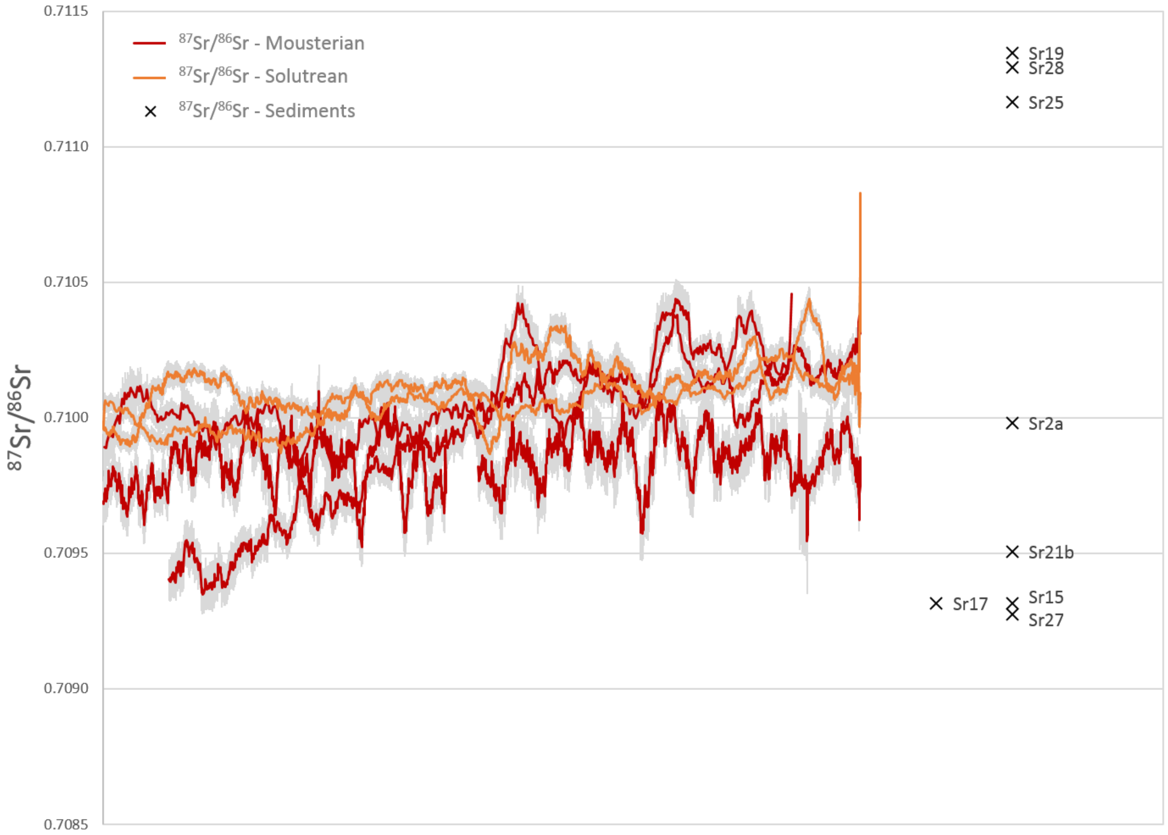


Figure 74. Combined strontium isotope data for the Mousterian and Solutrean horse molars from Gruta da Oliviera and Gruta do Caldeirão.

The *Equus sp.* sample in this study is made up entirely of loose indeterminate molars and premolars, and as such it is not possible to carry out a temporal comparison of isotope data from M1, M2 and M3 specimens. Instead, all strontium isotope profiles were normalised and plotted together in Figure 74. Because of the lack of directly comparable molars, the oxygen isotope data are not plotted for comparison. No Magdalenian horse teeth were available for analysis due to the scarcity of equid remains in Magdalenian deposits at the study sites, and as such, temporal comparisons can only be made between Mousterian and Solutrean horse molars from Gruta da Oliviera and Gruta do Caldeirão.

All molars exhibit relatively homogenous $^{87}Sr/^{86}Sr$ profiles along their growth axes. The average $^{87}Sr/^{86}Sr$ values of all Mousterian and Solutrean individuals are 0.709946 ± 0.000105 and 0.710085 ± 0.0000454 respectively; suggesting that these individuals subsisted upon the same geological substrate during both the Mousterian and Solutrean periods. The strontium isotope profiles of all individuals fall between the measured $^{87}Sr/^{86}Sr$ sediment samples Sr-21b and Sr25, with the average $^{87}Sr/^{86}Sr$ values of all Mousterian and Solutrean individuals highly consistent with the $^{87}Sr/^{86}Sr$ value of sediment samples taken along the banks of the Tagus River. The lack of heterogeneity in the strontium isotope profiles may be reflective of sedentary lifestyles spent occupying a permanent home range, consistent with extant wild horses whose home ranges have been observed to be in the order of approximately 10km^2 (Tyler, 1972). It is possible for individuals to move across significant geographical distances without altering the ^{87}Sr/^{86}Sr composition of their tissues if their movements take place over vast swathes of unchanging geology, but since the geology of Portuguese Estremadura is highly varied over short distances, it seems unlikely that the homogenous strontium isotope profiles seen in these individuals are indicative of such a scenario. Nevertheless, because the sample is primarily made up of single indeterminate molar fragments, these data will likely reflect shorter periods of time than the M1, M2 and M3 sequences of the red deer and ibex. Furthermore, variations in the amount of occlusal wear may truncate varying portions of enamel, hence limiting the amount of strontium isotope data retrievable. As such, it is important to note that although the profiles do not appear to reflect significant mobility across geological substrates, there is the possibility that any of the individuals may have been mobile across the study area during the formation of other teeth, and these variations in ^{87}Sr/^{86}Sr are not visible due to the aforementioned caveats.

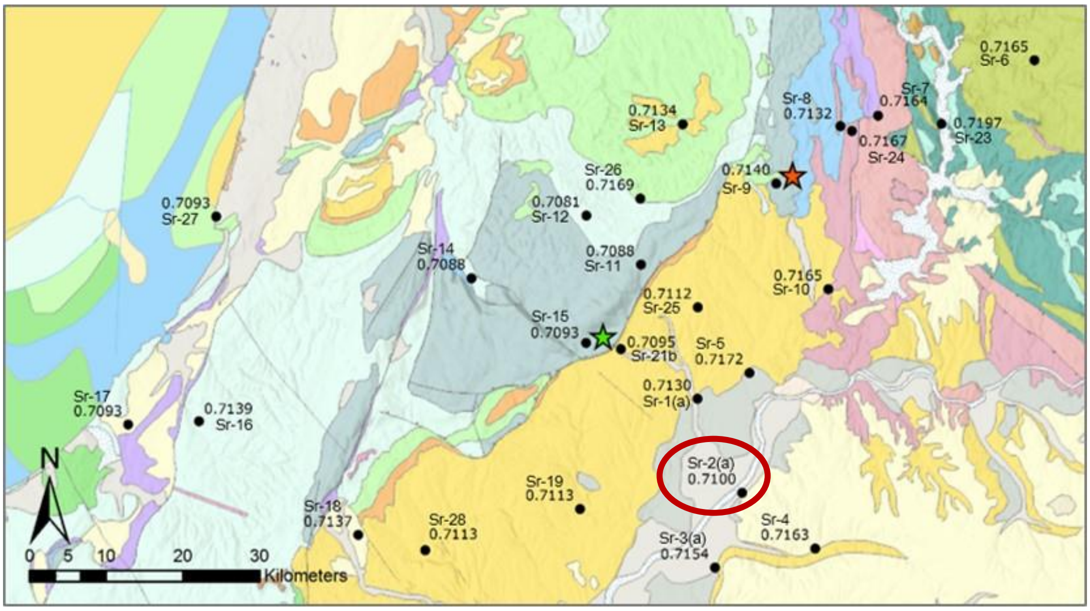


Figure 75. Strontium isotope map of the study area showing the region (red circle) likely occupied by *Equus* individuals over the course of the Mousterian and Solutrean based on their strontium isotope profiles. The green star denotes the location of the Almonda karstic system, and the orange star denotes the location of the Gruta do Caldeirão.

10.1.4 Temporal change?

Do the mobility patterns of large mammalian fauna change over time during the course of the Pleistocene in Central Portugal? This question bears relevance to our understanding of the effects of climate change upon the ecosystems and ecologies of the western reaches of Europe, and in turn the effects of any such changes on the subsistence strategies of human groups occupying this region over the span of the Middle and Upper Palaeolithic. The current study seeks to examine whether or not the most common large mammal species recovered from Gruta da Oliviera, Gruta do Caldeirão and Lapa dos Coelhos - primarily red deer, ibex and horse - adapted their mobility patterns over time. This represents the first dedicated attempt to track the mobility patterns of different large mammal species over the course of the Middle and Upper Palaeolithic periods in Europe, through the sequential isotopic analysis of tooth enamel.

Ideally, temporal changes would be best addressed by examining the isotope profiles of the same dental sequences of multiple, directly dated individuals of the same chronological age and species, and then comparing those isotopic data with those derived from the same dental sequences of the same species but dating to earlier and later periods. Whether or not this is possible is entirely dependent on the availability and abundance of dental specimens in each archaeological assemblage, the representation of species over time, and the chronological resolution of the site in question. The current study is limited primarily by the limited sample size and availability of dental specimens, as certain faunal assemblages are particularly small (for example, the Magdalenian deposits at Lapa dos Coelhos), and even in those assemblages that are larger, the availability of molar arcades is variable. Furthermore, as the radiocarbon dating of individual dental samples is beyond the scope of the current study, chronological assignments are made based on stratigraphic association and as such may not be accurate. As a result of these factors, it was not possible to include Mousterian red deer or Magdalenian horse in comparisons, and because the sample size for each archaeological period rarely exceeded two individuals for each species, it is not possible to reach conclusions about wider populations. Despite the limited sample size, however, some tentative observations about mobility patterns over time can be made.

Red deer molars recovered from Solutrean and Magdalenian deposits at the study sites exhibit sequential isotope data indicative of seasonal mobility over a distance of tens of kilometres within the Tagus River basin, and although the sample size is too small for comparative statistical analysis, there do not appear to be significant differences between the strontium and oxygen isotope profiles across the molars of both Solutrean and Magdalenian individuals. If these data are representative of the wider Upper Palaeolithic red deer population, it seems plausible to suggest that this species engaged in small-scale, seasonal mobility within the study area and was an available food resource year-round.

Sequential strontium isotope data obtained through the analysis of ibex molars recovered from Mousterian, Gravettian, Solutrean and Magdalenian deposits reveal heterogeneous patterns indicative of mobility, but very little inter-individual consistency in mobility patterns is evident - even among individuals of the same archaeological age. The strontium isotope data nevertheless imply that all individuals occupied geological substrates within a radius of approximately 60km of the Almonda karstic system (including higher altitudes along the Central Limestone Massif), and oxygen isotope data suggest that all individuals experienced seasonal extremes in temperature (i.e, did not migrate large distances). Without a larger sample, it is difficult to determine whether the inter-individual differences in strontium and oxygen isotope profiles are a function of temporal shifts in group behaviour over time, or purely differences in individual behaviour. It is clear, however, that these ibex were present within the study area throughout the Middle and Upper Palaeolithic periods.

The lack of availability of determinable horse molars resulted in an inability to carry out a similar comparison of strontium and oxygen isotope profiles for this taxon over time, but based on the homogenous strontium isotope profiles of the available indeterminable molars, it appears that Mousterian and Solutrean equids did not engage in mobility across the study area. Based on average $^{87}Sr/^{86}Sr$ values of both Mousterian and Solutrean individuals, this group of individuals likely occupied fixed home range consistent with the $^{87}Sr/^{86}Sr$ signature seen in sediment sample Sr-2a; the lowlands along the Tagus River. Heterogeneity in the corresponding oxygen isotope profiles suggests that these individuals were present in the study landscape throughout the year, making them a permanent feature in the landscape throughout the Mousterian and Solutrean periods. *Equus sp.* specimens are scarce in Magdalenian deposits at the study sites, and as such no comparable data is available for the Late Upper Palaeolithic.

Whilst this study is somewhat limited by small sample sizes in its ability to address questions of temporal shifts in animal mobility, it offers an excellent preliminary insight into the large mammal ecology of the Middle and Upper Palaeolithic period in Central Portugal. It further illustrates the feasibility of this method where larger faunal assemblages and access to direct radiocarbon dating are available.

10.2 Human mobility: mobility pattern and range size

Can sequential strontium isotope analysis of human tooth enamel via laser ablation MC-ICP-MS provide insights into whether the mobility patterns of individuals occupying the study area are characteristic of logistical or residential mobility patterns? Can range size be determined through the same means? These questions are explored in the following section.

10.2.1 *Neanderthals*

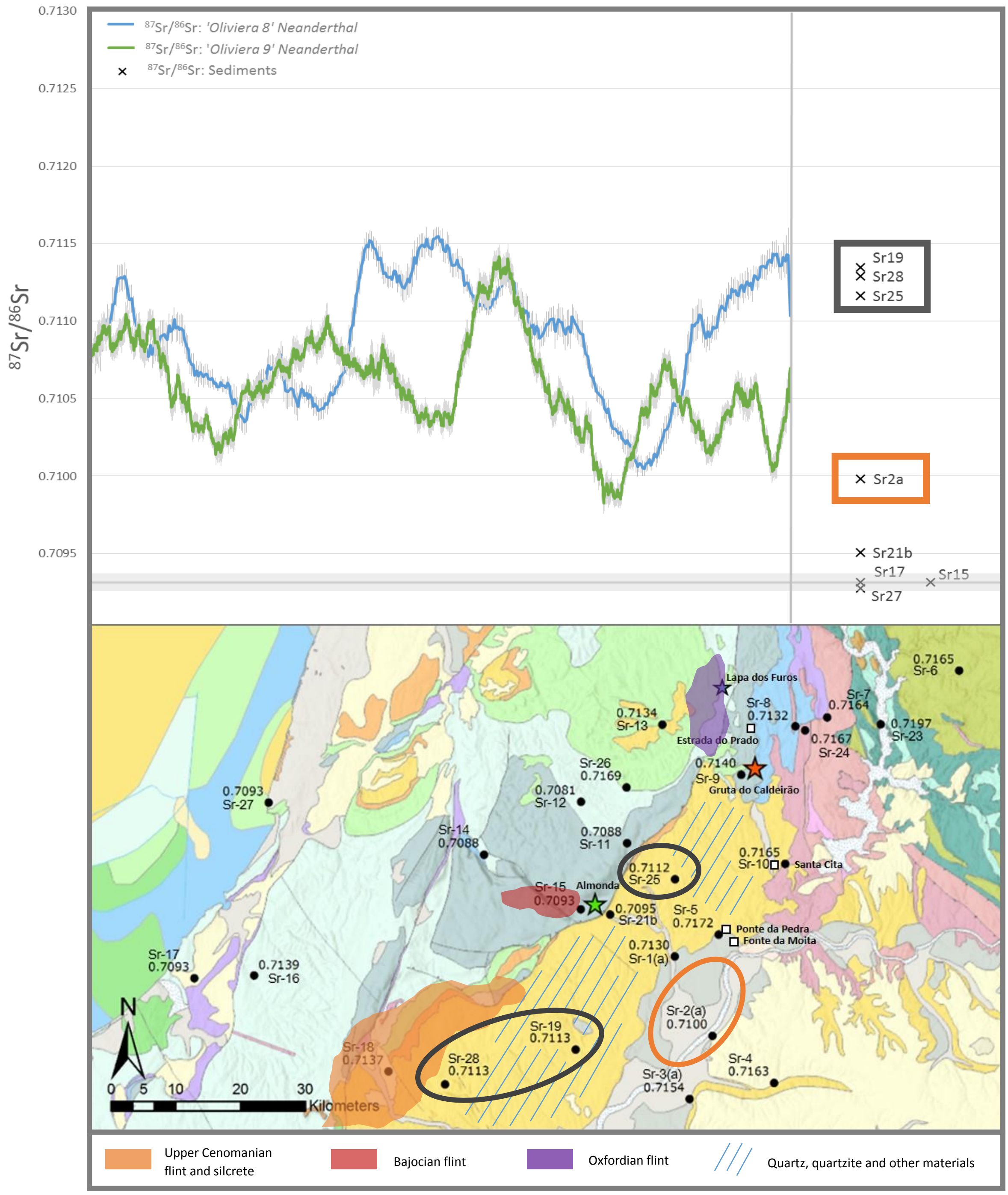


Figure 76. Sequential strontium isotope data for the Oliveira 8 and Oliveira 9 individuals, plotted alongside the sediment $^{87}Sr/^{86}Sr$ values for the study area (Pike et al. unpublished). The grey line denotes the average dentine Sr value at Gruta da Oliveira. The map provides the probable source locations of the raw materials identified in Layer 14 at Gruta da Oliveira (Matias, 2016: 12). White squares denote Mousterian open-air sites, and stars denote cave sites. Dark grey and orange ovals represent regions with $^{87}Sr/^{86}Sr$ values consistent with those observed in the strontium isotope values of the Neanderthal individuals.

Two Neanderthal teeth, Oliviera 8 and Oliviera 9, were selected to undergo sequential strontium isotope analysis via laser ablation MC-ICP-MS by Pike et al. (unpublished). The results of the analyses are presented in Figure 76 and are plotted from left (top of crown) to right (dentine-enamel junction). Both individuals exhibit inhomogeneous strontium isotope profiles, with $^{87}Sr/^{86}Sr$ values oscillating between 0.7100 and 0.7115. The most radiogenic values observed in the profiles of both individuals, 0.7112 to 0.7115, are consistent with the sedimentary basin that lies between the Central Limestone Massif and the Tagus River (Sr-19, Sr-25 and Sr-28). The least radiogenic values, approximately 0.7100, are consistent with strontium isotope values observed in plant samples from Quaternary sedimentary deposits along the banks of the Tagus River (Sr-2a). The strontium isotope profiles of both individuals also point to the occupation of two other geologically distinct regions, with values of 0.7105 and 0.7110 respectively, but none of the comparative sediment and plant $^{87}Sr/^{86}Sr$ values currently available are consistent with these signatures. The following interpretations are the author's, based on all available evidence.

The home range implied by the existing strontium isotope data (a radius of approximately 30km from the Almonda karstic system) is consistent with inferences made based on the study raw material provenience in Layer 14 (Matias, 2016). Quartz and quartzite, the most abundant lithic raw material in Layer 14 of Gruta da Oliviera, are abundant and readily available in the siliciclastic deposits of the Tagus Basin, though the Levallois artefacts are produced using a fine-grained quartzite available approximately 5 km to the northeast of the site. The flint, however, is geologically restricted to specific outcrops, and as such offers a greater insight into the mobility patterns of the Gruta da Oliviera Neanderthals. 62% of the flint from Layer 14 at Gruta da Oliviera was derived from Cretaceous sources identified within the Tagus Basin, whilst 12% was derived from local Bajocian sources less than 5 km away from Gruta da Oliviera itself. Oxfordian flint, the primary source of which is located approximately 20 km away from the site (in the vicinity of Gruta do Caldeirão), comprises 24% of the Layer 14 flint assemblage. Matias (2016) argues that the high percentage of flint obtained from Tagus Basin sources may reflect the long-term residence of the Neanderthal population on the Neogene plains of the Tagus Basin, with the Almonda karstic system acting as a short term camp that was somewhat residential in nature. During its use, the nearby Bajocian flint deposits were likely exploited, despite being of lower quality than the Cretaceous flint outcrops utilised preferentially. Matias (2016) also suggests that the presence of Oxfordian flint may reflect a degree of seasonal mobility, whereby the Nabão River Valley acted as a corridor between the Central Limestone Massif and the Sicó Massif further to the north.

What kind of settlement pattern is reflected in these data? The enamel of both individuals appears to record, in total, the $^{87}Sr/^{86}Sr$ values of four isotopically distinct geological substrates. This implies that either *a)* the individuals occupied the Gruta da Oliviera as a multi-seasonal base camp, hunting and gathering resources with distinct strontium isotope values that were brought back to the site for consumption (logistical mobility with 'central place provisioning'), or *b)* that these individuals moved between camps located within regions of distinct underlying geology, consuming local resources with each move (residential mobility). In light of this, the repetition of specific $^{87}Sr/^{86}Sr$ values along the strontium isotope profiles correspondingly suggests either that *a)* certain resources were consumed repeatedly over time, perhaps on a seasonal basis, or *b)* that certain camps were repeatedly visited over time. The strontium isotope profiles alone are perhaps not sufficient to differentiate between these two possible models of mobility, and it should be borne in mind that in reality, these individuals may have engaged in a mixture of both.

In order to make further observations about both the nature of these Neanderthals' mobility and the function of Gruta da Oliveira, it is necessary to combine the strontium isotope data with existing data derived from the lithic and faunal assemblages the wider study area. A number of open air sites are located within the Estremadura landscape (see Figure 76), and they offer an excellent insight into the use of the landscape by its human inhabitants. Though faunal remains are often not preserved at open air sites in the region due to hostile burial conditions, the lithic assemblages recovered at nearby sites located within the Tagus Basin such as Santa Cita, Ponte da Pedra and Fonte da Moita are incredibly valuable. At the MIS3 open air site of Santa Cita, approximately 20km to the west of the Almonda karstic system on the banks of the Nabão River, characteristically Mousterian lithic assemblages and post-hole-like features are considered to be evidence for use of the site as a logistical camp site, utilising a strategic position with plentiful local resources (Cardoso, 2006). No faunal remains are preserved in the Mousterian levels, but the large lithic assemblages reflect the opportunistic use of local quartz pebbles, as well as exhausted non-local flint cores (from two locations approximately 10km apart). All stages of the Chaîne opératoire are represented, with numerous refitting pieces. Five features interpreted as post holes are argued to represent a trapezoidal habitation structure, perhaps utilised as a form of shelter (Cardoso, 2006) on what may have been shorter-term residential stays.

Approximately 10km to the southeast of Santa Cita are two more important open air sites, Ponte da Pedra and Fonte da Moita. The use of these sites is suggested by Cura (2013) to be linked to the occupation of the caves sites within the Almonda karstic system; both are located on a riverside plain at which lowland species could be hunted and raw materials could be collected. The former, Ponte da Pedra is situated on the right-hand bank of the Atalaia stream, and excavations that took place on the lower terrace (associated with OIS3) led to the discovery of a palaeo-soil with a fireplace and a characteristically Mousterian lithic assemblage. The lithic assemblage is dominated by worked pebbles

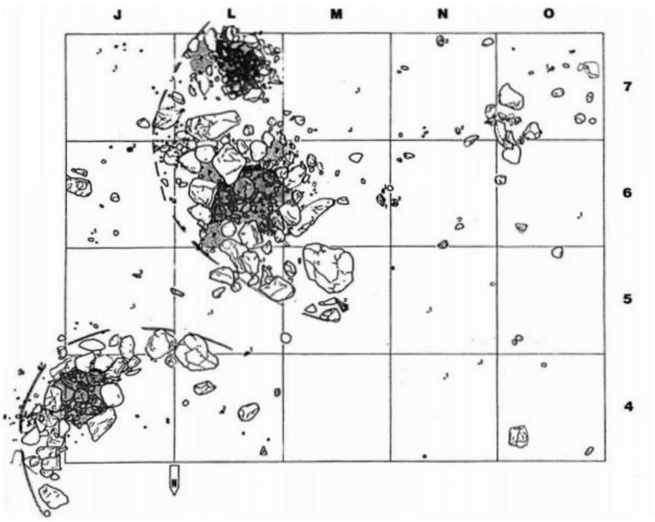


Figure 77. Wind breaks at Vilas Ruivas, with firehearths (dark zones) and possible postholes (grey zones). From Cardoso (2006: 28).

and blanks; including both cortical and non-cortical flakes (some of which are retouched), primarily produced using local quartzite fluvial pebbles. The presence of exhausted pebbles cores and débitage points to all stages of the Chaîne opératoire taking place at the site; indeed, evidence for the use of Levallois and disc-core techniques is present, though bifaces are rare. Notched tools and scrapers make up the remainder of the tool assemblage. The use of local raw materials for an overarchingly short and expeditious Chaîne opératoire points to the immediate and repeated use of the site for technical reasons, championing the quantity of lithic production over the quality. Because the fresh edges of recently knapped flakes are the most efficient for cutting meat and skin from carcasses, and because the crude notches and scrapers are well-suited for the processing of hides, the site has been interpreted a temporary camp situated strategically along the river bank for the hunting of species that were not available at higher altitudes, and for the collection of good-quality fluvial quartzite pebbles. Fonte de Moita, another open air site situated just 2km to the southwest of Ponte da Pedra, produced a similar lithic assemblage to the latter, again characterised primarily by worked pebbles and blanks made using local fluvial quartzite pebbles. The predominance of simplistic, opportunistic technologies associated with butchering and processing activities has led to suggestions that, like Ponte da Pedra, the site was utilised as a logistical camp for the exploitation of nutritional resources and raw materials that were territorially available (Rosina and Cura, 2010; Cura, 2013).

Further important examples of open air sites with similar functions are located further upstream on the banks of the Tagus River, approximately 70km to the west of the Almonda karstic system near to the Spanish border**.** Both Vilas Ruivas and Foz do Enxarrique have produced characteristically Mousterian lithic assemblages; the former dated to MIS4 (54,000 years + 12,000; -11,000 years BP) through TL dating of thermoclast elements, and the latter dated to MIS3 (33,600 years BP ± 500) years based on three U/Th dates of horse and aurochs teeth. At Vilas Ruivas, the remnants of two arched structures have been preserved, suggested by Zilhao (2001) to reflect 'hunting blinds' or windbreaks considering their proximity to the remains of fire structures. Akin to the lithic assemblages of Santa Cita, Ponte da Pedra, Fonte de Moita, the lithic assemblages of Vilas Ruivas and Foz do Enxarrique are characterised by flakes, chips and worked tools such as scrapers and denticulates, made from locally-sourced quartzite pebbles (Cardoso, 2006). At Foz do Enxarrique, the remains of fauna including deer, horse, aurochs, rhinoceros, rabbit and elephant have been recovered, with 90% of the deer, horse, aurochs and rabbit remains bearing clear signs of anthropogenic cutting and breaking. The larger species, elephant and rhinoceros, are suggested to have accumulated naturally (Cardoso, 2006), but considering the presence of *Stephanorhinus sp.* remains in the Mousterian Cone at the Gruta da Oliviera, it is possible that those taxa were also scavenged or hunted. The location of the site, situated on a small platform where the Enxarrique stream is met by the Tagus River, is likely to have flooded seasonally – suggesting that it could only have been in use by human groups for part of the year (Cardoso, 2006). This information, combined with the presence of butchered deer bones, hunting blinds and combustion structures, suggests that the Vilas Ruivas and Foz do Enxarrique sites may correspond to camps that were occupied residentially on a short term, perhaps seasonal basis.

If sites such as Santa Cita, Vilas Ruvias and Foz do Enxarrique were short-term occupations that were relatively residential in nature, and Ponte da Pedra and Fonte da Moita were indeed logistical sites, positioned strategically near territorial resources within the Tagus Basin, how did Gruta da Oliveira fit into the picture? Did it act as a logistical camp, or something more akin to a residential site? This is difficult to discern without more detailed analyses of the lithic assemblage and the faunal assemblage, which are still underway.

10.2.2 Solutrean humans

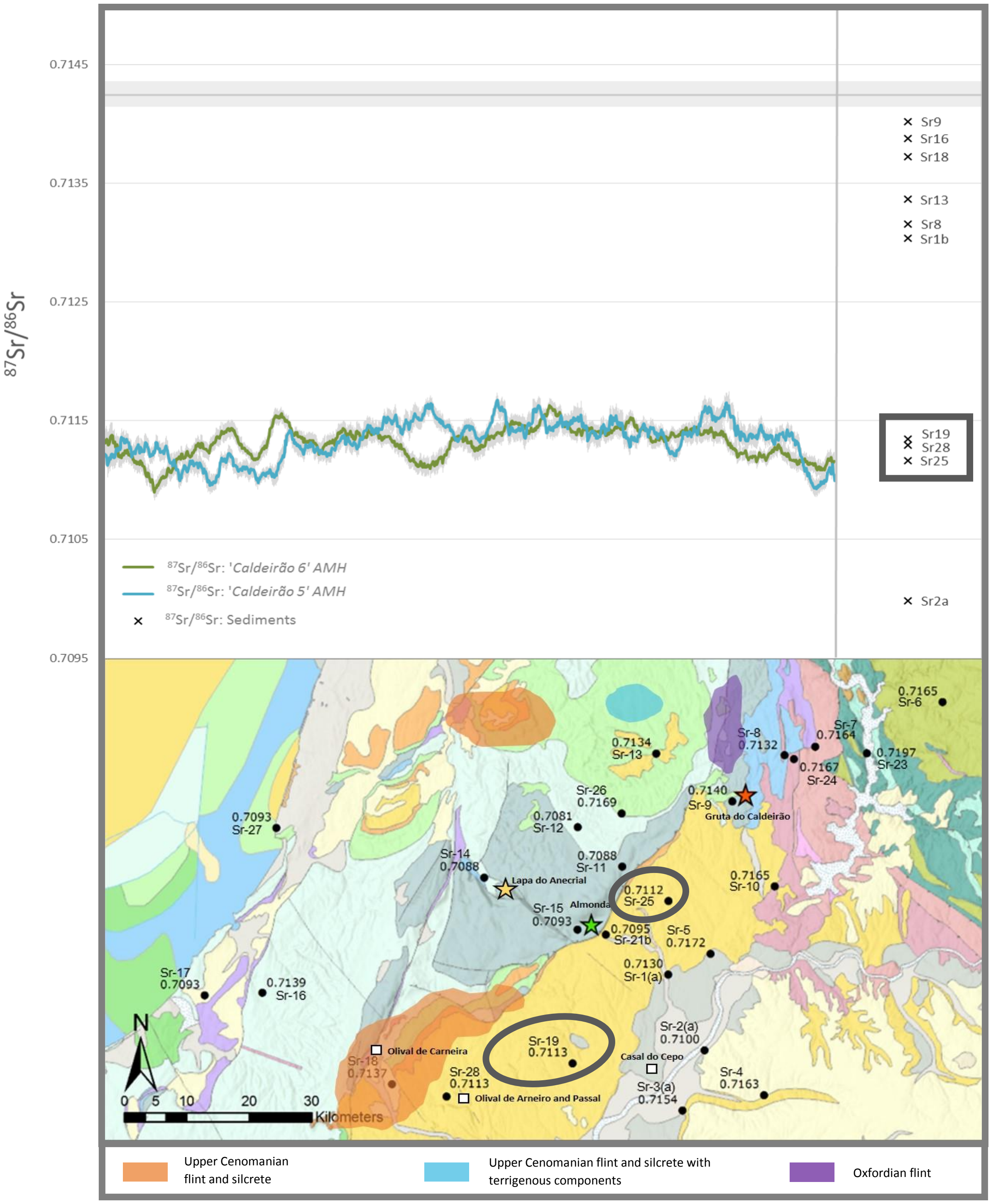


Figure 78. Sequential strontium isotope data for the Caldeirão 5 and 6 individuals, plotted alongside the sediment ^{87}Sr/^{86}Sr values for the study area (Pike et al. unpublished). The grey line denotes the average dentine Sr value at Gruta da Oliveira. The map provides the probable source locations of the raw materials identified in in Solutrean levels at Gruta do Caldeirão. White squares denote Solutrean open-air sites, and stars denote cave sites. The dark grey ovals represents regions with ^{87}Sr/^{86}Sr values consistent with those observed in the strontium isotope values of the Caldeirão individuals.

The strontium isotope profiles of the two Solutrean human teeth, designated Caldeirão 5 and Caldeirão 6, are presented alongside the bioavailable strontium map of the study area in Figure 78. Unlike the Neanderthal and AMH specimens from Gruta do Oliviera and Galeria da Cisterna, the Caldeirão 5 and 6 teeth are incisors (both have been identified as right I2). Based on observations of modern human tooth crown formation times, these profiles represent approximately four years of growth, between the ages of 1.5 and 5.5 years (AlQahtani, Hector and Liversidge, 2010). The profiles of both individuals are homogenous, showing little variation that might be considered to be indicative of little mobility, or the exploitation of multiple resources across varied geological substrates. Both profiles are also highly consistent with one another, with average $^{87}Sr/^{86}Sr$ values between 0.7114 and 0.7115. These data match three of the sediment samples (Sr-19, Sr-25 and Sr-28) taken within the Neogene sedimentary basin (Figure 78).

It is difficult to discern from this data what the range size of these Solutrean individuals may have been, and whether they engaged in any degree of mobility. The bioavailable strontium sample consistent with the human $^{87}Sr/^{86}Sr$ averages and the farthest away from the Gruta do Caldeirão, Sr-28, is approximately 60km to the southeast of the site, though the underlying geology extends further south beyond the range of the current map. The closest sample, Sr-25, lies approximately 20km to the southeast of the site. The individuals need not have travelled as far as either of these locations for $^{87}Sr/^{86}Sr$ values like these to be recorded in their tissues, however, as the same Neogene sediments are present just a few kilometres to the south of the site itself. Therefore, based solely on the strontium isotope data, it appears that nutritional resources were primarily obtained within a 5-60km range to the south of the Gruta do Caldeirão during the formation of these tooth crowns. This is consistent with a study of the lithic raw material sourcing for Solutrean levels at Gruta do Caldeirão, which determined that 3 of the 5 flint types have sources within 30km from the site (Matias, Aubry and Zilhao, 2019).

Whether the individuals were mobile within this range is especially difficult to discern based on the strontium isotope data alone, as any movement across the sedimentary basin could have been undertaken without alteration of the $^{87}Sr/^{86}Sr$ values of their tissues. It is important, therefore, that the data be considered in light of archaeological data from the region. A number of sites within the study area exhibit evidence for a Solutrean presence (Marks *et al.*, 1994), but only a few of these sites provide data from which settlement patterns and subsistence strategies can tentatively be derived – namely the open air site of Casal do Cepo, the Lapa do Anecrial, and the deposits of the Gruta do Caldeirão itself.

The open air site of Casal do Cepo is situated within the Tagus Basin, on a platform overlooking the valley of one of the tributaries of the Almonda River, approximately 40km to the south of the Gruta do Caldeirão. The emergency excavation, carried out in 1990, covered an area of approximately 50m^2 and yielded a characteristically Solutrean lithic assemblage - though no direct dates are available. Based on typological comparisons with similar assemblages, Casal do Cepo is estimated to date to approximately 20, 500 radiocarbon years BP. The lithic assemblage is composed primarily of flint, which makes up 90% of the raw material composition. Quartzite and quartz constitute 7% and 1% respectively, whilst other materials contribute the final 2% of all raw materials. Locally collected quartzite and quartz pebbles appear to have been used primarily for the production of flakes, some of which were retouched into tools such as scrapers, notches and denticulates. The flint assemblage is dominated by scrapers, points (including laurel leaves), knives and other side-edged utensils. The source of the raw flint remains undetermined as of yet, but it is suggested by Zilhão (1997) that it was likely collected from secondary deposits within the Tagus Basin. One particularly interesting aspect of the assemblage is the high occurrence of burnt broken points, perhaps implying that hafted tools whose points were broken during hunting activities were held over fires, thereby melting the resin so that broken bases could be removed from the wooden hafts. New points could then be reattached for later use. The presence of all stages of the Chaîne opératoire (including cores, débitage and tools), combined with the evidence for the repair of tools suggests that Casal do Cepo may have been residential in nature; a site at which raw materials were processed into tools that were subsequently used, broken, repaired and eventually abandoned. The use of local raw materials to produce flakes and tools ideal for butchering activities, when considered in light of the site's tactical proximity to the Tagus River, may suggest that the site was occupied with the intent of exploiting nearby nutritional resources, perhaps on a seasonal basis.

Lapa do Anecrial is located on the south slope of the Alvados' polje, approximately 340m above the modern day sea level and positioned within one of the natural passageways between the Atlantic Ocean and the Tagus Basin. Almeida *et al.* (2007) report on the excavation of the site and the subsequent discoveries. Gravettian, Terminal Gravettian and Solutrean levels have been identified; the latter is directly AMS dated to 20,520 ± 100 BP and corresponds to level Fa at Gruta do Caldeirão. The Solutrean assemblage is ephemeral, comprised of two lithic artefacts (a blade and a bifacial preform), four marine shells; one of which is pierced and burnt (saltwater periwinkles, *Littorina obtusata*), a small collection of ibex bones (though these may be derived from the underlying Terminal Gravettian layer), and a hearth filled with charcoal fragments and rabbit bones. Although small, the evidence from this layer may point to the use of the site as temporary waypoint for expeditions to the coastline from the Tagus Basin, and vice versa. The presence of the *Littorina* shells certainly implies the exploitation of coastal resources, and may even offer a tentative link to the Solutrean levels at Gruta do Caldeirão, where shells of this species have also been identified (Callapez, 2003). It likely acted as a temporary camp, at which local species such as rabbit were exploited.

Whilst studies of the lithic and faunal assemblages at Gruta do Caldeirão are ongoing and currently incomplete, the preliminary evidence supports the notion that the site was not an ephemeral campsite used briefly for hunting, but like Casal do Cepo, was occupied for longer periods of time. Results of analysis (Zilhão, 1997) report the presence of all stages of lithic reduction, suggesting that much of the Chaîne opératoire was carried out at the Gruta do Caldeirão. Few blanks are present, implying that tools were not brought to the site in a form ready to be used or adapted. On the contrary, nodules of flint were recovered from Solutrean layers, supporting the notion that raw materials were collected and brought back to the site to be processed. It follows that the site was likely occupied for longer periods of time, and indeed this is supported by the presence of the skeletal and dental remains of humans of a variety of ages, including adults, adolescents and infants. The presence of a range of human remains of varying ages – in particular, infants - suggests that family groups frequented the site, not just teams of specialised hunters (Zilhão, 1997).

Several other sites in the study region imply a human presence during the Solutrean phase, but their assemblages are too small to attempt to derive conclusions about their function and wider role in settlement patterns during the Solutrean. Nevertheless, it is worthwhile to briefly discuss their nature and location, in the context of both the strontium isotope data and the archaeological data discussed above. Located in the village of Arrudo dos Pisões are two open air sites, Passal and Olival de Arneiro. Excavated in the mid-19th Century, they were originally thought to represent Solutrean occupations, but under further investigation it was found that the majority of the lithic assemblages from both sites was accumulated during the Chalcolithic (Zilhâo *et al.*, 1995). Nevertheless, two patinated and typologically distinct barbed and tanged arrowheads from Passal imply a Solutrean presence in the area, and interestingly the sediment strontium isotope value nearby the location (Sr-28) is consistent with the average $^{87}Sr/^{86}Sr$ value of the enamel profiles of the Caldeirão 5 and 6 humans. Although the tools are unlikely to be in situ, this does imply a human presence (likely with the intention of hunting, considering that the tools are arrowheads) in this part of the Tagus Basin. Less than 20km to the northwest of Passal and Olival de Arneiro and within the Upper Cenomanian flint and silcrete catchment outlined by Matias, Aubry and Zilhao (2019) is Olival de Carneira, another open air site that has produced Solutrean material. Flint comprises 97% of the lithic raw materials in the Solutrean, with local quartz and quartzite comprising the remainder. All stages of the Chaîne opératoire are represented and are indicative of the production of flakes, blades and tools such as scrapers, though no laurel leaves or bifacially worked points were recovered. The assemblage is too small to derive further information about site function, but nevertheless points to a human presence in the vicinity of a major flint source within the Tagus Basin during the Solutrean, suggested by Matias *et al.* (2019) to be a potential flint source exploited by the human occupants of Gruta do Caldeirão.

One potential, alternative explanation that should also be borne in mind is the possibility that the strontium isotope profiles of these individuals may, in fact, be reflective of nursing signals. Whilst the weaning ages for human populations in the Upper Palaeolithic have not yet been investigated, it seems reasonable to suggest that children may have been nursed up to or beyond the age of five years. Indeed, Wickes (1953) observed that in modern Hawaiian indigenous groups, weaning does not occur until approximately five years of age, and that in Inuit communities, a weaning age of approximately seven is not unusual. A possible weaning age of six years is further suggested by (Dettwyler, 1995) for our hominid ancestors, based on the natural weaning ages of modern great apes. If these individuals were nursed for an extended period of time, the Caldeirão 5 and Caldeirão 6 strontium isotope profiles might be expected to be partly reflective of the $^{87}Sr/^{86}Sr$ signals of the mothers' milk, though the contribution of breast milk to the overall diet would likely be relatively minimal. Data pertaining to the source of calcium in human breast milk during nursing suggests that 5-10% of the skeletal mineral content is metabolised over a period of six months of continuous lactation (Kovacs, 2005), and as such it seems reasonable to hypothesise that the strontium isotope signature of the milk is likely to be homogenised due to mixing.

10.2.3 Magdalenian human

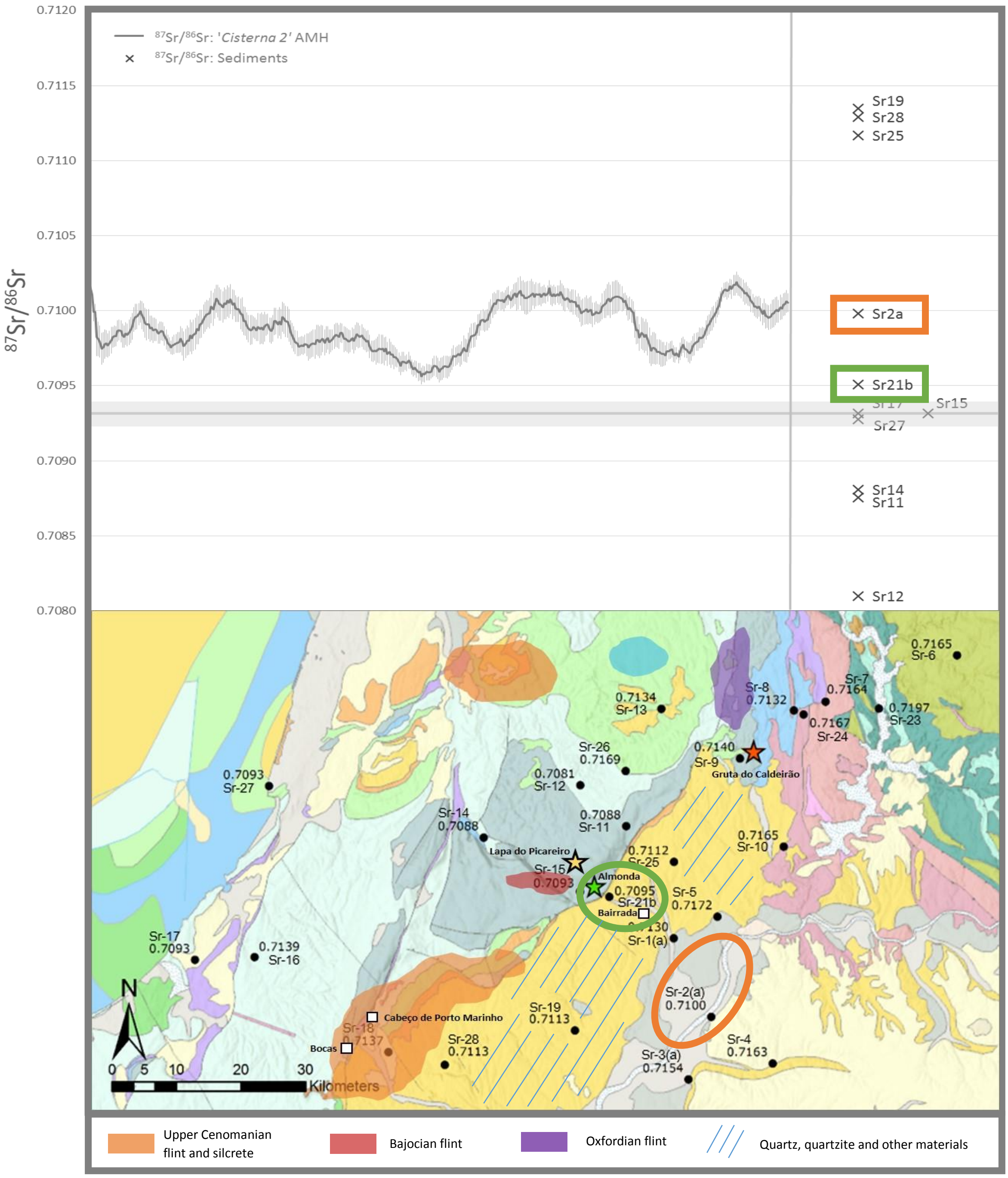


Figure 79. Sequential strontium isotope data for the Cisterna 2 individual, plotted alongside the sediment ^{87}Sr/^{86}Sr values for the study area (Pike et al. unpublished). The grey line denotes the average dentine Sr value at Gruta da Oliveira. The map provides the probable source locations of the raw materials available nearby. White squares denote Magdalenian open-air sites, and stars denote cave sites. The green and orange ovals represents regions with ^{87}Sr/^{86}Sr values consistent with those observed in the strontium isotope values of the Cisterna 2 individual.

The sequential strontium isotope data for the Magdalenian individual from Cisterna 2 is presented in Figure 79 alongside the geological map of the study area. Based on observations of human tooth formation times, this P_3 profile is likely to reflect three years, from the ages of 3.5 to 6.5 years (AlQahtani, Hector and Liversidge, 2010). The $^{87}Sr/^{86}Sr$ values repeatedly oscillate between approximately 0.7095 and 0.7100, suggesting that this individual consumed resources from two geologically distinct regions. Based on the $^{87}Sr/^{86}Sr$ values of the sediment and plant samples gathered in the study area, the peaks and troughs of the Cisterna 2 strontium isotope profile are consistent with the source of the Almonda River (Sr-21b) and the banks of the Tagus River (Sr-2a). The range size of the Magdalenian individual therefore falls within 30km of the Galeria da Cisterna (Figure 79). This is consistent with observations of raw material procurement in the contemporaneous Magdalenian layer at Lapa dos Coelhos (Gameiro, Aubry and Almeida, 2008). 62% of the lithic assemblage is comprised of flint, whilst the rest is made up of quartz and quartzite abundantly available in the direct vicinity of the site, in the form of rolled pebbles. Cenomanian flint, an excellent quality flint available within 15-20km of the site, accounts for 70% of all flint and 50% of total raw material in the Layer 3 assemblage. Oxfordian flint, which is available approximately 30km from the site in the Nabão Valley near Tomar, represents 12% of all flint and 7% of the total raw materials present. The origin of less than 1% of the flint assemblage could not be precisely identified, but belongs to the Bajocian and Bathonian types more than 50km from the site. Very little débitage of this type was recovered, suggesting it was worked elsewhere. As comparatively poor quality material, dedicated retrieval forays were perhaps not deemed worthwhile, with those artefacts that are present at Lapa dos Coelhos reflecting pieces occasionally collected by individuals during trips, and brought back to the Almonda karstic system. The isotopic and lithic evidence, then, supports a range size of approximately 30km at the outside, with the presence of flint from further afield offering the possibility that further distances were occasionally travelled, or that trading of materials took place.

The oscillation between two specific values may be reflective of seasonal mobility, whereby temporary sites were occupied in order to exploit specific resources depending on their seasonal availability. This inference is consistent with artefactual evidence from the Magdalenian layers at the Lapa dos Coelhos (adjacent to the Galeria da Cisterna), where fish hooks and broken projectile points combined with the presence of fish vertebra and the pierced terrestrial mammal bones hint at the hunting of specific quarry. Very little lithic refitting could be achieved, and this - combined with the fact that a high proportion of blanks were recovered from Magdalenian levels - suggests that much of the Chaîne opératoire was carried out elsewhere, with flakes brought to the site to be retouched for specific functions. The same is evident at Galeria da Cisterna itself, where the most abundant artefact type in the late Magdalenian layer (Layer 3) is flakes.

Further evidence for this form of mobility pattern can be found at the cave site of Lapa do Picareiro, located just a few kilometres away from the Almonda karstic system at an altitude of 540 metres above sea level. Described in depth by Bicho *et al.* (2006), the cave itself is a space measuring 10 metres in length and 8 metres in width, and contains layers corresponding to the Upper Palaeolithic through to the Holocene. Two layers (F and G) date to between 12,300 and 11,800 BP, correspond to the Magdalenian deposit (Layer 3) at Galeria da Cisterna. These deposits are characterised by the presence of hearths, situated at the back of the cave in both levels - in Level F, the hearth is accompanied by thousands of rabbit bones, hundreds of medium and large ungulate bones, and hundreds of pieces of charcoal, whilst in layer G, the hearth is accompanied by same array of bones and artefacts, but also numerous fish vertebra from sardine or shad. The lithic and faunal assemblages are suggested to be evidence of the use of the cave as a specialised, temporary camp.

The faunal assemblages from both levels are varied, including the remains of rabbits, red deer, roe deer, wild boar, aurochs, ibex, fish, and shellfish. Numerically, rabbit remains are the most abundant in layers F and G (which contain approximately 73% of the total rabbit specimens at Lapa do Picareiro). A good representation of all the skeletal elements, a high occurrence of long bones with the epiphyses removed, and an equally high occurrence of burning all suggest that this taxon was cooked and consumed on site, perhaps roasted over hot coals. The large mammal remains, on the other hand, illustrate the transport of certain elements out of the cave. In Layer F, the cranial elements of the wild boar are better represented than other elements, suggesting that the bulky heads were removed and the rest of the carcass was carried away from Picariero, with some on-site consumption reflected by the presence of infrequent lower limb bones. Red deer appear to have been processed differently, with a greater representation of limb bones except for the femur, whilst ribs, pelves and vertebrae are less well represented. It is suggested that meat may have been removed from the limb bones for the process of drying or smoking to be transported elsewhere, as well as for the removal of marrow from the medullary cavities, which is evidenced by fracture patterns. The presence of fish remains at such a high altitude site is suggested to be indicative of hunters carrying small, light 'snack foods' that could sustain them during the trip. Skeletal representation of these remains is reminiscent of ethnographical examples of hunter gatherer consumption of fish, whereby the vertebrae are removed and the heads and flesh are consumed (Bicho, Haws and Hockett, 2006).

The lithic assemblage provides further evidence for the use of the site as a temporary camp. 80% of the assemblage in Layers F and G is composed of chips smaller than 4mm, which are indicative of tool sharpening and retouching. Very few cores are present at the site, suggesting that the early reduction phases of the Chaîne opératoire were carried out elsewhere. Those that *are* present are small, and appear to have been brought to the cave ready-shaped for the production of standardised blanks.

Weaponry tips make up 40% of the retouched tools; many of which show clear signs of impact fractures. Their presence in the Tardiglacial deposits of Lapa do Picariero imply that they were brought into the cave after being broken; perhaps still imbedded within the animal carcasses. The lithic assemblage, then, suggests that early reduction sequences were not carried out at the site, and hence it was not likely to have been occupied for significant lengths of time. The broken projectile points imply that the cave may have had a specific function, likely such as targeted hunting of specific species (Bicho, Haws and Hockett, 2006).

When considered together, the faunal and lithic assemblages point to the use of the site as a temporary camp, used for the processing of red deer and wild boar carcasses that, considering the altitude of the site, were likely killed elsewhere. They were subsequently butchered and transported elsewhere; perhaps to a more residential site. Rabbits were likely hunted or trapped in the vicinity of the cave, and as evidenced by the frequency and representation of the skeletal remains, many were consumed at the site. Very little primary reduction took place at the site, but tools appear to have been sharpened and retouched for use, with many points broken in high impact situations. Cementum annuli and osteological evidence from fauna Layer F suggests that both the red deer and wild boar were killed in late autumn and early winter; implying that Lapa do Picareiro may have been repeatedly visited and used as processing camp for the butchering of seasonally available species (Bicho *et al.*, 2000). Lapa do Picareiro, Lapa dos Coelhos and Galeria da Cisterna may all represent sites with similar functions.

If the above sites are indicative of temporary seasonal camps in the study area, is there any evidence of sites more akin to residential camps in Portuguese Estremadura? Several sites in the study region do exhibit evidence for more prolonged occupation during the Tardiglacial period, including the open air site of Cabeço de Porto Marinho (CPM) and the cave site of Bocas. At CPM, 18 separate Magdalenian occupations have been identified across multiple loci, with those in the upper level of CPMIII and the middle level of CPMIIIS dating to between 12 000 BP and 11 000 BP (Bicho, 2002). A high density of undifferentiated combustion structures spread laterally is suggested to represent a palimpsest of successive occupations, reminiscent of repeatedly-built earth ovens observed ethnographically in hunter-gatherer societies. All stages of the lithic reduction sequence are present, with production geared towards the creation of flakes, microliths and blades, suggesting that the site must have been occupied for a more substantial amount of time. The proximity of the site to a reliable water source, raw material sources and hunting grounds would have made this a valuable tactical location, that was perhaps occupied seasonally (Zilhão, 1997). Similar characteristics are observed at the open air, multi-loci site of Bairrada, approximately 2km away from the town of Torres Novas. Like CPM, the site is situated in a strategic location in close proximity to drinking water and hunting grounds. The lithic assemblage at Bairrada exhibits aspects of every stage of the Chaîne opératoire, and a great quantity of burnt material similar in nature to the ‘earth ovens’ of CPM, pointing to the occupation of Bairrada as more residential in nature than the type of occupation observed at the nearby cave sites of the Almonda karstic system (Zilhão, 1997). Further evidence comes from the cave site of Abrigo das Bocas in the Rio Maior region, where two Magdalenian levels, ‘Fundas’ and ‘1’ are considered to reflect residential occupations. High concentrations of lithic material, including all stages of the Chaîne opératoire and a considerable presence of quartz and quartzite cores are thought to be indicative of residential occupation; particularly in light of the large accumulations of estuarine shells that accompany them. The position of the site within a deep canyon perhaps hints at its function, as the narrow valley acts as both a passage between the Tagus Basin and the coast, and as an ideal ambush for taxa such as red deer, ibex and aurochs (Bicho, 1993).

The archaeological data available for the Tardiglacial period in Portuguese Estremadura points to the use of two types of sites by human groups – short term, specialised camp sites such as Lapa dos Coelhos, Lapa do Picareiro and Galeria da Cisterna, and longer-term residential sites such as CPM, Bairrada and Abrigo das Bocas. The residential sites appear to be positioned close to major raw material sources, where primary lithic reduction stages took place. The smaller, temporary sites at higher altitudes appear to have been utilised mainly as carcass butchering sites, where the meat, hides and bones of taxa hunted nearby were processed for transport to the larger residential sites. In light of this, how might the Cisterna 2 strontium isotope profile be interpreted? The data appear to show fluctuation between values of approximately 0.7095 and 0.7100, which reflect the geological substrates in the locality of the Almonda karstic system and the banks of the Tagus River, respectively. These data could reflect mobility between two seasonally occupied residential sites, whereby food was hunted nearby and processed at logistical camps such as Lapa do Picareiro, before being brought back to the base camp. Alternatively, the data could reflect the permanent occupation of one residential site year-round, with seasonal forays to logistical camps either in the Serra d’Aire or along the banks of the Tagus River. It is difficult, however, to discern this without complimentary oxygen isotope data for the Cistern 2 individual, and without seasonality data for residential sites such as CPM, Bairrada and Bocas.

10.3 Combining human and faunal data

Are the mobility patterns of human groups occupying Portuguese Estremadura during the Middle and Upper Palaeolithic periods the result of the mobility and availability of fauna? Is it possible to associate certain geographical regions with the presence of certain species of fauna at specific times of the year, and if so, is it possible to detect seasonal human exploitation of these resources? The following section aims to address these questions by assessing the strontium isotope profiles of the Mousterian, Solutrean and Magdalenian human tooth enamel samples, in light of the sequential strontium and oxygen isotope data gathered through the analysis of the molars of contemporaneous terrestrial mammal species.

Without sequential oxygen isotope data for the human tooth enamel, it is not possible to seasonally constrain the strontium isotope profiles for the Neanderthal and AMH individuals. However, by examining the $^{87}Sr/^{87}Sr$ profiles of the humans and comparing it with those of the fauna (and considering these data in the context of the archaeological evidence discussed in the previous chapter), it may be possible to begin to explore the relationships between these human groups and the terrestrial mammals with whom they shared the Pleistocene landscape.

10.3.1 Mousterian

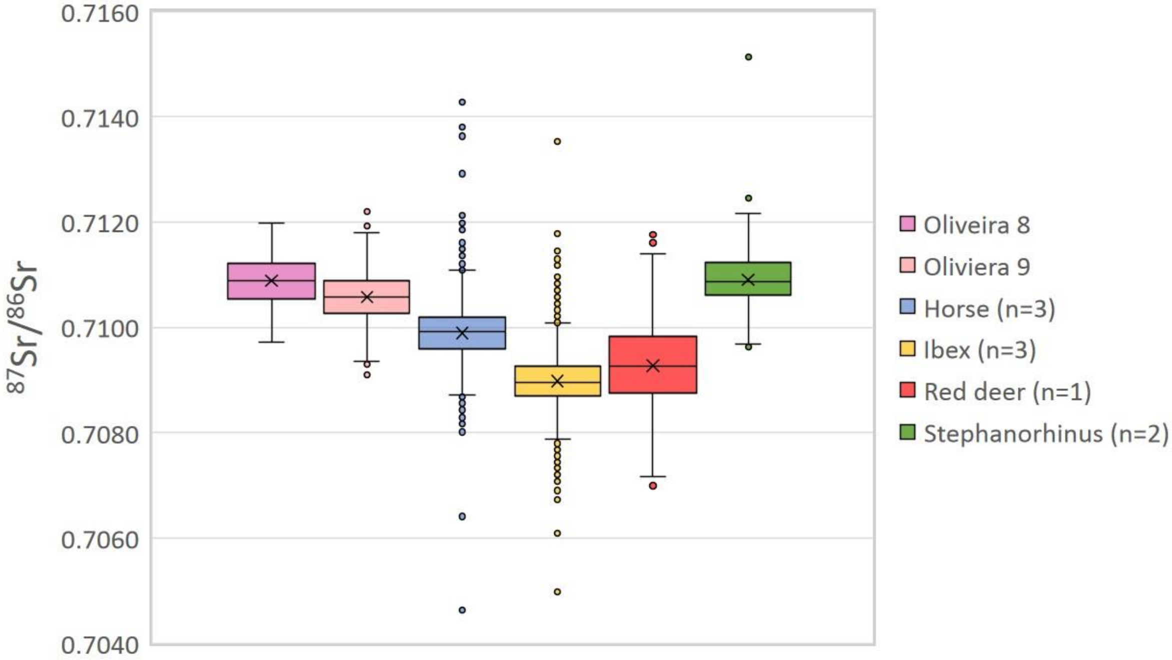


Figure 80. Box plot of strontium isotope data for Mousterian humans and fauna.

In total, the strontium and oxygen isotope profiles for the teeth of two Neanderthal individuals (Pike *et al.* unpublished), one *Cervus elaphus* individual, two Caprids, three *Equus sp*. individuals and two Stephanorhinus individuals have been gathered. All of the tooth crowns from the Mousterian fauna analysed in this study exhibit enamel strontium isotope profiles with $^{87}Sr/^{86}Sr$ values consistent with the bioavailable strontium isotope values in the study region, between approximately 0.7080 and 0.7200. Each species, however, appears to exhibit varying degrees of mobility during this period (see Figure 80).

Red deer remains often dominate Middle Palaeolithic faunal assemblages; indeed they are the most abundant species present in Mousterian deposits at Gruta da Oliviera. The strontium isotope profile of the second and third premolars of the *Cervus elaphus* individual recovered from Layer 20 implies consumption of vegetation from an area in the direct vicinity of the Almonda karstic system, whilst the fourth exhibits much less radiogenic values that do not appear to be reflective of any geological deposit in the study area. Potential evidence for seasonal movement can be observed in the P2 and P3, where summer peaks in $\delta^{18}O$ coincide with less radiogenic $^{87}Sr/^{86}Sr$ values, and winter $\delta^{18}O$ troughs coincide with the most radiogenic $^{87}Sr/^{86}Sr$ values. However, since only one Mousterian *Cervus elaphus* individual was successfully analysed, it is not possible to know whether the strontium and oxygen isotope profiles of this individual are representative of wider red deer behaviour during the Middle Palaeolithic. As such, it is difficult at this stage to investigate the relationship between the Neanderthal occupants of Gruta da Oliviera and the *Cervus elaphus* population, and to what degree it may have been seasonal in nature.

The three ibex molars from Layer 20 at Gruta da Oliviera exhibit strontium isotope profiles that are consistent with mobility between higher altitudes along the Serra d'Aire, and lower altitudes in the vicinity of the modern day coastline and/or the Almonda karstic system itself; essentially subsisting within a 40km radius of the site. The sinusoidal nature of the strontium isotope profiles implies repeated alternating consumption of vegetation growing at higher and lower altitudes; consistent with observations of the seasonal altitudinal mobility of modern Iberian ibex populations. The oxygen isotope profiles exhibit several annual cycles, suggesting that the individual(s) were present in the study area year-round, and would have likely been a valuable nutritional resource for the Neanderthal occupants of the cave. Interestingly, though, neither of the Neanderthal tooth crowns from Layer 22 exhibit $^{87}Sr/^{86}Sr$ values similar to those exhibited by the ibex individuals; indeed, the former are more radiogenic and perhaps reflective of the Eocene sedimentary basin as opposed to the higher altitudes of the Serra d'Aire. This may suggest that the Neanderthal individuals did not regularly consume plant-based resources from the same locations as the ibex, though the presence of ibex remains within the Mousterian deposits at the site does suggest that this species was exploited by Neanderthal groups either for food (in which case the low strontium contribution from ibex meat would likely not be visible in the Neanderthal enamel) or for their hides, or both. Because all three molars were recovered from the same layer, they may belong to the same individual, and as such this is too small a sample size to infer the behaviour of the wider ibex population during the Mousterian.

The strontium isotope profiles of the three horse molars recovered from Layers 22 and 24 at Gruta da Oliviera are relatively consistent with one another, giving average $^{87}Sr/^{86}Sr$ values consistent with geographical samples taken on the banks of the Tagus River and the Neogene sedimentary basin. Based on the observed behaviour of modern wild and feral horses, which are known to occupy home ranges that provide a water source, shelter and suitable grazing areas, the Tagus basin was likely an ideal territory for *Equus* populations during the Mousterian. The oxygen isotope profiles of the molars do not exhibit the degree of variation observed in other species, perhaps due to physiological differences or differences in water intake behaviours. No clear seasonal signals can be observed, and it is difficult, therefore, to discern whether or not these individuals were present in the study area year-round. Nevertheless, the strontium isotope values across both of the Neanderthal tooth crowns are consistent with those exhibited by the horse crowns, suggesting that either the two species subsisted upon the same geological substrates and consumed vegetation from the region, and/or that the Neanderthal population consumed the meat of equids on a regular basis. The existence within the Tagus Basin of open air sites such as Ponte da Pedra, Fonte da Moita and Santa Cita suggests a Neanderthal presence less than 10km from the suspected habitat of the equids analysed in this study. Based on lithic evidence, those sites are frequently interpreted to be logistical camps, and as such it seems reasonable to suggest that if they were contemporaneous with the occupation of Layer 22 at Gruta da Oliviera, they may well have acted as camps from which herds of horses were hunted for subsequent butchery and transportation. The presence of butchered horse bones at Foz do Enxarrique, a Mousterian open air site situated further upstream on the banks of the Tagus, lends support to the notion that camps such as Ponte da Pedra and Fonte da Moita could have been used to target species such as horse on a seasonal basis.

The *Stephanorhinus sp.* individuals exhibit strontium isotope profiles consistent with occupation of the Eocene sedimentary basin and the banks of the Tagus River. Extant rhinoceros are perhaps the most suitable analogues in terms of mobility and behaviour, though it must be borne in mind that they occupy areas such as wildlife reserves that are, in places, affected by human activity. White rhinoceros (*Ceratotherium simum simum*) are generally solitary, occupying marked territories. Male and female territories differ in their extent, with females in Kruger National Park observed to occupy overlapping territories of 7-45 km^2, whilst males in the same region occupy smaller territories of 6-14 km^2 (Pienaar, 1994). The range of $^{87}Sr/^{86}Sr$ values observed in the strontium isotope profiles of both individuals are consistent with home ranges of up to 45km in the Eocene sedimentary basin, which is relatively homogenous geologically. In female white rhinoceros, a degree of seasonal movement has been observed in the northern white rhino (*Ceratotherium simum cottini*), but in Kruger National Park the only seasonal change in territory appears to be a reduction in range size during the dry season amongst females (ibid). One of the two Mousterian *Stephanorhinus sp*. molar specimens appears to show vry slight covariance between the strontium and oxygen isotope profiles, with higher $\delta^{18}O$ values appearing at the same position in the tooth crown as less radiogenic strontium isotope values, and lower $\delta^{18}O$ values coinciding with more radiogenic strontium isotope values. This may reflect a slight degree of local mobility according to season, or perhaps a shift in the availability of suitable vegetation, but without a larger sample size it is difficult to explore this further. Nevertheless, both *Stephanorhinus sp*. molars exhibit $^{87}Sr/^{86}Sr$ values that are consistent with subsistence within the study area for the duration of the formation period of the tooth crown, and as such would have been readily available prey for the Neanderthal occupants of the Almonda karstic system. The strontium isotope profiles of the *Stephanorhinus sp*. and both Neanderthal individuals overlap considerably in places, suggesting that the Oliviera Neanderthals and the *Stephanorhinus sp.* individuals consumed vegetation growing upon the same geological substrates in the Tagus river basin, or that the Neanderthals consumed fauna that subsisted upon those substrates, or both. Again, the open air sites of Ponte da Pedra and Fonte da Moita provide evidence that the regions reflected in the strontium isotope profiles of the Oliveira 8 and Oliveira 9 individuals were indeed utilised by human groups, likely as camps from which hunting activities could take place. At Foz do Enxarrique, rhinoceros remains are suggested to have accumulated naturally, but it seems reasonable - considering the presence of *Stephanorhinus* remains in the craggy caves of the Almonda karstic system – to suggest that open air sites such as Ponte da Pedra and Fonte da Moita may have played a part in the hunting or scavenging of this taxon.

Because the study of the faunal remains and lithics recovered from Gruta da Oliviera has not yet been completed, it is difficult to fully understand its function on an archaeological basis alone. Nevertheless, the strontium and oxygen isotope data available here, when considered in light of the archaeological evidence from nearby sites, may offer new insights into the role of the site as part of the wider Neanderthal settlement patterns. Neither of the Neanderthal individuals appear to have subsisted significantly upon the resources available in the craggy habitats of the Central Limestone Massif during the time of molar formation, since their strontium isotope profiles exhibit consistently more radiogenic values than those observed in both the Serra d'Aire sediment samples and in the ibex specimens. Since ibex remains are present in the Mousterian deposits of Gruta da Oliviera, it seems likely that they were consumed locally, but perhaps not on such a considerable basis as other resources. Interestingly, even though red deer are the most abundant anthropogenically accumulated taxon in these deposits, the strontium isotope profiles of the Neanderthals and red deer do not overlap considerably. This implies that red deer and Neanderthal groups did not consume vegetation from the same geological region, but since the remains of red deer are so abundant at the site, they certainly seem to have comprised a significant part of the diet (or, alternatively, were heavily exploited for other resources such as hides). Consuming red deer meat would not necessarily result in the passing of noticeable strontium isotope signals into the body plasma of Neanderthals, since meat contains low concentrations of strontium (see Section 4.1.1.2). Nevertheless, the analysis of further Mousterian red deer specimens would provide a better insight into the geographical range of herds. It seems, then, that the Gruta da Oliviera Neanderthals subsisted to a considerable extent upon the geological substrates of the Tagus Basin and along the banks of the river itself; supported both by the consistency of the $^{87}Sr/^{86}Sr$ data observed in their molars and sediment samples, and by the presence of Mousterian open air sites in those localities. Whilst they may not be entirely contemporary with the Mousterian occupation of Gruta da Oliviera, these sites do suggest that camps were occupied in close proximity to the locations of species such as horse and *Stephanorhinus*, as suggested by the strontium isotope data. Since such a range of prey species were identified both within the Mousterian deposits at Gruta da Oliviera and at the open air site of Foz do Enxarrique, and since features considered to be indicative of possible habitation structures have been identified at open air sites such as Santa Cita, it seems reasonable to suggest that Neanderthal groups occupied these areas not just in ephemeral camps, but perhaps in slightly more residential ways. Based on the strontium isotope profiles of both individuals, some areas appear to have been revisited during the formation period of the molar crowns, though whether this was on a seasonal basis remains to be seen without accompanying oxygen isotope data from the same teeth. Nevertheless, as observed at the Bavarian site of Sesselfelsgrotte (Richter, 2006), the available isotopic and archaeological evidence in Portuguese Estremadura seems to tentatively point to a mixture of residential and logistical mobility, where tactical sites were inhabited for long enough to hunt local species and leave behind traces of habitation structures, but where food and lithic raw materials were also transported from their origins in the Tagus Basin to sites such as Gruta da Oliviera in the Serra d'Aire.

10.3.2 Solutrean

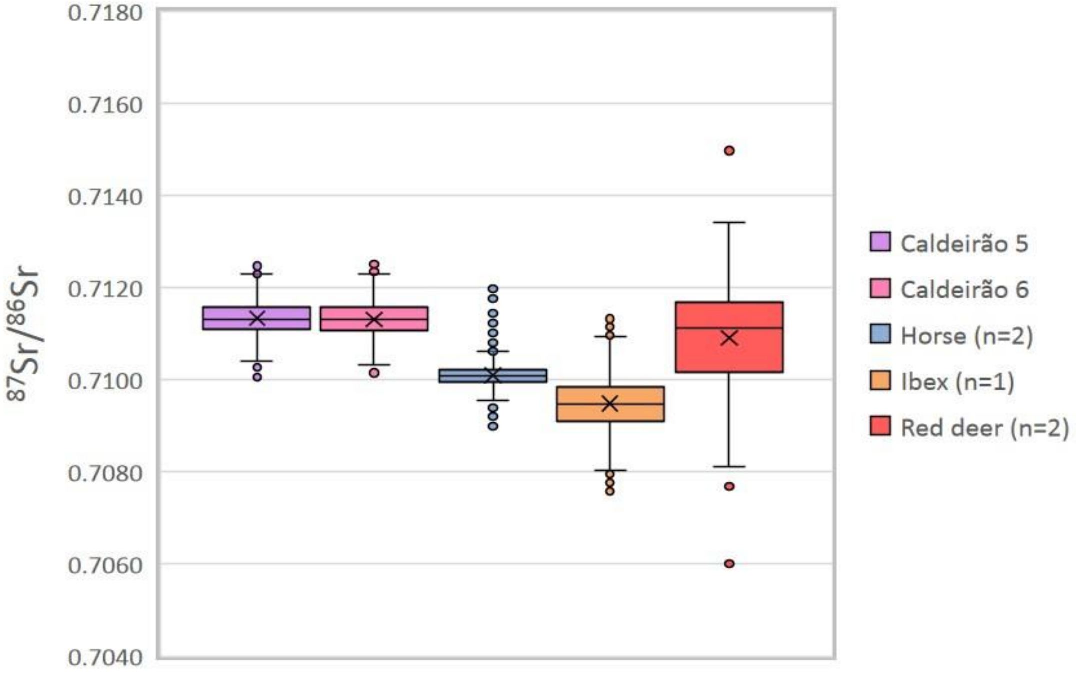


Figure 81. Box plot of strontium isotope data for Solutrean humans and fauna.

The strontium isotope profiles of two *Equus* individuals, one *Capra* individual and two *Cervus elaphus* individuals are available for comparison for the strontium isotope data acquired for the Caldeirão 5 and Caldeirão 6 humans (Figure 81). As discussed previously, all of these tooth crowns exhibit $^{87}Sr/^{86}Sr$ values that are consistent with the study region. The relatively homogenous strontium isotope profiles of the two Solutrean human individuals may imply mobility limited to the Neogene sedimentary basin during the formation of the tooth crowns or, alternatively, little mobility at all.

Of the fauna, only the red deer strontium isotope profiles overlap considerably with those of the Gruta do Caldeirão humans, at $^{87}Sr/^{86}Sr$ values between approximately 0.7110 and 0.7113. Such values are consistent with the Neogene sedimentary basin, suggesting that the human individuals from the Gruta do Caldeirão may have consumed resources from the same region upon which the red deer subsisted for at least part of the year, and/or that the humans directly consumed significant quantities of red deer meat after hunting them across the Tagus Basin. Open air sites such as Casal do Cepo on the west bank of the Tagus River, occupied around 20, 500 BP, certainly imply that human groups were present in the area – and, as evidenced by the high concentration of broken projectile points, seem to have hunted in the area. Since both Casa do Cepo and Gruta do Caldeirão have been interpreted as residential sites that were likely occupied for significant amounts of time, perhaps on a seasonal basis, it seems reasonable to suggest that the homogenous strontium isotope profiles of the Solutrean humans do indeed reflect mobility, primarily within the geology of the Tagus Basin. Considering the fact that red deer comprise 52% of the Solutrean faunal assemblage (Davis, 2002), it seems reasonable to suggest that their movements and behaviour may have had significant bearing on the subsistence strategies, settlement organisation and mobility patterns of the human population. The oxygen isotope data gathered for the Solutrean red deer certainly seem to suggest that these animals were moderately mobile on a seasonal basis – perhaps they were targeted at specific times of the year in order to exploit them in a particular tactical region, or in order to take advantage of the seasonal condition of individuals within the herd.

The strontium isotope profiles of the two Solutrean horse individuals are relatively homogenous, with average strontium isotope values of approximately 0.7100 (much like the Mousterian equids from Gruta da Oliviera). Based on the bioavailable strontium isotope map of the study area, the most likely area of occupation for these individuals is therefore the banks of the Tagus River. The strontium isotope profiles of the Gruta do Caldeirão humans do not seem to exhibit the same values, suggesting that plant-based resources were not shared by humans and horses across this geology. The location of the site of Casal do Cepo does imply, however, that the banks of the river were occupied by human groups during the Solutrean period, and horses would have been available nearby. Even if horses were consumed, their meat likely did not contribute enough to the overall diet of the Gruta do Caldeirão humans for the $^{87}Sr/^{86}Sr$ values of their enamel to be significantly swayed towards values consistent with the banks of the river, since meat has such a low strontium concentration (see Section 4.1.1.2). The overall decrease in the representation of equid remains from the Mousterian through to the Magdalenian at Gruta do Caldeirão suggests that they may not have contributed significantly to the diet - indeed, equid remains make up only 9% of the faunal remains in Solutrean levels at Gruta do Caldeirão (Davis, 2002).

The strontium isotope profile of the Solutrean ibex exhibits considerably less radiogenic values than the Gruta do Caldeirão humans, perhaps reflecting occupation of the higher altitudes and rocky crags of the Central Limestone Massif. The $^{87}Sr/^{86}Sr$ values measured in the Solutrean human tooth enamel, however, do not seem to reflect the same underlying geological substrate, and as such they likely did not consume plants from this area. Since Caprid remains were recovered from the Solutrean deposits at Gruta do Caldeirão, comprising approximately 16% of the assemblage, it does appear that these animals contributed to the diet (or alternatively were targeted for their hides, or other non-consumable resources). The presence of sites located along the Central Limestone Massif, such as Lapa do Anecrial, does imply that these areas were occupied during the Solutrean, and perhaps locally adapted species such as ibex were targeted and processed for one or both of these reasons.

Because red deer and rabbit remains are the most abundant species at in the Solutrean deposits at sites such as Gruta do Caldeirão (Davis, 2002), it seems reasonable to suggest that these resources, and the availability of raw materials for the production of tools, may have affected the decisions made by humans in regards to where settlements and camps were positioned. Although other taxa such as horse and ibex are present in the Gruta do Caldeirão assemblage, they do not seem to have been as important as red deer and rabbit at the site in terms of exploitation – though it should be borne in mind that this does not necessarily reflect the overall diet of the Solutrean human inhabitants of the study area, as other resources may have been targeted elsewhere. Carbon and nitrogen table isotope analysis of the Caldeirão 5 and 6 individuals (Section 3.5.3) suggests that aquatic protein may have contributed to their diets, despite the scarcity of fish bones at the site – one may speculate that if fish were caught and consumed, their skeletal remains could have either been abandoned elsewhere, or may simply not be represented in the archaeological record due to poor preservation. Nevertheless, the strontium isotope data seem to suggest that the Neogene sedimentary basin was a particularly important region for these individuals, and as the $^{87}Sr/^{86}Sr$ values are consistent with those observed in the red deer, this may be a result of systematic hunting of the species. The seasonal behaviour of the red deer may have, in turn, prompted seasonal residential moves across the isotopically homogenous Neogene sedimentary basin.

10.3.3 Magdalenian

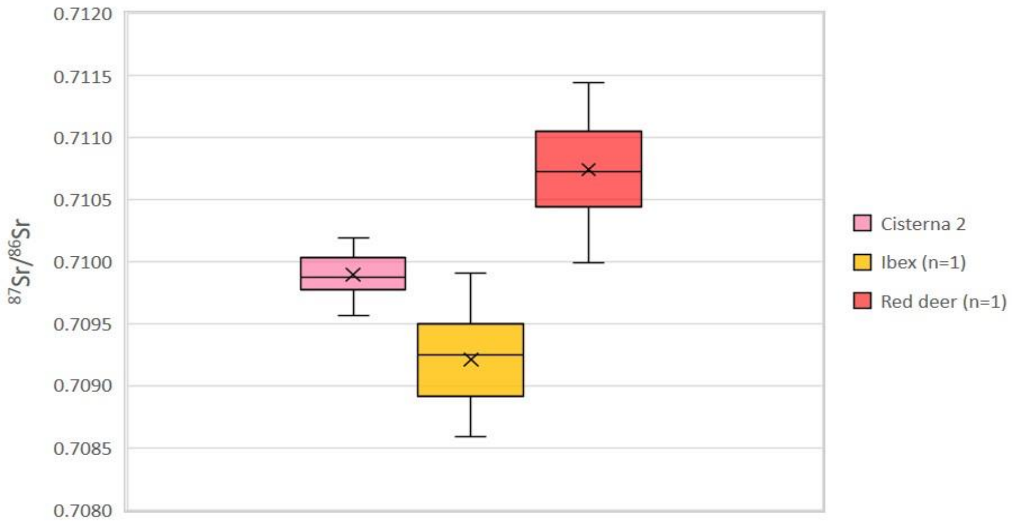


Figure 82. Box plot of strontium isotope data for Magdalenian humans and fauna.

The faunal assemblage from the Magdalenian contexts at Galeria da Cisterna is relatively limited, and as such, strontium isotope data from only two specimens (one *Cervus elaphus* and one *Capra sp*.) are available for comparison with the contemporaneous human premolar, 'Cisterna 2' (Figure 82). The red deer individual appears to be mobile between two geological substrates in the lowlands of the study area; the Neogene sedimentary basin and the banks of the Tagus River. Oxygen isotope data suggest that this mobility is seasonal in nature, with the individual subsisting upon vegetation along the banks of the Tagus during the summer months, and the Neogene sedimentary basin during the winter months. These $^{87}Sr/^{86}Sr$ values are available within a 30km radius of the site, and as such, the individual was likely present in the study area year-round. The *Cervus elaphus* $^{87}Sr/^{86}Sr$ values are, on the whole, more radiogenic than those of the Cisterna 2 human, suggesting that they mostly consumed foods from different geological substrates. Interestingly, however, the most radiogenic $^{87}Sr/^{86}Sr$ values observed in the strontium isotope profile of the Cisterna 2 human overlap slightly with the summer $^{87}Sr/^{86}Sr$ values of the red deer. This could **a)** be a result of both species consuming plant foods from the same geological area, and/or (considering the dominance of red deer remains in large herbivore assemblages of nearby Magdalenian deposits), **b)** reflect the targeted hunting of red deer groups during the summer and autumn months – though without oxygen isotope data for the Cisterna 2 individual, and without cementum annuli data for the determination of season of death, this is purely speculation.

The Iberian ibex exhibits considerably less radiogenic $^{87}Sr/^{86}Sr$ profiles across the molar array than the red deer, consistent with bioavailable strontium samples taken in the vicinity of the Serra d'Aire and likely reflecting occupation of rocky outcrops along the Jurassic limestones of the Central Limestone

Massif. The observed inter-tooth variation in $^{87}Sr/^{86}Sr$ implies a degree of mobility between the higher altitude regions of the massif and the lower altitudes in the direct vicinity of the mouth of the Almonda River was undertaken by the individual, consistent with observations of extant wild goat engaging in seasonal altitudinal mobility. Whilst the strontium isotope profile of the ibex is generally less radiogenic than that of the Cisterna 2 human, the profiles of both species do overlap between 0.7095-0.7097 (Figure 82). These $^{87}Sr/^{86}Sr$ values are consistent with bioavailable strontium isotope values in close proximity to the Almonda karstic system itself. If the strontium isotope profile of the Iberian ibex presented here is representative of the local ibex population, then this certainly suggests the presence of humans and ibex in the same geological catchment, and as such may reflect the use of the Galeria da Cisterna as a location for the targeted hunting of Iberian ibex. Without oxygen isotope data for the Cisterna 2 individual, however, it is difficult to know whether humans and ibex occupied the same space at the same time of the year.

Equus sp. are not represented in Magdalenian faunal assemblages at Galeria da Cisterna or Lapa dos Coelhos. It is possible that this is due to taphonomic processes and the relatively small very assemblage sizes, but their absence is consistent with an overall reduction in the representation of horses in Magdalenian layers at Gruta do Caldeirão, where they represent only 6% of the large herbivores (Trinkaus *et al.*, 2011).

Taking into account the archaeological evidence gathered from other Magdalenian sites in the study area, it seems likely that the mobility of the Cisterna 2 human was driven at least in part by the seasonal availability or condition of the red deer. Stable isotope data for the Cisterna 2 individual (Linscott, 2015; and Chapter 2) suggests that terrestrial herbivores certainly comprised a large part of their protein intake, alongside freshwater fish. At Lapa do Picareiro, cementum annuli analysis of Magdalenian red deer and wild boar imply autumn or early winter deaths, and considering the specialised nature of the lithic toolkits identified at sites such as Lapa so Picareiro and Lapa dos Coelhos, it seems feasible that certain sites were utilised by Magdalenian groups as temporary camps for the exploitation of seasonal resources. Since the Magdalenian red deer individual from Lapa do Coelhos exhibits a strontium isotope profile consistent with occupation of the Tagus Basin, and human presence is documented at nearby open air sites such as Bairrada, it may be that lower altitude sites were utilised as camps from which red deer hunting parties could be launched, whilst higher altitude sites in the Serra d'Aire were used in order to target species such as ibex. Use of these sites may have been highly seasonal, with some resources such as locally trapped or hunted rabbits and fish consumed on site, and the bulk of the large mammal carcasses transported away to larger residential camps, as observed at Lapa do Picareiro.

10.4 Temporal change in human mobility

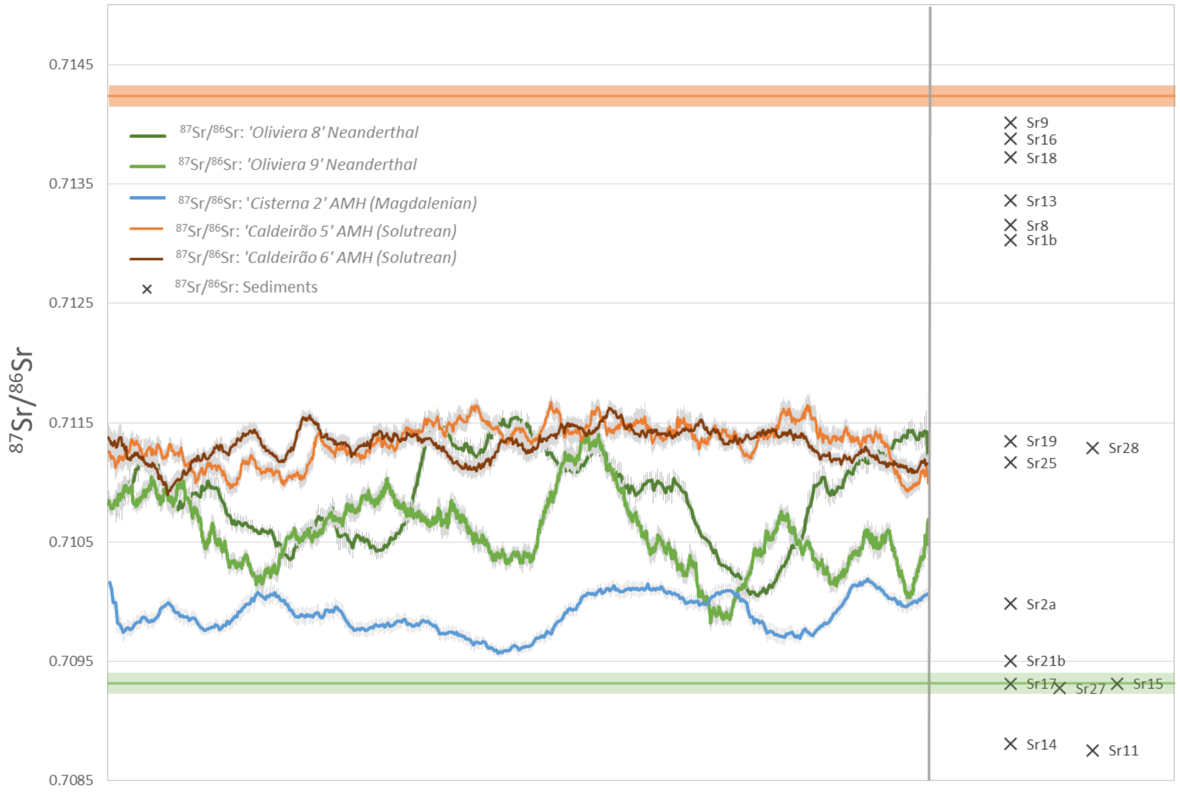


Figure 83. Strontium isotope profiles of the two Neanderthals from Gruta da Oliveira, two Solutrean humans from Gruta do Caldeirão, and the Magdalenian individual from Galeria da Cisterna. The green band represents the local 87Sr/86Sr value of the Almonda karstic system, and the orange band represents the local 87Sr/86Sr value of Gruta do Cadleirão.

Do the mobility patterns and subsistence strategies of Neanderthals and anatomically modern humans differ between the Middle and Upper Palaeolithic of Portuguese Estremadura? This has wider implications for our understanding of cognition, ecology, and behaviour of human groups through the Middle and Upper Palaeolithic, and has the potential to further inform our understanding of the apparent disappearance of the Neanderthals in the region. In order to address this question, it is necessary to bring together all available strands of archaeological and isotopic evidence across sites in the study area.

Over the last several decades, zooarchaeological evidence and bulk collagen stable isotope data have led many to argue that the diversification and expansion of the diets of anatomically modern humans during the Upper Palaeolithic to include, fish, plants and small game alongside large terrestrial herbivores gave them a selective advantage over Neanderthals, who elsewhere across Europe are suggested to have obtained much of their calorific intake from large mammals such as mammoth, aurochs, reindeer and horse (Richards and Trinkaus, 2009; O'Connell; 2006; Hoffecker, 2009). The zooarchaeological evidence from Middle Palaeolithic sites in Portuguese Estremadura such as Gruta da Oliviera and Foz do Enxarrique certainly lends support to the notion that large game such as red deer, horse and rhinoceros contributed significantly to the diet of the Neanderthal occupants of the region, but the presence of burnt tortoise shell remains in the lower levels of the Mousterian deposits at Gruta da Oliviera suggest that the exploitation of small game was not solely the domain of anatomically modern humans. Nevertheless, in the Upper Palaeolithic deposits of sites of Gruta do Caldeirão, Lapa dos Coelhos, Lapa do Picareiro and Galeria da Cisterna, the dominance of rabbit remains and apparent decline over time in large terrestrial mammals such as horse and ibex in comparison to Mousterian deposits at Gruta da Oliviera does seem to point to an increasing reliance upon small game from the Mousterian through to the Magdalenian periods. It has been suggested that this resource diversification may have been a response to a decrease in the availability of large game as a result of climate change – hence, with the reduced availability of large prey mammals required to adequately sustain human groups, subsistence strategies shifted to focus upon small game and intensive carcass processing such as bone marrow extraction and grease rendering (Manne *et al.*, 2012; Hockett and Haws, 2009b). Preliminary evidence from Portuguese Estremadura seems to support his notion, but since there is ample evidence for Neanderthal exploitation of small game and aquatic species in Portugal, it seems pertinent to suggest that rather than AMH populations targeting a previously unexploited range of resources, that Neanderthals and anatomically modern human groups *both* took advantage of a similarly wide range of resources -but the relative importance of these resources in the diet changed through time.

If this is the case, what does the isotopic and lithic evidence tell us about the way in which these resources were acquired through time? The strontium and oxygen isotope profiles of Mousterian fauna from Gruta da Oliveira, although limited in sample size, indicate that red deer, ibex, horse and rhinoceros were available within a 30km range of the site. Horses appear to have primarily occupied the banks of the Tagus River, and the presence of their remains at the Gruta da Oliveira suggests that they were likely hunted from open air sites in the Tagus Basin (such as Ponte da Pedra or Fonte da Moita), and then brought back to the cave either partially or as whole carcasses, though the former seems more likely. The same may apply to the extinct rhinoceros *Stephanorhinus,* which based on the strontium isotope data appears to have moved short distances within the vicinity of the Tagus Basin and the banks of the Tagus River. Again, these animals may have been hunted or scavenged at open air camps such as Santa Cita, and parts brought back to the Almonda karstic system for processing and consumption. Red deer are the most abundant large mammal species represented in the Mousterian deposits at Gruta da Oliveira, and it seems reasonable to assume based on the zooarchaeological evidence at Foz do Enxarrique and the relatively unchanging strontium isotope profiles of red deer in the Tagus Basin through time, that these animals were hunted seasonally at semi-residential base camps. Ibex occupied the craggy outcrops of the Central Limestone Massif, and were likely hunted locally to the Gruta da Oliveira itself. The sinusoidal nature of the Oliveira 8 and Oliveira 9 Neanderthal strontium isotope profiles suggest that these individuals subsisted upon certain geological substrates repeatedly, but whether this behaviour is seasonal remains to be seen without complimentary sequential oxygen isotope data for the same teeth. The lithic evidence from Mousterian sites in the study region points to heavy use of locally available quartz and quartzite pebbles, with full representation of all stages of the Chaîne opératoire apparent at the open air sites. Flakes and tools were created at the sites themselves, suggesting that whilst occupation was not necessarily permanent, it may have been more residential in nature. The presence of hearths and features interpreted as hunting blinds and habitation structures at sites such as Vilas Ruivas and Santa Cita imply a presence that was likely more substantial than just ephemeral campsites.

Taken together, the isotopic and archaeological evidence could be considered to reflect a mixture of residential and logistical mobility for the two Neanderthal individuals from Layer 22 at Gruta da Oliveira. Certain sites, such as the open air locations in the Tagus Basin, were perhaps repeatedly visited on a temporary (but somewhat residential) basis in order to exploit specific, nearby animal and raw material resources. Based on the fact that horse remains are present in the faunal assemblage at Gruta da Oliviera, it seems that a certain degree of carcass transportation took place, perhaps in some form of central place provisioning.

During the Gravettian, Solutrean and Magdalenian periods, the high occurrence and wide range of small game species in faunal assemblages compared to those of the Mousterian, and concurrent shift in technological trends has led some to argue that a change in subsistence strategies towards more intensive and specialised practices (and hence altered mobility patterns) took place during the transition from the Middle to Upper Palaeolithic in Central Portugal (Hockett and Haws, 2009). The isotopic evidence from Gruta do Caldeirão seems to support the notion that the study area was utilised differently by its anatomically modern human occupants during the Solutrean period, as opposed to its Neanderthal occupants during the Mousterian. Unlike the latter, the Solutrean humans from Gruta do Caldeirão appear to have primarily subsisted upon the Neogene sedimentary substrates of the Tagus Basin, with their strontium isotope profiles consistent with those of the red deer from the same archaeological deposits. Other large mammal species identified within Solutrean deposits at the Gruta do Caldeirão were, based on the strontium isotope data, available within a radius of between 30-50km of the site, either in the Tagus Basin itself (red deer and horse) or at higher altitudes on the Central

Limestone Massif (ibex). Open air sites further afield but still positioned upon the same geological substrates suggest that human groups occupied residential sites such as Casal do Cepo, Olival de Carneira, Olival de Arneiro and Passal. If these groups moved between such sites and used utilised them as bases from which to target local game such as red deer and rabbit, the strontium isotope values of their tissues could potentially still produce the homogenous profiles observed in the incisor enamel of the Caldeirão 5 and 6 individuals. It also seems likely that human groups moved between the Tagus Basin and the coastline, with temporary camps such as the cave site of Lapa do Anecrial offering evidence for passage between the two regions in order for marine resources such as shellfish to be exploited. Based on isotopic, zooarchaeological and lithic evidence, it seems that the mobility of the Solutrean humans at Gruta do Caldeirão was relatively residential in nature, with the primary focus upon resources gathered in the Tagus Basin. This strategy of mobility appears to be echoed at sites further afield such as Vale Boi in the Algarve, where evidence points to the seasonal residential occupation of a coastal cave, at which rabbits and red deer were exploited intensively alongside shellfish (Manne *et al.*, 2012). When considered in light of the apparent subsistence strategies Neanderthal population that occupied the landscape during the Middle Palaeolithic, the Solutrean human population appears to have engaged in a similar form of potentially seasonal residential mobility, but with across a more limited range of geological substrates and with a greater focus upon the consumption of fauna such as rabbit and red deer.

The archaeological and isotopic evidence available for the Magdalenian period in Portuguese Estremadura points tentatively to a shift to a different form of mobility and subsistence strategy. Whilst the focus upon the exploitation of small game such as rabbit and intensive processing of larger mammals such as red deer remains consistent with the preceding Solutrean period, the mode of exploitation appears to shift to something more logistical in nature. The strontium isotope profile of the Cisterna 2 premolar appears to reflect regular, perhaps seasonal shifts between the consumption of resources from two regions: the direct vicinity of the Almonda karstic system and the banks of the Tagus River. Although the sample size is limited, the red deer from Lapa dos Coelhos appears to have behaved seasonally, moving between the Tagus Basin and the banks of the river. The ibex individual also appears to have moved seasonally between higher and lower altitudes on the Central Limestone Massif. Both species were likely available in the study area year-round, but may have been targeted during certain times of the year in response to varying nutritional value, variation in ease of exploitation, or perhaps in order to target individuals of specific age ranges. Magdalenian groups may have hunted red deer in the Tagus Basin during specific seasons; potentially late autumn to early winter, based on cementum annuli (Bicho *et al.,* 2000) using tactical cave sites such as Lapa do

Picareiro as temporary bases, where the carcasses could be butchered and processed. Some body parts were likely eaten on site, whilst others were likely transported to sites that were more residential in nature, perhaps such as Bairrada. Ibex may also have been targeted seasonally, as seems to have been the case at cave sites in the Rio Maior region where the narrow canyon would have been an ideal location in which to ambush ibex. Rabbits were likely available across the landscape, and could have been trapped or hunted locally.

In short, the available zooarchaeological, lithic and isotopic evidence seems to suggest that in Central Portugal the Mousterian and Solutrean periods are characterised primarily by seasonal residential mobility, whilst the Magdalenian period appears to be characterised by a more logistical form of mobility. In terms of shifts (or lack thereof) in the resources exploited by Middle and Upper Palaeolithic groups, the preliminary zooarchaeological evidence does point to some changes. Between the Mousterian and Solutrean in Portuguese Estremadura, the importance of species such as rabbit appears to have increased, whilst focus shifted away from megafauna such as rhinoceros, which became extinct in the Upper Palaeolithic. Large herbivores such as red deer nevertheless remained central to the diet. During the subsequent Magdalenian, red deer and wild boar were likely targeted seasonally and intensively alongside small game such as rabbit and fish, with many cave sites used as temporary logistical camps from which to launch hunting parties. If this is the case, what could be the underlying reason(s) for **a)** the apparent shift in focus upon small game from the Middle to Upper Palaeolithic, and **b)** the change in mobility patterns from the Solutrean to Magdalenian?

There is some suggestion that shifts in subsistence strategies and increasing focus upon intensification and specialisation in small game exploitation was a result of population-resource imbalances, brought about either by overhunting of larger prey as a direct response to increased human population, or by the alteration of habitats and therefore resource availability due to climate change during the course of the Middle to Upper Palaeolithic (Hockett and Haws, 2009b). In the diet breadth model, diversification is expected to occur once staple resources begin to ‘fall beneath and economic threshold for net caloric return, thus leading to the inclusion of new items” (Hockett and Haws 2009: 2). Generally, increasing dietary breadth and intensification are seen as indicators of nutritional stress - so is there evidence in Central Portugal for increasing environmental and/or population pressure during the Solutrean that might offer an explanation for the apparent increase in the exploitation of rabbit when compared to the Mousterian?

The Proto-Solutrean and Solutrean technocomplexes in Central Portugal appear coincidentally with the onset of the HE2 cold event approximately 26.5 ka years ago. Central and southern Portugal underwent a climatic deterioration during this time, observing a strong expansion of semi-desert steppe vegetation and a reduction in Scots pine forests, which survived only in patches amongst the expanding heathland. The picture is one of an increasingly arid and open environment, but despite these environmental changes, the availability of prey species appears relatively consistent. Whilst certain megafauna such as *Elephus* and *Stephanorhinus* had disappeared by this time, taxa such as red deer, horse and aurochs appear to have remained abundant throughout the Solutrean, with populations of cold-adapted ibex and chamois more frequent than in the preceding periods (Bicho *et al.*, 2017). The environmental conditions in Central Portugal would have been particularly ideal for the endemic European rabbit, which thrives in grasslands and heathlands characterised by a low density of woody vegetation and the availability of herbaceous matter and forbs (Haws, 2012). Hockett and Bicho (2000) suggest that the extensive exploitation of rabbit despite the presence of larger prey at Solutrean sites in Portuguese Estremadura was therefore more likely due to their ubiquity and ease of capture, as opposed to environmental and climatic pressures placed upon human groups. Their high reproduction rates, abundance throughout the landscape and ease of detection and capture with technologies such as snares and nets likely made them an attractive target for Solutrean hunters in the arid heathlands of post-HE2 Estremadura. Furthermore, rabbit meat offers a comparable amount of protein per gram and a greater concentration of vitamins and minerals than that of red deer and wild boar; suggesting that rather than being a 'starvation food', it would have been a reliable, widespread and nutritionally valuable resource (Hockett and Bicho, 2000). The hunting of rabbit likely presented less of a risk to health and a lower likelihood of failure in comparison to the hunting of larger prey, providing good returns of meat and fur.

If subtle environmental changes created ideal and widespread habitats for European rabbit populations in Central Portugal, did an increase in human population during the Upper Palaeolithic also prompt the increased exploitation of the species? Straus, Bicho and Winegardner (2000) documented an increase in the concentration of sites per millennium from the Gravettian to the Solutrean in Iberia, which is suggested to have been a result of groups moving southwards into Iberian refugia during the climatic downturn of the LGM, and this also appears to be reflected in the archaeological record of Central Portugal (Hockett and Haws, 2009). Haws (2012) suggests that whilst factors such as loss of habitat, climate change and range restriction are likely to have played a role in the extinction of megafauna and certain carnivores during the Late Pleistocene, hunting pressure due to increasing human population density may have also contributed significantly to their eradication.

In Central Portugal, the disappearance of species with whom Middle Palaeolithic humans had coexisted such as elephant (*Elaphas*), rhino (*Stephanorhinus hemitoechus*) and striped hyena (*Hyaena prisca*) coincides with the appearance and expansion of anatomically modern humans into Iberia (Haws *et al.* 2020). The fact that these species had previously been relatively stable in Iberia despite climatic fluctuations (Rodríguez, 2004) has led some to suggest that increasing human predation may have led to their disappearance. This loss, alongside the climatic shifts of HE2, may have further restructured the local vegetation, helping to create ideal environments for species such as rabbit. Local extinctions of slow moving, easily captured game such as tortoise may be the result of over-hunting during the Middle Palaeolithic – indeed, evidence at sites such as Gruta da Oliveira suggest they had been heavily exploited during the Mousterian. Factors such as this may have further prompted the shift to rabbit exploitation during the Upper Palaeolithic.

Are similar environmental and population changes the driving force behind the shift in mobility pattern between the Solutrean and Magdalenian? The onset of the Magdalenian in Central Portugal coincides with the end of the LGM; at which time a climatic amelioration began. Where the environment had previously been dominated by heathland, scrub and receding arboreal cover, Mediterranean forests now expanded and species such as pine and oak took hold. Cold-adapted mammal species such as chamois and ibex decreased, whilst species suited to forested environments such as wild boar became more abundant (Bicho *et al.*, 2017). Bicho and Haws (2012) argue that the development of logistical mobility patterns may have been a response to increased biomass and forest cover, which in turn may have resulted in a reduction in the availability of desirable species such as red deer. If this is the case, adaptive technologies and logistical settlement patterns suited to specific resource distributions were likely adopted in order to maximise economic return. The selection of sites based on their proximity to raw material sources and tactical value is evident in the Magdalenian archaeological record in Central Portugal (Bicho *et al.,* 2006), with a clear variety of temporary rockshelter camps and lowland residential bases. Marine, riverine and estuarine resources, evidence for the exploitation of which can be found in multiple sites in the Tagus Basin, may have been targeted in response to the increased forest density and subsequent reduced availability of medium-sized mammalian prey, as well as in response to increased upwelling and marine productivity during MIS2 (Bicho and Haws, 2008). Bicho (2013) further suggests that a degree of forest clearing by managed fire may have been employed during the Magdalenian as a means to create suitable habitats for species such as red deer and wild boar, for ease of hunting – potentially with the assistance of canids.

The primary underlying cause of the apparent shift in settlement patterns from more a complex mix of residential and logistical mobility to more dedicated logistical mobility from the Middle to the Upper Palaeolithic, then, may have been changes in the degree of arboreal cover and vegetation during the climatic oscillations of the Middle and Late Pleistocene. Whilst the exploitation of medium-sized mammals remained relatively consistent and throughout these periods, and the range of taxa targeted were equally as diverse, the relative abundances of certain species including red deer, horse, ibex and wild boar likely fluctuated to an extent in response to climatic changes. The open heathlands of the Proto-Solutrean and Solutrean were ideal habitats for rabbit and cold-adapted caprids such as ibex, with taxa such as red deer, horse and aurochs readily available in the Tagus Basin. The increased arboreal cover during the climatic amelioration that followed the LGM, however, may have restricted the distribution of red deer and increased the availability of wild boar whilst cold-adapted species such as ibex declined, leading human groups to develop technologies and settlement patterns suited for the specialised hunting of these prey. Meanwhile, other small game, plants and aquatic resources that were abundantly available in the warmer, Mediterranean environments of post-LGM Portugal became equally valuable components of the diverse Magdalenian diet.

11. Conclusion and future work

The subsistence strategies and mobility patterns of Upper Palaeolithic human groups remains a topic of great importance and intrigue, particularly when viewed in light of the oscillating climatic conditions that characterised Pleistocene Europe. Isotopic analysis, and in particular studies that incorporate high-resolution sequential strontium and oxygen isotope analyses of contemporaneous animal and human tooth enamel, can offer a comprehensive and direct new insight into the land use and settlement patterns of Neanderthals and anatomically modern humans at the individual scale – where previously, inferences could only be made from the lithic and faunal assemblages of the sites themselves. Together, isotopic, faunal and lithic data can be synthesized to obtain a much more comprehensive view of the mobility behaviours of these groups, and hence their responses to the environment around them.

This study represents the first successful, extensive isotopic and archaeological study of human and animal mobility across the Middle and Upper Palaeolithic in Europe, and outlines the potential for future work in this area. Based on isotopic, faunal and artefactual evidence from Portuguese Estremadura, Middle and Upper Palaeolithic humans in Portuguese Estremadura appear to have exploited the landscape in slightly different ways, perhaps in response to changing arboreal cover during the climatic oscillations of the Mid to Late Pleistocene. Based on the lithic assemblages and strontium isotope data, both Neanderthal and anatomically modern human groups living within Portuguese Estremadura appear to have obtained the majority of their resources within a 30km radius of the sites at which they were buried, though the presence of marine shells and 'exotic' flints implies forays further afield, or long-distance social and trade networks.

The first aim of this project was as follows:

a) *To investigate whether the mobility of major prey species such as red deer, ibex and horse in central Portugal is seasonal in nature, and establish whether their mobility patterns change over time during the Pleistocene.*

Through the sequential $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ analysis of tooth enamel, it has been possible to investigate whether the mobility of major prey species such as red deer, ibex, horse and extinct rhinoceros in central Portugal is seasonal in nature, and establish whether their mobility patterns change over time during the Pleistocene. Strontium isotope data suggest that all of these species subsisted upon the local geology consistently through time, and in the case of the red deer and ibex, the oxygen isotope data suggest that seasonal extremes were experienced annually, implying that they were present in the study area year-round. Horse and *Stephanorhinus* oxygen isotopes did not exhibit the same high degree of $\delta^{18}O$ variation, but since their strontium isotope profiles are consistently homogenous and appears to be limited to specific local $^{87}Sr/^{86}Sr$ signals, it seems likely that the lack of $\delta^{18}O$ variation is a result of physiological differences or water intake behaviours that could be investigated in future work.

The second aim was:

b) *To investigate the mobility patterns of anatomically modern humans and Neanderthals in central Portugal, and determine whether range size and seasonal behaviour can be observed through the sequential strontium isotope analysis of tooth enamel;*

The application of the same kind of high resolution $^{87}Sr/^{86}Sr$ analysis vis LA-MC-ICP-MS to Middle and Upper Palaeolithic human teeth, both in this study and in previous work (Pike *et al.* unpublished) has successfully enabled the direct investigation the mobility patterns of anatomically modern human and Neanderthal individuals in central Portugal. The degree of variation in the $^{87}Sr/^{86}Sr$ profiles of the tooth enamel of these human groups has provided new insights into the patterns of land use in the region, enabling us to establish the extent of their ranges - at least in terms of food procurement.

The third aim was subsequently:

c) *To examine whether the mobility patterns of these two human groups are the result of the availability of local fauna, and potentially establish links between certain geographical regions and the exploitation of specific resources at certain times of the year;*

Considering that the $^{87}Sr/^{86}Sr$ values measured in the tooth enamel of both humans and fauna are consistent in many cases, we can infer that they likely occupied and subsisted upon the same geologies – and, although more oxygen isotope data is required to investigate this further, we can speculate that these joint occupations may have occurred simultaneously at times, and perhaps even as a result of targeted hunting on the part of the human populations.

The final aim was as follows:

d) *To investigate whether the mobility patterns and subsistence strategies of Neanderthals and anatomically modern humans differ between the Middle and Upper Palaeolithic of Portuguese Estremadura - this has wider implications for our understanding of the cognition and ecology of these populations.*

By combining the isotopic data gathered here with available zooarchaeological, lithic and climatic data for the study region, it has been possible to begin to piece together a picture of diachronic change in mobility patterns and landscape use in Portuguese Estremadura. This may have been a result of environmental changes (in particular, arboreal cover) brought about by oscillating climatic conditions during the Pleistocene.

Such syntheses could certainly be carried out in other archaeological contexts following similar methods and protocols. Nevertheless, there are limitations to both the method and the current work that must be borne in mind. Such limitations present the opportunity for future refinement and progress, which are discussed alongside the caveats in the following section.

11.1 Analytical caveats

Whilst this study has provided new insights into the behaviour and mobility of both humans and fauna across central Portugal during the Middle and Upper Palaeolithic, there are undoubtedly limitations to the methodology that must be borne in mind when considering the data.

11.1.1 *Enamel formation*

Arguably, the greatest limitation to the current study is the absence of detailed data regarding human tooth enamel mineralisation rates. As discussed in Chapter 5, many researchers have put forward different hypotheses regarding the process and rate of enamel mineralisation, and to date the topic is still debated. Due to difficulties in obtaining appropriate modern or historical human tooth samples with which mineralisation rate could be investigated, such work was beyond the scope of this study. As such, the interpretations drawn from the strontium isotope data gathered for the Palaeolithic humans analysed here are tentatively made based on existing data regarding overall human crown formation times and comparative primate mineralisation rate studies. Research into the enamel mineralisation process and rates carried out for species such as horse, sheep and cattle illustrates that maturation geometry and mineralisation rate can vary slightly depending on species, and as such, using other species as guidance is inadvisable unless no other options are available. A detailed study of human tooth enamel geometry and mineralisation will, in the future, enable the data produced in the current study to be re-visited in light of a more comprehensive understanding of the timescales represented by the strontium isotope profiles.

11.1.2 *Isotope incorporation*

Our limited understanding of the incorporation of isotope ratios into human tooth enamel is also a matter for consideration. In particular, the role of bone resorption in contributing to the strontium isotope ratio of a given section of enamel is, at current, poorly understood. If significant amounts of calcium (and therefore strontium) are routed from the bone mineral to the enamel during its formation, signals from ingested food and water may be dampened. If this is the case, then the degree of routing from the calcium body pool may also be dependent on the quality of the diet, and whether sufficient calcium is introduced to the body directly through the diet. As of yet, no dedicated studies have addressed this problem, and future work will likely better inform the interpretations made in this work.

11.1.3 *CaPO interference*

Although careful manipulation of plasma conditions (following the method of de Jong, 2013) has the potential to reduce oxide formation significantly and produce data within the precision of TIMS, it is still not possible to completely eliminate the problem. In tooth enamel samples with very little remaining biogenic strontium, the impact of CaPO interference has the potential to become increasingly significant. Re-tuning the instrument for low-oxide formation also requires a significant amount of time, and often has to be done several times a day.

11.1.4 *Interpretation of strontium isotope data*

One critical factor that should be borne in mind is that isotopic mixing likely takes place to varying extents, depending on how many resources are consumed from different geological substrates at any given moment in time. Take, for example, a human individual subsisting upon resources gathered purely upon one geological substrate. As long as those resources are plants, or animals with small home ranges limited to the geographical extent of the geological substrate in question, the body tissues of the human individual would be expected to reflect the $^{87}Sr/^{86}Sr$ value of the underlying geology - until the point at which the individual moves to a new area and commences consumption of new local resources. In a hypothetical residential mobility pattern, then, the strontium isotope profiles of human tooth enamel should reflect undamped signals from the underlying geology (assuming that strontium from the body pool is not incorporated). On the other hand, an individual engaged in a degree of central place provisioning (such as is observed in a logistical mobility system) might take in strontium from a variety of different sources at any given time, hence incorporating a mixed value depending on the diversity of the geologies upon which the resources are gathered. For example, if fauna and flora are collected from multiple locations with different underlying geologies, and brought back to the central place for consumption at the same time, then the isotope values incorporated into the human individual's tissues will be a mixed value weighted towards the resource that provides the greatest proportion of strontium at a given time. With comprehensive data regarding the proportions of animal (and plant) remains consumed during the time of enamel formation (i.e, estimates based on faunal assemblages from contemporary sites), and with strontium isotope data for the each of those resources, it may be possible in the future to produce a mixing model with which to reconstruct an 'undamped' strontium isotope profile – but with so many variables at play, and the unlikelihood of determining the origins of plant food and their contribution to the diet, this may be unrealistic. Nevertheless, it is important to bear in mind that a degree of isotopic mixing and damping is likely to play a part in the measured isotope ratios in human tooth enamel, and should be considered during the interpretation of sequential strontium isotope data until further advances are made in our understanding of this.

It is also important to note that the 'strontium weighting' of different components of the diet may skew strontium isotope profiles towards the values of heavier weighted resources. Salt, which contains particularly high concentrations of strontium, is required to be consumed only in small mounts in order for it to begin to distort $^{87}Sr/^{86}Sr$ of the overall diet (de Jong, 2013). Plants also contain high concentrations of strontium, and bones certainly contain higher concentrations of strontium than flesh. In a diet made up predominantly of meat, it is feasible that the majority of the strontium contribution could be from plants and drinking water (Haverkort, Bazaliiskii and Savel'ev, 2011). Likewise, in a population where the consumption of fish takes place (for example, during the Magdalenian occupation of the Almonda karstic system), even a relatively minimal nutritional input of fish could skew the resultant strontium isotope profiles; particularly if the bones are consumed.

11.2 Archaeological caveats

11.2.1 *Chronological resolution*

One of the primary chronological limitations of the current study is the absence of direct radiocarbon dates for the animal and human samples selected for analysis. At present, sample ages are assumed based on stratigraphic association, but it should be noted that at numerous cave sites such as Gruta do Caldeirão, a degree of bioturbation and stratigraphic mixing is known to have taken place in certain areas of the cave, particularly as a result of burrowing species such as badger and lynx. In the future, direct radiocarbon dating of the samples analysed in this study would prove (or disprove) that these specimens are truly in situ and have not been moved up or down the stratigraphic column as a result of bioturbation, strengthening the chronological resolution.

The study is further limited by the availability of lithic and zooarchaeological data from the study area. At a number of sites, most notably Gruta da Oliveira, detailed lithic and faunal studies are still underway, and as such the strontium isotope data gathered from Neanderthal and animal teeth cannot be considered in light of the wider archaeological context of the site. Since such work is beyond the scope of the current study, the hope is that the data presented here will be revisited at such a time when lithic and faunal reports are completed, so that a more detailed picture of site function can be drawn. Of course, in some cases, differential preservation conditions and varying excavation techniques may have an effect on the available data – for example, in deposits that were not sieved, small bones from species such as fish and amphibians may have been lost. To this end, it is important to bear in mind that absence of evidence is not necessarily evidence of absence – this also applies to our wider understanding of site distribution throughout the Palaeolithic in Portuguese Estremadura, where conditions may have entirely prevented preservation. Many sites may also be undiscovered as of yet (for examples, along the submerged prehistoric coastline), and in the future may further inform our understanding of subsistence and mobility patterns in the Middle and Upper Palaeolithic.

11.2.2 *Sample size*

Although this study presents the largest sequential strontium and oxygen isotope analysis of a Palaeolithic faunal assemblage carried out to date, the relatively small sample size for each species in each archaeological context does act as a considerable limitation, because the behaviours inferred from the analysed individuals may not be reflective of their wider populations. This is in part due to the limited number of individuals available for analysis at the study sites, as the faunal assemblages recovered from several of the cave deposits (such as Lapa dos Coelhos and Galeria da Cisterna in particular) are relatively small. Even in larger assemblages where a greater number of individuals are represented, such as Gruta da Oliveira, the number of teeth that are actually suitable for analysis (i.e, not deciduous) in each context further restricts the overall sample size of the study. With a larger sample size incorporating a greater number of sites (perhaps, for example, Lapa do Picareiro), it will be possible to expand the current data set and develop a broader picture of animal mobility across the landscape.

It is also worth noting that the current sample does not include all of the prey species represented at the sites. Species of particular interest in future work would be rabbit and wild boar, the remains of which become more common in Upper Palaeolithic anthropogenic deposits in Central Portugal. Strontium isotope analysis of the tooth enamel of rabbits, which have very small home ranges (less than 0.1 km^2), would enable us to determine whether rabbits were hunted locally to the sites at which they were consumed, or because of their ease of transport were carried to the sites from further afield. Similarly, strontium isotope data for wild boar (which appears to have played a more significant part in the diet of the Magdalenian occupants of Portuguese Estremadura) may shed light on where and how this species was hunted. If it could be proven that no diagenetic alteration had taken place, strontium isotope data for the abundant tortoise remains recovered from Mousterian layers at Gruta da Oliveira would also aid in establishing how locally they were hunted - but since tortoises do not have teeth, it is unlikely that it would be possible to obtain a reliable value, since bone or shell would be the only material available for analysis. Sulphur isotope analysis of tortoise bone collagen would perhaps help to elucidate their origin.

11.2.3 *Suitability of study area*

The methods outlined in this study are unlikely to be a suitable approach for mobility studies in areas with widespread, homogenous geologies. In such locations, individuals (both human and animal) may be able to range for hundreds of miles without encountering a different geological unit, and as such their tooth enamel would not reflect mobility despite extensive movement. Similarly, the method is likely not suited for studies of modern human populations, since global food networks enable individuals to access resources from a myriad of different countries and continents despite residing in a single area. Any movement is likely to either be masked by the consumption of imported foods from geologically different regions.

Whilst it is possible, to an extent, to model the bioavailable strontium isotope values of a region (for example, (see Willmes *et al.*, 2018), it is advisable to actively collect samples and directly measure the $^{87}Sr/^{86}Sr$ values for the study area in question. This involves locating suitable sample points across different geological substrates, from which sediment and/or plant specimens can be collected. If this is not possible, then the interpretation of human and/or animal strontium isotope profiles may be limited. It should also be borne in mind that even if the direct sampling of the study area is possible, modern agricultural practices and the deposition of aeolian sediments may alter the strontium isotope values of modern soils, producing an isotopic baseline that is unrepresentative of the bioavailable strontium present during the archaeological period under study. Future expansion of the bioavailable strontium isotope map created by Pike *et al.* (unpublished) that forms the basis of the current study will help to inform the interpretation of the human and faunal strontium isotope data gathered here.

11.2.4 *Suitability of samples for seasonality studies*

In a study such as this, methods for determining season of death such as cementum annuli and tooth wear analysis have the potential to aid in the interpretation of site function. Whilst cementum annuli analysis of the faunal tooth specimens from the Almonda karstic system and Gruta do Caldeirão was included in the initial proposal, it was not possible to identify any teeth with surviving cementum due to the relatively poor degree of preservation. This is unfortunate, as such data may have aided in the interpretation of the functions of the sites included in this study, and by implication the interpretation of the human strontium isotope profiles. Factors such as this should be borne in mind in future project proposals, where poorly preserved specimens (particularly of greater age) may not be suitable for cementum annuli or tooth wear analysis.

11.2.5 *Suitability of human tooth samples*

Because human tooth enamel is secreted and mineralised prior to birth, through childhood and into adolescence, the mobility reflected within the strontium isotope profiles may not be representative of the mobility of the subsistence behaviour of adults. This is a potential limit to the conclusions we can draw about the mobility of prehistoric human groups, since children and adolescents may not necessarily engage in the same behaviours until biological maturity. Of course, we cannot know at what age younger members of these groups took on adult activities, or the extent of any age-related division in behaviours at all – but these factors should be borne in mind when considering the archaeological implications of strontium and oxygen isotope data recovered from human tooth enamel.

Despite the above caveats, however, the application of this novel approach to a large sample of Middle and Upper Palaeolithic humans and fauna has demonstrated its immense value as a tool for the investigation of mobility and seasonality; not just in archaeological contexts but also in the fields of ecology and conservation. High resolution analysis via laser ablation MC-ICP-MS permits the efficient measurement of strontium isotope ratios at the micron scale, enabling the recovery of biogeochemical information from increasingly small, incrementally-formed tissues such as tooth enamel and otoliths with minimal destruction and precision comparable to standard TIMS measurements. When combined with sequential $\delta^{18}O$ analysis, such data can be anchored to season, permitting the reconstruction of seasonal mobility and seasonal landscape use in both humans and fauna. Future work combining these techniques may shed new light on the ecology of species both past and present.

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