Multi-Isotope investigations of ungulate bone and teeth from El Castillo and Covalejos caves (Cantabria, Spain): implications for paleoenvironment reconstructions across the Middle-Upper Palaeolithic transition

Jennifer R. Jones, Michael P. Richards, Hazel Reade, Federico Bernaldo de Quirós and Ana B. Marín-Arroyo*

*Corresponding author: marinab@unican.es

Abstract

The Cantabrian region of Northern Spain was an important area of human settlement during the Middle and Upper Palaeolithic as the rich archaeological record demonstrates. The environmental conditions experienced by late Neanderthals and Anatomically Modern Humans (AMH) in the region during MIS3 are still poorly known, but are crucial to understand the role climatic instability could have had on the adaptations of these populations. In this study, a series of archaeological levels with Mousterian and Aurignacian artefact assemblages, dating between 49-35ka uncal. BP, from the sites of El Castillo and Covalejos caves in Cantabria were studied using multi-isotope techniques including bone collagen δ^{13}C, δ^{15}N and δ^{34}S analysis and a pilot study based on tooth enamel δ^{18}O and δ^{13}C. Results at Covalejos indicate a large range in δ^{15}N values observable within both Mousterian Level D and Aurignacian level B, suggesting the presence of different micro-environments within the local area at certain times during each temporal period. Within Aurignacian Level C and Mousterian Level J, the δ^{15}N values range is much smaller, but shows consistency in the parts of the landscape being exploited during both times. Neanderthals and AMH appear to have been procuring animals from isotopically similar zones (isozones) when they occupied Covalejos. The variations in δ^{34}S values between levels analysed at Covalejos also implies the use of a variety of hunting locations by both late Neanderthals and AMH. At El Castillo, δ^{13}C and δ^{15}N values show great consistency between levels, indicating that animals were being hunted from the same isozones when the site was presumably occupied by the two human species. The mosaic landscapes of Cantabria may have buffered the sharp environmental fluctuations that occurred during late MIS3 throughout Europe, providing suitable habitats for the key prey ungulate species that were routinely exploited throughout the Middle and Early Upper Palaeolithic. This environmental buffering may explain why this southerly, oceanic region was consistently occupied throughout this time of bio-cultural transition.

1. Introduction

The circumstances contributing to the extinction of the Neanderthals and their subsequent replacement by Anatomically Modern Humans (AMH) is a hotly debated subject (e.g., Villa and Roebroeks 2014). Neanderthal extinction is now known to have been relatively rapid and followed a regional pattern rather than a uniform pan-European one (Higham et al 2014), meaning that climatic change cannot be claimed as a homogeneously driving factor for their extinction in a single event across the continent, as previously considered (D’Errico and Goñi 2003; Jiménez-Espejo et al. 2007; Banks et al. 2008, 2013). Whilst in some parts of Europe radiocarbon dating has recently indicated an overlap of Neanderthal and AMH populations of ±5,000 years (Higham et al. 2014), with indications of Neanderthal and AMH interbreeding based on skeletal morphology (Trinkaus 2007) and genetic evidence (Fu et al. 2016; Prüfer et al. 2014), in the Cantabrian region of northern Iberia (Figure 1), major archaeological deposits with evidence of Neanderthal and AMH occupation levels suggest that there was a swift replacement of Neanderthals by AMH (Marín-Arroyo et al. in press). The reasons behind this change in population are not been well understood and the environmental conditions experienced by both species require further exploration to evaluate how they could have influenced the adaptations of the two populations. Ascertaining the extent to which regional and temporal patterns of climatic variation account for the final extinction of Neanderthals in the Cantabrian region needs further research.
MIS3 is known to have been a period of significant climatic change in Europe, with cyclical oscillations among warmer, cooler, wetter and drier environments, as reflected in ice (Dansgaard et al. 2003) and marine cores (Sanchez Goñi et al. 2000), with millennial-scale fluctuations observable in deep-sea pollen core records from off the coast of northwestern Iberia (d’Errico and Sánchez Goñi 2003; Naughton et al. 2009). How these conditions were expressed in the local vicinities of archaeological sites occupied during this time is not well known. Palaeoenvironmental indicators available for the Cantabrian Region, such as pollen (Ruiz-Zapata and Gil 2005), charcoal (Uzquiano 2005; 2007; 2008), microfauna (Sesé 2005) and macrofauna (Yravedra et al. 2017), can be influenced by preservation biases, depositional hiatuses and diagenetic processes (Bryant et al. 1983; Coles et al. 1989). A significant part of Neanderthal and AMH diets would have come from large and medium-size ungulates, as zooarchaeology (e.g. Chase et al. 1986; Discamps et al. 2011; Gaudzinski-Windheuser and Niven 2009; Morin et al. 2014; Stiner 1994; Yravedra 2013) and stable isotope analysis of human remains have revealed, with a broadening in dietary behaviour among the early AMH population in comparison to that of the Neanderthals (Richards and Trinkaus 2009). However, the main Cantabrian ungulate species were ecologically adaptable to both warm and cold environmental conditions (Altuna 1992) and their presence in the archaeological record does not necessarily give a detailed reflection of environmental and climatic conditions. Microfauna can be more sensitive to local environmental shifts, but the relationship between their remains and the human occupation of cave sites is not necessarily straightforward. These lines of evidence alone are not enough to establish a cause-effect relationship between climate and evolutionary outcome and the actual role of climate in subsistence and survivorship is still under debate. Therefore, other methods are sought to provide a higher resolution and more detailed understanding of the conditions experienced by both human species inhabiting this region.

Stable isotope analyses of ungulate remains have proven to be a valuable method for palaeoenvironmental and palaeoclimatic reconstructions and as locational tools for investigating animal mobility. Analysis of bone collagen δ13C and δ15N values of animal informs on the conditions experienced throughout the period of bone growth (Hedges et al. 2007; Stenhouse and Baxter 1979) and have been well-established as a method for reconstructing European Palaeolithic environments (e.g. Bocherens et al. 2014; Britton et al. 2012; Drucker et al. 2003; 2008; 2011; 2015; Richards and Hedges 2003; Stevens and Hedges 2004; Hedges et al. 2004; Szpak et al. 2010). Collagen δ13S analysis can be used as a locational tool, with values directly linked to local geology and soil type, proximity to the sea and rainfall (Nehlich 2015). Sulphur stable isotope analysis has been applied in investigations of the movement of animals including reindeer, red deer and mammoths during the Upper Palaeolithic (Drucker et al. 2015; 2012; 2011). In ungulate teeth, the isotopic compositions of fossil tooth enamel (isotopes of δ18O and δ13C) can also be used to explore palaeoenvironmental conditions contemporaneous with the period of tooth mineralisation (e.g. Fricke et al. et al., 1998; García García et al., 2009 Navarro et al., 2004; Nelson, 2005), having been successfully applied in Palaeolithic archaeological contexts (Pellegrini et al. 2008; Pryor et al. 2013). This methodology has never been attempted in the Cantabrian region of Northern Spain in relation to this time period (although has been applied to late Pleistocene studies in the nearby Burgos Meseta-see Feranec et al. 2010). The Cantabrian Region is climatically distinctive with year-round rainfall and limited seasonal temperature variation (Peel et al., 2007) and there is a need to explore whether these conditions will be reflected in the tooth enamel carbonate record.

The abundance of the archaeological remains related with human activity in the Cantabrian Region provides an exceptional opportunity with which to use a multi-stable isotope methodology and to evaluate the relationships that late Neanderthals and AMH had with the environment and why the region was repeatedly occupied across the Middle to Upper Palaeolithic transition. El Castillo and Covalejos are important archaeological sites within the Cantabrian Region (Figure 1), with stratigraphic sequences including both Mousterian and Aurignacian artefact assemblages. Recent radiocarbon dating at the sites and new dates undertaken in this study has revealed coherent chronologies for the transition (Maroto et al. 2012; Wood et al. 2016; Marín-Arroyo et al. in press), making the present research timely.
This paper aims to explore the environmental conditions faced by both human populations and how climatic fluctuations may have affected the availability of ungulate species during this period. The existing MIS3 regional palaeoenvironmental information (pollen, macrofaunal, microfauna) will be examined at a site-specific level and will be correlated with the stable isotope analysis of the ungulates consumed by humans, to explore past environmental conditions.

2. The archaeological sites

Covalejos cave (Velo, Pielagos, Cantabria), located not far from the city of Santander, lies 48m above sea level in the lower part of the Pas valley on the coastal plain, 7 km from the Holocene shoreline and 13 km from the -100m isobath contour line. The site was discovered in 1872 and was first excavated in 1879. Recent excavations were directed by R. Montes and J. Sanguino between 1997–1999 and in 2002 (Sanguino and Montes 2005). The cave contains a sequence dating from the Early Middle Palaeolithic through the Aurignacian. Materials recovered during the latter work were analysed. The uppermost levels (B and C) were interpreted as being Early Aurignacian and Archaic Aurignacian, respectively based on lithic and bone tool typology present within each level. Below these, a sequence of Mousterian levels (D to M) have been identified based on the characteristic material culture (Sanguino and Montes 2005).

In this study, levels with secure stratigraphic position, were selected for analysis from Mousterian (J and D) and Aurignacian (C and B) levels. Radiocarbon dates from Level J (with a Quina Mousterian assemblage of stone tools) date it to beyond the limits of the method: >45,000 (Maroto et al. 2012; Table 1: Published and newly obtained radiocarbon dates in this study from El Castillo and Covalejos for the levels analysed, with laboratory codes, sample preparation methods used and a summary of the specimens per site and level sampled. For radiocarbon method AF=collagen extracted using the ultrafiltration method prior to analysis, AMS=dated using accelerated Mass Spectrometry, C14=dated using traditional radiocarbon methods.

*Denotes a date that extended beyond the limit of the calibration curve. All calibrations presented are those published in the original radiocarbon date reference. Calibrations for the new dates achieved from Covalejos and for the Sanguino and Montes (2005) dates were completed using OxCal v4.3, using the IntCal 13 curve.

) and Level D, representing the uppermost Mousterian occupation at the site (with discoid core technology), dates to 43,050 +750/-550 uncal. BP (Maroto et al. 2012). After the formation of Level D, there is a chronological gap in deposition, which is followed by the first evidence of Aurignacian activity represented by Level C, which was initially dated to 32,840+280/-250 uncal. BP (Sanguino and Montes, 2005) using traditional radiocarbon dating and a subsequent AMS date (on tooth enamel) of 37,940 +400/-350 was achieved (Maroto et al. 2012). Level C is then followed sequentially by Level B, which is also Aurignacian and was originally dated to 30,380±250 (Sanguino and Montes, 2005). New radiocarbon dating of bone collagen undertaken as part of this study by ultrafiltration, a methodological improvement in the radiocarbon method that removes young carbon contamination from old organic samples (Higham et al. 2006; Higham 2011), crucial at the limit of ~50,000 years ago because it is the time where transition took place (Fig. 1). Results yielded two dates for Level C of 34,850 ±600 (OxA-32512) and 35,250± 700 (OxA-32511) and for Level B of 34,350 ±600 (OxA-32549) and 35,150 ±650 (OxA-32513), indicating a close succession in the formation of these levels (Table 1: Published and newly obtained radiocarbon dates in this study from El Castillo and Covalejos for the levels analysed, with laboratory codes, sample preparation methods used and a summary of the specimens per site and level sampled. For radiocarbon method AF=collagen extracted using the ultrafiltration method prior to analysis, AMS=dated using accelerated Mass Spectrometry, C14=dated using traditional radiocarbon methods. *Denotes a date that extended beyond the limit of the calibration curve. All calibrations presented are those published in the original radiocarbon date reference. Calibrations for the new dates achieved from Covalejos and for the Sanguino and Montes (2005) dates were completed using OxCal v4.3, using the IntCal 13 curve.

).
El Castillo cave was one of the most consistently human occupied sites during the Middle and Upper Palaeolithic in the Cantabrian Region (Cabrera Valdés 1984). It is located above the town of Puente Viesgo in Cantabria, in the upper part of the Pas valley at 190 m.a.s.l. It is at the southern edge of the Santander coastal plain in the first foothill range of the Cantabrian Mountains, 17 km from the Holocene shore and 24 km from the -100m isobath line. It is about 14 km up the River Pas from Covalejos. While famous for having a rich, complex assemblage of Upper Palaeolithic cave art (Alcalde del Rio et al. 1912; Leroi-Gourhan 1965; Valladas et al. 1992; Pike et al. 2012; García-Díez et al. 2015), it is equally well-known for an 18m-thick stratigraphic sequence excavated in the early 20th century, first by its discoverer, H. Alcalde del Rio and then by an international team led by Hugo Obermaier. The occupation deposits at the entrance of the cave hold extensive evidence of Neanderthal and AMH activity (Cabrera Valdés 1984; Cabrera Valdés et al. 2006), mainly excavated between 1910-14 by Obermaier (Cabrera Valdés 1984). Between 1980-2011 new excavations of the early Upper Palaeolithic and late Middle Palaeolithic levels by Cabrera Valdés and Bernaldo de Quirós at the front of the cave mouth have revealed further evidence of human occupations (Cabrera Valdés 1984; Cabrera Valdés et al. 2006). All in all, El Castillo contained 26 sedimentological units with archaeological assemblages ranging from the early Middle Palaeolithic to the Azilian often separated by archaeologically sterile units (Cabrera Valdés 1984; Cabrera Valdés et al., 2006). Massive dating programs at the site have provided robust chronologies for the Middle to Upper Palaeolithic transition and have generated considerable discussion of the whole question of the El Castillo chronology, including a most recent review of the archaeology, lithic and bone assemblages from Obermaier and modern collections (Wood et al. 2016).

Relevant to this study, Level 20C contained clear evidence of Mousterian lithic technology and has two dates of 48,700±3400 (OxA-22204) and 49,400±3700 (OxA-22205) uncal BP. Level 19 did not contain any lithic or bone artefacts with evidence of human manipulation and is described as archaeologically sterile; a bone fragment found within this level was dated to 44,900±2100 ka uncal BP (OxA-21974) (Wood et al. 2016, Table 1). This level was subdivided archaeologically during excavation into ‘19 upper’ and ‘19 base’. El Castillo Levels 18B and 18C are technologically attributed to the Transitional Aurignacian (Cabrera Valdés et al., 2001; Maíllo and Bernaldo de Quirós, 2010; Wood et al. 2016, Table 1) and recent dates ranging between 42,000±1500 (OxA-22203) and 46,000 ± 2400 (OxA-21973) were achieved (Table 1). Level 17 was sterile and due to its placement in the stratigraphic sequence a red deer bone of Level 16/17 is dated to 39,100±1000 (OxA-22201) (Wood et al. 2016; Table 1). Level 16 is attributed to the Proto-Aurignacian and dated to 38,600±1000 uncal BP (OxA-22200) (Wood et al. 2016; Table 1).

The sites of Covalejos and El Castillo, both located in the same river valley and with well-dated late Mousterian and Aurignacian deposits, provide a valuable opportunity with which to understand the nature of this cultural transition in terms of the relationship both human populations had with their environment and its food resources. There is an important caveat, namely that no diagnostic human remains have been found in any of the the latest Mousterian or earliest Aurignacian levels in Cantabrian Spain, although it is widely assumed that Neanderthals were the authors of the Mousterian and AMH of the Aurignacian industries (Marín Arroyo et al. in press; Straus 2015). Regardless of this, this study provides valuable insights into the past environments when both caves were occupied.

3. Materials

For the bone collagen analysis, red deer (Cervus elaphus) bones were sampled, as it was the most commonly hunted mammal species regionally during the Late Pleistocene (e.g. Altuna 1972; Marín-Arroyo 2010). A total of 79 bones with evidence of anthropogenic modification, such as cut marks and/or marrow extraction breakage, were sampled. All bones were zooarchaeologically assessed to be adult, based on bone fusion, to prevent analysis of juveniles that may have prevailing weaning signatures (Schurr 1997; 1998).
From Covalejos a total set of 34 red deer bones were selected from Mousterian Levels J (n=9) and D (n=11) and Archaic Aurignacian Levels C (n=6) and B (n=8). These levels were selected because they were well-defined stratigraphically, contained diagnostic lithic and bone artefacts and had previously been successfully dated and could be attributed to distinct chronological timeframes. Of the 34 bones studied, those samples that had δ¹⁵N values from the lower, middle and upper ranges of the dataset and yielded the >5mg of collagen needed for analysis were analysed for sulphur stable isotope analysis (11 samples from the four levels) to explore possible differences in hunting locations near the site.

In total 45 bone specimens from El Castillo were selected from the recent excavations to ensure stratigraphic integrity, cultural attribution and with reliable dating evidence. Mousterian (Level 20C, n=8), Transitional Aurignacian (Levels 18B, n=9 and 18C, n=7) and Proto-Aurignacian (Level 16, n=3) were sampled, including in between sterile Level 16/17 (n=1), Level 17C (n=3) and Level 19 (base, n=7 and upper n=7). A pilot study of 4 horse teeth from Mousterian Level 20E (n=2) and Aurignacian Level 18B (n=2) was undertaken to establish whether the technique holds potential in the region for exploring Palaeolithic palaeoenvironments. In this study, two horse P3 and two P4 teeth were analysed. Horses were selected as they are obligate drinkers and are more likely to reflect local precipitation, humidity and rainfall (Longinelli 1984; Luz et al. 1984; D'Angela and Longinelli, 1990). The potential to infer climatic and environmental information from the enamel isotopes of this species has been previously demonstrated in both modern and fossil samples (Higgins and MacFadden 2004; Hoppe et al. 2004; Fabre et al. 2011), but has not previously been attempted in the Cantabrian region for the period studied. One of the main aims of this analysis were to determine whether the Cantabrian region provided the strongly seasonal conditions required to be registered in the tooth carbonate and to assess the potential of the technique for further palaeoenvironmental and palaeoclimatic reconstruction.

4. Methods

4.1. Bone collagen extraction and analysis

Collagen extraction was undertaken following procedures outlined in Richards and Hedges (1999) with an ultra-filtration step (Brown et al. 1988). Bone fragments between 0.6-0.8g were cleaned using aluminium oxide air abrasion, before demineralisation in 0.5M HCl at 6-8 °C for between 3-10 days and were washed using de-ionised water. Samples were gelatinised in a weak acidic solution (pH3 HCL) at 70 °C for 48 hours, then filtered with 5–8 μm Ezee® filters (cleaned for 20 minutes using deionised water in an ultrasonic bath), prior to ultrafiltration to separate out the larger >30ka collagen chains, indicative of well-preserved collagen. Ultrafilters were cleaned in the centrifuge using NaOH solution, before being rinsed 3 times with deionised water. The >30ka fraction was frozen and lyophilized for 48 hours before analysis using a Delta XP mass spectrometer coupled to a Flash EA 2112 elemental analyser at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). The δ¹³C values and δ¹⁵N values are reported relative to the V-PDB and AIR standards. International and internal standards were used to calculate analytical error which was ± 0.1‰ (1σ) or better. The mean difference observed between duplicate measurements was 0.03 for δ¹³C and 0.01 for δ¹⁵N. All samples were analysed in duplicate and the mean values of these are discussed within this manuscript. All specimens discussed further had %C values above 35 and %N values above 15% and C: N values of 2.9-3.4 indicative of in vivo collagen (De Niro 1985). Results from bone collagen δ¹³C and δ¹⁵N analysis of five modern day wild red deer are also presented to help contextualise the findings of this study. The δ¹³C values of the modern deer have not been corrected for the Suess effect, which is the decrease in atmospheric carbon isotope values as a result of modern anthropogenic processes (e.g. industrial pollution) (Keeling 1979), and direct comparisons of δ¹³C values of the modern specimens with the archaeological animals is not attempted.

Specimens were analysed for δ³⁴S values at the University of British Columbia Stable Isotope Laboratory in Vancouver using a MicroCube IsoPrime 100 DI mass spectrometer. Samples were weighed into tin capsules with 1 mg of V2O5 (Microanalysis, U.K.) to facilitate combustion (Morrison et al. 2000). Quality indicators of
atomic C:S collagen values between 600±300 and atomic N:S collagen values between 200±100 (Nehlich and Richards 2009).

Data were analysed statistically using a Mann-Whitney U test, with a post-hoc Holm-Bonferroni correction (Holm 1979). A p-value of <0.05 or less was deemed to be indicative of a statistically significant result. Tests were undertaken using statistical package PAST (Hammer et al. 2001). All raw data and quality indicators for each sample are included in the Supplementary Material.

4.2. Enamel sampling and analytical techniques

The surface of each tooth was cleaned by abrasion using a diamond coated drill bit mounted on a variable-speed handheld drill, prior to sampling to minimize contamination. Powdered enamel samples were collected using a 1mm-diameter cylindrical diamond tipped drill bit held perpendicular to the crown-root axis on the side of the tooth where the enamel was best preserved and samples were taken at 2-3 mm intervals. Each sample was taken through virtually the entire depth of enamel, avoiding the innermost portion of the enamel to prevent dentine contamination. For each sample, approximately 7mg of powdered enamel was prepared following (Balasse et al. 2002). Samples were treated with 3% NaOCl for 24 hours (0.1ml/mg sample), and thoroughly rinsed with deionised water, before a reaction with 0.1M acetic acid for 4 hours (0.1ml/mg sample). Samples were then thoroughly rinsed, frozen and freeze-dried. Isotopic analysis was performed at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge. Enamel powder samples were reacted with 100% orthophosphoric acid for 2 hours at 70°C in individual vessels in an automated Gasbench interfaced with a Thermo Finnigan MAT 253 isotope ratio mass spectrometer. Results are reported with reference to the international standard VPDB calibrated through the NBS19 standard (Coplen 2011) for which the precision is better than ±0.11‰ for 18O/16O and ±0.08‰ for 13C/12C.

3. Results

3.1. Covalejos

Preservation at Covalejos was excellent with 33 of the 34 bone specimens studied containing sufficient collagen for analysis. The mean bulk collagen δ13C values is -20.5‰ for Levels J, D and C and -20.6‰ for Level B (Table 2, . Redrawn from J. Rios.

Figure 2), showing great similarity through time and no statistically significant differences were observed between these Mousterian and Aurignacian samples (Table 3). Within Level D a greater range in the δ13C (1.6‰) is seen in comparison with the other levels, which typically have a range of 0.9‰ or less (Table 2). The mean bulk collagen δ15N values ranges between 4.0‰ in Level D and 5.3‰ in Level B (Table 2). The mean δ15N value for Level B is around 1‰ higher than is observed in the lower Levels C, D and J, although no statistically significant differences were observed between levels (Table 3). Within Levels B and D, a higher range in δ15N values is seen, of 5‰ and 5.9‰ respectively, with maximum values of up to 7.9 ‰ in Level B and 7.3‰ in Level D (. Redrawn from J. Rios.

Figure 2; Table 2 ).

The two δ34S values for Mousterian Level J are 7.9 and 9.7‰ (Figure 4). The δ34S values for Level D are higher than seen in Level J, lying between 11.9-13.0‰ and are consistent with those seen in Level C, ranging between 10.0-12.8‰). The δ34S values for Aurignacian Level B are slightly lower, ranging between 8-8.2‰ which are similar to those seen in Level J (Figure 4). No correlation between the δ34S and δ13C or δ15N values for the sample individuals was observed (Figure 4).

3.2. El Castillo
All 45 bone samples analysed yielded viable results within the parameters for in vivo collagen. Due to the large number of levels analysed at this site, full results are presented in Table 2 and Figure 5 and 6 and a summary of these findings are presented within the text. The mean bulk collagen values of δ¹³C and δ¹⁵N are remarkably similar within each level and no statistically significant differences between the red deer bones was observed, indicating a degree of homogeneity in the δ¹³C and δ¹⁵N values over this temporal range (Table 4).

In the lower part of the sequence, Level 20C was notable for having a minimum δ¹³C value of -21.4‰ (Table 2, Figure 5) and there is a range of 1.3‰ for the δ¹³C values within this level, which is slightly higher than seen for other levels. Within Level 19 Upper the largest range in δ¹³C_coll is observed (range= 2.2‰) among all the levels analysed, with one individual (CAS29) having a δ¹³C value of -18.6‰, which is the highest value seen at the site and falls outside 1.5 times the interquartile range of δ¹³C values at both sites, statistically making this individual an outlier. This value is also inconsistent with any other individual studied at either site, as seen at nearby El Mirón Cave (Stevens et al. 2014) and from other late Pleistocene sites within Northern Spain (Domingo et al. 2014; Rofes et al. 2015). It is possible that this bone was exposed to post-depositional or curational processes, that produced δ¹³C value inconsistent with other individuals, such as the use of consolidates or conservation treatments. In fact, the presence of glue was noted on the specimen, although care was taken during sampling to avoid treated areas. In the upper part of the stratigraphic sequence, larger δ¹³C_coll ranges are seen within Level 16 (range=1.1‰) (Table 2, Figure 5).

The δ¹⁵N values at El Castillo are typically lower than those seen at Covalejos. The maximum value within the sequence is 5.2‰ corresponds to Level 18B, meaning that this level has a range of 4‰ (Table 2, Figure 6). For the other levels the range in the δ¹⁵N values is typically around 2‰.

For the tooth enamel analysis, the δ¹²C_coll values of the two horse teeth from Transitional Aurignacian Level 18B (CAS58 range -11.5 to -11.8 ‰, CAS59 range -11.0 to -11.7‰) are higher than those in Mousterian Level 20E (CAS60 range -11.5 to -12.5, CAS61 range -12.4 to -12.1‰) (Table 5, Figure 7). There is also a small change in the δ¹⁸O values, with the specimens from Mousterian Level 20E that exhibit slightly lower values than those seen in Transitional Aurignacian Level 18B. The range of within-tooth δ¹⁸O variation ranges from 1.2‰ (CAS59) to 1.8‰ (CAS85), likely reflecting seasonal variations in the isotopic composition of drinking water, linked to precipitation.

4. Discussion

4.1. Environmental conditions at Covalejos and El Castillo

No collagen δ¹³C value at either El Castillo or Covalejos is lower than -22.5‰, suggesting that the red deer are consistent with consuming C₃ plants in a relatively open landscape, according to Drucker et al. (2008), outside of the influence of the canopy effect (van de Merwe and Medina 1991). At Covalejos and El Castillo δ¹³C values are constant through time, suggesting that there was very little change in tree cover, climate, or atmospheric δ¹³C in the region during the late Middle and early Upper Palaeolithic (late MIS3) and a predominantly open landscape prevailed. A summary of available environmental proxies available for both sites is included in Summary statistics for δ¹⁸O and δ¹³C values of each tooth analysed by level 20E and 18B at El Castillo.

Table 6.

At El Castillo, pollen evidence from Level 20 showed that steppic taxa were common and noted the presence of arboreal pollen including pine (Dari and Renault-Miskovsky 2001; Summary statistics for δ¹⁸O and δ¹³C values of each tooth analysed by level 20E and 18B at El Castillo.
Table 6). Within Levels 18B and 18C, a slight reduction in tree pollen is evident, including a decrease in pine and an increase in steppic vegetation (Dari and Renault-Miskovsky 2001; Summary statistics for δ¹⁸O and δ¹³C values of each tooth analysed by level 20E and 18B at El Castillo.

Table 6). Charcoal evidence shows a similar effect, with a disappearance of pine and increase in Sorbus between Levels 20 and Level 18B/C that could indicate a climatic improvement (Uzquiano 2005; 2008). In Covalejos Level J, slightly cooler environments are suggested by the presence of pine and birch and steppe shrubs being common, with more steppe species and temperate species within Levels D and C, followed by a cooler environment in Level B with pine and land shrubs increasing, based on the limited charcoal fragments available (Uzquiano 2005; 2008). These environmental changes are not clearly observable in the δ¹³C bone collagen record, suggesting that they were not sufficiently strong to impact on the long-term bone collagen record of the red deer populations. Whilst there may have been small changes in the level of tree cover at both sites, they are not observable in the stable isotopes analysed, which suggests that these changes were not necessarily dramatic and the impact on these changes on the niches of herbivore populations were minimal. Differences in the palimpsests of time represented by the archaeological levels and by the bone collagen record versus the timescales registered in the pollen record, may also explain why subtle changes in vegetation cover are not necessarily observable.

The horse tooth enamel δ¹³C_car results show higher isotopic values in Transitional Aurignacian Level 18C in comparison to Mousterian 20E, which could indicate a small change in local vegetation, climate or atmospheric δ¹³C, although these results have to be viewed with caution based on the small sample size studied. The shorter time frames represented by the teeth may allow subtle seasonal differences to be identified, which will not be reflected in the longer-term averaging of diet/environment in the bone collagen record. The continued and consistent human exploitation of species such as red deer, aurochs and horse throughout the archaeological sequences of both sites (Summary statistics for δ¹⁸O and δ¹³C values of each tooth analysed by level 20E and 18B at El Castillo).

Table 6) suggests that despite these small changes in local environment, those herbivores must have successfully persevered in the region and represented an important resource throughout the late Mousterian and Early Aurignacian, as attested by their numerous presence in the zooarchaeological record (Altuna, 1972; Marín-Arroyo 2010).

The incremental variation in enamel δ¹⁸O may also indicate seasonal variations in local climate, particularly in temperature and rainfall-amount mediated variations in the δ¹⁸O of precipitation. Although no consistent pattern of within tooth variation can be observed between different specimens, this may be because differences in the season of birth and/or differences in the timing and duration of mineralisation result in different patterns within teeth, between teeth and in addition to producing inter-individual variation (Bendrey et al., 2015). The relatively small magnitude of within-tooth δ¹⁸O variation reflects the dampening of the input signal through the contribution of atmospheric δ¹⁸O to the enamel signature, the turnover time of oxygen in the body and the process of enamel mineralisation (van Dam and Reichart 2009). The influence of climate on enamel δ¹⁸O can be further weakened through animal behaviour, such as drinking from large bodies of freshwater, which are less responsive to seasonal variations in δ¹⁸O_presh. However, the location of both sites within the Pas valley would imply a limited access to large bodies of freshwater. While this is only a small sample, it does highlight some of the complexities in using tooth enamel δ¹⁸O as a proxy from past climate in the mountainous Cantabrian Region. Further exploration of this method is limited by the availability of well-preserved equid teeth within the regional late Mousterian and Aurignacian (Altuna, 1972).

4.2. Microenvironments in the Late Middle and Early Upper Palaeolithic

The larger ranges in the δ¹⁵N values of individuals from Levels B and D at Covalejos requires further investigation to explore the causes of these differences. All individuals analysed were assessed to have been adults and thus not influenced by weaning signatures (Schurr 1997; 1998). Short-term stress episodes such
as starvation or pregnancy can impact on δ¹⁵N values within an individual (Fuller et al. 2005; Hauber et al. 2005), but they will not affect the long-term bone collagen record. Instead, environmental and geographic factors are more likely to have played a role, with multiple nitrogen isotopic zones (or ‘isozones, an isotopically distinct area within a landscape, which together form an ‘isoscape’) or microenvironments potentially being represented at the levels that contain the wide-ranging values. The effect of differing microenvironments representing isotopically distinct regions has been demonstrated as a concept, among archaeological animal populations in prehistoric southern Britain (Stevens et al. 2013) and might represent a reasonable hypothesis for this high-relief, coastal region. A plethora of factors, linked to geography, lithology and environment, can influence baseline δ¹⁵N values in a region. Locations that are part of an active floodplain versus areas where river flooding is uncommon can cause differing values. In European riparian environments, areas with higher water tables (c. -10 to -30cm) exhibit denitrification, reducing soil nitrogen availability compared to lower water tables where higher nitrogen values are seen (Hefting et al. 2004).

Differences in local soil types and the nitrogen fixing mycorrhizae linked to differing vegetation cause variations in δ¹⁵N values (Szpak 2014), observable on a variety of spatial scales (Craine et al. 2009; Hobbie and Högberg, 2012). A positive correlation between soil pH and δ¹⁵N ratios is seen within plants (Mariotti et al. 1980; Handley and Raven 1992), which can pass up the food chain to consumers (Rodière et al. 1996). Annual rainfall and aridity can also affect δ¹⁵N values in plant communities (Ambrose and DeNiro 1986; Cormie and Schwarcz 1996; Gröcke et al. 1997; Heaton et al. 1986), but due to the geography of the Cantabrian region this would imply movement over much greater distances to the South, indeed outside of the rain shadow effect of the Cantabrian Cordillera, into the Old Castilian Plateau. Alternatively, climatic oscillations between wetter and dryer conditions could explain the pattern (see the case of SW France in Bocherens et al 2014). These changes would not need to be dramatic to be recorded in the δ¹⁵N signature, with no major changes in vegetation cover as suggested by the homogenous δ¹⁵C_COT trend.

Animals habitually feeding in areas with differing baselines within the hunting ranges of a residential camp could result in individuals with higher and lower δ¹⁵N values accumulating within the same archaeological levels. In this scenario sufficient heterogeneity in the vicinity of the site would need to be experienced within the usual distance for optimally hunting animals. For the Magdalenian in the region, hunting ranges are observed to be around 10km in the region (see Marín-Arroyo, 2009a), although this was a period of greater economic specialisation and technological advancement and during the Middle and earlier Upper Palaeolithic, hunting ranges may have been slightly larger but would have still been relatively small due to the distinct topographic characteristics of the Cantabria province where the distance between the high mountains and the north Atlantic sea is only 30-35km. The presence of microenvironments in the region is further attested by isotopic results achieved at El Mirón cave, also in the same province. The highest red deer δ¹⁵N value observed in El Mirón within the Palaeolithic samples analysed was 3.8‰ (Stevens et al. 2014), which is lower than the maximum values observed in either El Castillo or Covalejos. El Mirón is located at 260 masl and 20 km from the shore (and 25 km from the -100 m isobath line) in the upper part of the nearby Asón valley in easternmost Cantabria (Figure 1), which again could represent an isotopically distinct area from Covalejos and El Castillo, which are both located in the lower and upper part of the Pas valley. The geology of the Pas valley and environs is varied, with lower cretaceous rocks framed by late cretaceous outcrops towards the coastal areas and pockets of Triassic and Jurassic rocks further south (Heredia et al. 1990). Spatial variation of plant δ¹⁵N values and subsequently δ¹⁵N values of the animals that consume them, can vary depending on localised factors relating to soil profile depth, bedrock and pH, amongst others (e.g. Högberg 1997; Hobbie and Ouimette 2009; Szpak 2014), which are in part dictated by the geology and lithology of the local area. The geological variations in this part of Cantabria, particularly in the northern area of the Pas valley could provide sufficiently distinct conditions to explain the variations in the isotope values observed. The wild modern red deer analysed (n=5) for this region have a mean value of 3.2‰ and a maximum value of 4.0‰ (n= Table 2), which is lower than the majority of the Pas Valley samples from El Castillo and Covalejos, although these values are viewed cautiously, as they are subject to a range of modern processes (e.g. the use of agricultural fertilizers) that may affect nitrogen baselines.
The results achieved in this study hint at a mosaic landscape in Cantabria with distinct isozones, offering different microenvironments with distinctive isotopic baselines, that were both exploited by Neanderthals and AMHs. In Covalejos Levels C and J, the lower ranges of nitrogen and the consistency within the stable isotope values observed show the use of one isotopically similar zone being exploited by both human species, whereas during the formation of Levels B and C, multiple nitrogen isozones appear to be represented. Due to the palimpsestic nature of Palaeolithic deposits, it is difficult to determine whether these patterns relate to the exploitation of different hunting zones through time or several hunting events within each level.

The δ34S analysis was undertaken on samples within Covalejos to explore the larger ranges in δ15N values observed and to determine if differing hunting locations were being used by human populations. That variation in the range of δ15N values was not observed in El Castillo and sulphur isotopic analysis was not attempted on samples from this site in this research, although future analysis would be beneficial in enhancing our understanding of sulphur isotope systems in the region. In Covalejos there is no statistical correlation between the δ34S and δ15N values. The systems controlling sulphur values in organisms are dictated by rainfall, rock type and seawater inclusions (Richards et al. 2003; Nehlich 2015), whereas nitrogen systems are controlled predominantly by a complex web of factors affecting plant-soil systems (Szpak 2014). This may explain why the δ34S and δ15N values are not necessarily connected. The results suggest that if animals from different nitrogen isozones were being exploited, they were not necessarily from the same sulphur zones.

Independently, the δ34S values also suggest exploitation of different zones in the landscape. In Covalejos, the lower δ34S values observed within Aurignacian Level B and Mousterian Level J indicates that these individuals were procured from an isotopically distinct zone than those obtained within Mousterian Level D and have values elevated by at least 4 ‰. Sulphur studies of modern ecosystems note 1.9 ‰ difference within living animal populations and a higher variability within archaeological studies of 2.4 ‰ variation have been observed (Nehlich 2015:8), although in reality this may be higher within Palaeolithic faunal assemblages (Drucker et al. 2015). However, even bearing this variability in mind, the individuals within Level B are substantially lower in δ34S than those seen in Level D and suggests that the red deer, from both levels, could be from isotopically different δ34S regions. Larger sample sizes would be needed in the future to determine whether these differences are statistically significant.

The macromammal spectrum at Covalejos shows a predominance of red deer, with some horse and bovids (Yravedra et al. 2016). The presence of wild boar and roe deer within Levels B, C, D and J (Yravedra et al. 2016) could indicate relatively temperate conditions The predominance of red deer is mirrored at El Castillo in both Mousterian and Aurignacian levels (Dari 1999; Luret 2017) (Summary statistics for δ18O and δ13C values of each tooth analysed by level 20E and 18B at El Castillo.

Table 6) and it is consistent with the patterns of exploitation seen within the wider Cantabrian region during Late Pleistocene (Altuna, 1972; Marín-Arroyo 2010). Information on seasonality of occupation is limited for these sites. Initial cementum studies at El Castillo suggest that there were similarities in the seasonal occupation patterns at El Castillo (Pike-Tay et al. 1999). To date, no information about the seasonality of occupation at Covalejos is available to explore whether there is a link between the season of occupation and the different isozones that were being exploited.

In Cantabria, the archaeofaunal record shows that, depending on the topographic location of the site in question, there is a predominant exploitation of red deer and/or ibex and more rarely of larger mammals (Marín-Arroyo 2009b). In El Castillo, red deer has been shown to be the predominant prey hunted and skeletal profiles indicate a complete transport of the carcass to the cave which suggest that red deer are found close to the site. In Covalejos, the complete nature of transported red deer carcasses is suggested by the presence of appendicular and cranial elements (Yravedra 2016). This suggests that animals were being hunted locally at both sites, which is why they are being brought back almost complete to the site. The exploitation of different isozones during certain episodes of time at Covalejos may represent periods of
environmental or climatic stress, which would force the need to seek for alternate hunting areas further afield. Additionally, during times of increased population sizes, hunting ranges may have been further increased to find sufficient food for the population. This would explain the patterns seen both in the δ³⁴S and δ¹⁵N values seen. At Covalejos, both Neanderthals and AMH specialised in the exploitation of red deer (Yravedra et al. 2016) and according to this study they were exploiting similar isozones within the landscape during the Mousterian and the Aurignacian.

The range of microenvironments in the Cantabrian region, suggests a mosaic landscape, likely created by the natural barriers represented by the Cantabrian Cordillera to the south, the North Atlantic Sea to the north and the steep-sided closed valleys in mountains that often drop directly to the ocean or onto only narrow coastal plains. The environmental variability in this particular Iberian region may have somewhat buffered the effects of the larger climatic events exhibited throughout MIS3 in Europe, enabling the successful survival of the most commonly consumed temperate ungulate species and may help to explain why this region was consistently occupied throughout the late Middle and during the vast majority of the Upper Palaeolithic and likely contributed to the importance of the region as a refugium for humans and animals (Consuegra et al. 2002; Fu et al. 2016; Meiri et al. 2014; Straus 2015a) in the later stages of the Upper Palaeolithic.

5. Conclusions

Multi-isotope analysis at the key Middle to Upper Palaeolithic transitional cave sites of El Castillo and Covalejos was undertaken to explore environmental conditions and the landscape exploitation by late Neanderthal and early AMH populations inhabiting the Cantabrian region between c.49-34ka uncal BP. Results in the bone collagen record show little change in δ¹³C Ernst values through time at both sites, indicating that there was stability in the relatively low density of late MIS3 tree cover in the region. Small changes in vegetation are observable in charcoal and pollen evidence, but this is not clearly observable in the long-term bone collagen record and temperate species continued to prevail. This suggests a level of stability in the environment in terms of vegetation. Initial analysis of horse teeth at El Castillo indicated a difference in δ¹³C Ernst values earlier than 42 ka cal BP, suggesting that this technique has potential for providing information on seasonal changes in vegetation or environment in this region, although achieving sufficient sample sizes of ungulate teeth for this time period would be a challenge.

Large inter-individual variations in δ¹⁵N values were observed within Levels B and D at Covalejos and it is likely a product of animals being procured from different isozones surrounding the site, which might indicate a mosaic of microenvironments. Whilst no link between δ¹⁵N and δ³⁴S values was observed, inter-level differences in δ³⁴S values within Covalejos suggested that animals came from different sulphur regions, confirming that animals were being procured from different areas of the landscape, likely adjacent, but geologically distinct areas within traveling distance of the site, as attested by almost complete carcasses being found within the archaeological deposits at El Castillo and by the presence of appendicular and cranial elements within the zooarchaeological record at Covalejos. The capture of ungulates from distance further from the cave may correspond to periods of higher resource pressure or increased population density where larger hunting ranges were needed to obtain sufficient animal resources.

The suite of ungulates identified within each archaeological level was similar, with red deer dominating the faunal record, indicating that these resources were readily available throughout the temporal period studied and the presence of microenvironments may have contributed to the success of these species. The distinctive conditions in the Cantabrian region likely tempered the larger environmental changes seen during late MIS3 in Europe, enabling these resources to be consistently and predictably exploited during the Late Middle Palaeolithic and Early Upper Palaeolithic and would have made the region a desirable location for Neanderthals and subsequently AMH.

Acknowledgements
This research was funded by the European Commission through a Marie Curie Career Integration Grant (FP7-PEOPLE-2012-CIG-322112), by the Spanish Ministry of Economy and Competitiveness (HAR2012-33956 and Ramon y Cajal-2011- 00695), the University of Cantabria and Campus International to ABMA. This research is also included within the HAR2015-70652-P project. Radiocarbon dating at ORAU was funded by HAR2012-33956 project. J.R.J was supported by a Marie Curie Individual Fellowship (H2020-MSCA-IF-2014-656122). Laboratory work, associated research expenses and isotopic analysis were generously funded by the Max Planck Society to M.R. We are grateful to the Museo de Prehistoria y Arqueología de Cantabria (Gobierno de Cantabria) for facilitating access to the fauna material analysed. Annabell Reiner provided essential lab support in the MPI-EVA laboratories and Sven Steinbrenner supported the mass spectrometry aspects of the project. Reba McDonald and Megan Wong at the UBC stable isotope laboratory were invaluable in their help with the sulphur analysis. James Rolfe and Louise Butterworth at Cambridge University are thanked for their laboratory and analytical assistance with the tooth δ18O and δ13C analysis. We thank Lawrence Straus for reading and editing the manuscript. We would also like to thank the two anonymous reviewers for their constructive comments and feedback that helped to improve the manuscript.

List of Figures

Figure 1. Sites located in the Cantabria province, Northern Spain cited within the text. 1: El Castillo; 2: Covalejos; 3: El Mirón. Redrawn from J. Rios.
Figure 2. Red deer δ13C values and the mean population value per level at Covalejos
Figure 3. Red deer δ15N values and mean population value per level at Covalejos
Figure 4. Plots showing of red deer δ13C and δ34S values (left) δ15N and δ34S values (right) plotted per level at Covalejos.
Figure 5. Red deer δ13C and mean population values per level at El Castillo
Figure 6. Red deer δ15N and mean population values per level at El Castillo
Figure 7. Horse tooth sequential δ18O and δ13C values from Levels 20E and 18B at El Castillo

List of Tables

Table 1: Published and newly obtained radiocarbon dates in this study from El Castillo and Covalejos for the levels analysed, with laboratory codes, sample preparation methods used and a summary of the specimens per site and level sampled. For radiocarbon method AF=collagen extracted using the ultrafiltration method prior to analysis, AMS=dated using accelerated Mass Spectrometry, C14=dated using traditional radiocarbon methods. *Denotes a date that extended beyond the limit of the calibration curve. All calibrations presented are those published in the original radiocarbon date reference. Calibrations for the new dates achieved from Covalejos and for the Sanguino and Montes (2005) dates were completed using OxCal v4.3, using the IntCal 13 curve.

Table 2. Summary statistics of bone collagen δ13C and δ15N values for each level at Covalejos and El Castillo and for modern deer from Cantabria Modern deer δ13C values are not corrected for the Suess effect.

Table 3. Results of Mann Whitney U test for δ13C and δ15N values between each level at Covalejos. No values yielded statistically significant results.

Table 4. Results of Mann Whitney U test for δ13C and δ15N values between each level at El Castillo. No values yielded statistically significant results.

Table 5. Summary statistics for δ18O and δ13C values of each tooth analysed by level 20E and 18B at El Castillo.
Table 6. Summary of available environmental proxies for levels analysed in this study at El Castillo and Covalejos. Grey shading denotes where information was not available.

Bibliography


Drucker, D.G., Bridault, A., Cupillard, C., Hujic, A., Bocherens, H., 2011. Evolution of habitat and environment of red deer (Cervus elaphus) during the Late-glacial and early Holocene in eastern France (French Jura and the western Alps) using multi-isotope analysis ($\delta^{13}$C, $\delta^{15}$N, $\delta^{18}$O, $\delta^{34}$S) of archaeological remains. Quat. Int. 245, 268–278.


Higham, T. 2011. European Middle and Upper Palaeolithic radiocarbon dates are often older than they look: problems with


