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Stable isotope analysis of Late Upper Palaeolithic human and faunal remains from Grotta del Romito (Cosenza), Italy

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ABSTRACT

Stable isotope analysis of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) were carried out on one of the largest assemblages of Late Upper Palaeolithic human remains in Southern Europe, at Grotta del Romito (Cosenza), Italy. The burials were stratigraphically dated from ca. 18,000 to 13,000 cal BP, which was confirmed by a series of new AMS dates made directly on the bone collagen. Dietary reconstruction from carbon and nitrogen stable isotopes revealed that eight of the nine individuals analysed, dating to the Final Epigravettian, had very consistent diets, rich in terrestrial animal protein, regardless of their age or sex. These included two individuals who were suffering from severe pathologies. A single individual, dating to the Evolved Epigravettian had a more variable diet, which was significantly enriched in protein from marine or freshwater fish compared to the later burials. Overall, the results are consistent with the very limited number of other studies which describe a change to more specialised and less variable subsistence strategies, in this case the hunting of large herbivores, towards the end of the Palaeolithic period. Sulphur isotope values of all of the nine burials and several faunal samples were notably consistent, showing no evidence of long-distance migration to the site from a different geological zone.

1. Introduction

Grotta del Romito, with its rare examples of rock art and multiple burials, is one of the most significant Upper Palaeolithic archaeological sites on the Italian peninsula. The cave was occupied during the Palaeolithic from ca. 24,000 to 10,000 BP (Martini, 2002, 2006b; Martini et al., 2004, 2007; Martini and Lo Vetro, 2005a,b, 2007), through a period of considerable climate change that had marked impacts on the local environment (Colonese et al., 2007; Ghinassi et al., 2009). To date, nine intact, well preserved burials have been recovered from stratigraphic layers dating from ca. 18,000 to 11,000 BP, the majority of burials corresponding to a period of climatic amelioration from ca. 15,000 to 13,000 cal BP (Final Epigravettian). This period corresponds to a greater intensity of occupation of the site, inferred from numerous fireplaces and extensive accumulations of bone and lithics, and is thought to correspond to specialization of hunting and gathering strategies (e.g. Martini et al., 2007).

Here we report on the stable isotope analysis of the human remains from Grotta del Romito, in order to extract direct palaeodietary information from each of the individuals. Our aims were to examine dietary differences through the sequence, between individuals buried at the same time or in close succession and to compare these data with those from other Palaeolithic Mediterranean sites.

The analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of bone collagen is a well established method for elucidating past diets; the technique and its application are well described in several review papers (e.g. Katzenberg, 2000; Sealy, 2001). Carbon isotopes clearly distinguish the consumption of marine (^{13}C enriched) from terrestrial foods (^{13}C depleted) and C_4 pathway plants (^{13}C enriched) from C_3 plants (^{13}C depleted). Nitrogen isotopic values ($\delta^{15}N$) increase by 3–5‰ with increasing trophic level (Hedges and Reynard, 2007) and therefore are useful for distinguishing animal-rich diets from plant-rich diets as well as the

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consumption of high trophic level marine and freshwater fish. Nitrogen in collagen is directly obtained from dietary protein and therefore will only reflect dietary protein sources consumed. Carbon in bone collagen is also preferentially obtained from dietary protein, but can be derived from other dietary carbon sources (carbohydrate and lipid), depending on dietary protein levels (e.g. Ambrose and Norr, 1993; Howland et al., 2003; Jim et al., 2006). As collagen turns over relatively slowly in bone, collagen stable isotope values reflect average diet over a long period of time; thought to be at least 10–15 years prior to death (Hedges et al., 2007). Stable isotope data therefore provides direct dietary information relating to a specific individual which contrasts with other methods more commonly used to study the Palaeolithic economy, such as the examination of faunal remains, the interpretation of the use of stone implements and, in rare preservational circumstances, the identification of plant remains (e.g. Weiss et al., 2004) all of which indirectly reflect diet at different intensities and over different periodicities.

Stable carbon and nitrogen isotope analyses have already been applied to Upper Palaeolithic human and Neanderthal specimens to characterise hunting strategies (Drucker and Bocherens, 2004; Richards et al., 2000, 2001a; Richards and Trinkaus, 2009; Stevens et al., 2010). These studies revealed the importance of marine and freshwater resources to anatomically modern humans from the mid-Upper Palaeolithic period, in addition to a range of other food resources (Richards et al., 2001a; Pettitt et al., 2003), indicative of a broad spectrum economy. Yet, relatively few human remains dating to the late Upper Palaeolithic or Final Epigravettian (15,000–11,000 cal BP) have been analysed. Stable isotope analysis of human remains from Kendrick's Cave (ca. 12,000 BP), a coastal British site, revealed significant consumption of marine foods (Richards et al., 2005), whilst other isotopic studies from British late Upper Palaeolithic inland sites have shown that diets were dominated by animal protein, in this case, indicative of a specialised hunting economy (Richards et al., 2000; Stevens et al., 2010). Here, the dietary evidence has been used to suggest the beginning of a narrowing in food procurement strategies, leading to the further specialism in the Mesolithic period and finally to food production (Richards et al., 2005). However, it is unknown whether this emerging trend is observable isotopically in other parts of Europe, highlighting the need for further isotopic investigations. In total only five Late Upper Palaeolithic samples have been analysed from the entire Mediterranean region (Francalacci, 1989; Garcia Guixé et al., 2009), with those from the coastal site of Arene Candide showing no significant marine consumption (Francalacci, 1989). In this region, there is some doubt whether the relatively low productivity of the Mediterranean Sea, and particularly the lack of extensive intertidal zones, could support a specialised marine economy (Bailey and Flemming, 2008; Fa, 2008). Whilst Grotta del Romito is located some 25 km from the current coastline, and much further from the late glacial coastline the presence of marine shell ornaments at the site suggests a relationships with the coastal area.

In addition, it has been proposed that Upper Palaeolithic burials such as those of Grotta del Romito were selective, specialised and drawn from a geographically extensive catchment (Formicola, 2007), albeit based on very little archaeological evidence. One indication of whether the individuals buried at Romito were non-local is to examine variation in bone collagen sulphur isotope values $\delta^{34}S$ compared to local range inferred from fauna found at the site. This approach has been used to investigate mobility and the geographic origin of human remains interred together (e.g. Linderholm et al., 2008; Vika, 2009). In addition, sulphur isotopes also relate to diet and can be used with carbon and nitrogen isotopes to identify marine, terrestrial and freshwater consumers (Richards et al., 2001b; Privat et al., 2007; Nehlich et al., 2010). Environmental sulphate is assimilated by plants and reduced to organic forms, amino acids and sulpholipids with only a small isotopic fractionation (−1 to −2‰; Trust and Fry, 1992). Plant sulphur containing amino acids are subsequently incorporated into the consumer's tissues with only negligible further fractionation (Petersen et al., 1985). Sulphur isotope ratios of bone collagen therefore reflect environmental sulphate values which vary considerably (ca. −20‰ to +20‰; Petersen et al., 1985) in all but marine environments, depending on geological sources of sulphate (e.g. pyrites, evaporates) and the degree of microbial reworking, which is significant particularly in anaerobic lake and river sediments. Marine sulphate values are much more uniform (ca. +20‰; Peterson et al., 1985) and therefore marine organisms, marine consumers and plants and animals from coastal areas affected by sea spray and precipitation high in marine sulphate can usually be distinguished from freshwater and terrestrial consumers. Due to the extensive variation of sulphur isotope values in freshwater and terrestrial ecosystems and by geological setting it is important to define the local range in the areas of interest. However, as this is the first archaeological application of sulphur isotopes to ancient human remains in Italy and no other ancient reference values are available, we have conducted the analysis only to see if there was a difference between the individuals which might relate to either a difference in diet or indicate that the individuals came from regions with different $\delta^{34}S$ signatures due to geological or meteorological variation.

1.1. Grotta del Romito: the site and its setting

Grotta del Romito (39° 54′N, 15° 55′E) is located in Southern Italy, 275 m above sea level, and ca. 25 km away from the Tyrrhenian coast of Calabria, in the Lao Valley (Fig. 1). The region has a rugged, high-relief topography with mountain peaks over 2000 m high descending steeply towards the coast. The cave is situated at the toe of a rocky cliff on the right side of a narrow creek tributary of the Lao River, which itself is less than 1km away. The cave is in a Jurassic limestone containing reddish-brown mudstone interbeds, underlain by a darkish-grey Triassic dolostone, the dominant

Fig. 1. Map showing location of Grotta del Romito.

rock type of the area, and overlain by Miocene calcarenites that form the upper part of the local topography. A deeper bedrock, comprising brownish-grey schist with thin metaquartzite beds, crops out in the upper reaches of the creek. Boulders that lie on slope in front of the entrance (Fig. 2b) are the collapsed remains of a rockshelter, which would have connected to the main cave during the Palaeolithic period to form a large living space (Martini, 2002). The site has a long history of excavation which has produced a detailed 13,000 year record of Palaeolithic occupation (Martini, 2002; Cattani et al., 2004; Colamussi, 2004; Martini et al., 2004, 2007; Martini and Lo Vetro, 2005a,b; Colonese et al., 2007; Colonese and Martini, 2007). During the Last Glacial, from ca. 23,000 to 16,000 BP, human occupations were quite sporadic due to intense episodes of water runoff into the cave (Ghinassi et al.,

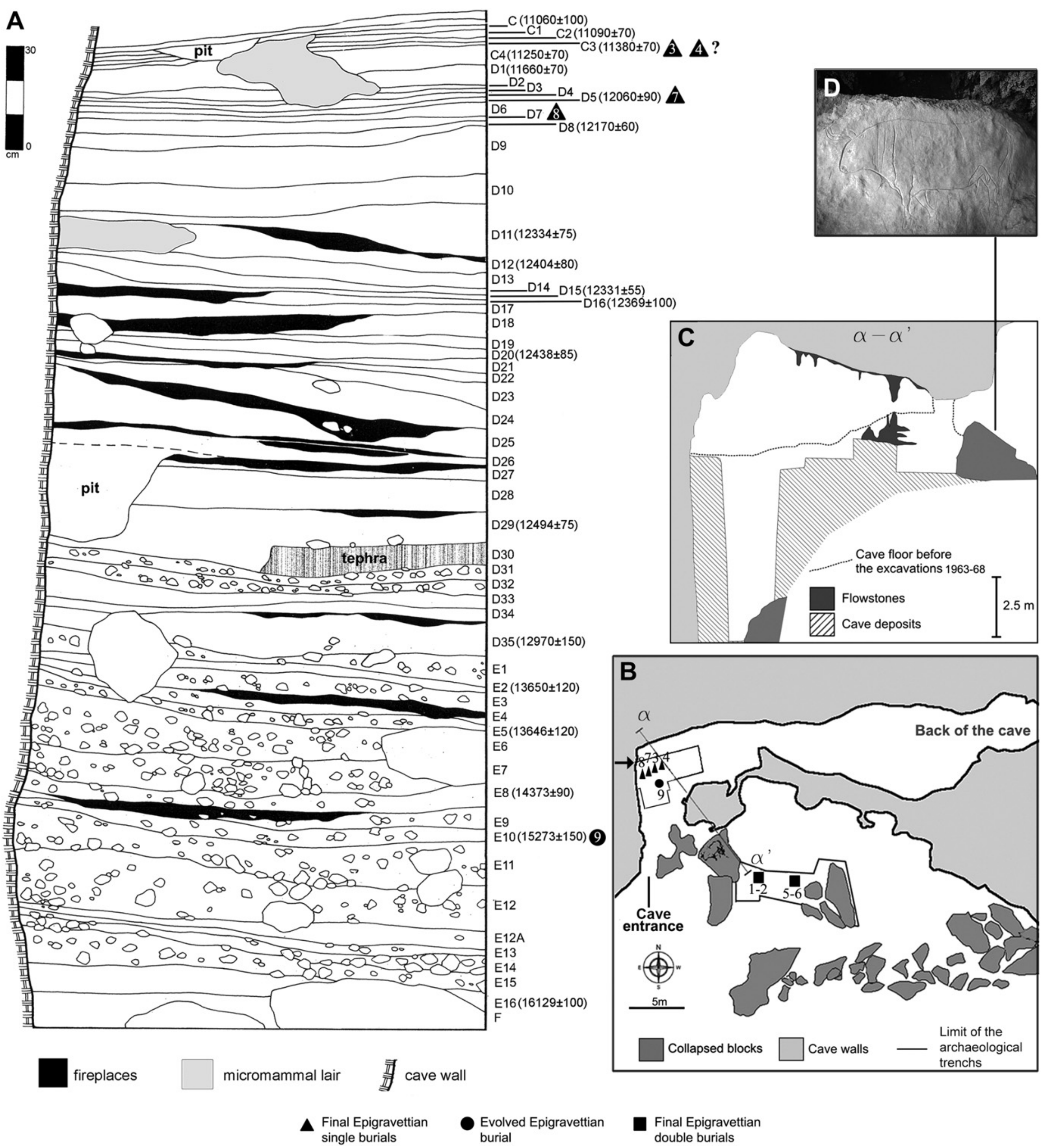

Fig. 2. Grotta del Romito showing: A: The Stratigraphic sequence (layers A–F) drawn from the section north of the trench excavated inside the cave (indicated with the arrow in diagram B). For calibrated ^{14}C dates see Table 1. The symbols indicate the position of the burials. Triangles with question mark (?) shows the probable stratigraphic position of Romito 3 and Romito 4 burials found by Graziosi in 1964. B: Plan of the site showing the distribution of the burials. C: cross-section of the site and of the excavated area along the axis α–α′ (indicated in diagram B). D: representation of a *Bos primigenius* engraved on a large rock in the rockshelter.

2009). This period was followed by a warm, moist period at ca. 15,000 cal BP until a further cooling corresponding to the Younger Dryas stadial from ca. 12,800 cal BP to 11,200 cal BP. At the regional scale, the oxygen isotopic composition of land snail shells suggests the area also experienced an abrupt and short-term climatic oscillation from ca. 14,200 to 13,900 cal BP, consistent chronologically with the Older Dryas (Colonese et al., 2007).

Fig. 2A shows the Epigravettian stratigraphic sequence of recent archaeological investigations within the cave detailing the main Final Epigravettian deposits, which correspond to the majority of the burials. In Southern Italy and Sicily Final Epigravettian burials are much simpler, often devoid of grave accompaniments, and more standardized than the previous phases (Gravettian and Early Epigravettian) which are well documented, especially in northern Italy (Liguria). The change is probably related to an increase in conceptual complexity of the funerary practices, enriched by a new element of abstraction and symbolism (Giacobini, 2006; Martini, 2006a,b,c, 2008; Palma di Cesnola, 2006). An abundance of perforated marine shell ornaments (particularly of *Columbella rustica* and *Cyclope* sp.), lithic and bone artefacts have been recovered from these layers (Cilli et al., 2005; Martini et al., 2004, 2007). The lithic assemblage shows an abundance of microlithic backed points and blades. Burins, denticulates, end-scrapers and flake and blade scrapers are also present (Martini et al., 2007). Raw lithic materials, such as radiolarites, were sourced up to 40 km from the cave in adjacent mountainous regions (Martini et al., 2006).

The faunal assemblage from the Final Epigravettian deposits is dominated by ibex (*Capra ibex*) and wild boar (*Sus scrofa*) and to a lesser extent by red deer (*Cervus elaphus*), roe deer (*Capreolous capreolous*) and chamois (*Rupicapra* sp.). Aurochs (*Bos primigenius*) and horse (*Equus ferus*) remains are much less abundant. Overall the assemblage points to a preference for hunting in woodland areas followed by steep slope environments (Martini et al., 2007).

In total, nine adult humans were recovered from the deposits at Grotta del Romito during excavations in 1965–1967 and 2000–2009 (Fabbri et al., 1989; Graziosi, 1971; Martini, 2002; Martini et al., 2004). In the rockshelter (Fig. 2B), two double burials (Romito 1 and 2) and (Romito 5 and 6) were excavated by Graziosi in the 1960's and are related to an human presence at the site between 13,290 and 10,700 cal BP (2σ), dates obtained from charcoal in stratigraphic context (Alessio et al., 1966; Graziosi, 1965; R-298, R-300; re-calibrated using Ox Cal V. 4.0). This was confirmed by a new AMS date of 12,700–12,900 cal BP (2σ) made directly on the femur of Romito 5 at the CEDAD radiocarbon facility in Lecce (Table 1). In addition, five single burials (Romito 3, 4, 7, 8 and 9) were recovered from inside the cave (Fig. 2B). Romito 3 and 4 were excavated in the 1960s and although there is uncertainty regarding their stratigraphic position they most likely correspond to layer C3 (Fig. 2A). These burials were originally associated with charcoal dating to the early 14th millennium cal BP (Alessio et al., 1966; Graziosi, 1965; R-221; re-calibrated using Ox Cal V. 4.0) and this is confirmed by direct AMS dates of Romito 4 and charcoal from layer C3 (Table 1, Fig. 2A). Romito 7 (layer D5) and 8 (layer D7) were dated to the late 14th millennium cal BP from AMS dates of charcoal in these layers (Table 1, Fig. 2A). The oldest burial, Romito 9, is related to an Evolved Epigravettian layer (E10; Fig. 2A); this cultural attribution is based on the archaeological and chronostratigraphical context in accordance with charcoal dating between 18,890 and 18,100 cal BP (Table 1). More dating is needed to resolve the apparent contradiction with the direct AMS date of this specimen of 17,000–16,150 cal BP (Table 1). All the remains were generally well preserved (e.g. Fig. 3) and in most cases age at death and sex could be determined (Mallegni and Fabbri, 1995; Table 2). It was also evident that Romito 2 suffered from dwarfism, most likely as a result of the rare inherited condition of acromesomelic dysplasia (Mallegni and Fabbri, 1995; Frayer et al., 1987).

Table 1
New AMS dates of human remains and charcoal from Grotta del Romito.

Sample, human remains	Layer	Laboratory code	Radiocarbon date (bp)	Calibrated date BP (2σ)
Romito 4	C3?	LTL3032A	11,340 ± 90	13,390–13,060
Romito 5	Rockshelter	LTL3033A	10,862 ± 70	12,940–12,780
Romito 9	E10	LTL3034A	13,915 ± 70	17,000–16,150
Charcoal	C	Beta-160295	11,060 ± 100	13,158–12,853
	C2	Beta-160296	11,090 ± 70	13,131–12,886
	C3	Beta-160297	11,380 ± 70	13,375–13,119
	C4	Beta-160298	11,250 ± 70	13,266–12,986
	D1	Beta-160300	11,660 ± 70	13,695–13,345
	D5	Beta-160302	12,060 ± 90	14,113–13,745
	D8	LTL234A	12,170 ± 60	14,173–13,857
	D15	LTL608A	12,331 ± 55	14,663–14,036
	D16	LTL601A	12,369 ± 100	14,877–14,036
	D20	LTL602A	12,438 ± 85	14,921–14,137
	D29	LTL1050A	12,494 ± 75	14,973–14,202
	D35	LTL1052A	12,970 ± 150	15,859–14,921
	E2	LTL1046A	13,650 ± 120	16,735–15,790
	E5	LTL1047A	13,646 ± 120	16,730–15,784
	E8	LTL1590A	14,373 ± 90	17,732–16,739
	E10	LTL1591A	15,273 ± 150	18,886–18,105
	E16	LTL1592A	16,129 ± 100	19,476–19,067

Samples were calibrated using Ox Cal version 4.

2. Materials and methods

2.1. Samples available for stable isotope analysis

Eight of the nine human remains were available for stable isotope analysis (Romito 2–9; Table 2) and all the samples were taken from the mid-shaft of either femurs or humeri. In order to provide comparative data to interpret the human isotope data, 28 adult mammalian bones were selected for stable isotope analysis (Table 3). With the exception of two aurochs which pre-dated the burials (from layers G and H), all of these samples came from contexts that were stratigraphically associated with human remains recovered in the most recent series of excavations (Table 3).

Fig. 3. Photograph of Romito 8 taken during excavation.

Table 2
Stable isotope measurements and collagen quality control indicators of humans from Grotta del Romito.

Sample	Sex	Age (years)	Layer	wt. %C	wt. %N	wt. %S	C:N	C:S	N:S	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	wt. % Collagen
Romito 1	F	25–30	Rockshelter										*n/a*
Romito 2	F?	ca. 17	Rockshelter	45.3	15.0	0.19	3.5	651	185	−20.0	10.3	12.2	2.4
Romito 3	M	25–30	C3?	44.7	15.9	0.19	3.3	626	191	−19.3	10.1	11.2	5.2
Romito 4	F	18–20	C3?	41.7	14.8	0.17	3.3	666	203	−19.6	10.0	11.1	2.2
Romito 5	F	25–30	Rockshelter	42.7	15.1	0.19	3.3	599	181	−19.7	9.3	10.6	2.4
Romito 6	M	adult	Rockshelter	46.6	16.1	0.21	3.4	605	179	−19.5	8.9	10.4	2.3
Romito 7	M	18–20	D5	46.6	16.8	0.20	3.2	636	197	−19.1	9.7	13.2	5.4
Romito 8	M	adult	D7	47.9	17.1	0.20	3.3	649	199	−19.5	9.7	13.7	12.4
Romito 9	?	adult	E10	42.0	15.2	0.17	3.2	653	203	−18.9	12.4	12.6	4.5
Bovine control (*n*=27)				47.6 ± 4.6	17.5 ± 1.8	0.17 ± 0.0	3.1 ± 0.1	747	235	−23.0 ± 0.2	6.2 ± 0.1	8.3 ± 0.1	12.4 ± 4.0

2.2. Collagen extraction and isotope ratio mass spectrometry

Each of the archaeological human and animal bone samples were cleaned by abrasion and pulverized to a coarse powder. Collagen was extracted, following a modified Login method (Brown et al., 1988), from 0.5 g of each of these samples and modern bovine bone which served as an extraction control (bovine control). The resulting 30 kDa collagen fraction was measured in triplicate by continuous flow isotope ratio mass spectrometry (Thermo Finnigan Delta Plus XL) in the Department of Archaeological Sciences, University of Bradford, to determine carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values. The results are reported using the delta scale in units of 'per mil' (‰) relative to internationally accepted standards, VPDB and AIR respectively. Analytical error, calculated from repeated measurements of each sample and twenty seven repeated measurements of the bovine control from multiple extracts, was <0.2‰ (1σ). Sulphur stable isotope values ($\delta^{34}S$) were determined by IRMS at Iso-Analytical (Crewe, UK) on all of the available human remains and on six of the mammalian bones. Bone collagen (ca. 8 mg) was combusted with V_2O_5 and the resulting gases SO and SO_2 were analysed on a Europa Scientific 20–20 isotope ratio mass spectrometer (Crewe, UK). The results are presented in per mil notation (‰) vs. the primary $\delta^{34}S$ isotope standard V-CDT (troilite of the Canyon Diablo meteorite). The inorganic international standards IAEA-SO-5 ($\delta^{34}S_{V\text{-}CDT} = 0.5$‰) and IA-RO36 (Iso-Analytical working standard barium sulphate, $\delta^{34}S_{V\text{-}CDT} = +20.74$‰) traceable to NBS-127 (barium sulphate, $\delta^{34}S_{V\text{-}CDT} = +20.3$‰) were analysed in triplicate along with the samples. Only single $\delta^{34}S$ measurements were made on Romito 2, 3, 7, 9 due to insufficient collagen. In all other cases the analytical error on duplicate samples and the bovine control was <0.5‰. Isotopic integrity of the extracted collagen was assessed against accepted quality indicators for carbon and nitrogen (DeNiro, 1985; van Klinken, 1999) and sulphur (Nehlich and Richards, 2009).

3. Results and discussion

The isotopic data, collagen quality indicators, and some information on the sampled individuals are summarized in Table 2 for the human bones and Table 3 for the animal bones. The carbon and nitrogen data for the animals and humans are plotted in Fig. 4.

3.1. Bone collagen preservation

All the humans bones contained more than 2% collagen by weight and had atomic C:N ratios within the acceptable range (DeNiro, 1985; van Klinken, 1999). Four of the animal bones contained <1% collagen but still had acceptable C:N ratios (Table 3). The atomic C:S and atomic C:N ratios of the human and faunal samples met the quality criteria of 600 ± 300 and 200 ± 100 respectively for mammalian bone collagen established by Nehlich and Richards (2009).

3.2. Carbon and nitrogen stable isotope analysis of faunal remains

The $\delta^{13}C$ values of the mammals range from −19.0‰ to −21.3‰ (Table 3, Fig. 4) and are characteristic of animals feeding on plants

Table 3
Stable isotope measurements and collagen quality control indicators of animals from Grotta del Romito.

ID	Species	Layer	wt. %C	wt. %N	wt. %S	C:N	C:S	N:S	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	% Collagen
3198	*Bos primigenius*	D11	44.8	16.1		3.2			−20.0	6.7		2.3%
1095	*Bos primigenius*	E10	51.9	18.6	0.16	3.2	851	262	−19.7	5.1	14.6	3.3%
P757a	*Bos primigenius*	G1	44.2	15.9		3.2			−19.7	6.9		0.8%
P186a	*Bos primigenius*	H4	43.3	15.8		3.2			−19.6	7.2		0.8%
3606	*Capreolous capreolous*	D12	41.2	14.4	0.16	3.3	707	212	−20.6	4.6	12.2	2.3%
Coll 5	*Capra ibex*	D5b	48.4	17.7	0.16	3.2	813	255	−20.2	5.6	12.5	3.4%
Coll 6	*Capra ibex*	D5b	45.5	16.8		3.2			−20.4	3.8		5.4%
Coll 7	*Capra ibex*	D5b	45.3	16.7	0.15	3.2	794	251	−20.5	4.2	15.0	5.5%
Coll 13	*Capra ibex*	D5b	45.8	16.5		3.2			−19.8	5.0		3.6%
Coll 2	*Capra ibex*	D7	43.4	14.7		3.4			−20.1	3.7		0.7%
1100	*Capra ibex*	E10	46.8	16.7		3.3			−19.6	4.1		1.6%
1088	*Capra ibex*	E10	48.8	17.9		3.2			−19.5	5.2		8.1%
1092	*Capra ibex*	E10	47.8	17.4		3.2			−19.0	4.5		8.3%
Coll 8	*Cervus elaphus*	D5b	43.4	15.2		3.3			−21.3	5.6		1.0%
Coll 9	*Cervus elaphus*	D5b	44.2	15.9		3.2			−21.3	6.0		0.6%
Coll 12	*Cervus elaphus*	D5b	47.3	17.3		3.2			−20.3	5.8		1.0%
1099	*Cervus elaphus*	E10	42.3	15.2	0.17	3.2	663	205	−20.2	5.2	13.8	4.4%
1097	*Cervus elaphus*	E10	48.7	17.6		3.2			−19.6	4.6		4.0%
Coll 4	*Sus scrofa*	D5b	49.3	18.2		3.2			−20.5	4.3		2.8%
Coll10	*Sus scrofa*	D5b	45.2	15.7		3.4			−21.0	6.3		3.1%
Coll 11	*Sus scrofa*	D5b	44.8	16.1		3.3			−20.7	6.1		2.3%

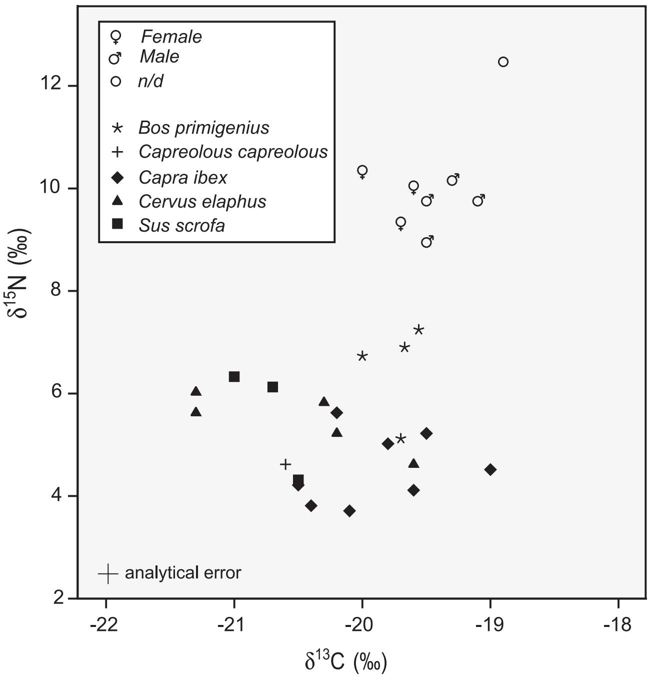

Fig. 4. Bone collagen carbon and nitrogen stable isotope measurements of humans and fauna at Grotta del Romito. The human data are discriminated by sex. Maximum analytical error is shown at one standard deviation from the mean.

with a C_3 photosynthetic pathway. The ibex and aurochs are significantly enriched in ^{13}C compared to the wild boar and roe deer (independent Student's t-test, $t = 3.85$, $p = <0.05$), and may have inhabited a more open environment, as closed forest canopy can cause a depletion in ground level plant ^{13}C content (Vogel, 1978). The red deer show most variation in δ^{13}C, consistent with variability observed in some modern populations, which cannot be attributable to a particular habitat (Stevens et al., 2006).

A relatively wide range (4‰) of δ^{15}N values were also obtained from the herbivores (Fig. 4). Of these, three of the aurochs specimens were significantly enriched compared to the other mammals (independent Student's t-test, $t = 4.09$, $p = <0.05$). A similar pattern has been observed from analysis of Upper Palaeolithic mammal assemblage from Grotta Paglicci also in Southern Italy (Iacumin et al., 1997), and could be due to a physiological difference or a dietary difference between forest or mountain dwelling species (such ibex, roe deer and wild boar) feeding on ^{15}N depleted woody shrubs, and open dwelling species (such as aurochs) on ^{15}N relatively enriched grasses (Delwiche et al., 1979). Overall, substantial intra-species variability may be due to the procurement of animals from different habitats that were subsequently brought to the cave. However, we note that similar isotope variability has been observed within a single modern population of red deer (Stevens et al., 2006) and a more detailed analysis of the Romito faunal samples, perhaps combined with strontium and oxygen isotope analysis would be needed to determine the extent of the site's catchment.

No significant trend in the δ^{13}C and δ^{15}N values was evident from fauna from the different layers at Grotta del Romito. In north-western Europe and other areas proximal to the ice sheets of the last glacial period, a significant depletion of ^{15}N in herbivore bone collagen has been recorded and attributed to depletion of ^{15}N in plants (Garcia Guixé et al., 2009; Richards and Hedges, 2003; Stevens and Hedges, 2004). At Grotta del Romito, the oldest samples analysed, two aurochs bones from layers G1 and H4 and stratigraphically associated with charcoal with uncalibrated radiocarbon dates of 19,351 ± 180 BP (LTL236A) and 20,210 ± 245 BP (LTL604A) respectively were not significantly different to the aurochs bone from the much younger layers (independent Student's t-test, $t = 6.31$, $p = 0.14$; Table 3), suggesting that climate variation had a much less of an impact on the ^{15}N composition of Mediterranean flora (see also Iacumin et al., 1997; Drucker and Bocherens, 2004).

3.3. Carbon and nitrogen stable isotope analysis of human remains

The majority of human nitrogen isotope values are enriched compared to the animals by 3–5‰ (Fig. 4) indicating that they derived the majority of their dietary protein from the large herbivores found in the cave. The exception is Romito 9, where the measured δ^{15}N value is >5‰ higher than even the maximum herbivore value suggesting that this individual regularly consumed protein from an additional dietary source that was of higher trophic level, such as freshwater or possibly even marine fish. Similarly enriched δ^{15}N values, indicative of significant aquatic (marine or freshwater) protein (interpreted to be >20%), have been observed for mid-Upper Palaeolithic humans from Europe (Richards et al., 2001a; Pettitt et al., 2003; Fig. 5). Neither freshwater nor marine fish were found at the site but either or both were an important dietary source for Romito 9, based on the interpretation of the relatively high δ^{15}N value. Considering the sites location, freshwater river fish may seem the most likely and the δ^{13}C value of Romito 9 is consistent with archaeological carp and sturgeon collagen values from the Danube (Nehlich et al., 2010), and seemingly inconsistent with the range of values recently reported for archaeological and recent Mediterranean marine fish bone collagen (Garcia Guixé et al., 2010) or recent fish from Eurasian lakes (Dufour et al., 1999). However, as previously discussed, the interpretation of the δ^{13}C is complicated by the potential for incorporation of carbon into collagen from other dietary sources, such plant carbohydrates, which may lead to an underestimation of ^{13}C enriched marine food in the diet (see also Hedges, 2006 for comment). Nevertheless, Romito 9 was less enriched in ^{13}C compared to the majority of individuals

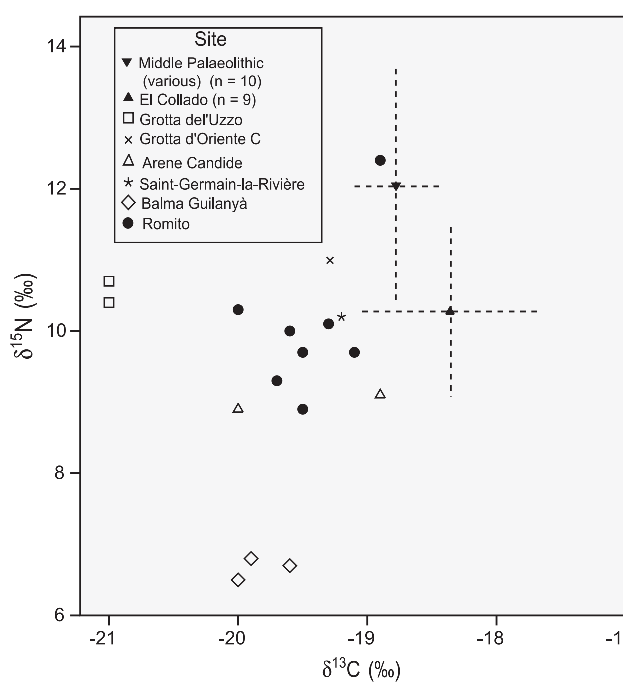

Fig. 5. Comparison of carbon and nitrogen stable isotope data from Grotta del Romito with other European Palaeolithic and Mesolithic sites. See Table 4 for information regarding these sites.

buried at the late Mesolithic site of El Collado, situated close to Mediterranean coast (Garcia Guixé et al., 2006; Fig. 5).

In contrast, the other individuals at Grotta del Romito had terrestrial diets with nitrogen isotopes 3–5‰ enriched compared to the herbivores, which were the most likely source of dietary protein. Similarly enriched isotopes have also been observed for other Late Upper Palaeolithic and Mesolithic human remains from Southern Europe (Table 4 and Fig. 5). At Balma Guilanyà in the Pre-Pyrenees, the human nitrogen isotope values are depleted compared to the Italian and French remains but still relatively 3–5‰ enriched compared to terrestrial herbivores found at this site (Garcia Guixé et al., 2009). Thus in all cases, it appears that terrestrial herbivores were the most important source of dietary protein, mirroring findings based stable isotope analysis of Late Upper Palaeolithic human remains from inland sites in the UK (Richards et al., 2000; Stevens et al., 2010). However, here one must exercise some caution, as many foraged plant foods, excluding nuts and some legumes, have much lower nitrogen content than herbivore tissues and therefore would be unlikely to be observed isotopically even if they were consumed. Furthermore, at Grotta del Romito any contribution of plant carbohydrate derived carbon to the human collagen is not likely to be distinguishable due to the wide range of herbivore $\delta^{13}C$ values.

Of the later burials, there is no clear isotope evidence that the diet Romito 2, who suffered from dwarfism, was different to the other humans who were buried in the area of the actual rock-shelter at approximately the same time (Romito 5 and 6). One rather fanciful view is that Romito 2 was revered or perceived as different and therefore afforded a 'special burial' in the rock-shelter (Frayer et al., 1987; Dettwyler, 1991), or possibly ritually sacrificed (Formicola, 2007). At least from a dietary perspective, it seems that this individual was not treated any differently during life. Another view is that due to her short stature, Romito 2 was unable to pursue regular economic activities, particularly hunting (Frayer et al., 1987). If so, this did not significantly alter her diet, as may have been expected if meat was less available. Food may indeed have been provided for Romito 2 which implies that she was simply cared for by other members of group, whilst not negating her ability to perform other roles within the community.

Similarly, the $\delta^{13}C$ and $\delta^{15}N$ values of the other burials (Romito 3–8), including the double burial were remarkably similar (Fig. 4). There was no indication of different status or that they had had access to different types of foods during life. No statistical differences were evident between the sexes (U Mann–Whitney test, P [$\delta^{13}C$] = 0.57; P [$\delta^{15}N$] = 0.63). Recent examination of the remains of Romito 8 indicates that this individual had suffered severe physical trauma whilst living to a mature age (Martini, 2006b). Heavy wear patterns on the teeth of this individual relative to the others may suggest that they pursued different daily activities within the group, such as the manipulation of animal hide (Martini, 2006b), whilst consuming a broadly similar diet.

3.4. Sulphur stable isotope analysis

Sulphur isotope values (Tables 2 and 3) obtained from both the animal and human bones from Grotta del Romito were relatively uniform (=12.4 ± 1.5 [1σ]). More variable $\delta^{34}S$ values have been recently reported in 10 Late Bronze Age individuals from Chicha in south-western Siberia (Privat et al., 2007), 19 individuals from the Danube Gorges (Nehlich et al., 2010) and 19 Viking age individuals from Birka on the west coast of Sweden (Linderholm et al., 2008; Fig. 6). At Chicha and the Danube Gorges $\delta^{34}S$ variation is attributed to dietary differences, particularly the differential consumption of freshwater fish, while at Birka it is attributed to a heterogeneous origin of the individuals buried in the cemetery. The relative consistency of $\delta^{34}S$ values in the Grotta del Romito does not indicate a diverse geographical origin for the humans. The values overlap considerably with terrestrial animals measured at the site, including species such as ibex, aurochs and roe deer, which are also unlikely to have migrated long distances. However, we cannot conclude that the individuals were local without greater knowledge of the range of $\delta^{34}S$ values from wider region and beyond. Interestingly, the range of $\delta^{34}S$ values at Romito is similar to the majority of Bronze Age humans from the city of Thebes in Greece (Vika, 2009; Fig. 6). This indicates that $\delta^{34}S$ values in humans with terrestrial diets can be homogenous over a wide geographical area, especially in regions where specific geologies, such as limestones and dolomites, are so dominant.

Table 4
Mean carbon and nitrogen measurements of human remains from other Palaeolithic and Mesolithic sites in Southern and Central Europe.

Site(s)	Approximate date (calibrated years BP)	*n*	Location	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Reference
European Middle Palaeolithic (various)	33–21k	10	Inland	−18.8	12.0	Richards et al., 2001a,b
Saint-Germain-la-Rivière	19k	1	Inland	−19.2	10.2	Drucker and Henry-Gambier, 2005
Arene Candide (Liguria, Italy)	13k	2	Coastal	−19.5	9.0	Francalacci, 1989
Balma Guilanyà (Pre-Pyrenees, Spain)	12–13k	3	Inland	−19.8	6.7	Garcia Guixé et al., 2009
Grotta d'Oriente C (Sicily)	13k	1	Coastal	−19.3	11.0	Craig et al., Unpublished
Grotta dell'Uzzo (Sicily)	9k	2	Coastal	−21.0	10.6	Francalacci, 1989
El Collado (Valencia, Spain)	8.5k	9	Coastal	−18.4	10.3	Garcia Guixé et al., 2006
Romito (Southern Italy)	18–13k	8	Inland	−19.5	10.0	This study

The ranges are plotted in Fig. 5.

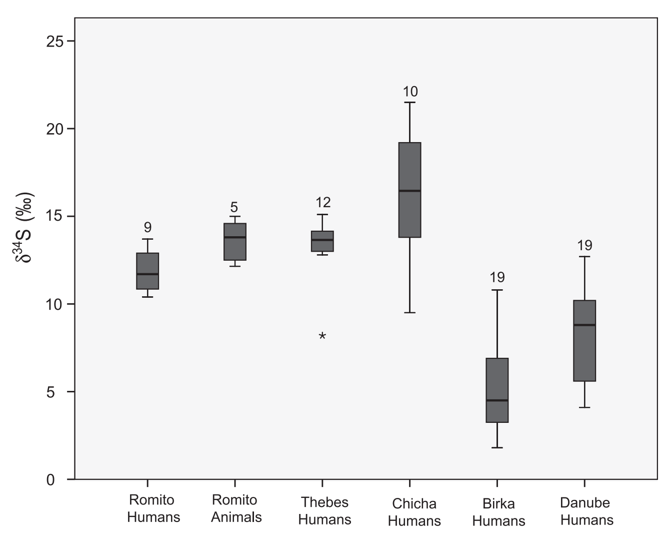

Fig. 6. Box plot of sulphur stable isotope data from Grottal del Romito data, Bronze Age Thebes (Greece), Late Bronze Age humans from Chicha (Russia), Viking Age humans from Birka (Sweden) and Mesolithic and Neolithic individuals from the Danube gorges. The number of individuals analysed are shown on the chart.

The $\delta^{34}S$ value of Romito 9 is also within the range of the other individuals and animals at the site, and therefore there is no indication that this individual consumed more marine or freshwater fish, as indicated by the $\delta^{15}N$ value. However, it is also plausible that no difference in $\delta^{34}S$ would be expected, since there may be considerable overlap between the $\delta^{34}S$ ranges of fish and terrestrial animals, as previously noted by Privat et al. (2007). In addition, duplicate $\delta^{34}S$ measurements made on a single marine fish bone (*Thunnus* sp.) from a Roman site in the Tyrrhenian coast of Campania (Sample Velia 1180; Craig et al., 2009) produced a value of 15.9‰ (±0.17 [1σ]; Craig unpublished). This value is only slightly enriched compared to several of the human values from Romito and again falls within the anticipated range of freshwater fish $\delta^{34}S$ values. Further work is needed to characterise ancient sulphur isotope variation in Mediterranean terrestrial, freshwater and marine ecosystems but it might be the case that sulphur isotopes are not useful for identifying individuals with variable residencies in this region, especially given its geological homogeneity.

4. Conclusions

In this study, we have substantially increased the stable isotope data set of Late Upper Palaeolithic human remains from the Mediterranean region. Most of the individuals had a very similar terrestrial diet. The exception is Romito 9, dating to several millennia earlier, who had a more mixed diet, comprised of freshwater and/or marine fish in addition to terrestrial animals. Whether this is typical of a more general dietary change during the Late Upper Palaeolithic cannot be ascertained by considering the dietary histories of just nine individuals. But this should be a priority for further studies, as such a change may be related to a reduction in the territories that people habitually used due to increased productivity and in turn related to climate amelioration. We can conclude that the inhabitants of Romito were unlikely to have frequented the coast very often during this period, at least for food, rather they focused on specialised and intensive hunting. Instead, we may expect that coastal resources were exploited by people who lived and buried their dead nearer the contemporaneous shoreline, which for the most part is now submerged. Interestingly however, marine dominated diets were not found through stable isotope analysis of humans at the Late Upper Palaeolithic layers of Arene Candide in Liguria or at Grotta d'Oriente (Oriente C burial, Lo Vetro and Martini, in press) in Sicily, both of which are located much nearer the Late Glacial coastline. This perhaps reflects the low productivity of the Mediterranean Sea compared to the Atlantic Ocean, which we know did support humans with marine diets during this period (Richards et al., 2005). Our results also show considerable dietary stability and consistency throughout the group regardless of age, sex and, in two cases, severe pathologies.

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