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# Strontium isotopic evidence for the provenance of occupants and subsistence of Sarakenos Cave in prehistoric Greece

Xiaoran Wang<sup>a</sup>, Xingxiang Zhang<sup>a,\*</sup>, Anchuan Fan<sup>a,\*\*</sup>, Adamantios Sampson<sup>b</sup>, Xiaotong Wu<sup>d</sup>, Jun Gao<sup>a</sup>, Fang Huang<sup>c</sup>, Zhengyao Jin<sup>a</sup>

<sup>a</sup> USTC Archaeometry Laboratory, University of Science and Technology of China, Hefei, Anhui, 230026, China

<sup>b</sup> Department of Mediterranean Studies, University of the Aegean, 1 Demokratias Ave, 85100, Rhodes, Greece

c Laboratory of Seismology and Physics of Earth's Interior, School of Earth and Space Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China

<sup>d</sup> Department of Archaeology, Shandong University, Jinan, 250100, China

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## ABSTRACT

The Sarakenos Cave in Greece, which preserves a series of cultural phases from the Middle Paleolithic to the Middle Helladic (approximately 1600 BCE), provides an ideal site for studying transitions among prehistoric phases. We analyzed the strontium isotopes of various materials unearthed from the site, providing results that fill gaps in relevant data about the Boeotia area within the Sub-Pelagonian zone on the <sup>87</sup>Sr/<sup>86</sup>Sr signature map of the Aegean region. The results show that the <sup>87</sup>Sr/<sup>86</sup>Sr values of the human teeth from different phases generally fall within with the local <sup>87</sup>Sr/<sup>86</sup>Sr range; thus, no migrants were identified at any period, indicating the people were either all locally born or moved from a region without evident geological variations compared to the study site. The results also imply that the foraging patterns of the equids and bovines were obviously different. The <sup>87</sup>Sr/<sup>86</sup>Sr values of the equids tended to be more "nonlocal" than those of most bovines. The intratooth variation reflected by equid individuals generally follows three main patterns, with a tendency of premortal adaption to the local dietary conditions, and some equids probably foraged somewhere outside the distribution of Karst predominant in Kopais Basin. This study also suggests that the shells were most likely collected from Kopais Lake, which possibly had a slightly lower <sup>87</sup>Sr/<sup>86</sup>Sr value than the present local value.

## 1. Introduction

The Sarakenos Cave (38.4527N, 23.2045E) is the most suitable archaeological site for studying ancient human settlement in the high concentration of caves within the limestone boundaries of the eastern part of the Kopais Basin (Sampson et al., 2009, Fig. 1). Research about the cave based on macroscopic scale dating has shown that it was continually used from the Middle Palaeolithic to the Middle Helladic (Sampson, 2008a). While in terms of the whole chronological span of the cave, the use tends to be discrete, with the existence of an archaeologically significant hiatus between the Paleolithic and Mesolithic phases, the Mesolithic and Neolithic phases, and the Neolithic and Bronze Age phases (Mavridis and Jensen, 2013; Sampson et al., 2009). There is a striking contrast between the Mesolithic occupations, which used "primitive" technology adapted to the local raw materials and had a subsistence economy based on fowling and plant gathering, and the Early Neolithic groups, which imported raw materials, practiced animal breeding and used macroblade technology (Sampson et al., 2009). Thus, the Sarakenos Cave is crucial for the study of the Mesolithic/Early Neolithic interface in particular. In addition, the provenance of the occupants is one of the key points for interpreting the transition between different archaeological phases and between different economic patterns.

The cave is at an altitude of 180 m, has a spacious entrance with a width of 25 m, and has an interior floor that declines only to the NE side. The systematic excavation of the Sarakenos Cave, aiming at establishing a chronological sequence of the cave and the economic modes of different periods, started in the mid-1990s and is still ongoing. The stratigraphic layers inside the cave preserve a succession of distinct cultural phases, which have been dated from the Middle/Upper Palaeolithic to the Middle Helladic (Sampson, 2008a; Sampson et al., 2009).

Because the stratigraphic sequence presented in Trench A is the most complete one available, this research used it to exemplify a brief

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<sup>\*</sup> Corresponding author.

<sup>\*\*</sup> Corresponding author.

E-mail addresses: zhangxx@ustc.edu.cn (X. Zhang), anchuan@ustc.edu.cn (A. Fan).



**Fig. 1.** The location of the Sarakenos Cave site (indicated by the red triangle, modified after Okuda et al., 2008). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

span of the complex. Complex II of trench A was excavated between 1994 and 2012, and 12 layers in this complex were classified as Middle Paleolithic, Epipalaeolithic, Mesolithic, Initial Neolithic, Early Neolithic, and younger deposits (Middle and Late Neolithic, Early Bronze Age) (Kaczanowska et al., 2016a; Bochenski et al., 2017).

In the Early and Middle Neolithic periods, Melian obsidian was regularly used, indicating that the Sarakenos Cave somehow maintained contact with the Cyclades. Moreover, the discovery of figurines from the Sarakenos Cave in the Late Neolithic II phase of the cave is the first time a high concentration of numerous figurines has been found in a mainland cave, which implies that one usage of the cave was probably related to cult activities (Sampson et al., 2009).

During the excavations, numerous and varied taxa of mollusks, fishes, amphibians, reptiles, birds and mammals have been identified and studied in all layers (e.g., Kaczanowska et al., 2016b; Bochenski et al., 2017). Although no evident grave features have been found in Sarakenos Cave, human bones were unearthed from the Final Palaeolithic, Mesolithic, Middle Neolithic, Late Neolithic I and other phases (Sampson, 2008b; Kaczanowska et al., 2016c).

Therefore, these noteworthy features of this cave with archaeological significance motivate further investigations on the transitions between different phases, the subsistence patterns in different periods and the potential agent behind them, i.e., human mobility. The analysis of strontium isotope ratios has been applied in the investigation of population mobility in Hellenic areas in several cases (Richards et al., 2008; Nafplioti, 2008, 2009, 2010; Triantaphyllou et al., 2015). As to the function and the use of the cave, Bochenski et al. (2017) concluded that during the Final Paleolithic and Mesolithic, the cave was only incidentally used as a short-term hunting shelter, while in the Initial and Early Neolithic, it played the role as a short-term shelter for herdsmen.

The provenance of Neolithic people in mainland Greece is often discussed in the context of the Neolithic transition of the Mediterranean area, for which strontium analysis may provide more clues. Thus far, neither the local bio-available <sup>87</sup>Sr/<sup>86</sup>Sr signatures nor the relevant <sup>87</sup>Sr/<sup>86</sup>Sr data of archaeological materials in Boeotia have been published. In this study, we used strontium isotopic analysis on various archaeological materials from Sarakenos Cave, including shells and human and animal teeth, as a pilot investigation of their provenance, expecting the results to provide more supportive data to extend an already published isoscape map of the Aegean area (Nafplioti, 2011), and for determining the immigrant situation of the site. In addition, the shell and animal remains covered multiple food resources of the Sarakenos people, and this research also aimed to detect the dietary

sources to reconstruct the geographic range of the ancient human activities and paleo-subsistence patterns of Sarakenos Cave.

#### 2. Methods and materials

### 2.1. Methods

The application of radiogenic strontium isotope analysis has been established as a proven method for reconstructing the paleo-mobility of people (Bentley et al., 2003; Haverkort et al., 2008; Standen et al., 2017), animals (Bendrey et al., 2009) and other artificial materials (Henderson et al., 2005). Moreover, strontium isotopic analysis on animal tooth enamel has made great contributions to detecting food and water intake sources and tracing potential seasonal movements and spatial mobility patterns (Copeland et al., 2016; Valenzuela-Lamas et al., 2016; Lugli et al., 2017). For samples from historic period sites, associated ranching practices and the trade and supply of livestock have been investigated (Bendrey et al, 2009; Arnold et al., 2016; Grimstead and Pavao-Zuckerman, 2016).

Strontium has four isotopic forms (<sup>84</sup>Sr, <sup>86</sup>Sr, <sup>87</sup>Sr and <sup>88</sup>Sr), among which radiogenic <sup>87</sup>Sr is formed over time by the decay of rubidium (<sup>87</sup>Rb, half-life ~  $4.7 \times 10^{10}$  years); in addition, the strontium isotope ratios in the Earth's crust vary with the age and type of rock (Price et al., 2002). The strontium isotopic composition of humans and animals is formed by the plants and water they consume through the chain, during which the Sr is not measurably fractionated because of the small difference in mass between <sup>87</sup>Sr and <sup>86</sup>Sr (Graustein, 1989). As an acellular and avascular tissue that will not remodel, enamel mineralizes during different time intervals with no appreciable change in later life; thus, the composition of enamel generally contains the <sup>87</sup>Sr/<sup>86</sup>Sr ratios reflecting one's diet catchment from infancy to adolescence (Montgomery, 2010). Therefore, animals and humans occupying the same territorial ranges should be characterized by similar strontium isotope signatures, and animals and humans occupying different ranges should be characterized by different strontium isotope signatures (Waterman et al., 2014). Thus, the systematic investigation of the strontium isotope signatures of a region with geological variations can provide relevant reference data (Bentley and Knipper, 2005; Kootker et al., 2016).

#### 2.2. Materials

Most samples measured were taken from Trench F of the Sarakenos Cave, including all the shells and mammal teeth, as well as one human tooth from a female individual of the Bronze Age. Trench F was dug in square G4 of the grid covering the interior of the Cave and excavated recently, but the formal report of the excavation has not been published yet.

We measured 8 dental enamel samples from 7 human individuals of the Mesolithic phase, Neolithic phase and Bronze Age from Trenches A, D, E, F, and H. Except for GR 1 and GR 1A, which were from a complete skeleton of a Caucasian female of the Bronze Age, the other teeth samples were unearthed singly from different layers, some of which were fragmented, so information about the sex and age of the individuals is not available (Table 1).

As for the mammal teeth, we measured a total of 14 samples unearthed from a bone assemblage in the Upper Palaeolithic layer, which coexisted with lithic tools. The teeth included 5 bovine teeth and 9 hypsodont equid teeth. They were unearthed in fragmentation from an assemblage (Fig. 2) for which more information about taphonomy has not yet been investigated.

In particular, we conducted sequential sampling on the hypsodonts, except for GR 7, a premolar (Table 1), due to its incompleteness. Three enamel bands with a band width of approximately 10 mm were vertically taken along one side from the tip (adjacent to the occlusal surface) towards the base (adjacent to the base) and were marked as the Cusp

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#### Table 1

List of 87Sr/86Sr ratios and relevant information for the samples measured in this paper collected from the Sarakenos Cave (human/animal enamel samples, shells and plants) and the 87Sr/86Sr ratios of the international standard.

Lab No.	Sample No.	Location	Phase	Material	Dentition/Taxon	<sup>87</sup> Sr/ <sup>86</sup> Sr	$\text{SE}\times 106$
ZY-9246	GR TA	Trench A Laver 32. square 4	Mesolithic	Human enamel	Lower M2	0.708289	6
ZY-9247	GR TF	Trench F Layer 15, square 40	Late Neolithic	Human enamel	Upper M2	0.708493	8
ZY-9248	GR TD8	Trench D8 Layer 12, Square 9	Late Neolithic II	Human enamel	Unclear	0.708541	17
ZY-9249	GR TD	Trench D	Late Neolithic II	Human enamel	Unclear	0 708234	10
ZY-9250	GR TE	Trench F	Late Neolithic	Human enamel	Unclear	0.708655	5
21 9200	GIUIE	Spit 6 square18	Lute Reontine	Human chamer	oncieur	0.7 00000	0
ZY-9251	GR TH	Trench H Spit 10, square 14	Late Neolithic	Human enamel	Unclear	0.708475	8
7V-9617	GR 1	Trench F	Bronze Age	Human enamel	Left Upper I1	0 708549	12
7V-9618	GR 1A	Trench F (The same body with GR 1)	Bronze Age	Human enamel	Lower M1	0.708555	8
7V-9386	Diant 1	Near Sarakenos Cave	Modern	Local plant	Asteraceae	0.708732	6
Z1-5300	Plant 2	Near Sarakenos Cave	Modern	Local plant	Olive	0.708200	7
Z1-936/	Plant 2	Near Sarakanas Cave	Modern	Local plant	Calium	0.700200	/ 0
Z1-9300	Plant 4	Near Sarakenos Cave	Modern	Local plant	Gulluin Distania Isatisaus	0.709236	0
Z1-9389	Plaint 4	Near Sarakenos Cave	Modern	Local plant	Pistacia tentiscus	0.708247	0
ZY-9390	Larva (n = $10$ )	Near Sarakenos Cave	Modern	Larva	-	0.708211	/
ZY-9391	Plant 5	Near Sarakenos Cave	Modern	Local plant	Pistacia lentiscus	0.708383	9
ZY-9604	F' Layer 8	Trench F	Neolithic	Shell	Unio sp.	0.708010	15
ZY-9605	F' Layer 9	Trench F	Neolithic	Shell	Unio sp.	0.707995	8
ZY-9606	F' Layer 10	Trench F	Neolithic	Shell	Unio sp.	0.707999	10
ZY-9607	F' Layer 11	Trench F	Neolithic	Shell	Unio sp.	0.708103	12
ZY-9608	F' Layer 12	Trench F	Neolithic	Shell	Unio sp.	0.708160	15
ZY-9609	F' Layer 14	Trench F	Neolithic	Shell	Unio sp.	0.708107	8
ZY-9610	F' Layer 15	Trench F	Neolithic	Shell	Unio sp.	0.708208	9
ZY-9611	F' Layer 16	Trench F	Neolithic	Shell	Unio sp.	0.708182	10
ZY-9612	F' Layer 17	Trench F	Neolithic	Shell	Unio sp.	0.708113	9
ZY-9613	F' Layer 18	Trench F	Neolithic	Shell	Unio sp.	0.708191	10
ZY-9614	F' Layer 19	Trench F	Neolithic	Shell	Unio sp.	0.708101	10
ZY-9615	F' Layer 33	Trench F	Mesolithic	Shell	Unio sp.	0.708101	8
ZY-9616	F' Layer 35	Trench F	Mesolithic	Shell	Unio sp.	0.708099	10
ZY-9620	GR 2-Cusp	Trench F	Upper Palaeolithic	Equid	Lower Right I1	0.708798	11
ZY-9619	GR 2-Mid	Trench F	Upper Palaeolithic	Equid	Lower Right I1	0.709018	11
ZY-9621	GR 2-Base	Trench F	Upper Palaeolithic	Equid	Lower Right I1	0.708843	6
ZY-9623	GR 3-Cusp	Trench F	Upper Palaeolithic	Equid	Lower Left 12	0.708802	9
ZY-9622	GR 3-Mid	Trench F	Upper Palaeolithic	Equid	Lower Left 12	0.708699	7
ZY-9624	GR 3-Base	Trench F	Upper Palaeolithic	Equid	Lower Left 12	0 708656	9
ZY-9626	GR 4-Cusp	Trench F	Upper Palaeolithic	Fauid	Lower Left 12	0 708792	7
7V-9625	GR 4-Mid	Trench F	Upper Palaeolithic	Fauid	Lower Left 12	0.708769	, 7
7V-9627	GR 4-Base	Trench F	Upper Palaeolithic	Fauid	Lower Left 12	0.708667	, Q
Z1-9027	CP 7	Trench F	Upper Palaeolithic	Equid	Lower Left P2	0.708770	6
Z1-9030	CR 10 Cum	Trench F	Upper Palacolithia	Equid	Lower Dight M1	0.708779	0
Z1-9034	CR 10 Mid	Trench F	Upper Palaeolithia	Equid	Lower Right M1	0.708842	9
Z1-9033	CR 10 Reso	Trench F	Upper Palacolithia	Equid	Lower Right M1	0.708872	0
Z1-9033	CR 11 Cuer	Trench F	Upper Palacolithia	Equid	Lower Loft D2 M2	0.708870	8
Z1-9037	CD 11 Med	Trench F	Upper Palaeolithic	Equid	Upper Left P3-M2	0.700004	/
Z1-9030	GR 11-MIG	Trench F	Upper Palaeonunc	Equid	Upper Left P3-M2	0.708772	8
Z1-9038	GR 11-base	Trench F	Upper Palaeonunc	Equid	Upper Left P3-M2	0.708802	9
ZY-9640	GR 12-Cusp	I rench F	Upper Palaeolithic	Equid	Upper Right P3/P4	0.708839	8
ZY-9639	GR 12-Mid	Trench F	Upper Palaeolithic	Equid	Upper Right P3/P4	0.708831	8
ZY-9641	GR 12-Base	Trench F	Upper Palaeolithic	Equid	Upper Right P3/P4	0.708640	7
ZY-9644	GR 14-Cusp	Trench F	Upper Palaeolithic	Equid	Lower Right P3	0.708815	7
ZY-9643	GR 14-Mid	Trench F	Upper Palaeolithic	Equid	Lower Right P3	0.708710	8
ZY-9645	GR 14-Base	Trench F	Upper Palaeolithic	Equid	Lower Right P3	0.708723	6
ZY-9647	GR 15-Cusp	Trench F	Upper Palaeolithic	Equid	Lower Right P3/P4	0.708933	8
ZY-9646	GR 15-Mid	Trench F	Upper Palaeolithic	Equid	Lower Right P3/P4	0.709104	6
ZY-9648	GR 15-Base	Trench F	Upper Palaeolithic	Equid	Lower Right P3/P4	0.708753	7
ZY-9628	GR 5	Trench F	Upper Palaeolithic	Bovine	Upper Left dP4	0.708499	7
ZY-9629	GR 6	Trench F	Upper Palaeolithic	Bovine	Upper Right dP4	0.708762	6
ZY-9631	GR 8	Trench F	Upper Palaeolithic	Bovine	Upper Left P3	0.708457	6
ZY-9632	GR 9	Trench F	Upper Palaeolithic	Bovine	Lower Right M2	0.708476	8
ZY-9642	GR 13	Trench F	Upper Palaeolithic	Bovine	Upper Left P3/P4	0.708466	8
NBS987				International standard		0.710248	8
NBS987				International standard		0.710245	8
NBS987				International standard		0.710248	6
NBS987				International standard		0.710248	7
NBS987				International standard		0.710249	7
							-

part, Mid part and Base part (Table 1).

The 13 shells were all fluvial and were taken in the order of the stratigraphic sequence [layer (8) to layer (35)] from the north wall of Trench F discontinuously; most of the shells belonged to the Neolithic

phase, and only 2 of them belonged to the Mesolithic (Table 1), and each shell sample represents a single individual because they were sampled from different layers of different periods (Fig. 8).

Moreover, to estimate the local strontium isotopic range



Fig. 2. Plan and topographical grid of the excavation (left), and the excavated position of the shells and the bovine and equid remains (right).



Fig. 3. Some of the samples from Trench F. a: GR 1, human tooth (Bronze Age); b: GR 9, bovine tooth; c: F' layer 8, the shell piece under the digital microscope (KEYENCE VHX-1000); d: GR 12, equid tooth.

surrounding Sarakenos Cave, we also analyzed 6 modern samples in periphery, on the slope or on the foothill of the mountain where the cave was situated, including 5 plants and 1 mixed set of larvae (n = 10). The plants we sampled were all from wild shrubs and trees growing without artificially added fertilizers, but the effects of pollution from the atmosphere could not be excluded. This methodology has been adopted in many similar cases (Rich et al., 2016; Wong et al., 2018). In addition, the use of modern plants in this study was determined to be the most feasible, although it would have been better if samples from more distant regions had been included in the analysis to represent strontium signatures across a broader area.

#### 2.3. Procedure

Different preprocessing methods were chosen for various materials. The preprocessing and measurement processes were all conducted in a clean laboratory. For the enamel samples, before the strontium isotope analysis was conducted, any visible dirt or contamination were removed by a dental drill with a diamond bit (approximately  $100 \,\mu$ m). A total of 20–25 mg of teeth enamel was taken from each sample. It was ensured that there was no dentine attached to the enamel by microscopic observation. The enamel samples were then cleaned in 5% acetic acid and MilliQ water with ultrasonic sonication several times and dried.

We selected shell samples whose color was unaltered, avoiding blackened shells with diagenesis contamination, and any visible dirt attached to the shells was removed. Then, the shells were fired in quartz crucibles in a muffle furnace with the cleaned and lyophilized plant and larva samples to a temperature of 825  $^\circ C$  for 8 h.

Approximately 10-25 mg of each sample was weighed, and then 8 M and 3 M HNO3 were (for secondary purification) added, and the samples were dissolved on a hot plate (above 200 °C) repeatedly. The plant and shell solutions were centrifugalized, and then the supernatant liquor was taken. In addition, the Teflon columns filled with Eichrom Sr-specific resin (mesh 100–150  $\mu$ m) were rinsed with MilliQ water and 3 M HNO3 multiple times to eliminate any Sr produced in the process of resin manufacturing. The purified Sr was extracted using cation exchange chromatography with a 3 M HNO<sub>3</sub> medium in the mobile phase (Zhang et al., 2018). Subsequently, we heated the Sr solution to dry the samples, and then we sealed them after they had cooled. Part of the purified Sr was taken and diluted with 2%  $HNO_3.$  The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured on a Neptune Plus MC-ICP-MS at the CAS Key Laboratory of Crust-Mantle Materials and Environments, School of Earth and Space Sciences, University of Science and Technology of China. The Sr carbonate standard NBS 987 yielded а value of  ${}^{87}\text{Sr}/{}^{86}\text{Sr} = 0.710248 \pm 0.000012$  (2SD, n = 99). All the data are listed in Table 1.

#### 3. Geological setting and local strontium isotopic range

# 3.1. Geological setting and available <sup>87</sup>Sr/<sup>86</sup>Sr signatures

The Palaeo-Kopais Lake, now drained, was inside the synonymous

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Fig. 4. The geological map of the relevant area (including the Kopais basin) with different lithologic distributions and available <sup>87</sup>Sr/<sup>86</sup>Sr signatures within the area (modified after Higgins and Higgins, 1996; the data of the sites in Euboea were cited from Nafplioti, 2011).

Basin, in Boeotia, Greece. As a "polje" situated at an altitude of approximately 95 m above sea level in the geotectonic zone of Eastern Greece (Sub-Pelagonian zone), the Kopais basin is the consequence of tectonic depression during the Pliocene and Pleistocene periods and of the dissolution of the calcareous rock caused by underground water. From a geological point of view, the most prevalent rock in the region is limestone dating from the Mesozoic era (Papadopoulou and Vrynioti, 1990) (Fig. 4), and Karstic dissolution played a significant role in forming the regional geomorphology (Sampson, 2008b).

The basin is overall covered by lacustrine deposits with intercalated peat beds in places. The surrounding mountains are composed of variable rocks, and the following stratigraphic series, from older to more recent formations, is present in the region: a) Upper Triassic limestones and dolomites; b) Jurassic limestones; c) a shale-chert-ophiolite complex with intercalated layers of limestone; d) a discontinuous iron ore horizon; e) limestones of Cenomanian to Paleocenic age; f) flysch of Paleocenic (paleocene) age; and g) Quaternary deposits. The shale-chert-ophiolite complex comprises sediments consisting of clays, marls, sandstones, conglomerates, limestones, cherts, and serpentinized ultramafic rocks (Sampson, 2008b). Since the general geological setting has stayed the same as the previously described series, the local signature values could be measured for reference.

The systematic investigation of the local bio-available  ${}^{87}$ Sr/ ${}^{86}$ Sr signatures in the Aegean area was conducted by Nafplioti (2011) and the relevant data for the Kopais basin are presented below. The northeast of the Kopais basin belongs to the Pelagonian zone, dominated by Triassic and Jurassic limestone, which contains the Manika and Tharrounia sites in Euboea and Kitsos Cave in southeastern Attica. The  ${}^{87}$ Sr/ ${}^{86}$ Sr values in Manika had an average of 0.708971 (3 sheep and 2 cow teeth enamel samples), and those in Tharrounia had an average of 0.708761 (1 pig and 1 sheep teeth enamel samples). Similarly, the  ${}^{87}$ Sr/ ${}^{86}$ Sr values of the collection from Kitsos Cave ranged from 0.708400 to 0.709307, averaging 0.708900 (2 pig and 1 sheep

teeth enamel samples) (Fig. 4).

In the southwest part of the Kopais basin, there is an area crossed by the Parnassos zone covered by flysch and late Eocene sediments. Taking the Kranidi site in the Argolid and the Perachora site in Corinthia as examples, the local bio-available strontium of Kranidi had an average value of 0.708451 (multiple modern snails), and that of Perachora had an average value of 0.708657 (1 pig tooth enamel) (Nafplioti, 2011). Since in the map of bioavailable strontium isotope ratio signatures in Greece generated by Nafplioti (2011) (Fig. 5) there is a lack of strontium data from the Sub-Pelagonian zone in mainland Greece, where the Sarakenos Cave is located, the measurements in this study provide relevant data to supplement that map. These measurements are also compared to similar data from the islands of Kos and Rhodes in the south-eastern Aegean (Nafplioti, 2011), which are also crossed by the Sub-Pelagonian zone.

# 3.2. Estimates for local strontium isotopic range surrounding Sarakenos Cave

Many studies have recommended that strontium isotope analysis should incorporate small animal samples for comparative purposes whenever possible (Price et al., 2002; Bentley, 2006; Waterman et al., 2014; Tafuri et al., 2016). Although constrained by the availability of the samples, we took 5 local plant samples and 1 mixed set of larvae (n = 10) attached to a local plant for the preliminary estimate of the local strontium isotopic range surrounding Sarakenos Cave. Since it has been demonstrated that plant-assimilated  ${}^{87}$ Sr/ ${}^{86}$ Sr may vary according to rooting depth (Nakano et al., 2001; Maurer et al., 2012), the plant samples we took belong to different species, including shrubs and trees, and each single plant sample covered various parts (leaves, branches, and flowers or seeds) of the plant to obtain an average value of the composite mixture. The results yielded  ${}^{87}$ Sr/ ${}^{86}$ Sr ratios ranging from 0.708200 to 0.709300, with an average of 0.708505 ± 2sd,



**Fig. 5.** Map of the <sup>87</sup>Sr/<sup>86</sup>Sr signatures and geologic zones of the Aegean. After Nafplioti (2011, Fig. 3). Marked on it is the site of Sarakenos Cave (the red triangle). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sd = 0.000419. Excluding an outlier of 0.709258, the values of the remaining 5 samples ranged from 0.708200 to 0.708732, with an average of 0.708355  $\pm$  2sd, sd = 0.000223. Thus, following Price et al. (2002), the local strontium isotopic range surrounding the Sarakenos Cave is 0.707909–0.708801. The average ratio is highly similar with those of Kos and Phodes, which are also in the Sub-Pelagonian zone in the published map, were analyzed with measurements of snail or sheep/goat material (Fig. 5), and have  ${}^{87}$ Sr/ ${}^{86}$ Sr ratios averaging out at about 0.7083 (Nafplioti, 2011), which, to some degree, probably increases the credibility of the local strontium isotopic range determined by the current study.

We should not take the values of equids and bovines as the data representing the local bioavailable strontium isotope, since their provenance is thus far unclear and there is no evidence indicating that they were born locally. Future studies should focus on increasing the quantity of the plant and soil leachate samples, between which the variations in the  ${}^{87}$ Sr/ ${}^{86}$ Sr values should be compared.

## 4. Results and discussion

#### 4.1. Human teeth

The  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  ratios of the 8 human teeth enamel samples from 7 individuals range from 0.708234 to 0.708655, with an average value of 0.708474 (sd = 0.000133). They are all within the strontium isotopic range surrounding the Sarakenos Cave defined by the plant samples (Fig. 6); thus, no migrants were identified at any period, indicating the people measured were either all locally born or were born in areas with homogeneity to the geological situation of the study site because they also matched the average values of part of Peloponnesus predominantly the Parnassos zone and Pindos zone (Nafplioti, 2011).

Perspectives about the provenance of the inhabitants of prehistoric Greece tend to be controversial. In the 19th century, the hypothesis of demic diffusion was proposed to interpret the transition from the Mesolithic to the Neolithic, which assumes that the immigration of farmers from Anatolia to Southeast Europe was the agent of the process of "Neolithization" in Greece. In addition, coastal Mesolithic sites, such as Sidari and Franchthi, have been used to support the idea that the Mesolithic inhabitants of Greece may not be indigenous (Van Andel and Runnels, 1988; Runnels, 1995). However, some evidence from the perspectives of physical anthropology and from the similarities among the lithic industries also support the indigenist hypothesis, particularly at sites such as Theopetra and Cyclops Cave (Perlès, 1990; Runnels, 1995, 2004; Sampson, 2014).

Although the study has its own limitations and it could not give a clear interpretation of such a big topic above, e.g., the <sup>87</sup>Sr/<sup>86</sup>Sr analysis can only distinguish the first generation of immigrants and is unlikely to distinguish immigrants from different areas with geological homogeneity, but more data and the researches alike are still necessary



Fig. 6. The  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  ratio ranges of various sorts of materials unearthed from the Sarakenos Cave and the local strontium isotopic range from the region around the site.

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and significant to be accumulated. In short, the results of the strontium analysis on the human teeth sampled in this paper do not show any indications of migrants in Sarakenos Cave. More evidence could probably be found with a higher quantity of samples or with DNA methods.

#### 4.2. Mammal teeth (equid and bovine)

The results of the inter- and intra-teeth enamel strontium analysis yielded 30 values for the 14 mammal teeth sampled in this study, ranging from 0.708457 to 0.709104, with an average of 0.708762 (sd = 0.000148). Among these, the 25 values from the 9 equid teeth, including the previously mentioned 8 sequentially sampled teeth and 1 broken tooth, range from 0.70864 to 0.709104, with an average of 0.7088077 (sd = 0.000104); the 5 values from the 5 bovine teeth range from 0.708457 to 0.708762, with an average of 0.708532 (sd = 0.000116) (Table 1).

There have been studies investigating equid teeth enamel samples with relevant implications on the timing of bio-mineralization, variable growth rates, the time-averaging lag and other factors, promoting better strategies for the prescreening and selection of specimens (Tornero et al., 2013; Bendrey et al., 2015; Guiry et al., 2016), which is crucial for a more rigorous understanding and interpretation of the data.

Hoppe et al. (2004) provided the mean growth rates of equid teeth, reporting that, on average, the M1 of modern horse teeth mineralizes from 0.5 ( $\pm$ 1) to 23 ( $\pm$ 3) (in months), P3 mineralizes from 14 ( $\pm$ 1) to 36 ( $\pm$ 3) (in months), and P4 mineralizes from 19 ( $\pm$ 3) to 51 ( $\pm$ 2) (in months). In addition, they also reported that the enamel collected from a region of  $\sim$ 3–4 vertical cm will produce bulk samples representing the enamel mineralized during an entire year of growth. However, the work by Bendrey et al. (2015) demonstrated enamel apposition and maturation advance at an exponentially decreasing rate, thus indicating that the times represented by same-sized bulk samples of enamel from different parts of a tooth are not equivalent.

The values of the three bulks on each tooth from the tip (adjacent to the occlusal surface) towards the base (adjacent to the base) are presented on a short polyline (Fig. 7). The result clearly shows intra-teeth fluctuations among the values, although our sampling strategies did not aim at reconstructing a high-resolution strontium isotopic span. Generally, the early growing Cusp part (a) represents the relatively earlier stage of the individual's life, followed by the Mid part (b), and the later life stage closer to the individual's death is reflected by the Base part (c) (Fig. 7). According to the exponentially decreasing tooth growth rate in the equid teeth (Bendrey et al., 2015), the Cusp bulk (a) with a faster growth rate covers a shorter time than the Mid bulk (b), and the Base bulk (c) covered the longest average period.

These results indicate a tendency for the sampled equids to adapt to the local dietary conditions before their death, because mostly the polylines of each intra-sample presents a descending tend approaching to the local bioavailable <sup>87</sup>Sr/<sup>86</sup>Sr range, except for GR 10 (Fig. 7). While sample GR 10 was taken from a tooth classified as M1, and according to the average age at which tooth enamel begins and ends mineralization in modern horse teeth (Hoppe et al., 2004), the values of GR 10 should reflect a relatively early period of the equid's life at 0.5



Fig. 7. The fluctuations in the strontium isotopic values of the sequentially sampled equid teeth.

(  $\pm$  1) to 23 (  $\pm$  3) (in months), thus it could not reflect the geographical source of the diet at later period of the equid's life.

Overall, the equid values are higher than the ranges of the other materials and the local range, indicating they initially foraged elsewhere. The intra-tooth variation reflected on Fig. 7 broadly follows three main patterns: Pattern A includes GR 2 and GR 15; Pattern B covers GR 3, GR 4 and GR 12; and Pattern C includes GR 11 and GR 14. For the Pattern A, there are respectively peaks during the middle of the polylines, which indicated that the equids had access to places with higher environmental <sup>87</sup>Sr/<sup>86</sup>Sr signatures, and they probably foraged somewhere in the Pelagonian zone east to the Sarakenos Cave, including the Southeastern Attica and Euboea areas, which both have mean values of bio-available <sup>87</sup>Sr/<sup>86</sup>Sr of 0.708900 (Nafplioti, 2011) (Fig. 5). As to the Pattern B, the polylines went down all along, reflecting their active pane were approaching local area all the time. While during the lives of Pattern C, their active pane was initially getting close to the local area, but changed to somewhere else with a slightly higher <sup>87</sup>Sr/<sup>86</sup>Sr value.

The variation of the  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  of each Base bulk (c) (the third point of each polyline reflecting the  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  of the final active location in their lives) indicates the resources of equids accessed by Sarakenos people were diversified. These clues do not provide enough evidence for us to judge whether the changes in  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  ratios in the intra-teeth enamel resulted from human behavior, while perceived changes in biogeographical ranges have been one of the indirect approaches to differentiate between wild and domestic horse populations (Bendrey, 2012). Thus, it is safe to say that we cannot exclude the possibility that people hunted the equids elsewhere and then brought them in situ and kept them for a short period, especially for the pattern B whose  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  ratios of the Base bulk (c)s matched the local strontium isotopic range.

In contrast, the <sup>87</sup>Sr/<sup>86</sup>Sr values of the bovines tend to be more concentrated and close to those of human teeth, indicating a local-source subsistence pattern (Fig. 6). The isotopic methodology could contribute to detecting the possible presence of initial husbandry practices. Since the differences in the strontium analysis between the equids, as well as those between the equids and bovines suggested the variations of their feeding locale (active pane). Thus it is indicated that they foraged in different patterns, and it may be possible to infer the variations were spontaneous or resulted from human hunting or management combining the context, which provides a potential method to study the initial husbandry behavior.

## 4.3. Shells

The 13 shell samples yielded  ${}^{87}$ Sr/ ${}^{86}$ Sr ratios ranging from 0.707995 to 0.708202, with an average of 0.708103 ± 2sd (sd = 0.000070). The strontium isotopes of the shells in Trench F from various spits define a narrow range, regardless of whether the shells are from the early Mesolithic phase or the late Neolithic phase (Fig. 8). The values in the shells are within a lower band than the local range (0.707908–0.708801), and it is evident that they are also slightly lower (but statistically significant) overall than the values in other sorts of materials (Fig. 6).

Aquatic resources of the Hellenic area have been exploited over the long term by the prehistorical inhabitants who lived by the rivers and the lakes. The earliest evidence for the systematic exploitation of aquatic resources using dates back to Mesolithic period (Trantalidou, 2011; Sampson, 2014). Marine resource exploitation, mainly fishing, also played an important role in the life of Mesolithic people, both in mainland and island sites in Greece (Sampson et al., 2012, 2014; Mylona, 2010). In fact, these activities were not only important in the Hellenic area, as there were many coastal groups of the Mesolithic period that heavily relied on fish and shellfish as food sources throughout Europe and elsewhere (Trantalidou, 2011). In the Neolithic period, the adoption of a sedentary, agro-pastoral way of life led to a

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**Fig. 8.** The sequential profile of the unearthed position of the shell samples on the north wall of Trench F and their corresponding  ${}^{87}$ Sr/ ${}^{86}$ Sr ratios. The "layers" here could also be presented as "spits", a unit of archaeological excavation with an arbitrarily assigned measurement of depth (10 cm in this case).

reduction in the intensity of fishing and shellfish gathering. Their importance as economic resources remained high only in certain regions of rich, eutrophic waters, such as the Neolithic lake-side settlement at Dispilio (Mylona, 2014; Trantalidou, 2011).

Regarding the Sarakenos Cave, which was studied in this work and is a mainland cave in Boeotia, the shell use in the cave depicts a continuous sequence from the first periods of the Greek Stone Age (Karali, 1999). In addition to serving as a subsistence resource, some of the shells were also processed into ornaments, such as beads and pendants. A total of 1884 shells belonging to 15 species (11 marine species, 1 fresh water and 3 land mollusks) have been identified in Sarakenos Cave. The most frequently encountered species and the species that all the samples in our study belong to is the fresh water *Unio* sp. (number = 1853) (Kaczanowska et al., 2016d).

Generally, the <sup>87</sup>Sr/<sup>86</sup>Sr ratios of seashells are consistent with the value of the ambient seawater for the parallel period (Elderfield, 1986;

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Reinhardt et al., 1998; Brand et al., 2003). A similar case has been associated with freshwater shells. The preliminary data in the investigation of Nakano and Hiroshi. (1991) strongly suggest molluscan shells attained a Sr isotopic equilibrium with both the ambient lacustrine water and seawater.

Regardless of the fact that the <sup>87</sup>Sr/<sup>86</sup>Sr ratios of the shells were overall slightly lower than those of the local range for other sorts of materials, they were the most likely to be collected from the vicinity of the site, or just from the former Kopais Lake. The formation of the Kopais basin resulted from the reactivation of older faults in the periphery of the basin during the Upper Pliocene. Thus, at about the end of the Lower Pleistocene, the homonymous lake was created. The major river flowing into the Kopais basin was Kephissos. Other minor river streams were Melas in the north and Erika, Phalaros, and Lophis in the south (Sampson, 2008b). Therefore, the composition of the lake strontium isotope ratio was a consequence of the mixture from multiple sources of rivers flowing across various underlying basements. Thus, there could be slight differentiation of strontium ratios between the former lake water and that of the local context surrounding the cave. However, due to the lack of available geological strontium isotopic data for the rivers contributing to the former lake or for the relevant areas surrounding the basin, it is hard to make assumptions about the exact trend in the <sup>87</sup>Sr/<sup>86</sup>Sr value of the former lake. As a result, these results do not contradict the hypothesis that the humans frequenting the cave were collecting mollusks from the lake (Kaczanowska et al., 2016d). Although the data match the <sup>87</sup>Sr/<sup>86</sup>Sr ratio of the central Argolid area in the existing bio-available <sup>87</sup>Sr/<sup>86</sup>Sr signature map (Figs. 5), 0.70822 (n = 12) (Nafplioti, 2011), considering the transport distance, the expiration of shells, and the stability of the <sup>87</sup>Sr/<sup>86</sup>Sr values of the shells, the hypothesis that the central Argolid represents the source of the shells should be excluded.

In conclusion, there is no significant variance among the shell materials unearthed from Sarakenos Cave, reflecting a stable and uniform source for the fresh water mollusks consumed and exploited by generations of Sarakenos people.

## 5. Conclusions

To summarize, for the Sarakenos Cave (ca. 12000 BP  $\sim$  3000 BP), the results of the strontium isotope analysis show that the  ${}^{87}\text{Sr}/{}^{86}\text{Sr}$  values of the equids tend to be more variable than the values of the bovines and humans.

The results of the human enamel samples in the cave from different phases overall fit with the local  ${}^{87}$ Sr/ ${}^{86}$ Sr range, which indicates that no migrants were identified at any period, although we cannot exclude the possibility that they moved from an area with similar geology. The intra-tooth variation reflected by equid individuals follows different patterns, and some equids probably foraged somewhere out of Karst area of the site, and most equids tended to adapt to the local dietary range before they died. All the  ${}^{87}$ Sr/ ${}^{86}$ Sr values of the bovines were within the local range. The differences in the strontium analysis between the equids and bovines suggest that they foraged in different patterns, which suggests that the isotopic methodology could contribute to detecting the possible presence of initial husbandry practices.

The research also indicates that the shells were most likely collected from the local Kopais lake, which possibly had a slightly lower  ${}^{87}$ Sr/ ${}^{86}$ Sr value than the current local value. The results fit with a multisource subsistence pattern adopted by people with a stable source of shell supplement.

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