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PALAEOCLIMATIC EVOLUTION IN LOUTRA ARIDEAS CAVE (ALMOPIA SPELEOPARK, MACEDONIA, N. GREECE) BY STABLE ISOTOPIC ANALYSIS OF FOSSIL BEAR BONES AND TEETH

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Abstract

Carbon and oxygen stable isotope values ($\delta^{13}C$, $\delta^{18}O$) were obtained from structural carbonate in the bioapatite of bear bones (Ursus ingressus) from Loutra Arideas cave, Almopia Speleopark, Macedonia, N. Greece. Samples of Late Pleistocene bear bones were studied for palaeoclimatic reconstruction of the area. The age range of the fossil layers is from 32ka BP to a maximum of 38ka BP. Generally, the palaeoclimatic proxy is correlated with literature data for climatic variations in the area during Late Pleistocene, whereas dietary behavior was investigated taking into account possible diagenetic processes that may have affected the carbonate matrix of the bones.

Key words: *Cave bears, stable isotopes, bioapatite, palaeoclimate, Almopia, Greece.*

1. Introduction

Recent palaeoclimatic studies include several different proxies for reconstruction of the Late Pleistocene unstable climatic and environmental conditions. This frame of research gets even more complicated in the transition climatic zone of Eastern Mediterranean region, which experienced several alterations during the last 40ka. Fossil bones offer a suitable material for such study and their isotopic composition has been widely used. In this study, fossil bones of cave bear from N. Greece have been used. However, in order to render fossil materials as reliable for palaeoclimatic and palaeodiet interpretation, diagenetic effects should be investigated.

Cave bears are considered to be endemic in Europe (Kurten, 1976). The diet and the physiology of this species are not well known. However, the morphology of teeth, skull and mandible indicate a basically herbivorous regime (Kurten, 1976; Mattson, 1998; Rabeder et al., 2000; Grandal-d'Anglade et al., 2005). The metabolism of this species is usually considered similar to this of the American black bear (*Ursus americanus*) or of the brown bear (*Ursus arctos*), according to Fernández-Mosquera et al. (2001). The fact that the cave bears are thought to have been up to 3 times larger than modern brown bears led Hilderbrand et al. (1996) to hypothesize that they were omnivores like most other temperate zone bears,

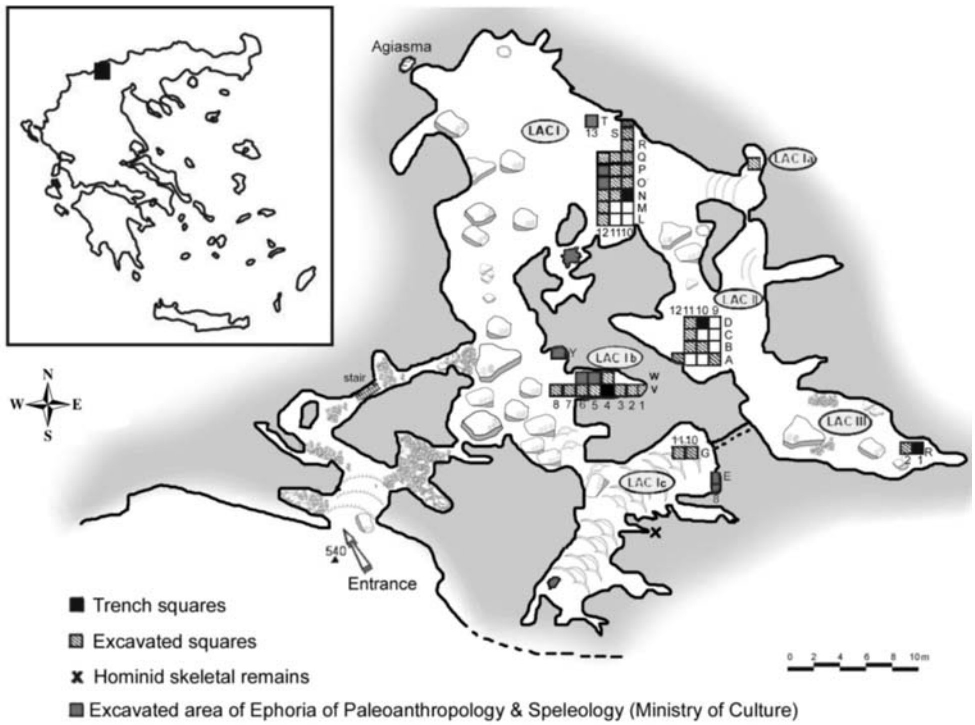

Fig. 1: Ground plan of the Loutra Arideas bear cave with the excavated block of squares of the various chambers [based on the topographic plan (Kambouroglou and Chatzitheodorou, 1999; Kambouroglou et al., 2006) modified in Tsoukala et al. (2006)].

but because of their size were specialized for feeding on megafauna, including carrion. The shortage of data in literature concerning cave bears isotopic analyses in combination with the burden of the difficulties in spotting and sampling such rare materials makes difficult to compare the results of a study.

Bones consist of an inorganic and an organic part. The inorganic part of the bone is mainly crystalline minerals in the form of bioapatite ($Ca_{4.5}[(PO_4)^{2.7}(HPO_4)^{0.2}(CO_3)]^{0.3}(OH)_{0.5}$, Hoppe, 2006), which is composed of both $-CO_3$ and $-PO_4$. The organic part of matrix is mainly composed of collagen; however, in this study just the inorganic part of bioapatite was analyzed. Oxygen stable isotope ($\delta^{18}O$) composition of both $-CO_3$ and $-PO_4$ is used for palaeoclimatic reconstruction while carbon stable isotope ($\delta^{13}C$) of bioapatite ($-CO_3$) provides information for both palaeoclimatic conditions in the habitat of the animals and their palaeodiet habits. Furthermore, diagenetic effects of the fossil bones can be identified by $\delta^{13}C$ variations.

2. Setting of the area

The material was excavated from Loutra Arideas cave in Northern Greece (Macedonia) (Fig. 1). The cave is located on the slopes of the Voras Mountain, at an altitude of 540m. The abundance of the milk teeth shows clearly that bears used the cave as a den. The fossils originate from other places or cavities, but they were moved by floodwater to their present sites, somewhere between 34 ka and 32 ka B.P. while according to radiocarbon dating these cave bears lived at least 37 ka BP (Rabeder et al., 2006). According to Rabeder and Hofreiter (2004), the bear findings belong to the species *Ursus ingressus*.

3. Materials and Methods

Fossil bone samples were collected during excavations from B11 square (Fig. 1). The material went under a chemical procedure to isolate the inorganic phase of the bone. In many cases, the protocol used for the isolation of the inorganic material has some variations between the published studies. Each researcher adapts the method in accordance to the samples' particularity, after performing a number of tests and analyses (Bocherens, 1992; Koch et al., 1989; Fricke et al., 1998; Iacumin et al., 2004, Hoppe, 2006). Studies have been done on the effects of these adaptations (Garvie-Lok et al, 2004).

The cleaned material was manually powdered by agate mortar. 20 mg of the material was soaked in 30% H_2O_2 to eliminate the organic material and afterwards in 1M acetic acid buffered solution to remove as much as possible the labile carbonate present in the sample. Finally the samples have been dried in an oven at 60°C, before the analyses.

The isotopic analyses of the bones took place in the Stable Isotope Unit of Institute of Materials Science (NCSR Demokritos, Athens) on a ThermoScientific Delta V Plus mass spectrometer, after reacted with ortho-phosphoric acid (99%) at 72°C, to produce CO_2 (GasBench II device). Results are reported in standard delta (δ) notation and units are reported per mil (‰).The standards used for comparison were NBS 19 and NBS 18 carbonates and an internal Carrara marble standard (VPDB).

4. Data and Results

4.1 Oxygen isotopic analysis

The period between Upper Pleistocene until the Holocene is characterized by a general climatic instability (Dansgaard et al., 1993). According to Iacumin et al. (1996) the difference between oxygen isotopic values from PO_4 ($\delta^{18}O_P$) and from CO_3 ($\delta^{18}O_C$) of bioapatite is about 9.2‰, conclusion given through research on bone and tooth samples of modern mammals. Similar value is given by Longinelli et al. (1973), in a study on modern carbonate shells, a completely different material by a biological point of view. Other more recent studies (Bryant et al., 1996, Zazzo, 2001) confirm these equations. The $\delta^{18}O_C$ VSMOW values of B11 square were used to estimate approximate values of $\delta^{18}O_P$ by using the value of Iacumin et al. (1996). Then, the equation $\delta^{18}O_P$=0.64* $\delta^{18}O_W$+22.37 after Longinelli (1984) was used, in order to calculate an estimation value of the $\delta^{18}O_W$ of the time of bears living (table 1).

Table 1.

Depth (cm)	*$\delta^{18}O_C$ vs SMOW (‰)*	*$\delta^{18}O_P$ vs SMOW (‰)*	*$\delta^{18}O_W$ vs SMOW (‰)*
88-103	22.21	13.22	-14.29
103-113	20.59	11.62	-16.79
113-123	22.63	13.63	-13.65
123-128	23.24	14.23	-12.71
128-133	22.88	13.88	-13.26
133-138	23.53	14.52	-12.26
138-143	22.07	13.08	-14.51

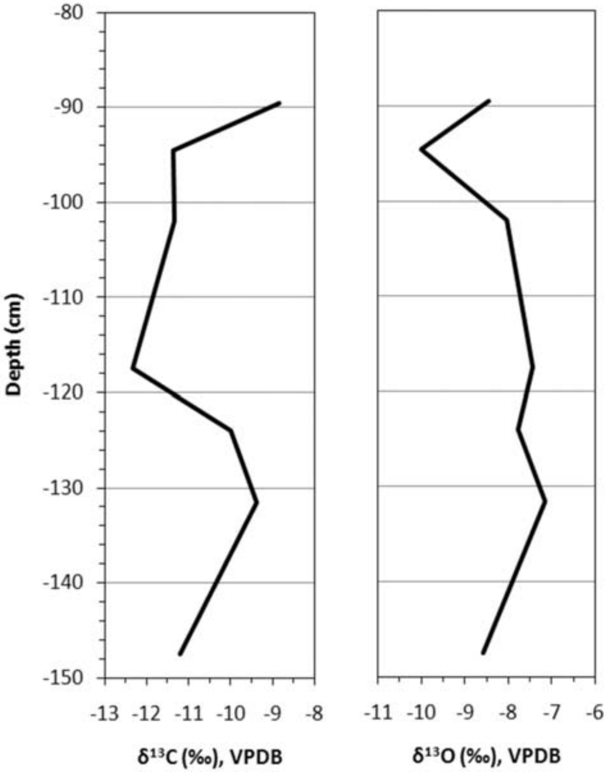

Fig. 2: Isotopic composition ($\delta^{13}C$ and $\delta^{18}O$ vs. VPDB) of cave bear bones from square B11 (Loutra Arideas cave, Almopia Speleopark) versus depth from cave floor surface.

4.2 Carbon isotopic analysis

The study of extinct species lead in most cases to approaches that eliminate at least one parameter. For instance, the results of an extinct species study are compared either to current ones, underrating the climatic conditions and accepting the similarity of metabolisms or to different species that lived under the same climatological conditions rejecting physiological conditioning (Fernández-Mosquera et al., 2001). This intriguing fact led this study to the path of elimination of as many concessions as possible. Fernández-Mosquera et al. (2001) collected isotopic data of cave bear bone collagen from literature, from the same period (Late Pleistocene), by nearby areas in the Eastern Alps, covering a wide range of altitudes (878 – 2800 m). This material is closer to the one of the present study. Both materials belong to the same species and lived in relatively close geographical areas. To achieve this comparison, it is necessary an established relationship between collagen and apatite isotope values provided by Krueger and Sullivan (1984):

$$\delta^{13}C_{apatite} = \delta^{13}C_{collagen} + 7(‰) \text{ for herbivores} \quad (1)$$

$$\delta^{13}C_{apatite} = \delta^{13}C_{collagen} + 3(‰) \text{ for carnivores} \quad (2)$$

The study of Fernández-Mosquera et al. (2001) showed that altitude is not a parameter that affects the carbon isotopic values in a large and incomparable scale. Using (1), carbon isotopic composition of bioapatite was converted to $\delta^{13}C$ of collagen and the values ranged from -19.3 to -15.8‰. An average deviation between them and the data from Fernández-Mosquera et al. (2001) is approximately 4‰ higher. The isotopic composition of the samples ($\delta^{13}C$ and $\delta^{18}O$ vs PDB) is shown in figure 2.

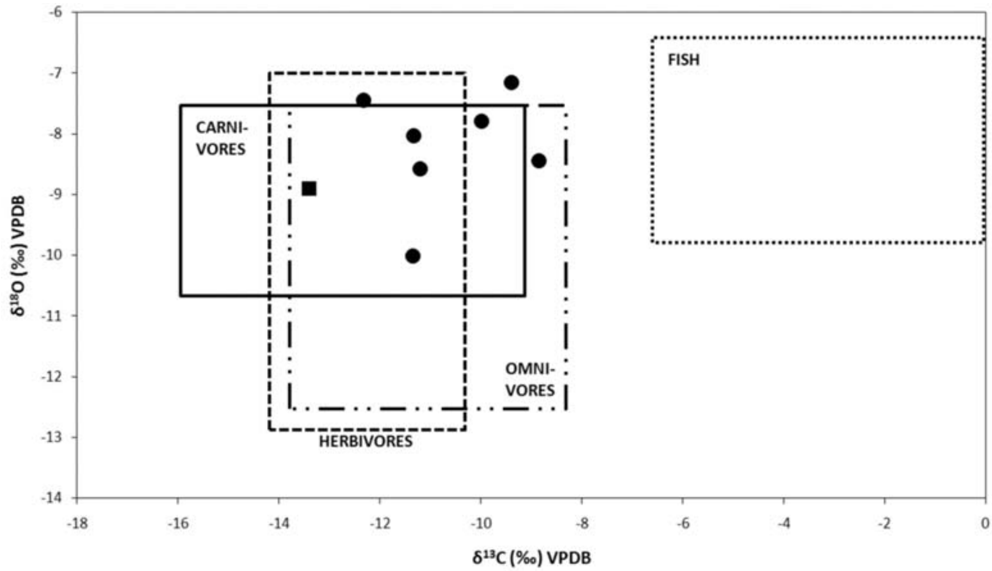

Fig. 3: Plot of $\delta^{13}C$ and $\delta^{18}O$ values (vs. VPDB) from bone structural carbonate of cave bear from Loutra Arideas cave (black circles). Also, bivariate plot of median isotopic composition of brown bear (omnivore) bone structural carbonate (black square). Frames facilitate the identification of the position of each dietary behavior/habitat preference in the plot (modified from Bösl et al., 2006).

5. Conclusions

Based on present isotopic data from local precipitation (Dotsika and Lykoudis, in press), the oxygen isotopic composition of precipitation in the area of Voras mountain (Almopia Speleopark area) is between -8 and -9.5‰. The depleted $\delta^{18}O_W$ estimated in this study by the fossil samples (average -13.92‰) in comparison to the modern oxygen isotopic composition of local meteoric waters (-8 and -9.5‰) indicate probably colder climatic conditions in relation to modern climate in the area. The radiocarbon dating of the samples (ca. 38 ka BP) set their respective populations living during the middle of Marine Isotope Stage 3 (MIS 3, 60-24 ka BP) (Dansgaard et al., 1993). MIS 3, although is considered a relatively warm period of the Würm glaciation, it is still characterized by much more cold and arid conditions in relation to MIS 1 (modern climate). Based on palaeoclimatic data for the region (Digerfeldt et al., 2000; Karkanas, 2001; Tzedakis et al., 2002), the relative colder climatic conditions indicated by the results of this study at 38 ka BP may be correlated with the cold and arid H4 event, assenting wider time limits of that phase due to dating uncertainties.

Studies have shown that depending on the unstable climatic conditions during the living time of the cave bears, they could be either herbivores, omnivores or even carnivores (Richards et al., 2008). These climatic variations probably pushed the cave bear to move southwards to the northern parts of the modern Greek territory. Bösl et al. (2006) distinguished the nutritional habits of different Late Neolithic vertebrates from Bavaria, Germany, including brown bear, using $\delta^{13}C$ and $\delta^{18}O$ of the bone structure carbonate (Fig. 3). Plotting Loutra Arideas cave bear samples' isotopic composition on figure 3, it can be noted that there is a general good correlation between the data and the plotted areas. However, the brown bear isotopic fingerprint seems to fall in different location in the diagram, indicating either distinguished nutritional habits in relation to cave bear or minor diagenetic effects of Loutra Arideas cave findings which may influence their isotopic composition. In fact, carbon stable isotope is possible to be enriched during diagenetic processes due to interaction with "dead" carbon from carbonate rocks and sediments ($\delta^{13}C \approx 0$). Other than that, it is difficult to differentiate the specific nutritional habits of the cave bears, as the overlapping plotted areas do not offer high precision. The estimated $\delta^{13}C_{collagen}$ of the cave bear from Loutra Arideas shows enriched values in relation to other studies (Fernández-Mosquera et al., 2001). This could indicate different nutritional habits (less herbivore), different vegetation in the area (C4 plants, arid conditions) or effects from minor diagenetic processes. From the above, the most likely combination seems to be some differentiation in cave bear diet and some diagenetic effects that influenced the carbonate matrix of the bones. This speculation should be further examined by collagen isotopic analyses and for diagenesis examination (e.g. C/N ratio, mineralogical identification).

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