# Mobility and Hibernation in *Ursus spelaeus* from Cueva de Guantes (Santibáñez de la Peña, Palencia, Spain)

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**Abstract**

This study analyzed the isotopic carbon:oxygen ratio in tooth enamel and carbon:nitrogen ratio in bone collagen of cave bear (*Ursus spelaeus)* remains of cubs and adults from "Galería 1" of Cueva de Guantes (Palencia, Spain). The results obtained from the carbon isotope ratios in enamel and collagen indicated that bears had a slight variation in their food sources and lived in a habitat dominated by $C_3$ plants. Oxygen isotopic values suggest that the water sources consumed were isotopically similar, indicating little displacement and no migration. Finally, $^{15}N$ values from collagen exhibited a wide range of values in both cubs and adults, probably from urea recycling during hibernation. Taken together, the analyses of carbon, nitrogen, and oxygen isotopic values indicated that cave bears inhabiting Cueva de Guantes may have had a limited ability to move across long distances from the hibernation cave and little variations in the diet, at least for the individuals inhabiting these caves. These factors may have been key for the survival of cave bear populations in the northern Iberian Peninsula during the Late Pleistocene.

*Keywords*:

Isotopic analyses

Migration

Mobility

Cave bear

Paleodiet

Paleoecology

Hibernation

Europe

Pleistocene

## 1. INTRODUCTION

The cave bear, *Ursus spelaeus* (Rosenmüller, 1794), was a member of the Quaternary megafauna in Europe, distributed from southern Siberia to Greece and from the Caucasus to northern Spain (Mackiewicz *et al*., 2017). The oldest records of this species date from 300 Kyrs BCE (Before Common Era), and the most recent specimens date from shortly before the Last Glacial Maximum (LGM), 26 Kyrs BCE (Llona *et al*., 2005; Charters *et al.*, 2022).

There is evidence that *Ursus spelaeus* had hibernation habits similar to those of present-day species of ursids, probably involving the same metabolic processes (Nelson *et al*., 1998; Llona *et al*., 2005; Grandal-d'Anglade and Fernández-Mosquera, 2008). Cave bear fossils come from karst environments due to their habit of using deep caves with large rooms as hibernation sites (Grandal d'Anglade and Vidal Romanı' 1997; Philippe and Fosse, 2003; Robu, 2016; Gimranov and Kosintsev, 2022).

Previous studies indicate that the diet of this species was strictly herbivorous (Bocherens *et al*., 1990; Bocherens *et al*., 1994; Ábelová, 2007; Pacher and Stuart, 2009; Münzel *et al*., 2011; Bocherens *et al*., 2014; Naito *et al.*, 2016; Naito *et al*., 2020). However, recent research using carbon and nitrogen isotopic ratios in collagen and dental wear analysis at the micro level has found a possible consumption of animal protein and, therefore, has opened up a debate on their carnivore-omnivore dietary flexibility (Pinto-Llona, 2013; Krajcarz *et al*., 2016; Robu *et al*. 2018; Ramírez-Pedraza *et al*., 2019 and 2020). Nelson et al. (1998), Grandal-d'Anglade and Fernández-Mosquera (2008), and Grandal-d'Anglade et al. (2019) consider that in this species, nitrogen isotopic ratios may be altered due to metabolic processes during hibernation, which would lead to increased $\delta^{15}N$ values. Lactation is also an important factor, since cubs would obtain nitrogen from breast milk and, given the fractionation of this element in milk proteins when metabolized, lactating cubs would exhibit higher $\delta^{15}N$ values than their mother (Nelson *et al*., 1998; Metcalfe *et al.*, 2010; Charters *et*

*al*., 2022). As a result of these processes, nitrogen isotopic ratios in certain biological structures, such as collagen, tend to be more positive due to urea recycling and bone remodeling, falsely suggesting animal protein sources in the diet.

Although there are several studies on the cave bear, there is little work on their ability to move territorially from the hibernation cave they inhabited. Present-day hibernating bears are highly mobile. For example, the brown bear (*Ursus arctos*) moves across long distances to mate (Dahle and Swenson, 2003; Todorov *et al.*, 2020), and the black bear (*Ursus americanus*) maintains a seasonal migration pattern throughout its life (Noyce and Garshelis, 2011 and 2014; Proctor *et al*., 2020). By contrast, the scarce evidence currently available suggests that *U. spleaeus* could not migrate or move over long distances. Furthermore, they apparently were extremely attached to the cave where they were born and continued hibernating in it generation after generation (Abelova, 2006; Fortes *et al.*, 2016; Grandal-d'Anglade *et al.*, 2019).

Multiple remains of large mammals have been found in "Galería 1" inside Cueva de Guantes, Palencia, Spain; particularly, this cave includes a level where more than 40 individuals of cave bears have been recovered (Mateos *et al*., 2014; Rodríguez and Mateos, 2014). According to these authors, taphonomic analyses have suggested that bears used this cave as a shelter for hibernation for a prolonged time of undetermined duration. In this paper, we analyzed the isotopic carbon:oxygen ratios in the tooth enamel of nine individuals (four cubs and five adults) and the carbon:nitrogen ratios in bone collagen of 10 individuals (five cubs and five adults) from the Galería 1 site. The objectives of this study were, first, to provide new data on the territorial mobility and variation in the diet in this population; second, to obtain evidence contributing to resolve the current debate on the dietary flexibility of cave bears.

*1.1. Carbon*

The metabolism of plants to process atmospheric $CO_2$ can be differentiated into three types: the Calvin Cycle ($C_3$ plants), the Hatch-Slack pathway ($C_4$ plants), and Crassulacean Acid Metabolism (CAM plants). The abundance of $C_4$ plants, which includes mainly grasses, is correlated with high temperatures and low humidity (Stowe and Teeri, 1978; Morales-Puente *et al*., 2012). For their part, $C_3$ plants are distributed in areas with temperatures below 25 °C, and most of these plants are trees and shrubs (Medrano and Flexas, 2000). $C_3$ and $C_4$ plants are isotopically different; $C_3$ plants exhibit $\delta^{13}C$ values ranging from -22 ‰ to -30 ‰, while $C_4$ plants possess values from -10 ‰ to -14 ‰. Finally, CAM plants exhibit intermediate values between $C_4$ and $C_3$ plants (Farquhar *et al*., 1989; Steuter *et al*., 1990; Cerling *et al*., 1997).

When herbivorous mammals feed, they incorporate carbon into their mineralized tissues, so their values will reflect the type of plant consumed (Koch, 2007). However, in dental enamel, carbon content may vary as a result of the physiology, metabolism, and body size of each animal (Tejada-Lara *et al*., 2018). On the other hand, collagen presents an enrichment of 3 ‰ to 5 ‰ in $\delta^{13}C$ values relative to levels in the plant consumed (DeNiro and Epstein, 1978; Palmqvist *et al*., 2003).

Carnivores obtain carbon from their prey, which has already undergone isotopic fractionation when incorporated by herbivores from the original food sources. In addition, carnivores can consume different types of prey and tissues. Accordingly, some studies consider that carbon values obtained from bone collagen may show enrichment of up to 2 ‰ relative to their prey, and this value increases for each step up the trophic levels (Bocherens and Drucker, 2003; Palmqvist *et al*., 2008).

*1.2. Nitrogen*

Herbivorous mammals do not take up nitrogen directly from the soil or air, but from the plants consumed, while carnivores acquire it from their prey. Nitrogen in plants is incorporated into the structure of many animal tissues, including collagen (DeNiro and Epstein, 1981). The amino acids in collagen proteins have nitrogen isotopic values enriched by 3 ‰ to 5 ‰ relative to the amino acids consumed by the animal, and this value increases for each step up the trophic levels (Bocherens and Drucker, 2003; Fox-Dobbs *et al.*, 2008). Thus, carnivores would show higher $\delta^{15}N$ values than their prey and these, in turn, would exhibit higher values than their food sources (Hilderbrand *et al*., 1996; Ambrose, 2002; Sponheimer *et al*., 2003), with herbivores commonly possessing the lowest $\delta^{15}N$ values (Wada *et al.*, 1986; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Ambrose, 1993; Sponheimer *et al.*, 2003).

Nitrogen isotopic values can be affected by environmental factors and the preferred consumption of some types of plants that may contain high $\delta^{15}N$ values, such as nitrogen-fixing plants (Delwiche *et al*., 1979; Ambrose and DeNiro, 1986; Koch *et al*., 1994). In addition, plants that thrive in arid areas with low rainfall also tend to have higher $\delta^{15}N$ values (Heaton, 1987; Sealy *et al*., 1987). On the other hand, when animals undergo severe dehydration, urea increases and, thus, $\delta^{15}N$ values (Ambrose and DeNiro, 1986).

### *1.3. Oxygen*

Oxygen from water ingested by organisms is incorporated as carbonates ($CO_3^{2-}$) and phosphates ($PO_4^{3-}$) into the bioapatite of mineralized tissues (Koch, 2007; Morales *et al*., 2012). However, $\delta^{18}O$ values of water can vary according to environmental factors such as altitude, temperature, and precipitation (Epstein *et al*., 1951; Epstein and Mayeda, 1953; Rozanski and Araguás-Araguás, 1995; Higgins, 2018). Thus, differences in $\delta^{18}O$ values in the

water consumed may indicate migrations or certain environmental conditions (Sponheimer and Lee-Thorp, 1999; Schwarcz *et al*., 2010; Morales *et al*., 2012).

## 2. MATERIAL AND METHODS

### *2.1. Study site*

Cueva de Guantes is part of a karst system developed in a marine limestone substrate of the Upper Cretaceous (IGME cartography, 1982). It is located on the southern slope of Sierra del Brezo, northwest of Palencia, Spain, at coordinates 42°47'8.53"N and 4°46'39.36" W at 1145 meters above sea level (m asl) (Figure 1).

The lower floor of Cueva de Guantes, which corresponds to the current vadose zone, has two entrances: Boca Norte and Boca Sur. The Galería 1 deposit is located in a gallery located 6.5 meters above the entrance level of Boca Sur and about 40 meters inland. The fills of Galería 1 comprise several levels that contain archaeological and paleontological materials. The cave bear fossils recovered amount to more than 2000 pieces from the stratigraphic unit 206-207-303 (Mateos *et al*., 2014; Mateos and Rodriguez, 2019; Rodriguez and Mateos, 2014), corresponding to a minimum of 41 individuals (Gallego, 2021). More than half of these individuals died before reaching one year of age. The skeletons are disarticulated, although some skeletal elements were occasionally anatomically connected. Likewise, in stratigraphic unit 206-207-303, radiocarbon dating evidenced an age greater than 45 Kyrs BCE (Late Pleistocene). This warranted a more accurate dating method (more than 50 Kyrs); therefore, a valid dating for this stratigraphic unit is still missing (Rodríguez and Mateos, 2014; Mateos *et al*., 2019).

Mateos and Rodríguez (2019) confirmed that Galería 1 was used as a hibernation site by cave bears during the Late Pleistocene and that the remains of these animals allow establishing the continued occupation of this gallery by bears over multiple generations. The

attritional death profile evidenced from the state of wear and eruption of the dentition (Mateos *et al*., 2019; Gallego, 2021), dominated by infantile individuals and those under one year of age, indicates natural death during hibernation.

*2.2. Sample collection*

We processed samples of dental enamel and bone collagen from *Ursus spelaeus* remains found in stratigraphic unit 206-207-303 of Galería 1, inside Cueva de Guantes, which are deposited in the Museo de Palencia, Palencia, Spain. Nine dental pieces were selected, four belonging to bears less than one-year-old and five to adult bears. Rib fragments from five adult bears and one cub were also selected, as well as four vertebrae fragments belonging to cubs (Tables 1 and 2). For dental pieces, age at death was determined from the estimates of Rodriguez and Mateos (2014) based on Debeljak (1996), where the degree of development, eruption, and wear of the dental pieces are observed. The age cohorts from dental wear defined by Stiner (1988) were also considered. As for rib and vertebrae fragments, the age of each individual was estimated by observing the epiphysis fusion process (Marks and Erickson, 1966); consequently, it was only possible to determine whether they were adult or immature specimens.

*2.3. Enamel isotopic analysis*

The nine dental enamel samples obtained were prepared using a technique based on Koch et al. (1997), as follows. The surface of each tooth was cleaned with fine sandpaper and reagent-grade ethanol; then, enamel samples were obtained with a Dremel 4300 drill with a carbide drill bit. Subsequently, each sample was thoroughly ground to a fine powder in an agate mortar with a pestle and sieved through a 125-micrometer mesh; the powder obtained was then deposited in separate properly labeled tubes. Afterward, 10 mL of 30 % hydrogen

peroxide was added to each sample, which was kept under constant stirring for two hours. Later, hydrogen peroxide was discarded, and the sample was washed three times with "type-I" water (18.2 MΩ). A single washing process is performed as follows: the sample is centrifuged at 8000 rpm for 10 minutes at 20 °C; then, type-I water (18.2 MΩ) is added and later discarded. Once the sample washing was completed, 4 mL of 1.0 M acetic acid-calcium acetate buffer solution was added to adjust the pH to 4.75, leaving it for nine hours under constant stirring. Afterward, samples were washed again with type-I water (18.2 MΩ) three times. Finally, reagent-grade ethanol was added to each sample, which was then oven-dried at 90 °C for 24 hours.

The samples were analyzed at the Stable Isotope Laboratory of the *Laboratorio Nacional de Geoquímica y Mineralogía* (National Laboratory of Geochemistry and Mineralogy; LANGEM in Spanish) at the *Instituto de Geología* (Institute of Geology), *Universidad Nacional Autónoma de México* (UNAM). The carbon:oxygen isotopic ratios of carbonates in samples were measured using a Finnigan MAT 253 mass spectrometer with a double input system and a GasBench auxiliary equipment with a GC Pal autosampler with a temperature-controlled aluminum plate attached to the mass spectrometer. The results of the isotopic analysis of carbonates in dental enamel were expressed as $\delta^{13}C_{VPDB,}$ $\delta^{18}O_{VPDB,}$ and ‰. These results were normalized to the Vienna Pee Dee Belemnite (VPDB) scale using NBS-19, NBS-18, and LSVEC, and with the corrections proposed by Coplen (1988), Werner and Brand (2001), and Coplen et al. (2006), with a standard deviation of 0.2 ‰ in the oxygen and carbon isotopic analyses (Brand *et al*., 2014).

*2.4. Collagen isotopic analysis*

The ten samples obtained from rib and vertebrae fragments of cave bears were processed using a technique based on Koch et al. (1997), as follows. First, the surface of each

rib and vertebra fragment was cleaned with acetone. Then, a Dremel 4300 drill with a 1 cm diameter trepanner was used to remove a bone sample weighing approximately 400 mg. Subsequently, each sample was ground to a fine powder in an agate mortar with a pestle and sieved through a 125-micrometer mesh; then, no less than 1 gram of each powder sample was placed in tubes. Afterward, 10 mL of 0.5 M hydrochloric acid (HCl) was added to reach a pH lower than 1; each tube was stirred for 30 minutes, as with the dental enamel samples, and three washes were carried out. A pH of 7 was checked using litmus paper strips.

To remove any humic acids in the sample, 5 mL of 0.1 M NaOH was added to each tube, followed by constant stirring for one hour. Then, it was centrifuged at 8000 rpm for 10 minutes at 20 °C, discarding the supernatant. Each sample was then washed three times, checking that the pH was 7. Subsequently, 5 mL of 0.5 M HCl was added, followed by constant stirring for one hour, then centrifuged again at 8000 rpm for 10 minutes at 20 °C. Once this procedure was completed, each sample was washed three times, checking that the pH was 7.

Once the above procedure was completed, 12 mL of type-I water (18.2 MΩ) was added, and the pH of the solution was adjusted to 3 by adding 2 mL of 0.01 M HCl. This solution was then placed in an oven at 80 °C for 20 hours; this process causes the collagen to dissolve as "gelatin". Finally, the solution was filtered (hot) using silver filters of 25 mm diameter and 0.45 μm pore size: afterward, the solution was placed in tubes and freeze-dried for 24 hours.

Once the samples were processed, they were analyzed at the Stable Isotope Laboratory, LANGEM, Institute of Geology, UNAM. The results of the isotopic analysis of bone collagen were expressed as $\delta^{13}C_{VPDB}$. $\delta^{15}N_{AIR}$, and ‰. Carbon results were normalized using the standards mentioned above, and nitrogen results were normalized with the reference

materials IAEAN1, USGS 26, USGS 40, and USGS 41, which have a 0.2 ‰ precision (Morales-Puente *et al*., 2012).

*2.5. Statistical analyses*

To verify that collagen samples had not been altered by diagenetic processes, C/N ratios were compared with the range of values proposed by DeNiro (1985), Schoeninger et al. (1989), and Ambrose (1990): 2.6–3.5.

Subsequently, we calculated the maximum, minimum, and average values of the carbon:oxygen isotope ratios for dental enamel and the carbon;nitrogen ratios for collagen. Given the number of data recorded and to identify potentially significant differences between the isotopic C:O ratio for enamel and the C:N ratio for collagen of adults and cubs, the Shapiro-Wilk normality test was run to check the normality of the samples and the Mann-Whitney U test to make comparisons between ursids. The software used for all statistical tests was IBM SPSS Statistics version 20.

To obtain the oxygen isotopic values of the water consumed by bears, $\delta^{18}O_{VPDB}$ values were first converted to $\delta^{18}O$ values in the VSMOW scale using the proposal of Faure (1977):

$$\delta^{18}O_{VSMOW} = 1.03091(\delta^{18}O_{VPDB} + 30.91)$$

Then, $\delta^{18}O_{VSMOW}$ values were calculated using the formula by Iacumin et al. (1996):

$$\delta^{18}O_{\text{ingested water (VSMOW)}} = (\delta^{18}O_{\text{VSMOW from CO3}}) - 33.63)/0.998$$

Finally, these values were compared with those obtained from site precipitation water obtained with the online tool "OIPC: The Online Isotopes in Precipitation Calculator" (Bowen and Revenaugh, 2003; Bowen *et al*., 2005; IAEA / WMO, 2015; Bowen, 2017) (Table 3).

## 3. RESULTS

*3.1. Dental Enamel*

The mean, maximum, and minimum $\delta^{13}C_{VPDB}$ values obtained for enamel were -14.1 ‰, -12.9 ‰, and -15.6 ‰, respectively (Table 1 and Figure 2). The nonparametric Mann-Whitney U test yielded a significance value of 0.624, i.e., no significant differences in $\delta^{13}C$ values between adults and cubs.

Regarding $\delta^{18}O_{VPDB}$, the mean, maximum, and minimum values were -5.6 ‰, -5.0 ‰, and -5.9 ‰, respectively (Table 1 and Figure 2). The Mann-Whitney U test yielded a significance value of 0.105 (>0.05), also indicating a non-significant difference between adult and cub values. The estimate of the oxygen isotope ratio in the water ingested by each individual is shown in Table 1, with a mean of -8.5 ‰.

### *3.2. Bone collagen*

The C:N ratios indicate that eight samples fall within the range of values proposed by DeNiro (1985), Schoeninger et al. (1989), and Ambrose (1990); only two samples (O-5 and O-10) showed evidence of diagenesis and, therefore, were excluded from this study (Table 2). In the case of carbon isotope ratios in collagen, the mean, maximum and minimum values were -21.7 ‰, -21.3 ‰, and -22.6 ‰, respectively (Table 2 and Figure 3). The Mann-Whitney U test yielded a significance of 0.043 for $\delta^{13}C_{VPDB}$; although this is a statistically significant difference between the two age groups, adult and cub values are distributed over a 1.3 ‰ interval. For $\delta^{15}N_{AIR}$, the mean, maximum, and minimum values were 4.9 ‰. 9.7 ‰ and 2.2 ‰, respectively. The Mann-Whitney U test yielded a significance of 0.663, i.e., no significant differences in the nitrogen isotope ratio between adults and cubs (Table 2 and Figure 3).

## 4. DISCUSSION

### *4.1. Carbon and Oxygen*

Carbon isotope ratios ranged between -5.0 ‰ and -5.9 ‰ in tooth enamel and between -22.6 ‰ and -21.3 ‰ in bone collagen, regardless of the age group (adult or cub). This is consistent with previous studies indicating that this species consumed $C_3$ plants (Llona *et al*., 2005; Grandal-d' Anglade, 2010; Robu *et al*., 2013; Martini *et al*., 2014; Heteren *et al*., 2016; Bocherens, 2019; Terlato *et al*., 2019; Naito *et al*., 2020). Separately, the oxygen isotopic values indicate that bears drank water from sources with similar $\delta^{18}O_{VPDB}$ values, which could have been the same water bodies.

Tooth enamel samples exhibit the oxygen isotopic value in water consumed during the formation of the enamel crystal lattice, thus providing information pertaining to months or even years (Müller *et al*., 2019; Plomp *et al*., 2020). This result suggests that the group of bears studied did not change their diet or water sources for a long time, remaining in areas close to Cueva de Guantes (Epstein *et al*., 1951; Epstein and Mayeda, 1953; Rozanski and Araguás-Araguás, 1995; Higgins, 2018).

Fortes et al. (2016) indicated that at least in northern Spain, each cave housed only a single lineage of cave bears with very stable maternal social groups, which were extremely attached to their cave of birth, which they would use as a hibernation site. The results obtained in the present study likely reflect the attachment of these ursids to Cueva de Guantes. These results are also consistent with studies based on strontium isotopes in cave bears from eastern Slovakia, demonstrating the low migration capacity of this species (Abelova, 2006).

Terlato et al. (2019) found evidence that the cave bear probably maintained its feeding preferences unchanged from ancient to the most recent populations, which may have significantly contributed to its extinction. Likewise, evidence from mitochondrial DNA and radiocarbon dating indicate that the gene diversity of cave bears decreased about 50 Kyrs BCE ago as a consequence of the reduction of their population size due to the marked climatic and vegetation changes that occurred since 40 Kyrs BCE in Europe (Mondanaro *et al*., 2019).

In view of the above, our results for Cueva de Guantes indicate little dietary variation and a limited ability to move to areas far from the cave, which may have been crucial for the survival of the species in northern Spain.

*4.2. Nitrogen*

Collagen $\delta^{15}N_{AIR}$ showed a wide range of values in both cubs and adults. This finding does not agree with what would be expected for strictly herbivorous mammals, which have the least positive $\delta^{15}N_{AIR}$ values within a community (Palmqvist *et al.*, 2008). On the other hand, higher $\delta^{15}N_{AIR}$ values would be expected in cubs than in adults, as the former would still feed on milk and should be one trophic level above their mothers. Likewise, the combined results for nitrogen and carbon suggest that although these bears had the same food sources, some individuals likely were secondary or tertiary consumers since they showed values one or even two trophic levels above other bears (Hilderbrand *et al*., 1996; Ambrose, 2002; Sponheimer *et al.*, 2003). However, the study of nitrogen isotope ratios in this species is complex due to the great uncertainty regarding dietary flexibility in *Ursus spelaeus*, so testing this assertion based on biogeochemical analyses alone would be misleading (Wada *et al*., 1986; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Ambrose, 1993; Sponheimer *et al.*, 2003).

For example, recent studies performed by Naito et al. (2020) in *Ursus spelaeus* using nitrogen isotopic analysis of individual amino acids (glutamate and phenylalanine) in collagen exhibited high $\delta^{15}N_{AIR}$ values. These findings are evidence of a marked dependence on plant consumption, which may explain the unusual values frequently found in these organisms. On the other hand, Ramírez-Pedraza et al. (2019 and 2020), using dental wear analysis at the micro level, indicated that the diet consumed by *Ursus spelaeus* during its last days or weeks before death showed a microwear pattern corresponding to carnivores and omnivores.

Therefore, they proposed that this species may have consumed food with high caloric content, such as meat and bones, just before hibernation. In contrast, Ramírez-Pedraza et al. (2020) found individuals with $\delta^{15}N_{AIR}$ values corresponding to strictly herbivorous mammals, such as *Cervus elaphus*.

On the other hand, it has been proposed that during hibernation, *Ursus spelaeus* had metabolic processes similar to those of current hibernating ursids (Nelson *et al*., 1998; Fernández-Mosquera *et al*., 2001). These processes are characterized by efficient urea recycling (up to 99.7 % of urea) during this period, consisting of urea hydrolyzation and a combination with glycerol to produce amino acids in blood during the prolonged fasting period (Barboza *et al*., 1997; Hellgren, 1998; Llona *et al*., 2005; Stenvinkel *et al*., 2013). It has been estimated that cave bears would display very long hibernation periods, lasting up to eight months (Nelson *et al*., 1973; Ramsay, 1993; Germonpré and Sablin, 2001). More than one-third of the total individuals in "Galería 1" died when they were between 6 and 8 months old, suggesting a long-lasting hibernation period in cave bears (Gallego, 2021). These individuals were born inside the cave and remained there with their mothers until their death. If we assume that blastocyst implantation occurred after the pregnant female began hibernating and a gestation period of 60 days, the hibernation of pregnant females would have lasted between 8 and 10 months, by analogy with grizzly bears (Friebe et al. 2014).

Protein breakdown during hibernation selectively removes $^{14}N$ from catabolized tissues, leading to an increase in the proportion of $^{15}N$; likewise, anabolism and protein synthesis during this period produce high $\delta^{15}N_{AIR}$ values in plasma amino acids (Lee *et al*., 2012). Consequently, $\delta^{15}N_{AIR}$ values increase 18 % to 20 % after several months of hibernation (Lohuis *et al*., 2007).

Therefore, high $\delta^{15}N_{AIR}$ values may be a direct result of prolonged hibernation periods from increased isotopic fractionation caused by urea recycling in amino acid synthesis

(Grandal-d' Anglade and Mosquera, 2008). Therefore, differences in $\delta^{15}N_{AIR}$ values between adult individuals may be more related to the time elapsed between the onset of hibernation and the death of the individual than to its diet and, in cubs, would be related to their age at death. Adults dying at the onset of hibernation and yearlings dying at an earlier age would have lower $\delta^{15}N_{AIR}$ values than those that died after several months in the cave.

*4.3. Paleobiological repercussions for bears of Cueva de Guantes*

The results from the isotopic analysis of carbon in enamel and collagen and oxygen in enamel suggest that bears that inhabited "Galería 1" of Cueva de Guantes maintained similar feeding habits and consumed water from the same sources. This finding indicates that it is unlikely that the bears ranged far from their hibernation cave. This is consistent with taphonomic interpretations of the site described by Mateos et al. (2019), who suggest that "Galería 1" would have been used repeatedly for a long time as a hibernation site by cave bears, including pregnant females.

Also, the range of nitrogen isotopic values in collagen could be a product of metabolic processes occurring during amino acid formation in the fasting period and, therefore, would be consistent with death during hibernation. In cubs, it is important to consider that cave bears had a particularly long lactation time, of approximately two years, which could increase the $\delta^{15}N_{AIR}$ values in cubs and would be affected by the metabolic processes in the hibernating mother (Liden and Angerbjörn, 1999). Lactation alone would cause nitrogen isotopic values to increase by one trophic level relative to the mother (Jenkins *et al*., 2001). Therefore, our observations in the present study regarding nitrogen isotopic analyses in cubs may well reflect the combination of hibernation and lactation.

It has been estimated that the active period in cave bears would probably occur from May–June to October, based on brown bear hibernation and the age at death of cave bears in

Goyet Cave (Belgium) (Germonpré and Sablin, 2001; Nelson *et al*., 1973, 1975; Grandal-d'Anglade and Mosquera 2008). The oxygen present in the skeletons of bears comes from water ingested only during the months of activity, as they do not drink during hibernation. The current $\delta^{18}O_{VSMOW}$ value of meteoric water in the surroundings of Cueva de Guantes in summer months is -5 ‰, while the value obtained for bear fossils is -8.5 ‰. July, August, and September are currently the warmest months with the lowest precipitation of the year in the province of Palencia (Table 3) (Bowen and Revenaugh, 2003; Bowen et al., 2005; IAEA / WMO, 2015; Agencia Estatal de Meteorología, 2016; Bowen, 2017). The lower $\delta^{18}O_{VSMOW}$ values in cave bear fossils suggest cooler and wetter climatic conditions than today, consistent with the environmental conditions proposed for the Late Pleistocene in Spain.

Likewise, the $\delta^{18}O_{VSMOW}$ results obtained in the present study support the proposal of Abelova (2006) indicating a limited ability to move far from the hibernation cave and no migration in *Ursus spelaeus.* Cave bear mDNA analyses by Fortes et al. (2016) and Grandal-d'Anglade et al. (2019) found that this species may have been extremely attached throughout their lives to the cave where they were born. Genetic analyses suggest that males and females from a single population used the same cave as a hibernation site year after year, generation after generation (Fortes *et al.*, 2016). Other data suggest that these bears preferred large caves because they hibernated collectively (Philippe and Fosse, 2003; Quilès *et al*., 2006). Such gregarious behavior would be a major difference with respect to brown bears, which hibernate in small caves no more than 6 m deep and are always alone, except for females with cubs (González-Bernardo *et al*., 2020). Another major ethological difference between the two species may be their dispersal behavior. In Scandinavia, 97% of male and 41% of female brown bears leave the territory where they were born when they become independent from their mother (Zedrosser *et al*., 2007).

## 5. CONCLUSIONS

$\delta^{13}C_{VPDB}$ values in bone collagen and dental enamel and $\delta^{18}O_{VPDB}$ values in enamel indicate that the sources of food and water ingested by bears inhabiting Cueva de Guantes probably were very similar and, moreover, that they died at a short distance from their birthplace. $\delta^{15}N_{AIR}$ values obtained in bone collagen showed great variability, which may be the result of the physiological and metabolic processes during the hibernation of *Ursus spelaeus*. These results are consistent with the hypothesis that the low variation in their diet and the decline and isolation of their population at the end of the Pleistocene were crucial for the extinction of this species in northern Spain and, probably, in other parts of Europe.

However, to improve the paleodietary inference for this species in future research, it is necessary to carry out dental wear studies combined with the analysis of stable isotopes of carbon, oxygen, and nitrogen in the same individuals. Additionally, an analysis of strontium isotope ratios in dental enamel and collagen could support the theory of the high attachment of these bears to the cave where they were born. These approaches will contribute to a better understanding of the paleobiology of this species in Cueva de Guantes.

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906 **Table and Figure captions**

907

908 **Table 1.** Dental specimens selected for carbon and oxygen isotopic analyses. The estimated
909 age for each specimen is shown (Debeljak, 1996; Rodríguez and Mateos, 2014).

910

911 **Table 2.** Vertebra and ribs selected for carbon and oxygen isotopic analyses.

912

913

914 **Figure 1**. Location of Cueva de Guantes (Santibáñez de la Peña), north of Palencia, Spain.

915

916 **Figure 2**. Values of $^{13}C_{VPDB}$ and $\delta^{18}O_{VPDB}$ obtained from tooth enamel samples. Triangles and
917 circles represent cubs and adults, respectively. Each point on the graph shows the number of
918 bears.

919

920 **Figure 3**. Results of the isotopic analysis of bone collagen. Triangles and circles represent
921 cubs and adults, respectively.

922