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Diet at the onset of the Neolithic in northeastern Iberia: An isotope–plant microremain combined study from Cova Bonica (Vallirana, Catalonia)

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The emergence of Neolithic societies was transformative, impacting many aspects of life, particularly diet. The process of Neolithization in Iberia is increasingly understood as the arrival of new people from the Central Mediterranean, who dispersed along the Iberian coasts introducing cereal production, herding, and Cardial pottery and associated material culture. Although research has clarified aspects of the cultigen-dominated economy of these new people, questions remain due to the limitations of conventional archaeobotanical and archaeozoological methods that tend to produce indirect evidence. The extent to which these early farmers adopted Mesolithic staples, which are often difficult to detect with other methods, remains unclear. Furthermore, questions surround the nature of methods of food preparation Cardial Neolithic people used when incorporating grains into their diet. In this study, we examined direct evidence of the diet from the Iberian Cardial Neolithic site of Cova Bonica (Vallirana, Baix Llobregat, Catalonia) using CN stable isotopes on bone and plant microremains trapped in dental calculus from six human individuals and associated fauna. Isotopes show a diet based on terrestrial C₃ resources, with no isotopic evidence of aquatic or C₄ resource consumption. Plant microremains (starches and phytoliths) provide evidence of cereal use, as well as of other plant foods. However, perhaps due to Bonica's early farmers' choice of grain variety, their grain processing methods, or due to specific dental calculus formation factors, the grain assemblages are rather limited and provide scarce information on food preparation.

KEYWORDS

Neolithic, diet, stable isotopes, plant microremains, Western Mediterranean

1 Introduction

1.1 Combining stable isotope and plant microremain analysis for dietary studies

The study of stable isotopes on both bone collagen and plant microremains in dental calculus is useful for recovering information on past diets and has been used individually as a direct means to trace critical aspects of prehistoric populations such as dietary patterns. While carbon and nitrogen stable isotope analysis is a well-established and frequently used method to obtain information on average protein consumption (Lee-Thorp, 2008), the study of plant microremains on dental calculus to recover information on plant food consumption is still not as frequently used as the latter (Power et al., 2015); the combination of both proxies is even scarcer. However, by combining these two techniques, there is a potential for achieving a wider picture of dietary subsistence patterns during prehistoric times (Goude et al., 2019; Goude et al., 2020; Salazar-García et al., 2021).

Isotopic diet reconstruction is based on the principle that as animal and human tissue grows, it records the isotopic composition of the diet through predictable isotope fractionation (Schoeller, 1999). For this analysis, bone collagen is usually the preferred substrate because it is both the only considerable nitrogen source from skeletal remains (Salazar-García et al., 2014a) and has accepted quality indicators to assess its isotopic integrity (De Niro, 1985; Van Klinken, 1999). However, it is necessary to consider when interpreting results that stable isotope ratios from bone collagen reflect only the main dietary protein sources consumed several years prior to death (Hedges et al., 2007; Katzenberg, 2012) rather than that of a diet as a whole, especially for nitrogen (Ambrose and Norr, 1993) since carbon may be derived from other dietary macronutrients like sugars and fats (Howland et al., 2003; Jim et al., 2004).

On the other hand, dental calculus is an oral plaque mineralized by salivary calcium. During the accumulation process, dietary and environmental information from plant and animal foods, including starch grains, phytoliths, lipids, proteins, and DNA, can become trapped and embedded. Dietary remains retrieved from dental calculus are a useful additional source of information on human diet and behavior. Amongst dietary reconstruction methods, it is a helpful complement to other methods because it offers direct information on food consumed and substances that enter the mouth (Armitage 1975; Henry and Piperno 2008; Power et al., 2014; Warinner et al., 2014; Leonard et al., 2015; Power et al., 2015). In this sense, while isotope analysis gives a more quantitative approach on the origin of consumed protein, plant microremains on dental calculus give more qualitative data on specific foods consumed. Likewise, while stable isotope analysis gives long-term averaged dietary information on protein consumption (Lee-Thorp, 2008), plant microremains

can give information on other types of consumed non-protein food resources, even if less frequently eaten (Leonard et al., 2015; Power et al., 2015). Therefore, combining these two techniques provides complementary information on past diets and should be implemented in dietary studies when possible (Salazar-García et al., 2021).

The application of these analytical techniques in Mediterranean Iberia is, however, unequal between them. While studies of stable isotopes have significantly increased during the past decade in prehistoric periods (Salazar-García et al., 2018) creating an important corpus of prehistoric dietary data from the Mesolithic onward in the region and its hinterland, plant microremain studies on dental calculus are almost non-existent. Isotope studies from the Mesolithic (García-Guixé et al., 2006; Fernández-López de Pablo et al., 2013; Salazar-García et al., 2014b), Neolithic–Chalcolithic (Fontanals-Coll et al., 2015; Fontanals-Coll et al., 2017; García-Borja et al., 2013; Gibaja et al., 2016; McClure et al., 2011; Salazar-García, 2009; Salazar-García, 2011; Salazar-García, 2014; Salazar-García et al., 2013; Salazar-García et al., 2016; Salazar-García et al., 2017; Villalba-Mouco et al., 2018a; Villalba-Mouco et al., 2018b; Villalba-Mouco et al., 2019), Bronze Age (McClure et al., 2011; García-Borja et al., 2013; Salazar-García et al., 2017), and Iron Age (Salazar-García et al., 2010) periods have been performed in Mediterranean Iberia. On the other hand, plant microremain studies on dental calculus have only been performed so far on one Paleolithic site (Salazar-García et al., 2013) and one Chalcolithic site (Power et al., 2014) in prehistoric Mediterranean Iberia.

1.2 The site studied: Cova Bonica

Cova Bonica (41°22′10.29″N, 1°53′38.64″E) is located in the vicinity of Vallirana, near Barcelona (northeast of the Iberian Peninsula). The site was discovered and first excavated in 1936; however, the archaeological artifacts were not published until the 70s (Baldellou, 1974). Current archaeological excavations are conducted by Grup de Recerca del Quaternari (Universitat de Barcelona) and produced genetic (Olalde et al., 2015) and archaeological data (Oms et al., 2017; Daura et al., 2019). Amongst the archaeological data published by Daura et al., 2019, macrobotanical evidence of diet from flotation consists only of the remains of *Arbutus unedo* charcoal, which produces edible fruits, and some taxa charcoal with aromatic and medicinal properties such as *Laurus nobilis* or *Rosmarinus officinalis*.

The cave consists of a principal chamber (SP) and two lateral chambers, one next to the entrance (SL1) and another in the inner area (SL2). The cave and sediments were partially destroyed by a sparry calcite mine, but intact Cardial infillings have been preserved in two separated layers: IV₂ preserved in SP-Grao 1 and IV in SP (Figure 1).

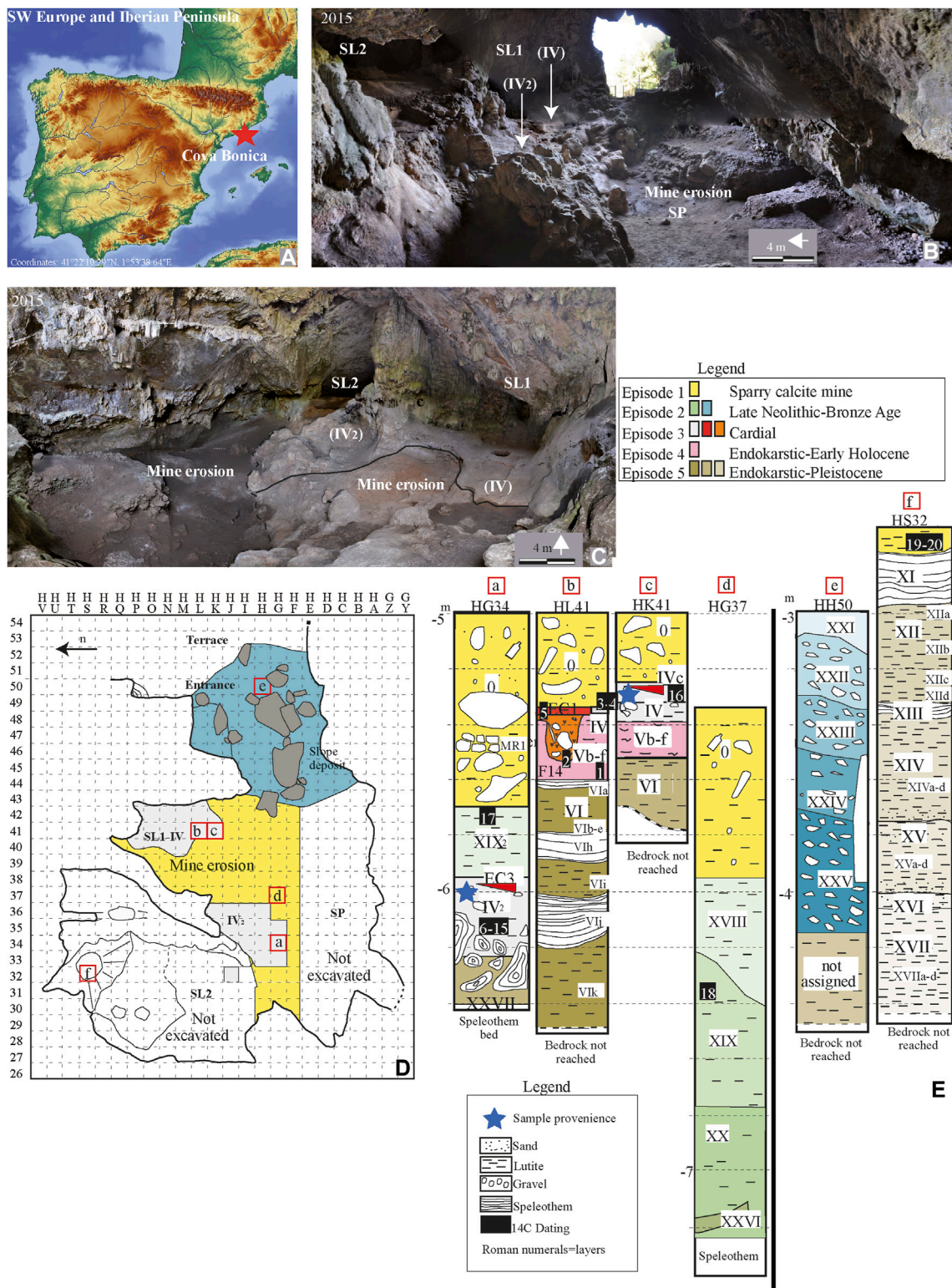


FIGURE 1 Cova Bonica. (A) Location of Cova Bonica in the NE of the Iberian Peninsula. (B) Current view of the cave entrance and main areas. (C) Neolithic area excavated. (D) Plan of the site indicating the excavated area. (E) Stratigraphy of the site's profiles (cave -a to -d-, terrace -e-, and SL2 -f-).

The stratigraphic succession of Cova Bonica is complex and can be grouped into five episodes (1–5). Episodes 5 and 4 represent endokarstic deposits and a ceiling collapse with a restricted entrance and scarce archaeological materials. Episode 3 corresponds to the Cardial Neolithic horizon and is formed by layers IV and IV₂. Layer IV is located in SL1, laying above the stalagmitic flowstone of layer V and contains a thermoaltered sediment labeled as EC1 and 14 post-holes, one of them yielding a radiocarbon age of 6,340 ± 34 BP. Layer IV contains scarce archaeological artifacts and human remains probably displaced from layer IV₂; a sheep bone shows a ¹⁴C age of 6,158 ± 32 BP, and one human individual shows an age of 6,395 ± 39 BP. Layer IV₂ lies in a depression in the cave's bedrock and between large collapsed speleothems and contains a minimum number of individuals (MNI) of 7 (ca. 315 bones): 6 individuals defined osteologically, and a seventh one defined by aDNA. The archaeological remains also include personal ornaments, lithics, bone tools, Cardial Neolithic pottery, faunal remains, and charcoal. Radiocarbon dating of the human remains recovered from layers IV and IV₂ indicates a homogeneous assemblage and a short or synchronic time span for the deposition of the human corpses around ca. 6400 BP (5,470–5,320 ka cal. BP). Episode 2, recorded in several areas of the cave, represents archaeological occupation spanning from the late Neolithic to the Bronze Age.

2 Materials and methods

For this study, we analyzed for stable isotopes 12 faunal and 6 human remains and assessed 5 samples of human dental calculus, one sediment and two associated blank controls. Human and faunal remains analyzed in the present work were recovered during the 2008–2017 field seasons conducted by Grup de Recerca del Quaternari (University of Barcelona). Materials were previously identified and studied in the Guixera laboratory (Castelldefels City Council) (Figures 2, 3). Human remains were scattered and often it was not possible to associate elements (e.g., long bones, ribs, teeth) as pertaining to the same individual with certainty.

2.1 CN stable isotope analysis

Bone samples from 6 human individuals and 12 faunal specimens from five species were sampled from Cova Bonica's Early Neolithic layer (IV₂) (Table 1) to obtain information on their diet by carrying out C and N stable isotope ratio analysis. The consumption of C₃ and C₄ terrestrial resources is distinguishable by the ^δ¹³C stable isotope ratio (Van der Merwe and Vogel, 1978). Isotopic signals also help define the input in the diet of terrestrial and marine foods (Chisholm

et al., 1982), although if freshwater or estuarine fish are involved, the interpretation of ^δ¹³C values becomes more complex (Salazar-García et al., 2014b). The ^δ¹⁵N stable isotope ratio increases by 3–5 ‰ up the food chain with each trophic level and is usually used to indicate the position of an organism in the food chain (Minagawa and Wada, 1984). Even if this quantification is less straightforward than previously thought (Hedges and Reynard, 2007), based on the exact values of the nitrogen ratio, it is potentially possible to differentiate between individuals that consumed more animal resources from those who consumed very little animal proteins (Fahy et al., 2013). Furthermore, the fact that aquatic food chains tend to contain more trophic levels than terrestrial ones, and therefore, show an increase in ^δ¹⁵N, helps to discriminate between the consumption of marine or C₄ terrestrial foods when samples are ¹³C enriched (Schoeninger and DeNiro, 1984).

Prior to analysis, visible contaminants were removed with aluminum oxide powder abrasion. Methods outlined by Richards and Hedges (1999) were followed to extract collagen for the C and N isotope ratio analysis at the Stable Isotope Facilities of the University of Cape Town (Cape Town, South Africa). Whole bone fragments weighing ca. 300 mg obtained from each of the specimens were demineralized in a 0.5 M HCl solution at 5°C. They were then rinsed three times with deionized water until the pH became neutral and gelatinized over 48 h at 70°C before being filtered and ultra-filtered using 9-ml EZEE® filters to remove small particles (<8 μm) and >30 kDa Amicon® ultrafilters, respectively. Finally, the purified solutions were frozen and lyophilized before being weighed into tin capsules and loaded into mass spectrometers.

The resultant collagen product was combusted to N₂ and CO₂. The carbon and nitrogen isotope ratios in collagen were measured in duplicate (reproducibility better than 0.1 ‰) using a Finnigan Delta plus XP continuous-flow isotope ratio mass spectrometer (Thermo Fisher Scientific, United States) after being combusted in an elemental analyzer Flash EA 1112 interfaced with it (Thermo Fisher Scientific, United States), at the Isotope Facilities of the University of Cape Town (South Africa). Stable carbon isotope ratios were expressed relative to the VPDB scale (Vienna PeeDee Belemnite) and stable nitrogen isotope ratios were measured relative to the AIR scale (atmospheric N₂), using the delta notation (δ) in parts per thousand (‰). Repeated analysis of internal and international standards (methionine, Merk Gelatin, Valine, and seal bone) determined an analytical error <0.1 ‰ (1σ) for ^δ¹³C and ^δ¹⁵N.

2.2 Dental calculus microremains

For samples prepared for this study, teeth were evaluated and sampled for dental calculus with a scalar in the field (Table 2).



FIGURE 2

Faunal remains from layer IV₂. **(A)** (Site # 3475) equid lower deciduous tooth (dp3). **(B)** (Site # 2969) brown bear metatarsal. **(C)** (Site # 3166) bovid calcaneum. **(D)** (Site # 3148) ovicaprid humerus. **(E)** (Site # 3147) ovicaprid humerus. **(F)** (Site # 2760) ovicaprid humerus. **(G)** (Site # 2444) bovid calcaneum. **(H)** (Site # 3167) ovicaprid humerus. **(I)** (Site # 2872) ovicaprid humerus. **(J)** (Site # 3279) ovicaprid humerus.



FIGURE 3

Human remains from layer IV₂. (A) (Site # 3029) humerus. (B) (Site # 2996) humerus. (C) (Site # 3121) humerus. (D) (Site # 3066) frontal bone. (E) (Site # 3075) mandible. (F) (Site # 2517) scapula. (G) (Site # 2777) vertebra. (H) (Site # 2303) lower canine. (I) (Site # 2793) lower incisor. (J) (Site # 2595) upper premolar. (K) (Site # 3260) canine. (L) (Site # 3155) canine. (M) (Site # 2618) upper molar. (N) (Site # 2583) upper premolar. (O) (Site # 3334) upper incisor. (P) (Site # 2406) upper canine. (Q) (Site # 3439) premolar. (R) (Site # 3343) incisor. (S) (Site # 3158) upper incisor.

TABLE 1 Cova Bonica $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from fauna and humans, human sex and age, chronology, collagen control indicators (yield, % C, % N, and C:N), S-UCT number, sampled bone, and archaeological context.

S-UCT	Site #	Archaeological context	Species	Element	Sex	Age	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	% col.	% C	% N	C:N
19257	2777	Layer IV ₂	Human	Vertebra	Indet.	8–9 yo	−19.4	8.5	1.7	39.6	14.1	3.3
19258	3066	Layer IV ₂	Human	Frontal	Indet.	2.5–3 yo	−19.1	11.0	1.5	41.7	14.8	3.3
19259	2517	Layer IV ₂	Human	Scapula	Indet.	4–5 yo	−19.7	8.8	4.4	42.0	14.9	3.3
19260	3121	Layer IV ₂	Human	Humerus	Indet.	12–13 yo	−19.8	8.7	1.6	39.9	14.0	3.3
19261	2996	Layer IV ₂	Human	Humerus	Indet.	Adult	−19.5	9.3	3.6	41.7	14.8	3.3
19262	3029	Layer IV ₂	Human	Humerus	Indet.	Adult	−19.6	9.2	1.0	41.4	14.4	3.4
19263	3100	Layer IV ₂	Ovicaprid	Humerus	*	Adult	−20.9	4.5	0.8	33.4	11.5	3.4
19264	2970	Layer IV ₂	<i>Ovis aries</i>	Humerus	*	Adult	−19.7	4.0	1.3	42.4	15.1	3.3
19265	3328	Layer IV ₂	Ovicaprid	Humerus	*	*	−20.0	3.6	1.7	42.1	14.9	3.3
19266	3148	Layer IV ₂	Ovicaprid	Humerus	*	Perinatal/ fetal	−20.1	5.0	2.7	42.5	14.7	3.4
19267	3167	Layer IV ₂	Ovicaprid	Humerus	*	Immature	−19.5	4.7	1.9	42.7	14.2	3.5
19268	3279	Layer IV ₂	<i>Capra hircus</i>	Humerus	*	Adult	−19.1	3.7	1.0	42.4	14.7	3.4
19269	3147	Layer IV ₂	Ovicaprid	Humerus	*	Fetal	−20.0	5.8	1.0	38.5	12.9	3.5
19270	2872	Layer IV ₂	Ovicaprid	Humerus	*	Neonatal	−19.6	5.1	0.7	41.4	14.4	3.4
19271	2444	Layer IV ₂	<i>Bos taurus</i>	Calcaneus	*	Immature	−21.0	6.4	4.1	42.7	15.2	3.3
19272	3166	Layer IV ₂	<i>Bos taurus</i>	Calcaneus	*	*	−19.7	5.0	4.6	42.5	15.1	3.3
19273	3475	Layer IV _{2b}	Equid	Deciduous tooth	*	Immature	−21.0	4.2	1.7	32.1	11.3	3.3
19274	2969	Layer IV ₂	<i>Ursus arctos</i>	Metatarsal	*	Adult	−19.8	8.8	3.0	42.4	14.9	3.3

Samples were subsampled in the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). When possible, we sampled chunks of supragingival dental calculus by prying them off, rather than scraping off a powder but a significant number of samples were of powder. The samples were then processed in a Heraeus safety-controlled airflow cabinet at the labs of the Department of Primatology in the Max Planck Institute for Evolutionary Anthropology. We used EDTA to decalcify the sample (Power et al., 2015; Tromp et al., 2017).

After the decalcification, the sample was rinsed twice with distilled water after centrifugation at 2,000×g for 10 min (Roth Mini-Centrifuge). Then, after the first decanting, the tubes were refilled with a 25% glycerin solution. Then we mounted 20 μL of the solution on a slide with an 18 × 18 mm or a 24 × 24 mm coverslip. During the preparation of samples, we prepared slides using an identical mounting procedure using 25% glycerin solution but without any dental calculus, to serve as a control blank to expose environmental contamination. We counted starches, phytoliths, and all other microremains irrespective of the type at 200–400× magnification using an absolute counting method with a light microscope under brightfield and cross-polarized light on an Axio Scope A1, Zeiss microscope with AxioVs40 V 4.8.2.0 software. Microremains were classified according to standard conventions (ICPN 2.0, Neumann et al., 2019).

3 Results

3.1 CN stable isotope analysis

Samples from six humans and 12 faunal specimens from five different species (*Ovis aries*, *Capra hircus*—ovicaprid when not assigned to species taxon—, *Bos taurus*, equid, and *Ursus arctos*) were taken for stable isotope analysis. All samples yielded sufficient collagen in the >30 kDa fraction for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis in duplicate. All of them met published collagen quality controls: appropriate CN elemental percentages together with C:N ratios between 2.9 and 3.6 (De Niro, 1985; Ambrose, 1990; Van Klinken, 1999). All isotope ratio results from Cova Bonica are given in Table 1 and shown in Figure 4.

Analyzing the carbon values of the 11 terrestrial herbivores, it can be seen that their $\delta^{13}\text{C}$ mean value is -20.1 ± 0.6 (1 σ) ‰ and its minimum and maximum values are -21.0 ‰ and -19.1 ‰, respectively. This herbivore value is compatible with a typical C₃ terrestrial ecosystem. Analyzing the nitrogen values, the herbivore mean $\delta^{15}\text{N}$ value is 4.7 ± 0.9 (1 σ) ‰ and has minimum and maximum values of 3.6 and 6.4 ‰, respectively, establishing the trophic baseline for human subsistence. The only available omnivore carbon value is -19.8 ‰, which is also consistent with the general herbivore values and a terrestrial C₃ food ecosystem. The $\delta^{15}\text{N}$ value of the omnivore specimen analyzed is 8.8 ‰, which is 4.1 ‰ higher than the

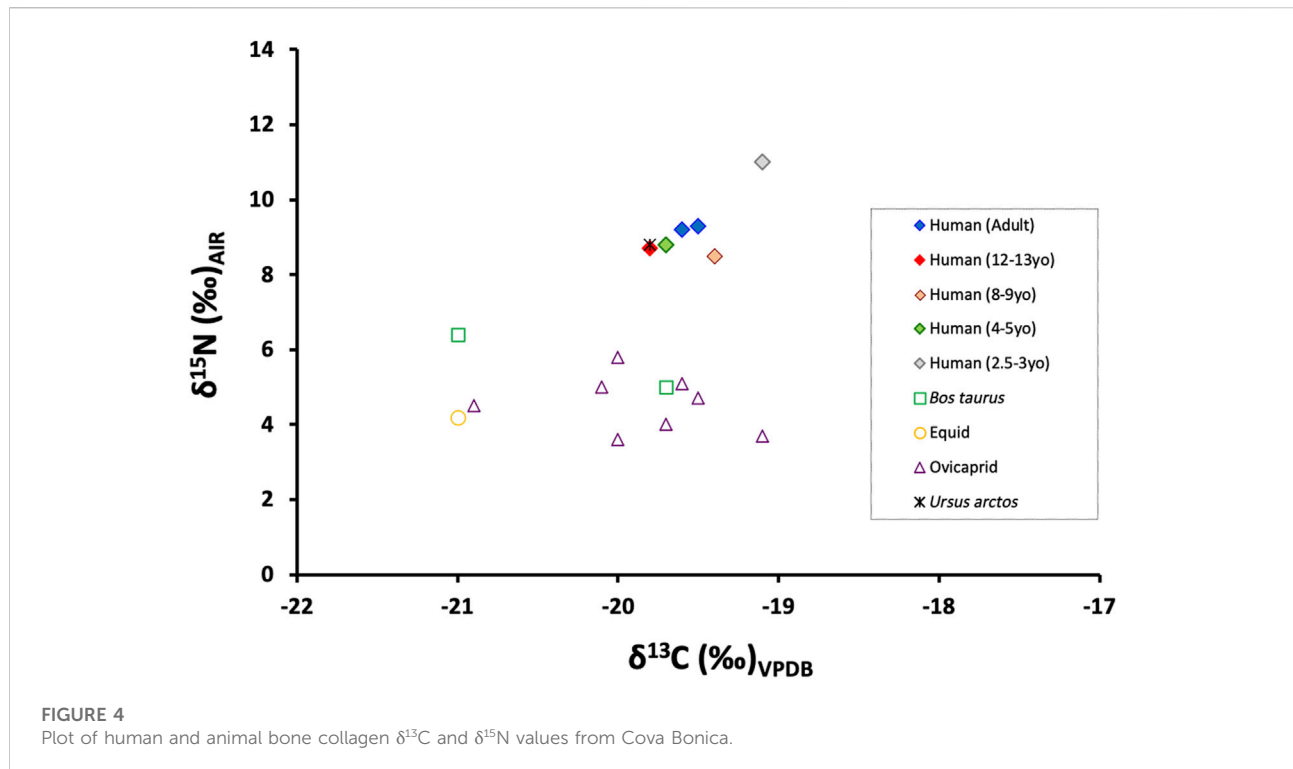


TABLE 2 Cova Bonica dental calculus samples taken for microremain microscopic analysis.

Sample code	Material	Dental piece code	Archaeological context	Species	Sex	Age	Tooth	Position	Weight (mg)
CB002	Dental calculus	3155	Layer IV ₂	Human	Female	Adult	Canine	Supragingival	4.139
CB003	Dental calculus	3260	Layer IV ₂	Human	Indet.	Adult	Canine	Supragingival	4.347
CB012	Dental calculus	3075	Layer IV ₂	Human	Indet.	Adult	Incisor	Supragingival	5.916
CB014	Dental calculus	3334	Layer IV ₂	Human	Indet.	8–9 yo	Incisor	Supragingival	7.137
CB019	Dental calculus	2618	Layer IV ₂	Human	Indet.	12–13 yo	Molar	Supragingival	2.114
CB008	Sediment	*	Layer IV ₂	*	*	*	*	*	0.987
CB002 b	Blank	*	*	*	*	*	*	*	*
CB012 b	Blank	*	*	*	*	*	*	*	*

herbivore mean value and situates the omnivore (*Ursus arctos*) almost one complete step higher than the herbivores in the food chain. Unfortunately, no aquatic resources are available for this site, and thus, the marine and freshwater-specific “baseline” is lacking for Cova Bonica.

All humans from the Early Neolithic period ($n=6$) have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values of -19.5 ± 0.2 (1σ) ‰ (min: -19.8

‰ and max: -19.1 ‰) and 9.3 ± 0.9 (1σ) ‰ (min: 8.5 ‰ and max: 11.0 ‰), respectively. These $\delta^{13}\text{C}$ values portray a diet based on terrestrial C_3 resources, while the $\delta^{15}\text{N}$ values place humans at almost one full trophic level over herbivores (4.6 ‰ higher) and only slightly higher than the one omnivore analyzed (0.5 ‰ higher). There is no isotopic evidence of marine protein consumption.

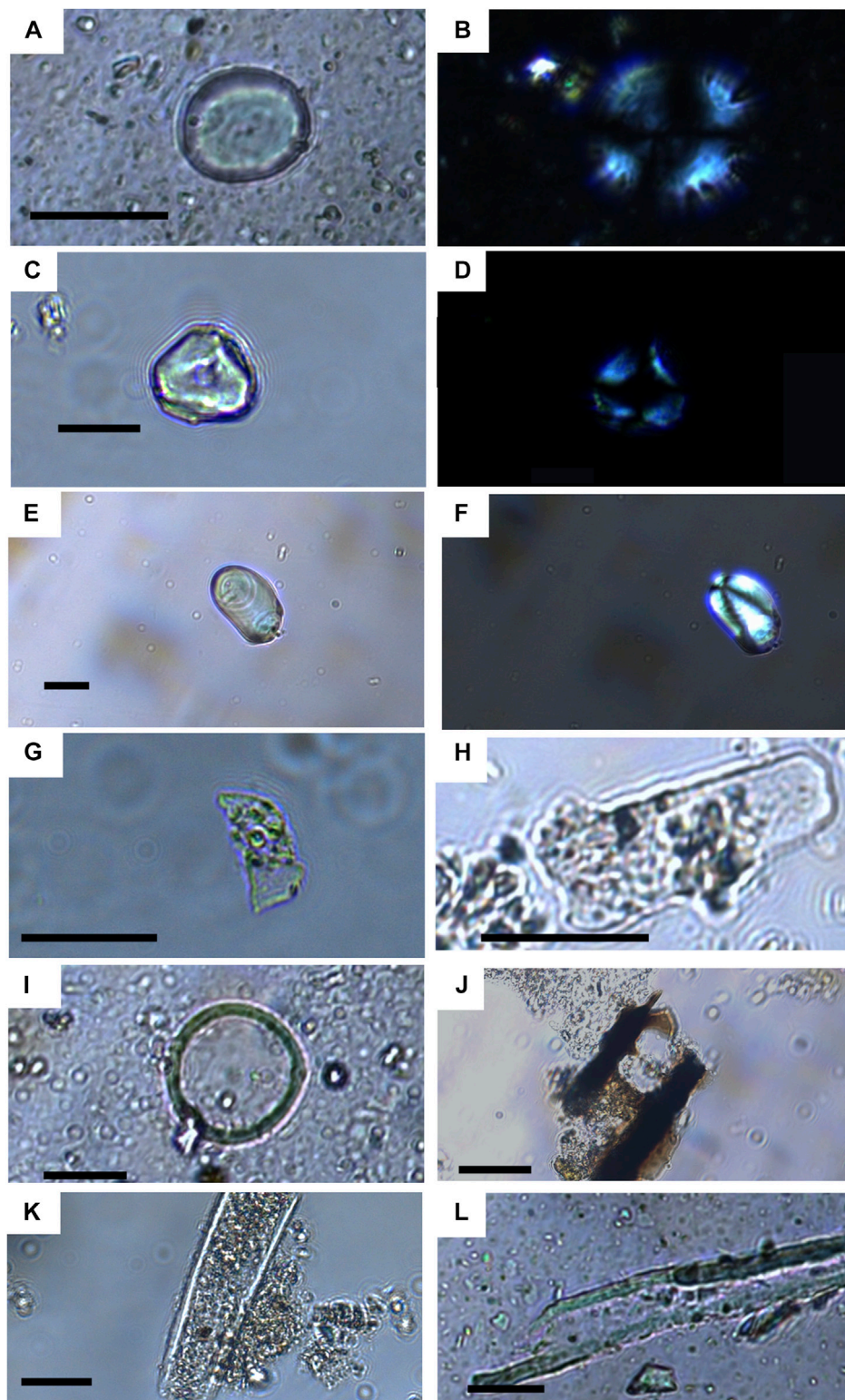


FIGURE 5

Micrographs of a selection of microremains. All scale bars represent 20 microns, (A) brightfield image of a lenticular starch in CB003, (B) cross-polarized image of the same starch, (C) brightfield image of faceted starch in CB003, (D) cross-polarized image of the same starch, (E) brightfield image of an eccentric starch in CB008, (F) cross-polarized image of the same starch, (G) trapezoidal (grass short-cell) phytolith in CB012, (H) elongate psilate thin phytolith, (I) indeterminate pollen from CB003, (J) charcoal particle in CB019, (K) broken probable non-human mammalian hair in CB003, and (L) plant fiber in CM003.

3.2 Microremain analysis

Microremains were limited in the control samples, and their assemblages were entirely composed of cellulose-type fibers. Plant remains were present in all the archaeological dental calculus samples (Table 2). They occur in significant numbers in most samples. Of the microremains, charcoal was the dominant group but starches and phytoliths were also common. Fibers, pollen, fungal spores, calcium oxalates, and a variety of other unknown particles were also present. Their abundance varied considerably between samples (Figure 5).

Phytoliths are well represented in the dental calculus samples, forming the most diverse category in the assemblage by occurring in five of the samples, totaling 23 phytoliths. Phytoliths from monocotyledon plants (wild grasses, domestic cereals, sedges, etc.) were the largest group, totaling 15 phytoliths (Table 3). Of these, Poaceae was dominant, comprising six phytoliths, present in five samples. A single Poaceae type was found in sediment (CB008). It cannot be determined if the assemblage is predominantly derived from the leaves/stems of wild or domestic. Grass short-cells (rondels and trapezoids) show grasses from the pooid subtribe which are consistent with, but not exclusive of, barleys and wheat ($n = 4$). Spheroid ornate/echinate is present in two individuals. Eudicots are represented in only two samples and represent leafy matter.

Starches are widespread in the assemblages, being present in four dental calculus samples totaling six grains. They occur in small numbers (5 or fewer starch grains). In contrast, in sediment sample CB008, they occur in a large concentration. These in CB008 are largely comprised of a highly eccentric simple starch, sub-spherical sub-ovoid types, typically with a birefringence, representing a different origin than the dental calculus starches. Lenticular starch indicating Triticeae that could derive from cultivated wheat or barley was only found in CB003. Polyhedral-type starches also occurred in CB008, and a related form is found in CB019.

Pieces of combustion products are present in all five dental calculus samples, totaling 389. They include long thin pieces of charcoal, indicative of charred grasses, rather than wood or other materials (Crawford and Belcher, 2014). They also include unidentified combustion products and the largest category, nonspecific charcoal, which is probably mostly wood charcoal in origin.

A total of five grains of pollen were found in four samples. Grains of pollen could not be identified. Calcium oxalates (6) were found in CB002 and CB012. Fungal spores totaling 8 of an unknown family were found in three individuals. A non-silica plant vessel was found in CB008, but is non-diagnostic. A mixture of cellulose-type fibers is present in the samples, totaling 32, and 4 in the sediment samples. These included colored fibers dyed red and blue (Table 3). A probable

mammalian hair, identified due to its birefringence, “surface scales,” and animal cell structure, was found in CB018. The medullar index indicates it is not human (Kshirsagar et al., 2009).

4 Discussion

Stable isotope and plant microremain studies are commonly used for past human dietary reconstructions. Until now, only few isotopic data of material directly dated to the earlier stages of the Neolithic are available in Eastern Iberia, unlike what is observed in Western Iberia (Guiry et al., 2016). No information on the diet inferred from plant microremains from dental calculus is available from the Early Neolithic. However, the Early Neolithic is a key period for understanding the process of Neolithization in Iberia, including dietary patterns and ecology. Mediterranean Iberia is also characterized by the low presence of human remains from this period compared with other regions, which makes the data from this study on the site of Cova Bonica even more valuable.

Previous paleogenetic data obtained from Cova Bonica (Olalde et al., 2015) support the evidence that new migrant populations introduced farming into western and central Europe. The presence of Cardial pottery, as documented in Cova Bonica, shows that the northeastern Iberian Neolithic is linked to the Italian Peninsula. It is in these regions that we see the earliest Cardial pottery and ovicaprid husbandry (Tresset and Vigne 2007; Vigne 2007) together with wheat and barley (Zapata et al., 2004; Antolín et al., 2015). Accordingly, the archaeological record (stratigraphy, radiocarbon dating, and artifacts) along the Mediterranean shoreline suggests a maritime colonization process (Zilhão 2001) that bypassed geographic obstacles using enclaves to rapidly settle lands optimal for farming (Bernabéu et al., 2015) (Figure 6A). Further insights into dietary patterns from these earlier stages of the Neolithic can help clarify the subsistence strategies of these first farming societies in the region.

The Early Neolithic individuals from Cova Bonica show a tight range of carbon isotope ratios, portraying a homogeneous protein diet based on C_3 terrestrial resources. While this is not unexpected for a Neolithic population, what is surprising is that there is no isotopic evidence whatsoever of marine protein consumption by any of the individuals. Taking into consideration that Cova Bonica is only 12 km away from the coastline (Figures 6.B,C), there is evidence at the same layers of the site of ornaments crafted from marine resources (Figures 6.H–J), and that there is jasper from the coast as well on the site (Figures 6.D–G), this population was aware of the marine environment. Of course, that there is no isotopic evidence of marine consumption does not mean necessarily that they did not eat any sea resources but that, if they ate them, they did so in such low amounts as to not be indicated by the collagen signal of their bones.

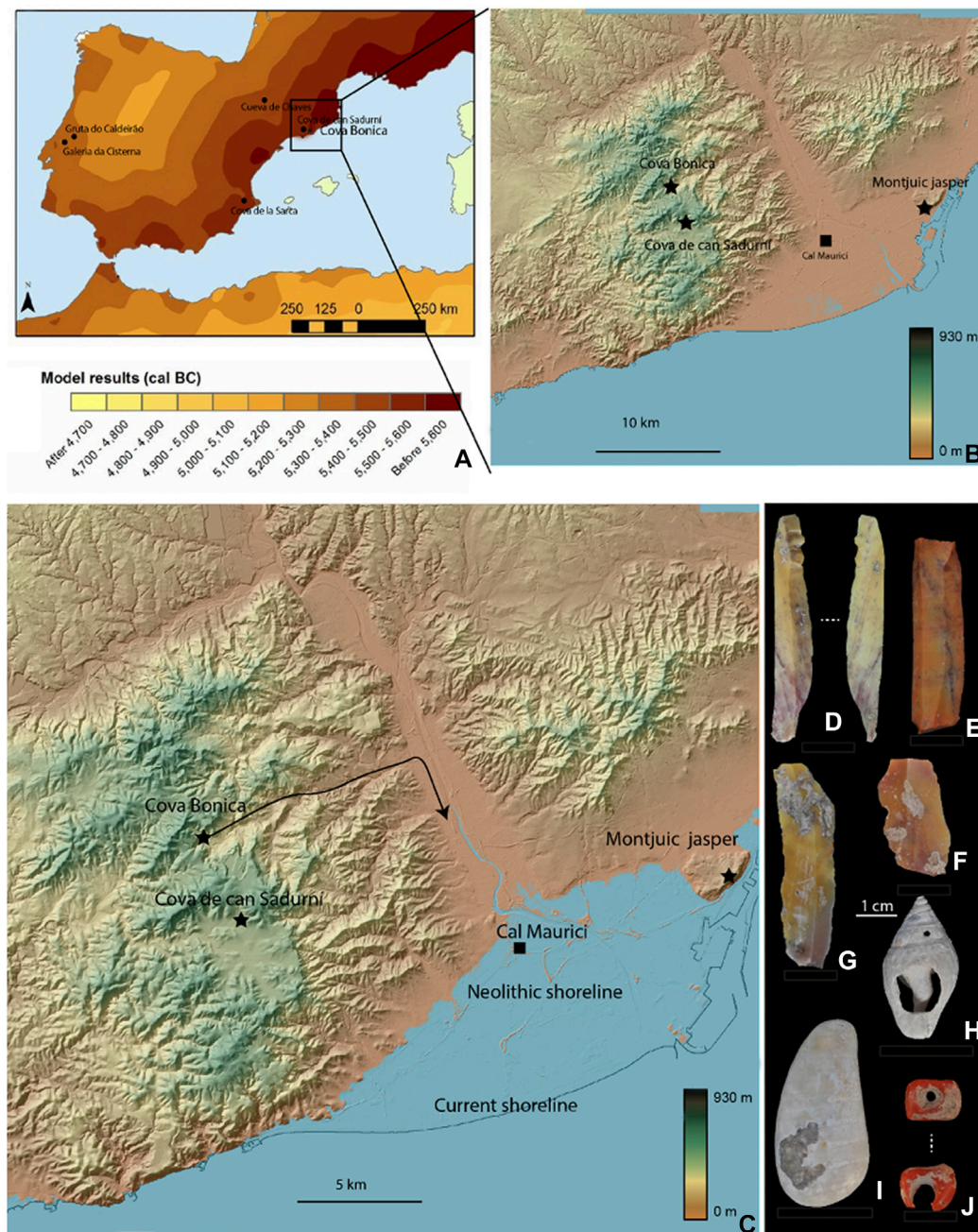


FIGURE 6

Cova Bonica in the context of regional settings. (A): Model predictions for the Neolithic expansion: leapfrog voyaging with jumps of about 350 km (Isern et al., 2017). (B–C): Digital elevation model (DEM) constructed using GIS version 3.24.2 and signaling current coastline (B) and neolithic shoreline (C). Neolithic shoreline has been established using data provided from the Cal Maurici site (Daura et al., 2016). (D–G): Jasper blades and flakes recovered at layer IV₂ coming from Montjuïc (Barcelona). (H–J): Marine ornaments recovered at layer IV₂ elaborated from *Columbella rustica* (H), polished shell (I), and red coral (J).

Having discarded any marine signal from the $\delta^{13}\text{C}$ values, the differences observed in the $\delta^{15}\text{N}$ values (range 8.5–11.0 ‰) must be considered under another light. This difference cannot be, at this point, attributed to sex, as all human individuals are of

indeterminate sex. However, the age of the different individuals analyzed should be considered, especially since four of the six total human individuals are not adults. As can be seen in Figure 3, the youngest individual analyzed (2.5–3 yo) is the one with the

TABLE 4 List of all Mediterranean Iberia sites with published carbon and nitrogen isotopic ratio analysis on human bone collagen from the Mesolithic up to the Late Neolithic–Chalcolithic period. Only samples from human adults for which both C and N results are available which meet collagen quality controls have been considered here.

Site (ca. distance from the coast on a straight line)	Archaeological chronological attribution	<i>n</i>	$\delta^{13}\text{C}$ <i>m</i> $\pm 1\sigma$ (‰)	$\delta^{13}\text{C}$ <i>max</i> (‰)	$\delta^{13}\text{C}$ <i>min</i> (‰)	$\delta^{15}\text{N}$ <i>m</i> $\pm 1\sigma$ (‰)	$\delta^{15}\text{N}$ <i>max</i> (‰)	$\delta^{15}\text{N}$ <i>min</i> (‰)	References
El Collado (7 km)	Mesolithic	9	-18.4 ± 0.7	-17.6	-19.5	10.3 ± 1.2	12.8	8.9	García-Guixé et al. (2006)
Santa Maira (30 km)	Mesolithic	2	-18.1	-18.0	-18.1	9.1	9.4	8.8	Salazar-García et al. (2014b)
Penya del Comptador (50 km)	Mesolithic	3	-18.5 ± 0.3	-18.2	-18.7	7.7 ± 0.1	7.8	7.6	Salazar-García et al. (2014b)
Casa Corona (80 km)	Mesolithic	1	-19.3	*	*	8.4	*	*	Fernández-López de Pablo et al. (2013)
Cingle del Mas Nou (70 km)	Mesolithic	4	-18.5 ± 0.1	-18.4	-18.6	8.8 ± 0.8	9.8	7.9	Salazar-García et al. (2014b)
Cova Bonica (12 km)	Early Neolithic	3	-19.6 ± 0.5	-19.5	-19.8	9.1 ± 0.3	9.3	8.7	This study
Costamar (0–1 km)	Early Neolithic	2	-19.1	-19.0	-19.1	8.2	8.5	7.9	Salazar-García (2009)
Cueva de Nerja (0–1 km)	Early–Middle Neolithic	3	-19.1 ± 0.5	-18.5	-19.4	9.0 ± 0.2	10.3	8.2	Salazar-García et al. (2017)
Feixa del Moro (200 km)	Early–Middle Neolithic	2	-20.3	-19.8	-20.7	8.7	9.1	8.2	Remolins et al. (2016)
Tossal de les Basses (0–1 km)	Middle Neolithic	11	-18.1 ± 0.6	-17.5	-19.1	11.2 ± 1.2	13.1	9.0	Salazar-García et al. (2016)
Pujolet de Moja (20 km)	Middle Neolithic	3	-19.3 ± 0.2	-19.2	-19.5	10.0 ± 0.5	10.3	9.4	Fontanals-Coll et al. (2017)
Hort d'en Grimau (27 km)	Middle Neolithic	3	-19.8 ± 0.3	-19.5	-20.1	9.2 ± 0.9	10.2	8.6	Fontanals-Coll et al. (2017)
Can Roqueta–Can Revella (15 km)	Middle Neolithic	6	-19.9 ± 0.5	-19.4	-20.4	8.4 ± 1.2	9.7	6.3	Fontanals-Coll et al. (2017)
Horts de Can Torras (27 km)	Middle Neolithic	2	-19.7	-19.6	-19.7	8.8	9.0	8.5	Fontanals-Coll et al. (2017)
Ceuró (125 km)	Middle Neolithic	3	-19.9 ± 0.2	-19.6	-20.1	8.9 ± 0.3	9.3	8.6	Fontanals-Coll et al. (2017)
Costa dels Garrics (120 km)	Middle Neolithic	2	-20.0	-19.7	-20.2	8.3	8.5	8.1	Fontanals-Coll et al. (2017)
El Llord (125 km)	Middle Neolithic	2	-19.8	-19.8	-19.8	9.9	10.2	9.6	Fontanals-Coll et al. (2017)
Puig d'en Roca (110 km)	Middle Neolithic	5	-20.3 ± 0.4	-20.0	-21.0	8.0 ± 1.2	9.1	6.1	Gibaja et al. (2016)
Can Gelats (35 km)	Middle Neolithic	1	-20.7	*	*	9.2	*	*	Gibaja et al. (2016)
Bòbila Madurell (25 km)	Middle Neolithic	36	-19.7 ± 0.3	-19.0	-20.6	9.2 ± 0.6	10.5	8.1	Fontanals-Coll et al. (2015)
Can Gambús (20 km)	Middle Neolithic	38	-19.6 ± 0.6	-16.8	-20.7	9.0 ± 0.5	10.2	7.9	Fontanals-Coll et al. (2015)
Costamar (0–1 km)	Late Neolithic	2	-18.0	-17.8	-18.2	10.1	10.4	9.8	Salazar-García (2009)
Cova dels Diablets (5 km)	Late Neo-Chalcolithic	8	-18.9 ± 0.3	-18.7	-19.5	10.0 ± 0.6	10.8	8.8	Salazar-García (2014)
Cova de la Pastora (40 km)	Late Neo-Chalcolithic	7	-19.3 ± 0.2	-19.0	-19.6	9.5 ± 0.8	10.6	8.1	McClure et al. (2011)
Avenc dels Dos Forats (40 km)	Late Neo-Chalcolithic	2	-19.1	-19.1	-19.1	10.2	10.4	10.2	McClure et al. (2011)
Coveta del Frare (75 km)	Late Neo-Chalcolithic	2	-19.0	-19.0	-19.0	9.7	9.8	9.6	García-Borja et al. (2013)
La Vital (0–1 km)	Late Neo-Chalcolithic	3	-18.7 ± 0.5	-18.3	-19.3	9.5 ± 0.8	10.3	9.0	Salazar-García (2011)
Cova del Rectoret (15 km)	Late Neo-Chalcolithic	1	-18.9	*	*	7.7	*	*	Miret Estruch et al. (2021)

highest $\delta^{15}\text{N}$ value (ca. 2 ‰ higher than the adults) as well as a slightly higher $\delta^{13}\text{C}$ value. All other sub-adult individuals (ages ranging from 4–13 yo) have isotopic values similar to those of the adults analyzed. This pattern could suggest that at the age of 2.5–3 yo, the individuals were still breastfed and in the process of being weaned (Fogel et al., 1989; Fuller et al., 2006; Herring et al.,

1998). Although the sample is small, there is scarce information about these social practices from Early Neolithic people which highlights the relevance of this finding.

For all individuals from 4 years old onward, nitrogen isotope ratios show that humans possess a higher trophic level than herbivores, but have similar $\delta^{15}\text{N}$ values to the omnivore (brown

bear). The archaeological record of Cova Bonica identified butchery activities mainly in adult ovicaprids, but also in other taxa, such as large bovids (Daura et al., 2019), demonstrating meat processing of domesticates. Moreover, the cave was used as a sheepfold indicating the relevance of husbandry practices for these individuals. All of this suggests that they consumed herbivore animal protein (possibly mainly ovicaprids), but also that they consumed plant foods (enough as to have similar values to the omnivore analyzed). While isotope analysis cannot shed further light on the type of plant resources consumed, the study of plant microremains trapped in dental calculus can.

The dental calculus assemblages show a distinct pattern that contrasts with the sparse remains in the control samples. This indicates the substantial integrity of the finds and that the microremains are archaeological with few phytoliths in the sediment sample. Poaceae is the dominant group of the assemblage, but counts are low relative to studies of many comparable grain-based economies (Scott et al., 2021). The only other Late prehistoric site from Mediterranean Iberia from which microremains from dental calculus have been studied is Camino del Molino (Murcia, Spain), although the site is from the Chalcolithic period (Power et al., 2014). The example of Camino del Molino does show a parallel with a relatively low-density assemblage missing expected morphotypes such as dendritics. At Camino del Molino, we do find some individuals with relatively higher numbers, as well as rare non-phytolith microremains such as sponge spicules and others. Given the purported grain-based Neolithic economy, the microremain counts in the Cova Bonica dental calculus are less than expected. They also lack unique husk types. The low Poaceae microremains count can be considered cryptic, but it could relate to limited cereal cultivation, free threshing versus non-free threshing varieties, or growing conditions that did not encourage phytolith formation in plant cells (Jenkins et al., 2011). Alternatively, it may be a product of specific grain processing methods that minimized phytolith intake.

There is evidence of eudicots, but they are rare and it is unclear if they had dietary significance. The occasional calcium oxalates we found may have also been derived from these eudicots, but they also could have originated from angiosperms. The starch assemblage is comparatively more numerous than the phytolith assemblage, but the non-dietary starches in CB008 drive this. Unidentified eccentric starches are predominant across the assemblage, representing plant storage organs, presumably from the C₃ pathway. Triticeae starch occurs, reinforcing the data from phytoliths of cereal use. The rich presence of charcoal indicates close contact with fires (Hardy et al., 2012; Buckley et al., 2021). It is unclear how close this contact was and whether these particles represent airborne charcoal inhaled, water-carried charcoal that was drunk, or the consumption of charcoal-covered or other sources.

The Cova Bonica isotope values complement the picture that the isotope analysis has already portrayed for Mediterranean Iberia from the Mesolithic up to the Chalcolithic (Table 4). Especially useful for this are the Early Neolithic individuals, as only very few have been published before for this chronology in the region. As we can see in the table, the values from all chronological periods (Mesolithic, Early Neolithic, Middle Neolithic, Late Neolithic, and Chalcolithic) are partially overlapping. Regarding marine protein, Mesolithic hunter-gatherers show intermixed isotopic values whether from coastal or inland sites, which is expected from a population with high mobility through different environments. Examples of this are the inland sites of Santa Maira and Cingle del Mas Nou and the coastal site of El Collado, as previous studies from all three reported individuals with both a full terrestrial isotopic signature and others with enough marine protein consumption to have it recorded in the collagen isotopic signal. However, during the Early Neolithic, not even individuals from coastal sites have a clear marine isotopic signal. All Early Neolithic individuals from the region studied, whether from the coastal (Cova Bonica, Costamar, and Cueva de Nerja) or inland sites (Feixa del Moro), show a terrestrial isotopic signature. It is from the Middle Neolithic onward, and especially during the last stages of the Neolithic and the Chalcolithic periods, when people start to live in more stable settlements and we could consider that the amount of marine resource consumption is many times linked to the proximity to the sea rather than to chronology or archaeological culture. Suggestive of this pattern is that most individuals reported with marine protein input in the diet are from sites directly located on the coastline (e.g., Tossal de les Basses). This same diachronic pattern is observed even in individuals from different time periods of a single same coastal site, Costamar, where its Early Neolithic individuals have a full terrestrial isotopic signal while its Late Neolithic individuals show isotopic evidence of marine protein consumption.

We must keep in mind that some of the differences observed between chronological periods and even sites of the same period could be due to non-dietary influences that modify the isotopic composition of resources consumed regardless of the resource type (i.e., factors that influence the food chain values themselves). For example, different environmental conditions between contemporary sites (e.g., coastal vs. inland; mountain vs. plain), or even between sites of a single geographic area but of different chronology, could have different isotopic food chain baselines (Goude and Fontugne, 2016). The use of complex husbandry practices and associated manuring in the region from the Late Neolithic onward could also influence crops, as well as domestic animals that feed on them, and, in turn, humans who consume the domestic crops or animals (Bogaard et al., 2007). Physiological traits of both the animals consumed (e.g. infantile vs adult specimens) and humans (age, nutritional status, and others), as well as the types of bones sampled (e.g., high vs.

low collagen turnover skeletal elements), also influence the isotopic values obtained from human bone collagen. Some of these “non-dietary” influencing factors are difficult to assess, as the archaeological assemblages provide limited information. These issues caution us away from rigid quantified interpretations when interpreting isotopic studies applied to archaeological material.

Conclusion

The combination of stable isotopes and plant microremains trapped in dental calculus in Cova Bonica brings, for the first time, the combined direct evidence of the diet of these Early Neolithic farmers in the NE Mediterranean area. The human stable isotope analysis and plant microremains from the Cardial Neolithic of Cova Bonica document a diet based on terrestrial C_3 resources. Although Cova Bonica is a mere 12 km from the current coastline, there is no isotopic evidence of marine protein consumption. The use of cereal and other plant foods is supported by plant microremains trapped in dental calculus.

The absence of marine and freshwater foods in the diet of Cova Bonica humans suggests that newcomers, at least initially, did not adapt to the fresh resources in the new land. In addition, the absence of a marine diet in people who moved through sea transport is, according to the coastal spread of the first farmers’ model, remarkable. Archaeological data from the early Neolithic suggest that farming people expand hand-in-hand with this “package:” pottery, domesticated plants, livestock, polished stones, and other archaeological materials. Thus, the Cova Bonica results support the notion that newcomers expanded bearing their own subsistence strategy, focused on terrestrial resources, such as husbandry of ovicaprines and domesticated plants, instead of exploring the alternatives that became available in new territories. This lifestyle is implemented in the regions that they arrived at with suitable lands (i.e., deltaic, estuarine, and lagoonal environments) for agriculture and animal husbandry.

Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Author contributions

DS initiated and coordinated the study. JD and MS conducted the archaeological/anthropological work and the curation of the archeological material. DS, RP, JD, and MS

participated in the sample management and gathering of contextual information. DS and RP organized the sample collection for analyses. DS and RP performed the laboratory work. All authors contributed to the interpretation of the data. DS wrote the first version of the manuscript, which was edited by RP, JD, and MS.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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