

Reconstructing the childhood diet of the individuals from the Middle/Late Bronze Age Bezdanjača Cave, Croatia (ca. 1430 - 1290 BCE) using stable C and N isotope analysis of dentin collagen

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Abstract

This paper investigates the childhood diet of 16 individuals from the Middle/Late Bronze Age (1430-1290 BCE) Bezdanjača Cave (Lika region, Croatia) using stable isotope analysis of dentin collagen from permanent first molars. Results from the analysis reveal that the individuals from Bezdanjača consumed notable quantities of C₄ plants during their childhood. The most common C₄ plant is millet, whose spread throughout Southern Europe was recently dated to the second half of the 2nd millennium BCE, which agrees with the results obtained in this research. Comparisons between the data collected for the individuals from Bezdanjača and other Middle and Late Bronze Age sites in Croatia suggest that only the individuals from the site of Veliki Vital (Middle Bronze Age, inland Croatia) exhibit similar isotopic values to those from Bezdanjača. Human isotopic values from coastal sites, however, reveal that during the Middle and Late Bronze Age people from the coast had diet that still predominantly contained C₃ plant-based foods, which appears to suggest that the dispersion of this crop in Croatia during the Bronze Age followed an east-west trajectory, appearing earlier (Middle and Late Bronze Age) in inland settlements such as Veliki Vital and Bezdanjača and only later (Late Bronze Age and mostly Iron Age) in coastal sites.

Introduction

The Bronze Age can be considered a pivotal time for the prehistory of Europe, as it saw the proliferation of new architectures as well as a change in the social structure of communities with a progressive hierarchization of the population and the birth of social elites. The production of bronze goods, the quest for raw materials (e.g., tin) and other luxury goods (e.g., amber, textiles, spices etc.), greatly boosted trades by sea and land. Food commerce is especially significant

during the Bronze Age as far as millet is concerned because its diffusion across Eurasia represents the first example of “*food globalization*” (Filipović *et al.*, 2020: 2). Broomcorn millet (*Panicum miliaceum*) was first domesticated and cultivated in northeast China around 6000 BCE (Early Neolithic). As recently proved by Filipović and colleagues (2020), the earliest millet findings in Europe date back to sixteenth-century BCE southwestern Ukraine and, one century later, the crop spread throughout the Middle Danube Region, most likely in Romania, Hungary, and probably Croatia and

northern Italy. By the thirteenth century BCE, millet was also present in central Europe, Greece, the whole Balkan region, reaching Germany and Poland only around the twelfth century BCE (*ibid*). Archaeobotanical data from coastal Croatia, in particular Istria and Dalmatia (Becker, 2001; Chapman *et al.*, 1996), confirm the presence of two different varieties of millet (broomcorn and foxtail [*Panicum italicum*]) in these regions throughout the Late Bronze Age and the Iron Age. Furthermore, archaeobotanical studies conducted by Reed (2012; 2016), also suggest that broomcorn millet was present in Croatia only starting from the Late Bronze Age. These data have been corroborated by Filipović and colleagues (2020) who have shown that increased trade networks across the Mediterranean during the Bronze Age allegedly promoted the commerce of millet towards Southern Europe, more specifically Italy and the Balkans (*ibid*).

Since millet is a drought-tolerant, fast-growing, and highly nutritional crop that survives well even on poor soils and adapts to various environments and climates and has a rather narrow growth span (Lightfoot *et al.*, 2014), the crop is considered to have been amongst the preferred cultivated plants of the Lika region (Western Croatia) dwellers from the Bronze Age onwards (Zavadny *et al.*, 2017). As a matter of fact, a study conducted by Zavadny and colleagues (2019) suggests that during the Middle and Late Bronze Age the Lika region was densely covered by forests with some clear areas for pasture. This heavy vegetation coverage most likely prevented the diffusion of light and oxygen on the forest floor, causing the area to suffer from the so-called “canopy effect” (Van der Merwe & Medina, 1991), which made the whole area rather unsuitable for intensive farming, and this might be one of the reasons why we can observe a rapid increase in the population of the region only from the beginning of the Late Bronze Age (1200-800 BCE), which corresponds with the occupation of the area by Iapodian cultural groups. Although the information regarding the Iapodians’ social, political, and economic structures as well as their material culture during the Late Bronze Age and Early

Iron Age is still rather scarce for this area, recent studies (Bakarić, 2010; Zavadny *et al.*, 2017) suggest that they employed a mixed farming strategy that also resorted to breeding. Evidence of dietary habits in this regard is primarily derived from small faunal assemblages excavated from house contexts. Stable isotope analysis were carried out by Zavadny and colleagues (2017) on both domesticated (cattle, sheep, goat, pig, dog, horse) and wild species (roe deer, red deer, chamois and hare) from the Middle Bronze Age sites of Jozgina Pečina (Ličko Valley), Ličko Lešće and Veliki Vital (Gacka Valley), as well as the Late Bronze Age settlements of Cvituša, Pipica and Miljača (Ličko Valley) and Hrvatsko Polje (Gacka Valley), all located in the Lika region¹. Conversely, little is known about agricultural practices in the Lika region because few paleobotanical remains have survived. According to Greek historian Strabo, however, the Iapodians were dependent on millet and spelt because of the poor agro-ecology of the land (*ibid*). This has recently been proven by stable isotope analysis carried out on seven adult individuals from the Bronze and Iron Age sites of Bezdanjača², Gospić-Lipe and Veliki Vital (*ibid*). Here, millet appears to have formed an important portion of diet, comprising about 20% of the overall intake, with Bezdanjača being chronologically the first site to present this dietary trend. Interestingly, the consumption of millet by the Lika region communities seems to increase up to 40% of the overall diet at the beginning of the Iron Age.

Dietary profiles for Bronze Age coastal Croatia have been reconstructed by Lightfoot and colleagues (2014). The individuals from the coastal sites of Koprivno, Radošić-Biluška Griža, Vučevica, Konjsko Polje, Matkovici, Veliki Vanik and Zavojane Ravča seem to have consumed little or no millet and to have relied on a pure C₃ plant-based diet instead. However, similarly to what has been observed for the Lika region, this trend changed, very gradually and slowly, at the beginning of the Iron Age, when isotopic signal from C₄ plants starts being detectable in individuals from the same sites. Based on the analysis presented by Lightfoot *et al.* (2014), it appears that millet was not

¹ The results from this study have been used to determine the faunal baseline of our research.

² In this case stable isotope analysis used bone collagen collected from four individuals.

available throughout coastal Croatia during the Middle and at least the beginning of the Late Bronze Age, but rather, it only started being significantly important to human diets during the Iron Age.

These works provide an important overview of the differences and similarities in both space and time of diet changes observed in different regions of Croatia during prehistory but focus mainly on adult diet. The aim of this study was therefore to investigate childhood diet in protohistoric Croatia using stable carbon and nitrogen isotope analysis of dentin collagen. In this paper the term “childhood” is used to refer to the age span that goes from birth to the onset of adolescence (0-15 years). The “infancy” period, which in bioarchaeology usually indicates the period between birth and the end of the first year (Scheuer & Black,

2000a,b; Lewis, 2007), has been arbitrarily incorporated in the “childhood” age category by the authors for convenience. We compared the results of our study with those available from other coastal and inland contemporary sites in Croatia in order to identify similarities and/or differences in isotopic dietary data, with a special focus on the consumption of millet.

Materials and methods

Bezdanjača Cave (**Figure 1**) is located on the Vatinovac hill, near Vrhovine in the Lika region in Croatia (**Figure 2**) (Malez & Nikolić, 1975). The cave was first recognized as a prehistoric site in October 1964 and the archaeological excavations began in July 1965 (Malez, 1979-80). The cave has been explored in all its branches for a total of 1176 meters, of which only the first 190 meters were of archaeological interest. The entrance to the cave is south of Zalužnica, above Brakusova Draga in the Stručnice region and is located 740 meters above sea level. The upper part of the entrance is funnel-shaped, and at the end of it there is a steep chasm which terminates in a stack of irregularly deposited material. The cave is naturally hidden and difficult to access (Malinar, 1998), which suggests that no one entered it or disturbed the material it contained after it ceased being used by the prehistoric communities. The overall length of the channels is estimated to be 1176 meters, while the difference in height between the highest and the lowest point of the cave is around 200 meters. At its base, the entrance, which today is inaccessible due to the danger of mines left from the 1991-1995 wars in Croatia, bifurcates in two main branches, namely the western channel and the eastern channel. The western channel of the cave is short and broad. Here, archaeologists could only open four excavation units (blocks 1-4), since the whole area was covered with several scattered wooden blocks and fallen rocks (most of which had collapsed near the channel may have been used as a burial space as well (Drechsler-Bižić 1979-80). The eastern channel is



Figure 1 Geographical location of Bezdanjača Cave (from Boljunčić, 1997)

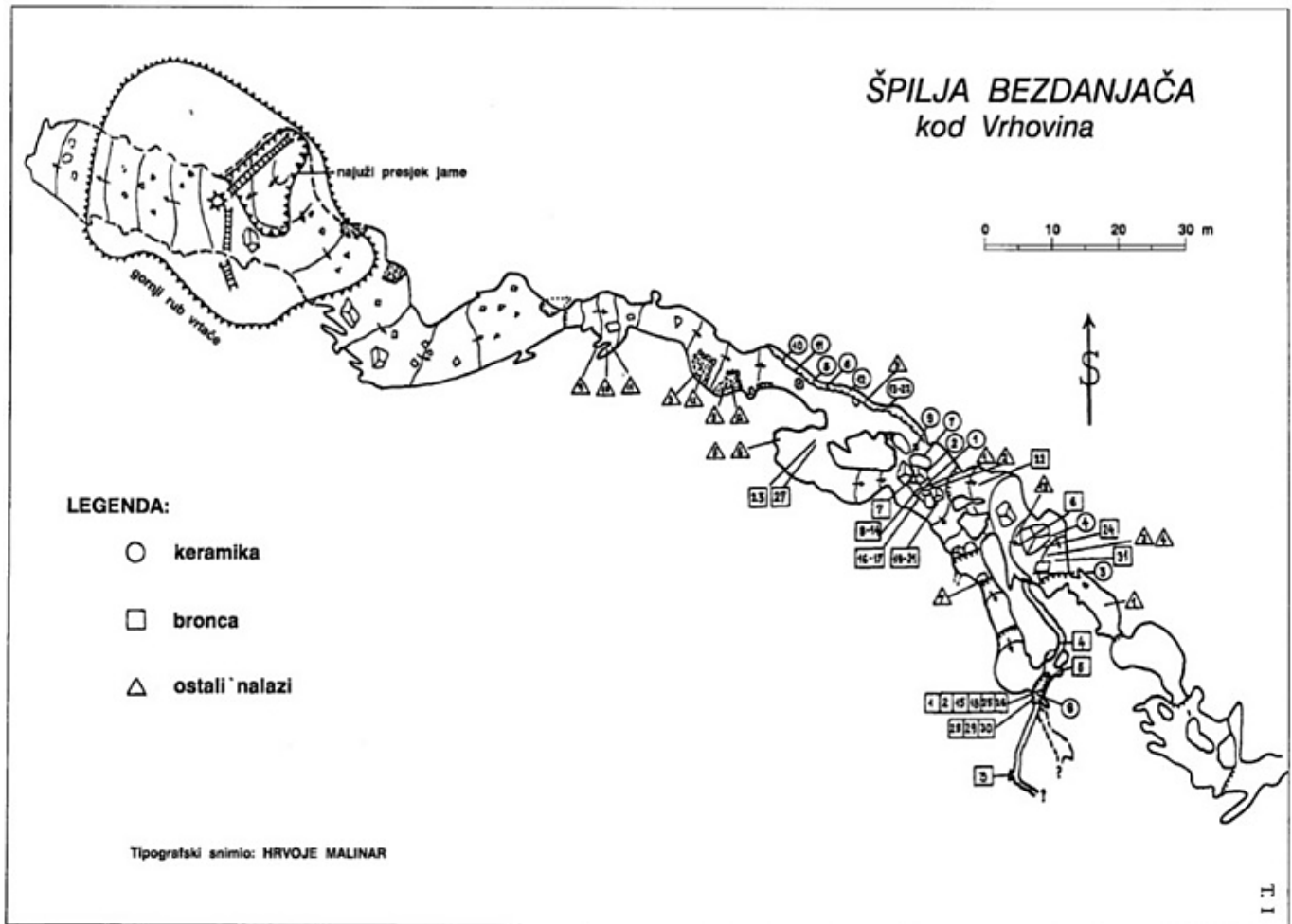


Figure 2 General topography of Bezdanjača Cave (from Malinar, 1998)

positioned at a 40° angle from entrance). Some fragments of animal bones and pottery were found among the rocks, suggesting that this the vertical entrance of the cave. It is much longer and morphologically different from the western channel, as it contains numerous side branches and levels. Seven excavation units were opened right at the entrance of this channel (blocks 5-11), where four disarticulated skeletons were found. Just over 70 meters further from the entrance of the eastern channel, archaeologists were able to open the greatest number of archaeological units (blocks 12-39), that revealed the presence of several well-preserved human skeletons. From block 27 departs a shorter and narrower channel, at the end of which more skeletal remains were found (Drechsler-Bižić 1979-80). In the eastern channel, archaeologists found architectural remains including numerous drywall and wooden structures (Malinar,

1998). Supposedly, these structures could have served to facilitate the entrance to the cave or as plateaus that may have functioned as working surfaces or beds. Traces of hearths, pottery, bronze artifacts, and remains of bracken and hay were found on the stone structures and the floor of the channel and were used in some cases to assess the chronology of the site.

In Benac's opinion (1993-94), based on the materials found in the cave, two cultural horizons can be distinguished at Bezdanjača: an older one, dating to the Middle Bronze Age (BrC/D, 1500-1200 BCE), and a more recent one, dating to the Late Bronze Age (BrD/HaA, 1200-1000 BCE). Drechsler-Bižić (1979-80) assigns both horizons to the Proto- Illyric period, which roughly corresponds to the turn of the second to the first millennia BCE.

This hypothesis agrees with that of Benac (1993-94), who suggests that the development of the Illyric tribal

communities settled in the southern part of the Sava river can be divided into four main phases, the second of which being the one called Proto-Illyric, which was to some extent influenced by the Danube area, the Carpathians, and the eastern Balkans. According to Benac (1993-94), Bezdanjača Cave, with regards to its cultural manifestations and rituals, perfectly fits this phase. Radiocarbon analysis of several wood samples from the site resulted in dates between 1350 and 1100 BCE (Sliepčević & Srdoč, 1979-80). Zavadny and colleagues (2017) provided radiocarbon dates for two individuals found in Bezdanjača Cave. New ^{14}C dates for two individuals from Bezdanjača have been recently carried out at the University of California, Santa Barbara as well (M. Novak, personal communication), and these agree with the previous radiocarbon dates from the same site.

Stable isotope analysis

A total of 49 *crania* from Bezdanjača have been considered for this research. The *crania* had been collected during the 1960s and stored at the Institute for Quaternary Paleontology and Geology of the Croatian Academy of Sciences and Arts. The analysis and the sampling were conducted in the Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb. In this research we used permanent first molars to reconstruct the diet during the early life of the individuals from Bezdanjača. The permanent first molar is the first of the permanent teeth to emerge. It begins to form and calcify at birth and completes its formation at approximately 9-10 years of age when the root is fully developed. For this reason, they are the best means to investigate diet during the early years of an individual's life. Since teeth form incrementally, they can be sampled in bulk to investigate the changes both in diet and environment that happened during their formation period (Miller *et al.*, 2018). Only 16 out of 49 individuals from Bezdanjača were used for stable isotope analysis as their dentition presented permanent first molars with a wear ≤ 3 (Smith, 1984). A low rate of wear likely indicates little (or no) loss of primary dentin due to dental attrition and/or abrasion, as well as limited development of secondary dentin,

which might interfere in the analysis blurring diet signal during childhood.

Dentin collagen was extracted following a modified version of the Leipzig protocol (Sealy *et al.*, 2014). All teeth were mechanically abraded using a Dremel sandpaper burr. This was done to remove superficial enamel to fasten the subsequent demineralization process. Intra-tooth sampling was performed using a diamond-coated circular blade. Only one half of each tooth was weighed out and used for the analysis, while the other half was stored for future analyses. The chosen tooth halves were then demineralized in 0.5 M aq. HCl at 4°C until demineralized. Samples were rinsed with de-ionized water and then gelatinized in acidic solution (pH 3) at 70°C for 48 hours. The liquid solution containing the gelatinized protein was frozen for 24 hours and then freeze-dried for 48 hours to obtain the final collagen product. The collagen from each tooth was weighed out and the samples were then shipped to the Iso-Analytical laboratory (Crewe, Cheshire, UK) where they were loaded into an auto-sampler on a Europa Scientific elemental analyzer for the mass-spectrometry analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

The carbon stable isotope ratio ($\delta^{13}\text{C}$) helps distinguish between diets based on C_3 plants from those based on C_4 plants, since atmospheric plants use different photosynthetic pathways that differently discriminate between ^{13}C and ^{12}C during CO_2 fixation. C_3 plants such as rice, various kind of grasses, all root crops, legumes, vegetables and fruits usually display variable $\delta^{13}\text{C}$ values that can range between -22/-24 to -36‰ (Burt & Amin, 2014; Dupras & Tocheri, 2007; Katzenberg & Saunders, 2008; Lee-Thorp, 2008), whereas C_4 plants (e.g. sorghum, millets, maize, sugarcane and tropical pasture grasses) show carbon values that range between -16 to -9‰ (Dupras & Tocheri, 2007; Katzenberg & Saunders, 2008). The $\delta^{13}\text{C}$ isotopic values of plants fixate into the consumer's tissues with an increase of circa +3-5‰ in large herbivores (Al-Bashaireh *et al.*, 2010) and of circa +1-2‰ in subsequent trophic levels (carnivores and omnivores including humans). It follows that the $\delta^{13}\text{C}$ values for humans are usually around -19‰ for diets based on C_3 plants and around -8‰ for diets based exclusively on C_4 plants (Dupras & Tocheri, 2007). The nitrogen found in bone and dentin collagen provides information about

the consumption of terrestrial and marine plant and animal proteins by an individual. Generally, $\delta^{15}\text{N}$ values increase by approximately +2/3‰ every step up in the food chain. In humans the nitrogen fractionation is variable and can be up to +6‰, although it is still unclear how and when nitrogen isotopic fractionation occurs in the body (Ambrose, 2000; Gannes *et al.*, 1997; Sealy *et al.*, 1987).

Osteological and paleopathological analysis

The determination of age at death on the adults' skulls of Bezdanjača was assessed using the method of ectocranial suture closure (Meindl & Lovejoy, 1985) and the observation of dental wear (Smith, 1984), whereas age at death of the subadults was carried out on the basis Ubelaker's dentition chart (1999). Determination of sex for the individuals from Bezdanjača was conducted exclusively on adult individuals using the morphological/observational method of skull traits since we lacked the postcranial portion of the skeletons. Paleopathological observation on the skulls were limited to the pathologies that interest the cranium and the teeth. Specifically, we looked for and/or observed: porotic hyperostosis, *cribra orbitalia*, scurvy, osteoma,

caries, abscesses, calculus, alveolar resorption, dental enamel hypoplasia. Osteological and paleopathological data are presented only for the 16 individuals used for stable isotope analysis.

Results

Stable carbon and nitrogen isotope values, as well as osteological and paleopathological data, for the 16 individuals from Bezdanjača are presented in **Table 1**.

The overall sample consists of two adult females (12.5%), seven adult males (43.7%), and seven subadults (43.7%). With regard to pathologies, six individuals presented signs of active *cribra orbitalia* (37.5%). Of these, five individuals showed lesions on both orbits (BzV 18a, BzV 18b, BzV 21c, BzV33h, BzV 30c), and one only on the left orbit (BzV X). Porotic hyperostosis has been detected on a total of four individuals (25%): one subadult (BzV 21b), one adult female (BzV 30a) and two adult males (BzV 30c and BzV X). All of these individuals, with the exception of BzV 30a (adult female), also presented *cribra orbitalia*. An indication of the only probable case of scurvy was the diffuse porosity observed on BzV Y's palatine bones.

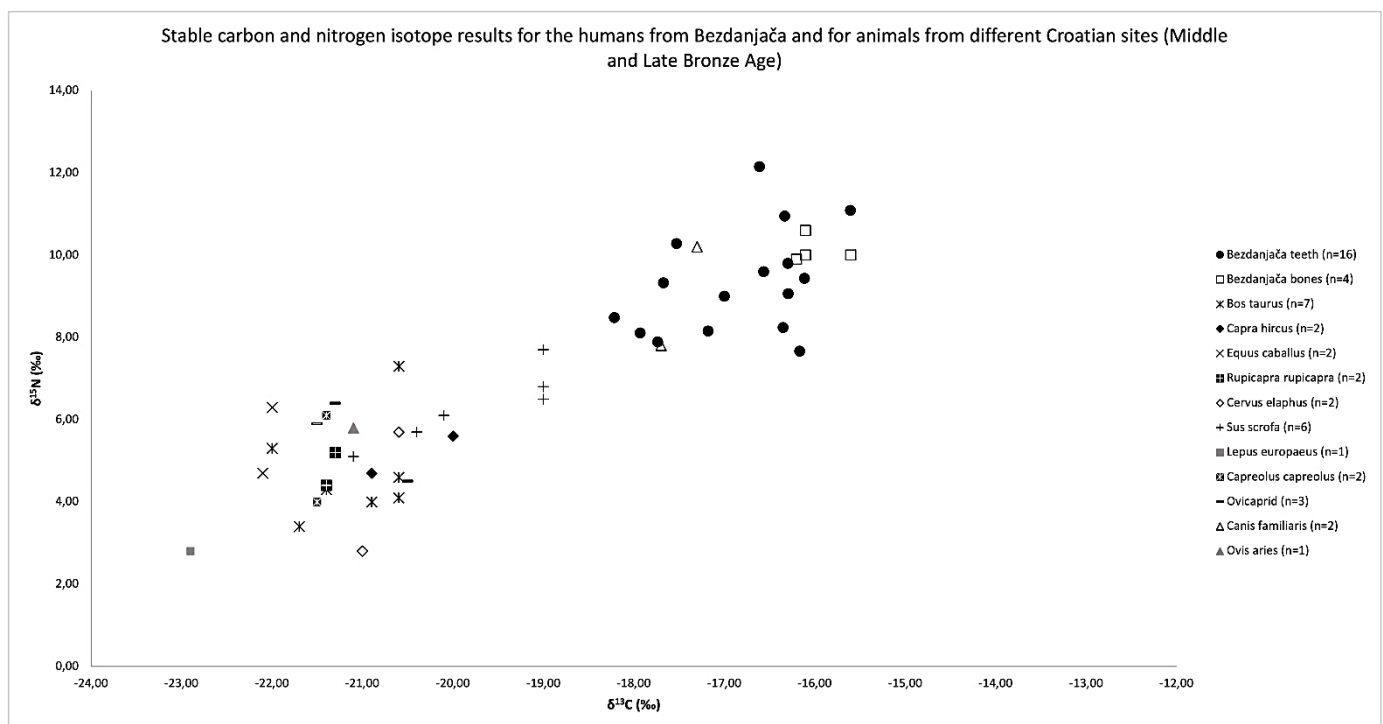


Figure 3 Stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for the individuals from Bezdanjača compared with the faunal baseline's isotopic values

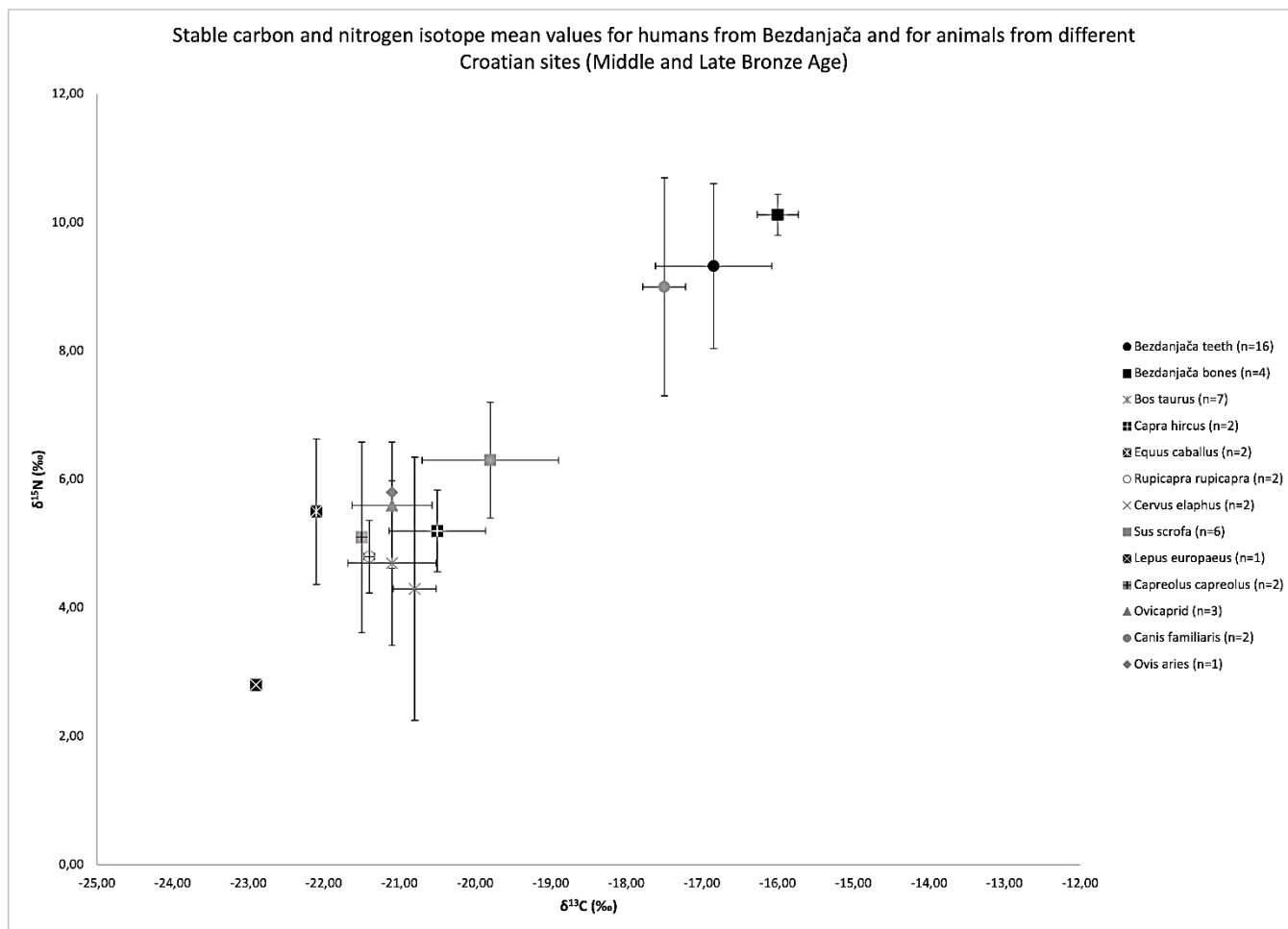


Figure 4 Stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope mean values for the individuals from Bezdanjača compared with the faunal baseline's isotopic mean values

However, lacking the postcranium, this diagnosis could not be confirmed.

16 individuals are -16.8‰ for $\delta^{13}\text{C}$ and 9.3‰ for $\delta^{15}\text{N}$ (Figure 4).

There were no major dental pathologies associated with the 16 individuals considered for this study. Four adult individuals (25%) presented enamel hypoplasia either on the upper canines (BzV 18a, BzV 33b, BzV X) or on the upper second incisor (BzV Y). Only BzV 33f-2, an adult male, showed slight signs of caries on the buccal side of the left M_2 .

Carbon and nitrogen isotopic values for the 16 individuals from Bezdanjača are shown in **Figure 3**. Isotopic data show rather high $\delta^{13}\text{C}$ values that range from -18.2 (BzV 30c) to -15.6‰ (BzV 30d), and high $\delta^{15}\text{N}$ values that vary between 7.7 (BzV 18a) and 12.1‰ (BzV 21c), whose nitrogen values represent the only outlier in the overall sample. The mean isotopic values for the

TABLE 1 Results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the individuals from Bezdánjača with osteological and paleopathological determinations.

Sample name	Sex	Age at death	Pathologies	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	C:N	s.d. $\delta^{13}\text{C}$	s.d. $\delta^{15}\text{N}$
BzV 18a	F	20-30 y	<i>Cribra</i> (L+R); osteoma on the occipital bone	-16.2	7.7			3.2		
BzV 18b	n.d.	8±2 y	<i>Cribra</i> (L+R)	-16.3	11.0			3.2		
BzV 21b	n.d.	11±2.5 y	Light porotic hyperostosis on the posterior part of the left parietal and some light porosity on the left side of the occipital	-16.1	9.4			3.2		
BzV 21c	n.d.	9±3 y	<i>Cribra</i> (L+R)	-16.6	12.1			3.2		
BzV 21d	M	30-40 y		-17.5	10.3			3.2		
BzV 27b	M?	30-40 y		-17.7	7.9			3.2		
BzV 30a	F	20-30 y	Diffuse light porotic hyperostosis on the posterior part of both temporals and on the occipital	-16.3	8.2			3.2		
BzV 30c	M	15-18 y	<i>Cribra</i> (L+R); porotic hyper. on both parietals and the occipital	-18.2	8.5	-16.8	9.3	3.2	0.77	1.28
BzV 30d	n.d.	10-12 y		-15.6	11.1			3.2		
BzV 33b	M	20-30 y		-17.9	8.1			3.1		
BzV 33e	n.d.	10±2.5 y		-16.3	9.1			3.1		
BzV 33f-2	M	25-35 y		-16.3	9.8			3.2		
BzV 33h	n.d.	9±3 y	<i>Cribra</i> (L+R)	-17.7	9.3			3.2		
BzV X	M	18-22 y	<i>Cribra</i> (L); Porotic hyperostosis on the frontal bone	-16.6	9.6			3.2		
BzV Y	M	18-25 y	Porosity on the palatine bones	-17.2	8.2			3.1		
BzV ?	n.d.	15±3 y		-17.0	9.0			3.2		



Discussion and Conclusion

The isotopic results from Bezdanjača seem to confirm Filipović's (2020) data by clearly showing that humans from this site consumed good amounts of C₄ resources during their childhood, given that all the individuals show $\delta^{13}\text{C}$ values $>-18\text{‰}$. (Filipović *et al.*, 2020). These enriched $\delta^{13}\text{C}$ values, however, likely suggest an early diet based on the inclusion of C₄ plants such as millet rather than marine foods. This argument is based on two main indicators. Firstly, Bezdanjača Cave is located far from the coast, and no fishbone and or shells have been found either at the site - except from two sea snail shells used as beads for a necklace (Malinar, 1998) - or at the other sites considered for this study, suggesting consumption of marine food by these communities during the Middle and Late Bronze Age. Secondly, at the study site, the $\delta^{15}\text{N}$ values do not seem to indicate an intake of marine proteins in the individuals' diet when compared to the $\delta^{13}\text{C}$ ones (Ambrose *et al.*, 1997). On the other hand, stable isotopic values related to faunal remains, although limited, suggest that the major part that the high $\delta^{13}\text{C}$ values are not a consequence of the livestock did not consume enough C₄ plants so that their presence in the animals' diet could be reflected by

their isotopic values, and it is therefore likely that millet was consumed exclusively by humans (Lightfoot *et al.*, 2014).

Previous research on paleodiet in Middle and Late Bronze Age inland Croatia (Zavodny *et al.*, 2017), shows both similar and different trends in subsistence strategies when compared to the individuals from Bezdanjača (**Figures 5 and 6**). Two individuals from the hillfort settlement of Veliki Vital, dated to the Middle Bronze Age, show similar isotopic values to those from Bezdanjača, with $\delta^{13}\text{C}$ values $\geq -18\text{‰}$, which suggests an intake of C₄ plants in their diets during adulthood. The single adult individual sampled from the Late Bronze/Early Iron Age necropolis of Gospić-Lipe, on the other hand, shows isotopic values that are consistent with a C₃-predominant diet in which a possible consumption of C₄ plants would have been limited after childhood. Although the number of samples from other inland settlements was smaller than from Bezdanjača, comparisons between different sites of inland Croatia during the Middle and Late Bronze Age seem to suggest that the spread of millet, which reached Croatia in the second half of the second millennium BCE, was more consistent in some sites than in others. It must however be noted that both Bezdanjača and Veliki Vital are

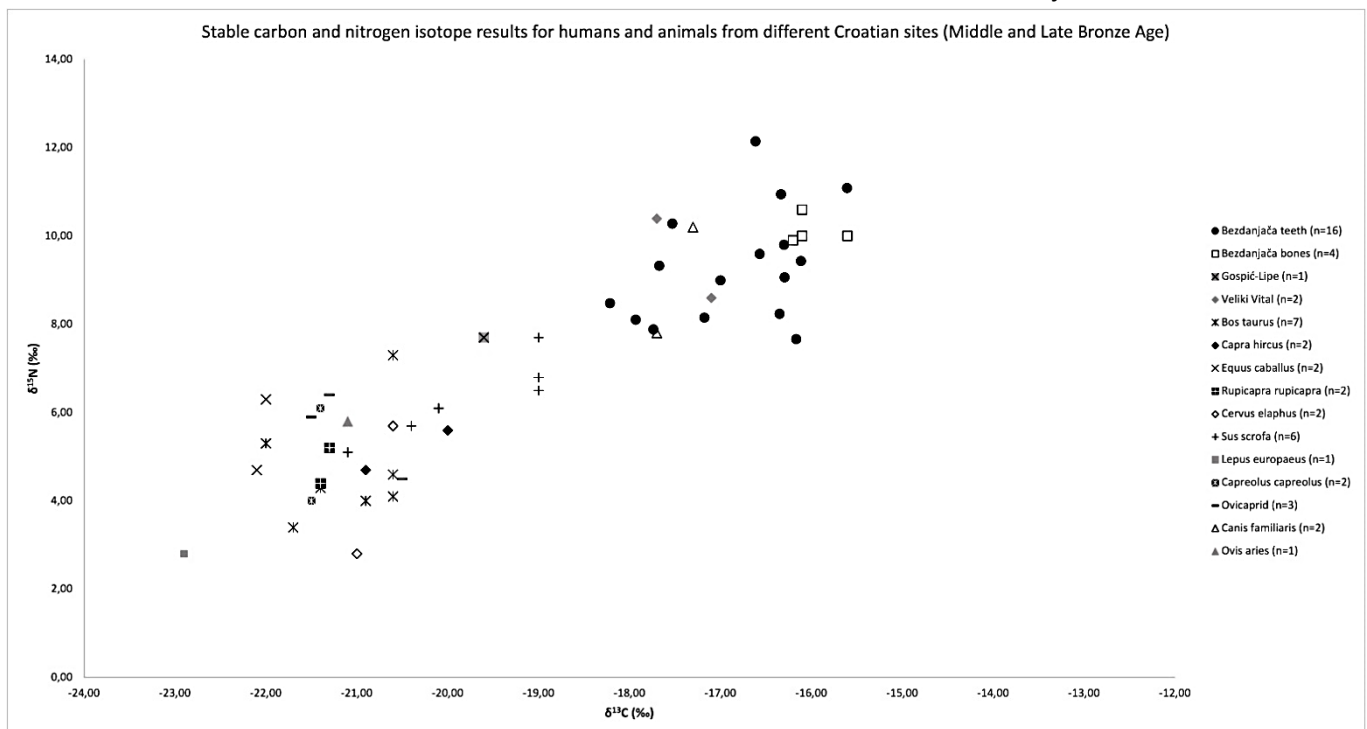


Figure 5 Stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for the individuals from Bezdanjača compared with other inland sites and with the faunal baseline's isotopic values.

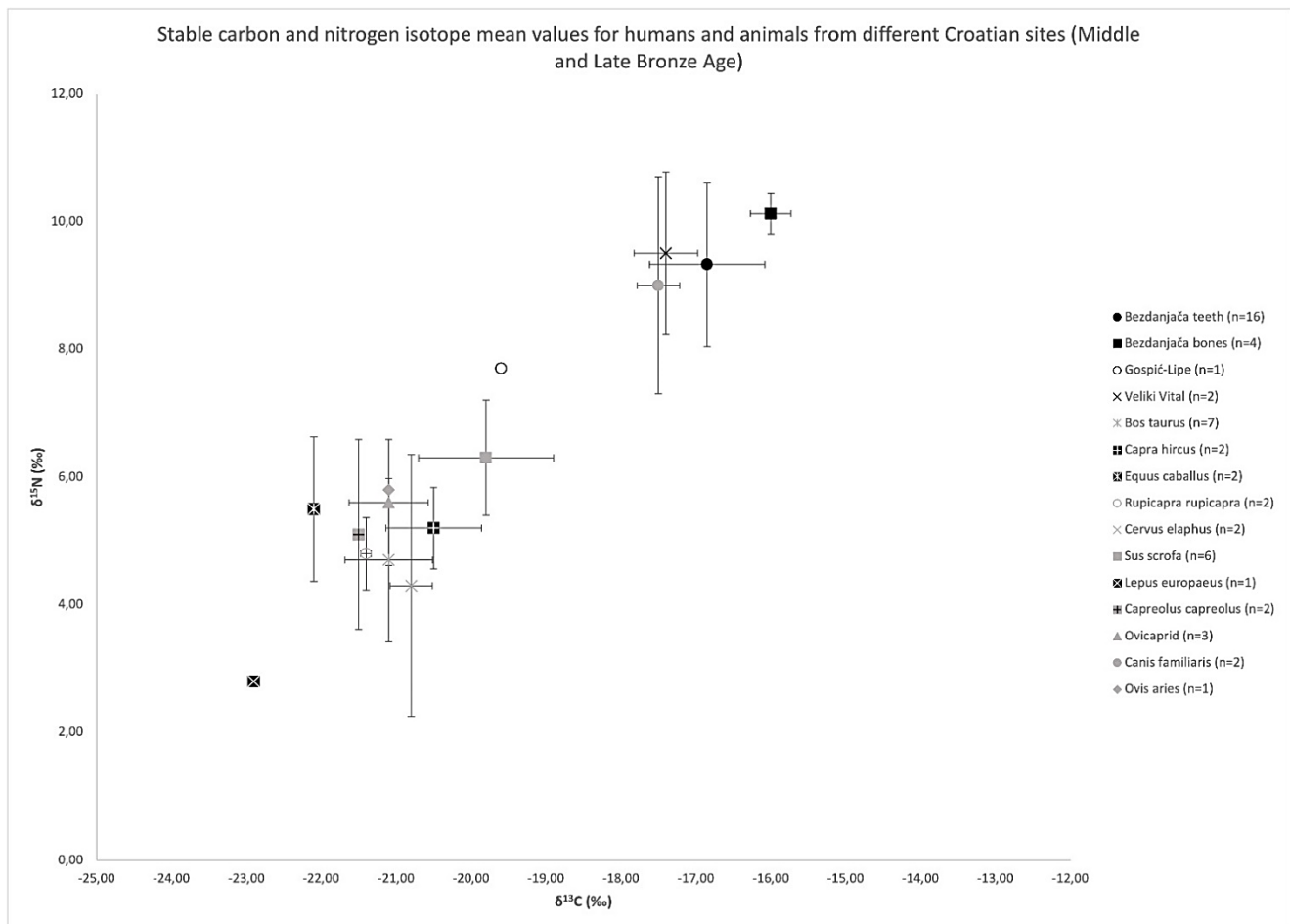


Figure 6 Stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope mean values for the individuals from Bezdanjača compared with the individuals from other inland sites and with the faunal baseline's isotopic mean values.

geographically close to each other, and this may be the reason why the individuals from these sites show similar dietary stable isotope values. Gospić-Lipe, on the other hand, although positioned in inland Croatia, is located south, towards the coast. Isotopic values for the only individual recovered from this site are, in fact, closer to the diet of the individuals from coastal sites of Croatia in the same period, who also show a C_3 plant-based diet, with little or no consumption of millet (Figure 7). The research carried out by Zavodny *et al.* (2017) also indicates that the consumption of millet grew steadily in inland settlements particularly from the Early Iron Age onwards, with a clear switch from a C_3 -based diet to a consistently mixed C_3/C_4 diet in this period.

Lightfoot and colleagues (2014) also draw the same conclusion about coastal Iron Age sites, but neither the isotopic values nor the information recently provided us by Filipović (2020) actually support that hypothesis. As

a matter of fact, the $\delta^{13}\text{C}$ values for individuals from the Iron Age Croatian coast are all $< -18\text{‰}$ and therefore point towards a diet with a seemingly exclusive intake of C_3 plants. Additionally, as stated by Filipović and colleagues (2020), some dietary stable isotope studies of humans from Iron Age Coastal Croatia seem to indicate how some communities from this area did not consume millet, unlike their neighbors. It is therefore possible to assume, in accordance with Filipović's (2020) data, that the spread of millet in Croatia from the Middle Bronze Age onwards most likely followed an east-west axis over land, was first introduced to individuals from northwestern inland Croatia and later to southeastern coastal sites.

Some differences can be seen in $\delta^{15}\text{N}$ values between both inland and coastal sites of Croatia during the Middle and Late Bronze Age, but some authors (Lightfoot *et al.*, 2014) have been reluctant in ascribing

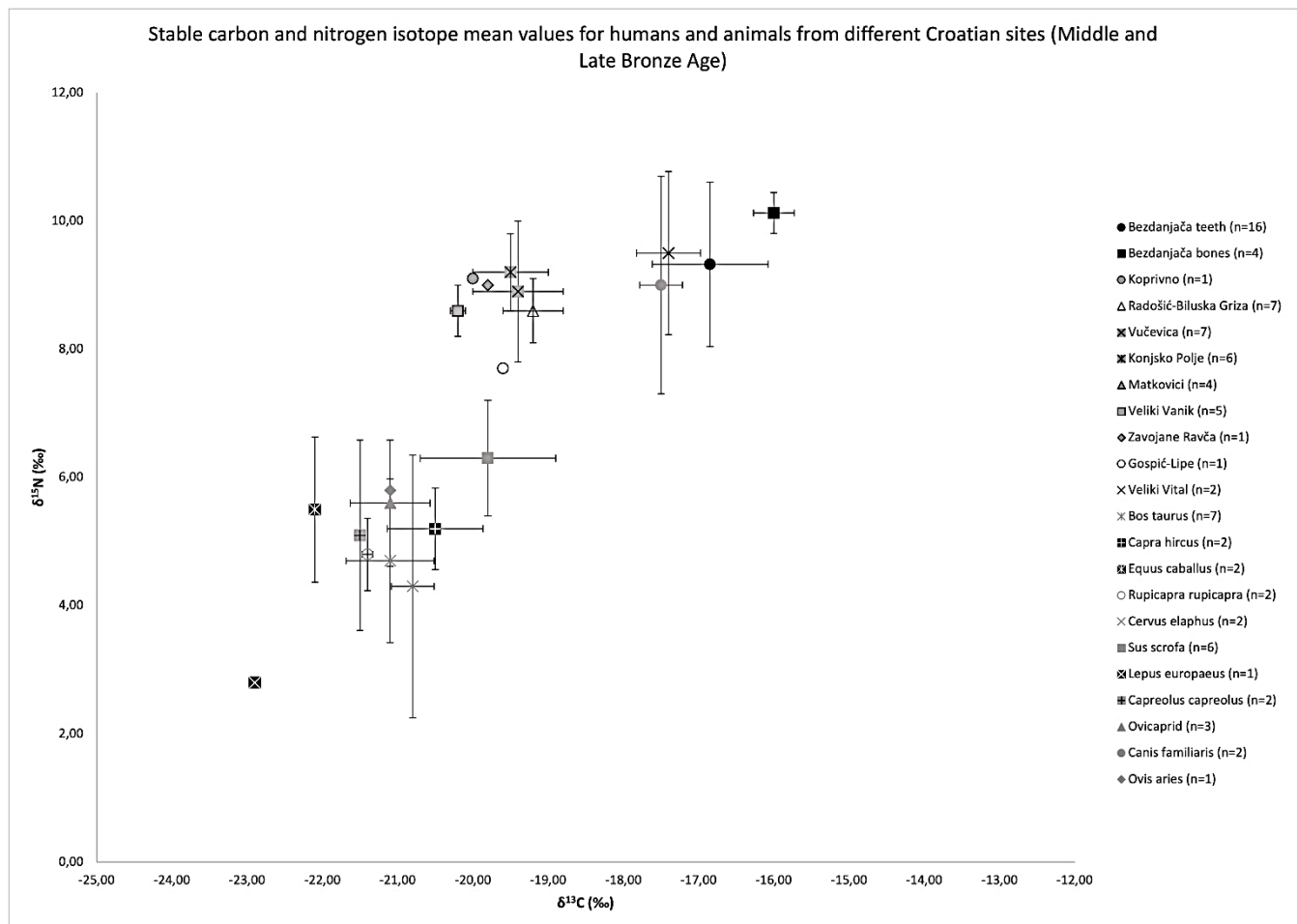


Figure 7 Stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope mean values for the individuals from Bezdanjača compared with other Croatian sites and with the faunal baseline's isotopic mean values

these changes to dietary differences exclusively, considering also possible changes in the environmental baselines. The nitrogen values for the individuals from Bezdanjača is generally slightly higher compared to those of the individuals from other contemporary sites and that can be ascribed to a reverberation of the signal from the weaning period given that we are considering childhood diet. As a matter of fact, it is known that during the breastfeeding period the consumption of maternal milk places an infant on one trophic level above that of its mother in the food chain (Beaumont *et al.*, 2015). This means that if a child was still being breastfed when it died, its isotopic values will show an enrichment in $\delta^{15}\text{N}$ of approximately +2.5‰ and in $\delta^{13}\text{C}$ of ~1‰ (Ventresca Miller *et al.*, 2016; Laffranchi *et al.*, 2018). Infant $\delta^{15}\text{N}$ values decrease during the weaning period through progressive substitution of breast milk with supplementary foodstuffs. When the breastfeeding has fully ceased and the infant is fully

weaned, the reduction to maternal levels is allegedly more rapid for $\delta^{13}\text{C}$ values than for $\delta^{15}\text{N}$ values, which lower more slowly over time (Howcroft *et al.*, 2012). Nonetheless, among the 16 samples from Bezdanjača, sample BzV 21c, belonging to a 9-11-year-old individual, exhibits unusually high $\delta^{15}\text{N}$ values (12.1‰) for Bronze Age communities in Croatia (Figure 8). Such a value places individual BzV 21c even above the normal values for animals that have a strictly carnivore diet and whose $\delta^{15}\text{N}$ values are usually between 8-12‰ (Codron *et al.*, 2007; 2016). Paleopathological observations on the skull of this individual revealed the presence of active *cribra orbitalia* on both the right and the left orbits. Moreover, it must be noted that the dentin sampled from BzV 21c was the first to complete the demineralization process in the hydrochloric acid, which means that the hydroxyapatite of the tooth was likely very frail or almost absent. Considering that: **1**) we sampled BzV 21c's permanent first molar, whose

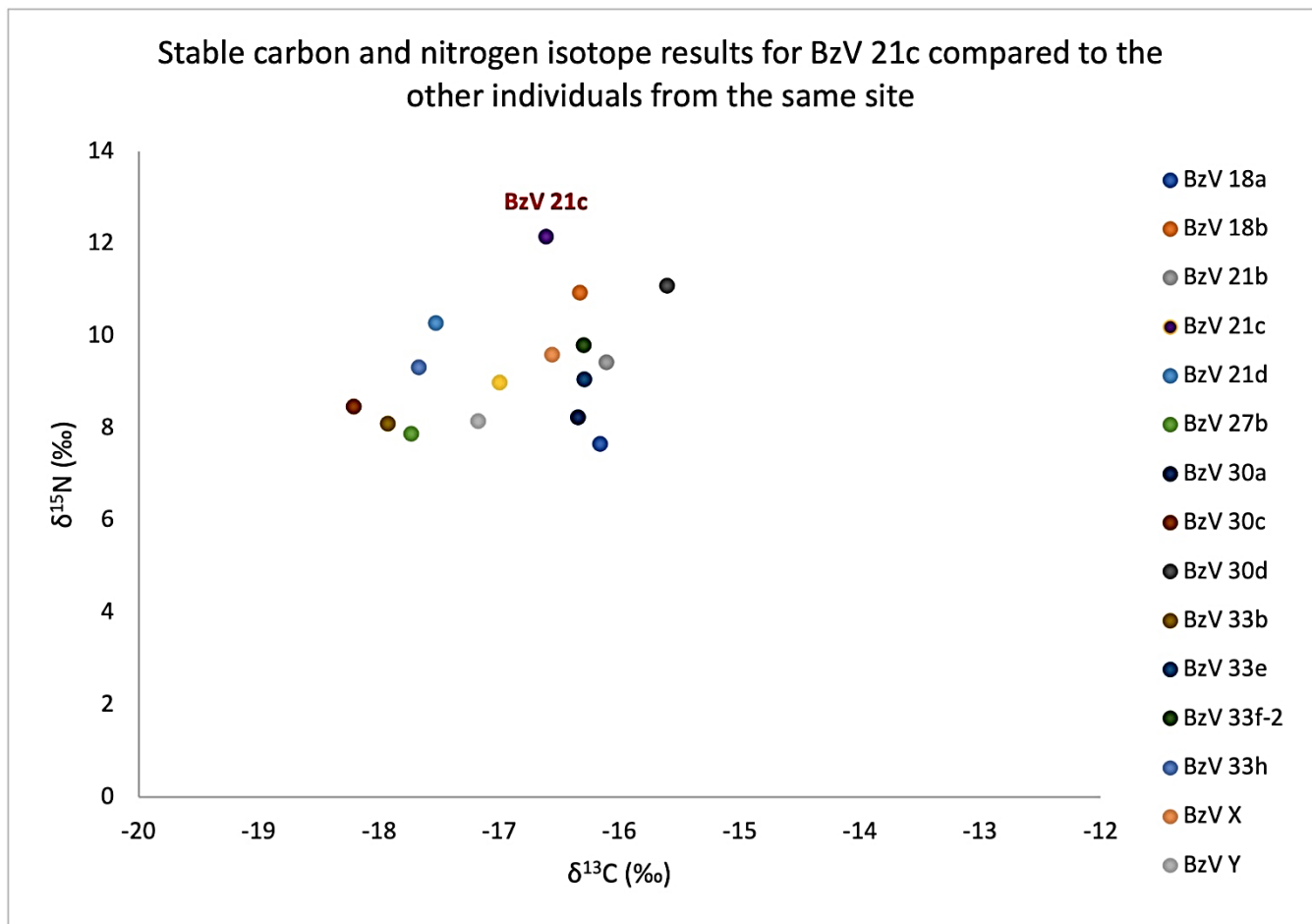


Figure 8 Stable carbon and nitrogen isotope results for BzV 21c compared to the other individuals from the same site

mineralization occurs between birth and 3 years of age; **2)** the age at death of BzV 21c was around 9-11 years; and **3)** BzV 21c's dentin did not undergo a tissue turnover because secondary dentin is usually not observable in individuals <28 years of age (Gustafson, 1950), it can be assumed that the high nitrogen levels exhibited by individual BzV 21c are a consequence of the preservation of the isotopic signal related to its breastfeeding and weaning. However, if we look at the overall trend in the nitrogen values for the individuals from Bezdanjača we can see how they do not differ much - or at all - from those of the individuals from other contemporary sites, most of whom are adults, meaning that in their case, the slightly high nitrogen values cannot be linked to a resonance of nursing signals.

Given the presence of *cribra orbitalia* and the quick demineralization of its dentin it seems more plausible that individual BzV 21c suffered from bad health, was most likely severely malnourished and was probably

affected by other pathologies that, however, were not observable on the skull. Therefore, it is not implausible to think that BzV 21c's high $\delta^{15}\text{N}$ values could be a consequence of a cellular process occurring when lysosomes degrade intracellular components and known as *autophagy* (Cuervo & Macian, 2012). Autophagy is not only indispensable for cell physiology but is also a common conservative and protective process in our body, since it can affect the immune response against viruses, bacteria, and some forms of cancerous cells. Additionally, the breakdown products of autophagy are usually recycled for important cell functions, particularly during periods of stress or starvation (He *et al.*, 2018). From an isotopic point of view, it is likely that the process of "autoconsumption" of the body's own proteins and tissues could cause the nitrogen levels to rise. Cells can adapt to face nutrient deficiencies through various nutritional sensors or regulators (*ibid*). When amino acids (which affect the growth and survival of cells) are deficient in the cell

milieu, "cellular protein synthesis and mitosis will cease immediately. More importantly, with a shortage in amino acids, the autophagic signaling pathway will be activated to release amino acids by degrading proteins in order to maintain the availability of amino acids pool for vital protein synthesis" (He *et al.*, 2018: 498). There are two different types of amino acids that regulate the nitrogen ratio in our bodies. Particularly, non-essential amino acids are those amino acids that are not absorbed by the organism through the consumption of food but are produced by the body itself. These AA usually have a nitrogen isotope ratio which differs greatly from that of dietary proteins. Autophagy, with the subsequent production of new amino acids by the body itself to face starvation or malnutrition and degradation of the enamel, could therefore be an explanation for BzV 21c's unusually high levels of nitrogen.

Conclusion

The earliest archaeobotanical remains of millet were found in China and date back to the Early Neolithic (6000 BCE), whereas the first isotopic evidence for the consumption of millet in Europe was found from sixteenth-century BCE southwestern Ukraine (Filipović *et al.*, 2020). From here, millet spread through Europe following most likely an east-southwest axis over land. In Croatia millet appeared from the fifteenth/fourteenth century BCE. The data presented in this study suggest that the spread of this crop in Croatia during the Bronze Age also followed an east-west trajectory, appearing earlier (Middle and Late Bronze Age) in inland settlements such as Veliki Vital and Bezdanjača and only later (Late Bronze Age and mostly Iron Age) in the sites located along the coast, thus corroborating Filipović and colleagues' (2020) data. Nonetheless, there are some notable dietary differences amongst prehistoric Croatian populations with regards to millet consumption. Data suggest that the individuals from Bezdanjača had a mixed C₃/C₄ diet during their childhood and therefore their intake of millet was higher than it was for the individuals from the nearby contemporary settlement of Veliki Vital whose diet, although still slightly mixed in adult years,

did not make much use of millet. In contrast, isotopic values for the Bronze Age individuals recovered from coastal sites reveal an adult mixed diet with more negative values than those that could be observed for inland sites. This suggests that during the Middle and Late Bronze Age millet either had not voluntarily been adopted as a crop or had not yet reached coastal Croatia. These hypotheses may be confirmed by further isotopic analysis conducted on Iron Age individuals from other coastal sites (Lightfoot *et al.*, 2014) which show a clear shift from a mixed diet with a predominant intake of C₃ plants to a mixed diet with a higher intake of C₄-based foods, but further research is needed to corroborate this.

Although the number of samples gathered and studied from both inland and coastal Croatian settlements is much smaller than the number of individuals from Bezdanjača, and although they take into consideration values referred to adulthood diet, which makes the comparison between these settlements not fully representative and definitive, so far it can be concluded that: **1)** the individuals from Bezdanjača not only started consuming C₄-based foods already from childhood, but they also consumed higher quantities of C₄ plants than other contemporary sites in Croatia; **2)** the isotopic data for the reconstruction of the diet for individuals from both inland and coastal Croatia seem to corroborate Filipović and colleagues' work (2020) regarding the timing and the routes of the spread of millet through Europe via land following an east-west axis; **3)** the millet was welcomed differently in different parts of Croatia. Supposedly, it was first used by inland settlements located east (such as Bezdanjača) beginning from the Middle-Late Bronze Age and only later (LBA and especially EIA) started being consumed by people from the coast (west and southwest).

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