



# Investigating Past Livestock Mobility Using $\delta^{34}\text{S}$ Stable Isotopes: Three Preliminary Case Studies From Prehistoric Croatia

RESEARCH PAPER

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## ABSTRACT

The benefit of including sulfur ( $\delta^{34}\text{S}$ ) stable isotopes in studies of past human diet and migration is increasingly clear, but  $\delta^{34}\text{S}$  analyses remain underutilized in addressing other patterns of mobility, animal management, and environmental change in the archaeological record. Here we evaluate the ability of  $\delta^{34}\text{S}$  isotope values to act as proxies for prehistoric environments in three distinct regions of Croatia: northern Dalmatia, Lika, and central Croatia. We then assess if  $\delta^{34}\text{S}$  isotope values can highlight differences in herding and management practices of livestock in these areas, specifically those that encourage the movement of herds into various parts of the landscape (e.g., transhumance vs. localized grazing). Analysis of faunal stable isotope values from these geographically diverse sites constitute the first step in building an environmental database for Croatia and addressing questions of how  $\delta^{34}\text{S}$  can be applied to questions about animal husbandry in the archaeological record.

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## 1. INTRODUCTION

Stable isotope analyses of bone and teeth have become routine in the study of prehistoric animal management strategies. The successful applications of carbon, nitrogen, and oxygen isotope analyses in particular have expanded our knowledge of past livestock foddering, penning, seasonal herding, and reproductive practices (e.g., Balasse et al. 2017; Berthon et al. 2018; Blaise and Balasse 2011; Bocherens et al. 2001; Gerling et al. 2017; Makarewicz and Tuross 2012; Marciniak et al. 2017; Price et al. 2017). In recent years, sulfur ( $\delta^{34}\text{S}$ ) isotope analyses have become a frequent addition to these types of studies (Nehlich et al. 2010; Sayle et al. 2013, 2016), though the full potential of this methodology is still being explored.

In this paper we evaluate the ability of sulfur isotopes to contribute to archaeological studies of past livestock husbandry practices, specifically transhumance or other patterns of landscape mobility. We present new stable sulfur isotope values alongside previously reported stable carbon and nitrogen measurements from archaeological contexts in three distinct environmental regions of

Croatia: northern Dalmatia, Lika, and central Croatia (Figure 1). Sulfur isotope signatures of domesticated (sheep, goat, cattle, pig) and wild (deer, boar) animal species are first used to establish isotopic baselines for each region. We then examine the suitability of these values as predictors of regional environments and differences in local management strategies. We specifically examine the possible seasonal movement of ovicaprid herds between the coast and mountains in Dalmatia during the Neolithic (~6000–4000 cal BC).

## 2. ARCHAEOLOGICAL APPLICATIONS OF $\delta^{34}\text{S}$

Stable isotopes are naturally occurring variants of elements and are continually cycled between organisms and the environment during an individual's lifespan. Anticipated differences in stable isotope values between species and over time can be attributed to changing patterns of diet, mobility, and residence. By extension, stable isotope studies also offer a systematic approach



**Figure 1** Map of study areas and sites.

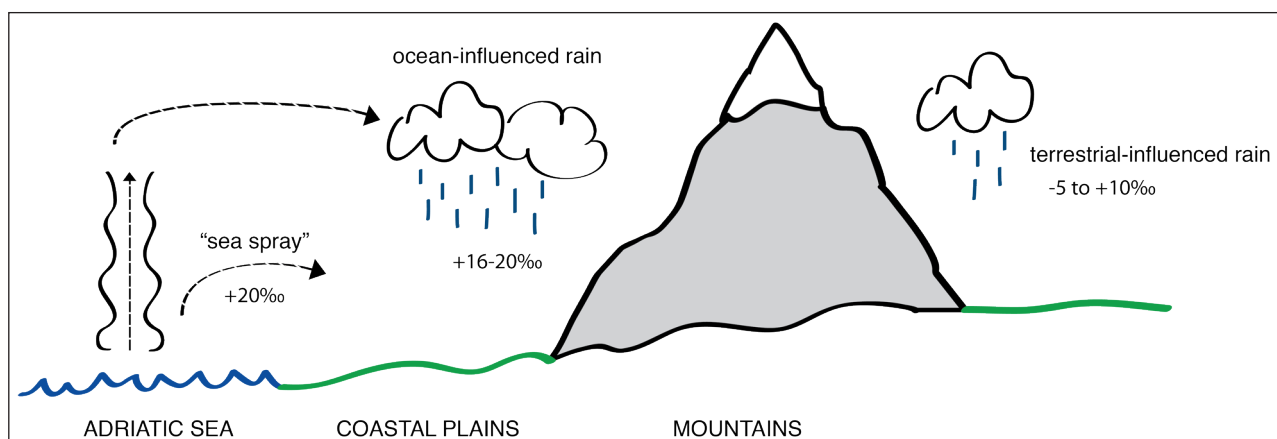
to mapping both temporal and geographic changes in animal management strategies as human communities invested more time and energy into livestock husbandry.

Stable carbon and nitrogen isotope values from bulk collagen are a common proxy for diet because they reflect the average dietary protein consumed during the last eight to ten years of an organism's life (DeNiro and Epstein 1978, 1981; Schoeninger and DeNiro 1984). Carbon stable isotopes are especially useful in determining the plant contributions to diet because of their different photosynthesizing processes;  $C_4$  pathway plants are  $^{13}C$  enriched compared to  $C_3$  pathway plants (DeNiro and Epstein 1978; Pearson et al 2007; van der Merwe 1982; van der Merwe and Vogel 1978). Dietary input can be further inferred according to the isotopic composition of food at the trophic level of foods consumed, with  $^{15}N$  becoming enriched at each subsequent level (Ambrose 1991; Schoeninger and DeNiro 1984). Consequently, the anthropogenic manipulation of animal diet or range can be visible isotopically and differences between species or over time can highlight changing patterns of management (Bocherens et al. 2015; Bogaard et al. 2013; Makarewicz 2014; Makarewicz and Tuross 2012; Szpak 2014).

$\delta^{34}S$  values are similarly informative about past trends in diet and mobility.  $\delta^{34}S$  measures the amount of the essential amino acid methionine in bone collagen. Organisms cannot produce methionine on their own; rather, they acquire it via their diet through the methionine-containing sulphates present in groundwater, rain, and atmospheric sulfurous gases (Doyle and Muir 1979; Walton et al. 1982). Consequently, an individual's  $\delta^{34}S$  signature is heavily influenced by region-specific environmental (Drucker et al. 2011, 2012) and geochemical (Nehlich et al. 2011; Sayle et al. 2013) processes, including the weathering of rock types and minerals, water circulation, and even modern pollution (Bocherens et al. 2011; Case and Krouse 1980; Richards et al. 2001). The small amount of  $\delta^{34}S$  in bone collagen (<1%) and longer replacement rates means that values can reflect over a decade or longer of dietary averages (Nehlich 2015), masking short-term changes in diet

or geographic location. Additionally, the fractionation of sulfur and its trophic shifts within food webs are smaller than those calculated for carbon and nitrogen (Hobson 1999; Krajcarz et al. 2018; Krouse et al 1996; Trust and Fry 1992). Nehlich (2015) has suggested that the difference between archaeological humans and terrestrial fauna is a mere  $0.8 \pm 2.5\text{‰}$ , compared to 1–3‰ for carbon and 3–5‰ for nitrogen (DeNiro and Epstein 1978; Schoeninger and DeNiro 1984). Despite these limitations, however, sulfur isotope analyses have been used frequently – and successfully – in paleodietary studies (e.g., Ebert et al. 2021; Nehlich et al. 2010; Privat et al. 2007; Vika 2009).

Given the strong environmental influence on individual sulfur values, archaeological studies have also now begun to apply the study of sulfur isotope values to issues of human and animal mobility in the past. These studies assume that organisms reflect the sulfur values of their local geology or environment. Any individual deviating from this established regional signature can be considered non-local. For instance, though freshwater and terrestrial environments vary widely in their sulfur signatures, ocean sulphate levels have remained largely uniform – approximately 20.3‰ – over the past one million years (Figure 2; Newton and Bottrell 2007). Marine organisms, or even individuals that eat exclusively marine resources, will have  $\delta^{34}S$  signatures within this range (Leach et al. 2003). This fixed value is useful when comparing the presence or frequency of marine versus freshwater dietary inputs, especially when used in conjunction with stable nitrogen isotope values. Furthermore, oceanic-influenced rainfall on the coast is known to create a “sea-spray effect,” or enrichment of  $\delta^{34}S$  values as far as 30 km inland (Figure 2; Cortecchi et al 2002; Mizota and Sasaki 1996; Wakshal and Nielsen 1982). In most cases a  $\delta^{34}S$  value exceeding 14‰ is a “coastal” signal (Richards et al. 2001). Regional studies have successfully identified and mapped the extent of this sea-spray effect in order to detect the movement of animals from coastal to interior landscapes (Hamilton et al. 2019; Sayle et al. 2013, 2016; Zazzo et al. 2011).



**Figure 2** Stylized depiction of the sea-spray effect and expected environmental values of  $\delta^{34}S$ .

The otherwise highly localized nature of  $\delta^{34}\text{S}$  values, however, requires archaeologists to create site-specific baseline values to contextualize their results. This need is compounded by the fact that pollution contamination makes comparisons between archaeological and modern material impossible. To this end, we present here the first sulfur isotope values from any archaeological context in Croatia. Though our sample size is small, initial results allow us to broadly evaluate the possibilities and limitations of  $\delta^{34}\text{S}$  as both an environmental indicator and a tool for reconstructing livestock mobility in the past.

### 3. ENVIRONMENT AND GEOLOGY OF CROATIA

The modern country of Croatia can be divided into multiple distinct environmental and geomorphological regions, of which three are discussed here: Dalmatia, Lika, and central Croatia (Figure 1).

Dalmatia encompasses the coastal plains and valleys between the town of Zadar in the north and Dubrovnik in the south. Its proximity to the Adriatic gives it a typically Mediterranean climate and environment, and the region as a whole is separated from the rest of the continent by the Dinaric Alps to the north and east. Low hills divide much of the region into long narrow valleys or *polja*. This undulating landscape is largely underlain by limestone and dolomite. Valley bottoms are the most fertile areas where the majority of agriculture is carried out today. Soil studies of Danilo polje in particular noted a well-developed karstic terrain covered with fine-grained soils high in carbon and calcite (Fadem et al. 2009). This study focuses on sites from northern Dalmatia (Figure 1).

To Dalmatia's north is Lika, a mountainous region characterized by an Alpine-like environment and climate. The Velebit mountains to the west and south and Kapela and Plješevica ranges to the north and east block any moderating climatic effects from the Adriatic or inner continent. Similar to Dalmatia, Lika's interior is also divided into a series of narrow karst *polja*. Bedrock is predominantly Mesozoic limestone and dolomite (Bašić 2013). Annual precipitation levels are high and valleys flood seasonally, but the limestone ensures that water drains quickly (Miko et al. 2000). The karst is also highly susceptible to erosion, and soils are subsequently thin, compacted, and nutrient-poor (Bašić 2013; Forenbaher 2011).

The flat and expansive plains of central Croatia form the southern edge of the Pannonian Basin. Unlike the predominantly karst landscape of Lika and Dalmatia, central Croatia's bedrock and soils are more heterogeneous and altered by different pedogenic processes. Sediments mostly date to the Quaternary and are hydromorphic, meaning the soil profile is continually saturated from either underground water or occasional regional flooding (Bačani et al. 1999; Halamić et al. 2012).

### 4. MATERIALS

$\delta^{34}\text{S}$  analyses were conducted on remaining ultrafiltered collagen from previous stable carbon and nitrogen isotope analyses and/or AMS radiocarbon dating of archaeological collections curated at the Archaeological Museum in Zagreb, the Museum of Lika in Gospić, and the Šibenik City Museum (Zavodny et al. 2014; Zavodny et al. 2019b; Zavodny 2020). Faunal remains had previously been identified to species at the Penn State Zooarchaeology Laboratory using comparative materials and published criteria for species differentiation, domestication status, and aging (Rowley-Conwy et al. 2012; Zeder 2006; Zeder and Lapham 2010; Zeder and Pilaar 2010). In addition to the 28 faunal remains sampled, we also analyzed human remains from Dalmatia ( $n = 1$ ) and Lika ( $n = 6$ ) to allow for trophic comparisons within each region. Though our ability to build a robust study assemblage equally representative of all regions and taxa was limited by sample availability, we argue that reporting these initial results – the first archaeological  $\delta^{34}\text{S}$  values in Croatia – is an important first step for establishing more comprehensive studies in the future.

#### DALMATIA

Seven cattle (*Bos taurus*), seven sheep (*Ovis aries*), one pig (*Sus domesticus*), one roe deer (*Capreolus capreolus*), and one red deer (*Cervus elaphus*) were selected from the assemblages of five open-air Neolithic settlements spanning most of the Neolithic period (6000–4700 cal BC): Čista Mala-Velištak, Danilo, Konjevrate, Krivače, and Pokrovnik (Figure 1). One human from Velištak was also sampled. Research suggests the inhabitants of these sites and other early farming societies practiced a mixed agro-pastoral subsistence strategy from dispersed village settlements along the coast (McClure et al. 2014). Seasonal movement of livestock into the nearby Dinaric Alps may have also begun during the Neolithic as a niche-expanding strategy (McClure 2013), but archaeological and isotopic evidence for this time period has so far been inconclusive (Zavodny et al. 2014, 2015).

#### LIKA

One goat (*Capra hircus*), two pigs (*Sus domesticus*), one boar (*Sus scrofa*), one roe deer (*Capreolus capreolus*), one red deer (*Cervus elaphus*), and one chamois (*Rupicapra rupicapra*) were chosen from hillforts dating to the Middle-Late Bronze (1400–800 cal BC; Veliki Vital) and Iron (800–100 cal BC; Lipova Glavica, Trošmarija) Ages (Figure 1). Six humans were sampled from two cemeteries: the Middle-Late Bronze Age cave site of Bezdanjača and the Iron Age tumulus of Sultanov grob. Groups in Lika during this period settled in or around centrally located hillforts and practiced mixed economic strategies aimed at minimizing environmental uncertainty, including cultivating millet as a fall-back crop and raising indigenous cattle breeds

specialized to withstand the rugged karstic landscape (Zavodny et al. 2017, 2019b). Seasonal movement of herds between valleys and mountains is attested to in the historical record (Forenbaheer 2011) but unconfirmed in earlier periods.

## CENTRAL CROATIA

One ovicaprid (*Ovis/Capra*) and three red deer (*Cervus elaphus*) were sampled from the Iron Age (~600–35 BC) settlement at the site of Sisak (Drnić 2020; Drnić and Groh 2018). Located roughly 40 miles southeast of Zagreb, Sisak is situated at the confluence of the Kupa, Sava, and Odra Rivers. Its prime position allowed both prehistoric and later Roman communities to participate in trade between the Adriatic and Danube regions (Šašel Kos 2005). Though there is no evidence of seasonal livestock movements at Sisak, it is possible that animals were imported from other regions as part of larger trade networks. The settlement was also likely supported by a strong agricultural economy (Drnić 2020).

## 5. METHODS

Faunal and human skeletal samples were analyzed using standard procedures for collagen extraction at the Penn State University Human Palaeoecology and Isotope Geochemistry Laboratory (Kennett et al. 2017; Zavodny et al. 2019a). Approximately 500 mg of dry bone were taken from each archaeological sample, with compact bone preferentially sampled to maximize collagen yield. Samples were crushed to increase the area of reactive surface, then washed in NanoPure water and demineralized in 0.5 N HCl at 5°C for several days. Samples were then prepared for collagen extraction and purification by the modified Longin (1971) method with ultrafiltration (Brown et al. 1988). Bone collagen samples that were too poorly preserved for ultrafiltration were processed using a modified XAD-purification method (Lohse et al. 2014; Stafford et al. 1988, 1991). Extracted gelatin was hydrolyzed in 1.5 mL 6N HCl for 24 hours at 110°C before being driven through a SPE column and 0.45µm Millex Durapore PVDF filter by syringe with an additional 10ml 6N HCl and dried under UHP N<sub>2</sub> gas while being heated at 50°C for 12 hours.

Carbon, nitrogen, and sulfur concentrations and stable isotope ratios were analyzed at the Yale Analytical and Stable Isotope Center.  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values are reported in standard ‰ notation with respect to VPDB, atmospheric nitrogen, and VCDT, respectively. Sample quality was evaluated with %C, %N and C:N ratios before further analysis. C:N ratios fall between 3.1 and 3.3, reflecting good preservation for isotopic analyses (see Table 1; DeNiro 1985; van Klinken 1999). Sulfur samples were further assessed using established quality controls (%S, C:S, N:S) for mammalian bone collagen (Nehlich

and Richards 2009; Nehlich 2015). Samples KRI-03, PK-01, PK-04, and PK-37 have %S values slightly above the recommended 0.35% S threshold but were included in this study because all other preservation standards were met.

Two-tailed t-tests assuming unequal variances (Ruxton 2006) were conducted to determine statistically significant ( $p < 0.05$ ) differences between groups under the assumption that the larger source populations for these groups were normally distributed.

## 6. RESULTS

Stable carbon and nitrogen isotope values are presented in Table 1 and Figures 3–5. Sulfur isotope values are also reported in Table 1 and displayed in Figures 4 and 5.

### DALMATIA

Dalmatian faunal samples have  $\delta^{13}\text{C}$  values between  $-21.6$  and  $-17.4$ ‰ and  $\delta^{15}\text{N}$  values between 4.0 and 6.3‰. These values are in range of those expected for herbivores living in a terrestrial C<sub>3</sub> environment (DeNiro and Epstein 1978, 1981; Richards and Trinkaus 2009) and are similar to other reported values for the region (Guiry et al. 2017). One sheep from Danilo (DA-13) is observably enriched in  $\delta^{13}\text{C}$  in comparison to its peers, with a  $\delta^{13}\text{C}$  value of  $-17.4$ ‰. There is no evidence for domesticated C<sub>4</sub> plants (e.g., millet) in Dalmatia at this time (Reed 2015), though it is possible that animals opportunistically foraged on infrequent wild C<sub>4</sub> plants. Other stable carbon and nitrogen isotope results have been discussed in-depth elsewhere, and there are no notable differences between species (Zavodny et al. 2014, 2015).

Faunal  $\delta^{34}\text{S}$  values range widely between 8.2 and 16.6‰. Sheep (*Ovis aries*)  $\delta^{34}\text{S}$  values are highly variable, ranging between 8.2 and 13.4‰ with an average  $\delta^{34}\text{S}$  of 10.6‰. The range of  $\delta^{34}\text{S}$  values for cattle (*Bos taurus*) is smaller, between 8.2 and 10.1‰ with an average of 9.3‰. The singular pig (*Sus sp.*) has a  $\delta^{34}\text{S}$  value of 8.5‰. The red deer (*Cervus elaphus*, KRI-27) and roe deer (*Capreolus capreolus*, PK-80) have the most elevated sulfur values at 15.4‰ and 16.6‰, respectively.

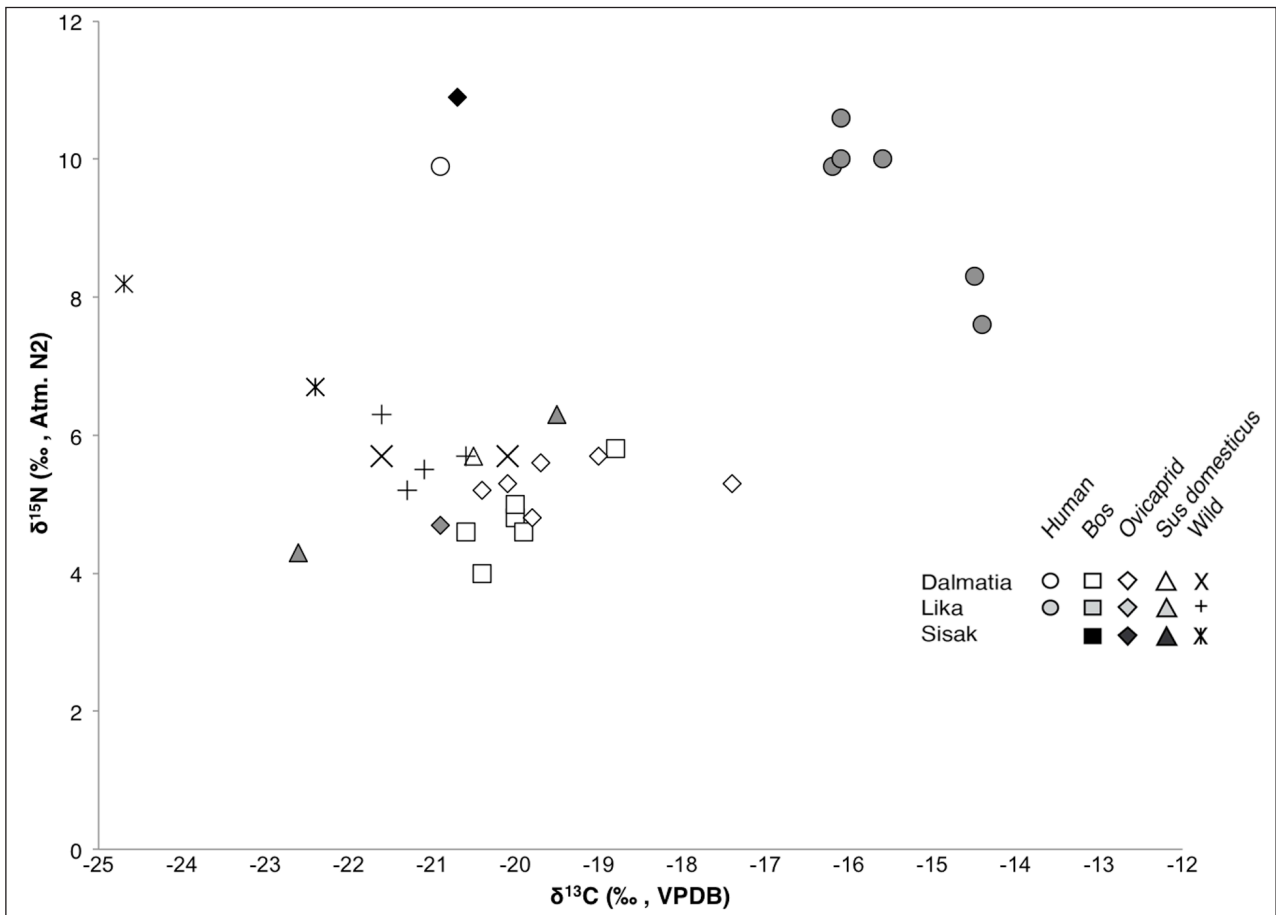
The Velištak human (CMV-8) has a  $\delta^{13}\text{C}$  value of  $-20.9$ ‰,  $\delta^{15}\text{N}$  value of 9.9‰, and  $\delta^{34}\text{S}$  value of 8.9‰. The  $\delta^{34}\text{S}$  value overlaps with the lower range of animal  $\delta^{34}\text{S}$  values. There is no evidence of C<sub>4</sub> plant consumption, though exploitation of marine and/or freshwater resources is possible. The individual's stable carbon and nitrogen values are also similar to those reported for humans from other Neolithic contexts (Guiry et al. 2017).

### LIKA

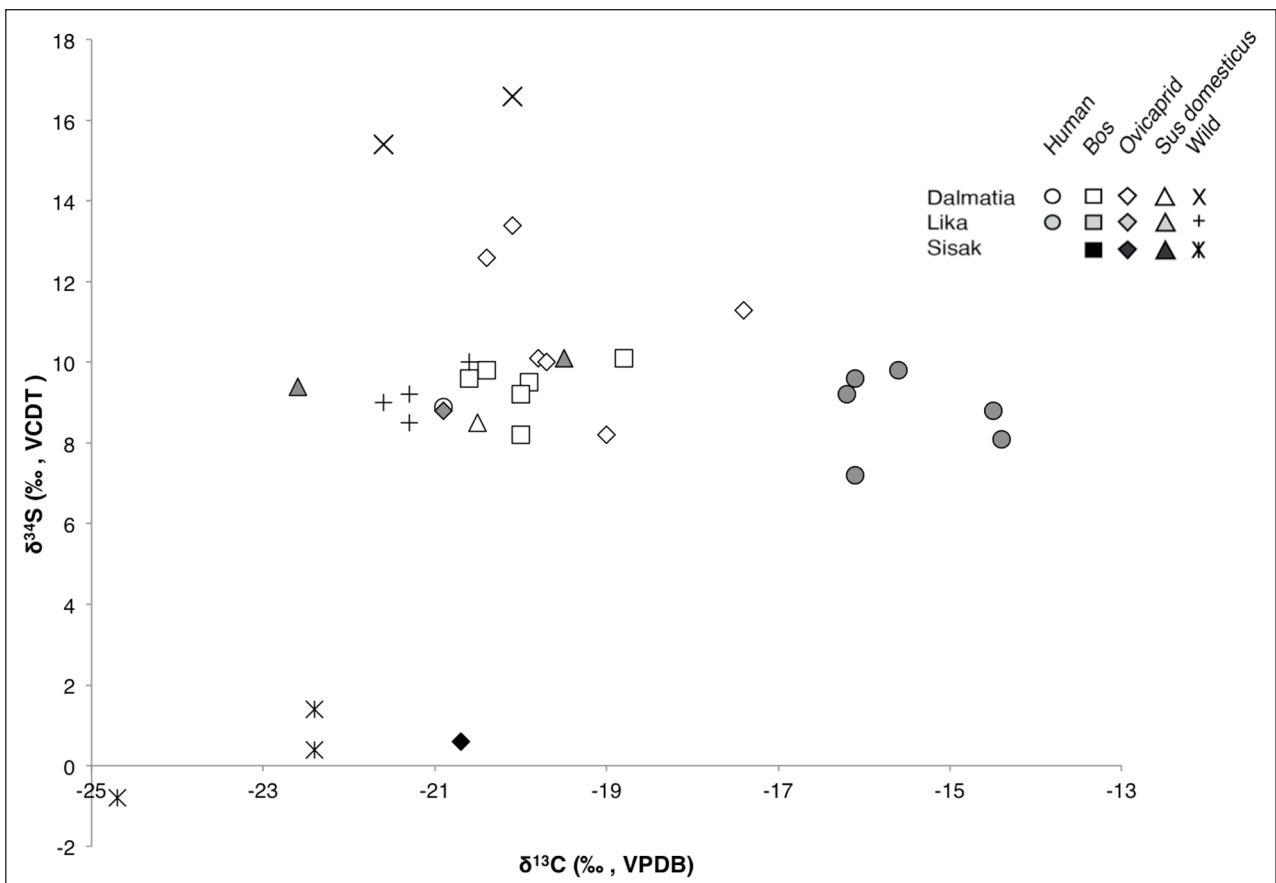
Lika faunal samples exhibit  $\delta^{13}\text{C}$  values between  $-22.6$  and  $-19.5$ ‰ and  $\delta^{15}\text{N}$  values between 4.5 and 6.3‰. These results have been discussed in-depth elsewhere (Zavodny et al. 2017, 2019b) and are typical of a terrestrial

SITE	PERIOD	LAB #	SPECIES	$\delta^{34}\text{S}$	%S	$\delta^{13}\text{C}$	%C	$\delta^{15}\text{N}$	%N	C:S	N:S	C:N
<b>Dalmatia</b>												
Čista Mala-Velišćak	Neolithic	CMV-03	<i>Bos taurus</i>	10.1	0.2	-18.8	47.1	5.8	17.5	702	224	3.1
		CMV-07	<i>Bos taurus</i>	8.2	0.3	-20.0	45.7	4.8	16.9	388	123	3.2
		CMV-12	<i>Homo sapiens</i>	8.9	0.3	-20.9	43.5	9.9	16.0	411	121	3.2
Danilo-Bitinj	Neolithic	DA-06	<i>Ovis aries</i>	8.2	0.2	-19.0	43.3	5.7	15.7	683	212	3.2
		DA-13	<i>Ovis aries</i>	11.3	0.2	-17.4	42.9	5.3	15.6	760	237	3.2
Konjevrate	Neolithic	KON-02	<i>Ovis aries</i>	10.1	0.2	-19.8	40.9	4.8	14.7	552	170	3.2
Krivače	Neolithic	KRI-02	<i>Sus domesticus</i>	8.5	0.2	-20.5	41.5	5.7	14.6	511	104	3.3
		KRI-03	<i>Bos taurus</i>	9.8	0.36	-20.4	47.7	4.0	17.8	380	121	3.1
		KRI-10	<i>Bos taurus</i>	9.5	0.35	-19.9	45.3	4.6	16.7	371	117	3.2
		KRI-18B	<i>Bos taurus</i>	9.2	0.3	-20.0	46.5	5.0	17.2	425	135	3.2
		KRI-19	<i>Bos taurus</i>	9.6	0.3	-20.6	47.5	4.6	17.0	402	123	3.3
		KRI-27	<i>Cervus elaphus</i>	15.4	0.2	-21.6	44.4	5.7	15.4	630	175	3.4
Pokrovnik	Neolithic	PK-04	<i>Ovis aries</i>	8.6	0.38	-19.8	45.8	5.7	16.9	341	108	3.2
		PK-15	<i>Ovis aries</i>	13.4	0.2	-20.1	42.4	5.3	15.6	667	210	3.2
		PK-19	<i>Ovis aries</i>	10.0	0.2	-19.7	42.6	5.6	15.7	710	224	3.2
		PK-21	<i>Ovis aries</i>	12.6	0.2	-20.4	43.5	5.2	16.0	725	229	3.2
		PK-37	<i>Bos taurus</i>	8.8	0.38	-20.0	43.5	5.3	16.0	328	103	3.2
		PK-80	<i>Capreolus capreolus</i>	16.6	0.2	-20.1	45.1	5.7	16.1	639	182	3.3
<b>Lika</b>												
Bezdanjača	Bronze	BZ-01	<i>Homo sapiens</i>	9.2	0.29	-16.2	53.3	9.9	18.7	521	157	3.3
		BZ-02	<i>Homo sapiens</i>	7.2	0.34	-16.1	45.5	10.6	16.2	379	116	3.3
		BZ-03	<i>Homo sapiens</i>	9.8	0.20	-15.6	45.5	10	16.2	643	197	3.3
		BZ-04	<i>Homo sapiens</i>	9.6	0.23	-16.1	45.6	10	16.4	562	173	3.3
Lipova Glavica	Iron	LG-03	<i>Sus domesticus</i>	10.1	0.3	-19.5	39.8	6.3	14.0	331	100	3.3
Sultanov grob	Iron	SG-02	<i>Homo sapiens</i>	8.8	0.23	-14.5	44.9	8.3	16.0	536	169	3.3
		SG-03	<i>Homo sapiens</i>	8.1	0.24	-14.4	45.4	7.6	16.1	521	163	3.3
Trošmarija	Iron	TR-13	<i>Sus scrofa</i> (wild)	8.5	0.3	-21.1	41.9	5.5	15.0	379	116	3.3
		TR-15	<i>Capreolus capreolus</i>	9.0	0.3	-21.6	43.7	6.3	15.6	382	117	3.3
		TR-17	<i>Sus domesticus</i>	9.4	0.3	-22.6	44.6	4.3	15.9	410	125	3.3
Veliki Vital	Bronze	VVA-01	<i>Capra hircus</i>	8.8	0.2	-20.9	43.7	4.7	15.7	565	174	3.3
		VVA-05	<i>Rupicapra rupicapra</i>	9.2	0.2	-21.3	44.5	5.2	15.7	720	218	3.3
		VVA-07	<i>Cervus elaphus</i>	10.0	0.2	-20.6	42.5	5.7	15.1	776	236	3.3
<b>Central Croatia</b>												
Sisak	Iron	SS-03	<i>Cervus elaphus</i>	-0.8	0.3	-24.7	47.5	8.2	16.7	391	118	3.3
		SS-09	<i>Cervus elaphus</i>	1.4	0.3	-22.4	41.1	6.7	14.6	385	117	3.3
		SS-10	<i>Cervus elaphus</i>	0.4	0.3	-22.4	48.1	6.7	17.1	412	126	3.3
		SS-15	ovicaprid	0.6	0.3	-20.7	45.3	10.9	16.1	436	133	3.3

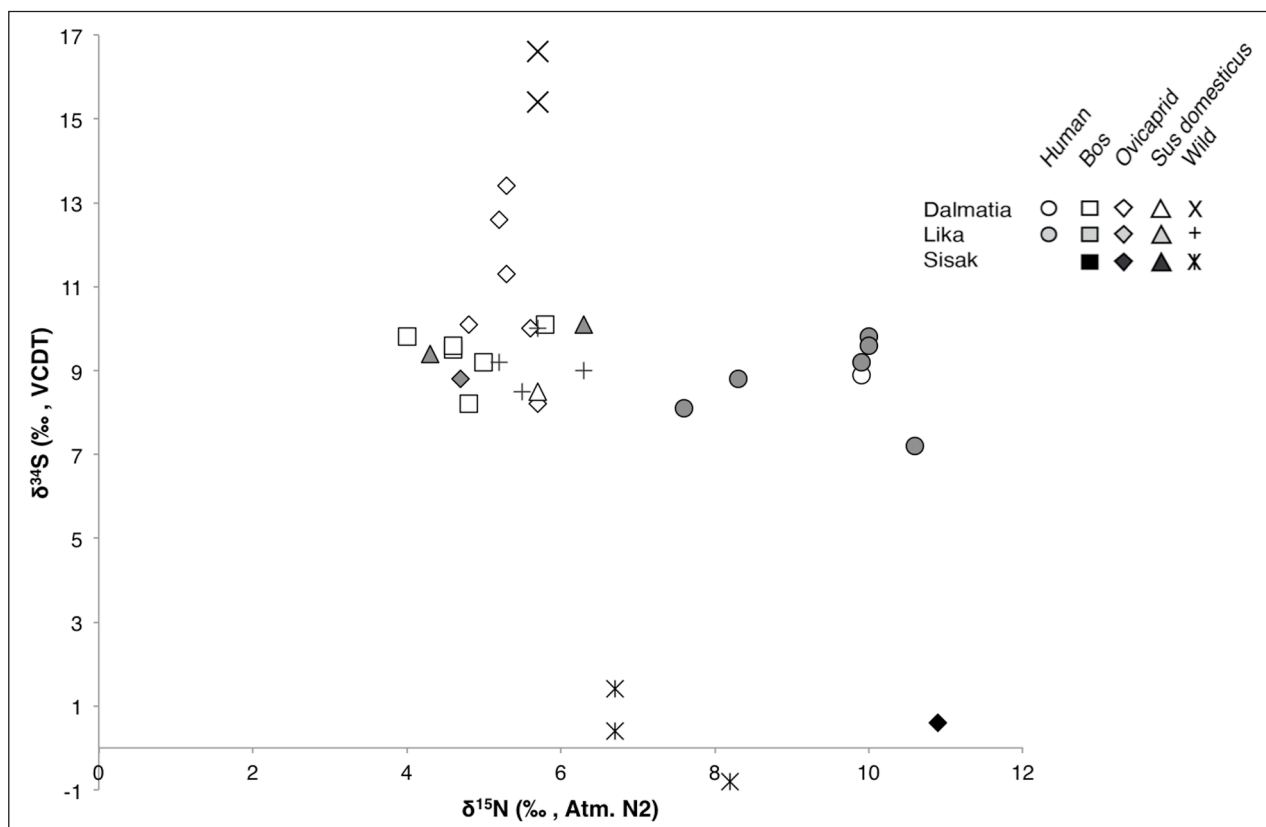
**Table 1** Stable isotope results for animals and humans sampled in this study.



**Figure 3** Stable carbon and nitrogen isotope values for samples in this study. Both red deer from Sisak (SS-09 and SS-10) have the same carbon and nitrogen values and appear in the graph as one data point.



**Figure 4** Stable carbon and sulfur isotope values for samples in this study.



**Figure 5** Stable nitrogen and sulfur isotope values for samples in this study.

$C_3$  environment. There are no significant inter-species differences in stable carbon or nitrogen isotope values. As a group, wild animals from Lika (red deer, roe deer, and boar) appear to have slightly lower  $\delta^{13}C$  values than their domesticated counterparts, which could be the result of frequenting more heavily forested areas, i.e. the “canopy effect” (Drucker et al. 2010; Zavodny et al. 2019b).

Humans have  $\delta^{13}C$  values between  $-16.2$  and  $-14.4$ ‰ and  $\delta^{15}N$  values between  $7.6$  and  $10.6$ ‰. At first glance, there is a wide spread in nitrogen values. However, the two adult individuals from Sultanov grob have lower  $\delta^{15}N$  values ( $7.6$  and  $8.3$ ‰) while the higher  $\delta^{15}N$  values are from Bezdanjača ( $9.9$  to  $10.6$ ‰). At least two of the individuals from Bezdanjača are identifiable as juveniles (BZ-01 and BZ-04) and may have eaten diets higher in meat or dairy than contemporary adults. Both populations ate a significant amount of  $C_4$  plants, likely millet. Comparison between human and faunal  $\delta^{13}C$  values suggest, however, that  $C_4$  plants were not used to systematically fodder livestock (Zavodny et al. 2017, 2019b).

Faunal  $\delta^{34}S$  values are narrowly distributed between  $8.5$  and  $10.1$ ‰. Despite the small sample size, it appears that there are no discernable differences in sulfur isotope values according to species or domestication status. Domesticated animals exhibit  $\delta^{34}S$  values between  $8.8$  and  $10.1$ ‰ with an average of  $9.4$ ‰. The range of  $\delta^{34}S$  values for wild animals is  $8.5$  to  $10.0$ ‰ with an average of  $9.2$ ‰. Human  $\delta^{34}S$  values overlap this range with values from  $7.2$  to  $9.6$ ‰. There is very little evidence for large amounts of sulfur in human diet indicative of freshwater

or marine resources, suggesting a mostly terrestrial diet. There is, however, a distinction to be made between the very similar values of the Sultanov grob burials ( $8.8$  and  $8.1$ ‰) and the wide range in values exhibited by the individuals at Bezdanjača ( $7.2$  to  $9.6$ ‰). More research is needed, however, to determine whether this pattern is representative of actual inter-population differences or the effect of a small sample size.

### CENTRAL CROATIA

Sisak faunal samples have  $\delta^{13}C$  values between  $-24.7$  and  $-20.7$ ‰ and  $\delta^{15}N$  values from  $6.7$  to  $10.9$ ‰, consistent with a terrestrial  $C_3$  environment and temperate climate. The three red deer (*Cervus elaphus*) from Sisak have much lower  $\delta^{13}C$  values than the ovicaprid. This separation in carbon values is likely the result of different habitats, specifically deep forest versus open fields (Drucker et al. 2010). Reported  $\delta^{34}S$  values fall between  $-0.8$  and  $1.4$ ‰. Only one domesticate, an ovicaprid, had enough collagen to sample for sulfur, but there is no observable difference between this individual and the deer from Sisak.

## 7. IMPLICATIONS FOR RECONSTRUCTING LIVESTOCK MOBILITY IN THE PAST

### ESTABLISHING REGIONAL $\delta^{34}S$ BASELINES

At the outset of our study we hypothesized that each region would exhibit clearly different  $\delta^{34}S$  values because



of wide variations in geology, environment, and climate. Values from Sisak are indeed significantly different than those from the karst landscapes of Dalmatia ( $p = 9.1E^{-08}$ ) and Lika ( $p = 1.2E^{-06}$ ; t-tests assuming unequal variances). Sisak's  $\delta^{34}\text{S}$  values, ranging from  $-0.8$  to  $1.4\text{‰}$ , are instead more comparable to those reported from other sites with similar environments and geology in the continental interior of Europe (Oelze et al. 2011; Richards et al. 2008). Low  $\delta^{34}\text{S}$  values have also been reported from other riverine floodplains where  $^{34}\text{S}$ -depleted freshwater sulphates are periodically deposited on the surrounding soils (Nehlich et al. 2011). The low  $\delta^{34}\text{S}$  values from Sisak may be an effect of occasional floods from the nearby Kupa and Sava Rivers, and the elevated  $\delta^{15}\text{N}$  values of the sampled individuals further suggest that both domesticated and wild animals grazed close to these rivers (Oelze et al. 2011).

We expected fauna from Dalmatia to exhibit the highest  $\delta^{34}\text{S}$  values within our study because of the enrichment of soils from the “sea-spray effect.” The two deer from Krivače and Pokrovnik do exhibit the highest sulfur signatures of any sample ( $15.4\text{‰}$  and  $16.6\text{‰}$ ). Though the sample size is small, both values are above the generally accepted “coastal” threshold of  $14\text{‰}$  (Richards et al. 2001) and suggest these individuals ranged close to the shore. The domesticated animals and human from Dalmatia, however, are not nearly as elevated and instead range in  $\delta^{34}\text{S}$  value from  $8.2$  to  $13.4\text{‰}$ . Rather than displaying a coastal signal, these individuals more closely resemble their landlocked contemporaries to the north in Lika (Figures 4, 5). In fact, despite differing proximities to the sea and marine-influenced rain, there is no clear  $\delta^{34}\text{S}$  threshold between values reported from Dalmatia and Lika. The overlapping ranges of  $\delta^{34}\text{S}$  values could be the effect of the limestone and dolomite bedrock present in both regions. It is also possible that the sea spray effect in Dalmatia was weak or diluted by rain (Heaton 1987), causing  $\delta^{34}\text{S}$  values in both regions to appear comparable despite different geological and climatic processes. Though the modern Adriatic coastline has been altered since the Neolithic, we assumed that villages would still have been close enough to the coast to result in coastal  $\delta^{34}\text{S}$  values. However, it is also possible that we underestimated the effect this geographic difference might have had on  $\delta^{34}\text{S}$  distribution across the landscape. For instance, the higher  $\delta^{34}\text{S}$  values of the two deer from Dalmatia could signal a “coastal” zone independent of the Neolithic settlements sampled in this study.

Regional distinctions in stable isotope values are clearer only when we include stable carbon and nitrogen isotope results in our analyses. For instance, carbon values are generally lower in Lika ( $p = 0.02$ ; t-tests assuming unequal variances), possibly because of a more densely forested landscape (Zavodny et al. 2019b). Regional averages of wild fauna  $\delta^{15}\text{N}$  isotope values are even more regionally discrete:  $3.6\text{‰} \pm 0.7$  in Dalmatia,  $5.6\text{‰} \pm 0.4$  in Lika, and  $7.2\text{‰} \pm 0.9$  at Sisak. Again, though these  $\delta^{15}\text{N}$

averages may be biased by small sample sizes, especially at Sisak, the trend is worth investigating further as a way of complementing sulfur baselines.

### **$\delta^{34}\text{S}$ AS A TOOL FOR IDENTIFYING SEASONAL MOBILITY OF HERDS IN DALMATIA**

Archaeological and historic evidence suggest that the seasonal transhumance of goats and sheep between villages on the coastal plains and pastures in the Dinaric Alps was a key feature of life in Dalmatia for centuries (Moore et al. 2007, 2019). Though previous stable carbon and nitrogen isotope studies to identify this practice in the Neolithic were inconclusive (Zavodny et al. 2014, 2015), more recent incremental sampling of ovicaprid tooth enamel does suggest vertical movement of livestock during part of the year at certain sites (McClure et al. 2018). We predicted sulfur isotope values could help identify this practice by varying according to species and level of movement across different landscapes. In this scenario, we anticipated that ovicaprids moved between pastures throughout the year should have lower  $\delta^{34}\text{S}$  values than the pigs and cattle kept near coastal settlements year round.

The majority of Neolithic sheep, cattle, and pig sampled, however, have  $\delta^{34}\text{S}$  signatures that fall within the narrow range of  $8.2$  to  $10.1\text{‰}$  (Table 1). The single human from Velištak also has a similar  $\delta^{34}\text{S}$  value of  $8.9\text{‰}$ . This low sulfur signature is consistent with a terrestrial diet, and corroborated by paleobotanical and zooarchaeological evidence from Neolithic settlements (Legge and Moore 2011; Moore et al. 2019; Podrug et al. *in press*; Podrug et al. 2019; Reed 2015). The low sulfur values of livestock also suggest that crops and animals were managed largely in areas unaffected by sea-spray, as discussed above. We suggest that the foddering of cattle and pigs was fairly uniform and their movement tightly controlled throughout the Neolithic. The overlap of cattle and pig  $\delta^{34}\text{S}$  values with the single human from Velištak further supports the idea that these animals were kept in close proximity to humans.

Sheep –the likeliest candidate for seasonal transhumance– possess both the widest range ( $8.2$ – $13.4\text{‰}$ ) and highest average ( $10.6\text{‰}$ ) of  $\delta^{34}\text{S}$  values (Figures 4, 5). Three sheep in particular exhibit high  $\delta^{34}\text{S}$  values of  $11.3$ ,  $12.6$ , and  $13.4\text{‰}$ , though these values do not quite exceed the so-called “coastal” threshold (Richards et al. 2001). Privat et al. (2007), identifying a similar pattern at an Eneolithic site in Ukraine, argued that  $^{34}\text{S}$ -enriched ovicaprid values could be evidence of imported livestock or multiple management systems. While we initially assumed herds would have wintered near the settlements in this study, the higher  $\delta^{34}\text{S}$  values of some sheep from Danilo and Pokrovnik could indicate they were held in other settlements or fields closer to the coast instead. Indeed, the  $\delta^{34}\text{S}$  values of two deer from Krivače and Pokrovnik hint at a potential “coastal” zone beyond the five sites represented in this study.

In a study of modern sheep, Zazzo et al. (2011) found that animals utilizing the same environment exhibited inter-individual variation of  $\delta^{34}\text{S}$  between 0.1–3.4‰. There is a 3.1‰ difference between the two Danilo sheep and a 4.8‰ spread among the four Pokrovnik individuals. This pattern strongly suggests that these individuals were not kept in the same area year-round. The wide range of  $\delta^{34}\text{S}$  values could reflect individual age or, more specifically, the number of times an individual sheep cycled between the coast and inland prior to death. For instance, lambs born in late winter or spring may not have survived long enough to set out for the mountain pastures in the summer. Hypothetically, then, they should have higher  $\delta^{34}\text{S}$  values than individuals who completed at least one seasonal cycle. Grazers feeding exclusively at higher arid elevations,

such as in the Dinaric Alps, should also have higher  $\delta^{15}\text{N}$  in relation to lowland grazers (Ambrose 1991). Presuming all individuals were weaned by the time of their death, those with elevated  $\delta^{15}\text{N}$  and lower  $\delta^{34}\text{S}$  values likely spent a significant amount of their lives in upland pastures while others with lower  $\delta^{15}\text{N}$  and elevated  $\delta^{34}\text{S}$  values would have spent the majority of theirs near the coast.

Approximate age at death for all seven sheep sampled from Dalmatia was estimated based on the stage of element fusion (Table 2; Zeder 2006): five individuals were at least six months old, one individual was likely between six and twelve months old, and another was older than eighteen months. A clear gradient can be seen among those individuals at least six months or older when sulfur and nitrogen values are plotted (Figure 6).

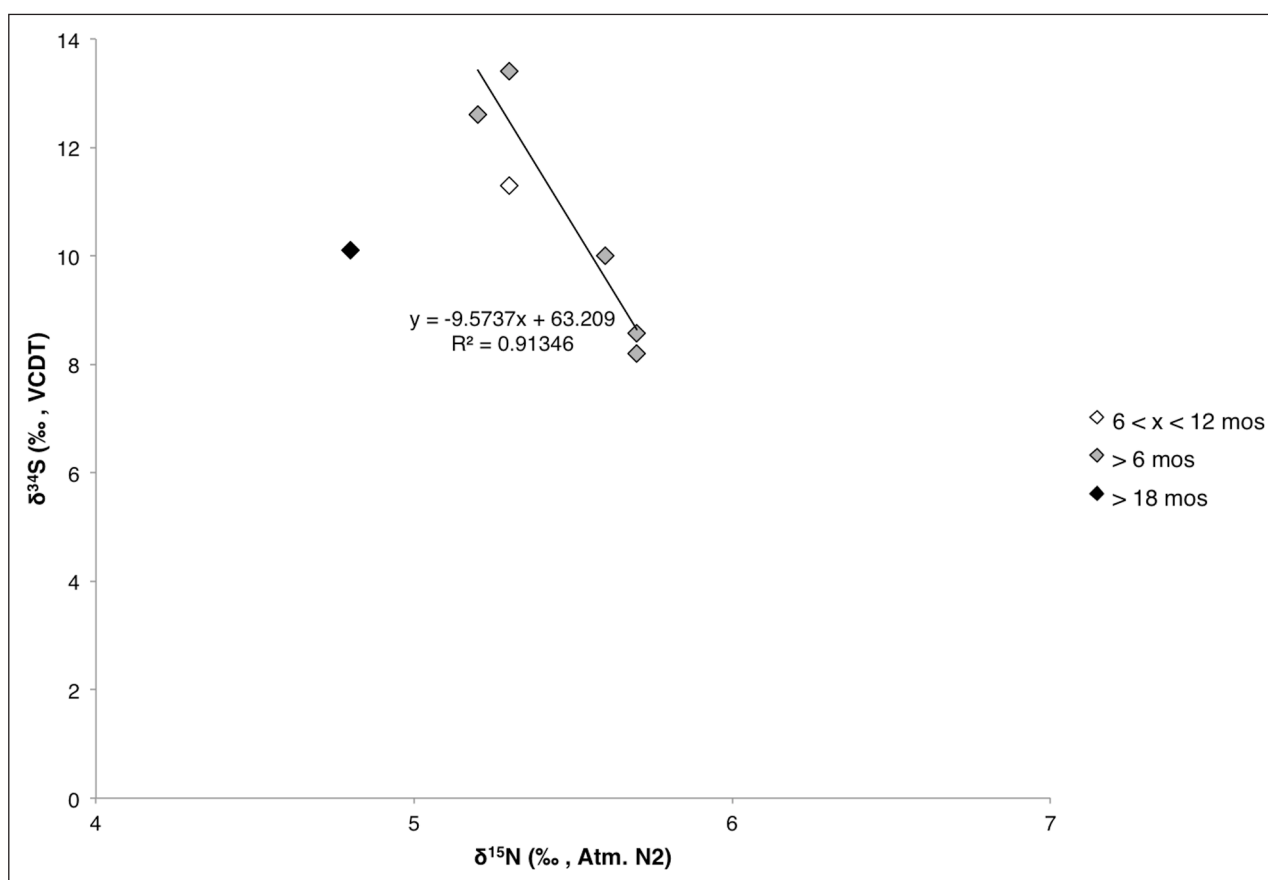


Figure 6 Stable carbon and nitrogen isotope values of sheep by estimated age at death.

SITE	PERIOD	LAB #	ELEMENT	AGE (MOS.)	$\delta^{34}\text{S}$
Danilo-Bitinj	Middle Neolithic	DA-06	humerus	>6	8.2
		DA-13	humerus	6–12	11.3
Konjevrate	Early Neolithic	KON-02	tibia	>18	10.1
Pokrovnik	Early Neolithic	PK-04	humerus	>6	8.6
		PK-15	humerus	>6	13.4
		PK-19	humerus	>6	10.0
		PK-21	humerus	>6	12.6

Table 2 Estimated age at death of Neolithic sheep in this study.

The sample from Konjevrate (KON-02), an individual 18 months or older, has a  $\delta^{34}\text{S}$  value that falls squarely within the middle of this gradient, similar to what we would expect for an individual that survived multiple transhumance cycles between coast and mountain. A linear regression trendline fitted to the other five individuals suggests a strong negative relationship between  $\delta^{34}\text{S}$  and  $\delta^{15}\text{N}$  (Figure 6). This pattern could indicate these individuals died before completing multiple cycles, or even possibly before completing just one full seasonal cycle. Furthermore, cattle do not exhibit this same  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  relationship, lending more support to the argument that sheep were more mobile than other livestock during the Neolithic. However, while these results are suggestive, the individual sheep in this study could not be assigned an exact age-at-death and may be much older. More research is needed to determine whether this age-dependent gradient truly exists within the population.

## 8. CONCLUSIONS

Our results demonstrate sulfur stable isotopes are a promising – though sometimes coarse-grained- tool for untangling animal management strategies and other questions of movement between environments in prehistory. In our study,  $\delta^{34}\text{S}$  worked best in interregional comparisons and would be most useful for tracking the movement of animals or people along trade or exchange routes between the continental interior and Adriatic coast. However, our results also show that  $\delta^{34}\text{S}$  values do not always reflect the precise nature of Croatia's environmental and geomorphological heterogeneity. There are problems in establishing differences between regions with similar bedrock and/or underlying soils, such as between Dalmatia and Lika. With our current understanding of regional  $\delta^{34}\text{S}$  isotopic baselines, identifying the presence of any movement of animals or people between Lika and Dalmatia using  $\delta^{34}\text{S}$  values alone would be difficult if not impossible. Future research should sample more aggressively within each region to determine whether these results reflect reality or are the bias of small sample sizes. Additionally, our study highlights how complementary  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  datasets can elevate  $\delta^{34}\text{S}$  studies and bring regional isotopic differences into better focus.

In our case study from Neolithic Dalmatia, we expected  $\delta^{34}\text{S}$  values to reflect differences in local management strategies (e.g., transhumance vs. local grazing). According to our results, communities primarily farmed and managed livestock in inland valleys despite the short distance to the coast. The possible correlation between  $\delta^{34}\text{S}$  values and age among sheep hints at possible seasonal movement on the landscape, but our sample size is small and should be expanded in order

to answer this question conclusively. In general, our study has shown that  $\delta^{34}\text{S}$  has great potential as a tool for archaeologists, but that there is still much work to be done in refining the methodology to address more nuanced questions about mobility, animal management, and environmental change in prehistory.

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## COMPETING INTERESTS

The authors have no competing interests to declare.

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EZ conceptualized the study and drafted the manuscript. All authors contributed data, conferred on methodology and results, and helped to edit and revise the manuscript.

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