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Abhandlung

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A multi-isotopic pilot study of the burial mound of Boyanovo

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Zusammenfassung: Im Rahmen einer Pilotstudie wurden fünf menschliche Skelette aus dem Grabhügel *Lozianska Mogila* nahe des heutigen Dorfes Bojanovo, Bulgarien, für Multi-Isotopenanalysen ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$) ausgewählt. Die Bestattungen datieren in die Früh- und Mittelbronzezeit. Einige der frühbronzezeitlichen Individuen zeigten Übereinstimmungen mit einem Bestattungsritual, welches für die Jamnaja- oder Grubengrabkultur charakteristisch ist. Die Ergebnisse der Strontium- und Sauerstoffisotopenanalysen am Zahnschmelz der untersuchten Skelette verweisen auf einen erhöhten Grad an Mobilität, der entweder als Indikator für Migrationen oder als Hinweis auf kleinräumige Bewegungen zwischen verschiedenen regionalen Siedlungen verstanden werden kann. Sie sind jedoch kein Beleg für Migrationen aus den osteuropäischen Steppenregionen, wie auf Basis des archäologischen Materials möglicherweise vermutet. Die Ergebnisse der Kohlenstoff- und Stickstoffisotopenanalysen sind relativ konsistent. Sie sind als charakteristisch für eine Ernährung anzusehen, die vornehmlich auf C_3-Nahrungsressourcen basiert mit einem geringen Einfluss an C_4-Nahrungskomponenten.

Schlüsselworte: Grabhügel; Bulgarien; Frühe und Mittlere Bronzezeit; Strontiumisotopenanalyse; Sauerstoffisotopenanalyse; Kohlenstoff- und Stickstoffisotopenanalyse

Résumé: Cinq squelettes humains provenant du tumulus funéraire de *Lozianska Mogila* près de Boyanovo en Bulgarie ont été sélectionnés pour une étude-pilote d'isotopes multiples ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$). Les sépultures datent de l'âge du Bronze Ancien et Moyen; les sépultures du Bronze Ancien ont certaines affinités avec les rites funéraires pratiqués par des communautés associées à la culture Yamna ou cultures des tombes à fosses. Les résultats des analyses isotopiques du strontium et de l'oxygène effectuées sur l'émail des dents indiquent une mobilité accrue, qui pourrait être due soit à une migration réelle, soit à de simples mouvements entre plusieurs habitats de la région. Ils ne sont pas suffisamment probants pour étayer une hypothèse de migration venant des steppes de l'Europe de l'Est, comme le suggèrent (en partie) les données archéologiques. Les résultats des analyses isotopiques du carbone et du nitrogène sont relativement cohérents et suggèrent une alimentation basée sur les plantes en C_3 et la viande, avec un apport mineur de plantes en C_4.

Mots-clefs: tumulus; Bulgarie; âge du Bronze Ancien; âge du Bronze Moyen; analyses isotopiques du strontium; analyses isotopiques de l'oxygène; analyses isotopiques du carbone et du nitrogène

Abstract: Five Bronze Age human skeletons from the burial mound of *Lozianska Mogila* near Boyanovo, Bulgaria, were selected for a multi-isotopic ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$) pilot study. The burials date to the Early and Middle Bronze Age, and the Early Bronze Age burials partly show affinities with the burial rite practised by communities associated with the Yamnaya or Pit Grave culture. The results of strontium and oxygen isotope analyses on tooth enamel indicate an increased level of mobility, suggesting either actual migration events or simply movement between various regional settlement sites. They do not provide sufficiently convincing evidence for migrations from the steppes of Eastern Europe as (partly) suggested by the archaeological record. The results of carbon and nitrogen isotope analysis are relatively consistent and indicate a diet based on C_3 plants and meat sources with a minor input of C*4* resources.

Keywords: burial mound; Bulgaria; Early Bronze Age; Middle Bronze Age; strontium isotope analysis; oxygen isotope analysis; carbon and nitrogen isotope analysis

Article note: This article is directly associated with the study by Daniela Agre, also published in this volume. Both contributions are the result of Research Group A2 "Spatial effects of technological innovations and changing ways of life" of the Excellence Cluster Topoi (2007–2012). Daniela Agre generously provided osteological material from her excavation for stable isotope analysis. She also published the find contexts and finds from the barrow *Lozianska Mogila*, which forms the foundation for the interpretation of the results obtained from stable isotope analysis presented in this article..

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Introduction

In order to better understand the structure of the burial mound *Lozianska Mogila* near Boyanovo we started an integrated scientific approach using strontium, oxygen, nitrogen and carbon isotope analyses. On the one hand, we tried to link mobility patterns and the buried skeletons, while on the other we tried to find connections between subsistence patterns and society.

The stable isotope study included four skeletons dated to the Early Bronze Age and one skeleton to the Middle Bronze Age from the Boyanovo burial mound as part of a wider ranging analysis of Bulgarian Eneolithic and Bronze Age 'Yamnaya-style' burials. These burials are characterized by skeletons, mainly buried in supine position with flexed legs and slightly flexed arms with a West-East orientation, in due correspondence to features also known from the contemporaneous graves in the North Pontic and adjacent regions[1]. The skeletons were placed in graves that are frequently covered by wood. The graves are mainly sparsely furnished and can contain burial objects like pieces of red ochre, or as ochre colouring on body parts, organic materials such as coloured blankets, ceramic vessels and spiral-shaped rings made of silver or other metals[2]. According to V. R. Dergachev, these are also features of the 'Lower Danube' or 'Dniester' variant of the Yamnaya culture[3].

An Eneolithic and Early Bronze Age steppe impact has been suggested by numerous researchers, and interpretations range from intrusive horse-riding steppe people into the Carpathian-Balkan region[4], or migrations of stockbreeders[5] to single infiltrations[6]. Major concentrations of burial mounds with Yamnaya characteristics outside the original distribution area are known from Romanian Moldova, the Dobrudzha, the lower Danube, the Carpathian Basin, and the Upper Thracian Plain in modern Bulgaria[7]. Early Bronze Age burial mounds in Bulgaria often show a mixture of Yamnaya and local cultural elements suggesting their temporary coexistence. According to I. Iliev[8], elements of the Yamnaya culture might have spread to Bulgaria already in the 4th Millennium BC but mainly after 2900 BC.

Previous studies using stable isotope analyses are generally rare for this region. Strontium and oxygen isotope analyses are carried out to investigate mobility patterns and residential changes. Previous $^{87}Sr/^{86}Sr$ studies on prehistoric humans from the same region are lacking to date[9]. A comparison in respect to $\delta^{18}O$ is provided by the study of Keenleyside *et al.*[10], who obtained data from oxygen isotope analysis on 60 human individuals from Kalfata-Budjaka, a necropolis associated with the ancient Greek colony of *Apollonia Pontica* on the Bulgarian Black Sea coast and dated to the 1st Millennium BC.

More intense work within a wider regional frame was done considering carbon and nitrogen isotope analysis, both providing evidence of dietary patterns. Several $\delta^{13}C$ and $\delta^{15}N$ studies concern the Mesolithic-Neolithic transition period in the Iron Gate region[11]. A moderate basis of comparison is given by the study of Honch *et al.*[12], in which the two cemeteries Varna I and Durankulak, both situated near the Black Sea coast and dated to the 5th Millennium BC, were investigated using a stable isotope-based approach.

Background

Strontium and to a lesser extent also oxygen isotope analysis is more and more commonly applied in archaeological studies to investigate the provenience and mobility patterns of past humans and animals.

Strontium isotope ratios vary geographically according to geology. The $^{87}Sr/^{86}Sr$ signature differs based on the rock's age and rubidium content and tends to be higher in regions of old rocks and lower in younger geological formations. In decay, the distinct $^{87}Sr/^{86}Sr$ signature of the underlying geology at a given location is transferred to soil and water and subsequently to plants[13]. In the course of the food chain it is incorporated into body hard tissues such as tooth and bone of animals and humans due to the similar properties of strontium and calcium[14]. This happens with negligible metabolic fractionation[15]; thus, the $^{87}Sr/^{86}Sr$ in humans and animals is distinct to the geological region where they spent a part of their lives

1 Alexandrov 2011; Heyd 2011; see also Agre in this volume.
2 Cf. Nikolova 1999, 369–389; Anthony 2007, 362–366.
3 Yamnaya culture or Pit Grave culture. Dergačev 1986; Alexandrov 2011, 315.
4 E.g., Gimbutas 1956; 1979; 1994.
5 E.g., Childe 1929, 132; Anthony 1986; 1990; 2007.
6 E.g., Häusler 1976; 1998.
7 Heyd 2011, 530–531; Nikolova 1999, 372.
8 Iliev 2009, 241–242.
9 by July 2014.
10 Keenleyside *et al.* 2011.
11 E.g., Bonsall *et al.* 2000; Borić *et al.* 2004; Nehlich *et al.* 2010; see also Borić/Price 2013 on results obtained using $^{87}Sr/^{86}Sr$ analysis.
12 Honch *et al.* 2006.
13 Graustein 1989.
14 Ericson 1985.
15 Bentley 2006.

and, thus, enables a distinction of movements of humans and animals between geologically differing regions. However, additional factors like rainwater and wind that transfer soil need to be taken into account[16]. Since tooth enamel forms and matures within the first years of life and does not undergo remodelling thereafter, its $^{87}Sr/^{86}Sr$ signature represents the geological background of this lifespan[17]. Comparison to the place, where an individual died, is enabled by the analysis of $^{87}Sr/^{86}Sr$ in local plants, water and soil, archaeological or recent faunal remains, and diagenetically altered tooth dentine and bone[18].

Oxygen isotope ratios are affected by geographic parameters such as the distance to the evaporation source, mainly the ocean, amount of precipitation, temperature, latitude and altitude, and vary systematically across the globe[19]. Since oxygen isotopes can be used as a proxy for temperature, they are widely applied to environmental and climate studies. They also have the potential of being applied to human and faunal skeletal remains in order to investigate their provenience and mobility patters[20]. This is due to the oxygen isotope composition in skeletal tissue, which is mainly derived from the ratio of ^{18}O to ^{16}O ($\delta^{18}O$) in drinking water, and to a minor degree to that in the atmosphere and in food[21]. From their content in drinking water to the carbonate and phosphate fractions in tooth enamel and bone, oxygen isotopes are subject to metabolic fractionation, which requires the application of drinking water conversion equations. Using the conversion equations proposed by Longinelli[22], Luz *et al.*[23], Levinson *et al.*[24], Daux *et al.*[25] and Pollard *et al.*[26], $\delta^{18}O$ in phosphate ($\delta^{18}O_p$) can be converted to $\delta^{18}O$ in drinking water ($\delta^{18}O_{dw}$). Another recent equation was published by Chenery *et al.*[27], which enables the direct conversion of $\delta^{18}O$ in carbonate ($\delta^{18}O_c$) to $\delta^{18}O_{dw}$. A comparison to published data in further archaeological studies and to those obtained from the GNIP stations[28] helps to decide whether a human being lived locally restricted or whether he/she obtained water from non-local sources. Uncertainties when transforming the data remain, however, and for larger data sets it is suggested to determine outliers on the basis of the data itself rather than further parameters[29].

Carbon and nitrogen stable isotope analyses enable the reconstruction of palaeodiets, as they provide information about an individual's main sources of dietary protein intake[30]. The analysis of carbon isotope ratios ($\delta^{13}C$) allows the distinction of terrestrial C_3 versus C_4 plant dominated food and freshwater and marine protein input to the diet. $\delta^{13}C$ varies due to differences in the photosynthetic carbon reduction pathways, which allows a distinction of C_3, C_4 and CAM plants[31]. Consumers of C_3 plants exhibit significantly more depleted $\delta^{13}C$ values than do those of C_4 plants. Marine food sources also result in less depleted $\delta^{13}C$ values in their consumers, whereas freshwater food tends to produce more negative but varying numbers[32].

Nitrogen isotope ($\delta^{15}N$) analysis enables the reconstruction of an individual's position in the food web[33]. While herbivores feature lower $\delta^{15}N$ values (typically 4–6 ‰) than carnivores (usually 10–12 ‰), omnivores are somewhere in between[34]. However, $\delta^{15}N$ varies due to parameters like climate, precipitation and temperature[35]. $\delta^{15}N$ analysis also enables the identification of aquatic, non-marine food sources[36]. Nitrogen isotope ratios in bone collagen mainly reflect the isotopic composition of the dietary protein intake of the last years to last decades of an individual's lifetime[37]. The reconstruction of food webs at a specific site also requires the analysis of a range of local archaeological fauna due to a large variation in both $\delta^{13}C$ and $\delta^{15}N$.

The study site of Boyanovo is located in Southeast Bulgaria in the valley of the river Tundzha and lies at the boundary of an area predominated by Upper Palaeozoic-Mesozoic (Permian-Middle Jurassic) rocks and Pliocene sediments[38]. Parts of the Palaeozoic and Mesozoic rocks are metamorphic plutonites. For this region the TRACE $^{87}Sr/^{86}Sr$ map features $^{87}Sr/^{86}Sr$ values for water and soil between 0.702 and 0.711[39].

16 Chenery *et al.* 2011.
17 Bentley 2006.
18 Cf. Price *et al.* 2002; Knipper 2004; Bentley 2006; Evans *et al.* 2010.
19 Dansgaard 1964; Bowen/Wilkinson 2002; Bowen *et al.* 2005.
20 Bowen *et al.* 2005.
21 Kohn 1996; Luz *et al.* 1984; Luz/Kolodny 1985.
22 Longinelli 1984.
23 Luz *et al.* 1984.
24 Levinson *et al.* 1987.
25 Daux *et al.* 2008.
26 Pollard *et al.* 2011.
27 Chenery *et al.* 2012.
28 IAEA/WISER 2008; Bowen 2010.
29 See Pollard *et al.* 2011.
30 E.g., Schwarcz/Schoeninger 1991; Ambrose 1993; Lee-Thorp 2008.
31 O'Leary 1981; Schwarcz/Schoeninger 1991; Ambrose *et al.* 1997; Lee-Thorp 2008.
32 E.g., Grupe *et al.* 2009; Fuller *et al.* 2012.
33 E.g., Minagawa/Wada 1984; Hedges/Reynard 2007.
34 Fizet *et al.* 1995; Bocherens/Drucker 2003.
35 Heaton *et al.* 1986; van Klinken *et al.* 2000.
36 Bonsall *et al.* 1997; Lillie/Richards 2000; Lillie *et al.* 2009.
37 Ambrose 1993; Ambrose/Norr 1993.
38 Asch 2005.
39 Voerkelius *et al.* 2010.

The site is characterised by moderate continental climatic conditions. Using the OIPC, the Online Isotopes in Precipitation Calculator[40], the mean annual $\delta^{18}O$ value in modern precipitation range around -6.8 ‰ (VSMOW) in the region of Boyanovo.

Samples

Five human skeletons were selected for $^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ analyses. Tooth and bone samples were obtained from four individuals (graves 5, 6, and 14 with skeletons 1 and 2) dated to the Early Bronze Age and one human individual (grave 4) dated to the Middle Bronze Age. Age and sex determination remain to be done[41].

For $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ analyses of tooth enamel, first and second permanent molars were chosen subject to availability. Two enamel samples from different tooth crowns (M1 and M2, M1 and M3) were extracted from the skeletons in graves 4 and 14/1 for the purpose of collecting data for different childhood stages. The first permanent molars mineralise during early childhood, approximately in the first 3 years of an individual's life. The crown formation period of the second molars covers 3 to 7 years of age and that of the third permanent molars approximately 9 to 14 years[42]. Thus, the sequential analysis of all three permament molars might provide a comprehensive isotopic view of a human's childhood.

Baseline information about the 'local' bioavailable $^{87}Sr/^{86}Sr$ was obtained from one soil, two faunal and three dentine samples. Archaeological fauna is a good material of choice for the establishment of the biosphere Sr as is soil[43]. Dentine is expected to feature mixed values between the $^{87}Sr/^{86}Sr$ of the tooth enamel and that of the burial environment due to the diagenetic alteration of dentine[44]. Thus, dentine can serve as a proxy for the bioavailable strontium isotopic signature in the absence of further references. Comparison regarding $\delta^{18}O$ was based on published data[45].

Bone collagen in long bone fragments from each of the five human skeletons was extracted for $\delta^{13}C$ and $\delta^{15}N$ analysis. Samples of terrestrial herbivorous faunal bones for the establishment of an estimated food web were not available from the site. Therefore, comparison was based on published stable isotope data in the same wider area[46].

Analytical protocols

Sample preparation for strontium and oxygen isotope analyses was carried out at the laboratory facilities of the Department of Archaeology and Anthropology at the University of Bristol. Vertical tooth enamel sections, representing the complete growth axis of the tooth, were separated using a dental drill and round diamond-encrusted drill bit. Samples were cleaned from contaminants and dentine remains, sequentially repeatedly rinsed with Milli-Q water and ultrasonically cleaned. Then the samples were dried down in an oven.

Samples for strontium isotope analysis were taken to the clean laboratory of the Department of Earth Sciences at the University of Bristol, where they were prepared following the method described in detail in Haak *et al.*[47], de Jong[48] and Gerling[49]. Samples were weighed into clean Teflon beakers and dissolved in 3 ml of 7M HNO_3 on a hotplate overnight, dried down and taken up in concentrated HNO_3. Samples were dried down again, taken up in 2 ml 3M HNO_3 and ultrasonicated. After their transfer to mini-spin tubes and centrifugation, aliquots representing 3 mg of enamel were loaded onto columns. Ion exchange chromatography with Eichrom Sr spec resin (50 to 100 µl) and 3M and 7M HNO_3 were used to separate Sr from other elements. The dried samples were taken up with a few µl 10% HNO_3 and loaded onto rhenium filaments preconditioned with 1 µl $TaCl_5$ and 1 µl of 10% H_3PO_4. Isotope ratios were determined using a ThermoFinnigan Triton Thermal Ionization Mass Spectrometer (TIMS). The data were corrected to NIST SRM 987 using the value of 0.710248[50], and typical precisions were ± 0.00001 (2 SE).

In an agate mortar samples for oxygen were ground to powder under methanol, weighed to pre-cleaned mini-spin tubes and transferred to the Research Laboratory for Archaeology and the History of Arts (RLAHA) in Oxford, where the procedure followed the method outlined in detail in Cahill Willson *et al.*[51] and Gerling[52].

40 Bowen 2010.
41 Cf. Agre in this volume.
42 Schroeder 1987, 28–29; Schumacher *et al.* 1990, tab. 3; Knipper 2011, fig. 8,3.
43 Price *et al.* 2002; Bentley *et al.* 2004; Bentley/Knipper 2005.
44 Budd *et al.* 2000; Trickett *et al.* 2003; Montgomery *et al.* 2007.
45 Keenleyside *et al.* 2011.
46 E.g., Bonsall *et al.* 2000; Borić *et al.* 2004; Honch *et al.* 2006; Nehlich *et al.* 2010.
47 Haak *et al.* 2008.
48 de Jong 2011.
49 Gerling 2012; 2015.
50 Cf. Thirlwall 1991; Avanzinelli *et al.* 2005.
51 Willson *et al.* 2012.
52 Gerling 2012; Gerling 2015; pers. comment P. Ditchfield.

Samples were rinsed with deionised water and freeze-dried at 60°C. In the Department of Earth Sciences at the University of Oxford, samples were analysed isotopically for $\delta^{13}C_c$ and $\delta^{18}O_c$ using a VG Isogas Prism II mass spectrometer with an on-line VG Isocarb common acid bath preparation system, where they were reacted with purified phosphoric acid (H_3PO_4) at 90 °C. The evolved CO_2 was pre-concentrated using a cold finger apparatus prior to admission to the mass spectrometer. Calibration to VPDB standard was against the Oxford in-house NOCZ Carrara marble standard. The reproducibility was better than 0.2 per mil (0.02 %). The $\delta^{18}O$ in the carbonate of tooth apatite are reported relative to the VPDB standard.

Sample preparation for stable carbon and nitrogen isotope analyses was carried out at the Research Laboratory for Archaeology and the History of Arts (RLAHA) at the University of Oxford. The preparation of the long bone fragments followed the method as outlined in Bronk Ramsey[53]. Bone samples were shot-blasted with aluminium oxide, ground to powder and demineralized with 0.5M HCl for 48 hours at < 10°C. The remaining residue was rinsed with deionised Milli-Q water, gelatinised with a pH 3 HCl solution at 75 °C for >48 hours, filtered using 5 µm EZEE© filters and freeze-dried for 48 hours. Samples were weighed in triplicate into tin capsules, which were analysed using an automated carbon and nitrogen analyser and a continuous-flow isotope-monitoring mass spectrometer (cf-irm-ms), an ANCA Roboprep linked to a 20/20 mass spectrometer, or a Carlo Erba carbon and nitrogen elemental analyser linked to a Europa Geo 20/20 mass spectrometer. $\delta^{13}C$ measurements were made relative to the VPDB, and $\delta^{15}N$ measurements relative to the AIR standard. The analytical error was ± 0.8 ‰ (1σ) for $\delta^{13}C$ and ± 0.2 ‰ (1σ) for $\delta^{15}N$.

Results

Strontium

The results of the strontium isotope analysis are listed in Tab. 1 and shown in Fig. 1. The seven tooth enamel samples obtained from five human individuals had a $^{87}Sr/^{86}Sr$ mean of 0.70867 ± 0.0007 (1σ, n = 7) with values ranging from 0.70771 to 0.70939. The $^{87}Sr/^{86}Sr$ mean value measured in the dentine is 0.70846 ± 0.00029 (1σ, n = 3). Environmental samples were obtained from the nearby surroundings of the site (soil and recent snails) and from

burial #	chronological date	burial pit construction	burial objects	skeletal position	orientation	specimen #	item sampled	$^{87}Sr/^{86}Sr$	Sr conc (ppm)	$\delta^{18}O_{carbonate}$ (VPDB; ‰)	$\delta^{13}C$ (VPDB; ‰)	$\delta^{15}N$ (AIR; ‰)
4	Middle Bronze Age	round burial pit*	ceramic vessel*	crouched on the right side	SW-NE	Bo 3	M1	0.70771	133	-3.75		
						Bo 11	M2	0.70930	59	-4.86		
						CG 60	bone				-17.23	9.68
5	Early Bronze Age	oval burial pit with wooden cover	2 pieces of red ochre, stone, organic sheet	supine on the back, flexed legs	W-E	Bo 1	M1	0.70867	88	-5.6		
						CG 58	bone				-16.71	10.52
6	Early Bronze Age	rectangular to ellipsoid pit with wooden cover	ochre piece, organic sheet*	supine on the back, flexed legs	W-E	Bo 4	M2	0.70879	80	-4.92		
						CG 59	bone				-17.13	10.93
14 skeleton 1	Early Bronze Age	rectangular to oval pit with wooden cover	traces of red ochre (near skeleton 1), silver hair ring (near skeleton 2), organic sheet*	supine on the back, flexed legs	SW-NE	Bo 2	M1	0.70939	52	-4.27		
						Bo 12	M3	0.70774	102	-4.41		
						CG 56	bone				-17.25	10.66
14 skeleton 2				semi-supine, flexed legs	SW-NE	Bo 5	M1	0.70910	93	-3.42		
						CG 57	bone				-16.96	10.06

Tab. 1: Burial, sampling and isotopic ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{15}N$, $\delta^{13}C$) data for the analysed human individuals from the barrow *Lozianska Mogila*. (* archaeological information partly differs from Gerling 2012 and Gerling 2015 due to varying notice)

53 Bronk Ramsey *et al.* 2004.

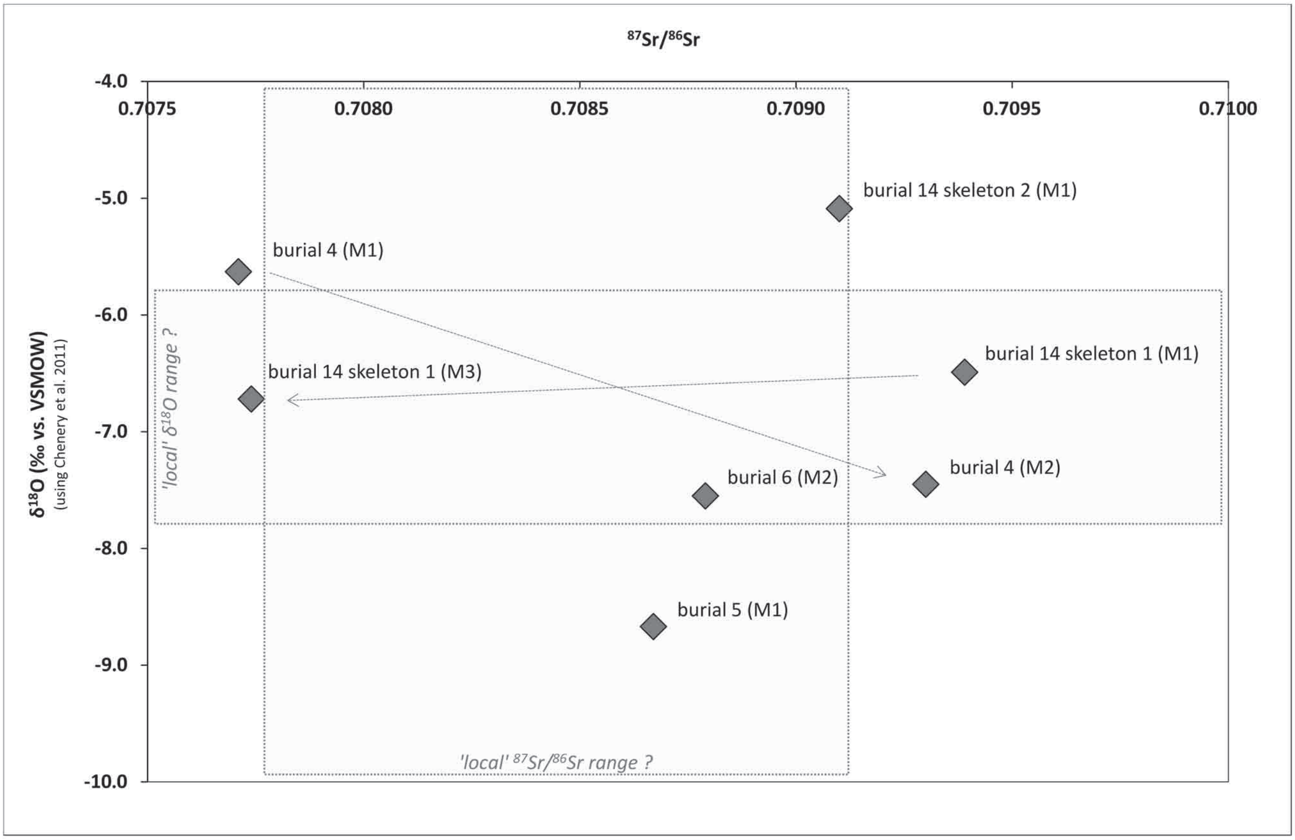

Fig. 1: Strontium and oxygen isotope data on humans from the barrow *Lozianska Mogila* and suggested 'local' $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ ranges (M1/2/3 = first/second/third permanent molar)

a distance of 10 km south of the burial mound (archaeological pig tooth); they featured a $^{87}Sr/^{86}Sr$ mean value of 0.70842 ± 0.00044 (1σ, n = 3), which is almost identical to the dentine $^{87}Sr/^{86}Sr$ mean that probably reflects the $^{87}Sr/^{86}Sr$ soil signature of the burial mound. On the basis of all baseline samples it can be suggested that the 'local' biologically available Sr is 0.70844 ± 0.00067 (2σ).

Oxygen

The results of the oxygen isotope analysis are displayed in Tab. 1 and Fig. 1. The $\delta^{18}O$ values in the carbonate fraction of the tooth enamel of five human individuals had a mean of -4.5 ± 0.7 ‰ (VPDB, 1σ, n = 7). Values ranged from -5.6 ‰ to -3.4 ‰. Calculated $\delta^{18}O_c$ (VSMOW) values feature a range of 25.1 to 27.4 with a mean of 26.3 ± 0.8 ‰ (1σ). The relationship between oxygen isotope ratios of tooth carbonate ($\delta^{18}O_c$) in humans and the respective $\delta^{18}O$ in meteoric water ($\delta^{18}O_w$), $\delta^{18}O_w = 1.59 \times \delta^{18}O_{c\ (SMOW)} - 48.634$, has been experimentally established[54]. This results in a $\delta^{18}O_w$ mean of -6.8 ± 1.2 ‰ (VSMOW, 1σ, n = 7) with a range from -8.7 ‰ to -5.1 ‰.

Carbon and nitrogen

The mean values for $\delta^{13}C$ and $\delta^{15}N$ triple analysis are shown in Tab. 1 and Fig. 2. The preservation of bone collagen was good. All 5 bone samples meet the recommended quality criteria for collagen with a mean of 3.3 for C/N ratios[55]. The human bone samples exhibit a mean $\delta^{13}C$ value of –17.1 ± 0.2 ‰ (VPDB, 1σ, n = 5) with a range of 16.7 to 17.3 and a mean $\delta^{15}N$ value of 10.4 ± 0.5 ‰ (AIR, 1σ, n = 5) with a range between 9.7 and 10.9.

54 Chenery *et al.* 2012.

55 DeNiro 1985; Ambrose 1990; van Klinken 1999.

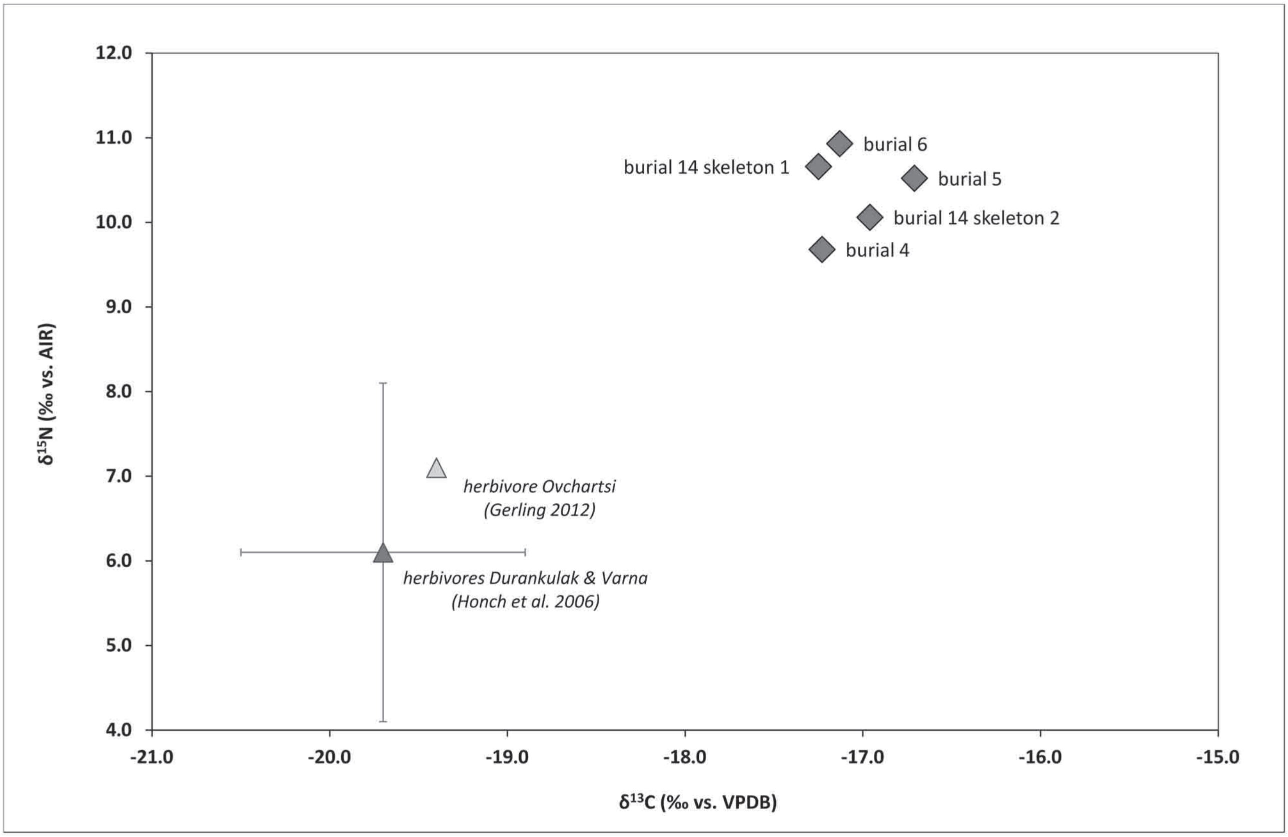

Fig. 2: Carbon and nitrogen isotope data on humans from the barrow *Lozianska Mogila* and additional comparative faunal data from Ovchartsi (Gerling 2012; Gerling 2015), and Durankulak and Varna (Honch *et al.* 2006)

Discussion

Human mobility

The interpretation of 'locality' and 'non-locality' of isotopic human data requires the estimation of an $^{87}Sr/^{86}Sr$ range that can be considered as a characteristic signature for a local settled community. A geological map can serve as a first indicator of the expected strontium isotope values for the site[56]. The immediate surroundings of the site are geologically varied and are predominated by Upper Palaeozoic to Mesozoic rocks and sediments deriving from the Pliocene in the valley of the river Tundzha. Late Cretaceous geological units occur within a 10 km radius; Palaeozoic geology, namely Carboniferous and Cambrian bedrock, occurs within 25 km around the site. Part of the Palaeozoic and Mesozoic rocks are metamorphic plutonites. According to the TRACE $^{87}Sr/^{86}Sr$ map[57], the 'local' $^{87}Sr/^{86}Sr$ signature for water and soil can be expected to range between 0.702 to 0.711 due to the mixture of Palaeozoic-Mesozoic metamorphic rocks and Cenozoic sediments. This is a very wide range. A second, and more precise, approach for the characterisation of 'local' biologically available strontium is the analysis of baseline or reference samples, typically archaeological faunal references, or modern plant, sediment and faunal material. As a cut-off value, the mean of these references ± 2σ was calculated[58], which allows the identification of differing and supposedly 'non-local' Sr composition. The 'local' $^{87}Sr/^{86}Sr$ range was established – in due dependence upon available sample material – by sediment, recent snail shells and three dentine samples and averaged in 0.7084 ± 0.0007 (2σ) with a 'local' range of biologically available Sr of 0.7077 to 0.7091. Sr isotope ratios for the geological formations in the Danube Gorge region were estimated to range between 0.7075 and 0.7090 for limestone (marine sediments) and between 0.7088 and 0.7092 for the Cenozoic alluvium of the Danube River[59]. Consequently, the 'local' range of biologically available

56 Asch 2005.
57 Voerkelius *et al.* 2010.
58 Cf. Grupe *et al.* 1997; Price *et al.* 2002; Bentley 2006.
59 Price *et al.* 2004; Borić/Price 2013.

strontium isotope signatures can probably be considered as the result of a mixed signal of the geological bedrock of a 10 km radius. Two human individuals, burial 4 and skeleton 1 in grave 14, can be considered as outliers, because they had marginally different $^{87}Sr/^{86}Sr$ values to the 'local' Sr range. Remarkably, the outlier individuals yielded comparable $^{87}Sr/^{86}Sr$ values, indicating that the places they moved to or from was similar in geology. Large intra-individual variability above 0.0015 is given in both individuals, which were analysed in duplicates. This large variation suggests a change in nutrition, in mobility behaviour and/or the place where they lived during the years of tooth enamel mineralization.

The $\delta^{18}O$ (VPDB) values in the tooth enamel carbonate of the five human individuals varied from -5.6 ‰ to -3.4 ‰ with an average of -4.5 ± 0.7 ‰ (1σ). This is significantly less depleted than the mean values of 60 human individuals from Kalfata-Budjaka on the Black Sea Coast published in Keenleyside *et al.*[60], which average at -5.8 ± 0.7 ‰ in first molars and -6.1 ± 0.7 ‰ in third molars. The basic isotopic variability in the region around Boyanovo today can be estimated by the Online Isotopes in Precipitation Calculator (OIPC)[61], which reveals a mean annual $\delta^{18}O$ values in meteoric water of -6.8 ‰ (VSMOW). The relationship between oxygen isotope ratios of tooth enamel carbonate ($\delta^{18}O_c$) in humans and the respective $\delta^{18}O$ in meteoric water ($\delta^{18}O_w$), $\delta^{18}O_w = 1.59 \times \delta^{18}O_c (VSMOW) - 48.634$, has been experimentally established[62], and results in a mean of -6.8 ± 1.2 ‰ (1σ) and a range from -8.7 to -5.1 ‰. Accounting for a 1 ‰ error three samples, the first molar of the burial 4 skeleton, burial 5 and skeleton 2 in grave 14 are highlighted as outliers. Five samples of water sources around Sozopol on the Bulgarian Black Sea coast averaging -11.1 ± 2.5 ‰ were analysed in the study of Keenleyside *et al.*[63] and can serve as a second line of comparison, although this seems rather depleted in comparison to both $\delta^{18}O$ values in modern precipitation and the human enamel values obtained in this study.

The first molar of the human in grave 4 is the only 'real' isotopic outlier considering both $^{87}Sr/^{86}Sr$ and $\delta^{18}O$, but none of the human samples lie far outside both 'local' isotope ranges. Although the results from graves 5 and 6 and grave 14, skeleton 2, fall outside the 'local' $\delta^{18}O$ range, the differences are minor and point to 'local' $^{87}Sr/^{86}Sr$ values. The first and third molars of the humans in graves 4 and 14, skeleton 1, vary gradually in their $^{87}Sr/^{86}Sr$ values. These huge intra-individual differences can probably be explained by changes of residence to places with different geological yet similar climatic conditions. This result hints to several places of residence that were visited during lifetime, but also to various residence places of the human individuals buried in this burial mound. However, according to the geology sites resulting in varying $^{87}Sr/^{86}Sr$ values of this kind might be located within a 10 km radius around the site.

Human migration

The graves dating to the Early Bronze Age, burials 5, 6, and 14 skeleton 1 and 2, are characterised by archaeological features that show similarities to the burial tradition typical for the North Pontic Yamnaya culture; the skeletons were placed in rectangular burial pits with wooden covers covered by an earthen mound. Furthermore, skeletons were laid down in supine positions with flexed legs, with orientations from West to East or Southwest to Northeast. In addition, the burials contained pieces of red ochre, the bottom of the graves were covered with organic materials (graves 5, 6) and a silver spiral ring (grave 14). The skeletal position and orientation, the ochre colouring, the organic materials on the pit floor, the wooden grave cover and the burial mound itself are all features that can be regarded as typical characteristics of the Yamnaya culture in the territory of modern Bulgaria. The generally sparse grave objects and the appearance of a silver spiral ring, also known from burial mounds in the West and Northwest Pontic region, provide further hints to connections to the Yamnaya cultural communities[64]. According to Alexandrov, this first stage of the Bronze Age North Pontic impact can be associated with the Dniester and Lower Danube groups in the Northwest Pontic and is also attested in North Bulgaria[65].

Applying the migration model of Tütken[66], the archaeological remains, and strontium and oxygen isotope analyses provide three independent lines of evidence for a migrating human individual. All human individuals can be considered 'migrants' in respect to the archaeological evidence, whereas only two individuals, burial 5 and the male skeleton in grave 14, provide additional stable isotopic evidence for migration. The $\delta^{18}O$ value of the individual in grave 5 is relatively negative and would imply a

60 Keenleyside *et al.* 2011.
61 Bowen 2010.
62 Chenery *et al.* 2012.
63 Keenleyside *et al.* 2011.
64 Alexandrov 2011.
65 *Ibid.*; cf. also Dergačev 1986; Panayotov 1989; see also Agre in this volume.
66 Tütken 2010, 45 fig. 6.

connection to an area farther north or northeast. Similarly depleted oxygen isotope values are reported for the North Pontic steppes; but since the $^{87}Sr/^{86}Sr$ value of this individual is consistent with the 'local' bioavailable Sr, there are also other possibilities to interpret these results. The outlier $^{87}Sr/^{86}Sr$ values of 14/1 do not necessarily point to very far varying geological regions since the site in located in a geologically diverse area and the measured isotope values can also be obtained by nearby regions of residence.

It can be concluded that the results of the strontium and oxygen isotope analyses do not or only partly support the archaeological evidence of 'foreign' human individuals, although the analysis of stable isotopes in tooth enamel enables the detection of first generation migrants only. Hence, we might be dealing with descendants of immigrants that held on to their old burial traditions.

The Middle Bronze Age burial: An outlier?

The presence of a ceramic cup, the lack of ochre and the skeletal position (crouched on the right side) dated grave 4 to the Middle Bronze Age[67]. The first and the third permanent molar featured highly varying results. The first molar was the only sample of the *Lozianska Mogila* that differed in respect to both isotopic systems; it was outside the 'local' $^{87}Sr/^{86}Sr$ range and was at the top end of the 'local' $\delta^{18}O$ range. However, the enrichment in $\delta^{18}O$ might be the result of a nursing effect. The third molar featured a result that is consistent with the 'local' $\delta^{18}O$, but elevated in comparison to the 'local' $^{87}Sr/^{86}Sr$ range. The large intra-individual variability points to a residence change between earliest and later childhood. Whether the results indicate a single movement or are related to a regular movement pattern cannot be concluded on this data basis. Remarkably, the isotope ratios of the two teeth are very similar to the ones of skeleton 1 in grave 14, dated to the Early Bronze Age. This might hint at similar residences or movement patterns during the two Bronze Age periods.

67 Cf. Alexandrov 2011; Agre in this volume.

Human dietary patterns

In absence of faunal samples from the same site or from an archaeological site in the proximity, we looked for comparisons from the wider geographic region. Several stable isotope studies were conducted at sites along the Danube Gorges dating to different time periods, mainly to the Mesolithic-Neolithic transition. Here, a large data set of faunal bones was analysed for $\delta^{13}C$ and $\delta^{15}N$. In agreement with the expectations for terrestrial C_3 grazers, herbivores provided mean values of $\delta^{13}C \sim -22$ ‰ and $\delta^{15}N \leq 7$ ‰[68]. In the context of their stable isotopic study of the Copper Age/Eneolithic sites of Durankulak and Varna I, Honch *et al.* found that the mean $\delta^{15}N$ values of the herbivorous species averaged at 6.1 ± 2 ‰ and mean $\delta^{13}C$ values at -19.7 ± 0.8 ‰[69]. A number of ovicaprids, however, featured results that were significantly enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$. Within the author's doctoral research project[70] one herbivorous animal from the near-by Early Bronze Age burial mound of Ovchartsi, located less than 50 km away, was available for stable isotope analysis, resulting in $\delta^{13}C$ -19.4 ‰ and $\delta^{15}N$ 7.1 ‰, which is in good agreement with the mean of the five ovicaprids analysed by Honch and colleagues.

The $\delta^{13}C$ and $\delta^{15}N$ results of the human individuals in Boyanovo are relatively consistent and give little indication of intra-population variation in diet. The spectrum of the $\delta^{13}C$ values covers a range from -17.3 to -16.7 ‰, which reflects a diet that was primarily based on the consumption of C_3 plants and their feeders, but shows a significant impact of C_4 plants. Due to the site's geographic location a major impact of marine resources seems unlikely, yet not impossible. Freshwater food results in more variable stable isotope values but is suggested to reveal a combination of normal to decreased $\delta^{13}C$ and increased $\delta^{15}N$ values. This was attested for the Mesolithic human skeletons (n = 31) from Vlasac in Serbia, which averaged in -19.4 ± 0.5 ‰ $\delta^{13}C$ and 14.2 ± 0.8 ‰ $\delta^{15}N$, and from Lepenski Vir (n =17), which featured values of -19.0 ± 0.6 ‰ $\delta^{13}C$ and 14.4 ± 1.8 ‰ $\delta^{15}N$, for example[71]. The combination of depleted $\delta^{13}C$ and elevated $\delta^{15}N$ values was interpreted as a decreased impact of freshwater fish at the onset of the Neolithic[72], while Borić *et al.*[73] argued that this was an indicator of a development of a more complex relationship

68 Borić *et al.* 2004, 224 tab. 1; Nehlich *et al.* 2010, 1136 tab. 3.
69 Honch *et al.* 2006.
70 Gerling 2012; 2015.
71 Borić *et al.* 2004, tab. 2.
72 Bonsall *et al.* 1997; 2000; 2004.
73 Borić *et al.* 2004.

between local communities and non-local individuals instead of a significant dietary change[74]. Less dense woodlands might also be considered as a reason for elevated $\delta^{13}C$ values[75]; the faunal remain from the site of Ovchartsi, however, features a significantly more negative value. Consequently, the most probable explanation would be to suggest an impact of C_4 plants, for example in connection with the consumption of plants like millet. Up to now however, consumption of millet on a larger scale has not been isotopically attested for the Eurasian steppes and adjacent regions during the 4th to 2nd Millennia BC[76]. $\delta^{15}N$ values range from 9.7 to 10.9 ‰, which is again consistent with a diet based on terrestrial C_3 plants and herbivores. Typical values for individuals of agricultural societies like the *Linearbandkeramik* communities investigated in the 'First Farmers of Europe' project average around 10.0 to 10.5 ‰ $\delta^{15}N$[77]. Values from Boyanovo are enriched compared to what we expect for temperate continental Europe, which is due to the warmer and drier climate of the Balkan Peninsula[78]. Similar values to those from Boyanovo were obtained from the human remains from the Copper Age cemeteries of Varna I and Durankulak on the Black Sea Coast. The 55 sampled humans in Varna I averaged at -19.3 ± 0.3 ‰ $\delta^{13}C$ and 10.0 ± 0.6 ‰ $\delta^{15}N$. The 78 humans from Durankulak gave comparable means of -19.1 ± 0.3 ‰ $\delta^{13}C$ and 9.3 ± 0.8 ‰ $\delta^{15}N$[79], which was interpreted as reflecting a diet based on C_3 plants with an important impact of terrestrial meat sources.

Values of $\delta^{13}C$ and $\delta^{15}N$ calculated from the five human individuals varied within standard variations of 0.2 and 0.5 (1σ). This is a relatively tight cluster, which might be due to the restricted sample size, yet it is surprising since the individuals do not form one community due to their differing chronological dates. Isotopic variations between the humans of varying stratigraphic grave positions and with different kinds of grave furniture remain statistically insignificant. Based on the absence of anthropological identifications, i.e. sex, age, pathologies, it is impossible to draw further conclusions.

74 Cf. Schulting 2011, 32.
75 Drucker *et al.* 2008.
76 Lightfoot *et al.* 2013.
77 Bickle/Whittle 2013, 362 tab. 9,7; for further LBK sites see also e.g., Dürrwächter *et al.* 2006; Nehlich *et al.* 2009; Oelze *et al.* 2011.
78 E.g., Honch *et al.* 2006.
79 Honch *et al.* 2006.

Conclusions

The data provides a first insight in life-ways and land-use of the people buried in the *Lozianska* burial mound using stable isotope analysis. These humans are associated with the Early Bronze Age communities in the Northwest and West Pontic regions, who shared similarities in the burial tradition with the contemporaneous populations of the Yamnaya culture in the East European steppes. The strontium and oxygen isotopic evidence suggests a certain level of movement within different periods of life. So far, it does not provide evidence of migrations from the East European steppes. The diet of these human individuals was probably based on terrestrial C_3 plants and meat sources with a minor impact of C_4 food sources.

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