Journal of Archaeological Science: Reports 14 (2017) 65–75

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Stable isotope palaeodietary analysis of the Early Bronze Age Afanasyevo Culture in the Altai Mountains, Southern Siberia

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ARTICLE INFO

Keywords:
Altai
Palaeodiet
Afanasyevo
Karakol
Bronze Age
Carbon
Nitrogen
Sulphur
AMS radiocarbon dating
Freshwater reservoir effects

ABSTRACT

This study represents the first stable isotope ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) palaeodietary data and AMS radiocarbon dates for Early Bronze Age humans and fauna (n = 24), and two modern fish from the Altai Mountains, Southern Siberia. The results show that the diet of the population was mainly C_3-based with heavy reliance on animal protein. Within the population, males are overall higher in $\delta^{15}N$ values which could be the result of better access to higher-trophic level foods, such as meat. Another important observation is that comparison of the results with the previous data for the Afanasyevo population from the Minusinsk Basin (Southern Siberia) suggests a difference in the amount of fish in the diet, with the Altai population virtually not using this food source. Modern fish (data from previous research) demonstrates a strong freshwater reservoir offset, although a human sample from a single archaeological terrestrial/human pair analysed does not appear to be affected. No significant offsets were detected between $\delta^{34}S$ values of archaeological humans and herbivores, and modern fish.

1. Introduction

Lately, considerable research has been focused on reconstructing past lifestyle of the prehistoric populations of Southern Siberia, including the nature of the region's economy over time, its role within the wider pattern of steppe pastoralism and its relationship to crop-based agriculture (e.g. Anthony, 2007, 2013; Frachetti, 2008; Levine et al., 1999; Scott et al., 2004; Murphy et al., 2013; Svyatko et al., 2013). Our study represents the first attempt to employ carbon, nitrogen and sulphur stable isotopes to investigate the diet of the Early Bronze Age populations of the Altai region of Southern Siberia, and compare these with the existing data for the Afanasyevo population of the Minusinsk Basin.

1.1. Geography

The Sayano-Altai Mountains, primarily located in the territory of the modern Altai Republic, are the highest highland area of Siberia. Geographically, the region is an interface between Southern Siberia, Eastern Kazakhstan, Western Mongolia and Northern China which has largely determined its important role in history. Since early prehistory, this territory appeared as a gateway for cultural exchange between Eastern and Western civilizations (e.g. Kuzmina, 2008).

The Altai Mountains region itself is a system of mountain ranges, river valleys, plateaus and intermontane basins ("steppes"). The altitude of the Mountains increases from 450 m in the north-west part to 4000 m in the south-east (Sidorenko, 1967). The region has an extensive hydrologic system, rich in fish, which includes the 665 km long Katun River and its tributaries which feed mainly from surface waters. The climate of the area is continental, with a mean annual temperature of 0 to +5 °C in river valleys and −6 °C in highlands. Annual precipitation varies between 200 mm in south-east part of the Mountains and 1500 mm in the south-west part. Due to climatic differences, the variety of the landscapes in the region (steppe, taiga and alpine) determines the variability of vegetation and fauna.

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http://dx.doi.org/10.1016/j.jasrep.2017.05.023
Received 17 October 2016; Received in revised form 15 May 2017; Accepted 16 May 2017

Fig. 1. Distribution of the Afanasyevo Culture type sites and finds (dashed) and location (numbers) of the Altai highlands sites analysed (12 and 13 are the localities of modern fish discussed in the text): 1. Ayrydash 1; 2. Elo-Bashi; 3. Inskoy Dol; 4. Kara-Koba 1; 5. Maliy Dugan; 6. Nizhniy Tyumechin 1, 5 and Perviy Mezhelik 1; 7. Nizhnyaya Sooru; 8. Ozernoye and Ozernoye 2; 9. Saldyar 1; 10. Tenga 2; 11. Uznezya 1; 12. Chuya R., Kurai Basin; 13. Katun R., Uimon Basin.
Intensified dashed area is the densest distribution of the Afanasyevo sites in Altai and the Minusinsk Basin (Vadetskaya et al., 2014).

1.2. Archaeology of the Afanasyevo Culture

The Early Bronze Age Afanasyevo Culture takes a special place among the Bronze Age archaeological cultures of Central Asia – the Afanasyevo population is believed to be the first in the region to have developed metal working and stock-rearing (Vadetskaya, 1986; Gryaznov, 1999), thus making Southern Siberia the earliest centre of metallurgy in Northern and Eastern Asia (Kyzlasov, 1992). The sites have been found across a wide area of Southern Siberia with the majority located along the Middle Yenisei River (ca. 45 sites excavated) and in the Altai-Sayan region (65 sites excavated; Stepanova, 2010; Vadetskaya et al., 2014; Fig. 1). Based on typological analysis, the Afanasyevo Culture is broadly dated to the end of the 4th mil. BC – beginning of the 2nd mil. BC (e.g. Tsyb, 1984; Molodin, 2002; Pogozheva et al., 2006). Radiocarbon chronology of the Afanasyevo sites is generally very broad – the dates from the Middle Yenisei sites belong to the 37th-22nd c. cal BC (Svyatko et al., 2009), and those of the Altai sites – to the 39th-21st c. BC (Polyakov, 2010). In particular, human individuals date to the 29th–25th c. cal BC in the Middle Yenisei and to the 36th–21st c. cal BC in Altai.

It is believed, that two distinct divisions of the Afanasyevo Culture coexisted in the Minusinsk Basin and Altai (e.g. Gryaznov and Vadetskaya, 1968, p. 159; Tsyb, 1984; Stepanova, 2010). The local variations primarily concerned funeral rites, structures and grave goods, as well as physical appearance of the people. Particular local specifics include the lack of collective burials and more frequent use of ochre in Altai (as opposed to the Middle Yenisei River sites), placing of several vessels into the grave in Middle Yenisei, and more often the occurrence of pestles and staffs in Altai (Stepanova, 2015).

1.3. Economy of the Afanasyevo population

Generally, the Altai Mountains are apt for stock-breeding. Only a small number of full-scale archaeological excavations have been performed in the region; all known Afanasyevo settlements (ca. 50) are located along rivers and near river mouths. The osteozoological analysis of the settlements implies that domestic fauna, especially ovicaprids, formed the basis of the Afanasyevo economy, with varying (possibly increasing with time) input of wild animals, especially roe-deer (Kosintsev and Stepanova, 2010). A detailed analysis of soil and zoological remains of the Afanasyevo Culture in Denisova Cave (North-West Altai) suggests that the cave was used by the Afanasyevo people as a sheep corral (Derevyanko and Molodin, 1994). From the archaeological data, cattle and horses were present in the Afanasyevo herd (Galchenko, 1994). The economical reliance of the Afanasyevo population on domestic animals is also supported by the anthropological data. Developmental disorders in a number of subadult humans have been interpreted as possibly related to cyclic productivity of livestock farming and the insufficient development of alternative economic sectors (Tur and Rykun, 2006). The prevalence of dental calculus and low caries rates in a number of Afanasyevo groups of Altai indicates high protein input in their diet (ibid.).

Hunting (especially roe deer; Kosintsev and Stepanova, 2010) apparently also played a role in Altai; however, the overall proportion of wild animal bones recovered from the Afanasyevo sites is small. The latter could partly be the result of butchering of the animal carcasses away from settlements – the practise still being used by modern local hunters.

Some forms of fishing are believed to have been practised in Altai since the Middle Palaeolithic (summarised in Soenov, 2001), however, fish remains are extremely rare in the Afanasyevo sites – possibly, they are under-represented in the archaeological record. Necklaces with fish vertebra have been recovered from two sites – Saldyar 1 (kurgan 36) and Ust-Kuyum graveyard (burial 18; Larin, 2005; Bers, 1974) and in both cases the burials were distinctive in comparison with the typical Afanasyevo sites. These may represent contact of the local population with different cultures (Pogozheva et al., 2006). Fish bones have also been found in the late Afanasyevo ritual site of Kara-Koba 1, enclosure 5 (Vladimirov and Tsyb, 1982), and in layers 11 and 12 of the Denisova Cave dated to the Afanasyevo Culture (Derevyanko and Molodin, 1994).

The presence of cereal cultivation in the Afanasyevo economy has been a long-standing issue, as is the role of cereals in the Eurasian steppes in general (see discussion in Gryaznov, 1999; Soenov, 2003; Svyatko et al., 2013). Although the majority of the sites are located in the zones where cereal cultivation would have been possible, to date no associated tools or cereal remains have been recovered (although no soil flotation has been conducted on the sites). Stone grinders which are believed to appear in the area only in the Afanasyevo time and are widely present at the Afanasyevo settlements and burial sites are the only argument in favour of crop processing, however these also could have been used for grinding wild plants or preparing dyes.

In the Minusinsk Basin, only a few Afanasyevo settlements have been investigated and as such the majority of data on the economy of these groups comes from burial sites. The role of hunting in the Afanasyevo economy is not clear, however, the grave goods occasionally include remains of sheep, cattle and horse meat cuts, as well as wild fauna (including deer; Vadetskaya et al., 2014). Domestic animal bones (horse, cattle and ovicaprids) have also been recovered from the Tepsei X settlement (Gryaznov and Komarova, 1979). Fishing is believed to have been practised in the area – scales and bones of fish, in particular pike and sturgeon, as well as freshwater mollusc shells, have been found in the sites of Afanasyeva Gora and Malye Kopeny II (Vadetskaya et al., 2014). No evidence for cereal cultivation, apart from pieces of grinding stones in several sites, has been found in the Minusinsk sites (Kiselev, 1951, 48; Vadetskaya, 1986, 21).

1.4. Stable isotope analysis

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis is extensively used in modern palaeodietary reconstructions, and the principles of the technique have been widely explained in the literature (see below). Briefly, for inland sites, $\delta^{13}C$ is used to estimate the proportions of C_3 (most temperate zone plants) and C_4 plants (more abundant in higher aridity and temperature and including such important agricultural staples such as millet and maize) in the diet. The bone collagen from herbivores that subsist only on C_3 grasses will result in a $\delta^{13}C$ value of ca. − 21.5‰; if the diet were based only on C_4 grasses then the value would be ca. − 7.5‰. The stable analysis of bone collagen principally reflects the consumption of protein-containing foods, although dietary lipids and carbohydrates also have an impact on consumer's $\delta^{13}C$ values (Newsome et al., 2014). Sampled collagen reflects the diet of the last 5–15 years before death, depending on the bone measured, and on the nutritional status of the individual. In some cases, $\delta^{13}C$ is also used to estimate the proportion of freshwater components in an individual's diet when aquatic systems show $\delta^{13}C$ levels differing from terrestrial ones (Keaveney and Reimer, 2012; Katzenberg and Weber, 1999). Non-dietary factors, such as the climate (higher plant $\delta^{13}C$ values as well as generally higher proportion of C_4 grasses with an increase in temperature and aridity; van Klinken et al., 1994) and the canopy effect (increase of leaf $\delta^{13}C$ values from ground to canopy; van der Merwe and Medina, 1991) may also affect $\delta^{13}C$ ratios of consumers.

Nitrogen isotope analysis is used to identify the trophic level of an individual with a 3–6‰ increase in each step of a food chain. The $\delta^{15}N$ values vary between 0 and 5‰ in most plants to around 9‰ for populations relying on terrestrial animal protein. The highest nitrogen isotopic levels can be seen in consumers of aquatic resources due to extended food chains in aquatic ecosystems. The most relevant non-dietary factors in consumers of terrestrial resources include a climatic effect (increase of plant $\delta^{15}N$ ratios in arid environments; Schwarcz et al., 1999; Chase et al., 2012) and the manuring effect (increase of the $\delta^{15}N$ ratios of manured soil and associated plants; Bogaard et al., 2007, 2013).

Sulphur ($\delta^{34}S$) isotopes have not been routinely employed as a palaeodietary proxy, although it has a potential for assessing the consumption of fish in studies of estuarine food webs where there is a pronounced difference between the isotopic composition of primary sulphur sources, such as aquatic, freshwater and terrestrial sulphate (e.g. Richards et al., 2003; Privat et al., 2007; Nehlich et al., 2010). The assessment of $\delta^{34}S$ in inland freshwater ecosystems is complex, due to its extreme variability in freshwater sediments and organisms; sulphur isotope values for freshwater sulphate can range between − 22 and + 20‰ (Krouse, 1980; Peterson and Fry, 1987). Sulphur isotopic values of terrestrial plants vary approximately between − 7 and + 8‰ (e.g. Chukhrov et al., 1980). Therefore, in the absence of marine food sources, high $\delta^{15}N$ collagen values in archaeological humans together with 'aquatic' $\delta^{34}S$ signatures could identify a freshwater input in the diet.

A substantial number of palaeodietary studies using stable isotope analysis have been undertaken on the prehistoric populations of Siberia and the Eurasian steppes, with a number of them focussing on the Early Bronze Age communities (Iacumin et al., 2004; Lillie et al., 2011; Schulting and Richards, 2016; Hollund et al., 2010; Shishlina et al., 2009, 2012, 2014; van der Plicht et al., 2016; Svyatko et al., 2013; Motuzaite Matuzeviciute et al., 2016; Katzenberg et al., 2012; Katzenberg and Weber, 1999; O'Connell et al., 2003; Motuzaite Matuzeviciute et al., 2015, 2016; Ventresca Miller et al., 2014). The main outcomes of this research are:

- an exclusively C_3 diet of the populations throughout Eurasian Steppes in the Early Bronze Age;
- a suggested emphasis on freshwater fish as an important, though variable, element of dietary protein for many of the analysed populations. This observation is somewhat contrary to archaeological data, as very few finds associated with fishing have been recovered in the Eurasian steppe region;
- strong links with climate (rainfall) documented in the Caucasus region, with the most enriched nitrogen isotopic values found in the dry steppe areas (Hollund et al., 2010).

To the best of our knowledge, only two studies involving $\delta^{34}S$ have been performed in the Eurasian Steppes. The study at the Late Bronze Age site of Chicha (South-Western Siberia; Privat et al., 2007) demonstrated the potential of using $\delta^{34}S$ in the area as an additional indicator for freshwater fish consumption, as a pronounced difference between terrestrial and aquatic signals was found. On the contrary, the $\delta^{34}S$ results from the Minusinsk Basin (Southern Siberia) did not appear to significantly differ between modern fish, Bronze to Early Iron Age herbivores and humans analysed and therefore could not contribute additional palaeodietary information (Svyatko et al., 2016). A similar situation (absence of significant difference in $\delta^{34}S$ between terrestrial and aquatic samples) has been recently observed in the prehistoric sites of the Baikal region (R Schulting, personal communication).

2. Materials and methods

2.1. Sites and materials analysed

For this study, 20 archaeological human and six faunal samples from 13 Altai sites have been used. The majority of sites are located in the Central Altai physiographical province in the valleys of medium altitude mountains (Fig. 1). Inskoy Dol is located in the North-West Altai province. Despite the relatively small distance between the sites (50–180 km), they are located in different landscape and climatic zones.

The most northern sites (settlements of Maliy Dugan and Uznezya 1) are located in the valleys of the Katun River right tributaries, near their mouths. The climate in the area is sharply continental and quite humid, with the annual mean precipitation of 400–600 mm. The ecology of the area is suitable for the major economic activities such as hunting, crop cultivation, stock-rearing, fishing and gathering.

The second group of sites (Saldyar 1 and Ayrydash 1) is located in the wide steppe regions of the Katun River valley. The climate of this area is also sharply continental, although somewhat drier; the mean annual precipitation is 200–300 mm. The region is favourable for the major economic activities with the exception of wide-scale crop cultivation.

The third group of sites (Elo-Bashi, Kara-Koba 1, Perviy Mezhelik 1, Nizhniy Tyumechin 1 and 5, Ozernoye, Ozernoye 2 and Tenga 2) is scattered around the Tenga-Elo intermontane tectonic basin on the shores of the Ursul River and its tributaries, and in the Karakol River valley (the settlement of Nizhnyaya Sooru). Modern day climate in the area is dry and cool, the mean annual precipitation is around 300 mm, and as such the area is suitable for stock-rearing and hunting. Gathering and fishing would be possible in the area while crop cultivation would be quite limited due to frosts and aridity.

The cemetery of Inskoy Dol is located in 180 km from the central group of sites in the Tenga-Elo Basin, on the Inya River shores of the modern Krasnoshchyokovsky District. The local relief is piedmont and low-mountain. The climate is continental with the mean annual precipitation of 430 mm.

The analysed sites can be clearly attributed to the Afanasyevo Culture, with the exception of Tenga 2 kurgan which belongs to the Early Bronze Age Aragol type (Mogilnikov, 1987; Stepanova 2000; Vadetskaya et al., 2014), later Afanasyevo Culture kurgan 2 of Nizhniy Tyumechin 5 which apparently represents the process of assimilation of the Afanasyevo people with those of different cultural traditions (Stepanova, 2009), and the cemetery of Ozernoye (not to be confused with the Afanasyevo grave 2 from Ozernoye 2 site) which belongs to the succeeding Karakol Culture and possibly coexisted for a certain period with the Afanasyevo Culture.

Most cemeteries consist of up to 45 detected stone enclosures (Soenov and Surazakov, 2005; Vadetskaya et al., 2014; Abdulganeev and Slavnin, 2004; Mogilnikov, 1987). The graves mainly represent single or double (adult and child) burials, with the exception of the Karakol Culture burial from the Ozernoye site which included five individuals. Some burials contain no grave goods while others included items such as pottery, ornaments, a bronze knife or a staff (Table 1). Two modern fish samples (grayling, *Thymallus thymallus*) were collected from Katun (Uimon Basin, 50°11′N 85°57′E) and Chuya Rivers (Kurai Basin, 50°13′56″N 87°55′59″E; Fig. 1) in September 2015.

2.2. Methods

The analyses were executed at Queen's University Belfast. EA-IRMS carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope measurements and AMS radiocarbon dating of bone collagen samples were performed in the ^{14}CHRONO Centre for Climate, the Environment and Chronology using a Thermo Delta V IRMS with Flash EA and NEC compact 0.5 MV AMS respectively. Sulphur ($\delta^{34}S$) measurements were performed at Stable Isotope Facility using a Thermo Flash 1112 coupled with a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS).

The pretreatment of all samples was performed in the ^{14}CHRONO Centre. Sample bone surfaces were cleaned. Preparation of collagen was based on an ultrafiltration method (Brown et al., 1988; Bronk Ramsey et al., 2004) and included bone demineralization (2% HCl), gelatinization (at 58 °C for 16 h), filtration, ultrafiltration (using Vivaspin 15S ultrafilters with MWCO 30 kDa; 3000–3500 rev/min for 30 min), and freeze-drying. The dried collagen was stored in a desiccator. Pretreatment of fish samples is described in Svyatko et al., 2017.

C, N and S isotopic measurements were made at least in duplicate (with the exception of samples SS ASU MD-3 and UBA 31086 where only single $\delta^{34}S$ measurements were made). The measurement uncertainty (1sd) of $\delta^{13}C$, $\delta^{15}N$, and atomic C:N ($C:N_{at}$) based on 6–10 replicates of seven archaeological bone collagen samples was 0.22‰, 0.15‰, and 0.2%, respectively. Sulphur concentrations were not available for the archaeological samples. The analytical precision of $\delta^{34}S$ measurements was ± 0.4‰ based on the standards analysed (NBS127 and IAEA-SO6). Results were reported using the delta convention relative to international standards – VPDB for C, AIR for N, and VCDT for S (Krouse and Coplen, 1997; Hoefs, 2009).

AMS radiocarbon ages were determined for 14 humans and two herbivores using the standard methodology (Reimer et al., 2015). The AMS-measured $\delta^{13}C$ is not presented in the summarising tables. The radiocarbon ages were calibrated using Calib 7.0 programme (Stuiver et al., 2013) and IntCal13 calibration curve (Reimer et al., 2013).

For evaluating statistical differences, we used the Microsoft Excel® *t*-test: Two-Sample Assuming Equal Variances (two-tailed). This is the method used in all cased where p values are reported. Data were checked for normality using Shapiro-Wilk Test except when there was an insufficient number of samples.

3. Results

3.1. Preservation of the samples

Two samples, ovicaprid/roe-deer from Maliy Dugan and a large herbivore from Uznezya 1 site (SS ASU MD 1 and SS Uzne1 respectively; not included in summary table), yielded no collagen. In other archaeological samples, the collagen content varied between 1.1 and 13.5%, and the $C:N_{atomic}$ ratio – between 3.1 and 3.4, which indicates a good to very good collagen preservation (van Klinken, 1999; DeNiro, 1985; Bronk Ramsey et al., 2004; Table 1). For the modern fish, %S in collagen was 0.55 for UBA-31085 and 0.43 for UBA-31086.

3.2. Faunal $\delta^{13}C$ and $\delta^{15}N$ isotope results

Faunal samples in this study are limited to only four herbivores (two ovicaprids, roe-deer and undeterminable herbivore). Overall, the results (Table 1 and Fig. 2) are typical for north temperate zones, as compared, for example, with herbivores from the Minusinsk Basin (Svyatko et al., 2013, also Fig. 5) or Baikal region (Weber et al., 2011); the low $\delta^{13}C$ levels (-19.9 ± 1.7‰) reflect primarily C_3-based mode of subsistence, while $\delta^{15}N$ ratios (5.2 ± 0.8‰) are characteristic for relatively humid climate (e.g. Schwarcz et al., 1999). The roe-deer sample from Maliy Dugan is ca. 1.5‰ lower in carbon isotope ratios than the rest of the animals analysed which is possibly due to the different nature of plants consumed by the species; the diet of roe-deer includes tree leaves, while ovicaprids feed mainly on steppe grasses. One can suggest that a canopy effect might be responsible for the slightly lower values for roe-deer, reflecting its preferred forest habitat.

3.3. Human $\delta^{13}C$ and $\delta^{15}N$ isotope results

Human $\delta^{13}C$ and $\delta^{15}N$ values cluster relatively close to each other, indicating the absence of major differences in diets within the population. Carbon isotopic levels (-18.9 ± 0.6‰) are indicative of a diet that did not include any significant amount of C_4 plants. Human $\delta^{13}C$ levels are also closer to ovicaprid values, rather than to that of roe-deer, which might indicate a heavier dietary reliance of humans on ovicaprids (which agrees with archaeological data), although the number of animals analysed is insufficient to make definitive conclusions. Human $\delta^{15}N$ values (10.5 ± 1.1‰) are on average 5.3‰ higher than the associated faunal values, which indicates a heavy reliance of the population on animal protein.

Human isotopic results also suggest slight inter-site dietary variations, however, the small number of individuals analysed does not allow assessing the significance of the differences and whether these were due to the diet or climatic variations between the locations.

3.4. $\delta^{34}S$ results

The resulting $\delta^{34}S$ values for humans, herbivores and one of the two analysed modern fish clearly overlap; human sulphur isotope levels vary between 0.9 and 4.1‰, herbivore levels are 2.0 and 2.2‰, and fish levels are 1.4 and -3.4‰ (Table 1; Fig. 3). The two modern fish show an offset in $\delta^{34}S$ of > 4‰, with the Chuya River (Kurai Basin) sample values closer to archaeological samples analysed. However, no significant correlation was found between overall $\delta^{34}S$ against $\delta^{13}C$ and $\delta^{15}N$ values. As such, from the available data, there is no clear $\delta^{34}S$

Table 1
$\delta^{13}C$ and $\delta^{15}N$ collagen values, $C:N_{at.}$ and % collagen yields of the analysed individuals from the Altai region (n = 26).

Lab. ID	$\delta^{34}S$, ‰	$\delta^{13}C$, ‰	$\delta^{15}N$, ‰	$C:N_{at.}$	% coll.	Provenance	Burial; grave goods	Sex, age/species	Bone
SS TSU-12[a]	2.79	−19.5	10.4	3.2	11.7	Ayrydash 1, encl. 15	Single; copper knife	♂, 35–40	Postcranial
SS TSU-1[a]	4.08	−18.5	11.3	3.2	13.5	Elo-Bashi, encl. 3	Double; pottery	♀, 35–40	L. humerus
SS TSU-2[a]	2.10	−19.1	10.2	3.3	5.8	Elo-Bashi, encl. 4	Single; no goods	♂, 30	Long bone
SS TSU-3	2.95	−18.7	11.1	3.2	13.3	Elo-Bashi, encl. 5	Double; pottery	♂, 35–40	Scapula
SS ASU ID 9[a]	–	−18.7	10.7	3.2	10.9	Inskoy Dol, k. 9	Single; no goods	♀ (?), 14–17	Postcranial
SS TSU-4[a]	2.49	−19.2	9.1	3.2	12.5	Kara-Koba 1, encl. 1	Double; pottery	♀, 20–25	L. pubic
SS TSU-5[a]	1.59	−18.7	10.1	3.2	9.7	Kara-Koba 1, encl. 3	Single; no goods	♀, adult	Hip bones
SS TSU-7	2.03	−18.9	10.4	3.2	11.6	Nizhniy Tyumechin 5, circle 2	Single; pottery	♀, 35–40	Vertebrae
SS TSU-8	2.09	−18.8	10.5	3.2	13.1	Nizhniy Tyumechin 1, encl. 9	Double; pottery, staff	♂, 50	Craneo
SS TSU-9	0.89	−19.2	10.2	3.2	10.1	Nizhniy Tyumechin 1, encl. 10	Single; pottery	♀, 50–55	R. humerus
SS TSU-10	2.65	−19.1	10.3	3.2	9.3	Nizhniy Tyumechin 1, encl. 12	Single; pottery	♀, 30–35	Craneo
SS TSU-6[a]	2.60	−18.3	11.3	3.3	4.4	Ozernoye 2, kurgan 1	Single; no goods	♂, 40–50	L. fibula
UBA-31090[a]	–	−18.6	10.9	3.2	13.1	Ozernoye, stone case, skel. 1	Multiple; no goods	♀, 35–55	Craneo
UBA-31091[a]	–	−18.5	10.4	3.2	11.0	Ozernoye, stone case, skel. 2	Multiple; no goods	♀, 35–55	Craneo
UBA-31092[a]	–	−19.2	10.8	3.2	12.9	Ozernoye, stone case, skel. 3	Multiple; no goods	♂, 25–40	Craneo
SS Sal 1.17[a]	–	−18.7	10.0	3.1	4.8	Saldyar 1, encl. 17	Single; pottery, grinder above the grave	♀, 20–25	Vertebra
SS Sal 1.31[a]	–	−19.1	10.2	3.1	3.3	Saldyar 1, encl. 31	Double; pottery, iron	♀, 18–20	Skull
SS Sal 1.36[a]	–	−19.4	10.0	3.1	1.1	Saldyar 1, encl. 36	Triple; ornaments made of animal teeth, fish vertebrae, stone beads etc.	?, 14–17	Skull
SS TSU-11	2.35	−18.8	11.3	3.1	7.9	Tenga 2, kurgan 1	Single; no goods	♂, 25–30	Craneo
PerMezh 1.12h[a]	–	−19.0	10.9	3.1	10.5	Perviy Mezhelik 1, encl. 12	Single; pottery, golden earring, stone ring and other	♂, 16–18	n/a
PerMezh 1.12a[a]	–	−19.3	5.2	3.1	3.3	Perviy Mezhelik 1, encl. 12		Ovicaprid/roedeer	n/a
SS ASU MD-2[a]	1.95	−19.7	4.8	3.3	5.8	Maliy Dugan	n/a	Ovicaprid	n/a
SS ASU MD-3[a]	2.18	−21.1	5.0	3.4	3.0	Maliy Dugan	n/a	Roe-deer	n/a
SS NiSo	–	−19.5	5.8	3.1	10.0	Nizhnyaya Sooru	n/a	Herbivore	n/a
UBA-31085[a,b]	1.4	−26.2	7.9	3.8	18.8	Chuya R., Kurai Basin	n/a	Grayling (*Thymallus thymallus*)	Spine
UBA-31086[a,b]	−3.4	−22.5	5.8	3.8	13.0	Katun R., Uimon Basin	n/a	Grayling (*Thymallus thymallus*)	Spine

[a] Samples subjected to AMS ^{14}C dating (see below).
[b] $\delta^{13}C$ and $\delta^{15}N$ data for modern fish samples have been taken from Svyatko et al., 2017.

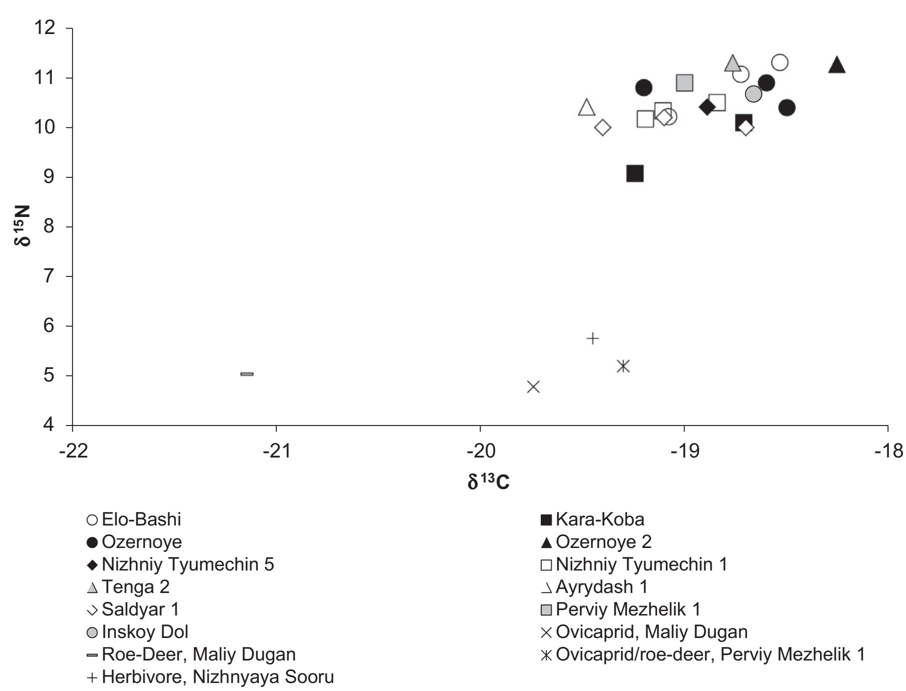

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ isotopic collagen values of the archaeological human (n = 20) and faunal (n = 4) samples from the Altai region.

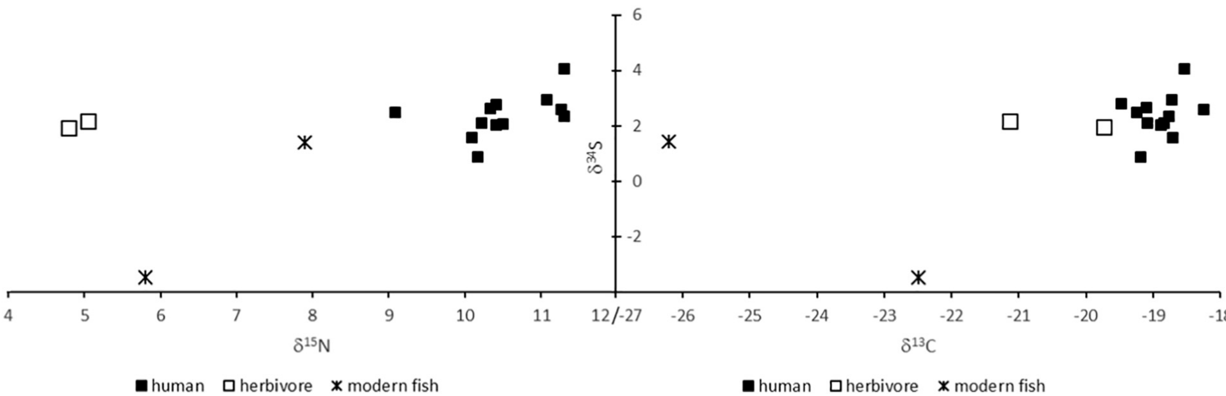

Fig. 3. Plot of $\delta^{34}S$ vs. $\delta^{13}C$ and $\delta^{15}N$ for the archaeological human (n = 12) and faunal (n = 2) samples, and modern fish (n = 2).

isotopic offset between terrestrial and aquatic signatures for the region, and it cannot be concluded whether the diet of the analysed humans included fish.

Furthermore, no significant differences in $\delta^{34}S$ between males and females analysed was found.

3.5. ^{14}C dating results and freshwater reservoir offsets

Among the ^{14}C dated Afanasyevo samples (n = 17; Table 2 and Fig. 4), the majority belongs to the 33rd–29th c. cal BC, two sites – Maliy Dugan and Inskoy Dol – date slightly later, to the 28th–26th c. cal BC. As expected, Karakol Culture site Ozernoye dates to a later period – 25th–22nd c. cal BC; the data do not imply any period of coexistence of the two cultures (although this is based only on three Karakol samples). For the analysed paired sample of associated human and herbivore from Perviy Mezhelik 1, the human bone does not appear to be affected by the reservoir effect. The human ^{14}C age is in fact 84 ^{14}C yrs. younger than that of the associated terrestrial herbivore but the dates are statistically indistinguishable (T' = 3.05, $\chi(0.05) = 3.84$; Ward and Wilson, 1978). A probable reason for the lack of the freshwater reservoir effect in the human sample is the absence of fish in its diet (see the discussion below).

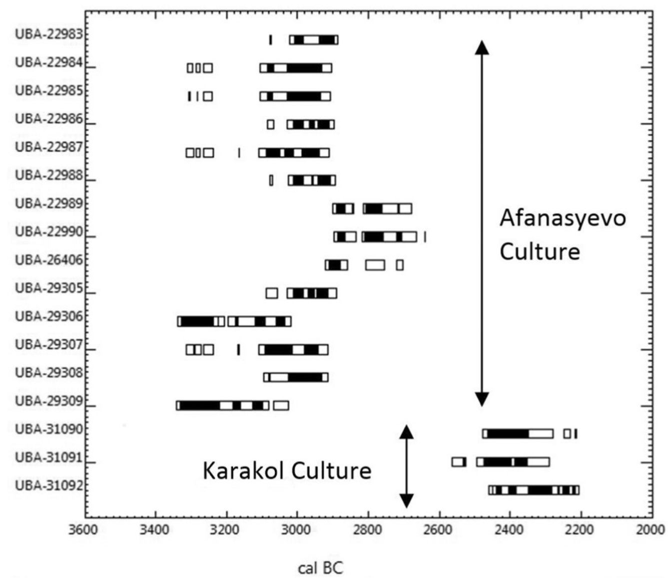

Fig. 4. Calibrated AMS ^{14}C dates of the analysed archaeological samples (n = 17).

Table 2
AMS ^{14}C dates of the analysed archaeological samples (n = 17) and modern fish (n = 2).
(*Data from Svyatko et al., 2017.)

ID	Provenance	Species	^{14}C BP	Calibrated age range (2 σ)
UBA-22983 (SS TSU-1)	Elo-Bashi, enclosure 3	Human	4322 ± 37	3077–2886 cal BC
UBA-22984 (SS TSU-2)	Elo-Bashi, enclosure 4	Human	4392 ± 40	3310–2905 cal BC
UBA-22985 (SS TSU-4)	Kara-Koba 1, enclosure 1	Human	4394 ± 37	3307–2907 cal BC
UBA-22986 (SS TSU-5)	Kara-Koba 1, enclosure 3	Human	4346 ± 35	3084–2895 cal BC
UBA-22987 (SS TSU-6)	Ozernoye 2, kurgan 1	Human	4404 ± 36	3314–2911 cal BC
UBA-22988 (SS TSU-12)	Ayrydash 1, enclosure 15	Human	4336 ± 34	3079–2893 cal BC
UBA-22989 (SS ASU MD 2)	Maliy Dugan	Ovicaprid	4209 ± 34	2900–2678 cal BC
UBA-22990 (SS ASU MD 3)	Maliy Dugan	Roe-deer	4197 ± 36	2896–2641 cal BC
UBA-29305 (SS Sal 1.17)	Saldyar 1, enclosure 17	Human	4344 ± 41	3088–2891 cal BC
UBA-29306 (SS Sal 1.31)	Saldyar 1, enclosure 31	Human	4462 ± 34	3339–3020 cal BC
UBA-29307 (SS Sal 1.36)	Saldyar 1, enclosure 36	Human	4409 ± 34	3315–2915 cal BC
UBA-29308 (PerMezh 1.12 h)	Perviy Mezhelik 1, enclosure 12	Human	4389 ± 33	3095–2913 cal BC
UBA-29309 (PerMezh 1.12a)	Perviy Mezhelik 1, enclosure 12	Ovicaprid/roe-deer	4473 ± 35	3340–3026 cal BC
UBA-31090	Ozernoye, stone case, skel. 1	Human	3900 ± 39	2478–2214 cal BC
UBA-31091	Ozernoye, stone case, skel. 2	Human	3925 ± 42	2565–2289 cal BC
UBA-31092	Ozernoye, stone case, skel. 3	Human	3851 ± 39	2459–2266 cal BC
UBA-26406 (SS ASU ID 9)	Inskoy Dol, kurgan 9	Human	4255 ± 35	2921–2704 cal BC
UBA-31085*	Kurai Basin	Fish modern (grayling)	790 ± 34	n/a
UBA-31086*	Uimon Basin	Fish modern (grayling)	270 ± 34	n/a

4. Discussion

4.1. Overall dietary assessment and consistency with the archaeological and stable isotope record

The main conclusion regarding the diet of the Afanasyevo population of the Altai region from our data is that in the 33rd-26th c. cal BC human diet did not include any significant amount of C_4 plants (such as millet) and relied heavily on animal protein. At the moment, neither nitrogen, nor sulphur isotope results can clearly demonstrate routine consumption of fish. The average δ^{15}N enrichment of humans over the associated herbivores in this study is 5.3‰. The commonly observed human-herbivore nitrogen trophic level increase is usually cited as 3–5‰ (DeNiro and Schoeninger, 1983, Minagawa and Wada, 1984; Bocherens and Drucker, 2003, etc.), with one recent study proposing that human trophic ^{15}N enrichment may be as high as 6‰ (O'Connell et al., 2012). As the human δ^{15}N values in this study are on the borderline of one trophic level offset, we can only speculate whether the diet of humans included fish. This needs to be further assessed in future research. The lack of the freshwater reservoir effect in the human bone from Perviy Mezhelik 1 (see below) might argue for the absence of aquatic component in its diet.

The results suggest the absence of major differences in diets between people from different sites and through time for the later Karakol Culture (cemetery of Ozernoye; 25th-22nd c. cal BC).

The isotopic data generally corresponds with the archaeological and anthropological evidence for the Afanasyevo population of Altai, indicating a largely protein-based diet (Pogozheva et al., 2006). The results are also consistent with previous stable isotope data from other northern Eurasian Steppe Eneolithic – Early to Middle Bronze Age populations (with the obvious exception of the population from the Lake Baikal region, see Katzenberg and Weber, 1999 for the details of this unique ecosystem) in that they indicate isotopically homogeneous diet of the groups, with no clear millet signal until the 14th c. BC for Southern Siberia, particularly the Minusinsk Basin (Svyatko et al., 2013). Despite animal protein, including fish, having played an important dietary role for many of the Eurasian Steppe Eneolithic – Middle Bronze Age inland populations, individuals analysed in the current research are generally low in δ^{15}N and suggest minimal, if any, consumption of fish.

4.2. Local variations in the Afanasyevo economy

The stable isotope analysis of the Afanasyevo individuals from two sites of the Minusinsk Basin to the north-east of the present study area (Svyatko et al., 2013) revealed significantly higher nitrogen values than those for the Altai group (on average, 1.4‰ higher; mean $\delta^{15}N = 10.5 \pm 1.2$‰ and 11.9 ± 1.4‰, respectively; $p < 0.01$ Fig. 5). This difference could hardly be explained by the climatic shifts for the sites where the two populations originate, as the associated herbivores do not show major variations in either δ^{13}C or δ^{15}N values (in all cases $p > 0.05$, data from Altai ($n = 4$) was not sufficient for normality check). The sites are also slightly different chronologically; both Minusinsk Basin cemeteries date to the 29-25th c. BC (Svyatko et al., 2009), while the analysed Altai sites to the 33rd-28th c. BC.

No major shifts in the economy between the various groups of the Afanasyevo people have yet been archaeologically recorded. This study highlights for the first time, the local differences in economy. Nitrogen isotopes demonstrate variations between the groups on their reliance on high-trophic level foods, such as fish. For the Minusinsk Basin groups, this was apparently related to their proximity to major water sources. The site of Afanasyeva Gora was located on the junction of two large rivers (the Yenisey River and its tributary Tashtyg), while Karasuk 3 was further up the smaller Karasuk River (Svyatko et al., 2013); thus, people from the Afanasyeva Gora possibly had better access to fish. Apparently fish did not play an important role for the analysed Altai group, which is somewhat surprising given that the Altai mountain rivers are suitable for fishing. Fish also did not seem to be part of the diet of a later Karakol population (site of Ozernoye); however, several Early Iron Age humans from Ukok Plateau in the Southern Altai yielded higher nitrogen isotopic levels suggesting regular fish consumption (O'Connell et al., 2003).

The importance of fish in the diet of the Eurasian Steppe populations has been often discussed in recent isotopic literature (e.g. Privat et al., 2005; Hollund et al., 2010; Lillie et al., 2011) and it has been summarised that fishing was "taking place practically everywhere in Central Eurasia, particularly during the earlier periods" and that "its importance is also recognised in the ethnographic literature" (O'Connell et al., 2003, p. 253). The presented data from the Altai region demonstrates that, even with the availability of this dietary source, some groups did not use it as a major staple (or perhaps they ate lower trophic level fish). Apparently, variations in the diet were related not only to the local geographical features, such as presence of major water bodies (and indicating the adaptation to various "foodscapes" across the area of Southern Siberia), but also to cultural preferences such as existence of dietary taboos.

4.3. Sulphur isotope analysis

Sulphur isotope analysis was employed for the Altai region for the first time to serve as an additional palaeodietary proxy, specifically for assessing the amount of fish in the human diet. However, the small number of modern freshwater samples, overlapping of the results from archaeological human and terrestrial herbivore samples, and partly modern fish, as well as the lack of relationship with either carbon or nitrogen isotopes means the data is unable to contribute additional information on freshwater fish consumption by humans. The values in humans appear to fit expectations based on mainly terrestrial diets (see e.g. Privat et al., 2007, Nehlich et al., 2010).

The new results for the Altai region contribute to the emerging database of sulphur isotopes in the Eurasian Steppe, currently only represented by the Minusinsk Basin (Svyatko et al., 2016). The latter study also demonstrated the lack of significant variation in δ^{34}S between modern fish, Bronze and Iron Age herbivores and humans analysed (thus also being inconclusive for the palaeodietary reconstructions). The comparison of the two datasets (Fig. 6) shows that Altai samples are slightly higher in sulphur isotope values than those from the Minusinsk Basin, which is likely related to fundamental variations in sulphur isotope ratios in geology of the two regions.

4.4. Dietary variations between the sexes

For the analysed Afanasyevo humans from the Altai region, males on average appear 0.5‰ higher in δ^{15}N values than females, although this is not the case for site by site comparison (the average δ^{15}N values, 10.8 ± 0.8‰ and 10.3 ± 1.2‰, respectively, were used in the comparison; Fig. 7). This difference borders on significant ($p = 0.048$). We can make a preliminary assumption that, within this population, males had a better access to higher-trophic level foods, such as meat (or possibly very minor amounts of fish), compared to females.

Interestingly, the higher frequency of dental enamel hypoplasia, Harris lines, heavy forms of periodontal disease and ante-mortem tooth loss in females in this population has also been explained as the result of protein deficiency (Pogozheva et al., 2006), which was probably related to the differential access for children of different sex to high-protein foods, or the existence of dietary taboos (particularly of meat) for women, also documented ethnographically.

No significant gender-related differences have been observed for the δ^{13}C values ($p > 0.05$). Neither have gender-related differences been detected in either δ^{13}C or δ^{15}N values of the Minusinsk population (Svyatko et al., 2013).

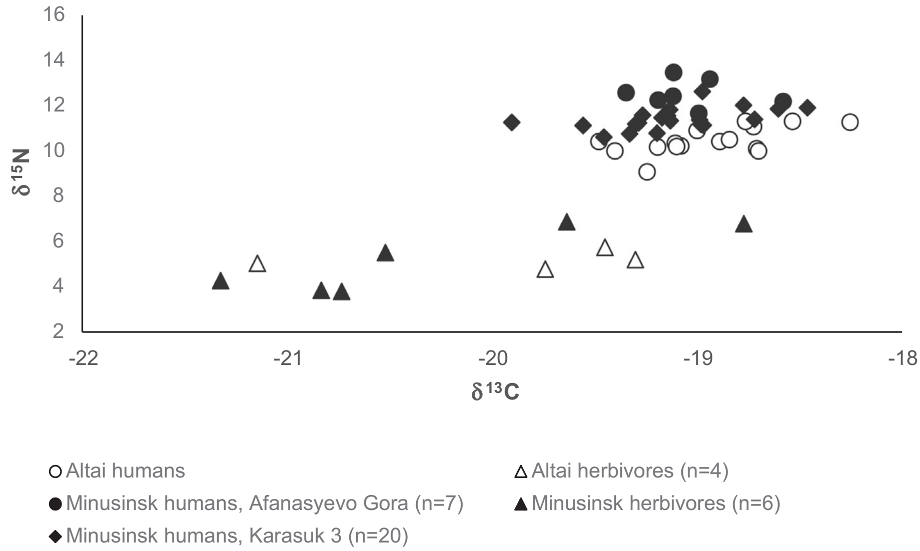

Fig. 5. $\delta^{13}C$ and $\delta^{15}N$ isotopic collagen values of the Afanasyevo human and faunal individuals from the Altai Mountains and Minusinsk Basin.

4.5. Positive correlation between human $\delta^{13}C$ and $\delta^{15}N$ values

Regression analysis reveals a statistically significant positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values of human individuals analysed ($n = 20$; $r = 0.549$; $p = 0.01$). Ecological/climatic variation in the region might have been one of the reasons for the isotopic variation of the human samples. The relationship between the increased aridity and elevated $\delta^{15}N$ of plants and faunal and human samples has been reported for a number of regions in Siberia and the Eurasian Steppe (e.g. Iacumin et al., 2004; Hollund et al., 2010; Shishlina et al., 2012). Generally, $\delta^{15}N$ in soils and plants may vary across a landscape depending on soil moisture (Handley et al., 1999; Chase et al., 2012), and 100 mm difference in the annual precipitation in a region can result in at least ca. 2‰ shift in the $\delta^{15}N$ and ca 1‰ shift in $\delta^{13}C$ of local plants and associated humans/fauna (Hollund et al., 2010; Shishlina et al., 2012 and Chase et al., 2012), although the limited number of herbivore samples in this study do not show this correlation ($n = 4$; $r = 0.368$; $p = 0.63$).

As the study area represents a distinctive patchwork of meadows and mountain forests, the analysed archaeological samples originate from several areas with humidity levels varying between 300 and 400–600 mm (see "Sites and Materials Analysed"). The correlation of carbon and nitrogen isotopes of these individuals against the humidity values for their provenance is very weak for humans ($R^2 = 0.1$, $p = 0.21$ for $\delta^{15}N$; $R^2 = 0.1$, $p = 0.12$ for $\delta^{13}C$) as compared to the four faunal samples ($R^2 = 0.6$, $p = 0.21$ for $\delta^{15}N$; $R^2 = 0.5$, $p = 0.27$ for $\delta^{13}C$). Further analysis of local terrestrial herbivores (both domesticated species being an immediate dietary source for humans despite

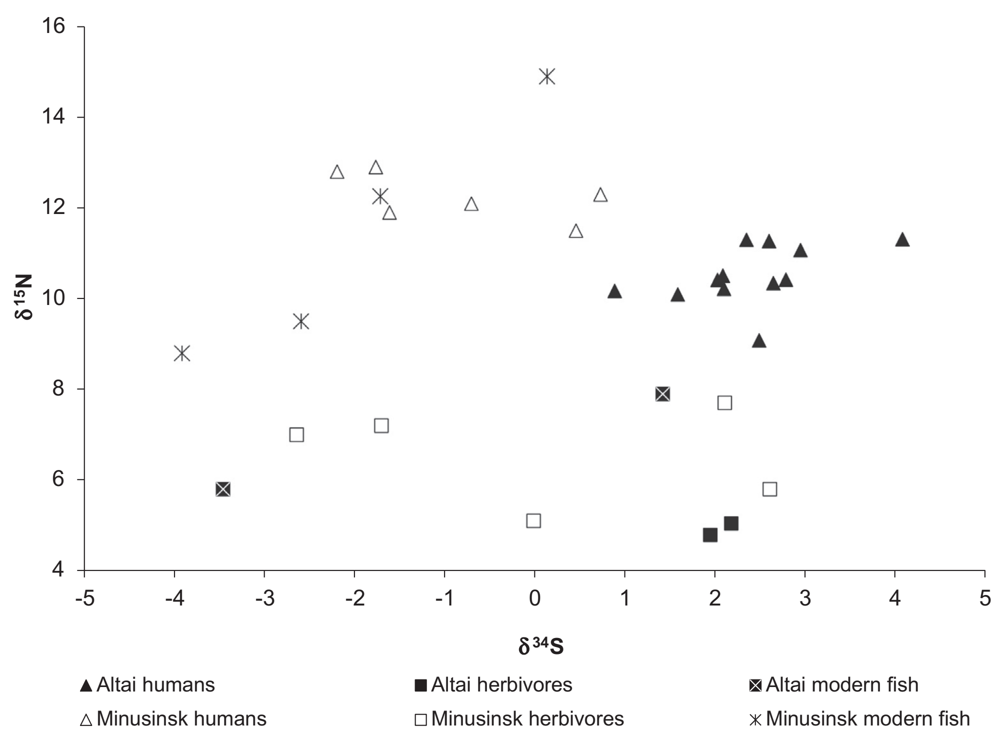

Fig. 6. Bone collagen $\delta^{34}S$ values plotted against $\delta^{15}N$ for the modern fish and archaeological human and terrestrial herbivore samples from Altai (current study) and the Minusinsk Basin (Svyatko et al., 2016).

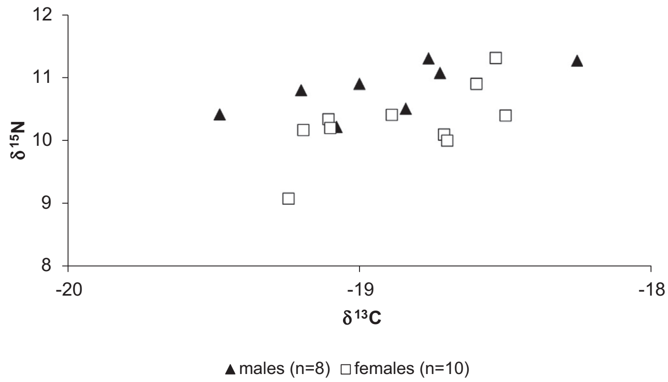

Fig. 7. $\delta^{13}C$ and $\delta^{15}N$ isotopic values of the analysed Altai individuals by sex.

possibly having been involved in transhumance, and wild ones as most precisely reflecting the local humidity) from various sites, as well as palaeoenvironmental data, are essential for supporting this hypothesis. It also needs to be noted, that the burial sites were not necessarily used by the people from a single settlement; individuals recovered from single cemeteries might have lived in various settlements/locations or have migrated to the region.

4.6. Freshwater reservoir effects

Freshwater reservoir effects are a source of dating uncertainty for individuals whose diet include non-atmospheric carbon. The presence of the effect, both modern and archaeological, has been detected for a number of sites across the Eurasian Steppes, with the nearest areas including Lake Baikal (Schulting et al., 2014, 2015; Nomokonova et al., 2013) and the Minusinsk Basin (Svyatko et al., 2016). The ^{14}C age of the modern fish used in the current study was 270 ± 34 BP for the Katun River sample and 790 ± 34 BP for the Chuya River sample, which indicates freshwater reservoir offsets of 578 ± 36 ^{14}C yrs. and 1097 ± 40 ^{14}C yrs., respectively (Svyatko et al., 2017). However, the present research does not confirm the presence of the effect in the archaeological material, as the single associated archaeological human-herbivore pair analysed does not show a statistical difference. The latter implies that the ^{14}C dates from the Afanasyevo humans analysed are likely to show the true (and not apparent) age. A similar situation has been observed for the Minusinsk Basin, with modern fish showing strong reservoir offsets while archaeological humans are identical in ^{14}C age or younger than associated herbivores (Svyatko et al., 2016).

5. Summary

The research highlights the following observations:

- ✓ the diet of the Afanasyevo population from the Altai region was C_3-based with heavy reliance on animal protein; this corresponds with the archaeological evidence and existing stable isotope data for Early Bronze Age populations from the Eurasian Steppe;
- ✓ $\delta^{34}S$ results do not suggest significant $\delta^{34}S$ isotopic offset between terrestrial and aquatic signatures for the region. As such, in our study neither nitrogen, nor sulphur isotope results clearly demonstrate routine consumption of fish by the population;
- ✓ for the first time, isotopic (and as such economic) variations have been detected between the Afanasyevo humans from the Altai region and Minusinsk Basin – these are likely due to variable amounts of fish consumed;
- ✓ the possible reason for slightly higher $\delta^{15}N$ values in the analysed males is their better access to higher-trophic level foods, such as meat, which also agrees with the existing palaeopathological data;
- ✓ the positive correlation between human $\delta^{13}C$ and $\delta^{15}N$ values can possibly be explained by the climatic (aridity) variation in the region;
- ✓ the majority of the Afanasyevo samples radiocarbon date to the 33rd-29th c. cal BC, two sites, Maliy Dugan and Inskoy Dol, date to the 28th–26th c. cal BC. The Karakol Culture site of Ozernoye dates to a later period, 25th-22nd c. cal BC. The data does not imply any period of coexistence of the two cultures but rather attests to their succession;
- ✓ despite very strong modern freshwater reservoir offsets detected in Kurai and Uimon Basins (1097 ± 40 and 578 ± 36 ^{14}C yrs. respectively), the effect has not been observed from the archaeological material.

Further analysis of local archaeological aquatic fauna, as well as a larger sample of humans and specifically terrestrial herbivores from various Altai locations is essential for addressing the issues of fish consumption among humans, and climatic variations in the isotopic results.

Acknowledgements

This work was supported by the Leverhulme Trust (UK, grant RPG-2014-08), Ministry of Education and Science of the Russian Federation (resolution N 220; contract No. 14.Z50.31.0010; also grant “The systems of Environmental Management and Production Technologies of Ancient and Traditional Societies of Altai Mountains”, code 536), and Russian Foundation for Basic Research (grant No. 15-06-01916A). We are also very grateful to Dr. Svetlana S. Tur and Prof. Piotr K. Dashkovskiy (Altai State University, Barnaul, Russia) for their expertise and for providing us with a number of samples.

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