



# A lack of freshwater reservoir effects in human radiocarbon dates in the Eneolithic to Iron Age in the Minusinsk Basin

Svetlana V. Svyatko<sup>1</sup> · Rick Schulting<sup>2</sup> · Andrey Poliakov<sup>3</sup> · Neil Ogle<sup>4</sup> ·  
Paula J. Reimer<sup>1</sup>

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**Abstract** A number of recent studies have highlighted the importance of freshwater reservoir effects (FRE) when dating human remains across large parts of Eurasia, including the Eurasian steppes. Here, we address this question in the context of the Early Bronze Age (Okunevo), Late Bronze Age (Karasuk) and Late Iron Age (Tashtyk culture) of the Minusinsk Basin, Southern Siberia. The issue is important given the large number of radiocarbon dates that have been published on human remains here, which have been used both to refine the cultural historical sequence (Svyatko et al. 2009), as well as to suggest a date of ca. 1400 BC for the appearance of millet agriculture (Svyatko et al. 2013). In these studies, it was argued that there was little or no freshwater reservoir effect to take into account, despite the likely consumption of freshwater fish. Subsequent work across the steppe raised a legitimate question concerning this assumption. Here, we present the first set of paired dates on late prehistoric humans and terrestrial fauna from the Minusinsk Basin, as well as data from modern fish for the region. The results, with one exception, show no clear evidence for a reservoir effect, with the human-fauna difference averaging  $-31 \pm 95$  <sup>14</sup>C years. Yet,

dating of modern fish from the Yenisei River and its tributary Karasuk River does show a variable but significant FRE. Either this effect has changed radically over time, or the contribution of fish to human diets in the Minusinsk Basin was less than previously thought.

**Keywords** Freshwater reservoir effects · Eurasian Steppe · Minusinsk Basin · Okunevo · Karasuk · Tashtyk · Carbon · Nitrogen and sulphur isotope analysis

## Introduction

The Minusinsk Basin of Southern Siberia represents one of the most explored regions in Siberia and in the Eurasian Steppe in terms of radiocarbon chronologies and palaeodietary isotopic analysis of Eneolithic to Early Iron Age populations. The large set of <sup>14</sup>C dates from human remains (more than 100) obtained for the region to date has been used both to refine the cultural historical sequence (Svyatko et al. 2009), as well as to suggest a date of ca. 1400 BC for the appearance of millet as an important element of human diet (ibid.). In these studies, it was argued that there was little or no freshwater reservoir effect (FRE) to take into account, despite the likely consumption of freshwater fish by humans. No solid evidence for absence of the FRE in the region was available at that time, and the assumption of little or no FRE was made based on the lack of relationship between the <sup>14</sup>C age of humans and their  $\delta^{15}\text{N}$  values. Subsequent work across the steppe raised a legitimate concern regarding this assumption, and hence, the reliability of existing radiocarbon dates from human bones and chronologies based on them.

Here, we present new evidence for the extent of modern freshwater reservoir effects in the Minusinsk Basin, as well as the first attempt to assess the influence of the FRE on

✉ Svetlana V. Svyatko  
[s.svyatko@qub.ac.uk](mailto:s.svyatko@qub.ac.uk)

<sup>1</sup> <sup>14</sup>CHRONO Centre for Climate, the Environment, and Chronology, Queen's University of Belfast, Belfast, Northern Ireland BT7 1NN, UK

<sup>2</sup> School of Archaeology, University of Oxford, 36 Beaumont Street, Oxford, England OX1 2PG, UK

<sup>3</sup> Institute for the History of the Material Culture, Russian Academy of Sciences, 18 Dvortsovaya nab., St Petersburg 191186, Russia

<sup>4</sup> Stable Isotope Facility, School of Natural and Built Environment, The Queen's University of Belfast, Belfast, Northern Ireland BT9 5AG, UK

archaeological human remains from the region through paired accelerator mass spectrometry (AMS)  $^{14}\text{C}$  dates from late prehistoric humans and associated terrestrial fauna from the same burial contexts.

### The FRE in Eurasia

Recently, a number of studies have been focused on the variability of freshwater reservoir effects and their effect on chronology of the prehistoric populations and events. The FRE occurs when part of the carbon in an individual's diet comes from a freshwater source with a lower-than-atmospheric  $^{14}\text{C}$  concentration, and this results in an offset (i.e. "older")  $^{14}\text{C}$  age for such a sample compared to  $^{14}\text{C}$  age of a contemporaneous purely terrestrial sample. One source of "old" carbon in freshwater is dissolved inorganic carbon from  $^{14}\text{C}$ -free carbonate minerals in groundwater (e.g. Godwin 1951; Deevey et al. 1954; Sveinbjörnsdóttir et al. 1995; Culleton, 2006). In the process of underwater photosynthesis, this carbon becomes incorporated into aquatic plants and algae, and is then transferred further up the food chain to aquatic fauna and terrestrial animals (including humans) that rely on aquatic sources. Freshwater reservoir offsets (FRO) can be highly variable within the reservoir depending on the type and age of the organism analysed, which is related to its particular habitat and diet (e.g. Fernandes et al. 2013). For example, because of greater carbon exchange between the atmosphere and the water, fish and shellfish living in shallow or well-mixed water will be affected by FRE to a lesser extent than bottom feeders in deep water. The FRE can also vary through time as a result of changes in the hydrological system (e.g. Ascough et al. 2010) or the reservoir or, presumably, climatic conditions, leading to the thawing of permafrost and the entry of "old" organic carbon into local reservoirs (see discussion in Schulting et al. 2015). The latter could be a factor in the Minusinsk Basin, as permafrost is present in Western and Eastern Sayan mountains, surrounding the depression.

The majority of FRE research has focussed on Europe (Cook et al. 2001, 2002; Fischer and Heinemeier 2003; Olsen et al. 2010; Keaveney and Reimer 2012; Loughheed et al. 2013; Fernandes et al. 2014; Meadows et al. 2016) and North America (Ingram and Southon 1996; Goodfriend and Flessa 1997; Culleton 2006), and only recently has the importance of freshwater reservoir effects been highlighted for Russia and the Eurasian steppe zone, including the upper Lena River and Lake Baikal region (Nomokonova et al. 2013; Schulting et al. 2014, 2015), Caspian steppes and lower Don River (Shishlina et al. 2007, 2009, 2012, 2014; Motuzaitė-Matuzevičiute et al. 2015), middle and lower reaches of the Dnieper River (Lillie et al. 2009), North-Eastern Kazakhstan (Svyatko et al. 2015), Sertejka River in Smolensk Oblast (Kulkova et al. 2015) and Kubenskoye Lake in Vologda Oblast (Wood et al. 2013). The main finding from

this research is that freshwater reservoir effects are extremely variable geographically (spanning from zero to several thousand years), and that when dealing with archaeological human remains "each population thought to be affected by a FRE must be examined individually" (Wood et al. 2013, p. 163). Here, we apply this admonition to the Late Bronze and Iron Age populations of the Minusinsk Basin.

### The Minusinsk Basin

The Middle Yenisei River region represents a group of isolated intermountain basins surrounded by the Kuznetsk Alatau mountain range, and by the Western and Eastern Sayan mountains. The combination of a rich hydrological system, vast steppes and climatic conditions are virtually ideal for stock rearing which became the basis of the economy for the ancient populations in the area from the Eneolithic, supported by hunting and fishing (Vadetskaya 1986).

Funerary sites prevail in the archaeology of the Middle Yenisei; this possibly explains the absence of remains associated with fishing. The clear exception are the sites of the Okunevo culture (twenty-fifth to eighteenth century BC) which have yielded tools associated with fishing (spears, needles for making nets, stone sinkers and fishing hooks; Vadetskaya et al. 1980; Lazaretov 1997; Kovalev 1997; Gotlib and Podolsky 2008) hinting at the possibility of an important role for fishing in the economy. No fishing tools have been found in the Karasuk (thirteenth to ninth century BC) and Tashtyk (first to sixth century AD) sites which may be explained by the absence of the tradition of offering utilitarian tools as grave goods (e.g. Vadetskaya 1986). Fish remains have only been recovered from one Karasuk and one Tashtyk site (Vadetskaya and Poselyanin 2015).

## Materials and methods

### Materials

In total, four modern fish from two sites and five groups of associated archaeological samples ( $n = 14$ ) from four sites have been analysed, including six humans, five animals, and three wood samples (Tables 1, 2).

Archaeological materials were collected from the following sites (Fig. 1):

1. Uibat-Charkov (excavations by I. P. Lazaretov, 2009)—an Okunevo culture (middle of third—first half of second mil. BC) cemetery which consists of three kurgans located 1 km NW from Charkov village (Ust-Abakanskiy region, Khakassia Republic), on a terrace above the Uibat River, in the piedmont of the Khazynsorakh mountains. Kurgan 1 contained 16 Okunevo and one Tashtyk grave. Okunevo grave 11 was located in the SW sector and contained the

**Table 1** Results of AMS  $^{14}\text{C}$  dating, stable C and N isotope analysis and calculated freshwater reservoir offsets (FRO) for modern fish

| Lab ID    | Species                           | Provenance    | % coll. | $F^{14}\text{C} \pm \sigma F^{14}\text{C}$ | FRO ( $^{14}\text{C}$ years) | $\text{C}/\text{N}_{\text{at}}$ | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ | $\delta^{34}\text{S}$ |
|-----------|-----------------------------------|---------------|---------|--|------------------------------|---------------------------------|-----------------------|-----------------------|-----------------------|
| UBA-29395 | Adult pike ( <i>Esox lucius</i> ) | Karasuk Bay   | 13.7    | $0.9614 \pm 0.0032$                        | $757 \pm 31$                 | 3.2                             | -23.9                 | 12.3                  | -1.71                 |
| SS MBF-1  |                                   |               | n/a     | —  | —                            | 3.2                             | -23.2                 | 12.4                  |                       |
| UBA-29396 | Small pike ( <i>Esox lucius</i> ) | Karasuk Bay   | 9.1     | $0.976 \pm 0.0033$                         | $636 \pm 31$                 | 3.5                             | -26.1                 | 12.6                  | 0.14                  |
| SS MBF-2  |                                   |               | n/a     | —  | —                            | 3.3                             | -25.3                 | 14.9                  |                       |
| UBA-29397 | Carp ( <i>Cyprinus carpio</i> )   | Karasuk Bay   | 19.9    | $1.0041 \pm 0.0032$                        | $408 \pm 30$                 | 3.1                             | -24.2                 | 8.9                   | -3.91                 |
| SS MBF-3  |                                   |               | n/a     | —  | —                            | 3.7                             | -23.1                 | 8.8                   |                       |
| UBA-29398 | Sazan ( <i>Cyprinus carpio</i> )  | Yenisei River | 13.1    | $1.0349 \pm 0.0033$                        | $165 \pm 30$                 | 3.2                             | -25.3                 | 9.3                   | -2.59                 |
| SS MBF-4  |                                   |               | n/a     | —  | —                            | 3.2                             | -23.9                 | 9.5                   |                       |

Note that fish samples underwent different pretreatment. For samples with lab IDs SS MBF, the  $\delta^{15}\text{N}$  values were taken from the sample not subjected to lipid removal, and the  $\delta^{13}\text{C}$  value was obtained by taking the carbon isotopic value of the sample which had undergone the lipid removal process and adding 1.5 ‰ for the Suess effect (see [Sample Pretreatment](#) section for details)

n/a not applicable

skeletons of a 30-year-old female (UBA-31076) and 12–14-year-old male (UBA-31077), two pots, a stone scraper, 14 sheep astragali (UBA-31075), a bronze awl, and a white perforated stone ball. The wood from the burial structure has also been sampled (UBA-31074).

2. Krasniy Kamen (excavations by I. P. Lazaretov, 2010) represents two kurgans of Okunevo culture located 2 km N of Krasniy Kamen village (Bogradskiy region, Khakassia Republic), on the left slightly sloping terrace of the Koksa River, 500 m from its bed. Kurgan 1 contained 12 graves. Grave 1 is located in the very centre of the enclosure and it represents the first burial of the kurgan. The grave had been plundered, but the remaining finds show that the grave held the remains of a 20–25-year-old female (UBA-31072), interred with, at minimum, a ceramic pot, 37 perforated deer teeth (UBA-31073), a bone comb and 36 stone beads and one bone bead.
3. Tes 9 (excavations by O. V. Kovaleva, 2006)—a Karasuk culture (thirteenth to ninth century BC) cemetery consisting of more than 500 kurgans located 3.5 km W from Tes village (Minusinsk region, Krasnoyarsk Krai), on the slope of Georgievskaya mountain in the valley of the Tuba River. Kurgan 50 is one of the largest, containing only one grave located in the very centre. The burial had been disturbed and it contained pottery fragments, human (45–50-year-old male, UBA-31311, and fragments of skull of another male apparently from a different grave) and animal (including cattle and sheep, UBA-31312) bones and a bronze temple ring.
4. Abakan 8 (excavations by P. B. Amzarakov, 2014–2015)—a multi-period cemetery located in a modern construction site in the central part of Abakan City. Kurgan 1 belongs to the Podgornovo phase (eighth to sixth century BC) of the Tagar culture, and it contained an intrusive burial of the succeeding Tashtyk culture (first to fifth century AD). As such, the kurgan held the remains of a

disturbed intrusive burial (wood, human and faunal remains sampled, UBA-31078-31080), and disturbed original earth grave containing a mummified inhumation and three cremations. Excavation 5 represents an area of ca. 500 m<sup>2</sup> in the central part of the construction site, encompassing a mainly nineteenth century cemetery and at least 32 prehistoric burials. Grave 30 represented a 2.85 × 2 m timber burial orientated to the W covered with bark. The grave had been plundered and it contained two partially disarticulated inhumations with trepanned skulls (skeleton 1 has been sampled, UBA-31082), one cremation, three ceramic pots, bone awl, animal bones (UBA-31083), golden foil and copper fragment. Wood from the burial structure has also been sampled (UBA-31081).

Notably, three of four analysed burials were disturbed. For the area of Minusinsk Basin, intact burials are generally very rare; however, in these cases, the association of human and faunal remains is considered to be reasonable.

Modern fish were collected from local fishermen in two locations, from the mouth of the Tuba River (Yenisei tributary) near Tepsei mountain (UBA-29398), and from the mouth of Karasuk River (Karasuk Bay, another tributary of Yenisei, UBA-29395-29397; Fig. 1). In all cases, the fish were cooked prior to sampling the bones (spine and ribs) for analysis. We do not expect this to have had a significant effect on the  $^{14}\text{C}$  dates.

### Sample pretreatment

Sample pretreatment was performed in the  $^{14}\text{C}$  CHRONO Centre for Climate, the Environment and Chronology (Queen's University Belfast). For bone samples, the surfaces were cleaned prior to collagen extraction. The extraction of collagen from archaeological samples was based on the ultra-filtration method (Brown et al. 1988; Bronk Ramsey et al.

**Table 2** Results of AMS <sup>14</sup>C dating, stable C and N isotope analysis and calculated freshwater reservoir offsets (FRO) for archaeological samples

| Lab ID    | Species <sup>a</sup>         | Provenance                    | Culture | % coll. | <sup>14</sup> C age (BP) | Cal age          | FRO<br>( <sup>14</sup> C years) | Pooled<br>mean<br><sup>14</sup> C age | χ <sup>2</sup> test    | C:N <sub>at</sub> | δ <sup>13</sup> C | δ <sup>15</sup> N | δ <sup>34</sup> S |
|-----------|------------------------------|-------------------------------|---------|---------|--------------------------|------------------|---------------------------------|---------------------------------------|------------------------|-------------------|-------------------|-------------------|-------------------|
| UBA-31072 | Human (♀, 20–25 years old)   | Krasnyy Kamen,<br>k. 1, g. 1  | Okunevo | 12.7    | 3777 ± 41                | 2339–2040 cal BC | -78 ± 59                        | 3815 ± 30                             | T = 1.8(5 % 3.8)       | 3.2               | -18.4             | 11.5              | 0.46              |
| UBA-31073 | Deer (tooth)                 |                               | Okunevo | 15.3    | 3855 ± 42                | 2462–2205 cal BC |                                 |                                       |                        | 2.2               | -19.1             | 5.1               | -0.01             |
| UBA-31074 | Wood                         | Uibat-Charkov,<br>k. 1, g. 11 | Okunevo | —       | 3889 ± 33                | 2470–2236 cal BC |                                 | 3927 ± 25                             | T = 3.0(5 % 6.0)       | —                 | —                 | —                 | —                 |
| UBA-31075 | Sheep                        |                               | Okunevo | 8.5     | 3971 ± 40                | 2578–2346 cal BC |                                 |                                       |                        | 3.2               | -19.3             | 5.8               | 2.61              |
| UBA-31076 | Human 1 (♀, 30 years old)    |                               | Okunevo | 3.5     | 3924 ± 39                | 2562–2292 cal BC | -47 ± 56                        |                                       |                        | 3.2               | -18.7             | 12.1              | -0.7              |
| UBA-31077 | Human 2 (♂, 12–14 years old) |                               | Okunevo | 12.6    | 3861 ± 50                | 2470–2154 cal BC | -110 ± 64                       |                                       |                        | 3.2               | -18.3             | 12.3              | 0.73              |
| UBA-31311 | Human (♂, 45–50 years old)   | Tes 9, k. 40, g. 1            | Karasuk | 12.3    | 2880 ± 51                | 1210–924 cal BC  | -81 ± 64                        | 2931 ± 31                             | T = 1.6(5 % 3.8)       | 3.2               | -18.2             | 11.9              | -1.61             |
| UBA-31312 | Ovicaprid                    |                               | Karasuk | 7.4     | 2961 ± 39                | 1287–1041 cal BC |                                 |                                       |                        | 3.2               | -19.3             | 7.2               | -1.7              |
| UBA-31078 | Wood                         | Abakan 8, k. 1                | Tashyk  | —       | 1908 ± 30                | 23–209 cal AD    |                                 |                                       |                        | —                 | —                 | —                 | —                 |
| UBA-31079 | Ovicaprid                    |                               | Tashyk  | 17.8    | 1744 ± 34                | 220–391 cal AD   | -31 ± 50                        | 1729 ± 25                             | T = 0.4(5 % 3.8)       | 3.2               | -19.5             | 7.0               | -2.64             |
| UBA-31080 | Human (♀, <i>senilis</i> )   |                               | Tashyk  | 16.2    | 1713 ± 36                | 244–400 cal AD   |                                 |                                       |                        | 3.2               | -15.6             | 12.8              | -2.19             |
| UBA-31081 | Wood                         | Abakan 8, exc. 5,<br>g. 30    | Tashyk  | —       | 1703 ± 31                | 252–404 cal AD   |                                 |                                       |                        | —                 | —                 | —                 | —                 |
| UBA-31082 | Human (sk. 1; ♂, young)      |                               | Tashyk  | 14.6    | 1893 ± 39                | 27–227 cal AD    | 154 ± 54                        | 1813 ± 27                             | T = 8.2(5 % 3.8) fails | 3.2               | -15.1             | 12.9              | -1.76             |
| UBA-31083 | Animal                       |                               | Tashyk  | 16.1    | 1739 ± 37                | 218–396 cal AD   |                                 |                                       |                        | 3.2               | -19.6             | 7.7               | 2.11              |

The alpha level for the chi<sup>2</sup> test is 0.05

<sup>a</sup> For humans, sex and age determinations were undertaken by N.I. Lazaretova (personal communication)

2004) with an additional sodium hydroxide (NaOH) wash step (Brock et al. 2010). Briefly, these included the following:

- Bone demineralization in 2 % HCl, followed by MilliQ® ultrapure water wash
- 0.1 M NaOH treatment for 15 min to remove humic acids, followed by MilliQ® wash
- 2 % HCl wash for 15 min, followed by MilliQ® wash
- Gelatinization in pH = 2 HCl at 58 °C for 16 h
- Filtration, using ceramic filter holders, glass filter flasks and 1.2-μm glass microfiber filters
- Ultrafiltration using Vivaspin® 15S ultrafilters with MWCO 30 kDa; 3000–3500 rpm for 30 min and
- Freeze-drying; the dried collagen was stored in a desiccator

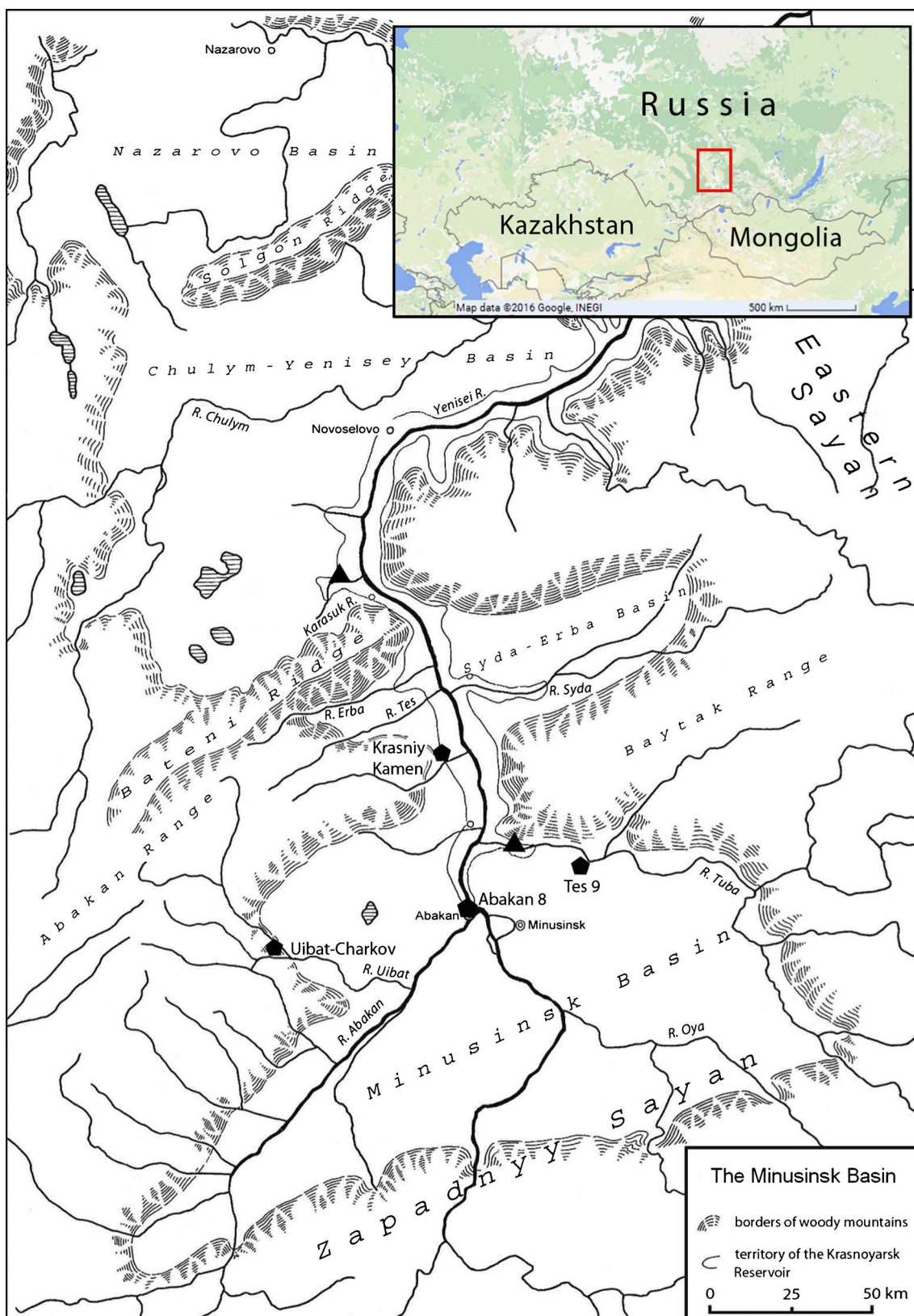
Modern fish bones were pre-treated for stable isotope analysis and <sup>14</sup>C dating at different times. For this reason, the pre-treatment procedure varied. For <sup>14</sup>C dating and sulphur isotopic measurements (lab IDs UBA), the procedure above was followed for pretreatment of modern fish bones, but omitting the NaOH step, as modern fish would not contain humic acids commonly affecting archaeological bones. For carbon and nitrogen stable isotope analysis (lab IDs SS MBF), two samples of collagen were prepared for each fish specimen so that the stable carbon (δ<sup>13</sup>C) and nitrogen (δ<sup>15</sup>N) values could be measured separately. The first sample of each fish was subjected to the lipid removal process following Bligh and Dyer (1959), as lipids are significantly lower in <sup>13</sup>C and thus the measured signal will not correspond to that for protein (e.g. Liden et al. 1995; Sotiropoulos et al. 2004; Mintenbeck et al. 2008). Afterwards, the process of collagen extraction described above (excluding the NaOH step) was applied to all samples. The δ<sup>15</sup>N values of the fish (introduced in Table 1 and Fig. 2) represent the sample not subjected to lipid removal, as this process is known to potentially alter nitrogen isotopic values (e.g. Sotiropoulos et al. 2004); the final δ<sup>13</sup>C values represent the sample which had undergone the lipid removal process plus 1.5 ‰ (as suggested by Tieszen and Fagre 1993) to offset for post-industrialisation changes in the atmospheric reservoir due to fossil fuel burning (the Suess effect).

Wood pretreatment follows the standard AAA procedure (Mook and Waterbolk 1985) and included 4 % HCl wash on an 80 °C hotplate for 2–3 h followed by 2 % NaOH wash for 2 h, and then by another 4 % HCl wash on an 80 °C hotplate for 2–3 h.

### Stable isotope analysis and AMS radiocarbon dating

Bone collagen stable carbon and nitrogen isotopes were measured in duplicate on a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) coupled to a Thermo Flash 1112 Elemental Analyzer (EA) peripheral in the <sup>14</sup>CHRONO





**Fig. 1** Location map for the investigated sites (polygons for archaeological samples and triangles for modern fish); adapted from Vadetskaya 1986

Centre for Climate, the Environment and Chronology (Queen’s University Belfast). The measurement uncertainty

(1sd) of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and atomic C:N ( $\text{C:N}_a$ ) based on 6–10 replicates of seven archaeological bone collagen samples was

0.22, 0.15 and 0.2 ‰, respectively. Sulphur isotopic measurements ( $\delta^{34}\text{S}$ ) were performed in triplicates using a Thermo Delta V IRMS with Thermo Flash 1112 EA in the Stable Isotope Facility of the School of Planning, Architecture and Civil Engineering (Queen's University Belfast), with the analytical precision of  $\pm 0.4$  per mil based on the standards analysed (NBS127 and IAEA-SO6). Results are reported using the delta convention relative to international standards: VPDB for  $\delta^{13}\text{C}$ , AIR for  $\delta^{15}\text{N}$  and VCDT for  $\delta^{34}\text{S}$  (Krouse and Coplen 1997; Hoefs 2009).

AMS  $^{14}\text{C}$  dating was performed in the  $^{14}\text{C}$ CHRONO Centre. For the AMS  $^{14}\text{C}$  measurements, prepared bone collagen and wood samples were sealed under vacuum in quartz tubes with an excess of CuO and combusted at 850°C. The  $\text{CO}_2$  was converted to graphite on an iron catalyst using a zinc reduction method (Slota et al. 1987). Pressed graphite “target” was then measured by 0.5 MV National Electrostatics Compact AMS. The sample  $^{14}\text{C}/^{12}\text{C}$  ratio was background corrected and normalised to the HOXII standard (SRM 4990C; National Institute of Standards and Technology). The  $^{14}\text{C}/^{12}\text{C}$  ratio, corrected for isotopic fractionation using the AMS-measured  $\delta^{13}\text{C}$ , is by definition fraction modern ( $F^{14}\text{C}$ ; Reimer et al. 2004). The  $^{14}\text{C}$  age and 1sd were calculated from  $F^{14}\text{C}$  using the Libby half-life (5568 years) following the conventions of Stuiver and Polach (1977). The radiocarbon ages were calibrated using the Calib 7.0 program (Stuiver et al. 2013) and the IntCal13 calibration curve (Reimer et al. 2013).

### Calculating the freshwater reservoir offset

For both archaeological and modern samples, FRO was calculated as a difference in the  $^{14}\text{C}$  ages between the “aquatic” (human/fish) and terrestrial (faunal/atmosphere) samples. Atmospheric  $^{14}\text{C}$  age and  $^{14}\text{C}$  age for the modern fish samples (conventionally given as >modern) were calculated using the following equation:  $^{14}\text{C}$  age =  $-8033 \ln F^{14}\text{C}$ .  $F^{14}\text{C}_{\text{atm}}$  for 2007 was taken as a mean of the monthly  $^{14}\text{C}_{\text{atm}}$  measurements for 2007 from Levin et al. (2013).  $^{14}\text{C}$  age uncertainties for the modern samples were calculated using the following formula:  $\sigma^{14}\text{C} = -8033 \times \ln(F^{14}\text{C} + \sigma F^{14}\text{C} - (-8033 \times \ln(F^{14}\text{C})))$  for each sample. FRO uncertainty was calculated using  $\sigma\text{FRO} = \sqrt{\sigma a^2 + \sigma b^2}$ , where  $\sigma a$  and  $\sigma b$  are  $^{14}\text{C}$  age uncertainties for aquatic (human and modern fish) and terrestrial (fauna and atmosphere) samples.

### Results

The majority of bone samples analysed demonstrated excellent collagen preservation with yields ranging between 3.5–19.9 % (van Klinken 1999; Tables 1 and 2). The C:N<sub>at</sub> ratio of the samples varied between 3.1 and 3.5, which is also within

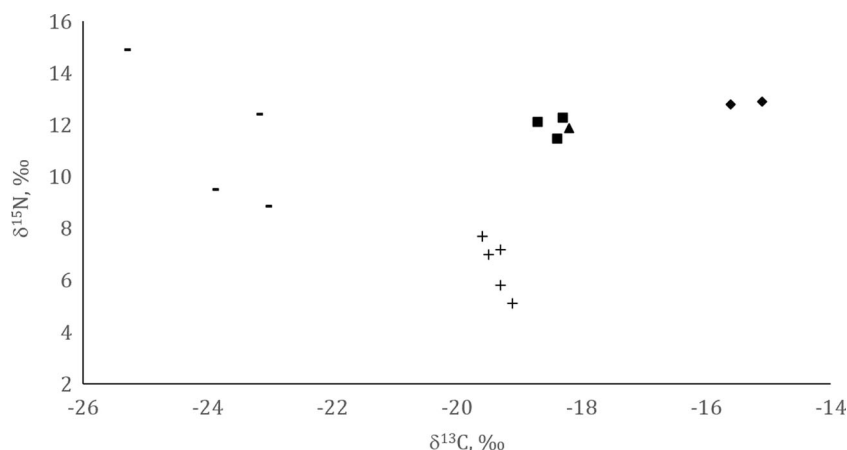
the accepted range characterizing well-preserved collagen (DeNiro 1985). At 3.7, the modern fish fell just outside this range, perhaps as a result of the retention of a small amount of lipid. This would not affect the  $^{14}\text{C}$  date.

Several observations can be made from the results of radiocarbon dating and stable isotope analysis of the samples. Firstly, in all cases, modern fish demonstrate a reservoir offset, with values differing significantly from  $165 \pm 30$  to  $757 \pm 31$   $^{14}\text{C}$  years (Table 1). The FRO values vary both between different reservoirs (with the tributary Karasuk having a higher offset compared to that of the main Yenisei River, although the latter is represented by only a single sample) and within the reservoir between fish depending on their species (the two pikes having the highest offsets) and age (larger pike being ca. 120  $^{14}\text{C}$  years “older” than the smaller one). Given the small sample size, the authenticity of these patterns needs to be further verified. Obviously, modern reservoirs in the Minusinsk Basin contain “old” carbon; however, its particular sources remain unclear at the moment.

Secondly, and surprisingly, archaeological humans do not appear to be affected by the FRE (Table 1). With one exception, the human radiocarbon determinations fall within 1sd of those of the associated terrestrial fauna within the same grave. The exception is grave 30 from excavation 5 of the Abakan 8 cemetery, where there is an offset of  $154 \pm 54$   $^{14}\text{C}$  years between the human and terrestrial samples. The association of faunal bones and timber with the interred skeleton is quite secure. This burial had been disturbed, however, very “carefully” as skeletons reportedly remained in articulation. The animal bone was located at the feet of the deceased, and its date can be successfully combined (Ward and Wilson 1978) with the date of timber used for the construction of the grave. Admittedly, among the studied sites, Abakan 8 cemetery is the only one located on the shore of the main Yenisei River while other sites are situated near its tributaries. One could suggest geographical variations in the extent of the FRE, with the main Yenisei waters carrying a larger FRE signal; however, it is then unclear why another human from the same cemetery (kurgan 1) is not affected, despite having similar  $\delta^{15}\text{N}$  values, which presumably reflect comparable contributions of high-trophic-level foods, i.e. fish.

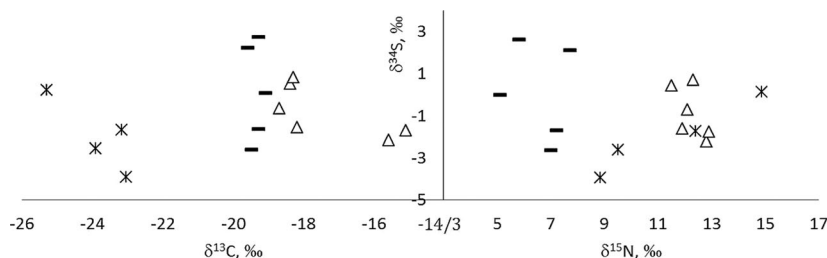
For stable nitrogen isotope values, the enrichment of human samples over mean terrestrial fauna  $\delta^{15}\text{N}$  values ( $6.6 \pm 2.1$  ‰) varies between 4.9 and 6.3 ‰ (with an average of  $5.7 \pm 1.1$  ‰; Fig. 2). The commonly observed human-herbivore nitrogen trophic level increase is usually cited as 3–5 ‰ (Minagawa and Wada 1984; Bocherens and Drucker 2003; DeNiro and Schoeniger 1983, etc.), with one recent study proposing that human trophic  $\delta^{15}\text{N}$  enrichment may be as high as 6 ‰ (O'Connell et al. 2012). As such, the human  $\delta^{15}\text{N}$  values in this study are on the borderline of one trophic level offset, and we can only speculate if the diet of humans included fish. Human-herbivore  $\delta^{15}\text{N}$  enrichment has a weak

**Fig. 2** Bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for archaeological human ( $n = 6$ ) and terrestrial herbivore samples ( $n = 5$ ), and modern fish ( $n = 4$ ). For fish samples (lab IDs SS MBF), note that the  $\delta^{15}\text{N}$  values were taken from the sample not subjected to lipid removal; the final  $\delta^{13}\text{C}$  value was obtained by taking the carbon isotopic value of the sample which had undergone the lipid removal process and adding 1.5 ‰ for the Suess effect (see [Sample Pretreatment](#) section for details)



to moderate relationship, but statistically insignificant, with human FRO values ( $R^2 = 0.45$ ;  $p = 0.13$ ). In fact, more of the associated faunal samples are actually “older” than the humans, though not significantly so, since all of these pairings were statistically the same (Table 2). The exception is again the aforementioned grave 30 at Abakan 8—this human individual is the single case showing a freshwater reservoir offset and it has the highest  $\delta^{15}\text{N}$  enrichment compare to other humans analysed ( $\Delta\delta^{15}\text{N} = 6.3$  ‰).

To further investigate the potential impact of aquatic sources in the human diet, stable sulphur isotopes were employed (Richards et al. 2003). The technique has not been routinely used in the region, and, to the best of our knowledge, only one study has been performed in the Eurasian Steppes, namely, at the Late Bronze Age site of Chicha (southwestern Siberia; Privat et al. 2007). The study demonstrated the potential of using  $\delta^{34}\text{S}$  in the area as an additional indicator for freshwater fish consumption, as a pronounced difference between terrestrial and aquatic signals was found. However, the  $\delta^{34}\text{S}$  results for the Minusinsk Basin do not appear to significantly differ from modern fish, archaeological herbivores and humans analysed (in all cases  $p > 0.05$ ; Tables 1 and 2; plotted against  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Fig. 3), and thus cannot contribute additional information on freshwater fish consumption by humans.



**Fig. 3** Bone collagen  $\delta^{34}\text{S}$  values plotted against  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for archaeological human ( $n = 6$ ) and terrestrial herbivore samples ( $n = 5$ ) and modern fish ( $n = 4$ ). For fish samples (lab IDs SS MBF), note that the  $\delta^{15}\text{N}$  values were taken from the sample not subjected to lipid removal; the final  $\delta^{13}\text{C}$  value was obtained by taking the carbon isotopic value of

Samples of wood were used in the study as an additional terrestrial reference; however, bearing in mind the possibility of “old wood effect” or potential re-use of wood, the results are treated separately from faunal samples. In two of three cases,  $^{14}\text{C}$  dates from wood are consistent with those for terrestrial fauna. Only for the kurgan 1 of Abakan 8 cemetery, is the wood sample approximately 160  $^{14}\text{C}$  years older than associated ovicaprid bone sample. The reason for the older wood age is that, due to the disintegration of the outer wood, the sample was taken from central part of what was apparently originally a very large log.

## Discussion and conclusions

The main observation from this study is that, with a single exception, and despite the FRE values observed for modern fish (from  $165 \pm 30$  to  $757 \pm 31$   $^{14}\text{C}$  years), archaeological humans do not appear to be significantly affected by the FRE. These are preliminary conclusions, which need to be checked by additional pairs of contemporaneous human and terrestrial samples.

The results for the graves are in agreement with the major previous conclusions on chronology and subsistence of Okunevo and Karasuk populations. The new  $^{14}\text{C}$  dates

the sample which had undergone the lipid removal process and adding 1.5 ‰ for the Suess effect (see [Sample Pretreatment](#) section for details). The  $\delta^{34}\text{S}$  values were taken from the sample also not subjected to lipid removal (lab IDs UBA)



(twenty-sixth to twenty-first century BC for Okunevo, thirteenth to tenth century BC for Karasuk, and third—early fifth century AD for Tashtyk individuals) support the existing chronology attributing Okunevo culture to twenty-fifth to eighteenth, Karasuk—to the fifteenth to ninth century BC, and Tashtyk—to the third—early fifth century AD (Svyatko et al. 2009; Pankova et al. 2010; Zaitseva et al. 2007).

The isotopic results also correspond well with the previously identified patterns of Bronze to Iron Age subsistence in the region, according to which the diet of the Eneolithic to Middle Bronze Age populations was primarily  $C_3$ -based and included large proportions of animal protein (and possibly fish), and only from the Late Bronze Age are  $C_4$  plants, most likely millet (as no other  $C_4$  plants suitable for a dietary staple are known in the area), incorporated into the diet in a systematic, isotopically detectable way (Svyatko et al. 2013). The Late Bronze Age Karasuk individual analysed here does not show  $\delta^{13}C$  enrichment, but this is consistent with previous results showing that this period was highly variable in the contribution of  $C_4$  plants, as was the succeeding Early Iron Age Tagar (ibid.). Elevated  $\delta^{13}C$  values of ca.  $-15.3\text{‰}$  for the Late Iron Age Tashtyk individuals clearly demonstrate the consumption of millet, confirming that systematic reliance on this crop continued in the region into the Late Iron Age (see also Shishlina et al. 2016). Moreover, we can be reasonably confident in proposing that millet was cultivated in the Basin, since any significant and persistent trade in a staple crop with regions outside the Minusinsk seems unlikely given the distances involved and the long-term average represented by stable isotope measurements of bone collagen. While relatively little archaeobotanical research has been undertaken in the region, millet and barley have been identified at the Early Iron Age Tagar cemeteries of Erbinskaya and Poylovo (Vadetskaya 1986). Although only two associated animal values are presented here, the fact that they are depleted in  $\delta^{13}C$  supports the direct consumption of millet by humans, rather than its use as a fodder crop. This is in keeping with previously reported results for the Late Bronze and Early Iron Ages in the Minusinsk Basin (Murphy et al. 2013; Svyatko et al. 2013).

There are three important outcomes for the mid- to late Holocene archaeology of the Minusinsk Basin from the present paper. The first is that the refined cultural historical sequence present in Svyatko et al. (2009) is likely to remain broadly correct. The second is that there is little reason to doubt the inferred date of ca. 1400 cal BC for the appearance of millet agriculture in the Basin. Note that this need not be the very first time millet itself appeared in the region, but rather the first evidence for its substantive use, as seen in significantly elevated human  $\delta^{13}C$  values (Svyatko et al. 2013). The third outcome is that our results raise questions concerning the extent to which freshwater fish were in fact consumed by Early Bronze Age to Late Iron Age communities in the Minusinsk

Basin, given that at least some modern fish collected for the Basin's rivers clearly do show a reservoir offset of up to three centuries. However, the effect is highly variable even within the Karasuk River, and seems to have some relationship with fish species, though why this should be the case in this particular context is difficult to understand. This in turn raises a wider issue that extends beyond the Minusinsk Basin, regarding the explanation for high  $\delta^{15}N$  values in humans, if not as a result of the significant consumption of fish. Further research, for example using  $\delta^2H$  measurements on bone collagen as another proxy for trophic level (Birchall et al. 2005), may be useful in addressing this question. Single amino acids also hold promise (e.g. Naito et al. 2013). Additional understanding of the spatial and potentially species variability in the FRO in the waters of Basin should also be a priority, alongside further paired archaeological human and faunal radiocarbon measurements.

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