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FRESHWATER RESERVOIR EFFECTS IN ARCHAEOLOGICAL CONTEXTS OF SIBERIA AND THE EURASIAN STEPPE

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ABSTRACT. In this paper we evaluate the extent of freshwater reservoir effects (37 samples across 12 locations) and present new data from various archaeological sites in the Eurasian Steppe. Together with a summary of previous research on modern and archaeological samples, this provides the most up-to-date map of the freshwater reservoir offsets in the region. The data confirm previous observations highlighting that FREs are widespread but highly variable in the Eurasian Steppe in both modern and archaeological samples. Radiocarbon dates from organisms consuming aquatic sources, including humans, dogs, bears, aquatic birds and terrestrial herbivores (such as elk feeding on water plants), fish and aquatic mammals, as well as food crusts, could be misleading, but need to be assessed on a case-by-case basis.

KEYWORDS: Eurasian Steppe, freshwater reservoir effects, radiocarbon dating.

INTRODUCTION

Freshwater reservoir effects (FREs) are increasingly acknowledged sources of offsets in radiocarbon (^{14}C) dates on human and some faunal remains. The nature of the effect is well described in the literature (e.g., Keaveney and Reimer 2012; Wood et al. 2013; Fernandes et al. 2016). Briefly, the FRE refers to the apparent, "older" age of samples when part of the carbon in the diet of an individual comes from freshwater resources (such as fish, waterfowl, etc.) with a reservoir offset compared to the ^{14}C age of a contemporaneous purely terrestrial sample obtaining its carbon from the atmosphere. FREs vary geographically and over time and can vary considerable within what are ostensibly single reservoirs, between and within species, and even within single organisms (e.g., Kulkova et al. 2015; Schulting et al. 2022), and herein lies the major challenge for chronological determinations and hence for the archaeological interpretations that rely on temporal sequence.

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Over the past several decades, a number of studies have highlighted the presence of FREs in various regions of Siberia and the Eurasian Steppe, featuring both modern fish and archaeological samples (shell, human, fish, dog and others; summarized in Svyatko et al. 2017a; also Figure 3). Attempts have also been made to analyze the relationship between diets and radiocarbon ages, and to develop regression and Bayesian models to calculate ^{14}C offsets using δ^{13}C and δ^{15}N values (Bronk Ramsey et al. 2014; Schulting et al. 2014, 2015, 2022). The analysis of modern aquatic fauna (Svyatko et al. 2017a, 2017b), as well as alkalinity of water in local reservoirs (Keaveney and Reimer 2012), are informative proxies to assess the extent of modern FREs; however, the values cannot necessarily be extrapolated to prehistoric contexts as the extent of the offsets can change over time (e.g., Ascough et al. 2010), and these proxies do not account for particular aquatic resources in past human or faunal diets.

Here, we evaluate the extent of the FRE associated with materials from various archaeological sites from Siberia and the Eurasian Steppe, introduce new data, and present an up-to-date summary of the existing FRE data in this region and beyond.

MATERIALS AND METHODS

In total, this study includes 12 locations across the Eurasian Steppe (Figure 3). The analyzed materials represent groups of synchronous samples of purely terrestrial *versus* aquatic/mixed origin. In cases where the contexts were disturbed (e.g., plundered), only specimens with reliable associations were sampled. The samples (37 in total) include 14 human bone, 1 wood, 15 terrestrial faunal bone, 2 plant macrofossil and 5 fish bone/scale samples.

The sampled archaeological sites date to various periods of the Bronze and Early Iron Ages. The sites are attributed to the Sintashta (21st–18th c. BC), Begazy-Dandybai (2nd mil. BC–8th c. BC), Andronovo (20th–9th c. BC), Samus (15th–13th c. BC), Bulan-Koba (2nd c. BC–5th c. AD), and Tasmola (7th–3rd c. BC) Cultures. The description of the sites and their cultural affiliations are presented in detail in SI 1.

All samples were analyzed in the ^{14}CHRONO Centre for Climate, the Environment and Chronology (Queen's University Belfast).

Sample Pretreatment

For bone samples, collagen extraction was based on the ultrafiltration method (Brown et al. 1988; Bronk Ramsey et al. 2004), which included the following steps: a) bone demineralization in 2% HCl, followed by MilliQ® ultrapure water wash; b) gelatinization in pH=2 HCl at 58°C for 16 hours; c) filtration, using ceramic filter holders, glass filter flasks and 1.2 µm glass microfiber filters; d) ultrafiltration using Vivaspin® 15S ultrafilters with MWCO 30 kDa; 3000–3500 rpm for 30 min; and e) freeze-drying. The dried collagen was stored in a desiccator.

Acid-only pretreatment was used for fish scale samples. The samples were placed in clean 100 mL beakers and immersed in hydrochloric acid (4%, 30–50 mL), followed by deionised water wash until neutral.

For the wood sample, a standard ABA procedure (Mook and Waterbolk 1985) was used. This involves a 4% HCl wash at 80°C, a 2% NaOH wash and another 4% HCl wash at 80°C (1 h for each step), followed by a final rinse in deionized water. For plant macrofossil samples, acid-only pretreatment was used, which included 4% HCl wash at 80°C, and a rinse in deionized water.

Stable Isotope Analysis

Bone collagen stable carbon and nitrogen isotopes were measured in duplicate on a Thermo Delta V Isotope Ratio Mass Spectrometer coupled to a Thermo Flash 1112 Elemental Analyzer peripheral. The measurement uncertainty (± 1SD) of $\delta^{13}C$ and $\delta^{15}N$ based on 6–10 replicates of seven archaeological bone collagen samples was 0.22‰ and 0.15‰ respectively. The reference standards used were IA-R041 L-Alanine, IAEA-N-2 Ammonium Sulphate, IA-R001 Wheat flour, IAEA-CH-6 Sucrose, and Nicotinamide. Results are reported using the delta convention relative to international standards: VPDB for $\delta^{13}C$ and AIR for $\delta^{15}N$ (Hoefs 2009). The results were calibrated using a regression based on the measured and known values of the standards (cf. Coplen et al. 2006).

AMS ^{14}C Dating

Prepared samples were sealed under vacuum in quartz tubes with an excess of CuO and combusted at 850°C. The CO_2 was converted to graphite on an iron catalyst using zinc or by the hydrogen reduction method (Slota et al. 1987; Vogel et al 1984). The pressed graphite "target" was then measured on a 0.5 MV National Electrostatics Compact AMS. The sample $^{14}C/^{12}C$ ratio was background corrected and normalised to the HOXII standard (SRM 4990C; National Institute of Standards and Technology). The $^{14}C/^{12}C$ ratio corrected for isotopic fractionation using the AMS-measured $\delta^{13}C$, is equivalent to fraction modern ($F^{14}C$; Reimer et al. 2004). The ^{14}C age and one-sigma error term were calculated from $F^{14}C$ using the Libby half-life (5568 years) following the conventions of Stuiver and Polach (1977). The statistical proximity of the paired dates was assessed using the Ward and Wilson (1978) chi-squared test in CALIB 7.0.

Calculating the Freshwater Reservoir Offset (FRO)

Freshwater reservoir offsets were calculated as the difference in the ^{14}C ages between the terrestrial (faunal/wood) samples and aquatic/mixed (human/fish). FRO uncertainty was calculated using $\sigma_{FRO} = \sqrt{\sigma_a^2 + \sigma_b^2}$, where σ_a and σ_b are ^{14}C age uncertainties for aquatic/mixed and terrestrial samples. Dates that passed the chi-squared test were interpreted as showing no FRO.

RESULTS AND DISCUSSION

Results

The collagen content of the bone samples varied between 1.3–19.7% (Table 1), meeting the recommended minimum of 1% (van Klinken 1999). Atomic C:N ratios were all within the accepted range of 2.9–3.6 (mean $C:N_{atomic} = 3.2 \pm 0.1$), indicating well-preserved collagen (DeNiro 1985). The isotopic results and observed freshwater reservoir offsets are presented in Table 1.

Stable Isotope Values

Stable isotope results (Figure 1) indicate predominately C_3-based ecosystems for the sampled sites of the Eurasian Steppe, as expected. Enrichment in both C and N isotopes can only be

Table 1 AMS ^{14}C dates, stable C and N isotope values, atomic C:N ratios and calculated FRO of the samples.

Site	Lab ID	Sample type	^{14}C BP	FRO (^{14}C yr)	δ^{13}C (‰) VPDB	δ^{15}N (‰) AIR	C:N_{at}	% collagen
		Southern Trans-Urals and Western Siberia						
Kamennyi Ambar								
Inner fortification (excavation 8), ditch, 140–145 cm	UBA-26185	Fish scales (KA G1-F)	3754 ± 43	276 ± 56	–25.4	5.9	—	n/a
	UBA-26186	Plant macros (KA G1-P)	3478 ± 36		—	—	—	—
Rubbish layer, 200–220 cm	UBA-26187	Fish scales (KA W6.1-4 F)	4060 ± 40	712 ± 54	–25.3	6.8	—	n/a
	UBA-26188	Plant macros (KA W6.1-4 F)	3348 ± 36		—	—	—	—
Utinka								
Burial 1	UBA-32611	Human	3896 ± 41	143 ± 55	–18.8	11.7	3.1	16.1
	UBA-32612	Sheep	3753 ± 37		–21.1	2.5	3.2	11.0
Eastern Siberia								
Kharga i settlement								
Midden	UBA-28384	Fish	6198 ± 40	250 ± 57	–12.8	9.5	3.2	2.6
	UBA-28385	Roe-deer	5948 ± 41		–20.6	6.1	3.3	4.0
Southern Siberia								
Verkh-uimon								
Kurgan 35	UBA-31087	Human	1601 ± 34	13 ± 48*	–19.3	9.7	3.2	19.7
	UBA-31088	Horse	1588 ± 34		–21.2	4.1	3.2	15.6
Kuraika								
Kurgan 21	UBA-32616	Human	1827 ± 44	–210 ± 56	–18.1	12.7	3.2	17.3
	UBA-32617	Sheep	2037 ± 34		–18.1	8.1	3.1	15.9
Kurgan 25	UBA-33252	Human	2335 ± 38	423 ± 51	–17.9	12.8	3.2	16.5
	UBA-33253	Sheep	1912 ± 34		–17.7	8.2	3.3	9.7
Kazakhstan								
Shat								
Structure 1	UBA-27481	Human	3078 ± 48	142 ± 69	–17.5	13.8	3.3	4.0
	UBA-27480	Horse	2936 ± 49		–21.1	6.4	3.4	2.8

Table 1 (*Continued*)

Site	Lab ID	Sample type	^{14}C BP	FRO (^{14}C yr)	δ^{13}C (‰) VPDB	δ^{15}N (‰) AIR	C:N_{at}	% collagen
Tegiszhol								
Mound 27	UBA-27484	Human	2463 ± 47	1071 ± 64	–17.7	14.4	3.3	1.5
	UBA-27483	Horse	1392 ± 44		—	—	—	1.3
Kenzhekol I								
Grave 49	UBA-32609	Fish	3727 ± 38	338 ± 54[1]	–21.9	11.5	3.3	5.1
	UBA-32608	Human	3389 ± 38		–18.5	12.6	3.2	10.9
Bestamak								
Pit 111	UBA-28980	Human	3451 ± 36	17 ± 51*	–19.1	12.1	3.2	10.0
	UBA-28981	Sheep	3434 ± 36		–20.0	7.3	3.2	13.5
Pit 123	UBA-28982	Human	3459 ± 37	–35 ± 52*	–18.9	12.9	3.2	8.5
	UBA-28983	Sheep	3494 ± 37		–19.4	7.8	3.2	13.8
Pit 130	UBA-28984	Human	3472 ± 37	68 ± 52*	–18.5	13.7	3.2	7.5
	UBA-28985	Herbivore	3404 ± 36		–19.1	7.2	3.2	3.4
Pit 140	UBA-28986	Human	3476 ± 39	38 ± 53*	–19.0	10.4	3.2	7.4
	UBA-28987	Horse	3438 ± 36		–19.9	7.8	3.2	4.2
Pit 170	UBA-28988	Human	3408 ± 38	–66 ± 55*	–18.6	11.3	3.2	5.9
	UBA-28989	Horse	3474 ± 39		–18.8	7.7	3.1	4.2
Halvai 3								
Pit 3	UBA-28992	Human	3536 ± 37	1942 ± 50	–19.4	10.2	3.1	5.3
	UBA-28993	Horse	1594 ± 34		–17.2	9.5	3.1	8.9
	UBA-28994	Wood	3458 ± 32	78 ± 49*	—	—	—	—
Halvai 5								
Pit 4	UBA-28998	Human	3461 ± 32	91 ± 45	–19.5	11.4	3.3	3.8
	UBA-28999	Horse	3370 ± 31		–20.0	4.2	3.2	8.8
Kesken-Kuyuk kala								
Element 286 (midden),	UBA-29369	Fish	1386 ± 29	263 ± 50	–22.4	6.9	3.2	6.3
–15 cm	UBA-29370	Herbivore	1123 ± 41		–16.6	12.1	3.2	14.9

Notes: The cultural affiliation of the sites and specific layers or burials the samples come from is presented in SI 1. The location map of the site is presented in Figure 3.

[1] This FRO was calculated between fish and human specimens, the latter possibly being not purely terrestrial sample.

**The dates are statistically indistinguishable at 95% level indicating no demonstrable FRO.*

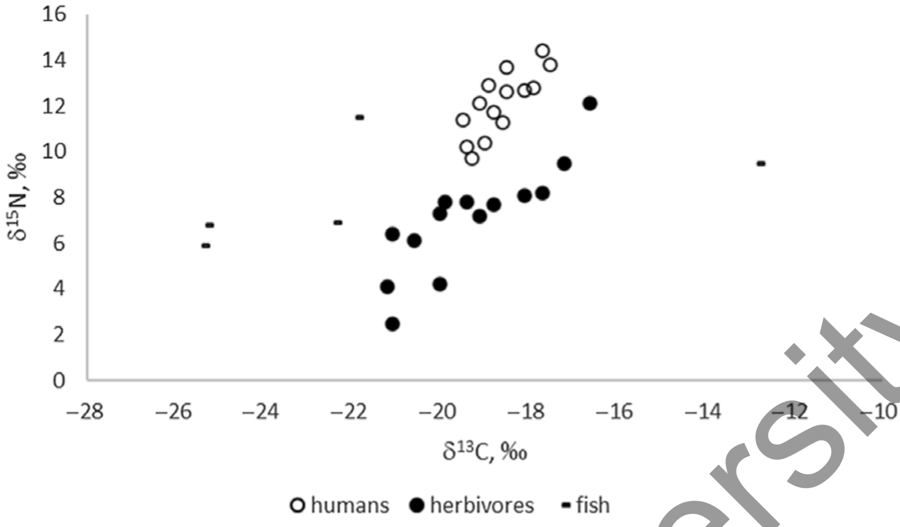

Figure 1 δ^{13}C and δ^{15}N values of the analyzed human and faunal samples (n=33).

observed in herbivores from the "marsh town" of Kesken-Kuyuk kala located in the delta (the ancient channel) of the Syr-Darya River. A wide range of nitrogen isotopic values (especially in herbivores) is likely to be the result of climatic variation, specifically aridity (e.g., Hollund et al. 2010), as a number of sites are located in arid areas of Kazakhstan. The positive linear correlation between δ^{13}C and δ^{15}N for both herbivores ($R^2 = 0.730$) and humans ($R^2 = 0.633$) is likely related to a comparable gradient (Hollund et al. 2010; Schulting and Richards 2016).

Archaeological fish values show great variability in δ^{15}N and δ^{13}C values, as is also the case for modern freshwater fish (Dufour et al. 1999; Svyatko et al. 2017a). While the variability in δ^{15}N values is likely related primarily to the trophic level of the fish, δ^{13}C values rather reflect the isotopic ecology of the particular reservoirs (e.g., Dufour et al. 1999; France 1995; Hecky and Hesslein 1995; Spies et al. 1989; Gu et al. 1996). It has been shown previously that freshwater reservoirs in the Eurasian Steppe may produce a wide range of δ^{13}C signatures (e.g., Katzenberg and Weber 1999; Svyatko et al. 2017a) depending on specific physical and biological factors, yet most reflect C_3 ecologies. Our results show elevated δ^{13}C for fish from the settlements of Kharga I (δ^{13}C= –12.8‰) in Eastern Siberia, which corresponds with elevated δ^{13}C data for modern fish from those areas (Svyatko et al. 2017a). The isotopic values and FROs of archaeological and modern fish in Siberia and the Eurasian Steppe are further discussed in detail elsewhere (Marchenko et al. 2021).

The mean δ^{13}C and δ^{15}N values for humans are –18.6 ± 1.3‰ and 12.1 ± 2.8‰, respectively; and for herbivores they are –19.3 ± 3.0‰ and 7.1 ± 4.8‰, respectively. We have not undertaken dietary modelling here because there is insufficient isotopic food source data for any of the sites to make this a realistic exercise. In the absence of representative sample sets of triple fish/human/terrestrial specimens, the human-herbivore pairs are not sufficient to provide a regional FRO value but only a minimum. It is impossible to be definite about the amount of fish consumption by humans based on stable isotope values alone, and, therefore, the FRO resulting from terrestrial/human pairs must be considered as a minimum value.

Freshwater Reservoir Effects

The results overall indicate a frequent occurrence of FROs from archaeological sites across the Eurasian Steppe, both for faunal and human samples. However, they are extremely variable, with the largest offset values reaching 1071 ± 64 ^{14}C years (human sample, site of Tegiszhol, Kazakhstan). An even larger FRO of 1942 ± 50 ^{14}C years, detected in pit 3 at Halvai 3, is likely the result of disturbance of the burial at a later period and intrusion of the animal bone. The ^{14}C date for the wood sample from the same burial is similar to the date from the human sample which indicates an absence of an FRO within the $2\sigma_{FRO}$ range (78 ± 49), however the dates of paired samples from Halvai 5 are statistically different indicating a potential, albeit small, FRO (FRO=91 ± 45).

Negative FRO values that are within the $2\sigma_{FRO}$ range, such as those from Bestamak, also indicate the absence of a FRO. The negative FRO values that are larger than $2\sigma_{FRO}$ indicate that terrestrial samples are older than those containing an aquatic component, which is not theoretically possible if the pairs are contemporaneous. These sample pairs need detailed consideration. This concerns a pair from the site of Kuraika (kurgan 21) in the Altai Mountains, where sheep bone appears to be 210 ± 56 ^{14}C years older than associated human sample. The graves had apparently not been disturbed (see SI 1). At the moment it is not clear why the ^{14}C date for the terrestrial sample is older than that of human here, but unrecognized disturbance, or the inclusion of residual material from an earlier grave or settlement, would seem the most likely explanations.

The FROs for archaeological fish vary between 250 ± 57 and 712 ± 54 ^{14}C years, with the highest value detected for the site of Kamennyi Ambar, where a measurement on another sample only showed a FRO of 276 ± 56, and underlines the differences that can occur even within the same site. There is often inconsistency in FROs between modern and archaeological fish samples within single areas. For example, within the Kharga I area, the FRO in archaeological fish is 250 ^{14}C years while the offset is only 15 ^{14}C years in modern fish from the associated lake (Figure 3), although we cannot rule out the possibility that the archaeological fish originated in a different reservoir. It is also possible that the Kharga basin itself exhibits variable reservoir effects (cf. Fernandes et al. 2015, tab. 4), or that the FRO has changed over time (cf. Ascough et al. 2010).

Logically, FRO values must be lower in humans than in the fish being consumed, as the extent of the human FROs depends on the proportion of fish in the diet. The maximal offset for a human sample determined within this study is 1071 ± 64 ^{14}C years (Tegiszhol, Kazakhstan), excluding the pair from Halvai 3 pit 3 discussed above. The results also indicate a moderate positive linear correlation between the size of FROs and both δ^{13}C ($R^2 = 0.381$) and δ^{15}N ($R^2 = 0.324$; Figure 2) for the human samples from this study, although the regressions are heavily weighted by the results from the undisturbed burial from Tegiszhol, Kazakhstan.

Yet, the major implication here is that the human isotopic values cannot reliably indicate the presence or absence of FROs across such a broad region. Neither does the presence of a FRO in associated archaeological or modern fish necessarily indicate the presence of the offsets in humans, since fish may not have been consumed to any great extent (e.g., 13 ^{14}C years in human from Verkh-Uimon versus 578 ^{14}C years in local modern fish from the Katun River).

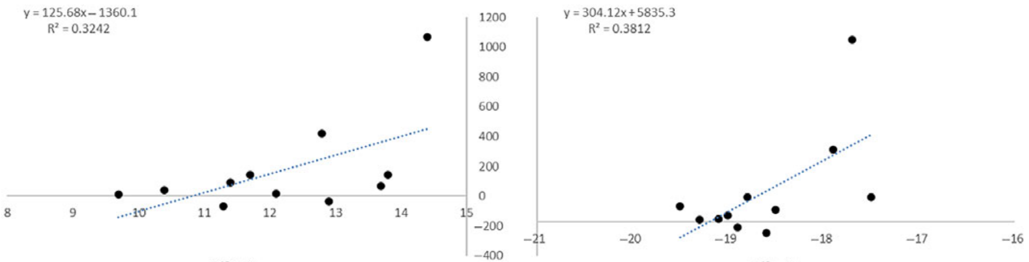

Figure 2 Human $\delta^{13}C$ and $\delta^{15}N$ plotted against their FRO values. Note that the pair from Halvai 3, and the pair from Kuraika (kurgan 21) with significant negative FRO values were removed, as this would be theoretically impossible.

SUMMARY

In recent decades, a number of freshwater radiocarbon offsets have been reported for various modern samples and archaeological sites of Siberia and the Eurasian Steppe region. Plotting our results together with existing FRO data for Siberia and the Eurasian Steppe (Figure 3), several observations can be made:

- FROs are common but highly variable across the Eurasian Steppe in both modern and archaeological samples including humans. Radiocarbon dates from individuals consuming aquatic sources, such as humans, dogs, bears, beavers, certain birds and terrestrial herbivores (such as elk *Alces alces* feeding on water plants; e.g., Philippsen 2019), fish and aquatic mammals, as well as food crusts (e.g., Hart et al. 2018), could be misleading;
- FROs between modern and archaeological samples are often inconsistent within single areas and even within sites, especially in fish;
- the presence of FROs in local archaeological or modern fish does not necessarily imply the presence of an offset in associated humans, i.e., fish or other aquatic resources do not always feature significantly in the diet;
- a weak positive relationship has been found between FROs and $\delta^{13}C$ or $\delta^{15}N$ values of human samples across the region.

From the outlined scenario, it is clear that, when using freshwater/mixed resources for chronological reconstructions, the presence and the variability of FREs need to be explored in depth in each individual area *and for each period* as the hydrology or carbon sources could change (Schulting et al. 2015). The latter could be related to a number of factors, such as melting of permafrosts releasing old ^{14}C-depleted carbon into the reservoir (Schulting et al. 2015), geothermal activity (e.g., Ascough et al. 2010), or even changes in the hydrological system of an area (e.g., Marchenko et al. 2021). Bearing this in mind is particularly important for archaeologists because, as mentioned earlier, human remains are very often sampled for ^{14}C dating, and without clear understanding of local FREs chronological reconstructions based on such dates may be unreliable. Without systematic research into the local food chain and isotopic baseline, it is very difficult to predict the extent (or even the presence) of a potential offset in human samples solely from $\delta^{13}C$ and $\delta^{15}N$ values. This would be especially the case when associated fish isotopic values are close to those for terrestrial fauna, in which case the consumption of fish would be isotopically

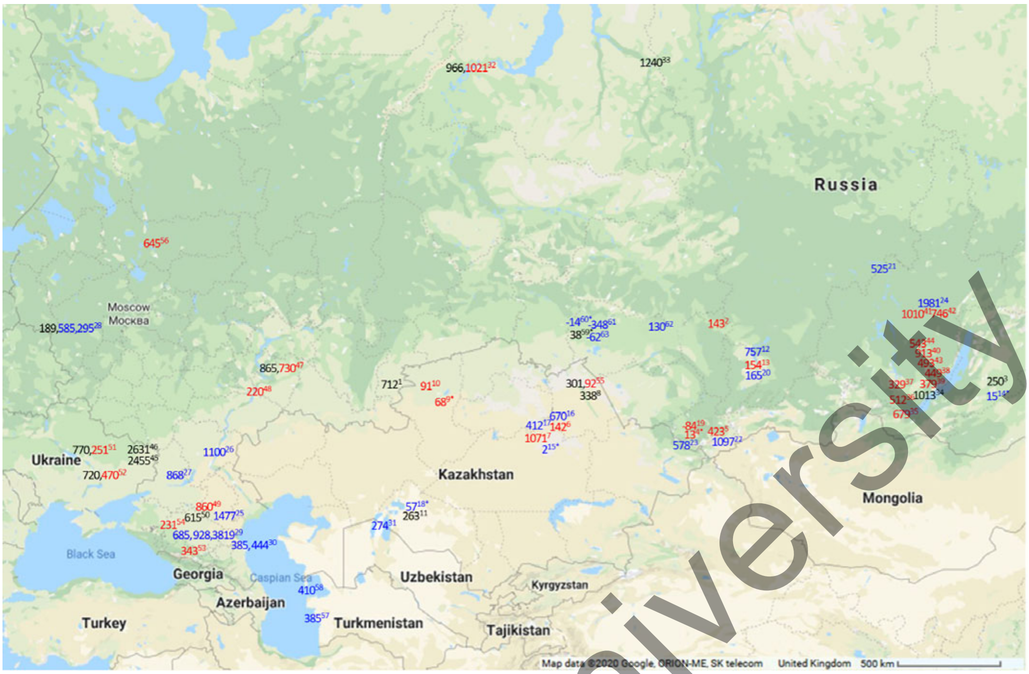

Figure 3 Map of maximal FROs in ^{14}C years for modern (in blue) and maximal observed FROs in archaeological (in red for humans and black for other) samples in Siberia and the Eurasian Steppe. Numbers 1–11 are the sites sampled for current study. Locations presented are approximate. For exact locations see the source studies. 1. Kamennyi Ambar, fish (present study); 2. Utinka, human (present study); 3. Kharga I settlement, fish (present study); 4. Verkh-Uimon, human (present study); 5. Kuraika, human (present study); 6. Shat, human (present study); 7. Tegiszhol, human (present study); 8. Kenzhekol 1, fish (present study); 9. Bestamak, human (present study); 10. Halvai, human (present study); 11. Kesken-Kuyuk kala, fish (present study); 12. Karasuk Bay, modern fish (Svyatko et al. 2017b); 13. Abakan 8, human (Svyatko et al. 2017b); 14. Kharga Lake, modern fish (Svyatko et al. 2017a); 15. Kyzylkoi River, modern fish (Svyatko et al. 2017a); 16. Shat River, modern fish (Svyatko et al. 2017a); 17. Nura River, modern fish (Svyatko et al. 2017a); 18. Syr-Darya River, modern fish (Svyatko et al. 2017a); 19. Perviy Mezhelik 1, human (Svyatko et al. 2017c); 20. Yenisei River, modern fish (Svyatko et al. 2017b); 21. Edarma River, modern fish (Svyatko et al. 2017a); 22. Chuya River, modern fish (Svyatko et al. 2017a); 23. Katun River, modern fish (Svyatko et al. 2017a); 24. Lena River, modern fish (Schulting et al. 2015); 25. Deed-Khulsun Lake, modern fish (van der Plicht et al. 2016); 26. Volga River, modern fish (van der Plicht et al. 2016); 27. Tsimlyansk city, modern algae (van der Plicht et al. 2016); 28. Serteya II and Serteyka River, food crusts, modern fish, aquatic plant (Kulkova et al. 2015); 29. Podkumok River, modern fish, aquatic plant matter and water HCO3 (Higham et al. 2010); 30. Tyuleniy Island, Sulak River mouth, seal, shell (Olsson 1980; Kuzmin et al. 2007); 31. Kuzhetpes Island, shell (Kuzmin et al. 2007); 32. Ust'-Polui, fish, human (Losey et al. 2018); 33. Mangazeya, fish (Kuzmin et al. 2020); 34. Sagan-Zaba II, seal (Nomokonova et al. 2013); 35. Shamanka II, human (Bronk Ramsey et al. 2014); 36. Lokomotiv, human (Schulting et al. 2014); 37. Ust'-Ida, human (Schulting et al. 2014); 38. Kurma XI, human (Schulting et al. 2014); 39. Khuzhir-Nuge XIV, human (Schulting et al. 2014); 40. Popovskii Lug 2, human (Schulting et al. 2015); 41. Turuka, human (Schulting et al. 2015); 42. Zakuta, human (Schulting et al. 2015); 43. Makrushino, human (Schulting et al. 2015); 44. Ust' Iamnaia, human (Schulting et al. 2015); 45. Starobelsk-II, shell (Motuzaite-Matuzeviciute et al. 2015); 46. Novoselovka-III, shell (Motuzaite-Matuzeviciute et al. 2015); 47. Lebyazhinka V, human, fish (Shishlina et al. 2018); 48. Khvalynsk II, human (Shishlina et al. 2014); 49. Peschany V, human (Shishlina et al. 2014); 50. Shakhaevskaya, fish (Shishlina et al. 2012); 51. Dereivka 1, fish, human (Lillie et al. 2009); 52. Yasinovatka, fish, human (Lillie et al. 2009); 53. Klin-Yar, human (Higham et al. 2010); 54. Aygurskiy, human (Hollund et al. 2010); 55. Shauke, human, fish (Svyatko et al. 2015); 56. Minino, human (Wood et al. 2013); 57. Cheleken Peninsula, shell (Kuzmin et al. 2007); 58. Garabogaz Spit, shell (Kuzmin et al. 2007); 59. Preobrazhenka 6, fish (Marchenko et al. 2015); 60. Tartas R., fish (Marchenko et al. 2021); 61. Lozhka L., fish (Marchenko et al. 2021); 62. Ob R., fish (Marchenko et al. 2021); 63. Kama R., fish (Marchenko et al. 2021). *The values are statistically non-significant at 95% confidence, indicating the lack of any detectable FRO.

invisible in humans. The application of other isotopes ($\delta^{34}S$, $\delta^{2}H$), as well as analysis of individual amino acids might help assessing the role of fish in the diet (e.g., Webb et al. 2015; Drucker et al. 2018; Schulting et al. 2018). Yet, even when the isotopic values suggest the consumption of freshwater resources, this would not necessarily imply the existence of FROs in human samples.

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SUPPLEMENTARY MATERIAL

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