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FRESHWATER RESERVOIR EFFECT ON REDATING OF EURASIAN STEPPE CULTURES: FIRST RESULTS FOR ENEOLITHIC AND EARLY BRONZE AGE NORTHEAST KAZAKHSTAN

Svetlana V Svyatko[1,2] • Ilya V Mertz[3] • Paula J Reimer[1]

ABSTRACT. Freshwater reservoir effects (FRE) can cause problems when radiocarbon dating human skeletal material from the Eurasian steppe. This article presents the first results of research into the extent of the FRE in the sites of Borly 4 (Eneolithic) and Shauke 1 and 8b (Early Bronze Age), northeastern Kazakhstan. Accelerator mass spectrometry (AMS) ^{14}C dating and stable isotope (δ^{13}C, δ^{15}N) analysis of associated groups of samples (32 samples, 11 groups in total) demonstrate the following: (a) the diet of the humans and fauna analyzed was based on the C_3 foodchain with no evidence of a C_4 plant (such as millet) contribution; aquatic resources apparently were a continuous dietary feature for the humans; (b) the first ^{14}C dates obtained for the Upper and Middle Irtysh River region attribute the Eneolithic period of the area to the 34th to 30th centuries BC, and the Early Bronze Age to the 25th to 20th centuries BC, with a ~450-yr hiatus between the two periods; (c) the maximum fish-herbivore freshwater reservoir offset observed equals 301 ± 47 ^{14}C yr. As such, ^{14}C dates from aquatic and human samples from the area need to be interpreted with caution as they are likely to be affected by the offset (i.e. appear older). The article also discusses the effect of a sodium hydroxide (NaOH) wash on δ^{13}C, δ^{15}N, C:N$_{\text{atomic}}$ levels and collagen yields of the bone samples. Our results indicate a minor but significant effect of NaOH treatment only on C:N$_{\text{atomic}}$ ratios of the samples.

INTRODUCTION

Archaeologists often sample human bone to radiocarbon date past societies especially when undertaking paleodietary studies. However, in many cases a major source of uncertainty, the reservoir effect, hinders the accuracy of these dates. The effect occurs when a portion of the carbon in the diet comes from a non-atmospheric reservoir with a lower ^{14}C level (e.g. marine/freshwater). The difference between the ^{14}C age of such a bone sample and that of a contemporaneous, purely terrestrial sample is termed the "reservoir offset."

The extent of the marine reservoir effect is well documented in terms of the average global marine reservoir correction (estimated as 400 yr; Stuiver and Braziunas 1993), regional differences (ΔR) (compiled in the marine reservoir correction database; www.calib.org/marine), and its impact on human/animal bone samples through marine-based diet (Ascough et al. 2005; Shishlina et al. 2007, 2009, 2012, 2014). In contrast, research on the extent of the freshwater reservoir effect (FRE) is rather scarce. Most FRE studies have been focused on Europe and the European part of Russia (Cook et al. 2001, 2002; Fischer and Heinemeier 2003; Olsen et al. 2010; Keaveney and Reimer 2012; Lougheed et al. 2013; Wood et al. 2013; Fernandes et al. 2014) and North America (Ingram and Southon 1996; Goodfriend and Flessa 1997; Culleton 2006), and only a few in Siberia and the Eurasian steppe (Shishlina et al. 2007, 2009, 2012; Lillie et al. 2009; Higham et al. 2010; Nomokonova et al. 2013; Schulting et al. 2014). The main conclusions from this research are that FRE is highly variable geographically, spanning from zero to several thousand years, and that "each population thought to be affected by a FRE must be examined individually" (Wood et al. 2013:163). However, exploring the phenomenon in particular areas, analyzing its extent or demonstrating its absence in unaffected regions can significantly improve chronological interpretations for these areas. The largest source of "old" carbon in freshwater is dissolved inorganic carbon from ^{14}C-free carbonate minerals in the groundwater, as many sedimentary rocks are composed of skeletal fragments of marine organisms that died millions of years ago (Sveinbjörnsdóttir et al. 1995; Culleton 2006). The

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1. ^{14}CHRONO Centre for Climate, the Environment, and Chronology, Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland, UK.
2. Corresponding author. Email: s.svyatko@qub.ac.uk.
3. A. Kh. Margulan Centre for Archaeological Research, Pavlodar State University n.a. S. Toraigyrov, 64 Lomov st., room 102, Pavlodar, 140008, Kazakhstan.

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extent of the FRE in most regions is closely related to the geological composition of the underlying substrate. High correlation between the FRE and carbonate content has been demonstrated for modern lakes in Britain and Ireland (Keaveney and Reimer 2012).

The main objective of this study is thus to improve the accuracy of ^{14}C dating of archaeological (primarily Bronze Age) human bone by assessing the extent and diversity of the FRE in the key areas of western and southern Siberia and Kazakhstan. We also aim to explore paleodietary and paleoeconomic patterns of the populations, specifically the role of freshwater resources in human diet.

Siberia and the Eurasian steppe represent an unmatched economic, political, and cultural interface between the East and West from early prehistory. The turbulent history of migrations and collisions of tribes and nations, rise of empires, and fall of civilizations in the region has forged its unique heritage, drawing enormous interest from the global academic community (Mair 2006; Anthony 2007; Kuzmina 2008; Beckwith 2009). A growing number of ^{14}C ages from archaeological human bone samples appear considerably older than expected from traditional or established archaeological chronologies based on associated wood and/or charcoal ^{14}C ages (Görsdorf et al. 2001; Alekseev et al. 2005; Hanks et al. 2007; Svyatko et al. 2009), possibly due to the FRE. Often, particular human burials are of interest, but they do not always have associated terrestrial material for dating. In many cases (e.g. plundered burials, graves without goods, or wooden structures), ^{14}C dating of human bone is the most feasible, and sometimes the only, way to date particular sites. Furthermore, there is a large number of human bone ^{14}C dates from past research, and it is important to assess their accuracy where a FRE correction is required. The refinement of ^{14}C dates through documenting the FRE in these regions is crucial for understanding the chronology of cultural transitions and development of these interactions. A number of recent studies (O'Connell et al. 2003; Privat et al. 2005; Shishlina et al. 2007; Svyatko et al. 2009; Murphy et al. 2013) have suggested a strong contribution of freshwater fish in the diet of prehistoric north Eurasian steppe populations, which complicates the interpretation of ^{14}C dates because of the possibility of a FRE.

Geographical Background

The current stage of our research is focused on archaeological sites of northeast Kazakhstan, in particular Pavlodar Oblast. Geographically, Pavlodar Oblast is a part of the Irtysh River plain with Irtysh River being the main water artery. The 10–15-km-wide river valley bears a large number of Irtysh tributaries and minor lakes and it is completely flooded during the spring high-water period. The area is also characterized by a large number of deflation basins filled with salt lakes. The substrate of the region is mostly composed of alluvial and lacustrine-alluvial Early to Middle Pleistocene deposits dominated by sandy Kastanozems, with a carbonate-rich layer at 30–50 cm depth (Esenov 1970). Modern alluvial deposits of various composition are the parent material of the Irtysh River floodplains; river terraces are widely composed of carbonate-rich sandy Kastanozems (Uryvaev 1959), while 30–50% of the lake basin deposits represent complex sandy Kastanozems (Esenov 1970).

Along the entire length of the Irtysh River, its water is carbonate rich and moderately hard (carbonate content generally varies within 100–160 mg/L throughout the year) and potable. The chemical composition of the river water is formed outside the Pavlodar Oblast, mainly in the highlands of the Upper Irtysh region (Uryvaev 1959). Melted snow constitutes a proportion of the river water.

The area is mainly covered by dry steppes; the modern climate is continental. Summers are hot (23–25°C mean; >40°C max.), with shower rains; winters are cloudless 17–21 days/month with little snow (22 cm thick by February), mean winter temperature varies between –10°C and –20°C (min. –47°C; Esenov 1970). The mean annual precipitation averages around 240 mm (Uryvaev 1959). The area is dominated by mixed and sod grasses across the plains and lake basins, with poplars (*Populus* sp.) and willows (*Salix* sp.) along the river floodplain (Esenov 1970).

Archaeological Background

Eneolithic (Copper Age; second half of the 4th to first half of the 3rd millennium BC) and Early Bronze Age (second half of the 3rd to first quarter of the 2nd millennium BC) are generally the least understood periods in the history of Kazakhstan. Indeed, this was the time of transition to a producing economy and spread of metallurgy in the area. In the Early Bronze Age, copper mining transformed with the use of artificial bronze alloys, and the ethnocultural environment in the region changed dramatically.

The majority of studied sites of this period are located along the Ishim, Turgai, and Irtysh rivers. The Eneolithic sites of the middle Irtysh Valley are represented by small, poorly stratified settlements without a clear cultural attribution. As such, the discovery of the Borly 4 settlement is particularly important as this site and has a potential of partly filling the existing archaeological "gap" in the region. The Early Bronze Age monuments in the region are represented by the sites of Yamnaya, Elunino, Afanasyevo, Chemurchek, and other cultures. The two recently excavated sites of Shauke 1 and Shauke belong to the Elunino culture. The pastoral European-type population of the Elunino culture apparently was the first in the Ob-Irtysh interfluve to practice bronze metallurgy (Grushin et al. 2009).

The economy of the populations is widely regarded as pastoral. Generally, the animal husbandry of Borly 4 is characteristic for the Eneolithic of the steppe zone of Kazakhstan (Gaiduchenko 2014). According to archaeozoological data, horse and cattle constituted more than 98% of the total meat component of the diet, with the rest (~1.7%) coming from wild game (including Przewalski-type horse, kulan, elk, wild boar, and roe deer; Gaiduchenko and Merz 2012). In addition, birds and fish (small *Cyprinidae* specimens) were procured, the latter apparently being sourced from local reservoirs (excluding saline Lake Borly with brine shrimp *Artemia* the only fauna presented) (Uryvaev 1959) and possibly the Irtysh River. The pastoralism pattern of the Elunino culture has been reconstructed as domestic-transhumant (Grushin 2013). The herd composition and the significance of particular species depended on environmental factors. For the steppe zone, horse was apparently the most significant source of meat, followed by cattle and then ovicaprids (despite the latter being the most numerous in the herd); the herd composition was defined on the basis of the minimal number of individuals identified (Kiryushin 2002; Grushin 2013). Faunal remains also suggest hunting and fishing playing a supplementary role in the Elunino economy (Kiryushin 2002; Grushin 2013).

To date, ^{14}C dates have only been obtained in the area for two Early Bronze Age sites of the Upper Irtysh River region (Kovalev 2009; Stöllner et al. 2013). As such, the Eneolithic and Early Bronze Age chronology of the region is vague and generally based on traditional archaeological dates. In this context, the use-time of particular monuments, as well as the internal periodization of different phases, are the main chronological issues of the area.

PALEOECONOMY RECONSTRUCTIONS IN THE REGION USING STABLE ISOTOPE ANALYSIS

Carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope analysis has been used in paleodietary and paleoeconomy reconstructions for several decades (particularly, investigating the role of marine/freshwater component in the diet, as well as maize and millet consumption) and is currently one of the state-of-the-art techniques of modern bioarchaeology. Recently, research has expanded to understanding nuances of subsistence strategies and complex economic patterns of various prehistoric populations of the Eurasian steppe (O'Connell et al. 2003; Privat et al. 2005; Shishlina et al. 2007, 2009; Svyatko et al. 2009; Murphy et al. 2013; Ventresca Miller et al. 2014). One of the topical and longstanding issues is the role of aquatic resources in their diet and economy, which is generally poorly understood, as very few associated archaeological remains have been recovered. Moreover, the isotopic signatures of freshwater systems in southern and western Siberia and Kazakhstan are themselves not well known. Traditional interpretations of the archaeological data for the prehistoric Eurasian steppe populations have emphasized the pastoral element of the economy; however, recent bioarchaeological research suggests that fishing and gathering continued to be important. Through stable isotope studies, the importance of freshwater resources in the subsistence of the populations is being increasingly acknowledged, although the conclusions are complicated by the possibility of the aridity/salinity effect as one of the explanations of elevated $\delta^{15}N$ values (e.g. Murphy et al. 2013; Ventresca Miller et al. 2014).

Methodological grounds of $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis are well described in recent literature. The analysis primarily concerns the protein part of the diet. Briefly, it is used to estimate the proportions of C_3 versus C_4 plants, proportion of marine and in some cases freshwater (e.g. Katzenberg and Weber 1999) components in the diet, and a trophic level of an individual. C_3 plants (most plants) are well adapted to temperate environments and are characterized by $\delta^{13}C$ values of about –26.5‰, while C_4 plants (far less common, but including millet) are better adapted to higher aridity and temperature and demonstrate $\delta^{13}C$ values of about –12.5‰ (e.g. Pyankov et al. 2000; Wang et al. 2005; Makarewicz and Tuross 2006). The bone collagen from herbivores that subsist only on C_3 grasses will give a $\delta^{13}C$ value of about –21.5‰. If the diet was based only on C_4 grasses, then the value would be about –7.5‰. There is a small trophic level offset of 0.5–2‰ in $\delta^{13}C$ for each step in a foodchain (e.g. Bocherens and Drucker 2003; McCutchan et al. 2003). The $\delta^{15}N$ values of most modern plants vary between 0 and 5‰; these increase by 3–6‰ with each step of a foodchain (e.g. Hedges and Reynard 2007; O'Connell et al. 2012), and are most elevated when relying on aquatic resources. Nondietary factors include the canopy effect (increase of leaf $\delta^{13}C$ values from ground to canopy; van der Merwe and Medina 1991), climatic factors (higher plant $\delta^{13}C$ and $\delta^{15}N$ values and generally higher proportion of C_4 grasses with increase in temperature and aridity; Ambrose 1991; van Klinken et al. 1994), and the manuring effect (increase of the $\delta^{15}N$ ratios of manured soil and associated plants; Bogaard et al. 2013).

SITES ANALYZED

The present research involves isotopic and ^{14}C analysis of three sites (Borly 4 and Shauke 1 and 8b) located in the middle course of the Irtysh River, on the territory of modern Pavlodar Oblast and excavated in 2010–2012 by V K Merz (Figure 1). The cemetery of Shauke 1 (52°26′5.85″N, 76°50′15.59″E) and the settlement of Shauke 8b (52°25′22.30″N, 76°50′16.50″E) are situated near the modern village of Shauke on the sandy dunes of the fluvial terrace of the Irtysh River right shore, 10 km north from the city of Pavlodar. The settlement of Borly 4 (51°49′28.9″N, 77°56′42.4″E) is located 35 km east of the river, on the western terrace above the Lake Borly floodplain.

The multilayer settlement of Borly 4 has a total area of 175 m^2. The site is well stratified and contains archaeological materials from the Middle Neolithic to Early Iron Age; however, the main cultural layer belongs to the Eneolithic period. The latter includes the remains of dwellings and household outbuildings containing a large number of stone, ceramic, and copper objects, together with horse and cattle bones. The obtained materials indicate a well-developed producing economy (Gaiduchenko and Merz 2012; Gaiduchenko 2013), which, coupled with a distinctive material culture, suggest the existence of a particular Borly culture.

The cemetery of Shauke 1 is located in a sand tapping point and consists of five graves spread in a 2100-m^2 area. Four graves contained disarticulated human and animal bones, pottery, and bronze and stone objects. The fifth tomb apparently represented a cenotaph as no human remains were found; cremated animal bones and charcoal were recovered from the filling of the grave. The site is now completely destroyed. The artifacts from the site have similarities to those from the Elunino sites of Steppe Altai and the northeastern Kazakh Uplands (Merz 2008; Grushin 2013); however, specific features of Shauke 1 (unusual artifacts such as a stone ax and foundry ladle, the presence of arsenic in bronze alloys, specifics of pottery and its similarity with that of Western Catacomb cultures) suggest its earlier chronological position.

The settlement of Shauke 8b is a single-layer site of the Elunino culture with a total area of 54 m^2. The average thickness of the cultural layer is 10–15 cm; the southern edge of the site was destroyed by a sand tapping point. The cultural layer was overlain by a 2-m dune containing a 20-cm-thick paleosol. The northern part of the cultural layer was destroyed by wind erosion. The settlements yielded remains of fireplaces, ash pits, faunal (including fish) bones, pottery, rare stone and metal artifacts, and copper ore. A particular feature of the site is the presence of large quantities of fish bones. No dwelling structures were found; apparently, the settlement represented a seasonal camp. The artifacts found have similarities with those of the Elunino culture. Single-layer sites like Shauke 8b are very rare in the region and can be used to refine the internal chronology of archaeological cultures.

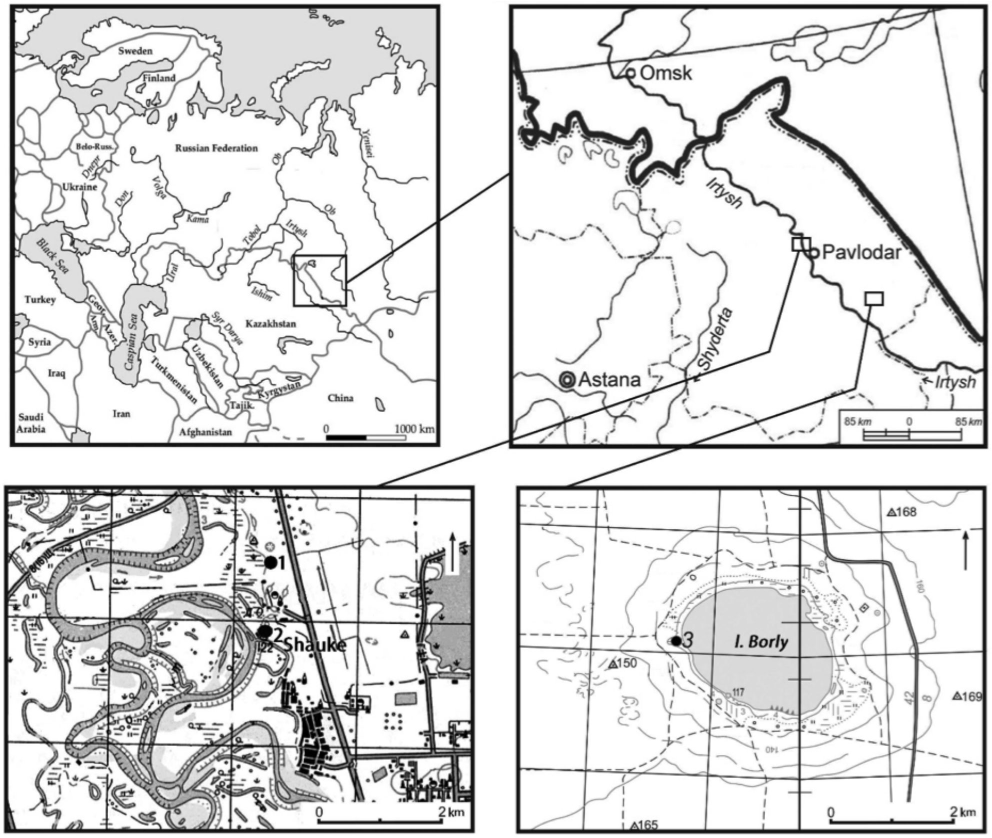

Figure 1 Provenance of test specimens: 1. Shauke 1; 2. Shauke 8b; 3. Borly 4

MATERIALS AND METHODS

For correcting the ^{14}C dates of human bones, the FRE can be calculated for the region by measuring contemporaneous terrestrial and freshwater samples and the proportion of aquatic diet in the humans is assessed by stable isotope measurements. As such, this study involves two major techniques:

a. AMS ^{14}C dating of synchronous archaeological samples (e.g. freshwater fish and/or human versus terrestrial herbivores or associated plant/charcoal samples) from strictly the same archaeological context. The difference in ^{14}C ages within these pairs/groups will indicate the extent of the freshwater reservoir offset (FRO) in the area and help to quantify the uncertainty in ^{14}C ages of human samples where δ^{15}N values indicate freshwater dietary input.

b. carbon and nitrogen (δ^{13}C, δ^{15}N) stable isotope analysis of bone collagen on archaeological samples for dietary and isotopic background assessment, in particular for estimation of the proportion of nonterrestrial material in the diet of the analyzed individuals.

Materials

In total, 11 groups of associated samples ($n = 32$) from three sites have been analyzed, including 4 human, 26 animal (5 are calcined; 7 are fish), and 2 charcoal samples (Table 1). One group from Shauke 8b did not include associated aquatic and human samples; however, the site was incorporated in the study for consistency of the archaeological chronological and isotopic paleoenvironmental record.

Pretreatment of Samples

Elemental analysis–isotope ratio mass spectrometry (EA-IRMS) δ^{13}C and δ^{15}N isotope measurements and AMS ^{14}C dating of samples were performed in the ^{14}CHRONO Centre for Climate, the Environment and Chronology (Queen's University Belfast, UK).

For bone samples, the surfaces were cleaned using a brush prior to collagen extraction. Extraction of collagen from archaeological samples was based on the ultrafiltration method (Brown et al. 1988; Bronk Ramsey et al. 2004) with an additional sodium hydroxide (NaOH) wash step (Brock et al. 2010). Briefly, these included:

- bone demineralization in 2% HCl, followed by MilliQ® ultrapure water wash;
- 0.1M NaOH treatment for 15 min, followed by MilliQ wash;
- 2% HCl wash for 15 min, followed by MilliQ wash;
- gelatinization in pH 2 HCl at 58°C for 16 hr;
- filtration, using ceramic filter holders, glass filter flasks, and 1.2-µm glass microfiber filters;
- ultrafiltration using Vivaspin® 15S ultrafilters with 30kD MWCO; 3000–3500 rpm for 30 min; and
- freeze-drying, with the dried collagen stored in a desiccator.

Being extremely fragile and in most cases very small in weight, archaeological fish bones were not subjected to NaOH treatment to avoid further collagen loss, as NaOH treatment has shown to decrease collagen yields of bone samples (Liden et al. 1995).

To explore the possible effect of the NaOH treatment on stable carbon and nitrogen isotope ratios of bone collagen, duplicates were prepared for 13 bone samples following the ultrafiltration method (Brown et al. 1988; Bronk Ramsey et al. 2004), and omitting the NaOH wash step.

Pretreatment of charcoal was performed following the standard AAA procedure (Mook and Waterbolk 1985) and included a 2% NaOH wash for 2 hr, followed by another 4% HCl wash on an 80°C hotplate for 2–3 hr. Cremated bone pretreatment procedure followed Lanting et al. (2001) and included bone crushing, 1.5% sodium hypochlorite (NaClO) wash for 48 hr, 1M acetic acid ($C_2H_4O_2$) wash for 24 hr, vacuum filtration, and drying overnight, followed by combustion with silver.

$\delta^{13}C$ and $\delta^{15}N$ Stable Isotope Analysis and AMS Radiocarbon Dating

$\delta^{13}C$ and $\delta^{15}N$ of bone gelatin were measured on a Thermo Delta V isotope ratio mass spectrometer (IRMS) with a Thermo Flash 1112 elemental analyzer (EA) peripheral. The measurements were made in duplicate. Results were reported using the delta convention relative to international standards, VPDB for C and AIR for N (Hoefs 2009). The measurement uncertainty (1σ) of $\delta^{13}C$, $\delta^{15}N$, and $C{:}N_{atomic}$ based on 6–10 replicates of seven bone collagen samples is 0.22‰, 0.15‰, and 0.2, respectively.

With the exception of fish bone samples, only bone samples subjected to the NaOH wash were measured for AMS ^{14}C. Prepared bone collagen and plant 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 the zinc reduction method (Slota et al. 1987). The graphite was then pressed to produce a target and this was measured using a 0.5MV National Electrostatics Compact AMS. The ^{14}C age and 1 standard deviation were calculated using the Libby half-life (5568 yr) following the conventions of Stuiver and Polach (1977). The ^{14}C ages were then corrected for isotopic fractionation using the AMS-measured $\delta^{13}C$. The ^{14}C ages were calibrated using the CALIB 7.0 program (Stuiver et al. 2013) and the IntCal13 calibration curve (Reimer et al. 2013). Statistical analyses (linear correlations, two-tailed paired *t* tests, significance level of 0.05) were performed using Microsoft Excel 2013.

Calculating the Freshwater Reservoir Offset (FRO)

Two ways of calculating FRO for the cases where more than two associated samples are compared (represented here by four groups of samples from Shauke 8b) have been used. The first approach is to combine the dates for the terrestrial samples using uncertainty-weighted means and then use this pooled ^{14}C date for a comparison with the aquatic sample (fish). Prior to combining the ^{14}C dates, their statistical proximity was assessed using the Ward and Wilson (1978) chi-squared test in CALIB 7.0. The FRO uncertainty is the sum of the squares of the uncertainty in the terrestrial and aquatic ^{14}C values. An alternative, second approach for calculating the FRO is to calculate a separate offset for each terrestrial sample vs. fish within the groups, and then combine these individual offsets using uncertainty-weighted means. In this case, the uncertainty is taken as the square root of the variance of the offsets. Table 1 presents the FRO values calculated using both methods.

RESULTS AND DISCUSSION

Preservation of Bone Samples

The majority of bone samples analyzed demonstrated very good collagen preservation with its content varying within 4.3–16.4% (van Klinken 1999; Table 1). Three samples from Shauke 8b and Borly 4 yielded no collagen, and one fish sample from Borly 4 (UBA-27721) yielded very low collagen content, only 0.7%. The collagen content of this latter sample (UBA-27721) is rather marginal and, as suggested by van Klinken (1999), “might or might not be suitable for analysis.” We include this sample into the discussion of the results; however, we admit that the interpretation for this sample is rather ambiguous (see below). The $C{:}N_{atomic}$ ratio of the samples varied between 3.1–3.4, which is also within the accepted range characterizing a well-preserved collagen (DeNiro 1985).

Table 1 Results of AMS ^{14}C dating, stable C and N isotope analysis of the samples, and calculated freshwater reservoir offsets (FRO). ^{14}C calibration is discussed in the text.

Lab ID (UBA-)	Sample	Provenance	^{14}C age BP	Calibrated age (2σ)	Combined terrestrial ^{14}C age BP	FRO with combined terrestrial ^{14}C age	FRO	Combined FRO	NaOH treatment applied				No NaOH treatment			
									% coll.	δ^{13}C (‰)	δ^{15}N (‰)	C:N$_{at}$	% coll.	δ^{13}C (‰)	δ^{15}N (‰)	C:N$_{at}$
Shauke 8b (52°25′22.30″N, 76°50′16.50″E)																
26189	fish	sq. A1, hearth 2	3802 ± 37	2435–2063 BC*	—	269 ± 69	—	260 ± 61	—	—	—	—	3.3	–24.3	8.7	3.4
26190	herbivore		3624 ± 48	2136–1884 BC	3533 ± 59		178 ± 61		n/a	–18.6	6.9	3.2	16.2	–19.2	7.9	3.2
26191	charcoal		3532 ± 34	1948–1754 BC			270 ± 50		—	—	—	—	—	—	—	—
26192	animal, calcined		3501 ± 29	1905–1744 BC			301 ± 47		—	—	—	—	—	—	—	—
26197	fish	sq. A3	3771 ± 39	2333–2038 BC*	—	157 ± 42	—	157 ± 16	—	—	—	—	1.7	–22.5	9.7	3.2
26198	ovicaprid		3603 ± 42	2130–1784 BC	3614 ± 16		168 ± 57		9.7	–18.4	7.2	3.2	5.7	–18.5	7.2	3.2
26199	animal, calcined		3626 ± 43	2134–1888 BC			145 ± 58		—	—	—	—	—	—	—	—
26200	fish	sq. a I	3775 ± 42	2339–2038 BC*	—	161 ± 85	—	154 ± 74	—	—	—	—	3.6	–24.2	8.8	3.3
26201	herbivore		3678 ± 41	2196–1945 BC	3614 ± 74		97 ± 59		12.5	–18.7	8.4	3.2	10.5	–18.9	6.4	3.2
26202	animal, calcined		3573 ± 33	2025–1780 BC			202 ± 53		—	—	—	—	—	—	—	—
26193	fish	sq. A 7, ash pit	—	—	—	—	—	—	0	—	—	—	—	—	—	—
26194	herbivore		—	—	—		—		0	—	—	—	—	—	—	—
26203	fish	sq. A II, pit	3810 ± 43	2456–2137 BC*	—	224 ± 47	—	225 ± 19	—	—	—	—	1.8	–22.9	9.4	3.2
26204	ovicaprid		3571 ± 36	2027–1777 BC	3586 ± 19		239 ± 56		5.7	–18.6	7.1	3.2	4.8	–18.1	7.1	3.2
26205	animal, calcined		3598 ± 32	2033–1884 BC			212 ± 54		—	—	—	—	—	—	—	—
Shauke 1 (52°26′5.85″N, 76°50′15.59″E)																
26890	human	grave 1	3763 ± 34	2289–2042 BC*	—	53 ± 53	—	—	4.3	–19.2	13.3	3.2	7.4	–19.2	13.4	3.2
26891	ovicaprid/deer		3710 ± 41	2271–1976 BC	—		—	—	12.2	–20.2	9.8	3.2	10.4	–20.1	9.9	3.2
26892	human	grave 2	3772 ± 33	2293–2047 BC*	—	66 ± 49	—	—	9.6	–19.2	13.5	3.2	2.0	–19.5	13.4	3.2
26893	sheep		3706 ± 36	2202–1980 BC	—		—	—	9.7	–19.3	6.8	3.2	13.0	–19.3	6.9	3.1
26894	human	grave 3	3782 ± 33	2334–2050 BC*	—	21 ± 52	—	—	3.7	–18.4	14.1	3.2	8.8	–18.5	14.1	3.2
26895	sheep		3761 ± 40	2292–2036 BC	—		—	—	4.3	–18.6	8.0	3.2	2.3	–18.8	8.0	3.2
26896	human	grave 4	3883 ± 37	2470–2212 BC*	—	92 ± 55	—	—	6.7	–19.3	13.8	3.2	9.9	–19.4	13.5	3.1
26897	sheep, juvenile		3791 ± 40	2427–2046 BC	—		—	—	6.5	–19.4	7.8	3.2	14.9	–19.8	7.1	3.2

*Calibrated ^{14}C dates from aquatic and human samples need to be interpreted with caution as they might require a FRO correction.

Table 1 Results of AMS ^{14}C dating, stable C and N isotope analysis of the samples, and calculated freshwater reservoir offsets (FRO). ^{14}C calibration is discussed in the text.

Lab ID (UBA-)	Sample	Provenance	^{14}C age BP	Calibrated age (2σ)	Combined terrestrial ^{14}C age BP	FRO with combined terrestrial ^{14}C age	FRO	Com-bined FRO	NaOH treatment applied				No NaOH treatment			
									% coll.	δ^{13}C (‰)	δ^{15}N (‰)	C:N$_{at}$	% coll.	δ^{13}C (‰)	δ^{15}N (‰)	C:N$_{at}$
26898	charcoal	grave 5	3863 ± 35	2463–2209 BC	—	n/a	—	—	—	—	—	—	—	—	—	—
26899	deer/ovicaprid, calcined		3883 ± 32	2468–2234 BC	—		—	—	—	—	—	—	—	—	—	—
26900	horse/cattle		104 ± 51	AD 1675–1941	—		—	—	16.4	–20.5	5.1	3.2	10.1	–20.5	4.9	3.1
Borly 4, house 3 (51°49′28.9″N, 77°56′42.4″E)																
27719	horse	main bone-bearing horizon in ash layer	4387 ± 34	3095–2912 BC	—	—	—	—	11.2	–19.6	6.2	3.3	—	—	—	—
27720	cattle		4507 ± 41	3360–3037 BC	—	—	—	—	4.9	–18.8	8.0	3.2	—	—	—	—
27721	fish		4241 ± 46	2925–2666 BC*	—	—	—	—	0.7	—	—	—	—	—	—	—
27722	fish		—	—	—	—	—	—	0	—	—	—	—	—	—	—
7723	dog	sq. Б-I, layer 3a	4607 ± 39	3517–3125 BC*	—	—	—	—	12.6	–21.2	11.4	3.2	—	—	—	—
27724	fox	sq. B-I, 53–56 cm	4346 ± 34	3083–2896 BC	—	—	—	—	12.1	–18.6	8.7	3.3	—	—	—	—

*Calibrated ^{14}C dates from aquatic and human samples need to be interpreted with caution as they might require a FRO correction.

Effect of NaOH Treatment on Bone Collagen $\delta^{13}C$ and $\delta^{15}N$

NaOH wash is often used in ^{14}C sample preparation to remove humic acids as one of the main contaminants of the bone collagen (Berglund et al. 1976; DeNiro and Epstein 1981; Brock et al. 2010); however, there have been many concerns regarding the possibility of preferential affecting specific amino acids and, as a result, alteration of overall $\delta^{13}C$ signal of bone collagen (see discussion in Liden et al. 1995). Recent data demonstrated minor but systematic alteration of $\delta^{13}C$ values of the samples as a result of NaOH wash (Jørkov et al. 2007); however, in this case, lower $\delta^{13}C$ of bone collagen samples that have not been subjected to NaOH wash were explained as possibly affected by preservation of some lipids, known to have more negative $\delta^{13}C$ values compared to those of protein. This study also demonstrated no systematic effect of NaOH wash on $\delta^{15}N$ values of the samples, slightly lower C:N atomic ratios compared to the samples treated with ultrafiltration method (Brown et al. 1988 modified in Richards and Hedges 1999; includes both ultrafiltration and filtration with Ezee® filter separators), and fairly consistent %C and %N values of 41.1–44.7% and 14–16%, respectively, suggesting that the use of NaOH removes the nonprotein contaminants. The data on the effect of NaOH treatment on collagen yields of bone samples generally demonstrates a decrease in collagen yields (Liden et al. 1995), although not as remarkable as the effect of ultrafiltration (Jørkov et al. 2007).

In our study, 13 samples have been prepared in duplicate to further explore the possible effect of NaOH treatment at the concentrations used for the ^{14}C pretreatment on stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios, $C{:}N_{atomic}$ and collagen yields (for % collagen comparison, only 12 pairs were available). Our *t* test results at a significance level of 0.05 indicate a minor but significant effect of NaOH only for $C{:}N_{atomic}$ ratios of the samples ($p = 0.014$; Table 2); samples treated with NaOH appear on average 0.02 higher than untreated.

Table 2 Comparative analysis of the differences in $\delta^{13}C$, $\delta^{15}N$, $C{:}N_{atomic}$, and collagen yields between samples pretreated with and without a NaOH wash step.

Testing	Nr of observations	Mean ±1σ (NaOH-treated)	Mean ±1σ	*t*	*p*
$\delta^{13}C$, ‰	13	–19.1 ± 0.6	–19.2 ± 0.6	1.429	0.179
$\delta^{15}N$, ‰	13	9.4 ± 3.0	9.2 ± 3.1	0.823	0.427
$C{:}N_{atomic}$	13	3.2 ± 0.0	3.2 ± 0.0	2.880	0.014
% collagen	12	8.4 ± 3.8	8.3 ± 3.8	0.090	0.930

Despite the presence of several outliers, generally $\delta^{13}C$ or $\delta^{15}N$ isotope ratios demonstrate significant linear correlation between the values of samples pretreated in different ways ($R^2 = 0.86$ for $\delta^{13}C$; $R^2 = 0.96$ for $\delta^{15}N$; Figure 2). No significant correlation has been observed for the $C{:}N_{atomic}$ levels of the samples ($R^2 = 0.22$); however, in both cases the values for the prepared gelatin range between 3.12 and 3.24, which indicates a very high degree of consistency. Collagen yields varied between 3.7% and 16.4% for samples subjected to NaOH wash, and between 2.0% and 14.9% for those not subjected to NaOH treatment. No significant correlation has been observed in this case ($R^2 = 0.07$). Hereafter, the discussion only concerns bone samples pretreated using NaOH (with the exception of fish samples).

$\delta^{13}C$ and $\delta^{15}N$ Stable Isotope Analysis: Paleodietary Implications

The study represents the first attempt to assess the human subsistence patterns for the northeastern Kazakhstan prehistoric populations. Unfortunately, precise species determinations were not available for a number of faunal samples, which complicates some aspects of interpretation of the results.

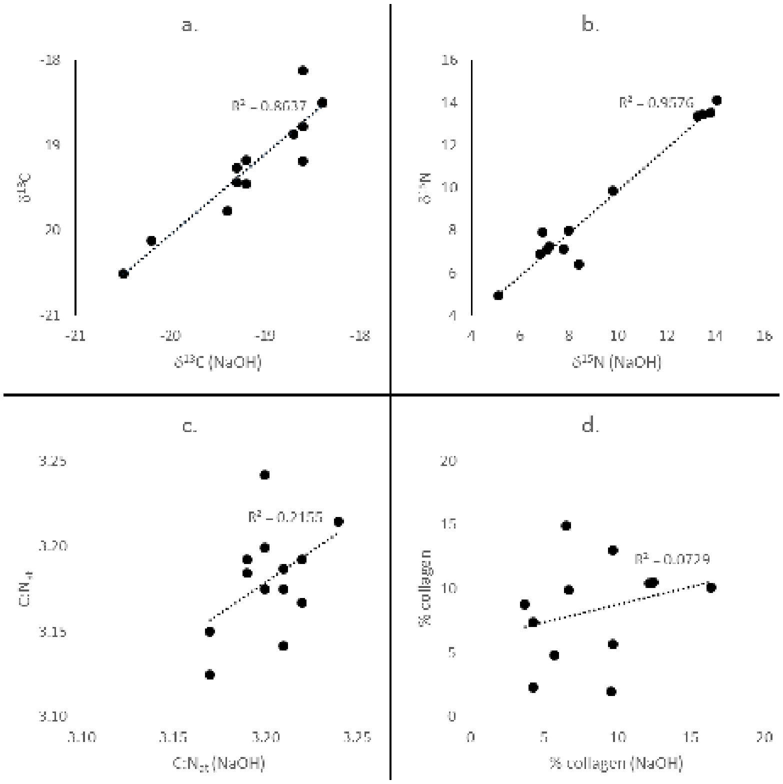

Figure 2 Plot showing the correlation between (a) δ^{13}C and (b) δ^{15}N isotope ratios, (c) C:N$_{atomic}$ levels, and (d) collagen yields of samples subjected to NaOH treatment and their NaOH wash-free duplicates.

Given the limited number of samples and general geographical and environmental similarity of the sites analyzed, the samples were plotted and discussed together.

In total, 1 dog, 1 fox, 4 fish, 11 domestic herbivore, and 4 human samples have been analyzed for stable isotope ratios. Overall, the results do not suggest major variations within these groups and indicate the C$_3$-based foodchain with no evidence of C$_4$ plant consumption (such as millet; Figure 3).

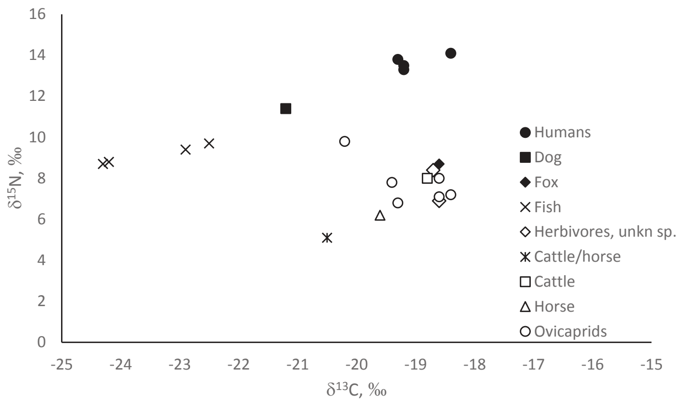

Figure 3 Bone collagen δ^{13}C and δ^{15}N values for human and faunal samples ($n = 21$)

The mean $\delta^{13}C$ and $\delta^{15}N$ values for freshwater fish (*n* = 4) from the settlement of Shauke 8b are –23.5 ± 0.9‰ and 9.2 ± 0.5‰, respectively. The low carbon isotopic values are typical for the inland freshwater reservoirs characterized by primarily C_3 foodchains and depleted $\delta^{13}C$ in the local fauna (unlike e.g. Lake Baikal ecology, Katzenberg and Weber 1999).

There are two outliers among the analyzed herbivores (*n* = 11). The horse/cattle sample from grave 5 of Shauke 1 (UBA-26900) clearly demonstrated the lowest $\delta^{13}C$ and $\delta^{15}N$ values among other herbivores analyzed (–20.5‰ and 5.1‰, respectively). As this sample is dated to cal AD 1675–1941, there is a possibility that it has been affected by modern changes in the atmospheric reservoir due to fossil fuel burning ("Suess effect"), although the $\delta^{13}C$ depletion from pre-industrial value of –6.5‰ until AD 1940 has only been 0.3–0.4‰ (Francey et al. 1999). We could also suggest that, belonging to a different period and culture, that animal was subjected to a different pasture strategy (e.g. in a more forested environment). Another clear outlier is an ovicaprid from grave 1 of Shauke 1 (UBA-26891), which has the highest $\delta^{15}N$ values among herbivores analyzed (9.8‰) and second lowest $\delta^{13}C$ values (–20.2‰). At the moment, it is not clear what could cause such unusual isotopic values. One of the suggestions would be the use of manured fodder (pasture) by this animal, as this could significantly increase bone collagen $\delta^{15}N$ ratios of the analyzed individual. However, this hypothesis needs to be tested against a larger number of herbivorous samples from the same culture. With the exclusion of the two outlying individuals, the analyzed herbivores (*n* = 9) have mean $\delta^{13}C$ and $\delta^{15}N$ values of –18.9 ± 0.4‰ and 7.4 ± 0.7‰, respectively.

The isotopic values of a single fox analyzed ($\delta^{13}C$ = –18.6‰; $\delta^{15}N$ = 8.7‰) are only 0.3‰ higher in $\delta^{13}C$ and 1.3‰ higher in $\delta^{15}N$ than the associated mean values for herbivores. This difference is smaller than commonly observed trophic level offset of 0.5–2‰ for $\delta^{13}C$ and 3–6‰ for $\delta^{15}N$ (see Introduction). However, at the moment no local wild herbivorous species (such as steppe rodents, reptiles or birds which would constitute the diet of a steppe fox) have been analyzed and as such we cannot make conclusions on the isotopic signature of this animal's diet.

All human samples analyzed (*n* = 4) come from the cemetery of Shauke 1. The mean $\delta^{13}C$ and $\delta^{15}N$ values for the humans are –19.0 ± 0.4‰ and 13.7 ± 0.4‰, respectively, which indicate a diet based predominately on C_3 foodchains and animal protein. Isotopically, humans cluster together quite tightly, indicating the absence of major variation in their diet. Human mean $\delta^{13}C$ and $\delta^{15}N$ ratios are respectively 0.1‰ and 6.3‰ higher than those of herbivores analyzed. The human-herbivore nitrogen offset is slightly larger than the commonly observed trophic level increase of 3–6‰ and suggests that herbivores may not represent the only source of dietary protein for humans; however, their diet may have also included fish, which is at a slightly higher trophic level than terrestrial herbivores analyzed due to the multiple steps in an aquatic food web. A similar conclusion could be drawn regarding the diet of a single dog analyzed; its isotopic signature ($\delta^{13}C$ = –21.2‰; $\delta^{15}N$ = 11.4‰) suggests the contribution of both domestic herbivores and fish into the diet. However, the amount of fish in the dog's diet was possibly slightly larger compared to the human diet as canine $\delta^{13}C$ values are closer to that of fish.

Recently, there has been a number of reports suggesting that, despite the traditional perception of steppe communities as pastoralists with similar herd-based economies, apparently depending on local climate, topography, and ecology, there was variation in subsistence strategies between the groups, with some of them relying on fishing and/or cultivation (e.g. Frachetti et al. 2010; Murphy et al. 2013). Archaeologically, fishing is regarded as playing only a minor role in the economy of the Early Bronze Age populations in the region, as very few associated artifacts have been recovered. However, the discovery of a large quantity of fish remains in the settlement of Shauke 8b, as well as current results of stable isotope analysis on humans from Shauke 1, suggest the opposite, that fishing was an accounted economical feature for this population. Undoubtedly, more samples, in particular human, need to be analyzed do draw more definite conclusions on the extent of fishing for various prehistoric communities of the area.

The majority of the samples analyzed for stable isotopes during the current study were fauna, which contributes to the reconstruction of the isotopic baseline for the region. Generally, the results indicate primarily C_3-based ecology (both terrestrial and aquatic) of the Pavlodar area, and also suggest the absence of millet cultivation in the area in the Middle Bronze Age (the latter conclusion is however based on extremely small number of human samples analyzed). Our data generally correspond with results for a number of Middle to Late Bronze Age sites in northern (Ventresca Miller et al. 2014) and central Kazakhstan (Lightfoot et al. 2014), and the Baraba forest steppe of southwestern Siberia (Privat et al. 2005), which isotopically document the appearance of millet consumption in central Kazakhstan only in the Late Bronze Age (Lightfoot et al. 2014).

AMS Radiocarbon Dating Results: FRO

Ten associated human–terrestrial herbivore or fish–terrestrial plant/herbivore groups from all three sites have been AMS ^{14}C dated to assess the freshwater reservoir offset (FRO) at the Pavlodar area. Unfortunately, precise fish species determinations were not available. The group from house 3 of the Borly 4 settlement demonstrated quite variable ^{14}C age ranges, which apparently represent several phases of use of the building (see discussion in the next section). As such, they apparently cannot be strictly associated with each other as suggested before the analysis, and used for the estimation of the FRO.

For other groups, FRO was calculated using the two methods described above. For the first method, all samples, except for UBA-26201 and UBA-26202 (which might possibly be explained by the small number of terrestrial samples analyzed), the combined dates from terrestrial samples were statistically the same at the 95% level [using the Ward and Wilson (1978) chi-squared test in CALIB 7.0]. In all cases, the resulting final FROs for the four groups calculated using the two methods show very little difference. In general, for Shauke 8B, FROs between fish and individual terrestrial samples range from 97 ± 59 to 301 ± 47 ^{14}C yr; the overall mean FRO (calculated by combining all individual offsets of terrestrial samples versus fish using uncertainty-weighted means) is 210 ± 65 ^{14}C yr.

Human-terrestrial herbivore pairs from Shauke 1 show less variation in FRO, which is not unexpected as the diet of human individuals would only partly be based on aquatic resources. As suggested earlier, the diet of the human individuals possibly included proportions of fish. The FRO values within the pairs vary from 21 ± 52 to 92 ± 55 ^{14}C yr; the general mean FRO for Shauke 1 (calculated by combining all individual human-terrestrial herbivore offsets using uncertainty-weighted means) is 57 ± 29 ^{14}C yr. The results for the human samples suggest negative correlation between FROs and $\delta^{13}C$ values ($R^2 = 0.790$; $n = 4$; Figure 4), which is to be expected with more depleted (negative) $\delta^{13}C$ values of fish yielding higher FRO values. This correlation is the strongest evidence for freshwater resources in the diet for these individuals. In comparison, no correlation was observed between human FRO and $\delta^{15}N$ values, which is possibly not surprising given only 1.8‰ difference between mean $\delta^{15}N$ values of fish and herbivores (unlike the 4.4‰ difference in $\delta^{13}C$). Furthermore, the human with the highest $\delta^{15}N$ value (14.1‰) also has the lowest offset value with the associated herbivore sample (21 ± 52 ^{14}C yr).

The results obtained represent the first data for the freshwater offset in Kazakhstan; no comparative material is available for the area at the moment. The geographically closest research, undertaken

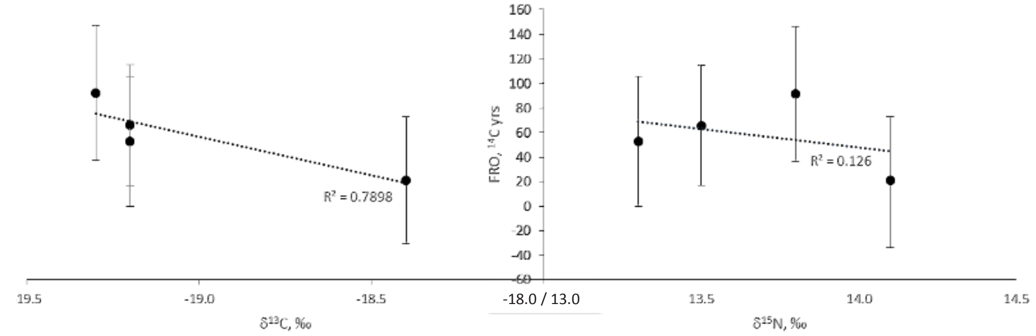

Figure 4 Linear regression plot of human δ^{13}C and δ^{15}N values and human-faunal ^{14}C offsets (means and 1σ)

in the Lake Baikal region, has the highest offset of ~700 ^{14}C yr for the endemic seals in the lake (Nomokonova et al. 2013), while in humans the highest detected offset is 622 ^{14}C yr (Schulting et al. 2014). Such a large value for the freshwater reservoir offset in the area is related to the nature of the lake itself, being the largest, deepest, and oldest freshwater lake in the world. However, there is still an ongoing discussion on the particular factors affecting the extent of the offset (Schulting et al. 2014). More distantly, in the North Caucasus site of Klin-Yar, the offset values between humans and historically dated artifacts have been found to be highly variable, ranging between 0 to ~350 ^{14}C yr (Higham et al. 2010). Even further away, in the Dnieper Rapids area, the FRO values for human-faunal/fish paired samples vary between 125–750 ^{14}C yr (Lillie et al. 2009). Among these three studies, only for the Lake Baikal region was it possible to calculate the correction to account for the FRO in humans as 77 ± 10 ^{14}C yr for each per mil increase in δ^{15}N (Schulting et al. 2014).

The FRO values observed in our study (maximum fish-herbivore offset of 301 ± 47 ^{14}C yr) generally correspond with the data above, although the highest values are much lower than in the Baikal and Dnieper Rapids regions, which is apparently due to a smaller proportion of dissolved old carbon in the water. More human samples need to be tested for ^{14}C and stable isotope values to assess the FRO correction for the area.

AMS Radiocarbon Dating Results: Archaeological Implications

In total, 28 dates have been obtained for the three sites analyzed. The horse sample from Shauke 1, dated to the modern period (UBA-26900; AD 1680–1940), is obviously intrusive in the burial and therefore is not discussed further in the text.

The group from the Borly 4 settlement demonstrated quite variable ^{14}C age ranges of samples, with cattle and dog samples being the oldest (3360–3040 and 3520–3130 BC, respectively) and the fish sample being the youngest (2930–2670 BC). Being an open complex (unlike closed complexes such as graves), the assemblage of house 3 apparently represents several phases of use—as a living space (a house) and, apparently some time later, as a midden (the midden was likely filled during a very short period of time as no signs of weathering have been observed on bones). At the moment, we can suggest that the older date of the dog sample is likely to be affected by the FRE rather than actually belonging to an earlier period of use of the building, as the bone was stratigraphically located above the cattle sample in the midden. However, the cattle sample bone analyzed was recovered from the very bottom of the midden; therefore, there is a chance that it could have belonged to the earlier phase of the site functioning. The interpretation of the ^{14}C date of the fish sample is rather complicated. The sample yielded very low % collagen (0.7%), which might be an indication of collagen degradation. It is also possible that contaminating humic acids were not removed since no

NaOH step was used. However, the date can also be interpreted as associated with the later Early Bronze Age phase of the site; the existence of multiple burrows suggests that the bone could have been relocated by animals. Furthermore, we cannot deny the possibility of FRO affecting the date of fish sample (i.e. the actual date of this sample being even younger). There is no direct evidence for the latter suggestion; however, there is a chance that the fish might have been taken from the Irtysh River and thus might be bearing an associated offset.

In general, dates obtained from fish, dog, and human bone samples are not included in the discussion below as they are likely to be affected by the FRO (see above). The remaining 18 dates cluster well together within the sites (Figure 5). The 2σ summed probability age ranges are 3340–2900 BC for Borly 4, 2470–2230 BC for Shauke 1, and 2130–1770 BC for Shauke 8b.

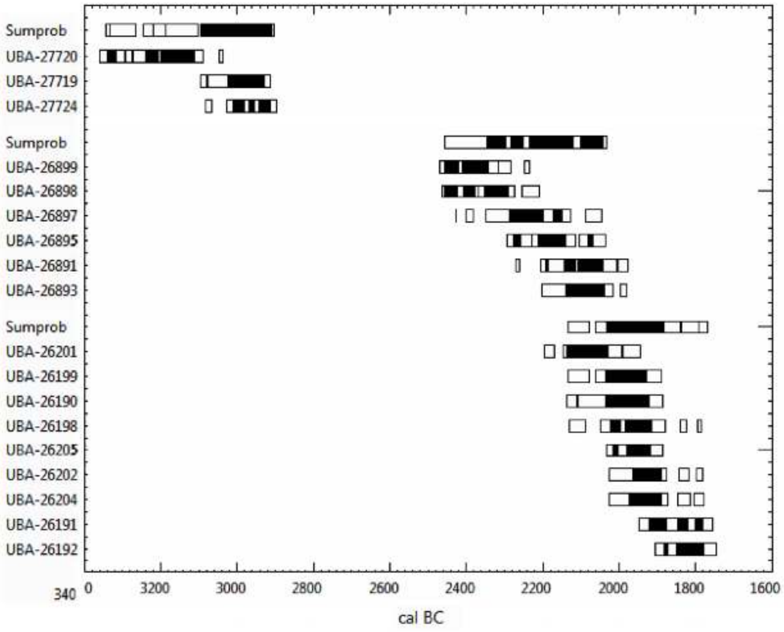

Figure 5 Calibrated age ranges of the samples analyzed (dates obtained from fish, dog, and human bone samples are not included as likely to be affected by the FRO).

The obtained AMS dates are the first for the Eneolithic of the Middle and Upper Irtysh River region and correspond with the archaeological chronology for the sites. The new ^{14}C dates of the Borly 4 settlement suggest that the site is generally synchronous to the Eneolithic sites of southwestern Siberia and Kazakhstan, including Novoilyinka 3 and 6 in the North Kulunda River area (38–25th centuries BC), sites of the Ust-Narym-Shiderty type in the northeast Kazakh Uplands (second half of the 4th to first half of the 3rd millennium BC; Merz 2008; Gaiduchenko and Kiryushin 2013), Tersek culture settlements of Kozhai 1 and Kumkeshu 1 in the Turgai region (41st to 25th centuries BC; Kaliyeva and Logvin 1997), Botai culture settlements of Botai and Krasny Yar in the forest-steppe Ishim River region (38th to 32nd centuries BC; Levine and Kislenko 2002), as well as to the Afanasyevo culture sites of the Altai Mountains and Yenisei River (38–25th centuries BC), and Yamnaya culture sites of the Volga-Ural region (39–27th centuries BC; Polyakov 2010; Morgunova 2014). Archaeologically, the aforementioned sites reveal similar economical features characteristic to early pastoral societies (Gaiduchenko 2013; Gaiduchenko and Kiryushin 2013). At this stage, it can be argued that the sites of the Botai and Tersek cultures are earlier than Borly 4 and possibly

Novoilyinka 3 and 6; however, we can suggest that, as a result of further research, the chronological dates of the Borly 4 settlement and generally the Eneolithic period of the region will be expanded, possibly towards the earlier period.

The Early Bronze Age period of the Middle Irtysh River region is mainly represented by the Elunino culture, which is ^{14}C dated to the 25–18th centuries BC (Grushin 2013). Previously, the earliest Elunino sites have only been found northeast of Sary-Arka (Kazakh Uplands, to the west of Irtysh River) and ^{14}C dated to 25th to 22nd centuries BC (Grushin 2013). The dates obtained from the Shauke 1 cemetery suggest that the site was used for extended period of time. The two earlier graves are dated to the 24th century BC, and currently these are some of the earliest dates for the Elunino sites in the Ob-Irtysh interfluve; they are synchronous to the dates from the cemetery of Shiderty 10 and the settlement of Shiderty 3 of northeast Sary-Arka (Merz 2008) and generally correspond with those from the Early Bronze Age kurgans of Aina-Bulak 1 cemetery in the Upper Irtysh River area (Kovalev 2009). The latter suggests that the formation and spread of the Elunino population occurred from west to east. The other three graves analyzed were built in the mid-22nd to early 21st centuries BC, and they appear to belong to the beginning of the second phase of the Elunino culture, i.e. synchronous to the classical Elunino sites of Berezovaya Luka and Elunino 1 (Grushin 2013). The dates obtained from the settlement of Shauke 8b (3000 to 2000 BC) attribute the site to the end of the second stage of Elunino culture, also making it synchronous to the sites of Berezovaya Luka and Elunino 1 (Grushin 2013).

In general, there is a ~450-yr hiatus between the ^{14}C dates of the Eneolithic complex of Borly 4 and Early Bronze Elunino site of Shauke 1, which at the moment is likely to be explained by the small number of excavated sites, as well as the lack of the ^{14}C research in the area. However, the ongoing archaeological work in the region (including the investigation of various buildings of the Borly 4 settlement) might result in discovery of the sites ^{14}C dating to the present chronological hiatus (i.e. 29–25th centuries BC) and synchronous to e.g. the Early Bronze Age site of New Shulba IX of the Upper Irtysh River region (first half of the 3rd millennium BC; Stöllner et al. 2013). Further ^{14}C research into the recently discovered sites of the Yamnaya culture type in the area (Merz and Merz 2010) might also fill the present chronological hiatus.

CONCLUSIONS

The present study makes significant contributions to several major areas across both environmental science and archaeology of NE Kazakhstan. It represents the first data on freshwater reservoir offsets for the entire area of Kazakhstan, the first ^{14}C chronology of the Middle Bronze Age sites of NE Kazakhstan, and the first paleoisotopic investigation of this area. The main observations of the study are as follows:

1. The diet of the humans and fauna analyzed was based on C_3 foodchains with no evidence of a C_4 plant (such as millet) contribution; no major dietary variations have been observed.
2. Aquatic resources apparently were a continuous dietary feature of the Eneolithic to Middle Bronze Age populations of NE Kazakhstan.
3. The first ^{14}C dates obtained for the Upper and Middle Irtysh River region attribute the Eneolithic period of the area to the 34–30th centuries BC, and Early Bronze Age to the 25–20th centuries BC. As such, the Eneolithic settlement of Borly 4 appears to be synchronous with the sites of Botai and Tersek culture and to some Eneolithic sites of southwestern Siberia.
4. At present, there is a ~450-yr hiatus between the ^{14}C dates of the Eneolithic complex of Borly 4 and Early Bronze Elunino site of Shauke 1.

5. The maximum fish-herbivore FRO observed in our study equals 301 ± 47 ^{14}C yr.
6. ^{14}C dates from aquatic and human samples from the area apparently need to be interpreted with caution as likely to be affected by the FRO (i.e. they appear older).
7. NaOH treatment of bones showed a minor but significant effect (0.02 increase in the ratio) on $C:N_{atomic}$ ratios of the samples. No significant effect of the treatment on $\delta^{13}C$, $\delta^{15}N$, and collagen yields of the samples has been found.

As a major implication for future work in the area, a larger number of human-herbivore paired samples need to be analyzed (both for ^{14}C and $\delta^{15}N$) to develop a regression model (equation) to predict FRE corrections for the human bone dates, as well as to explore in detail the contribution of fish into the human diet.

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