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USING δ^2H IN HUMAN BONE COLLAGEN TO CORRECT FOR FRESHWATER ^{14}C RESERVOIR OFFSETS: A PILOT STUDY FROM SHAMANKA II, LAKE BAIKAL, SOUTHERN SIBERIA

Rick J Schulting[1*] • Christophe Snoeck[2] • Ian Begley[3] • Steve Brookes[3] • Vladimir I Bazaliiskii[4] • Christopher Bronk Ramsey[1] • Andrzej Weber[5]

[1]School of Archaeology, University of Oxford, 1 South Parks Road, Oxford OX1 3TG, United Kingdom.
[2]Research Unit: Analytical, Environmental & Geo-Chemistry, Dept. of Chemistry, Vrije Universiteit Brussel, AMGC-WE-VUB, Pleinlaan 2, 1050 Brussels, Belgium.
[3]Iso-Analytical, Phase 3, The Quantum, Marshfield Bank, Crewe CW2 8UY, United Kingdom.
[4]Department of Archaeology and Ethnography, Irkutsk State University, Karl Marx Street 1, Irkutsk 664003, Russia.
[5]Department of Anthropology, University of Alberta, Edmonton, Alberta T6G 2H4, Canada.

ABSTRACT. There is increasing awareness of the need to correct for freshwater as well as marine reservoir effects when undertaking radiocarbon (^{14}C) dating of human remains. Here, we explore the use of stable hydrogen isotopes (δ^2H), alongside the more commonly used stable carbon (δ^{13}C) and nitrogen isotopes (δ^{15}N), for correcting ^{14}C freshwater reservoir offsets in 10 paired human-faunal dates from graves at the prehistoric cemetery of Shamanka II, Lake Baikal, southern Siberia. Excluding one individual showing no offset, the average human-faunal offset was 515 ± 175 ^{14}C yr. Linear regression models demonstrate a strong positive correlation between δ^{15}N and δ^2H ratios, supporting the use of δ^2H as a proxy for trophic level. Both isotopes show moderate but significant correlations ($r^2 \sim 0.45$, $p < 0.05$) with ^{14}C offsets (while δ^{13}C on its own does not), though δ^2H performs marginally better. A regression model using all three stable isotopes to predict ^{14}C offsets accounts for approximately 65% of the variation in the latter ($r^2 = 0.651$, $p = 0.025$), with both δ^{13}C and δ^2H, but not δ^{15}N, contributing significantly. The results suggest that δ^2H may be a useful proxy for freshwater reservoir corrections, though further work is needed.

KEYWORDS: Early Bronze Age, Early Neolithic, fisher-hunter-gatherers, stable carbon, nitrogen and hydrogen isotopes, freshwater reservoir effects.

INTRODUCTION

Stable carbon isotope measurements on human bone collagen are often used to estimate the amount of dietary protein deriving from marine foods and so can help correct radiocarbon (^{14}C) dates subject to marine reservoir effects (Barrett et al. 2000; Yoneda et al. 2002; Dewar and Pfeiffer 2010; Ascough et al. 2012; Craig et al. 2013). In freshwater aquatic systems, stable nitrogen isotopes have generally been found to be of greater utility (Cook et al. 2001; Shishlina et al. 2009; Wood et al. 2013; Bronk Ramsey et al. 2014; Fernandez et al. 2015; Schulting et al. 2014; 2015; Svyatko et al. 2015, 2017a, 2017b), sometimes in combination with stable carbon isotope ratios, in situations where these differ isotopically from terrestrial ecosystems (Katzenberg and Weber 1999; Yoshii 1999; Yoshii et al. 1999). The degree to which isotopic inferences concerning past diets are effective in correcting for marine and freshwater reservoir offsets in ^{14}C can be assessed through paired dating programs of human and terrestrial mammal bone—the latter usually not subject to significant reservoir effects—from the same graves. In many cases, however, the use of carbon and/or nitrogen isotope ratios still leaves much unexplained variation in observed offsets in ^{14}C years. Here, we present the results of a pilot study aimed at exploring the utility of stable hydrogen isotope ratios as an independent proxy for trophic position, in order to address freshwater reservoir offsets at the Early Neolithic and Early Bronze Age cemeteries at Shamanka II, Lake Baikal, southern Siberia (Figure 1). Any additional information that can be gained will contribute to improving the accuracy of radiocarbon determinations on human remains, which in turn provides the framework for an increasing

*Corresponding author. Email: rick.schulting@arch.ox.ac.uk.

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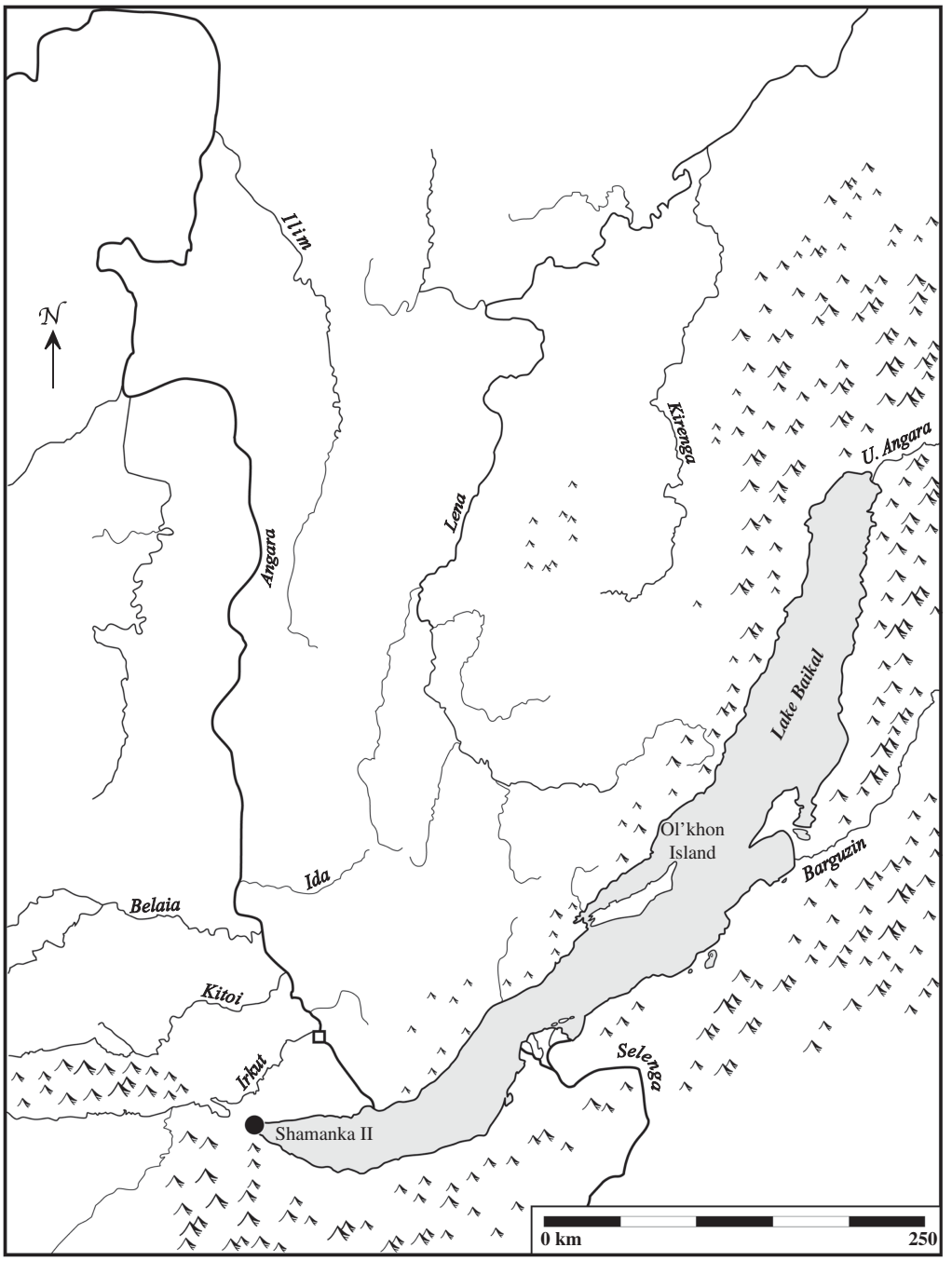

Figure 1 Map of the Lake Baikal region showing the location of Shamanka II.

number of bioarchaeological studies in the region (Waters-Rist et al. 2015; Lieverse et al. 2016; Weber et al. 2016a, 2016b). This is important since not all graves contain alternative materials suitable or available for dating (i.e., terrestrial mammalian bone/tooth), and so there is still a strong reliance on directly dating human skeletons. There is the potential for wider application

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in many other contexts where investigating trophic levels is of interest, whether or not these include the need for ^{14}C reservoir corrections.

Stable Hydrogen Isotopes

The use of stable carbon and nitrogen isotopes in archaeology is sufficiently common to require no detailed introduction, and many overviews are available (e.g., Lee-Thorp 2008). In the specific context of this paper, they have seen extensive use in the Lake Baikal region, both for palaeodietary reconstruction and for the investigation of freshwater reservoir effects (FRE) (Katzenberg and Weber 1999; Weber and Bettinger 2010; Weber et al. 2011, 2016a, 2016b; Katzenberg et al. 2012; Bronk Ramsey et al. 2014; Schulting et al. 2014; 2015). Important points to bear in mind are that (1) the subsistence economy of the Early Neolithic to Early Bronze Age cultures of the region was based entirely on fishing, hunting and gathering, with a variable but generally strong contribution from the aquatic resources of the lake itself and its surrounding rivers, and (2) Lake Baikal exhibits an unusually variable stable carbon isotope ecology, with fish bone collagen values from different zones ranging from –10‰ to –25‰ (Katzenberg and Weber 1999; Yoshii 1999; Yoshii et al. 1999; Weber et al. 2011).

Stable hydrogen isotopes (δ^2H, or δD), on the other hand, have seen relatively limited use in archaeology and so require additional discussion. They have been mainly used in forensic applications, food sourcing and ecological studies, particularly to trace migration patterns in animals. They often covary with $\delta^{18}O$ ratios and so are used to track mobility and seasonality (Hobson et al. 2004; Kirsanow et al. 2008; Bowen et al. 2009). But many studies have also demonstrated that δ^2H is subject to a marked trophic level enrichment, such that it combines climate (drinking water) and dietary signals (Birchall et al. 2005; Reynard and Hedges 2008; Soto et al. 2011; Peters et al. 2012; Topalov et al. 2013). Perhaps because it combines these two signals, δ^2H has been suggested to be particularly useful in distinguishing terrestrial and aquatic systems (Finlay et al. 2010). While a positive correlation with $\delta^{15}N$ ratios would be expected—as these are also a useful proxy for trophic level (Hedges and Reynard 2007)—there does seem to be the potential for additional information with δ^2H (e.g., Birchall et al. 2005, Figure 1). This may particularly be the case in dealing with situations where $\delta^{15}N$ ratios are affected by factors other than trophic level enrichment, such as aridity or manuring (Amundson et al. 2003; Bogaard et al. 2007), though we do not expect either to be relevant in the case of Lake Baikal hunter-gatherers.

MATERIALS AND METHODS

Paired dates were obtained on human bone and terrestrial faunal (marmot: *Marmota sibirica*; and red deer: *Cervus elaphus*) dentine collagen from 10 graves at the Early Neolithic (EN) and Early Bronze Age (EBA) cemeteries of Shamanka II, on the southwest shore of Lake Baikal. Of these, three had been previously dated and were included in a study of the region's FRE (Bronk Ramsey et al. 2014; Schulting et al. 2014). All the selected humans are of post-weaning age, with the youngest being aged 5–6 yr (see Waters-Rist et al. 2011). Eight of the graves were known to date to a large Early Neolithic (ca. 7500–6700 cal BP) cemetery based on their mortuary protocols, while two graves were selected from a smaller Early Bronze Age (ca. 4600–3700 cal BP) cluster of burials (Weber et al. 2016a, 2016b) in order to look at possible temporal variation in diets and ^{14}C offsets. A number of samples were dated multiple times. These are combined using the R_Combine function in OxCal 4.2 (Bronk Ramsey 2013).

Stable Isotope Measurements

Stable carbon and nitrogen isotope ratio measurements were undertaken using the same collagen preparation used for radiocarbon dating (see below). Measurements were made on a

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Sercon continuous flow IRMS, with a precision of ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. An alanine standard was used for drift correction on the IRMS, with additional alanine and glutamic acid standards (USGS40: $\delta^{13}C = -26.4‰$, $\delta^{15}N = -4.5‰$; USGS41: $\delta^{13}C = +37.6‰$, $\delta^{15}N = +47.6‰$) used in a three-point calibration of the results (Coplen et al. 2006). The values reported here are the means of measurements in triplicate.

The same prepared collagen was used for stable hydrogen isotope analysis. Measurement of $\delta^{2}H$ presents additional challenges, since a minor but not insignificant proportion (ca. 20% for collagen) of the hydrogen in proteinaceous tissues (including collagen and keratin) is prone to exchange with hydrogen in ambient water vapor found in the laboratory undertaking the analysis (Cormie et al. 1994; Wassenaar and Hobson 2000, 2003; Bowen et al. 2005; Reynard and Hedges 2008; Chesson et al. 2009; Meier-Augenstein et al. 2011, 2013). While there is a protocol in place for the analysis of keratin making use of international standards (Wassenaar and Hobson 2003; Bowen et al. 2005; Meier-Augenstein et al. 2011, 2013), there are as yet no such standards for collagen. A further issue has been noted recently affecting both keratin and collagen, involving hydrogen fractionation during the formation of HCN during measurement in the presence of nitrogen (Nair et al. 2015; Coplen and Qi 2016; Reynard and Tuross 2016). Given these problems, we report both the measured and exchange-corrected values of $\delta^{2}H$, using the latter in the analysis. Since the relationship between them is perfectly linear, the use of either set of values will give the same results in terms of our discussion.

The primary reference material used was IA-R002 (mineral oil, $\delta^{2}H_{VSMOW} = -111.2‰$), traceable to NBS-22 (mineral oil, $\delta^{2}H_{VSMOW} = -118.5‰$), an inter-laboratory comparison standard distributed by the International Atomic Energy Agency (IAEA). In addition, inter-laboratory comparison standard IAEA-CH-7 (polyethylene foil, $\delta^{2}H_{V\text{-}SMOW} = -100.3‰$) and FIRMS 221-1 (nylon, $\delta^{2}H_{VSMOW} = -160.9‰$) (http://www.lgcstandards.com) were measured for quality control. Also included in the runs were the keratin standards USGS42 (human hair, non-exchangeable $\delta^{2}H_{VSMOW} = -72.9 \pm 2.2‰$), USGS43 (human hair, non-exchangeable $\delta^{2}H_{VSMOW} = -44.4 \pm 2.0‰$), and Eurofins 11/2/C (casein, non-exchangeable $\delta^{2}H_{VSMOW} = -113.4 \pm 3.8‰$). The $\delta^{2}H_{VSMOW}$ ratios for inter-laboratory comparison standards USGS42 and USGS43 have recently been revised (Coplen and Qi 2016), and we employ the new values here. Eurofins 11/2/C is an inter-laboratory quality control sample provided by Eurofins Scientific. In addition, we included cow and bison collagen standards previously prepared at the Research Laboratory for Archaeology and the History of Art (RLAHA), Oxford (Reynard 2007), which underwent equilibration in our study with two waters with known $\delta^{2}H_{VSMOW}$ ratios (depleted Water A = –43.5 ± 0.21‰, and enriched Water B = +110.0 ± 1.42‰, calibrated against in-house standards IA-R053 and IA-R055 at Iso-Analytical), differing by more than 100‰ as recommended by Meier-Augenstein et al. (2011). Since the $\delta^{2}H$ ratios of the cow and bison (Table 1) do not entirely bracket the range of human values in our study, and therefore are not entirely appropriate for their calibration, we are in the process of preparing a new marine seal bone collagen standard regularly used as an internal standard for stable carbon and nitrogen isotope measurements at Oxford.

USGS42, USGS43 and Eurofins 11/2/C were weighed into open capsules and simultaneously equilibrated alongside the archaeological samples and collagen standards with ambient water vapor at Iso-Analytical (Crewe, Cheshire, UK) for 12 days prior to analysis (cf. Wassenaar and Hobson 2003). These were only sealed and added to the sample carousel once batch analysis had begun. They should therefore be subject to the same exchange between the different runs, adhering to the "Principle of Identical Treatment" (Werner and Brand 2001).

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Table 1 δ^2H results for standards $\delta^2H_{VSMOW(non\text{-}exchangeable)} = \delta^2H_{VSMOW(measured)} - (-36.752/0.892)$.

Standard	Material	Accepted value	±	δ^2H_{VSMOW} (measured)	±	δ^2H_{VSMOW} (non-exchangeable)	±	n
IA-R002	Mineral oil	–111.2		–110.4	1.3	—	—	35
IAEA-CH-7	Polyethelene	–100.3		–100.1	1.9	—	—	16
FIRMS 221-1	Nylon	–160.9		–160.2	3.5	—	—	16
USGS42	Human hair	–72.9	2.2	–102.5	2.7	–73.6	3.1	15
USGS43	Human hair	–44.4	2.0	–76.8	3.2	–44.4	3.6	15
Eurofins 11/2/C	Casein	–113.4	3.8	–137.4	2.8	–112.8	3.1	15
Bison	Collagen			–151.9	3.7	–129.1	4.2	12
Cow	Collagen			–79.6	3.1	–48.1	3.5	12
Seal	Collagen			–14.8	3.8	24.7	4.3	12

Samples were kept in a sealed glass container with dessicants until ready for analysis. Measurements were undertaken at Iso-Analytical by Elemental Analyser–Isotope Ratio Mass Spectrometry (EA-IRMS). Following equilibration, samples and references were placed in silver capsules and loaded into a zero-blank auto-sampler, flushed with 99.9992% helium at a flow rate of approximately 50 mL/min before and during the entirety of batch runs. The samples were then dropped into a furnace at 1080°C and thermally decomposed to H_2 and CO over glassy carbon. Any traces of water produced were removed by magnesium perchlorate, and any traces of CO_2 were removed via a Carbosorb™ trap. H_2 was resolved by a packed column gas chromatograph held at 35°C. The resultant chromatographic peak entered the ion source of the IRMS where it was ionized and accelerated.

The measured δ^2H ratios for USGS42, USGS43 and Eurofins 11/2/C were used to obtain a 3-point linear calibration ($y = 0.892x - 36.752$, $r^2 = 0.987$, $n = 36$), which was used for the exchangeable hydrogen correction. We assume that keratin and collagen powders will behave similarly in terms of exchangeable H, though this may not be valid because of differences in their amino acid composition (Reynard and Tuross 2016; Soto et al. 2017). We have not applied a separate calibration using the collagen standards, pending the dual water calibration of the seal collagen. While the reported values may thus be subject to revision, any changes will apply equally to all the archaeological samples and so will not affect our discussion here. It is worth noting that the sub-fossil bison collagen we employed as one of our standards is the same material used in a recent study by Reynard and Tuross comparing different protocols, which yielded a nearly identical measured $\delta^2H_{VSMOW\text{-}SLAP}$ ratio of $-151.4 \pm 1.7‰$ ($n = 5$) compared to that of $-151.9 \pm 4.2‰$ ($n = 12$) obtained in our study (Table 1), despite being analyzed in—and equilibrated to the atmosphere of—a laboratory on the eastern seaboard of the United States (Reynard and Tuross 2016: tab. 1). While on opposite sides of the Atlantic, the two laboratories are in zones sharing similar δ^2H and $\delta^{18}O$ precipitation values (Bowen 2010), and so their equivalence is not surprising but does provide additional confidence in the results. Measurement precision during the runs for the non-organic standards (mineral oil, polyethelene and nylon) was 1.9‰ ($n = 67$), and that for the organic standards (keratin and casein) was 2.9‰ ($n = 45$) (Table 1).

Radiocarbon Dating

Radiocarbon measurements were undertaken following the standard protocols in place at the Oxford Radiocarbon Accelerator Unit (ORAU) (Brock et al. 2007, 2010). Briefly, bone

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surfaces are cleaned by shot-blasting after which they are crushed and then demineralized using an acid-base-acid treatment (0.5M hydrochloric acid – 0.1M sodium hydroxide – 0.5M HCl). The resulting "collagen" is then gelatinized (after Longin 1971) and filtered with an Ezee filter. The filtrate then undergoes a 30 kD ultrafiltration step, in which the >30 kD gelatin fraction is retained, washed with milliQ ultrapure water, and freeze-dried pending conversion to CO_2 for ^{14}C AMS measurement (Brock et al. 2010).

RESULTS AND DISCUSSION

Results of the paired dating program are provided in Table 2. All C:N ratios fall between 3.1 and 3.4, indicating well-preserved collagen (DeNiro 1985; Ambrose 1990). The human-faunal offsets range between 0 (i.e., no offset) and 679 ^{14}C yr. The paired dating in Grave 104 showing no offset is clearly an outlier, the removal of which results in an average offset of 515 ± 175 ^{14}C yr. The individual in Grave 104 may have been an outsider who died and was buried at Shamanka II not long after arriving there. The fact that their $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values all suggest a significant contribution of aquatic resources from Lake Baikal itself (or a connecting river) is puzzling, since a ^{14}C reservoir offset would then be expected. It may be that other as yet unidentified river systems in the Cis-Baikal region are also ^{13}C-enriched, but lack a significant reservoir age.

The reported $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values are averages of measurements made in triplicate (or multiples thereof in the case of $\delta^{13}C$ and $\delta^{15}N$ where multiple dates were obtained on the same human skeleton). The average standard deviation of the triplicate calibrated $\delta^{2}H_{VSMOW}$ measurements on human bone is 3.9 ± 3.2‰. In one case, two different samples from the same individual (Grave 39) were also measured for $\delta^{2}H$, with the resulting six measurements giving very consistent ratios with a mean of –28.9 ± 2.0‰. In two cases, however, the triplicate measurements are more variable (Grave 75: +1.8 ± 7.9‰; and Grave 104: –41.8 ± 11.3‰). The considerable degree of variability in $\delta^{2}H$ between individuals at Shamanka II is apparent from the high coefficient of variation (Table 3), and is consistent with previous studies reporting particularly high variance in $\delta^{2}H$ values from carnivores (Topalov et al. 2013). This can also be seen in the values of –3.5‰ and +31.7‰ for two archaeological Baikal seals (nerpa, *Phoca sibirica*) from the site of Sagan Zaba II (Nomokonova et al. 2013), though these are both still substantially higher than the values of –97.1‰ and –83.3‰ obtained on two unidentified ungulates from the same site (Table 3). The human $\delta^{2}H$ average of –29.7 ± 21.9‰ is, as would be expected, intermediate between the seals and the ungulates, though closer to the former. As a point of reference, the waters of Lake Baikal exhibit extremely homogeneous $\delta^{18}O$ and $\delta^{2}H$ values horizontally, vertically and seasonally, averaging –15.8 ± 0.3‰ and –123.0 ± 1.2‰ (n = 32), respectively (Seal and Shanks 1998).

Considering the human stable isotope results, there is no significant correlation between either $\delta^{13}C$ and $\delta^{15}N$ ($r^2 = 0.138$, $p = 0.291$) or $\delta^{13}C$ and $\delta^{2}H$ values ($r^2 = 0.306$, $p = 0.098$). There is, however, a strong correlation between $\delta^{15}N$ and $\delta^{2}H$ values ($r^2 = 0.885$, $p = 0.000$) (Figure 2), providing further support for the use of $\delta^{2}H$ as a proxy for trophic level. This is not unexpected, since an estimated 60% of the non-exchangeable hydrogen in bone collagen derives from food, with the remainder coming from drinking water (Reynard 2007). But, to our knowledge, such a strong correlation has not previously been observed in humans. The explanation may lie in the coincidence of hydrogen from both food (i.e., fish) and drinking water from Lake Baikal. This relationship is carried through in the positive correlations with the observed human-faunal ^{14}C offsets. While there is no significant correlation in the case of $\delta^{13}C$ ($r^2 = 0.000$, $p = 0.991$) (see also Schulting et al. 2014), both $\delta^{15}N$ ($r^2 = 0.428$, $p = 0.040$) and $\delta^{2}H$ ($r^2 = 0.482$, $p = 0.023$) are

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Table 2 ^{14}C paired dating results on humans and fauna from Shamanka II. M = male; F = female; I = indeterminate. Samples with multiple dates have been combined in OxCal using the R_combine function; in all cases these passed χ^2 tests (Ward and Wilson 1978). Dates from graves marked * have been previously published (Bronk Ramsey et al. 2014; Schulting et al. 2014).

Grave-Ind	Master ID	Species	Age (yr)	Sex	OxA-	^{14}C yr	±	$\delta^{13}C$	$\delta^{15}N$	δ^2H	C:N	Offset	Species	Lab no.	^{14}C yr	±	$\delta^{13}C$	$\delta^{15}N$	C:N
Grave 22	SHA_2002.022	*H. sapiens*	19–22	M	24797, 24740	7083	26	–15.9	15.6	4.2	3.2	641	*Marmota sibirica*	OxA-31795	6442	33	–18.5	7.2	3.3
Grave 39*	SHA_2004.039	*H. sapiens*	40–44	M	24773, 24798, 26189, 26190	6895	18	–16.4	14.2	–28.9	3.2	533	*Marmota sibirica*	OxA-26299, 31922	6362	27	–19.5	5.5	3.3
Grave 47	SHA_2004.047	*H. sapiens*	20–25	F	24777, 24788, 26268	7027	19	–16.2	15.8	–5.4	3.2	468	*Marmota sibirica*	OxA-31761	6559	34	–19.6	3.9	3.4
Grave 56-2*	SHA_2004.056.02	*H. sapiens*	8–10	I	27052, 26446	6986	27	–15.7	15.5	11.4	3.2	679	*Marmota sibirica*	OxA-26300, 31763	6307	25	–19.7	8.2	3.3
Grave 73	SHA_2006.073	*H. sapiens*	16–18	F	21949	7010	45	–16.5	14.1	–28.4	3.3	617	*Marmota sibirica*	OxA-31765	6393	35	–20.0	5.9	3.3
Grave 75	SHA_2006.075	*H. sapiens*	25–29	M	26455	7093	37	–16.2	16.0	1.8	3.2	644	*Marmota sibirica*	OxA-31766	6449	34	–20.4	7.9	3.3
Grave 77	SHA_2006.077	*H. sapiens*	30–39	F	21549	7025	40	–17.6	14.1	–34.5	3.2	588	*Marmota sibirica*	OxA-31797	6437	37	–20.8	4.6	3.4
Grave 104*	SHA_2008.104	*H. sapiens*	20–35	F	21497, 27552, 28697	6334	20	–16.4	13.6	–41.8	3.2	0	*Marmota sibirica*	OxA-26302, 27553, 31843	6334	22	–19.7	5.5	3.2
Grave 103-2	SHA_2008.103.02	*H. sapiens*	5–6	I	21496	3746	28	–16.6	12.4	–50.1	3.1	294	*Cervus elaphus*	OxA-31846	3452	28	–19.9	4.9	3.3
Grave 111	SHA_2008.111	*H. sapiens*	18–20	M	22030	3700	33	–16.0	13.4	–37.6	3.2	171	*Cervus elaphus*	OxA-31847	3529	28	–19.9	5.3	3.3

Table 3 Human $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ results from Shamanka II. Both measured and "non-exchangeable" $\delta^{2}H$ are reported, with the caveat that the latter have not been corrected with like-for-like standards (i.e., collagen).

Period	Grave	$\delta^{13}C$	$\delta^{15}N$	C:N	$\delta^{2}H$ measured	±	$\delta^{2}H$ non-ex	±
EN	Grave 22	–15.9	15.6	3.2	–33.0	1.6	4.2	1.8
EN	Grave 39	–16.4	14.2	3.2	–62.5	1.7	–28.9	2.0
EN	Grave 47	–16.2	15.8	3.2	–41.6	2.7	–5.4	3.0
EN	Grave 56-2	–15.7	15.5	3.2	–26.6	1.8	11.4	2.1
EN	Grave 73	–16.5	14.1	3.3	–62.1	1.7	–28.4	1.9
EN	Grave 75	–16.2	16.0	3.2	–35.1	7.1	1.8	7.9
EN	Grave 77	–17.6	14.1	3.2	–67.5	1.4	–34.5	1.6
EN	Grave 104	–16.4	13.6	3.2	–74.1	10.1	–41.8	11.3
EBA	Grave 103-2	–16.6	12.4	3.2	–81.5	3.3	–50.1	3.7
EBA	Grave 111	–16.0	13.4	3.2	–70.3	3.2	–37.6	3.5
	Seal (E2008.143)	–20.9	15.6	3.4	–39.9	7.6	–3.5	8.5
	Seal (E2008.155)	–21.7	14.2	3.4	–8.5	2.8	31.7	3.1
	Ungulate (E2008.146)	–18.8	3.3	3.4	–123.4	4.3	–97.1	4.8
	Ungulate (E2008.147)	–18.9	5.0	3.4	–111.1	2.7	–83.3	3.0
	Human X	*–16.3*	*14.5*	*3.2*	*–55.4*	*3.5*	*–20.9*	*3.9*
	SD	*0.5*	*1.2*	*0.0*	*19.5*	*2.9*	*21.9*	*3.2*
	CV	*3.2*	*8.3*		*35.2*	*82.8*	*104.5*	

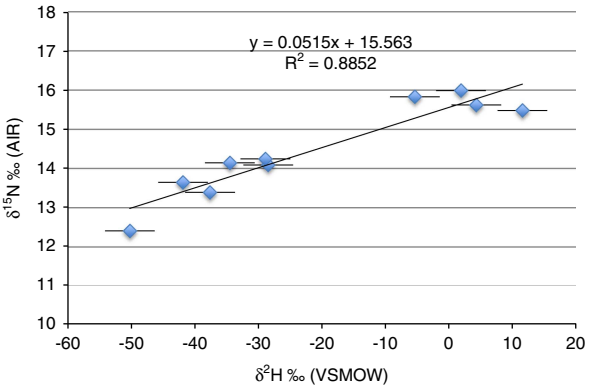

Figure 2 Scatterplot with linear regression of $\delta^{2}H$ and $\delta^{15}N$ ratios on human bone collaen, showing strong positive correlation ($r^2 = 0.885$, $p = 0.000$). Error bars show average ±1SD.

significant predictors of the ^{14}C offsets, accounting for approximately 45% of the observed variability (Figures 3 and 4).

The results are in fact strikingly similar, as might be expected given the strong correlation between the two stable isotopes, although the relationship with $\delta^{2}H$ is actually slightly stronger (although a regression model incorporating both $\delta^{2}H$ and $\delta^{15}N$ fails to attain statistical significance (adjusted $r^2 = 0.334$; $p = 0.100$)). This is confirmed in a multiple linear regression model (Equation 1), in which both $\delta^{13}C$ ($p = 0.034$) and $\delta^{2}H$ ($p = 0.036$) ratios contribute significantly to the predicted ^{14}C offset (adjusted $r^2 = 0.656$, $p = 0.024$), while, interestingly, $\delta^{15}N$ does not ($p = 0.199$). Re-running the regression model (Equation 2) excluding $\delta^{15}N$ reduces the contribution of $\delta^{13}C$ to the point where it just fails to attain significance ($p = 0.066$)—though

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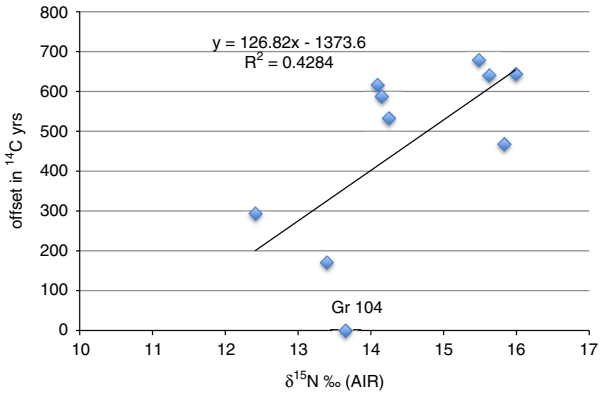

Figure 3 Scatterplot with linear regression model of human $\delta^{15}N$ values and human-faunal offsets in ^{14}C yr, showing a moderate positive correlation ($r^2 = 0.428$, $p = 0.040$). The outlier Gr 104 is identified.

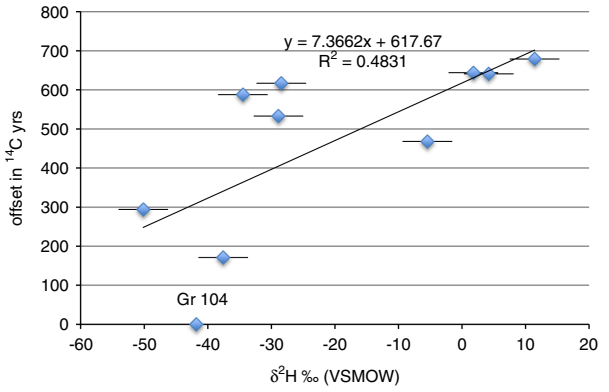

Figure 4 Scatterplot with linear regression model of human δ^2H values and human-faunal offsets in ^{14}C yr, showing a moderate positive correlation ($r^2 = 0.483$, $p = 0.023$). Error bars show average ±1SD for δ^2H.

nevertheless providing a significant improvement over the single isotope models (adjusted $r^2 = 0.603$, $p = 0.016$)—while retaining the significance of δ^2H ($p = 0.007$). For both models all standardized residuals are less than two standard deviations.

$$^{14}\text{C offset} = -1903.2662 - 340.8886(\delta^{13}\text{C}) - 189.8488(\delta^{15}\text{N}) + 21.5779(\delta^2\text{H})$$
$$F = 6.72,\ \text{adjusted}\ r^2 = 0.656,\ p = 0.024,\ S = 135.92,\ \text{n} = 10 \quad (1)$$

$$^{14}\text{C offset} = -3338.0028 - 246.2623(\delta^{13}\text{C}) + 10.5748(\delta^2\text{H})$$
$$F = 7.83,\ \text{adjusted}\ r^2 = 0.603,\ p = 0.016,\ S = 146.03,\ \text{n} = 10 \quad (2)$$

That the regression equations obtained here are less precise than those previously produced for the Cis-Baikal region, including Shamanka II (Bronk Ramsey et al. 2014; Schulting et al. 2014), is largely the result of the much-reduced range of variability in both the ^{14}C offsets and the dietary stable isotope data at this site. These previously published equations, therefore, are still preferred for the correction of ^{14}C dates on human remains in the Southwest Baikal and Angara micro-regions.

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While a comparison of the Early Neolithic and Early Bronze Age results is limited by the inclusion of only two graves from the latter period, it is worth noting that the EBA results fall below the entire range seen in the Early Neolithic individuals in terms of their $\delta^{15}N$ and $\delta^{2}H$ values as well as their ^{14}C offsets (with the exception of the abovementioned Grave 104). They do not differ, however, in their $\delta^{13}C$ values (Table 3).

CONCLUSIONS

The average offset of 515 ± 175 ^{14}C yr is consistent with that of 537 ± 80 yr previously reported for Shamanka II (Schulting et al. 2014). Linear regression models making use of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ both separately and in combination suggest that stable hydrogen isotope ratios are at least as useful a predictor of the ^{14}C offsets as stable nitrogen isotopes. At the same time, the greater difficulties involved in the measurement of $\delta^{2}H$ compared with that of $\delta^{15}N$, and the thus far limited additional information obtained, are factors that do need to be taken into account. Nevertheless, our results provide further support for the utility of $\delta^{2}H$ as a proxy for trophic position, and suggest that this isotope system holds promise for future investigations in the Baikal region and elsewhere. In terms of correcting dates on human remains, the previously published equations are still preferred (Bronk Ramsey et al. 2014; Schulting et al. 2014). This may be modified in future as new results become available.

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