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**Citation for published version:**

Cook, G, Bonsall, C, Hedges, R, McSweeney, K, Boronean, V & Pettitt, P 2001, 'A freshwater diet-derived  $^{14}\text{C}$  reservoir effect at the Stone Age sites in the Iron Gates gorge' *Radiocarbon*, vol 43, no. 2A, pp. 453-460., <https://journals.uair.arizona.edu/index.php/radiocarbon/article/view/3985>

**Digital Object Identifier (DOI):**

<https://journals.uair.arizona.edu/index.php/radiocarbon/article/view/3985>

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher final version (usually the publisher pdf)

**Published In:**

*Radiocarbon*

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# A FRESHWATER DIET-DERIVED $^{14}\text{C}$ RESERVOIR EFFECT AT THE STONE AGE SITES IN THE IRON GATES GORGE

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**ABSTRACT.** Human bones from single inhumation burials and artifacts made from terrestrial mammal (ungulate) bone found in direct association with the skeletons were obtained from the Stone Age site of Schela Cladovei situated just below the Iron Gates Gorge of the River Danube. The results of stable isotope analyses of the human bone collagen are consistent with a heavy dependence on aquatic protein while radiocarbon dating of the samples reveals an offset of 300–500 years between the two sample types, indicating a freshwater reservoir effect in the human bone samples. Since protein consumption is by far the major source of nitrogen in the human diet we have assumed a linear relationship between  $\delta^{15}\text{N}$  and the level of aquatic protein in each individual's diet and derived a calibration for  $^{14}\text{C}$  age offset versus  $\delta^{15}\text{N}$  which has been applied to a series of results from the site at Lepenski Vir within the gorge. The corrected  $^{14}\text{C}$  ages (7310–6720 BP) are now consistent with the previous  $^{14}\text{C}$  age measurements made on charcoal from related contexts (7360–6560 BP). In addition, the data indicate a change from a primarily aquatic to a mixed terrestrial/aquatic diet around 7100 BP and this may be argued as supporting a shift from Mesolithic to Neolithic. This study also has wider implications for the accurate dating of human bone samples when the possibility exists of an aquatic component in the dietary protein and strongly implies that  $\delta^{15}\text{N}$  analysis should be undertaken routinely when dating human bones.

## INTRODUCTION

“Iron Gates” is the term now most frequently used in referring to the 130 km gorge section of the River Danube between Baziaș and Gura Văii (Figure 1). This area is rich in later Stone Age sites which assume a special significance because they span the period when the shift from a foraging to a farming economy occurred. In archaeological terms, the best documented sites in this region are those at Vlasac, Lepenski Vir, and Schela Cladovei. Vlasac and Lepenski Vir are situated approximately 2.5 km apart in the central part of the gorge and occupy narrow terraces at the base of a steep sided valley while in contrast, Schela Cladovei lies in a more open section of the Danube Valley, approximately 65 km downriver from Lepenski Vir and 7 km below the gorge.

The radiocarbon dating of charcoal samples from Lepenski Vir has been the subject of considerable controversy (Bonsall et al. 1997). The samples were associated with houses assigned to Phases I and II and from contexts that lacked domesticated animal remains and showed little evidence for the use of pottery. For these reasons they were assigned to the Lepenski Vir Mesolithic culture, however, they produced  $^{14}\text{C}$  ages between 6560 and 7360 BP that Srejović (1972, 1989) rejected as approximately 500 years too young. Others (Whittle 1985; Chapman 1992; Radovanović 1996) have accepted the ages as valid and interpret Lepenski Vir I and II as representing the latest phases of the Mesolithic in the Iron Gates region. They view the gorge as a special environment that was rich in natural resources but not immediately attractive to farming societies and where foraging communities and early farming communities were contemporary in the lowland areas on either side of the Carpathian Mountains. Milisauskas (1978) proposed a further theory, namely that the structures comprising Lepenski Vir I and II were the remains of houses built by a sedentary farming community, the foundations of which were dug into earlier hunter-gatherer deposits, resulting in mixing of materials from Mesolithic and Early Neolithic occupations. Subsequent accelerator mass spectrometry (AMS) dating of human

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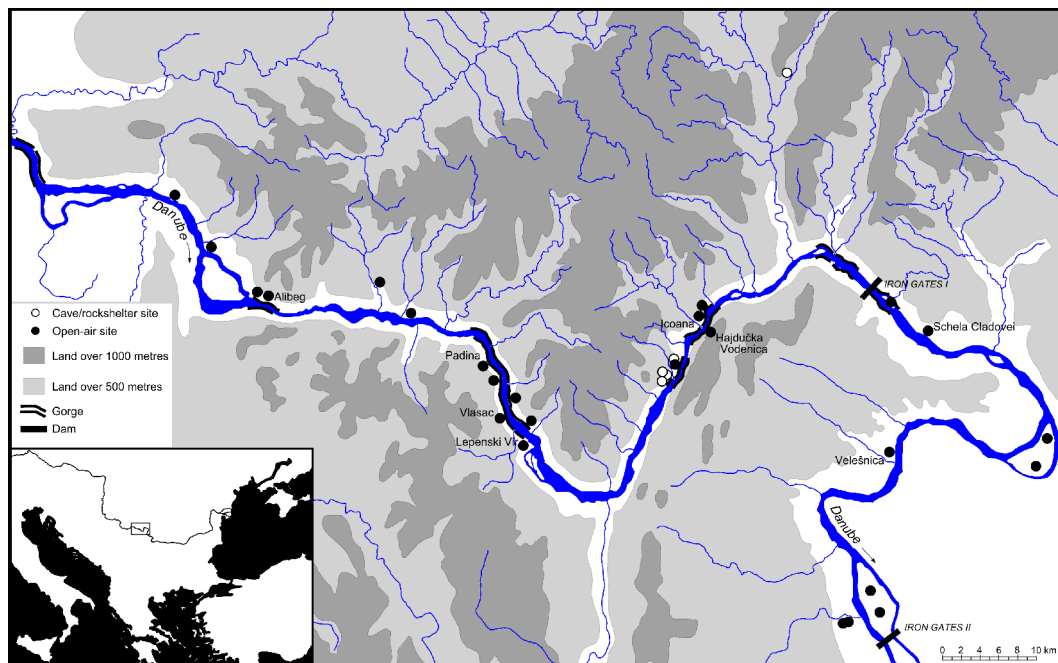


Figure 1 Mesolithic and early Neolithic sites in the Iron Gates

remains assigned to the later Phase III of Lepenski Vir produced ages of between 6910 and 7770 BP which puts them out of sequence with the charcoal samples assigned to Phases I and II (Bonsall et al. 1997). A similar pattern has been observed at both Vlasac and Schela Cladovei.

Bonsall et al. (1997) have suggested that the phasing of the burials at Lepenski Vir may be incorrect, however, even if the samples were contemporary in context, we would expect that either 1) the charcoals would produce similar  $^{14}\text{C}$  ages, if short lived species and/or roundwood were specifically selected for measurement, or 2) the charcoal would produce older ages because of the inclusion of long lived species or timber that had previously been used for a significant time in construction, etc. Therefore, if the charcoal ages are accepted as correct then the fact that they are consistently younger than the human bone samples suggests the possibility that the human diet may have included material from a reservoir that differed in  $^{14}\text{C}$  specific activity from the contemporary atmosphere.

The most comprehensive dataset of  $^{14}\text{C}$  and stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) measurements on human bone collagen is from Schela Cladovei. The  $\delta^{13}\text{C}$  values range from  $-19.2$  to  $-20.0\text{‰}$  (Bonsall et al. 1997) and are within the range that can be assigned to a predominantly terrestrial-based diet derived from  $\text{C}_3$  plants (Johansen et al. 1986; Mays 1998; Arneborg et al. 1999). In contrast, however, the  $\delta^{15}\text{N}$  values range from  $+14.9$  to  $+16.0\text{‰}$  and are indicative of a diet derived from a complex food web in which trophic level increases from primary producer to top carnivore are particularly pronounced, such as in marine ecosystems (Schoeninger and DeNiro 1984). Similar  $\delta^{15}\text{N}$  values have also been observed at Vlasac, and Lepenski Vir by Bonsall et al. (1997) who interpret the  $^{15}\text{N}$  data as evidence that the Mesolithic people had high protein diets in which the bulk of the protein was derived from riverine food sources. There is evidence of substantial fish consumption within the Iron Gates, however, successful stable isotope analyses of fish bone obtained from both Lepenski Vir and Schela Cladovei are at present limited to three analyses, giving a  $\delta^{15}\text{N}$  range of  $+8.2$  to  $+12.9\text{‰}$  (Bonsall et al. 1997).

If we employ a +3.4‰ trophic level shift (range 1.3 to 5.3‰) (Minagawa and Wada 1984) between the fish and human bone collagen, this certainly indicates that  $\delta^{15}\text{N}$  values for fish of approximately +12‰, or greater, could result in human bone collagen values of >+15‰. While this type of data for fish from the Iron Gates is limited, such values are not uncommon. Iacumin et al. (1998) and Pate (1998) report  $\delta^{15}\text{N}$  values of about +12‰ for Lake Nasser and South Australia fish, respectively. Doucett et al. (1999) quote a range of +7.4 to +16.8‰ for brook trout from what they considered to be a mixed anadromous/non anadromous population and Hobson and Welch (1995) report  $\delta^{15}\text{N}$  values for large char collected from a high Arctic lake of >+14‰. Furthermore, Lanting and van der Plicht (1998) have estimated that a 100% diet of freshwater fish would result in a  $\delta^{15}\text{N}$  value of approximately +16‰. The enrichment of any species will of course depend on the complexity of the food web and its trophic level within the web.

This reliance by Mesolithic populations on aquatic food obviously implies that the aquatic reservoir is the one that differs in  $^{14}\text{C}$  specific activity from the contemporary atmosphere and that an age correction may have to be applied to the human bones to take this effect into account. Freshwater reservoir ages have been reported for the Netherlands by Lanting and van der Plicht (1998) who demonstrated these by measuring the  $^{14}\text{C}$  activity of modern fish/shellfish flesh and fish bone collagen. They noted reservoir ages of up to 4430 years in canals and 2000 years approximately in rivers such as the Waal and Maas. Furthermore, they also demonstrated significant reservoir effects in the skeletons of the historically dated, 11th–13th century Counts of Holland which they ascribed to the consumption of freshwater fish.

Bonsall et al. (1997) have suggested that a freshwater reservoir effect for the Iron Gates stone age sites could most easily be tested by comparing age measurements on human bones with those on artifacts of terrestrial (ungulate) animal bone found in the same graves and indeed, Schela Cladovei provides material ideal for investigating this problem. The samples comprise human bones from single inhumation burials and artifacts made from terrestrial mammal (ungulate) bone found in direct association with the skeletons. The associations are of two kinds: 1) bone projectile points embedded in human bone, and 2) bone projectile points found immediately adjacent to bones of articulated skeletons (which may originally have been embedded in the soft tissue surrounding the bones). In all cases, the bone points may have been the actual cause of death. The samples may therefore be considered to come from secure archaeological contexts. In the case of the “embedded” projectile points, there can be no doubt about the reliability of the association between the human bones and the bone artifacts. In open-air archaeological sites where soil-forming processes have been active, there is always a possibility that very small objects can be moved from their original positions, e.g. by the actions of roots or earthworms. The bone points not embedded in human bones are thought not to have been so affected since at Schela Cladovei such artifacts are found almost exclusively with burials and are extremely rare in other contexts, and since the burials themselves appear undisturbed by soil processes. There is no reason therefore to doubt any of the associations between the projectile points and human remains.

Therefore,  $^{14}\text{C}$  analysis of the human bones and comparison with the ages derived for the directly associated ungulate bones would provide firm evidence of the existence of a dietary induced reservoir age. Table 1 lists the human samples and the contexts of the associated ungulate bones and Figure 2 illustrates one example of a projectile point embedded in the pelvis.

Table 1 Samples from Schela Cladovei for  $^{14}\text{C}$  analysis: human bones from single inhumation burials and artifacts made from terrestrial mammal (ungulate) bone found in direct association with the skeletons.

Human bone samples (yr excavated)	Associated terrestrial (ungulate) bone samples
1.1 Bone sample from skeleton (1967)	1.2 Bone point <b>embedded</b> in thoracic vertebra of skeleton
2.1 Bone sample from skeleton (1967)	2.2 Bone point <b>embedded</b> in lumbar vertebra of skeleton
3.1 Bone sample from skeleton (1991)	3.2 Bone point <b>embedded</b> in thoracic vertebra of skeleton
4.1 Bone sample from skeleton (1995)	4.2 Bone point <b>embedded</b> in left innominate of skeleton 4.3 Bone point found <b>adjacent</b> to skull of skeleton
5.1 Bone sample from skeleton (1996)	5.2 Bone point found <b>adjacent</b> to proximal end of femur of skeleton 5.3 Bone point found <b>between</b> lowermost vertebra and left innominate of skeleton

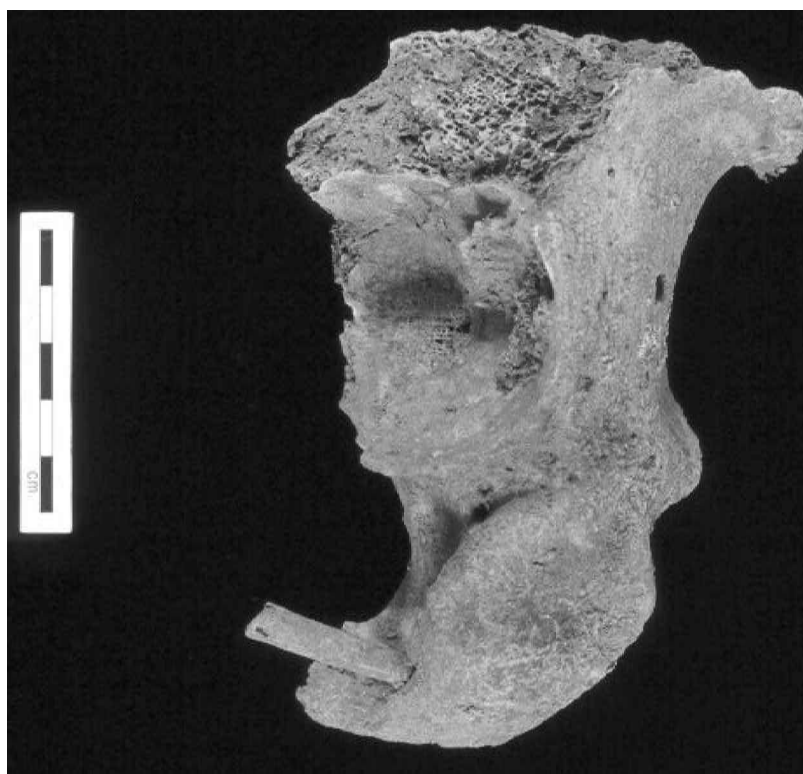


Figure 2 Bone projectile point embedded in pelvic bone of adult male

## MATERIALS AND METHODS

### Bone Sampling Procedure

The bone samples were either sandblasted on the area from which the sample was to be drilled (aluminium pellets at high pressure) or scraped thoroughly with a scalpel. Then, a sample of 100–500 mg was removed by drilling at low velocity, with a tungsten drill bit, onto aluminium foil. The bone powder was ultimately stored in glass vials.

### Collagen Extraction

Essentially this was by the method of Longin (1971). The samples were decalcified with HCl, the insoluble material was then washed with NaOH, which was then removed with further HCl. This was followed by gelatinization of the sample at pH3 followed by filtration and freeze drying. This procedure is done under continuous flow conditions, which we feel contributes strongly to removal of exogenous material.

### Stable Isotope Measurements

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured using a Europa CHN analyzer system coupled to a 20/20 stable isotope ratio mass spectrometer. The lyophilized collagen samples are combusted in a helium/oxygen stream in a furnace, and the gaseous products are separated by gas chromatography after oxides of nitrogen have been reduced to  $\text{N}_2$  and water has been removed. The time-separated pulses of  $\text{N}_2$  and  $\text{CO}_2$  are separately measured mass spectrometrically after splitting, and the remainder of the  $\text{CO}_2$  is trapped and converted to graphite. Isotope ratios are compared with standards that are included in the same combustion and measurement run.

### Graphite Preparation

Graphite is produced by the reduction of  $\text{CO}_2$  with  $\text{H}_2$ , catalyzed by iron powder at a temperature of about 550 °C. 1–1.5 mg of C are used and the resulting graphite is compressed into an expendable aluminium ion source target head for measurement in the AMS system.

### AMS Analysis

The  $^{14}\text{C}$  ages were determined by standard AMS procedures, using a tandem accelerator at 2 MV, with calibration samples derived from NIST standard oxalic acid and known age material included in the same run.

## DISCUSSION AND CONCLUSIONS

The results of the  $^{14}\text{C}$  and stable isotope analyses on the human and ungulate bone collagen samples are presented in Table 2. The human collagen  $\delta^{13}\text{C}$  values range from  $-18.2$  to  $-19.5\text{‰}$ , and are, on average, heavier by  $2.3\text{‰}$  than the associated ungulate samples. The  $\delta^{15}\text{N}$  values are in the range  $+13.2$  to  $+15.3$  and are, on average, heavier than the ungulate samples by  $8.6\text{‰}$ . Allowing for trophic level shifts, both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the human bone collagen are consistent with a heavy reliance on a freshwater aquatic diet.

The  $^{14}\text{C}$  results indicate that the human bone collagen samples are always significantly older than the ungulate samples by approximately 300–500 years. The weighted mean offset between the paired samples is  $440 \pm 45$  years and subtraction of this reservoir age will bring the age of the human bone samples closer to the true  $^{14}\text{C}$  age of the contemporary terrestrial biota, however, this necessarily assumes that individuals in the Mesolithic population all had the same proportion of aquatic protein in their diet. A more elegant approach would be to derive a relationship between each individual's level of aquatic diet and the magnitude of the age offset. Ambrose and Norr (1993) have concluded that  $\delta^{13}\text{C}$  values in bone collagen overestimate the amount of protein in the food because of the influence of non-protein components of the diet. This means that a linear mixing model between  $\delta^{13}\text{C}$  in bone collagen of the consumer and in the different components of the diet cannot be used in palaeodiet studies. However, this problem should not apply to nitrogen as a higher animal's principal source of nitrogen is the protein that it consumes in its diet (Conn and Stumpf 1972).

We have therefore derived a simple linear relationship between  $\delta^{15}\text{N}$  and age offset. The basic premise is that the more aquatic food intake an individual has, the greater the  $\delta^{15}\text{N}$  enrichment in their bone collagen and the greater the aquatic reservoir effect. To accomplish this, we have assumed an end-point of +8‰ for 100% terrestrial diet. This is based on: 1) the research of Ogrinc (1999) who studied dietary habits of Neolithic people living in Slovenia.  $\delta^{15}\text{N}$  values for adults in this study averaged approximately +7.5‰, and 2) Estimates by Mays (1998) of likely  $\delta^{15}\text{N}$  values resulting from a pure  $\text{C}_3$  terrestrial diet (+8 to +10‰). For 100% aquatic diet, we have assumed +17‰ which is the highest  $\delta^{15}\text{N}$  value measured in an adult from the Iron Gates region (Bonsall et al. 1997 and unpublished results).

Table 2  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements,  $^{14}\text{C}$  ages and  $^{14}\text{C}$  age offsets in human bone and associated ungulate bone samples from Schela Cladovei

Lab nr (OxA-)	Sample ID	Bone Type	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$^{14}\text{C}$ age (BP $\pm$ 1 $\sigma$ )	Age offset (BP $\pm$ 1 $\sigma$ )
8502	IG(D)1.1	Human	-18.6	13.2	8300 $\pm$ 60	
8579	IG(D)1.2	Ungulate	-20.6	5.0	7790 $\pm$ 100	510 $\pm$ 117
8547	IG(D)2.1	Human	-19.3	13.9	8240 $\pm$ 60	
8580	IG(D)2.2	Ungulate	-20.8	6.1	7770 $\pm$ 240	470 $\pm$ 247
8581	IG(D)3.1	Human	-19.5	15.1	8330 $\pm$ 75	
8582	IG(D)3.2	Ungulate	-22.0	9.4	7880 $\pm$ 290	450 $\pm$ 300
8583	IG(D)4.1	Human	-18.5	15.0	8380 $\pm$ 80	
8584	IG(D)4.2	Ungulate	-21.5	4.7	7915 $\pm$ 65	465 $\pm$ 103
8585	IG(D)4.3	Ungulate	-20.9	4.5	7780 $\pm$ 75	600 $\pm$ 110
8548	IG(D)5.1	Human	-18.2	15.3	8200 $\pm$ 70	
8549	IG(D)5.2	Ungulate	-20.4	7.7	7905 $\pm$ 60	295 $\pm$ 92
8550	IG(D)5.3	Ungulate	-21.2	4.7	7805 $\pm$ 70	395 $\pm$ 99

Of the five human bone collagen samples presented in Table 2, three have  $\delta^{15}\text{N}$  values that are statistically indistinguishable (+15.1, +15.0 and +15.3). The average (+15.1‰), based on the linear relationship between +8 and +17‰, corresponds to 79% aquatic diet. In turn, these samples have a weighted mean age offset from the associated ungulate bones of 425  $\pm$  55  $^{14}\text{C}$  years. Therefore, the reservoir age for 100% aquatic diet would approximate to 540  $\pm$  70  $^{14}\text{C}$  years. This gives us the potential to correct individual human bone ages from the Iron Gates, thereby producing age estimates that reflect more closely the terrestrial environment.

Table 3 illustrates a series of human bone ages and associated stable isotope values for burials attributed to Phase III of Lepenski Vir (Bonsall et al. 1997). These ages span a period during which the population appears to have undergone a significant change in dietary/subsistence patterns (aquatic to mixed aquatic/terrestrial) and can be divided into 2 groups as follows: 1) Samples OxA-5828, OxA-5831 and OxA-5829 have stable isotope values typical of a mixed diet, 2) Samples OxA-5827 and OxA-5830 are characterized by heavier  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, typical of a primarily aquatic diet. They are also significantly older. Calculation of the percentage aquatic diet indicates >80% in samples OxA-5827 and OxA-5830 and <45% in the other three samples. Leaving aside the question of whether this dietary shift represents the beginning of farming in the gorge, it is interesting to note the effect of correcting the age measurements. Application of the  $\delta^{15}\text{N}$ -derived aquatic reservoir correction results in the ages quoted in the final column of Table 3.

The important aspects to note here are:

1. There is now no significant age difference between the two groups of samples. The corrected  $^{14}\text{C}$  ages form a continuous sequence which is exactly what one would expect if sampling across a transition (in a continuous series).
2. The corrected ages indicate a dietary transition at around 7100 BP.
3. The corrected ages, which span the period from 7310–6720 BP, are now in good agreement with the previous radiometric measurements made on charcoal samples from house floors/ hearths at Lepenski Vir (Phases I and II), which spanned 7360 to 6560 BP. However, an important point to note is that many of the charcoal samples were oak which may include “old timber” and two were from an oak beam that had previously been used for some other purpose. Therefore, the charcoal ages are conceivably overestimates. Speculatively, a more realistic estimate would be 7200–6400 BP which then suggests that a significant proportion of the houses were constructed around or after the dietary shift.
4. If one accepts that the dietary shift represents a shift from Mesolithic to Neolithic then the data contradict the conventional view (Whittle 1985; Chapman 1992; Radovanovic 1996) of a population that did not adopt farming until several hundred years after its introduction into surrounding areas.

Table 3 Correcting human bone dates from Lepenski Vir for the aquatic reservoir age using the  $\delta^{15}\text{N}$  value to determine percentage aquatic diet

Lab nr (OxA-)	Sample context	$^{14}\text{C}$ age (BP)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Aquatic diet (%)	Corrected $^{14}\text{C}$ age (BP)
5827	Phase IIIb, sk 31a	7770 ± 90	-18.7	15.7	86	7310 ± 108
5830	Phase IIIb, sk 44	7590 ± 90	-18.9	15.3	81	7150 ± 106
5828	Phase IIIb, sk 32	7270 ± 90	-19.5	11.9	43	7040 ± 95
5831	Phase IIIa, sk 88	7130 ± 90	-20.2	10.9	32	6960 ± 93
5829	Phase IIIb, sk 35	6910 ± 90	-19.7	11.2	36	6720 ± 93

More generally, these results demonstrate that it is not only a marine diet that may induce a reservoir age in human collagen but that a freshwater aquatic diet can have a similar influence. While the routine measurement of  $\delta^{13}\text{C}$  may help to identify a marine diet (because of the potential for a large shift), it is unlikely to identify a freshwater diet. This reinforces the conclusion of Lanting and van der Plicht (1998) that routine measurement of  $\delta^{15}\text{N}$  should be undertaken when human bone samples are submitted for  $^{14}\text{C}$  age measurement.

## ACKNOWLEDGMENTS

We gratefully acknowledge radiocarbon dating support from the UK Natural Environment Research Council.

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