FISH RESERVOIR EFFECT ON CHARRED FOOD RESIDUE ^{14}C DATES: ARE STABLE ISOTOPE ANALYSES THE SOLUTION?

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ABSTRACT. In order to verify the relative dating based on pot type morphology and decoration of the Swifterbant pottery collected at the Final Mesolithic site of Doel "Deurganckdok" (Belgium) and of the Late Iron Age pottery excavated at Grijpskerke (the Netherlands), direct radiocarbon dates were obtained on charred food residue preserved on the inner surface of numerous potsherds. In addition, a number of indirect ^{14}C dates were obtained from samples of organic material. In the case of Doel, the results indicate an important incompatibility between the charred food residue dates and the other dates, the former being systematically older. This difference may be explained by a reservoir effect of the charred food residue, caused by the processing of (freshwater) fish. The ^{14}C dates for the Grijpskerke site are in agreement between the charred food residue and the organic material. The stable isotopes of the charred food residue were analyzed to demonstrate fish processing in the pottery, but the results were inconclusive.

INTRODUCTION

Charred deposits encrusted on archaeological ceramic vessels are an interesting phenomenon and can provide valuable information concerning pottery use. Lipid analysis of charred deposits, preserved on the inner surface of potsherds, has become a promising technique to obtain information about food processing and dietary habits (Dudd et al. 1998, 1999; Romanus et al. 2007; Evershed 2008).

Direct radiocarbon dating of charred food residue has become popular in archaeology due to its direct association with pottery use. Hence, it is generally expected that charred food residue yields reliable ^{14}C dates. Fischer and Heinemeier (2003) noticed that some charred food residue dates from Scandinavian Late Mesolithic sites are older than the dates obtained on terrestrial organic material and their archaeological context, suggesting that a reservoir effect caused by the processing of fish might be the cause. However, this hypothesis was contested through a critical assessment by Hart and Lovis (2007). They obtained substantial agreement between dates on food residue and those on other materials from the same contexts, consistent with the results of their assessment of accelerator mass spectrometry (AMS) dates on residue in northeastern North America (Hart et al. 2007).

In our study, charred food residue and organic material were dated from 2 sites, Doel (Belgium) and Grijpskerke (the Netherlands) to obtain more insight in this matter. Stable isotopes of the charred food residue were analyzed in an attempt to demonstrate a possible aquatic (marine or freshwater) processing in the ceramic vessels. According to Craig et al. (2007), stable isotope analysis has the advantage of providing information, albeit of lower resolution, on the origin of the total amount of carbon and nitrogen in the sample. In their study, the interpretation based on the bulk δ^{13}C analysis was in accordance with more specific molecular analysis. Therefore, it is likely that the carbon present in these samples is derived from vessel use and not from contamination during use by, for example, wood-smoke, or extensive alteration in the post-depositional environment. In view of these findings, it was suggested that δ^{13}C analysis is a suitable tool, at least in screening surface res-

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idue for possible marine components, and that when combined with bulk $\delta^{15}N$ analysis samples, it is also suitable for identifying the possible presence of freshwater fish. This is significant as it provides a quick and inexpensive method for identifying ^{14}C dates liable to inaccuracies resulting from the presence of marine or freshwater carbon (Craig et al. 2007).

MATERIALS AND METHODS

Sample Selection

Doel

Archaeological surveys during the construction of a new dock, the Deurganckdok at Doel (Belgium), situated in the Antwerp Harbor along the Lower Scheldt (Crombé 2005; Sergant et al. 2006), led to the discovery of 3 wetland sites yielding pottery of Swifterbant tradition in association with Final Mesolithic, hunter-gatherer-fisher settlement remains (lithics, burnt ecofacts, etc.). Stylistically, the pottery closely resembles the Early Swifterbant pottery from the Netherlands, dated to the first half of the 5th millennium cal BC.

Surface charred food residue were obtained from potsherds of the inland sites Doel B, Doel J/L, and Doel M, located in the sandy lowlands of the Scheldt River valley (NW Belgium). Faunal remains, namely cremated freshwater fish bones (mainly cyprinids) and cremated wild animal bones from red deer and wild boar were excavated in these sites.

From the complete list of seeds and fruits found, several species could have been useful to the inhabitants at the time. Hazelnuts, crab apple, sloe plums from blackthorn, oak acorns, and hawthorn berries could have been collected and eaten (Crombé 2005).

Grijpskerke

A rescue excavation in 2003 by the Stichting Cultureel Erfgoed Zeeland (SCEZ), along the Kievitshoekweg near the village of Grijpskerke (municipality of Veere, province of Zeeland, the Netherlands) (Jongepier 2003), yielded an isolated rectangular 7.5 m^2 pit with a content of 5 m^3. Post-excavation processing, in cooperation with Ghent University (Belgium) and the Flemish Heritage Institute (Belgium), is still in its early phase (Van Dierendonck and Jongepier 2009). From this pit, 700 kg of finds were recovered, largely consisting of handmade pottery (~670 kg), which can be stylistically dated to the Late Iron Age (250–12 BC) (Van Heeringen 1998, 1992; De Clercq 2009). The number of vessels represented is estimated to reach 220–375 specimens; a large number, part of which seem to have been carefully placed in the pit (De Clercq 2009) while other recipients were thrown in less carefully. Centrally positioned, the skeleton of a ~1-yr-old male dog was found (Van Dierendonck 2009). A human pelvis and corresponding lumbar vertebrae also formed part of the context in addition to other animal bones of cattle and sheep/goat. Other finds were also found, like querns, a polishing stone, spit supports, oven grids, pieces of cord, and a bronze bracelet.

The excavation showed that deposition took place as a single event and that the pit was filled and covered with the dug-out peat and clay shortly after its construction. The evidence gathered suggests that the feature excavated should be interpreted as a ritual deposition.

^{14}C Dating

In order to verify the relative dating of the Doel site, an extensive ^{14}C dating program was set up, aiming at dating different organic components from the same stratigraphic and spatial context. Direct dates were obtained on samples of charred food residue preserved on the inner surface of

numerous potsherds, while indirect dates were done on samples of carbonized hazelnut shells, charcoal, and burnt seeds originating from presumed surface hearths.

For the Grijpskerke site, the collagen of 2 animal bones (a dog and a cow) and charred food residue were ^{14}C dated. The bone collagen was extracted with the Longin method (Longin 1971) modified with an extra NaOH step. All the charred food residue and organic material (carbonized hazelnut shells, charcoal, burnt seeds) were pretreated with the acid-alkali-acid (AAA) method. Furthermore, the lipid extract from a charred food residue was ^{14}C dated. The residue was removed using a scalpel and crushed to a fine powder in a mortar with a pestle. The lipids of each sample were extracted ultrasonically with a 30-mL mixture of chloroform and methanol (2:1 v:v) for 30 min and filtrated afterwards. The solvent was partially evaporated and the remaining solvent was removed under a stream of nitrogen. All samples were converted into graphite and measured by AMS (Van Strydonck and Van der Borg 1990–91; Nadeau et al. 1998).

Stable Carbon and Nitrogen Isotope Analysis

$\delta^{13}C$, $\delta^{15}N$, and atomic C/N measurements were made on a number of the bulk residue samples and on the bone collagen. Acid-alkali-acid pretreatment of the charred food residue samples was undertaken prior to analysis, to remove carbonates, humic, and fulvic acids. Each sample was transferred in duplicate into tin capsules, which were analyzed using a ThermoFinnigan Delta Plus XL (continuous-flow type), interfaced with a Flash EA1112 elemental analyzer via a Conflo III interface. Both elements were measured together and a helium-dilution was applied for carbon as the amount of C in the crusts was often far in excess of the amount of N. Analytical precision was greater than 0.5‰ for both elements, as determined by duplicate measurements.

RESULTS AND DISCUSSION

^{14}C Dating and Stable Isotope Measurements

Doel

Uncalibrated ^{14}C ages, $\delta^{13}C$ and $\delta^{15}N$ values, and atomic C/N determined for the charred food residue samples are given in Table 1. Uncalibrated ^{14}C ages for the organic material are given in Table 2. ^{14}C dates calibrated using OxCal 3 (Bronk Ramsey 1995, 2001) and IntCal04 calibration curve date (Reimer et al. 2004) for the charred food residue and organic material are plotted in Figure 2.

The ^{14}C dates for the charred food residue are systematically older than the organic material dates (see Tables 1 and 2, Figure 1). Only charred food residue samples 416, 281, 304, and 2246 have ^{14}C dates that are comparable to the organic material ^{14}C dates. The older ^{14}C dates are probably provoked by freshwater fish processing because the site is located in the lowlands of the Scheldt River valley and cremated freshwater fish bones were excavated.

Craig et al. (2007) defined a bulk isotopic theory ($\delta^{15}N$ and $\delta^{13}C$) to distinguish aquatic products in the charred food residue. This theory can nominally discriminate charred food residue into 3 groups:

1. The first group has relatively heavy $\delta^{13}C$ values (> −25‰) and $\delta^{15}N$ values between +7 and +12‰, which suggest a marine origin.
2. The second group has relatively light $\delta^{13}C$ values (< −25‰) and $\delta^{15}N$ values between +6 and +10‰. Freshwater fish is a likely component of these charred food residues, and probably the major component for samples with high $\delta^{15}N$ (+8 and +10‰).
3. The last group has light $\delta^{13}C$ values (< −25‰) and $\delta^{15}N$ values between +1 and +5‰, and herbivore products and/or plant material were processed in these pots.

Table 1 ^{14}C dates for the charred food residue from the site of Doel.

Sample name	Lab code	^{14}C date (BP)	δ^{13}C (‰)	δ^{15}N (‰)	atomic C/N
Sector B					
3	KIA-33819	5865 ± 40	–27.0	7.9	7.7
87	KIA-33815	5820 ± 45	–27.4	3.5	12.2
109	KIA-12260	5980 ± 35	n.a.	n.a.	n.a.
251	KIA-33818	5820 ± 40	–26.7	4.1	13.0
277	KIA-33817	5790 ± 45	–28.2	8.8	12.2
279	KIA-33816	5825 ± 45	–27.3	4.5	n.a.
281	KIA-33820	5665 ± 40	–28.4	3.7	n.a.
291	KIA-29787	5810 ± 35	–27.2	2.8	n.a.
304	KIA-29788	5595 ± 40	–27.3	6.3	n.a.
416	KIA-33811	5685 ± 40	–26.7	8.3	n.a.
419	KIA-29789	5910 ± 40	–27.0	8.5	n.a.
430	KIA-33809	5875 ± 45	–27.4	5.7	13.0
440	KIA-33812	5880 ± 40	–26.5	6.7	n.a.
550	KIA-32599	5780 ± 40	–27.0	9.6	n.a.
742	KIA-14399	5835 ± 35	n.a.	n.a.	n.a.
W24/Z2(4)	KIA-20232	6015 ± 30	n.a.	n.a.	n.a.
Sector J/L					
J/L-46	KIA-20207	5900 ± 45	–27.3	7.7	n.a.
J/L-62	KIA-20233	5915 ± 45	–28.3	8.8	n.a.
Sector M					
2246	KIA-38961	5695 ± 45	–26.5	6.5	16.6
extracted lipids of 2246	KIA-38962	5885 ± 40	n.a.	n.a.	n.a.

Table 2 ^{14}C dates for the organic material from the site of Doel.

Sample name	Lab code	^{14}C date (BP)
Sector B		
W16/Z7(1)	KIA-17995	5635 ± 30
W19/Z1(4)h	KIA-17996	5595 ± 35
W20/Z2(1)	KIA-17994	5575 ± 35
W6/Z6(3)	KIA-17987	5570 ± 30
W19/Z1(4)	KIA-17997	5550 ± 35
W6/Z18(4)	KIA-17986	5400 ± 30
R069	NZA-12076	5220 ± 55
W16/Z8(1)	KIA-32618	5595 ± 35
Sector M		
hedera # 4	KIA-35774	5700 ± 35
viscum # 4	KIA-35770	5490 ± 40
hedera # 9	KIA-35771	5490 ± 40
corylus # 9	KIA-35786	5280 ± 40
corylus # 13	KIA-35769	5350 ± 40
cornus # 16	KIA-35772	5325 ± 35
viburnum # 17	KIA-36257	5385 ± 30
corylus # 17	KIA-36231	5305 ± 50
quercus # 27	KIA-35804	5570 ± 35

If this group discrimination is applied on the measured stable isotopes of Doel, the charred food residue can be divided into 2 groups (see Table 1):

1. The first group (samples 3, 277, 304, 416, 419, 430, 440, 550, J/L-46, J/L-62, 2246) has $\delta^{13}C$ values < –25‰ and $\delta^{15}N$ values between +6 and +10‰. This suggests that freshwater fish is a likely component of these charred food residues, and probably the major component for samples with high $\delta^{15}N$ (+8 and +10‰).
2. A second group (samples 87, 251, 279, 281, 291) has all $\delta^{13}C$ values < –25‰ and $\delta^{15}N$ values between +1 and +5‰. This indicates the processing of herbivore products and/or plant material in the pots.

Theoretically, a clear age difference should be expected between both charred food residue groups defined by the isotopic study. This is, however, not the case. All ^{14}C dates on the charred food residue generally form a coherent group except for the samples 416, 281, 304, and 2246. Their ^{14}C dates are closer to the organic material (see Figure 2) dates.



Figure 1 Inner charred food residue preserved in a pottery sherd from the site of Doel

The stable isotopes from the second group indicate that the ^{14}C dates of the charred food residue from this group should be in agreement with the ^{14}C dates of the organic material (see Tables 1 and 2, Figure 2). This is clearly not the case. The ^{14}C dates of the charred food residue, except sample 281, are systemically older than the ^{14}C dates of the organic material. Furthermore, the isotopic values of samples 304, 416, and 2246 (belonging to group 1) suggest freshwater fish as the major component of the residue. Yet the ^{14}C dates suggest a minimal reservoir effect or none at all.

To distinguish food products based on these observations, one concludes that analysis of stable isotopes of charred food residue should be applied very carefully for the demonstration of fish processing due to different reasons:

1. Thermal treatments induce a series of complex chemical reactions in foodstuffs, which can provoke changes in the stable isotope and atomic C/N values of charred food residue. Our thermal degradation tests revealed enrichment of ~1‰ in the $\delta^{15}N$ value and extreme C/N variations (see Table 3). Spangenberg et al. (2006) noticed different $\delta^{13}C$ fractionation when heating milk at different temperatures. Chemical reactions induced by thermal treatment are a) denaturation in protein; b) lipolysis, oxidation, and polymerization in lipids; c) gelatinization in carbohydrates; and d) reactions in various other substances such as enzymes. In the reactions involving proteins, lipids, and carbohydrates, the Maillard reaction is particularly important in food sci-

ence. The Maillard reaction, also known as the "non-enzymic browning reaction" is a complex series of chemical reactions that take place during the preparation of foods. More precisely, sugars, amino acids, and proteins react with each other to facilitate the formation of a large variety of compounds with differing volatilities and solubilities. Several variables are involved, e.g. structure and concentration of the components, pH, temperature, pressure, water activity, and the presence of catalysts or inhibitors as well as light and time. The Maillard reaction is dramatically increased by temperature (20,000-fold between 0° and 70°), and hydration plays an important role (Hardy et al. 1999). Prior knowledge of the resources cooked in the pot (Hart et al. 2007) and the cooking conditions (e.g. pH, temperature) are essential in order to interpret the $\delta^{13}C$, $\delta^{15}N$, and atomic C/N values of charred food residue.

2. The C/N ratio of the charred food residue is not in a certain range, e.g. it varies between 7.7 and 58.2 for the Doel samples (see Table 6 of Boudin et al. 2009). Hence, the C/N ratio cannot be established as a quality control indicator for stable isotope use of charred food residue.

Table 3 Stable isotope values for fresh and charred/heated modern reference material to study thermal degradation. The hazelnuts and the muscle tissue of the wild boar and bream were heated (200 °C) in industrial ceramics for 30 min until charred. Turnip and wheat were heated (100 °C) for 30 min on an electrical hotplate in a glass vessel.

Sample name	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Atomic C/N
Fresh wild boar	–20.6	2.4	2.2
Charred wild boar (200 °C)	–20.8	3.8	28.6
Fresh bream	–26.6	9.4	1.6
Charred bream (200 °C)	–26.3	10.9	39.4
Fresh hazelnut	–26.9	1.2	7.2
Charred hazelnut (200 °C)	–26.9	2.2	8.8
Fresh turnip	–30.3	7.5	62.8
Heated turnip (100 °C)	–29.8	7.7	44.0
Fresh wheat	–28.6	2.0	33.9
Heated wheat (100 °C)	–28.9	3.2	29.3

A lipid extract of a charred residue (sample 2246) was dated in order to verify the bulk charred food residue ^{14}C date. The ^{14}C date for the extracted lipids is older than the bulk ^{14}C date (see Table 1). One hypothesis is that the possible fish reservoir effect is more abundant in the lipid ^{14}C date than in the bulk ^{14}C date, where carbohydrate (the most abundant nutrient in plant material) degradation products are also dated. To confirm these findings, more ^{14}C dates should be obtained on bulk and lipid extract of several charred food residue samples.

Grijpskerke

In Grijpskerke, the ^{14}C date for the charred food residue is compatible with the ^{14}C dates for the animal bones (dog and cow) (see Table 4 and Figure 3). In this case, a reservoir effect on the ^{14}C date of the charred food residue can be excluded. This assessment is strengthened by: 1) the stable isotope values of the bone collagen suggest a mainly carnivore diet for the dog and a herbivore diet for the cow (see Table 4); and 2) the relative dating (250–12 BC), based on pot morphology and decoration, is also in agreement with the 3 ^{14}C dates (see Figure 3). If the stable isotope theory of Craig et al. (2007) is applied to the Grijpskerke charred food residue, a marine origin is suggested as the $\delta^{13}C$ value is > –25‰ and the $\delta^{15}N$ value is between +7 and +12‰ (see Table 4). However, this is in contradiction with the charred food residue ^{14}C date where no reservoir effect was observed.

Table 4 ^{14}C dates and stable isotope values from the site of Grijpskerke.

Sample name	Lab code	^{14}C date (BP)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Atomic C/N
Dog (collagen)	KIA-39442	2115 ± 30	–19.1	8.8	3.2
Cow (collagen)	KIA-39443	2155 ± 30	–20.3	5.4	3.3
Charred food residue	KIA-39445	2120 ± 30	–24.9	8.3	13.9

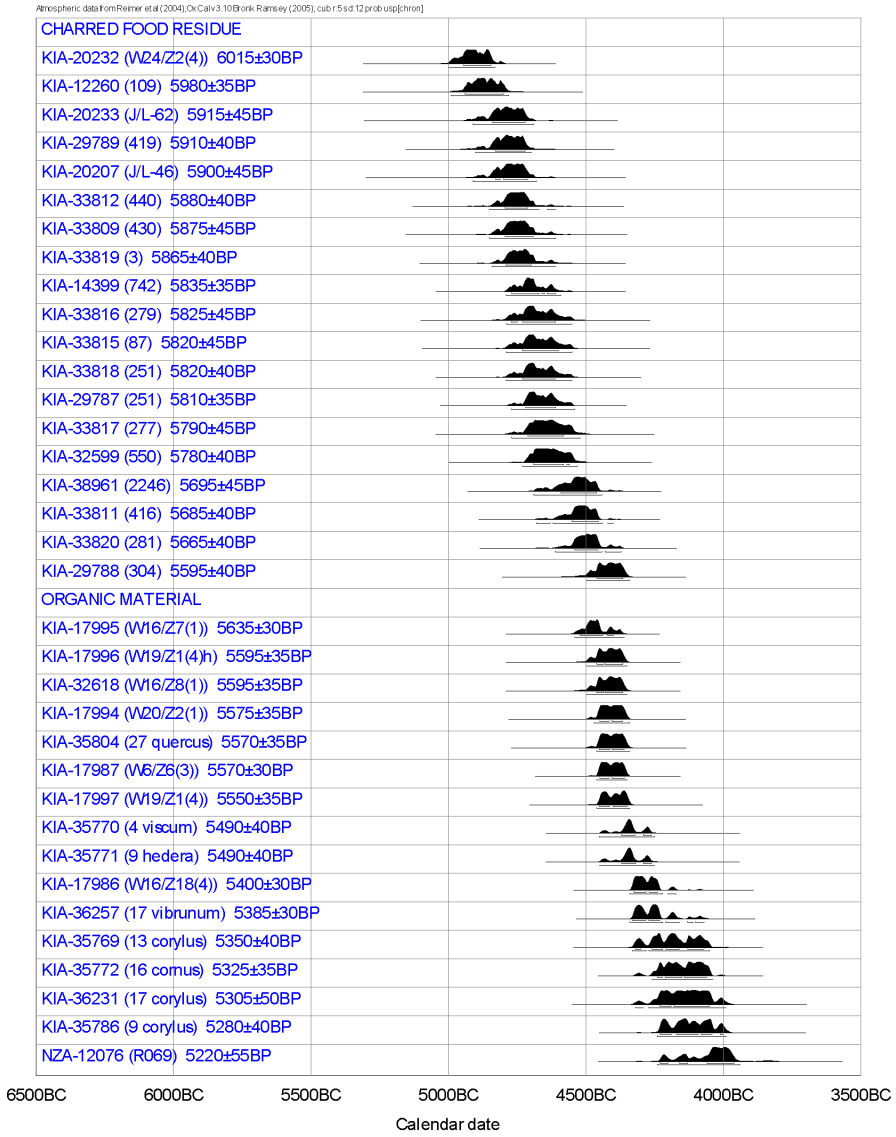


Figure 2 Calibrated ^{14}C dates for charred food residue and organic material for the site of Doel

The ^{14}C date of the charred food residue is in good agreement with the ^{14}C dates of the animal bone collagen and the typochronological date of the pottery (see Figure 3). This suggests that acid-alkali-acid is a valid pretreatment method for charred food residue. This should be confirmed by further experiments.

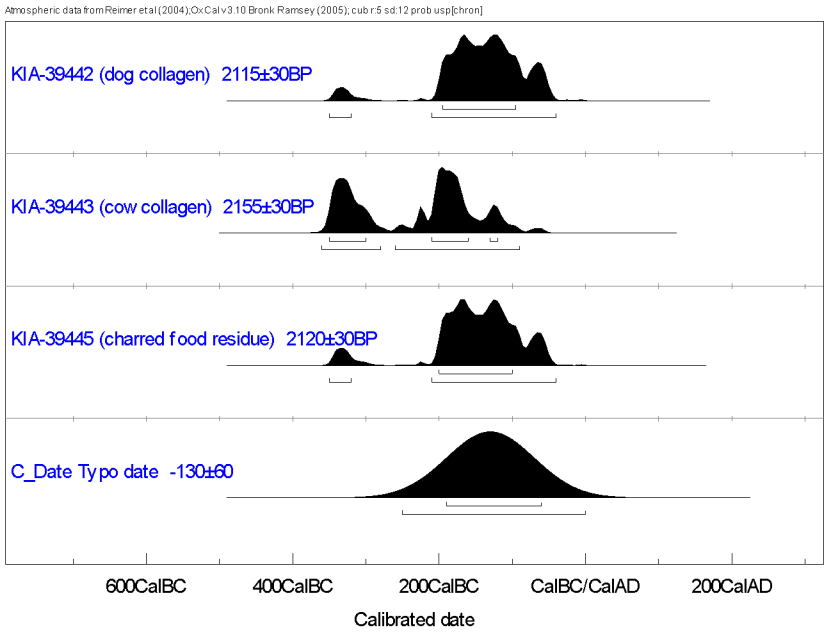


Figure 3 Calibrated ^{14}C dates for charred food residue and bone collagen from the Grijpskerke site. The typochronological date of the pottery is inserted according to Van Strydonck et al. (2004).

CONCLUSION

^{14}C dates for the charred food residue samples of Doel are systematically older than the organic material dates, except for the charred food residue samples 416, 281, 304, and 2246, which are comparable to the organic material ^{14}C dates. The older ^{14}C dates can be caused by a fish reservoir effect and, in the case of Doel, probably from freshwater fish. This can be assumed because the site is located in the lowlands of the Scheldt River valley and tiny fragments of cremated freshwater fish bones were excavated.

The ^{14}C dates for the charred food residue and animal bone collagen of Grijpskerke are in perfect agreement and demonstrates that acid-alkali-acid pretreatment of charred food residue results in reliable ^{14}C dates.

This study proves that ^{14}C dating of charred food residue can be a viable technique in cultural periods where the society was primarily agricultural such as in the Iron Age. Nevertheless, charred food residue ^{14}C dates should be treated with extreme caution in cultural periods such as in the hunter-gatherer-fisher Mesolithic, where fish consumption played an important role.

It is unlikely to detect aquatic (fish) processing in charred food residue by the use of the stable isotopes $\delta^{13}C$ and $\delta^{15}N$. This technique is not suitable for identifying ^{14}C dates liable to inaccuracies resulting from the presence of marine or freshwater carbon.

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