UNIVERSITY of York

This is a repository copy of *Finding Britain's last hunter-gatherers: A new biomolecular approach to 'unidentifiable' bone fragments utilising bone collagen*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/103636/

Version: Accepted Version

Article:

Charlton, Sophy Jessica Laura, Alexander, Michelle Marie orcid.org/0000-0001-8000-3639, Collins, Matthew James orcid.org/0000-0003-4226-5501 et al. (5 more authors) (2016) Finding Britain's last hunter-gatherers: A new biomolecular approach to 'unidentifiable' bone fragments utilising bone collagen. Journal of archaeological science. pp. 55-61. ISSN 0305-4403

https://doi.org/10.1016/j.jas.2016.07.014

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Finding Britain's last hunter-gatherers: A new biomolecular approach to 'unidentifiable' bone fragments utilising bone collagen

Sophy Charlton[1], Michelle Alexander[1], Matthew Collins[1], Nicky Milner[1], Paul Mellars[2], Tamsin C O'Connell[2], Rhiannon E Stevens[3] and Oliver E Craig[1]

[1] - Department of Archaeology, University of York, York, YO1 7EP
[2] - Department of Archaeology and Anthropology, University of Cambridge, Cambridge, CB2 3DZ
[3]- University College London, Institute of Archaeology, London, WC1H 0PY

Corresponding Author (Present address): Sophy Charlton, Natural History Museum, Department of Earth Sciences, Cromwell Road, London, SW7 5BD; sophy@palaeo.eu

Keywords: Mesolithic; Neolithic; collagen; stable isotopes; AMS dating; ZooMS; diet

Abstract

In the last decade, our knowledge of the transition from foraging, fishing, and hunting to agricultural food production has been transformed through the molecular analysis of human remains. In Britain, however, the lack of Late Mesolithic human remains has limited our understanding of this dietary transition. Here, we report the use of a novel strategy to analyse otherwise overlooked material to identify additional human remains from this period. ZooMS, a method which uses bone collagen sequences to determine species, was applied to unidentifiable bone fragments from 5th millennium deposits from the Late Mesolithic site of Cnoc Coig (Oronsay, Inner Hebrides) using an innovative new methodology. All samples bar one produced ZooMS results, with 14/20 bone fragments identified as human, and the remainder a mixture of pig and seal. 70% of bone fragments had sufficient collagen for stable isotope analyses, however none of three human bone fragments analysed had sufficient endogenous DNA. By conducting AMS dating and stable isotope analysis on this identified collagen, we provide new data that supports the view that the exploitation of marine resources partially overlapped with the earliest agricultural communities in Britain, and thus argues against the idea that forager lifeways in Britain were immediately replaced by agriculture c.4000 cal. BC. Unfortunately, we were unable to explore the genetic relationship between contemporaneous farmers and foragers. However, the more persistent bone protein could be used to identify species, determine date, and assess diet. This novel approach is widely applicable to other early prehistoric sites with fragmentary skeletal material.

42 **1. Introduction**

43 Archaeology, according to Kristiansen (2014), is experiencing its third scientific revolution,
44 driven by the application of new biomolecular methods. One of the most demonstrable signs
45 of this revolution has been to transform our understanding the transition from foraging,
46 fishing and hunting to agricultural food production. Previously, stable isotope analysis of
47 human bone collagen has been used to assess the degree of dietary change associated with the
48 shift to farming, AMS dating of bone collagen has been used to determine the speed and
49 trajectory of this shift, whilst ancient DNA analysis has provided new insights regarding the
50 extent of demographic change. In many parts of Europe, these methods have been applied
51 with spectacular success (e.g. Bramanti et al., 2009; Haak et al., 2010; Lidén et al., 2004;
52 Lightfoot et al., 2015; Michael P. Richards et al., 2003; Rasteiro and Chikhi, 2013; Shennan
53 and Edinborough, 2007) triggering new and better informed debates regarding this key phase
54 in human history (e.g. Richards, 2003; Rowley-Conwy, 2011; Rowley-Conwy, 2004; Tresset
55 and Vigne, 2011). However, in Britain their application has been hampered by the near
56 absence of human remains dating to the period immediately preceding the arrival of farming
57 c.4,000 cal. BC, i.e. the Late Mesolithic. Remarkably, the only directly dated sites from the
58 whole of the 5th millennium BC with human remains are from the small Inner Hebridean
59 island of Oronsay (Meiklejohn et al., 2011), severely restricting meaningful comparisons with
60 more abundant Neolithic remains found across Britain.
61
62 The paucity of human remains from the Late Mesolithic of Britain is puzzling. Although the
63 British Isles may have been less densely populated during this period compared to the
64 Neolithic (Collard et al., 2010), many bone bearing sites have been identified. One possibility
65 is that human remains became disarticulated and highly fragmented through cultural practices
66 (Gray Jones, 2011) that rendered them unidentifiable using conventional osteological
67 methods. Here, we revisit the site of Cnoc Coig, Oronsay, one of the few Late Mesolithic
68 sites with human remains known in Britain. Despite the identification of only six individuals
69 at the site (Meiklejohn et al., 2011, 2005), it has previously been pivotal to the argument for a
70 rapid dietary change with the arrival of agriculture in Britain (Richards et al., 2003; Schulting
71 and Richards, 2002). We apply an innovative method (ZooMS), which uses bone collagen
72 sequences to determine species, to investigate whether additional human remains can be
73 identified amongst 5th millennium deposits of small, fragmentary 'loose' bone. The study also
74 aimed to utilise this collagen to conduct AMS dating and stable isotope analysis on any
75 identified bone samples, to enhance our understanding of the diet of Britain's last forager
76 groups and their chronological relationship to the earliest evidence for agriculture, thereby
77 contributing to larger debates regarding the transition in Britain.

78 **1.1. Cnoc Coig**

79 The site of Cnoc Coig is one of five Mesolithic shell middens on the island of Oronsay, Inner
80 Hebrides. Cnoc Coig was first excavated in 1911-1912 (Wickham-Jones et al., 1982), and
81 then more extensively in 1973-1979 (Mellars, 1987). During the latter excavations, 49 pieces
82 of human bone were recovered, predominantly from the hands and feet, thought to represent
83 at up to six individuals (Meiklejohn et al., 2011, 2005; Meiklejohn and Denston, 1987).

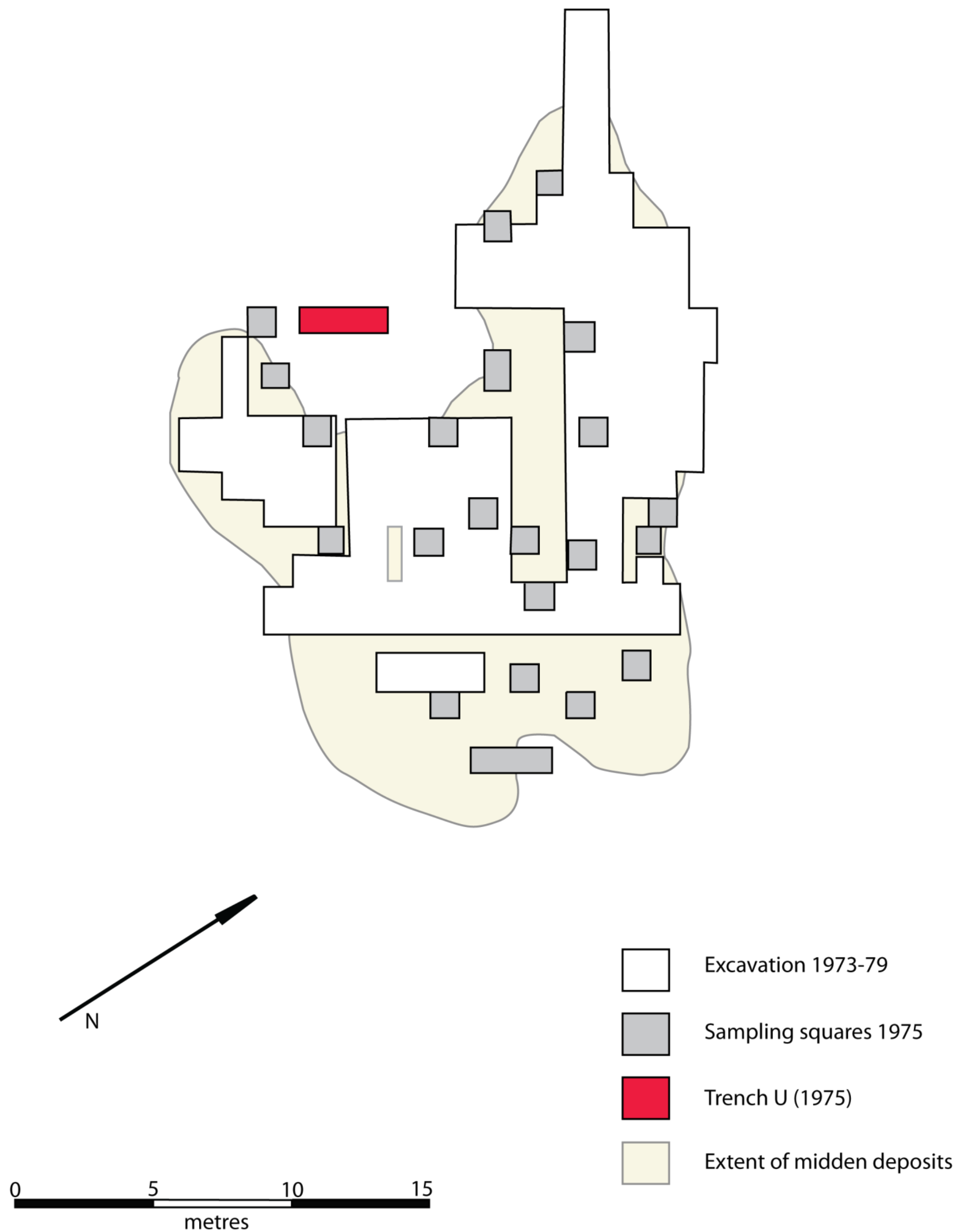
84 Spatial analysis has suggested these human remains fall largely into seven circumscribed
85 bone groups, although none are indicative of primary inhumation (Meiklejohn et al., 2005).
86 Critically, AMS dating of the samples revealed that they date to the late 5th millennium BC,
87 immediately prior to the emergence of agriculture in Britain; although slightly earlier dates
88 (4300 cal. BC) have been proposed for both Neolithic monuments and pottery on the West
89 Coast of Scotland (Sheridan, 2010). Although small and fragmented, the Oronsay remains
90 represent one of the only Late Mesolithic human skeletal assemblages in Britain and, as such,
91 have been subjected to a range of analyses aimed at establishing their date, circumstances of
92 deposition, and diet (e.g. Meiklejohn et al., 2011, 2005; Mellars et al., 1980; Richards and
93 Mellars, 1998; Richards and Sheridan, 2000; Wicks et al., 2014).
94
95 In particular, stable isotope analysis of the human bones from Cnoc Coig has shown a
96 strongly marine isotopic signature, in contrast to the terrestrial signatures observed for
97 humans from early 4th millennium sites along the west coast of Scotland and elsewhere in
98 Britain (e.g. Schulting and Richards, 2002; Hedges et al., 2008; Milner and Craig, 2009). In
99 the absence of other Late Mesolithic human remains, the Oronsay material has been pivotal
100 to the argument for a rapid dietary change with the arrival of agriculture in Britain (Richards
101 et al., 2003; Schulting and Richards, 2002), despite being based on a very small number of
102 individuals. However, from recent recalibration of the dates, it has been suggested the human
103 remains may instead date to the early 4th millennium BC (Milner and Craig, 2009) and are
104 therefore coeval with the earliest evidence for domestic crops and animals in Scotland and
105 other parts of Britain (Brown, 2007; Rowley-Conwy, 2004). Therefore, further identification
106 of human remains for dating and dietary analysis from Cnoc Coig has the potential to greatly
107 clarify our understanding of the transition in Western Scotland and more generally across
108 Britain.

109 **2. Materials and Methods**

110 **2.1. Samples**

111 New biomolecular techniques have opened up the possibility of the identification of bone
112 fragments to genera using collagen peptide mass fingerprinting (ZooMS; Welker et al.,
113 2015). Twenty fragments of disarticulated and heavily fragmented bone from the 1973-9
114 excavations, originally classified as ‘unidentifiable’ or ‘?human’, and which had therefore
115 remained unstudied, were utilised within this research (Fig. 1). Although the trench number
116 of the remains is known, little other contextual information is available. The majority of
117 fragments (n=15) derive not from the main midden structure, but instead lie just outside in a
118 single outlying trench (Fig. 2). The remaining five ‘unidentifiable’ bones were selected from
119 other areas within the main midden structure. This research was undertaken with permission
120 from National Museums Scotland, to whom the Oronsay assemblage has been allocated.
121

122
123 Figure 1: Selection of bone fragments from the Cnoc Coig assemblage used within this
124 research; highlighting the range of sizes, elements and preservation. From top, L-R, ZooMS
125 IDs: seal, pig, remainder human
126



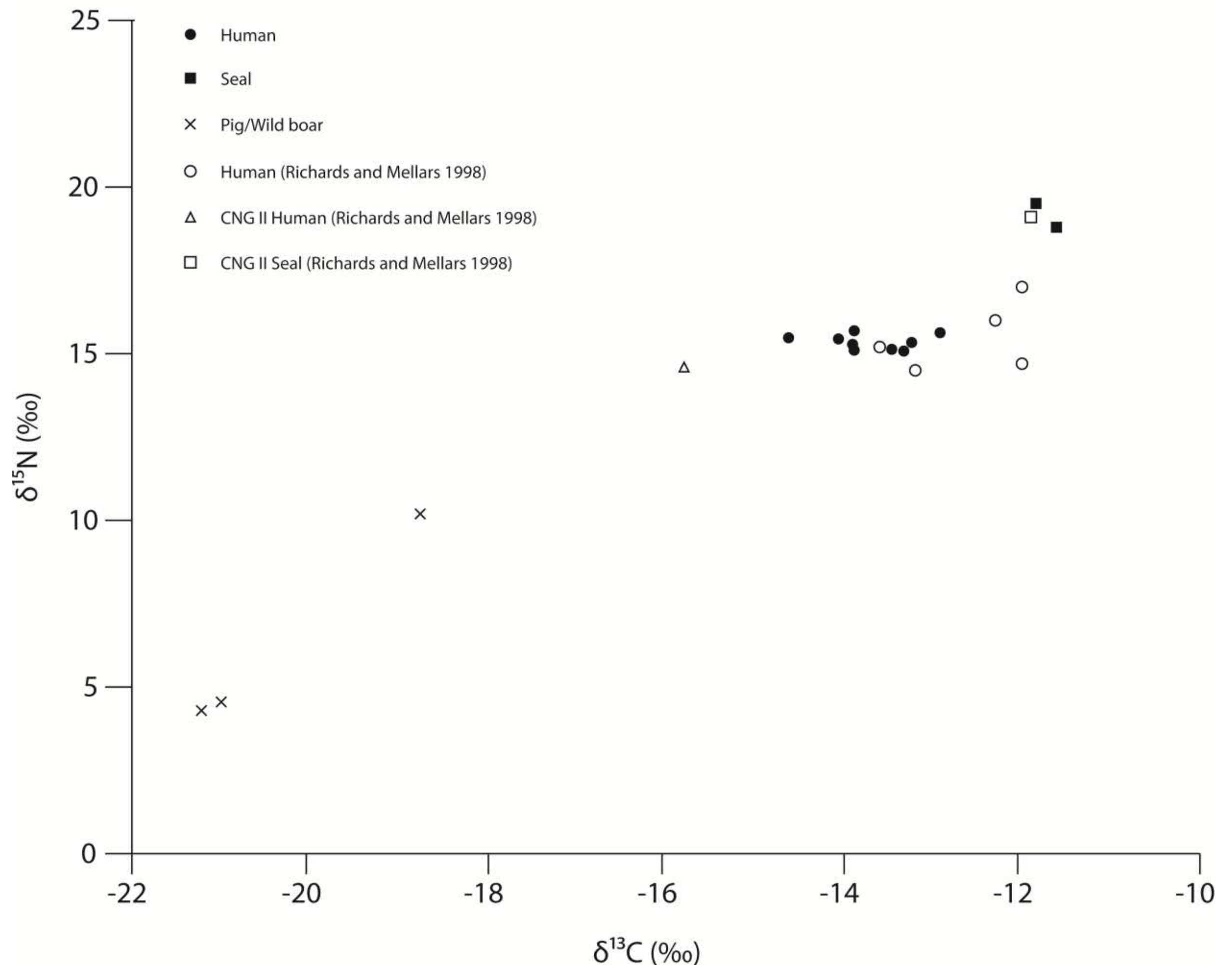
127
128 Figure 2: Plan of excavated areas of Cnoc Coig (adapted from Mellars 1987, 215), indicating
129 the extent of the midden as defined by Mellars. Trench U, where the human remains
130 identified in this study were found, is highlighted in red
131
132

2.2. A Combined Biomolecular Approach

A multi-methodological approach was adopted in the study of these bone fragments, combining ZooMS, stable isotope analysis and AMS dating (Fig. S1). Collagen was extracted and isotopically analysed using published protocols (Richards and Hedges, 1999; Colonese et al., 2015). ZooMS, a qualitative analytical technique for taxonomic identification of archaeological materials (Buckley et al., 2009; 2010), was undertaken on a sub-sample of the extracted collagen (<1mg), using a novel methodology, as outlined below. Four samples with adequate collagen preservation were submitted for AMS dating at the NERC radiocarbon facility (Oxford) and calibrated using the procedure detailed below. Three samples identified as human using ZooMS were also submitted for aDNA analysis. Protocols for each of the methodologies employed in this study are provided in the Supplementary Information.

3. Results and Discussion

Initially, collagen was prepared and extracted from all 20 bone fragments using previously published protocols (Richards and Hedges, 1999; Colonese et al., 2015), but yields varied, with only fourteen having sufficient collagen for $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis (Fig. 3). The range of $\delta^{13}C$ and $\delta^{15}N$ values obtained however indicated samples with both fully marine and fully terrestrial diets. van Doorn et al. (2011) have previously shown that it is possible to undertake ZooMS on samples soaked in ammonium bicarbonate buffer (AmBic), utilising macroscopic amounts of bone collagen. Due to this, we speculated that the emptied 15ml Falcon tubes previously utilised for lyophilisation following collagen extraction would have absorbed sufficient collagen to their surface to allow for ZooMS identification to be undertaken. Lyophilised collagen samples were therefore removed from Falcon tubes, and 75µl 50mM AmBic was added to each 'empty' tube used during ultrafiltration and digested with 1µl trypsin. Identification was based upon peptide matching as outlined in Welker et al. (2015). Nineteen of the twenty samples yielded identification information, including six that had insufficient collagen to undertake stable isotope analyses. Two fragments identified as Pinnipedia (seal) using ZooMS had $\delta^{13}C$ and $\delta^{15}N$ isotopic values indicative of a typical marine based diet expected of these animals. Indeed, all the seal bones analysed from Oronsay (Richards and Mellars, 1998; this study) are within analytical error (Pestle et al., 2014) and could therefore even be from the same skeleton.



164
165 Figure 3: Stable carbon and nitrogen isotope values from Cnoc Coig and Caisteal nan Gillean
166 human and fauna. The analytical error on data obtained in this study was <0.2‰ (1σ) for both
167 δ^{13}C and δ^{15}N. Data from this study and(Richards and Mellars, 1998)
168
169 Of the three bone fragments identified as Sus scrofa using ZooMS, two samples (at least one
170 individual) showed δ^{13}C and δ^{15}N values indicative of a terrestrial herbivorous diet, whilst the
171 third had isotope values consistent with a more marine/omnivorous diet, possibly deriving
172 from the consumption of refuse from the shell midden, or being purposively fed marine foods
173 by humans. Biometrically identified as wild boar (Grigson and Mellars, 1987), these animals
174 are likely to have been purposively brought to Oronsay from the mainland or larger
175 surrounding islands. As they had differential diets prior to death, we may hypothesise that
176 they inhabited different areas, derived from two (geographically) distinct populations, or
177 were managed differently prior to their death, mirroring interpretations of deer at the site
178 (Grigson and Mellars, 1987).
179
180 Remarkably, fourteen of the fragments were identified as human, increasing the number of
181 known human bone fragments from all five Oronsay middens from 55 (Meiklejohn et al.,
182 2005) to 74 (including five fragments recently recovered at NMS) (Sheridan, pers. comm.).
183 Of the fourteen bone fragments identified as human, nine yielded sufficient amounts of
184 collagen for δ^{13}C and δ^{15} N isotopic analysis (Fig. 3). Generally, the isotope values of the

185 human samples are similar to those from previous study, confirming marine protein rich diets
186 (Fig. 3; Table S1). However, variation in the $\delta^{13}C$ and $\delta^{15}N$ values indicates that the human
187 bone samples are unlikely to be from the same individuals as previously analysed. At least
188 two of the new human samples are outside the error expected by replicate analysis of a single
189 individual (Pestle et al., 2014). Conservatively, if we use these errors, combining this new
190 human isotopic data with previous analysis (Richards and Mellars, 1998) suggests a potential
191 minimum of seven human individuals are represented isotopically, from thirteen pieces of
192 bone (Fig. 3).
193
194 Given the scarcity of British Late Mesolithic human remains and the unique nature of these
195 samples, aDNA analysis was attempted at two independent laboratories on three fragments of
196 bone identified as human here using ZooMS. Unfortunately, this was unsuccessful due to low
197 endogenous DNA content and inhibition of the samples (Barnes & Brace, pers. comm.; Reich
198 & Harney, pers. comm; see Supplementary Information). However, this does highlight that in
199 sites which do not yield DNA, the bone protein collagen can still provide useful biomolecular
200 information, through identifying species, determining date, and assessing diet.
201
202 Finally, importantly, all the human remains identified here originate from outside the main
203 midden structure (Fig. 2) and may represent a different depositional event. This raises
204 interesting questions as to whether deposition in this location was intentional, or is a product
205 of taphonomic processes – and can perhaps contribute to discussions surrounding the
206 deposition of human remains in the late 5th-early 4th millennium BC in Britain. Given the
207 ubiquitous nature of disarticulated human remains with the Mesolithic burial record, potential
208 degrees of intentionality with regards to these kinds of deposits have previously been
209 discussed. Gray Jones (2011), for example, has suggested that 'loose bone' or disarticulated
210 remains may in fact be the result of deliberate acts, and thus a part of, rather than separate
211 from, other types of mortuary practice. Additionally, only one of the bone fragments
212 identified here as human appears to originate from the hands or feet, which have previously
213 been noted to be the dominant element types within the midden deposits, and have led to
214 suggestions of excarnation at the site (Meiklejohn et al., 2005).

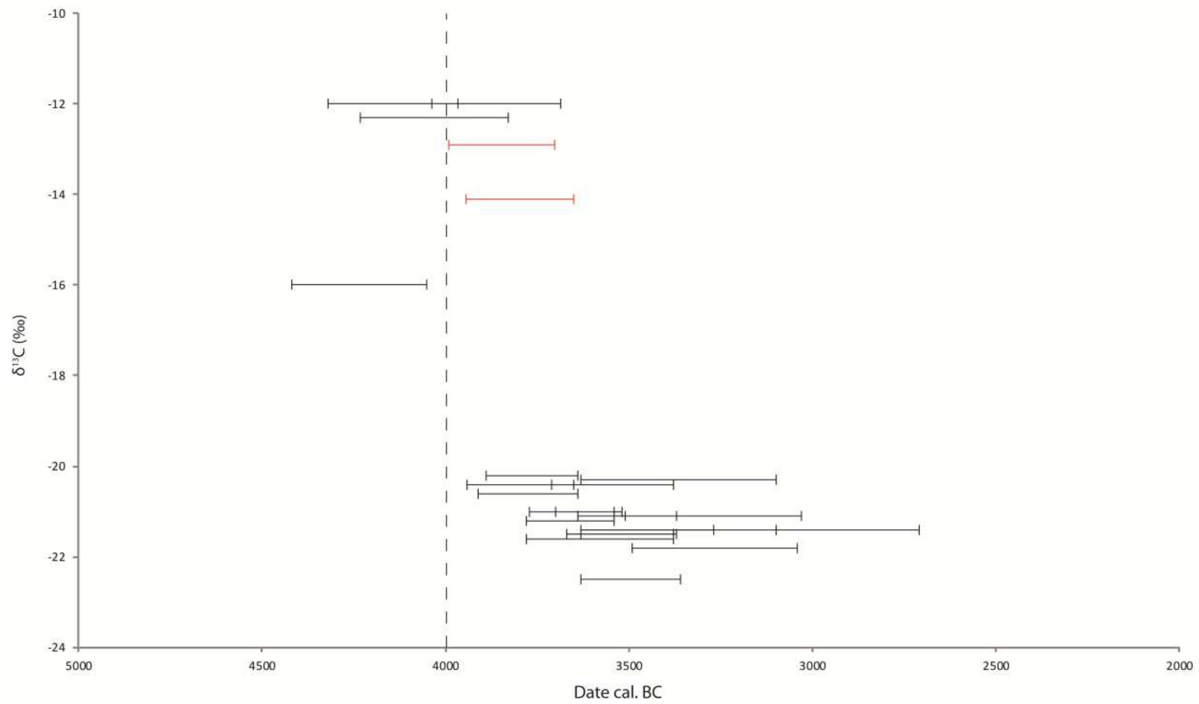
215 **3.1. Re-dating human remains at Cnoc Coig**

216 Previous AMS dates on human remains dated Cnoc Coig to 4300-3800 cal. BC (Milner and
217 Craig, 2009; Richards and Sheridan, 2000). However, the marine carbon isotope signatures of
218 the human remains mean they are subject to uncertainties associated with the marine
219 reservoir effect (MRE). Additional ^{14}C dates previously obtained from bulk charcoal (Switsur
220 and Mellars, 1987) could have derived from 'old wood' (Schiffer, 1986), adding to the
221 uncertainty regarding the dating of the site. The first dates on short-lived terrestrial mammals,
222 as identified by ZooMS, were therefore undertaken here, along with dates on the newly
223 identified human remains (Table 1).
224
225
226
227

Sample Number	Trench	ZooMS ID	Lab Ref. No.	^{14}C Date BP	Date cal. BC (95.4%)
8257	U III	Human	OxA-29939	5391 ± 30	3991-3702
8267	U III	Human	OxA-29938	5379 ± 29	3944-3649
10494	P (E)	Pig	OxA-29937	5122 ± 30	3982-3803
17050	H/ 13	Pig	OxA-29936	5117 ± 29	3977-3803

228 Table 1: AMS dates obtained within this study from newly identified humans and fauna. A
229 ΔR value of 47 ±52 was used to calibrate samples 8257 and 8267
230
231 Calibration of all dates was undertaken using OxCal v.4.2 (Bronk Ramsey, 2009). As the
232 human samples had marine isotopic signatures however, they were calibrated using a mixed
233 marine-terrestrial curve (Marine13/IntCal13, Reimer et al., 2103) in a proportion determined
234 by marine/terrestrial carbon contribution to collagen (as in Barrett and Richards 2004;
235 following best practice outlined in Cook et al. 2015). The latter was estimated for each
236 individual from their δ^{13}C values following linear interpolation from the observed marine and
237 terrestrial endpoints after Schulting and Richards (2002) (-12‰ and -21‰ respectively;
238 Table S1). We placed a 10% error on this value following Hedges (2004). Calibration of
239 AMS dates from Cnoc Coig using this approach has previously been successfully undertaken
240 by Gordon Cook (Milner and Craig 2009). MRE and ΔR values are known to vary both
241 temporally and geographically, caused by palaeoclimatic, environmental and oceanographic
242 changes (Ascough et al., 2007, 2004). As the MRE has not been assessed at Oronsay itself, a
243 calculated mean ΔR value for Scotland was utilised (ΔR= 47 ±52 14 C yr) (Russell et al.,
244 2015) following best practices (Cook et al., 2015). Although this value is calculated for the
245 west coast of Scotland 3500 BC onwards, it is at present the most conservative and suitable
246 ΔR offset to utilise for these samples (Ascough, pers. comm.). The two pig samples dated
247 were calibrated using only the terrestrial (IntCal13) calibration curve.
248
249 Intriguingly, the dates on two pig bone samples with purely terrestrial diets fall within the 4th
250 millennium BC at 95% confidence (Table 1). After calibration of both new dates presented
251 here and those previously obtained from Oronsay using the approach outlined above, it is
252 clear that all the humans overlap with the terrestrial fauna and fall within the early part of the
253 4th millennium BC (Tables 1 and S1; Fig. 4). This is a significant result as the Oronsay
254 human dates, with marine isotope signatures, overlap with humans from other parts of
255 Western Scotland with fully terrestrial isotope signatures (Fig. 4) and with the earliest
256 evidence for domesticated animals and plants in Britain. We suggest that there was
257 considerable heterogeneity in human diets in the early part of the Neolithic reflecting
258 specialisation in subsistence practices across the landscape, and the continuity of foraging,
259 hunting and fishing into the period traditionally associated with agriculture and pastoralism.
260 Sheridan (2010) argues for the arrival of a 'Breton Neolithic' in this region from around
261 4300-4200 cal. BC, and Collard et al. (2010) suggest that farming emerged in western
262 Scotland c.6100 cal. BP. These dates, combined with the data obtained here, would imply

263 that both hunter-gatherer-fisher and farming lifestyles potentially co-existed on the West
264 Coast of Scotland for several hundred years. However, it should be noted that we have very
265 little isotopic evidence for human subsistence practices in 5th millennium Britain.
266



267
268 Figure 4: Plot of $\delta^{13}C$ values against radiocarbon dates for humans from Scottish West Coast
269 sites, c.4500-3000 cal. BC (data from this study (Tables 1 and S1); Richards and Sheridan,
270 2000; Schulting and Richards, 2002). New human data obtained for Cnoc Coig within this
271 study is highlighted in red

272 **4. Conclusions**

273 This study adopted an innovative biomolecular approach to bone fragments from the site of
274 Cnoc Coig, and highlights the archaeological information which can be obtained from bone
275 protein alone. By combining a variety of scientific techniques (all of which target bone
276 collagen) and applying them in tandem to the same samples, this research has aimed to
277 illustrate the information which can be obtained from previously overlooked fragmented
278 material. As such, this study importantly highlights the research potential currently dormant
279 within osteologically unidentifiable bone fragments from prehistoric contexts. There is
280 consequently significant potential for future application of the method to other prehistoric
281 sites with fragmentary or loose bone, such as caves and middens. We therefore call for
282 widespread application of ZooMS to similar Mesolithic assemblages across Britain.
283
284 As demonstrated here for the first time, the ability to be able to obtain taxonomic information
285 from ‘empty’ tubes previously utilised within the collagen extraction process also appears to
286 hold great future potential - particularly as it does not require the use of collagen reserved for
287 isotopic analysis or AMS dating. It also presents a potential opportunity to retrospectively

analyse empty tubes previously utilised within collagen extractions to gain taxonomic information. This may be of particular use with samples which produce AMS dates or isotopic values that are distinctly different from what is anticipated.

Overall, this research has detected extremely rare human bones from the Mesolithic-Neolithic transition which can be used to further elucidate issues surrounding the period. In total, fourteen new fragments of human bone have been identified, increasing the number of known human bone fragments from the five Oronsay middens from 55 (Meiklejohn et al., 2005) to 74 (including five fragments recently recovered at NMS) (Sheridan, pers. comm.). The human remains identified here provide additional data comparable to isotopic analyses undertaken previously at Cnoc Coig. The isotopic results also provide additional evidence of a high marine protein diet along the west coast of Scotland – but AMS dates obtained from these samples suggest that this marine diet may have extended into the 4th millennium BC and the 'Neolithic' period. However, the presence of a marine isotopic signature within one Sus scrofa sample suggests the need for better characterisation of faunal baselines within the British Mesolithic, in particular when considering interpretations of marine resource consumption by humans.

Finally, unfortunately, insufficient endogenous DNA content within the samples analysed here meant that it was not possible to explore the genetic relationship between the Cnoc Coig humans and the earliest known agricultural communities in Britain. In future, analysis of the single human petrous bone known from Cnoc Coig may be worth exploration, as this element is known to provide significantly higher endogenous DNA yields (Pinhasi et al., 2015).

Acknowledgements

We thank Alison Sheridan and National Museums Scotland for providing permissions & comments on the manuscript, Frido Welker for assistance with ZooMS, Peter Rowley-Conwy for discussions on Mesolithic pigs, Emily Hellewell for discussions on the site & human remains, and Philippa Ascough & Nicola Russell for assistance with determining ΔR values. DNA data and methods were kindly provided by Selina Brace & Ian Barnes (Natural History Museum) and David Reich & Eadaoin Harney (Department of Genetics, Harvard Medical School). This research was funded by NERC (Ref.NE/K500987/1), and radiocarbon dates were funded by the NERC-AHRC National Radiocarbon Facility (NRCF).

References

Ascough, P.L., Cook, G.T., Dugmore, A.J., Barber, J., Higney, E., Scott, E.M., 2004. Holocene variations in the Scottish marine radiocarbon reservoir effect. Radiocarbon 46, 611–620.

Ascough, P.L., Cook, G.T., Dugmore, A.J., Scott, E.M., 2007. The North Atlantic marine reservoir effect in the Early Holocene: Implications for defining and understanding MRE values. Nucl. Instrum. Methods Phys. Res. B 259, 438–447.

330 Barrett, J.H., Richards, M.P., 2004. Identity, Gender, Religion and Economy: New Isotope
331 and Radiocarbon Evidence for Marine Resource Intensification in Early Historic Orkney,
332 Scotland, UK. European Journal of Archaeology 7, 249–271.
333 Bramanti, B., Thomas, M.G., Haak, W., Unterlaender, M., Jores, P., Tambets, K., Antanaitis-
334 Jacobs, I., Haidle, M.N., Jankauskas, R., Kind, C.-J., Lueth, F., Terberger, T., Hiller, J.,
335 Matsumura, S., Forster, P., Burger, J., 2009. Genetic discontinuity between local hunter-
336 gatherers and central Europe’s first farmers. Science 326, 137–140.
337 Bronk Ramsey, C., 2009. Bayesian analysis of radiocarbon dates. Radiocarbon 51, 337–360.
338 Brown, A., 2007. Dating the onset of cereal cultivation in Britain and Ireland: the evidence
339 from charred cereal grains. Antiquity 81, 1042–1052.
340 Brown, T.A., Nelson, D.E., Vogel, J.S., Southon, J.R., 1988. Improved collagen extraction by
341 modified Longin method. Radiocarbon 30, 171–177.
342 Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2009. Species identification by
343 analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight
344 mass spectrometry. Rapid Commun. Mass Spectrom. 23, 3843–3854.
345 Buckley, M., Whitcher Kansa, S., Howard, S., Campbell, S., Thomas-Oates, J., Collins, M.
346 2010. Distinguishing between archaeological sheep and goat bones using a single
347 collagen peptide. J. Archaeol. Sci. 37, 13-20.
348 Collard, M., Edinborough, K., Shennan, S., Thomas, M.G., 2010. Radiocarbon evidence
349 indicates that migrants introduced farming to Britain. J. Archaeol. Sci. 37, 866–870.
350 Colonese, A.C., Farrell, T., Lucquin, A., Firth, D., Charlton, S., Robson, H.K., Alexander,
351 M., Craig, O.E., 2015. Archaeological bone lipids as palaeodietary markers. Rapid
352 Commun. Mass Spectrom. 29, 611–618.
353 Cook, G.T., Ascough, P.L., Bonsall, C., Hamilton, W.D., Russell, N., Sayle, K.L., Scott,
354 E.M., Bownes, J.M., 2015. Best practice methodology for 14C calibration of marine and
355 mixed terrestrial/marine samples. Quat. Geochronol. 27, 164–171.
356 CR Wickham-Jones, MM Brown, TG Cowie, DB Gallager, JNG Ritchie, 1982. Excavations
357 at Druim Arstail, Oronsay, 1911-12. Glasgow Archaeological Journal 9, 18–30.
358 Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera,
359 C., García, N., Pääbo, S., Arsuaga, J.-L., Meyer, M., 2013. Complete mitochondrial
360 genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA
361 fragments. Proc. Natl. Acad. Sci. U. S. A. 110, 15758–15763.
362 DeNiro, M.J., 1985. Postmortem preservation and alteration of in vivo bone collagen isotope
363 ratios in relation to palaeodietary reconstruction. Nature 317, 806–809.
364 Gray Jones, A., 2011. Dealing with the Dead: Manipulation of the Body in the Mortuary
365 Practices of Mesolithic North West Europe. School of Arts, Histories and Cultures;
366 University of Manchester.
367 Grigson, C., Mellars, P., 1987. The mammalian remains from the middens. Excavations on
368 Oronsay: Prehistoric Human Ecology on a Small Island. Edinburgh University Press,
369 Edinburgh 24.
370 Haak, W., Balanovsky, O., Sanchez, J.J., Koshel, S., Zaporozhchenko, V., Adler, C.J., Der
371 Sarkissian, C.S.I., Brandt, G., Schwarz, C., Nicklisch, N., Dresely, V., Fritsch, B.,
372 Balanovska, E., Villems, R., Meller, H., Alt, K.W., Cooper, A., Members of the
373 Genographic Consortium, 2010. Ancient DNA from European early neolithic farmers
374 reveals their near eastern affinities. PLoS Biol. 8, e1000536.
375 Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G.,
376 Nordenfelt, S., Harney, E., Stewardson, K., Fu, Q., Mittnik, A., Bánffy, E., Economou,
377 C., Francken, M., Friederich, S., Pena, R.G., Hallgren, F., Khartanovich, V., Khokhlov,
378 A., Kunst, M., Kuznetsov, P., Meller, H., Mochalov, O., Moiseyev, V., Nicklisch, N.,
379 Pichler, S.L., Risch, R., Rojo Guerra, M.A., Roth, C., Szécsényi-Nagy, A., Wahl, J.,

380 Meyer, M., Krause, J., Brown, D., Anthony, D., Cooper, A., Alt, K.W., Reich, D., 2015.
381 Massive migration from the steppe was a source for Indo-European languages in Europe.
382 Nature 522, 207–211.
383 Hedges, R.E.M., 2004. Isotopes and red herrings: comments on Milner et al. and Lidén et al.
384 Antiquity 78, 34–37.
385 Hedges, R., Saville, A., O'Connell, T., 2008. Characterizing the Diet of Individuals at the
386 Neolithic Chambered Tomb of Hazleton North, Gloucestershire, England, Using Stable
387 Isotopic Analysis. Archaeometry 50, 114–128.
388 Jardine, W.G., 1978. Radiocarbon ages of raised-beach shells from Oronsay, Inner Hebrides,
389 Scotland: a lesson in interpretation and deduction. Boreas 7, 183–196.
390 Kristiansen, K., 2014. Towards a New Paradigm: The Third Science Revolution and its
391 Possible Consequences in Archaeology. Current Swedish Archaeology 22, 11–34.
392 Lidén, K., Eriksson, G., Nordqvist, B., Götherström, A., Bendixen, E., 2004. "The wet and
393 the wild followed by the dry and the tame" – or did they occur at the same time? Diet in
394 Mesolithic – Neolithic southern Sweden. Antiquity 78, 23–33.
395 Lightfoot, E., Boneva, B., Miracle, P.T., Šlaus, M., O'Connell, T.C., 2015. Exploring the
396 Mesolithic and Neolithic transition in Croatia through isotopic investigations. Antiquity
397 85, 73–86.
398 Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows–Wheeler
399 transform. Bioinformatics 25, 1754–1760.
400 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis,
401 G., Durbin, R., 1000 Genome Project Data Processing Subgroup, 2009. The Sequence
402 Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079.
403 Lindgreen, S., 2012. AdapterRemoval: easy cleaning of next-generation sequencing reads.
404 BMC Res. Notes 5, 337.
405 Meiklejohn, C., Chamberlain, A.T., Schulting, R.J., 2011. Radiocarbon dating of Mesolithic
406 human remains in Great Britain. Mesolithic Miscellany 21, 20–58.
407 Meiklejohn, C., Denston, B., 1987. The Human Skeletal Material: Inventory and Initial
408 Interpretation, in: Mellars, P. (Ed.), Excavations on Oronsay: Prehistoric Human
409 Ecology on a Small Island. Edinburgh University Press, Edinburgh, pp. 290–300.
410 Meiklejohn, C., Merrett, D.C., Nolan, R.W., Richards, M.P., Mellars, P.A., 2005. Spatial
411 relationships, dating and taphonomy of the human bone from the Mesolithic site of Cnoc
412 Coig, Oronsay, Argyll, Scotland, in: Proceedings of the Prehistoric Society. Cambridge
413 Univ Press, pp. 85–105.
414 Mellars, P., 1987. Excavations on Oronsay: Prehistoric Human Ecology on a Small Island.
415 Edinburgh University Press, Edinburgh.
416 Mellars, P.A., Wilkinson, M.R., Fieller, N.R.J., 1980. Fish Otoliths as Indicators of
417 Seasonality in Prehistoric Shell Middens: the Evidence from Oronsay (Inner Hebrides).
418 Proceedings of the Prehistoric Society 46, 19–44.
419 Meyer, M., Kircher, M., 2010. Illumina sequencing library preparation for highly multiplexed
420 target capture and sequencing. Cold Spring Harb. Protoc. 2010, db.prot5448.
421 Michael P. Richards, T. Douglas Price, Eva Koch, 2003. Mesolithic and Neolithic
422 Subsistence in Denmark: New Stable Isotope Data. Curr. Anthropol. 44, 288–295.
423 Milner, N., Craig, O.E., 2009. Mysteries of the middens: change and continuity across the
424 Mesolithic Neolithic Transition, in: Land and People: Papers in Memory of John G.
425 Evans. Oxbow Books Oxford, pp. 169–180.
426 Pestle, W.J., Crowley, B.E., Weirauch, M.T., 2014. Quantifying inter-laboratory variability in
427 stable isotope analysis of ancient skeletal remains. PLoS One 9, e102844.
428 Pinhasi, R., Fernandes, D., Sirak, K., Novak, M., Connell, S., Alpaslan-Roodenberg, S.,
429 Gerritsen, F., Moiseyev, V., Gromov, A., Raczky, P., Anders, A., Pietrusewsky, M.,

430 Rollefson, G., Jovanovic, M., Trinhhoang, H., Bar-Oz, G., Oxenham, M., Matsumura,
431 H., Hofreiter, M., 2015. Optimal Ancient DNA Yields from the Inner Ear Part of the
432 Human Petrous Bone. PLoS One 10, e0129102.
433 Rasteiro, R., Chikhi, L., 2013. Female and male perspectives on the neolithic transition in
434 Europe: clues from ancient and modern genetic data. PLoS One 8, e60944.
435 Reimer, P.J., Bard, E., Bayliss, A., Beck, W.J., Blackwell, P.G., Bronk Ramsey, C., Buck,
436 C.E., Cheng, H., Edwards, L.R., Friedrich, M., Haflidason, H., Heaton, T.J., Hoffmann,
437 D.L., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Staff, R.A., Turney,
438 C.S.M., van der Plicht, J., 2013. IntCal13 and Marine13 Radiocarbon Age Calibration
439 Curves. Radiocarbon 55, 1869–1887.
440 Richards, M., 2003. The Neolithic transition in Europe: archaeological models and genetic
441 evidence. Documenta Praehistorica 30, 159–167.
442 Richards, M.P., Hedges, R.E.M., 1999. Stable Isotope Evidence for Similarities in the Types
443 of Marine Foods Used by Late Mesolithic Humans at Sites Along the Atlantic Coast of
444 Europe. J. Archaeol. Sci. 26, 717–722.
445 Richards, M.P., Mellars, P.A., 1998. Stable isotopes and the seasonality of the Oronsay
446 middens. Antiquity 72, 178–184.
447 Richards, M.P., Schulting, R.J., Hedges, R.E.M., 2003. Archaeology: sharp shift in diet at
448 onset of Neolithic. Nature 425, 366.
449 Richards, M.P., Sheridan, J.A., 2000. New AMS dates on human bone from Mesolithic
450 Oronsay. Antiquity 74, 313–315.
451 Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., Reich, D., 2015. Partial uracil–DNA–
452 glycosylase treatment for screening of ancient DNA. Philos. Trans. R. Soc. Lond. B Biol.
453 Sci. 370, 20130624.
454 Rowley-Conwy, P., 2011. Westward Ho! The Spread of Agriculturalism from Central Europe
455 to the Atlantic. Curr. Anthropol. 52, S431–S451.
456 Rowley-Conwy, P., 2004. How the West Was Lost: A Reconsideration of Agricultural
457 Origins in Britain, Ireland, and Southern Scandinavia. Curr. Anthropol. 45, S83–S113.
458 Russell, N., Cook, G.T., Ascough, P.L., Scott, M. 2015. A period of calm in Scottish seas: A
459 comprehensive study of ΔR values for the northern British Isles coast and the consequent
460 implications for archaeology and oceanography. Quat. Geochronol. 30, 34–41.
461 Schiffer, M.B., 1986. Radiocarbon dating and the “old wood” problem: The case of the
462 Hohokam chronology. J. Archaeol. Sci. 13, 13–30.
463 Schulting, R.J., Richards, M.P., 2002. The wet, the wild and the domesticated: the
464 Mesolithic–Neolithic transition on the west coast of Scotland. European Journal of
465 Archaeology 5, 147–189.
466 Sealy, J., Johnson, M., Richards, M., Nehlich, O., 2014. Comparison of two methods of
467 extracting bone collagen for stable carbon and nitrogen isotope analysis: comparing
468 whole bone demineralization with gelatinization and ultrafiltration. J. Archaeol. Sci. 47,
469 64–69.
470 Shennan, S., Edinborough, K., 2007. Prehistoric population history: from the Late Glacial to
471 the Late Neolithic in Central and Northern Europe. J. Archaeol. Sci. 34, 1339–1345.
472 Sheridan, A., 2010. The Neolithization of Britain and Ireland: The “Big Picture,” in:
473 Finlayson, B., Warren, G. (Eds.), Landscapes in Transition. Oxbow, Oxford, pp. 89–105.
474 Strohalm, M., Kavan, D., Novák, P., Volný, M., Havlícek, V., 2010. mMass 3: a cross-
475 platform software environment for precise analysis of mass spectrometric data. Anal.
476 Chem. 82, 4648–4651.
477 Switsur, V.R., Mellars, P.A., 1987. Radiocarbon dating of the shell midden sites. Excavations
478 on Oronsay 139–149.

479 Tresset, A., Vigne, J.-D., 2011. Last hunter-gatherers and first farmers of Europe. C. R. Biol.
480 334, 182–189.
481 van Doorn, N.L., Hollund, H., Collins, M.J., 2011. A novel and non-destructive approach for
482 ZooMS analysis: ammonium bicarbonate buffer extraction. Archaeol. Anthropol. Sci. 3,
483 281–289.
484 van Klinken, G.J., 1999. Bone Collagen Quality Indicators for Palaeodietary and
485 Radiocarbon Measurements. J. Archaeol. Sci. 26, 687–695.
486 Welker, F., Soressi, M., Rendu, W., Hublin, J.-J., Collins, M., 2015. Using ZooMS to identify
487 fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les
488 Cottés, France. J. Archaeol. Sci. 54, 279–286.
489 Wicks, K., Pirie, A., Mithen, S.J., 2014. Settlement patterns in the late Mesolithic of western
490 Scotland: the implications of Bayesian analysis of radiocarbon dates and inter-site
491 technological comparisons. J. Archaeol. Sci. 41, 406–422.

Supporting Information

SI Materials and Methods

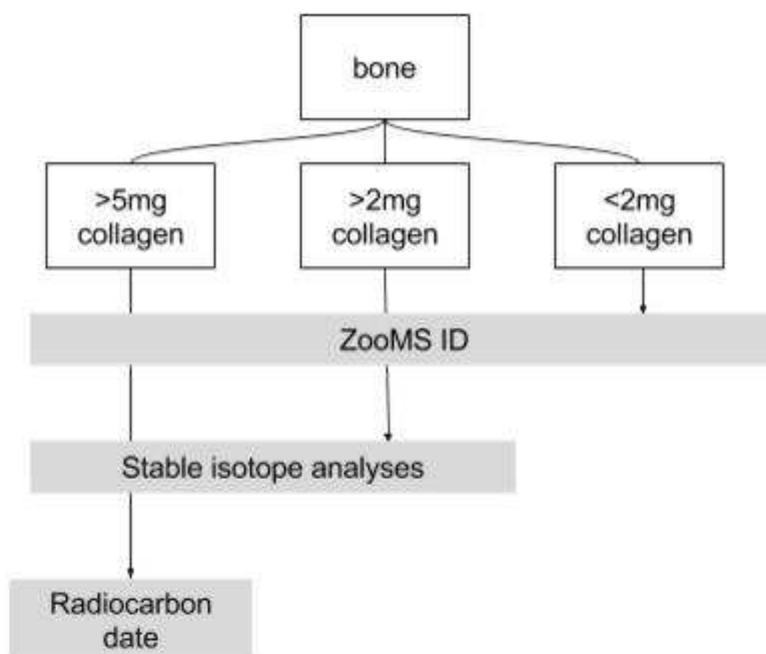


Figure S1: Schematic of analyses

ZooMS Analysis

The ZooMS methodology utilised involved a standard collagen extraction from c.500mg of bone (see below), followed by ZooMS analysis (as in Welker et al., 2015) being undertaken on the 'empty' tubes used for the lyophilisation of collagen – thereby utilising the macroscopically invisible amounts of collagen left adhering to the tube. The benefit of this novel ZooMS methodology lies in the fact that it can determine species identification from samples without the utilisation of collagen reserved for isotopic analyses. In effect, taxonomic information is being obtained from 'empty' tubes previously used within the collagen extraction process of these samples.

Briefly, lyophilised collagen samples (see below) were removed from falcon tubes and transferred into eppendorfs. 75µl 50mM AmBic (ammonium bicarbonate buffer, pH8.0) was added to each 'empty' tube used during ultrafiltration, vortexed and then centrifuged. 1µl trypsin (Promega) was then added to each sample, and digested for 16h at 37°C. Following this, samples were centrifuged at 13k RPM for 1 min and then 1µl 5% TFA was added to stop enzymatic digestion. Peptides were then extracted using C_{18} ZipTips (Agilent), which were eluted using 50µl 50% ACN in 0.5% TFA.

MALDI-TOF-MS analysis, using 1µl eluted peptides and 1µl α-cyano-4-hydroxycinnamic acid matrix solution (Buckley et al., 2009; Welker et al., 2015) spotted onto a ground steel

plate, was undertaken in triplicate for each sample on a Bruker Ultraflex III MALDI-TOF/TOF at the University of York. Spectral analysis was performed using the open-source cross-platform software mMass (Strohalm et al., 2010). Replicates were averaged for each sample and manually analysed for peptide markers following the protocol detailed in (Welker et al., 2015).

All samples bar one provided sufficient taxonomic information using this modified ZooMS protocol to allow for species identification (Table S1). Two samples (17050 and GEN1) however required the initial ZooMS identification obtained from the empty tubes to be clarified via secondary ZooMS analysis using the standard protocol(Welker et al., 2015) on 0.5mg of extracted collagen.

Isotopic Analysis

Isotopic analyses of $\delta^{13}C$ and $\delta^{15}N$ were undertaken following a modified Longin collagen extraction protocol using ultrafiltration on c.500mg of bone (Brown et al., 1988; Colonese et al., 2015; Richards and Hedges, 1999). Briefly, samples were initially cleaned manually using a scalpel, and then were demineralised in 0.6M aq. HCl solution at 4°C, and the resulting insoluble fraction gelatinised in pH3 HCl for 48h at 80°C. The supernatant solution was then ultrafiltered (30kDa MWCO, Amicon) to isolate the high molecular weight fraction, which was then lyophilised. Purified collagen samples (1mg) were analysed in triplicate by EA-IRMS on a Sercon GSL analyser coupled to a Sercon 20-22 Mass Spectrometer at the University of York. The analytical error, calculated from repeated measurements of each sample, a bovine control, and international standards, was <0.2‰ (1σ) for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values are presented here relative to the internationally defined standards of VPDB for $\delta^{13}C$ and AIR for $\delta^{15}N$.

Collagen quality fell within prescribed quality ranges (DeNiro, 1985; van Klinken, 1999). However, some variability was seen in the yields obtained from the samples, generally ranging from over 1% to over 3.5%. Only two samples fell below the 1% collagen yield from the retentate sample alone (samples 8260 and 8266), but both samples showed acceptable C:N ratios and so were still included within this study (Table S1). Furthermore, it has previously been noted that collagen yields calculated from retentate samples following ultrafiltration, as was undertaken here, contain only high molecular weight fractions and therefore quality criteria are actually more important than yields(Sealy et al., 2014). All samples with reported $\delta^{13}C$ and $\delta^{15}N$ values in this work have atomic C:N ratios of between 3.3-3.6 (Table S1).

Sample Number	ZooMS ID	Possible Element	δ13C (‰)	δ15N (‰)	Atomic C:N Ratio	% Collagen Yield	Estimated % contribution of marine C to collagen
8254	Human	Cranial frag?	-13.8	15.1	3.4	2.4	79%
8255	Human	Long bone?	-13.4	15.1	3.4	3.2	84%
8256	Human	Radius?	-13.3	15.0	3.3	2.1	85%
8257	Human	Cranial frag?	-14.1	15.4	3.3	3.6	77%
8258	Human	Cranial frag	-13.9	15.3	3.3	1.2	79%
8260	Human	Vertebrae	-14.6	15.5	3.6	0.9	71%
8266	Human	Vertebrae	-13.9	15.7	3.6	0.6	70%
8267	Human	Unknown	-12.9	15.6	3.3	1.4	90%
General Find 1 (GEN1)	Human	Metacarpal?	-13.2	15.3	3.2	3.7	86%
10420	Seal	Unknown	-11.8	19.5	3.5	1.2	-
10494	Pig	Long bone?	-21.2	4.3	3.4	2.9	-
10502	Seal	Long bone?	-11.6	18.8	3.3	2.9	-
17050	Pig	Long bone?	-21.0	4.6	3.3	2.3	-
'Unknown' (General find)	Pig	Unknown	-18.8	10.2	3.4	2.7	-
8259	Human	Rib	-	-	-	0.5	-
8261	Human	Vertebrae	-	-	-	0.8	-
8262	Unidentifiable	Unknown	-	-	-	0.5	-
8263	Human	Vertebrae	-	-	-	0.1	-
8265	Human	Rib	-	-	-	0.2	-
8268	Human	Vertebrae	-	-	-	0.8	-

Table S1: ZooMS species ID and collagen stable isotope values obtained. Estimated % marine carbon contribution to collagen for humans calculated from isotopic data after

Schulting and Richards (2002) using marine and terrestrial carbon end-points of -12‰ and -21‰ respectively, with an error ±10%

AMS Dating

AMS dating of four bone fragments identified using ZooMS and with associated isotopic information was also undertaken in an attempt to elucidate information about the chronology of the skeletal remains at Cnoc Coig. Dating of terrestrial faunal samples was undertaken to provide valuable reference points to evaluate the overall date of the site, which is currently based on marine and charcoal samples (Table S2), and to identify if the fauna studied were contemporaneous to the human remains. There have previously been no dates (or isotopic values) for terrestrial fauna from Cnoc Coig.

All AMS data were generated by the NERC radiocarbon facility based in the Oxford Radiocarbon Acceleration Unit. Calibration of dates (both those undertaken in this study, and dates previously obtained) was undertaken using OxCal v.4.2. As many of the bone fragments utilised in this study showed high marine isotopic values however (Table S1), this suggested the need for calibration of radiocarbon dates adjusted for a marine reservoir correction, with the appropriate ΔR offset. To do this, mixed marine/atmospheric calibration curves (Marine13/IntCal13, Reimer et al., 2103) were used in a proportion determined by marine/terrestrial carbon contribution to collagen (as in Barrett and Richards, 2004); following best practice as outlined in Cook et al. (2015). The latter was estimated for each individual from their δ^{13}C values following linear interpolation from the observed marine and terrestrial endpoints (Table S1), with an error ±10%. A ΔR value of 47 ±52 was also applied to human samples with marine isotopic signatures (Tables S1 and S2). This is a mean ΔR value calculated for the entirety of Scotland (Russell et al., 2105; Ascough, pers. comm.).

Material Dated	Lab Ref. No.	^{14}C Date BP	Original Published Date cal. BC	New Date cal. BC (with MRO) (95.4%)	Reference
Arctica islandica shell	Birm-326Z	7240 ±200	6400-5100	6144-5354	(Jardine, 1978; Mellars, 1987)
Arctica islandica shell	Birm-326Y	7290 ±120	6200-5450	6011-5527	(Jardine, 1978; Mellars, 1987)
Arctica islandica shell	Birm-326X	7610 ±150	6500-5650	6399-5760	(Jardine, 1978; Mellars, 1987)
Bulk charcoal	Q-1352	5430 ±130	4520-3970	-	(Switsur and Mellars, 1987)
Bulk charcoal	Q-1351	5495 ±75	4510-4070	-	(Switsur and Mellars, 1987)

Bulk charcoal	Q-1354	5535 ±140	4690-4040	-	(Switsur and Mellars, 1987)
Bulk charcoal	Q-1353	5645 ±80	4690-4340	-	(Switsur and Mellars, 1987)
Bulk charcoal	Q-3006	5675 ±60	4690-4360	-	(Switsur and Mellars, 1987)
Bulk charcoal	Q-3005	5650 ±60	4660-4350	-	(Switsur and Mellars, 1987)
Human bone (sample no. 17203)	OxA-8014	5495 ±55	4000-3800	4036-3686	(Richards and Sheridan, 2000)
Human bone (sample no. 17157)	OxA-8019	5615 ±45	4200-4000	4232-3830	(Richards and Sheridan, 2000)
Human bone (sample no. 18284)	OxA-8004	5740 ±65	4300-4000	4320-3966	(Richards and Sheridan, 2000)

Table S2: Radiocarbon dates previously obtained for Cnoc Coig. It is important to note that the previous dates on human bone were undertaken on collagen which had not been ultrafiltered. Also note the large standard deviations on both previous shell and charcoal BP dates. New calibration of dates was undertaken using mixed marine/atmospheric calibration curves (Marine13/IntCal13, Reimer et al., 2103), in a proportion determined by marine/terrestrial carbon contribution to collagen following linear interpolation from the observed marine and terrestrial endpoints, with an error ±10%. A ΔR value of 47 ±52 was also applied

DNA analysis

Sample 8256:

DNA extraction and library preparation were carried out in a dedicated ancient DNA laboratory at The Natural History Museum, London. 50mg of finely drilled bone powder was utilised, and DNA extracted following Dabney et al. (2013), but with the the Zymo-Spin V column binding apparatus replaced with a high pure extender assembly from the High Pure Viral Nucleic Acid Large Volume Kit (Roche). Library preparations followed a modified version of the Meyer and Kircher (2010) protocol: the initial DNA fragmentation step was not required; all clean-up steps used MinElute PCR purification kits (Qiagen). The index PCR step used AmpliTaq Gold DNA polymerase and the addition of 0.4mg/mL BSA. The index PCR was set for 20 cycles with three PCR reactions conducted per library. The library was sequenced on an Illumina NextSeq platform (The Natural History Museum, London) using a NextSeq 500/550 Mid-Output v2 Kit (150 cycles).

Bioinformatics

AdapterRemoval (Lindgreen, 2012) was used to trim residual Illumina adapter sequences and low quality bases, with paired end reads longer than 25 bases merged with a minimum overlap of 11 bases. Quality trimmed, merged only reads were then aligned to a human reference genome (hg19) using BWA (Li and Durbin, 2009), with minimum base quality set to phred scale 15. SAMtools (Li et al., 2009) was used to further filter the mapped reads by map quality value 30 and remove all duplicates. Endogenous DNA content was determined through the number of filtered, quality (30), non-duplicate reads aligning to the human genome divided by the total number of reads. The endogenous DNA content of sample 8256 was found to be 0.02% (Barnes and Brace, pers. comm.).

Samples 8257 and 8267:

DNA extraction and library preparation were carried out in dedicated ancient DNA facilities at Harvard Medical School, Boston, following a standard screening process previously reported (Haak et al., 2015). Samples were cleaned by removing the outer layer of bone using a Dremel sanding disk, followed by overnight exposure to UV light. Powder was produced by crushing the cleaned bone using a mortar and pestle. 75mg of powder was utilised, and DNA extracted following Dabney et al. (2013) but with the the Zymo-Spin V column binding apparatus replaced with a high pure extender assembly from the High Pure Viral Nucleic Acid Large Volume Kit (Roche). Library preparations were performed following Rohland et al. (2015), using a partial UDG treatment. This treatment repairs C->U damage in the interior of the DNA molecule, while leaving a fraction of damage intact at the terminal nucleotides of the molecule in order to enable tests of ancient DNA authenticity.

Following amplification, libraries were evaluated on a BioAnalyzer to assess library preparation success. Both samples exhibited signs of inhibition during library preparation, demonstrated by flat BioAnalyzer traces. The libraries were therefore not brought forward in the ancient DNA screening process, as inhibition of the library prevents the acquisition of useful DNA sequences from samples. Thus, no informative genetic data could be obtained from either sample (Reich and Harney, pers. comm.).