Lifeways at the Mesolithic-Neolithic Transition: Integrating New Biomolecular Approaches to Skeletal Material in Britain

Sophy Jessica Laura Charlton

PhD
University of York
Archaeology
February 2016
Abstract

The Mesolithic–Neolithic transition is a period which has long held fascination for archaeologists, and yet the lifeways of individuals at this time are still not fully understood – in part due to the lack of human remains in Britain from the period. This thesis therefore aimed to adopt a combined biomolecular approach to determine more information about the lifeways of both the Mesolithic and Neolithic of Britain, and of the transition between them, but utilising archaeological material not traditionally included within these debates – notably unidentifiable bone fragments, disarticulated skeletal remains, and dental calculus. Through analysis of these materials, the thesis focuses on five main areas of interest: identification, diet, mobility, chronology, and health/disease; utilising six different techniques: ZooMS, δ¹³C and δ¹⁵N stable isotope analysis, ⁸⁶Sr/⁸⁷Sr isotopic analysis, AMS dating, and metagenomic and metaproteomic analysis of dental calculus. As such, this marks the largest combined application of biomolecular techniques to British Mesolithic and Neolithic material to date. The results of this study highlight the value which may be held within previously overlooked early prehistoric archaeological materials, and the information which they may be able to contribute to existing discussions of Mesolithic and Neolithic lifeways. Overall, it can be seen that the main outcomes of this study are (i) that additional human remains may be present within early prehistoric ‘unidentifiable’ fragmented bone assemblages; (ii) dietary complexity in both the Mesolithic and Neolithic of Britain may be greater than previously thought; (iii) enhanced understanding of Neolithic mobility; (iv) a reconsideration of the approach to chronology at the Mesolithic–Neolithic transition; and (v) that dental calculus may provide a suitable and useful new medium via which to study prehistoric health and disease in future studies.
Table of Contents

Abstract................................................................................................................................. iii
Table of Contents.................................................................................................................. v
List of Figures......................................................................................................................... xi
List of Tables......................................................................................................................... xiii
Acknowledgements............................................................................................................... xxvii
Author’s Declaration............................................................................................................... xxxi

Chapter 1 – Lifeways at the Mesolithic-Neolithic Transition.............................................. 1
  1.1. Studying Prehistory in Britain ....................................................................................... 1
  1.2. Aims and Objectives .................................................................................................... 3
  1.3. Outline of Paper .......................................................................................................... 5

Chapter 2 – The Mesolithic and The Neolithic Periods in Britain ...................................... 9
  2.1. Categorisation of the Prehistoric Past ......................................................................... 9
  2.2. Defining the Mesolithic .............................................................................................. 10
  2.3. Defining the Neolithic ................................................................................................ 21
  2.4. Defining the Mesolithic-Neolithic Transition ............................................................ 28
    2.4.1. Dietary Change ..................................................................................................... 31
    2.4.2. Demographic and Population Change ................................................................. 36
    2.4.3. Health Change ..................................................................................................... 42
    2.4.4. Social Change ...................................................................................................... 45
    2.4.5. Change or Continuity? ........................................................................................ 49

Chapter 3 – Human Remains in the British Mesolithic and Neolithic ............................. 53
  3.1. Human Remains From the British Mesolithic ............................................................ 53
    3.1.1. Mortuary Practices Adopted in Mesolithic Britain ................................................ 54
  3.2. Why do we not have more human remains from the Mesolithic in Britain? .......... 69
  3.3. Neolithic Human Remains ........................................................................................ 70
    3.3.1. Mortuary Practices Adopted in Neolithic Britain .................................................. 71
  3.4. Mesolithic and Neolithic Mortuary Practices – Similarities and Distinctions ....... 85
3.5. Rest in Peace? The Ethics of Dealing With Prehistoric Human Remains .......... 87

Chapter 4 – An Integrated Scientific Approach ................................................................. 91
4.1. New Approaches to Studying the Mesolithic and Neolithic of Britain ............... 91
4.2. Identification .................................................................................................................. 92
4.2.1. Why identification is important ............................................................................... 92
4.2.2. Previous methods of species ID .............................................................................. 93
4.2.3. Proteomic Approaches to Species Identification ..................................................... 95
4.2.3.1. Collagen and bone structure ............................................................................. 96
4.2.3.2. Protein Mass Spectrometry and Peptide Mass Fingerprinting ......................... 99
4.2.3.3. ZooMS – Zooarchaeology by Mass Spectrometry ............................................ 101
4.3. Diet ............................................................................................................................... 105
4.3.1. The Longstanding Interest in Mesolithic and Neolithic Diets ......................... 105
4.3.2. Carbon and Nitrogen Isotopes ............................................................................. 106
4.3.3. Dietary Routing and Fractionation ....................................................................... 110
4.3.3.1. Carbon routing models and fractionation ......................................................... 110
4.3.3.2. Nitrogen models and fractionation ................................................................... 113
4.3.4. Isotope Ratio Mass Spectrometry ........................................................................ 114
4.3.5. Isotopes as a Palaeodietary Tool in Archaeology ................................................ 115
4.3.5.1. Previous Applications to Mesolithic and Neolithic Material ............................ 118
4.3.5. Mixing Models ........................................................................................................ 118
4.4. Mobility ........................................................................................................................ 121
4.4.1. Understanding Prehistoric Mobility and Demography ....................................... 121
4.4.2. Studying prehistoric mobility ............................................................................... 122
4.4.3. Strontium Isotopes ................................................................................................ 123
4.4.4. Using Strontium to Determine Mobility – Methods and Approaches .............. 125
4.4.5. Previous Applications of Strontium Isotopes to Mesolithic and Neolithic Material .................................................................................................................. 128
4.5. Chronology ................................................................................................................... 130
4.5.1. Understanding prehistoric chronologies ............................................................... 130
4.5.2. AMS dating and the radiocarbon revolution ........................................... 131
4.6. Health and Disease ..................................................................................... 133
4.6.1. Understanding prehistoric health and disease ........................................ 133
4.6.2. How dental calculus may help .................................................................. 135
4.6.2.1. Human dental calculus ........................................................................ 136
4.6.2.2. Previous studies of dental calculus ...................................................... 138
4.6.2.3. Metaproteomic and metagenomic study of dental calculus – and moving towards an understanding of the oral microbiome ........................................... 140
4.6.2.3.1. Metagenomic approaches ................................................................. 141
4.6.2.3.2. Metaproteomic approaches ............................................................... 143
4.6.2.3.2. The Oral Microbiome, Health, and Disease ...................................... 144
4.7. Standard Operating Protocols (SOPs) .......................................................... 149
4.8. The Advantages of a Multi-Methodological Approach – A New Means of Studying Prehistoric Archaeology? ................................................................. 150

Chapter 5 – Rediscovering Oronsay: Biomolecular Approaches to Skeletal Material from Cnoc Coig ............................................................................................................ 153

5.1. Introduction ..................................................................................................... 153
5.2. Cnoc Coig ........................................................................................................ 154
5.2. Materials and Methods .................................................................................. 155
5.2.1. Samples .................................................................................................... 155
5.2.2. A Combined Biomolecular Approach ...................................................... 158
5.3. Results and Discussion .................................................................................. 158
5.3.1. Identification ............................................................................................. 158
5.3.2. Isotopic Data and Dietary Inferences ....................................................... 161
5.3.2.1. Mesolithic Pigs on Oronsay ................................................................. 167
5.3.3. Re-dating human remains at Cnoc Coig .................................................. 170
5.4. Conclusions ................................................................................................... 173

Chapter 6 – Finding the Mesolithic: Using ZooMS to Identify Bone Fragments ...... 177
6.1. Finding the British Mesolithic .......................................................................... 177
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2. Using ZooMS to Identify Prehistoric Skeletal Remains</td>
<td>178</td>
</tr>
<tr>
<td>6.3. Blick Mead, Wiltshire</td>
<td>179</td>
</tr>
<tr>
<td>6.4. Cnoc Coig, Oronsay</td>
<td>180</td>
</tr>
<tr>
<td>6.5. Western Isles, Scotland</td>
<td>181</td>
</tr>
<tr>
<td>6.6. Material Sampled and Methodology</td>
<td>184</td>
</tr>
<tr>
<td>6.6.1. Blick Mead, Vespasian’s Camp</td>
<td>184</td>
</tr>
<tr>
<td>6.6.2. Cnoc Coig, Oronsay</td>
<td>185</td>
</tr>
<tr>
<td>6.6.3. Western Isles</td>
<td>185</td>
</tr>
<tr>
<td>6.7. Species Identified</td>
<td>185</td>
</tr>
<tr>
<td>6.7.1. Blick Mead</td>
<td>185</td>
</tr>
<tr>
<td>6.7.2. Cnoc Coig, Oronsay</td>
<td>188</td>
</tr>
<tr>
<td>6.7.3. Western Isles</td>
<td>191</td>
</tr>
<tr>
<td>6.8. Collagen preservation</td>
<td>193</td>
</tr>
<tr>
<td>6.9. Conclusions</td>
<td>195</td>
</tr>
<tr>
<td><strong>Chapter 7 – Banbury Lane, Northampton: A Large Scale Multi-Isotopic Study on an Unusual Neolithic Assemblage</strong></td>
<td>199</td>
</tr>
<tr>
<td>7.1. Introduction</td>
<td>199</td>
</tr>
<tr>
<td>7.2. The Banbury Lane Site</td>
<td>200</td>
</tr>
<tr>
<td>7.4. Banbury Lane – A Unique Human Bone Assemblage</td>
<td>205</td>
</tr>
<tr>
<td>7.5. Materials and Methods</td>
<td>207</td>
</tr>
<tr>
<td>7.6. Results and Discussion</td>
<td>208</td>
</tr>
<tr>
<td>7.6.1. Dietary Inferences</td>
<td>208</td>
</tr>
<tr>
<td>7.6.2. Trophic Levels and Mixing Models</td>
<td>215</td>
</tr>
<tr>
<td>7.6.3. Origins and Movement: $^{87}$Sr/$^{86}$Sr ratios</td>
<td>220</td>
</tr>
<tr>
<td>7.6.3.1. Characterising Biosphere $^{87}$Sr/$^{86}$Sr</td>
<td>221</td>
</tr>
<tr>
<td>7.6.3.2. $^{87}$Sr/$^{86}$Sr values from Banbury Lane</td>
<td>225</td>
</tr>
<tr>
<td>7.7. Conclusions</td>
<td>235</td>
</tr>
</tbody>
</table>
Chapter 8 – Biomolecular Analysis of Dental Calculus: A New Way to Discover Neolithic Diet and Disease ................................................................. 237
8.1. Determining New Sources of Prehistoric Disease Information: Metaproteomic and Metagenomic Study of Dental Calculus ........................................ 237
8.2. Sites ........................................................................................................ 238
8.2.1. Hambledon Hill .................................................................................. 238
8.2.2. Hazleton North ..................................................................................... 242
8.2.3. Coldrum ............................................................................................... 247
8.2.4. Banbury Lane ....................................................................................... 250
8.3. Materials and Methods .......................................................................... 251
8.5. Results and Discussion .......................................................................... 253
8.5.1. Calculus as a reservoir for early prehistoric aDNA ..................................... 253
8.5.2. Bacterial Composition and Pathogens ..................................................... 256
8.5.3. Sex Identification .................................................................................. 263
8.5.4. Genetic Population Affinities ................................................................. 268
8.5.5. Protein Concentration and Function ...................................................... 270
8.5.6. Neolithic Milk Drinking & The Archaeological Evidence ......................... 286
8.5.6.1. β-lactoglobulin (BLG) and Dental Calculus ........................................ 287
8.5.6.2. Neolithic Milk Drinking and Dental Calculus ...................................... 287
8.6. Conclusions ........................................................................................... 295

Chapter 9 – Discussion: Discovering Mesolithic and Neolithic Lifeways .............. 299
9.1. Identification: Revealing the Mesolithic ...................................................... 300
9.1.1. Detecting Faunal Species ...................................................................... 300
9.1.2. Detecting Human Remains ................................................................... 304
9.1.3. Utilising ZooMS as a Mesolithic Identification Tool ................................ 305
9.2. Chronology: The Mesolithic, the Neolithic, and the Transition ..................... 306
9.3. Mesolithic and Neolithic Diets and Dietary Change .................................... 308
9.3.1. The Isotopic Evidence .......................................................................... 308
9.3.2. The Proteomic Evidence ..................................................................... 312
9.3.3. How far do we understand Mesolithic and Neolithic diets in Britain? ......... 316
A.4.4.1.2. DNA Authentication ........................................................................382
A.4.4.2. Proteomic Data Analysis ..................................................................394
A.4.4.3. BLG Identification and Alignment .....................................................395

Appendix B – Isotopic Database .....................................................................397

Bibliography .......................................................................................................399
List of Figures

Figure 1: Schematic of methodologies and samples utilised within this research..........5
Figure 2: Past definitions of the Mesolithic period (Zvelebil 1986(a), 7) .....................12
Figure 3: Table depicting the periods and subdivision of the late glacial and post-glacial periods in Britain (Darvill 2010, xix) ..........................................................................................13
Figure 4: Stages of flooding of land-bridge between Britain and the mainland European continent, and approximate timings (Bradley 2007, 11) .................................................................14
Figure 5: Graph depicting a shift in δ^{13}C values at the onset of the Neolithic period in Britain. Squares represent individuals less than 10km away from the coast, and crosses are individuals from greater than 10km away from the coast (Richards et al. 2003) ...............33
Figure 6: Proposed routes of Neolithic colonisation of Britain, indicating multiple strands of colonisation (Sheridan 2010, 93) ........................................................................................................38
Figure 7: Economic and material culture changes between the Mesolithic and Neolithic periods (Rowley-Conwy 2004(a), S84) ........................................................................................................50
Figure 8: Mesolithic cemetery sites known across Europe (Schulting 1998, 207) ..........66
Figure 9: Demographic distribution of inhumations at West Kennet long barrow (Bayliss et al. 2007, 87) .................................................................................................................................76
Figure 10: Image highlighting the ZooMS methodology and the principle of peptide mass fingerprints. Species identification is determined through amino acid differences in collagen sequences (reproduced with kind permission of Matthew Collins) ..................101
Figure 11: Principle component analysis of bone collagen peptide masses of species (Collins et al. 2010, 10) .............................................................................................................................102
Figure 12: Variation in the δ^{13}C values of C_3 and C_4 plants (adapted from Taiz et al. 2015) ......................................................................................................................................................108
Figure 13: Schematic showing distribution of stable isotope ratios in biosphere (Schoeninger and Moore 1992, 257) .........................................................................................................................110
Figure 14: Predictions of collagen δ^{13}C with increasing contributions of marine protein in the diet. (a) 100% routing of protein to collagen; (b) 100% scrambling, carbon derived equally from all dietary macronutrients; (c) combined model of mixed routing and scrambling (50:50) (Craig et al. 2013, 349) .............................................................................................112
Figure 15: Variation of $\delta^{15}N$ with dietary animal protein fraction. (1) the ‘standard model’ (4‰); (2) cereal $\delta^{15}N$ is enriched to forage by 1‰; (3) in addition to (2), humans are enriched 1‰ to herbivores; (4) in addition to (3), humans on an all plant diet are enriched 1‰ than humans on an all meat diet (Hedges and Reynard 2007) .................................. 114

Figure 16: Graph depicting the isotopic variation of different foodstuffs within the diet (reproduced with permission of Oliver Craig)................................................................. 116

Figure 17: Schematic of the mixing concept utilised within the FRUITS model (taken from FRUITS manual, available via http://sourceforge.net/projects/fruits/) .................................. 120

Figure 18: Biosphere $^{87}$Sr/$^{86}$Sr isotope map of Britain (Evans et al. 2010)............... 125

Figure 19: Levels of calculus severity (Brothwell 1972, 150) .......................................... 139

Figure 20: Schematic depicting how gut bacteria may affect the brain and body (Gregoire 2015)................................................................................................................................. 147

Figure 21: Oral microbial diversity (Zijinge et al. 2010, 2) ........................................... 148

Figure 23: A selection of the twenty bone fragments obtained from the Cnoc Coig assemblage used within this research. The images highlight the range of sizes, element types, shapes and degrees of preservation condition seen within the bone fragment assemblage. From top, working left to right, these bone fragments were identified later using ZooMS as seal, pig, remainder human........................................................................ 156

Figure 24: Plan of excavated areas of the midden 1973-1979 (adapted from Mellars 1987, 215), indicating the extent of the midden as defined by Mellars. The trench highlighted in red denotes Trench U, from which the human remains identified come from – this can clearly be seen to fall outside of the defined limits of the midden structure ................. 157

Figure 25: MALDI-TOF spectra of (a) known human bone and (b) sample identified as human from Cnoc Coig fragments ................................................................. 159

Figure 26: Plot of stable isotope values from Cnoc Coig and Caisteal nan Gillean human and fauna, against previously obtained human isotopic data (from Richards and Mellars 1998)........................................................................................................ 161

Figure 27: Comparison of human isotopic data from Cnoc Coig plotted against data from other sites on the West coast of Scotland of a comparable date. Data obtained by the author (noted ‘SC’ in legend), and from Richards and Mellars 1998; Meiklejohn et al. 2005; 2011 ......................................................................................................................... 163

Figure 28: FRUITS model 1 for Cnoc Coig data, considering all terrestrial animals (herbivores and omnivores) as one food group ................................................................. 165
Figure 29: FRUITS model 2 for Cnoc Coig data, considering only terrestrial herbivores (no
omnivores) as a food group.................................................................166

Figure 30: *Sus scrofa* data from Mesolithic and early-mid Neolithic European sites (data
compiled from Schulting and Richards 2002(a); 2009; Borić et al. 2004; Eriksson 2004;
Craig et al. 2006; Bocherens et al. 2007; Fornader et al. 2008; Hedges et al. 2008;
Lightfoot et al 2011; Oelze et al. 2011; Fontanals-Coll et al. 2014; the author) ..............169

Figure 31: Plot of δ^{13}C values against recalibrated radiocarbon dates for humans from
Scottish West Coast sites, c.4500-3500 cal. BC (data from this study; Richards and
Sheridan 2000; Schulting and Richards 2002(a)). New human data obtained for Cnoc Coig
is highlighted in red..............................................................................173

Figure 33: Site location of Bágh an Teampaill (Temple Bay) and Northton, on the Isle of
Harris (Blake et al. 2012(b)) .......................................................................182

Figure 34: Site location of Tràigh na Beirigh (TNB) on the Isle of Lewis (Blake et al.
2012(a)) ..................................................................................................183

Figure 35: Examples of bone fragments used in this study. (Left = sample 1004; right =
sample 1008) .........................................................................................184

Figure 36: Site location map, indicating Northamptonshire within its East Midlands setting
(Holmes 2012) .........................................................................................200

Figure 37: Location of Banbury Lane site (indicated in red) within Northampton (Holmes
2012) .........................................................................................................201

Figure 39: Selection of the Banbury Lane teeth once sectioned, mounted in epoxy resin,
and polished, ready for LA-ICP-MC-MS analysis .......................................208

Figure 40: Plot of isotope values obtained from Banbury Lane humans and fauna ........209

Figure 41: Distribution plot of δ^{13}C values within the Banbury Lane human isotopic data
.................................................................................................................210

Figure 42: Distribution plot of δ^{15}N values within the Banbury Lane human isotopic data
.................................................................................................................211

Figure 43: Comparison of Banbury Lane isotope data with δ^{13}C and δ^{15}N values from
British Neolithic sites of a similar date (data from Schulting and Richards 2002; Hedges et
al. 2008; Schulting 2013; Montgomery et al. 2013; the author) .......................212
Figure 44: Cattle (blue) and pig (orange) values from British Neolithic sites (data from Richards 2000; Schulting and Richards 2002; Hedges et al. 2008; Milner and Craig 2009; Stevens et al. 2012; Montgomery et al. 2013; the author) ........................................213

Figure 45: Comparison of $\delta^{13}$C and $\delta^{15}$N stable isotope values from Banbury Lane with two early Bronze Age sites from the region .................................................................215

Figure 46: FRUITS model 1 for Banbury Lane data (considering both cattle and pigs as a combined food group) ..................................................................................................................219

Figure 47: FRUITS model 2 generated for Banbury Lane data (considering cattle and pigs as two separate food groups) ..................................................................................................................220

Figure 48: Strontium transfer between ecosystem components (Szostek et al. 2015, 139) .............................................................................................................................................222

Figure 49: Location of soil samples (indicated by blue pins) utilised within this research (Google Maps 2016) .............................................................................................................................................223

Figure 50: Map of bedrock geology of soil sample area (BGS 2015) .........................................................................................................................................................................................224

Figure 51: $^{87}$Sr/$^{86}$Sr values obtained from soil samples analysed. The green box indicates the biosphere value for Northampton (0.709-0.710) as calculated by Evans et al. (2010) 225

Figure 52: Plot of $^{87}$Sr/$^{86}$Sr values of Banbury Lane individuals. This figure has been colour-coded to correlate directly to the Sr map of the UK (Figure 18). Square icons represent enamel samples, and crosses represent dentine samples ..................................................................................227

Figure 53: LA-MC-ICP-MS plot generated for sample 201.001 .........................................................................................................................229

Figure 54: LA-MC-ICP-MS plot generated for sample 2003.039 .........................................................................................................................229

Figure 55: LA-MC-ICP-MS plot generated for sample 309.012 .........................................................................................................................230

Figure 56: LA-MC-ICP-MS plot generated for sample 128.10 .........................................................................................................................231

Figure 57: LA-MC-ICP-MS plot generated for sample 440.004 .........................................................................................................................232

Figure 58: Plan of Hambledon Hill monument complex (Mercer and Healy 2008, 5) .................................................................239

Figure 59: Plan of Hazleton North chambered tomb. The two internal chambered areas where human remains were recovered can be seen highlighted in the centre of the monument (Meadows et al. 2007, 46) .................................................................................................................................................................................................................................................................243

Figure 60: Plan of human bone from both the north and south chambered areas at Hazleton North (Saville 1990, 93 and 99) .................................................................................................................................................................................................................................................................................................................................245
Figure 61: Plan of Coldrum monument, with dolmen indicated in the centre of the rectangular long barrow (Ashbee 1998, 12) ................................................................. 248

Figure 62: Absolute abundance of bacteria (phyla level assignment) present within samples analysed within this research. From left, calculus samples BL201.1C (BA1; Banbury Lane), COL11038 (COL3; Coldrum), HH3181 (HH81; Hambledon Hill), and HN3793 (HN93; Hazleton North); bone samples BL201.1B (BA2) and BL201.2B (BA6) from Banbury Lane; and an extraction blank (EBK1) and library blank (LBL1) ......................... 258

Figure 63: Absolute abundance of bacteria (phyla level assignment) present within Hambledon Hill samples (generated from OneCodex analysis) ............................................. 260

Figure 64: Absolute abundance of bacteria (phyla level assignment) present within Hazleton North samples (generated from OneCodex analysis) ......................................... 260

Figure 65: Absolute abundance of bacteria (phyla level assignment) present within Banbury Lane samples (generated from OneCodex analysis) ......................................... 261

Figure 66: Absolute abundance of bacteria (phyla level assignment) present within Coldrum samples (generated from OneCodex analysis) ............................................. 261

Figure 67: Image of mandibular fragment of HH76 1916 (HH1916), osteologically identified as female (McKinley 2008). Photograph by the author ........................................... 267

Figure 68: Example of generated bammds plot, highlighting genetic affinities of a reference sequence ‘G’ to known populations ................................................................. 268

Figure 69: Sunburst diagram detailing phyla present within BL132.17 ............................ 275

Figure 70: Sunburst diagram detailing phyla present within BL309.12 ............................ 276

Figure 71: Sunburst diagram detailing phyla present within HH3 .................................... 277

Figure 72: Sunburst diagram detailing phyla present within HH610 ............................... 278

Figure 73: Sunburst diagram detailing phyla present within HH3188 ............................ 279

Figure 74: Sunburst diagram detailing phyla present within HN4786 ............................... 280

Figure 75: Sunburst diagram detailing phyla present within HN5037-1 ........................... 281

Figure 76: Sunburst diagram detailing phyla present within HN7387 ............................... 282

Figure 77: Sunburst diagram detailing phyla present within HN7656 ............................... 283

Figure 78: Sunburst diagram detailing phyla present within HN11456 ............................ 284

Figure 79: Sunburst diagram detailing phyla present within eBK (extraction blank) ........... 285
Figure 80: Total number of spectra matching BLG peptides within each dental calculus sample analysed here proteomically.................................................................293
Figure 81: Example of interpretation of resource representation at Meilgaard shell midden, Denmark (Bailey 1978, 47) ........................................................................................................302
Figure 82: Published δ^{13}C and δ^{15}N values on Mesolithic and Neolithic human remains from Britain. Data collated from Richards and Mellars 1998; Richards and Hedges 1999; Schulting and Richards 2000; 2002(b); Schulting 2005; 2009; 2013; Taylor et al. 2006; Hedges et al. 2006; 2008; Richards 2000; 2008; Schulting et al. 2008; 2010; 2015; Lightfoot et al. 2009; Meiklejohn et al. 2011; Bruning and Firth 2012; Milner and Craig 2009; 2012; Stevens et al. 2012; Montgomery et al. 2013 ......................................................318
Figure 83: Factors involved in host-microbiome interactions and microbiome composition (Tims et al. 2011) ..................................................................................................................336
Figure S1: Example of sectioned M1 tooth from the Banbury Lane assemblage ..........361
Figure S2: LA-MC-ICP-MS plot generated for sample 99.0 ........................................362
Figure S3: LA-MC-ICP-MS plot generated for sample 132.17 .................................362
Figure S4: LA-MC-ICP-MS plot generated for sample 138.003 ...............................363
Figure S5: LA-MC-ICP-MS plot generated for sample 141.00 .................................363
Figure S6: LA-MC-ICP-MS plot generated for sample 172.00 .................................364
Figure S7: LA-MC-ICP-MS plot generated for sample 201.002 ...............................364
Figure S8: LA-MC-ICP-MS plot generated for sample 240.005 ...............................365
Figure S9: LA-MC-ICP-MS plot generated for sample 302.8 .................................365
Figure S10: LA-MC-ICP-MS plot generated for sample 440.003 ............................366
Figure S11: LA-MC-ICP-MS plot generated for sample 462.7 ...............................366
Figure S12: LA-MC-ICP-MS plot generated for sample 551.00 ...............................367
Figure S13: LA-MC-ICP-MS plot generated for sample 838.3 ...............................367
Figure S14: LA-MC-ICP-MS plot generated for sample 838.4 ...............................368
Figure S15: LA-MC-ICP-MS plot generated for sample 901.00 ...............................368
Figure S16: LA-MC-ICP-MS plot generated for sample 1076.5 ...............................369
Figure S17: LA-MC-ICP-MS plot generated for sample 1078.008 ...........................369
Figure S18: LA-MC-ICP-MS plot generated for sample 1079.002 ...........................370
Figure S19: LA-MC-ICP-MS plot generated for sample 1303.01..........................370
Figure S20: LA-MC-ICP-MS plot generated for sample 1336.3..........................371
Figure S21: LA-MC-ICP-MS plot generated for sample 2005.213..........................371
Figure S22: LA-MC-ICP-MS plot generated for sample 2013.2..........................372
Figure S23: DNA concentrations in dental calculus post-extraction (ng of DNA/mg of dental calculus)................................................................................375
Figure S24: Schematic depicting shotgun library building (Image created by Jessica Hendy).................................................................................................376
Figure S25: MapDamage plot for sample BL201.1C .............................................383
Figure S26: MapDamage plot for sample BL201.1B (bone sample).....................383
Figure S27: MapDamage plot for sample BL201.2C .............................................384
Figure S28: MapDamage plot for sample BL201.2B (bone sample).....................384
Figure S29: MapDamage plot for sample BL309.12C ...........................................385
Figure S30: MapDamage plot for sample BL440.4B .............................................385
Figure S31: MapDamage plot for sample HH3 ......................................................386
Figure S32: MapDamage plot for sample HH1916 .................................................386
Figure S33: MapDamage plot for sample HH610 ..................................................387
Figure S34: MapDamage plot for sample HH3181 .................................................387
Figure S35: MapDamage plot for sample HN11456 ..............................................388
Figure S36: MapDamage plot for sample HN5037 .................................................388
Figure S37: MapDamage plot for sample HN4786 .................................................389
Figure S38: MapDamage plot for sample HN7656 .................................................389
Figure S39: MapDamage plot for sample HN3793 .................................................390
Figure S40: MapDamage plot for sample COL11033 ..........................................390
Figure S41: MapDamage plot for sample COL11035 ..........................................391
Figure S42: MapDamage plot for sample COL11038 ..........................................391
Figure S43: MapDamage plot for sample EBK1 (extraction blank 1)......................392
Figure S44: MapDamage plot for sample EBK2 (extraction blank 2)......................392
Figure S45: MapDamage plot for sample LBL1 (library building blank 1)..............393
Figure S46: MapDamage plot for sample LBL2 (library building blank 2).......................... 393

Figure S47: Sequence alignment of BLG in a range of fauna. Consensus sequences for each individual studied here are provided below the protein alignments. All BLG peptides identified within human dental calculus within this study are specific to the Pecora infraorder of Artiodactyla, with individual samples containing diagnostic polymorphisms for Bovidae and Caprinae (see Chapter 8)  396
List of Tables

Table 1: Number of key UK Mesolithic sites 19
Table 2: Number of key UK Neolithic sites ................................................................. 27
Table 3: Range of Mesolithic foodstuffs available at Star Carr (adapted from Milner 2006, 72) ................................................................. 32
Table 4: Dated British Mesolithic cave sites containing human remains (adapted from Conneller 2006, with additions) .................................................. 61
Table 5: Stable isotopes used within this thesis, their natural abundance, ratios, notation and standards (adapted from Schoeninger 1995) ..................................................... 107
Table 6: ZooMS species ID and collagen stable isotope values obtained from newly identified human and faunal remains from Cnoc Coig. Estimated marine % of diet for humans calculated from isotopic data obtained after Schulting and Richards (2002(a)) (using marine and terrestrial carbon end-points of -12‰ and -21‰ respectively) ............. 160
Table 7: Previously obtained δ^{13}C and δ^{15}N isotopic results on human bone from Cnoc Coig (adapted from Richards and Mellars 1998) ..................................................... 162
Table 8: δ^{13}C and δ^{15}N isotope values of food groups used within the FRUITS model generated for Cnoc Coig ................................................................. 165
Table 9: AMS dates obtained within this study from newly identified human and fauna. A ∆R value of 47 ±52 14C yr was used to calibrate the dates of samples 8257 and 8267 (Ascough, pers. comm.; Russell et al. 2015) ................................................................. 171
Table 10: 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. Information on new calibration of dates can be found below .......... 172
Table 11: ZooMS identifications of samples from Blick Mead ........................................ 187
Table 12: ZooMS identifications of samples from Cnoc Coig ........................................ 189
Table 13: ZooMS identifications of samples from Western Isles sites (TB11 = Bágh an Teampaill (Temple Bay); NT10 = Northton; TNB11 = Tràigh na Beirigh) ............. 191
Table 14: Radiocarbon values obtained for Banbury Lane, courtesy of Northamptonshire Archaeology (MOLA). Additional information on these dates can be found in Appendix A (OD = outer ditch; x% denotes date range with highest individual probability) .......... 204
Table 15: Determination of % animal protein in diet of individuals from Banbury Lane using the dietary models proposed by Hedges and Reynard (2007). Cattle and pig data from this study was combined with Towers et al. 2011 to increase sample size ..........216

Table 16: δ¹³C and δ¹⁵N isotope values of food groups used within the FRUITS model generated for Banbury Lane .................................................................................................................218

Table 17: Details of soil sample locations .................................................................................................................................223

Table 18: ⁸⁷Sr/⁸⁶Sr values obtained from soil samples analysed ..................................................................................................224

Table 19: Averaged Sr values of Banbury Lane individuals analysed. Osteological data from Caffell and Holst (2012). Dentine values were measured on a small number of individuals to provide an indication of the biosphere Sr value of the immediate burial environment ......................................................................................................................227

Table 20: Individuals sampled for dental calculus within this study from the Hambledon Hill assemblage (adapted from McKinley 2008). (Pathology: AMTL = ante-mortem tooth loss; PD = periodontal disease; OA = osteoarthritis; OP = osteophytes; MV = morphological variation). Sample numbers detailed here were created by the author for ease of analysis .................................................................................................................................241

Table 21: Human remains present in each area of the chambered tomb at Hazleton North. Information taken from Saville (1990) and Rogers (1990) .................................................................244

Table 22: Individuals sampled for dental calculus within this study from Hazleton North (osteological data from Rogers 1990). (Pathology: AMTL = ante-mortem tooth loss; PD = periodontal disease; OA = osteoarthritis). Sample numbers detailed here were created by the author for ease of analysis .................................................................................................................................246

Table 23: Individuals sampled for dental calculus within this study from the Coldrum assemblage. Age determination is based upon dental eruption/tooth formation following AlQahtani et al. (2010), undertaken by Sam Neil. AMS dates were provided by Sam Neil (pers. comm.) ..................................................................................................................................................249

Table 24: Individuals sampled for calculus in this study from Banbury Lane. Age, sex, and root mark information taken from Caffell and Holst (2012) .................................................................................250

Table 25: Samples utilised for aDNA analysis ..........................................................................................................................252

Table 26: Samples utilised for proteomic analysis .....................................................................................................................252

Table 27: DNA data obtained from all Neolithic samples ........................................................................................................254
Table 28: Comparison of osteologically determined sex identification (data from Rogers 1990; McKinley 2008; Caffell and Holst 2012) with aDNA sex identifications from endogenous DNA within the dental calculus (XX = female; XY = male). $R_Y$ 95% CI represents the fraction of sequences aligned to the Y-chromosome expressed as a ratio of the total number of sequences aligned to either sex chromosome, at a 95% confidence interval, as generated by the Skoglund et al. (2013) script ..................................................266

Table 29: Number of mapped and paired reads for bammds analysis .........................269

Table 30: Spectrum assignment for proteomic data obtained from dental calculus samples and an extraction blank (eBK) .................................................................271

Table 31: Spectrum assignment for ancient and modern dental calculus (using ProteinPilot v.4) in Warinner et al. 2014(a) ........................................................................271

Table 32: BLG peptides identified within dental calculus samples. All sequences have been verified for specificity by conducting a protein BLAST (blastp) search against the NCBI nr database, and sequences that uniquely match BLG are marked with an asterisk (*). Only samples with at least one spectrum uniquely matching BLG are considered BLG+. Excludes spectra with a Mascot ion score <25. Peptides identified more than once are followed by parentheses indicating the total number of observations. Species identification indicated with (‘) is done so as among Bovidae, Bovinae (cattle, yak and buffalo) are distinguished from Caprinae (sheep and goats) by N→D at residue 71. However, because N deamidation to D is a common post-mortem modification, it is uncertain if the D at this residue is authentic or a damage artefact, and therefore species identification can only be to Bovidae ..............................................................................................................291

Table S1: The diet to tissue offset and weight contribution of macronutrients towards dietary proxy signals. Values in parentheses represent uncertainty. Based upon data from Fernandes et al. (2014; 2015) ........................................................................348

Table S2: Concentration values for each food group (dry weight composition of macronutrients). Values in parentheses represent uncertainty (based on data in Fernandes 2015) ........................................................................................................348

Table S3: Food values used within FRUITS model 1. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%_o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%_o$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%_o$; and marine values using $\Delta^{13}C_{\text{protein-collagen}} = -1\%_o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%_o$ and $\Delta^{13}C_{\text{energy-collagen}} = -7\%_o$. Plant values were estimated using the offsets shown in Table S5 and terrestrial animal values, and $\Delta^{13}C_{\text{protein-energy}} = +1\%_o$ (Tieszen 1991; Fernandes 2015) ........................................................................................................349
Table S4: Food values used within FRUITS model 2. Herbivore macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%$; and marine values using $\Delta^{13}C_{\text{protein-collagen}} = -1\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -7\%$. Plant values were estimated using the offsets shown in Table S5 and herbivore values, and $\Delta^{13}C_{\text{protein-energy}} = +1\%$ (Tieszen 1991; Fernandes 2015) ....... 349

Table S5: Collagen stable isotope values obtained from human remains sampled from the Banbury Lane assemblage. All above values were taken from right femora. Layers referred to correspond to the original excavation layers – which were arbitrary spits taken through the pit, and were determined by the excavators – not archaeological or contextual layers ................................................................. 355

Table S6: Collagen stable isotope values obtained from fauna from Banbury Lane ........ 356

Table S7: Results from Bradford-York interlab comparison on bovine and cod standards used during IRMS analysis ................................................................. 357

Table S8: Results from Bradford-York interlab comparison on two human bone samples from the Banbury Lane assemblage ................................................................. 358

Table S9: The diet to tissue offset and weight contribution of macronutrients towards dietary proxy signals. Values in parentheses represent uncertainty. Based upon data from Fernandes et al. (2014; 2015) ................................................................. 359

Table S10: Concentration values for each food group (dry weight composition of macronutrients). Values in parentheses represent uncertainty ........................................ 359

Table S11: Food values used within the Banbury Lane FRUITS model 1. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%$, and plant energy values using $\Delta^{13}C_{\text{protein-energy}} = +1\%$ (Tieszen 1991; Fernandes 2015) ................................................................. 360

Table S12: Food values used within the Banbury Lane FRUITS model 2. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%$, and plant energy values using $\Delta^{13}C_{\text{protein-energy}} = +1\%$ (Tieszen 1991; Fernandes 2015) ................................................................. 360

Table S13: Post-extraction Qubit results for bone and calculus samples ..................... 374

Table S14: Sample adapter numbers, number of PCR cycles and post-iPCR Qubit quantification ........................................................................................................ 378

Table S15: Post-extraction (pre-trypsin digest) Qubit results for calculus samples...... 380
Table S16: Spectrum assignment for raw proteomic data obtained from dental calculus samples and an extraction blank (eBK) without quality controls
Acknowledgements

There are a huge number of people without whom this research would not have been possible. First and foremost, I would like to say a huge thank you to my two PhD supervisors, Oliver Craig and Michelle Alexander, both of whom have been truly fantastic throughout the past three and a half years, and have provided me with endless advice, support and opportunities. I also owe thanks to Steve Roskams for his chairing of my TAP meetings, and for providing consistently insightful comments on my research. I would also like to thank Matthew Collins for all of his wonderful help and advice throughout my time at York, and for introducing me to both ZooMS and dental calculus analysis – he has ultimately shaped and inspired my research hugely. I am also very grateful to Nicky Milner for countless conversations on the British Mesolithic, and for providing me with a chance to actually dig Mesolithic sites each summer in the Vale of Pickering. Similarly, I would like to thank Mark Edmonds for many fruitful conversations on the British Neolithic, and for allowing me to sit in on his fantastic seminars during the first year of my PhD. I also owe a huge deal of thanks to Camilla Speller for all her assistance and advice on my calculus research, and for very patiently teaching me how to command line script and analyse aDNA data. I am vastly indebted to Alistair Pike for always being so generous with his time and for all his help with strontium isotope analysis. I also owe thanks to Malin Holst for her constant support and encouragement over the last six and a half years. Beyond this, I would also like to say a huge thank you to Matt Von Tersch and Krista McGrath for laboratory support and advice, and to Alizon Holland for all her fantastic administrative help. I am also very grateful to all my colleagues at s-block for providing such a great working environment.

Alongside this, there are a large number of people who have provided me with samples, access to material, permissions, and information – and without them this research would not have been possible. For the Cnoc Coig material: Alison Sheridan, National Museums Scotland, Tamsin O’Connell, Rhiannon Stevens, and Paul Mellars. For the Blick Mead material: David Jacques and the Blick Mead excavation team (and a special thank you to David for his boundless enthusiasm for my research!). For the Western Isles material: Mike Church, Peter Rowley-Conwy, and Steph Piper. For the Banbury Lane material: Malin Holst, Anwen Caffell, York Osteoarchaeology Ltd., and Northamptonshire Archaeology (MOLA) – particularly Andy Chapman, Adam Yates & Mark Holmes. For
the Coldrum material: Marta Lahr, the Duckworth Laboratory (Cambridge), and Sam Neil. For the Hambledon Hill material: Dorset County Museum, Richard Breward and Jonathan Murden. For the Hazleton North material: Corinium Museum, Alison Brookes and Heather Dawson.

I also need thank NERC for providing me with the funding to undertake this research, and for providing additional training opportunities. The new Cnoc Coig AMS dates presented within Chapter 5 of this thesis were funded separately through the NERC-AHRC National Radiocarbon Facility (NRCF).

Finally, I would like to say a massive thank you to all my friends and family for their help and support throughout my PhD. Of my archaeologist friends, I would particularly like to thank Andy Needham, Becky Knight, Nick Overton, Aimée Little, Barry Taylor & Amy Gray-Jones for countless conversations on the Mesolithic, sharing a trench with me every summer, and the gin teapot; and also Ruth Whyte for always being at the end of the phone and never failing to make me laugh. Of my non-archaeologist friends, I owe special thanks to Sophie Thomas, Lauren Kilgallon, Leigh Spencer & Laurence Hobden for putting up with me for so many years, and for always reminding me that there is a world outside of archaeology and beyond the PhD. Lastly, and most importantly, there are two people to whom I owe extra special thanks: my parents, Sue (Suze) and Andy, for their unwavering faith in me, innumerable pep-talks, and constant fantastic support & guidance – without them I would not be where I am today.
Author’s Declaration

This thesis is the original work of the author. This work has not been submitted for any other degree or award at any other university or academic institution. All sources are acknowledged as references.

A modified version of Chapter 5 has been submitted to the *Journal of Archaeological Science* with the following reference:


- The text was written by SC with contributions from co-authors. All practical work and analysis was undertaken by SC. The ZooMS methodology utilised was developed by MC and SC. Contextual information was provided by PM, TCOC and SRE. Data interpretation was aided by OEC, MA and NM
Chapter 1 – Lifeways at the Mesolithic-Neolithic Transition

1.1. Studying Prehistory in Britain

“Where do we come from? What are we? Where are we going?”

(Gauguin 1897)

The above questions are taken from the title of a painting – but ultimately, these, are arguably the key questions which we seek to address through archaeology, and through the study of prehistory in particular. The desire to understand the past, and the processes of change over time which have resulted in our current modern day societies, have fascinated since the idea of evolution, and indeed ‘prehistory’, was posited by Darwin in 1859. Childe’s evocatively titled ‘Man Makes Himself’ (1936) encapsulates the idea that humankind has indeed created the world in which we live today – and whilst we must not view this in progressive evolutionist terms (i.e. creating a narrative moving from a barbarous, savage past to a perfect present), we can use archaeology to help us to understand our distant past, and the processes of change through time. Thomas (1999, 2) however writes that “any prehistory we write is a modern production” – in that when we write about the prehistoric past we choose to define what we deem as ‘significant’ evidence, we create new meanings, and we place our findings in the context of a narrative. Ultimately therefore, we write with our own (un)conscious preoccupations and preconceptions – and thus the study of the past can never be truly objective. Nonetheless, whilst interpretation of the prehistoric past will always be difficult, it will also always be fascinating, “for prehistory is the science of us… it is the discipline by which we study ourselves and the way we have come to be as we are” (Renfrew 2007, viii). Both Europe and the USA in particular have historically had a strong desire to identify their ‘origins’ – and this has therefore influenced national and colonial histories, and also approaches to archaeology (Gamble 2007, 22) – perhaps explaining in part our fascination with the prehistoric past.

British prehistory in particular has had a long history of study, of which a brief overview is provided in Chapter 2. However, as is highlighted within the chapter, certain areas of prehistory have tended to have been favoured over others – with the Mesolithic period in particular being seen as ‘the poor relation’, specifically when compared to the preceding Palaeolithic and the succeeding Neolithic periods. The transition between the Mesolithic
and Neolithic periods however is a time period which has long held fascination for archaeologists – heralding a change from mobile hunter-gatherer-fisher populations to more sedentary agriculturalists or pastoralists – and has consequently been heavily studied. This significant change in lifeways and subsistence is believed to have subsequently, or concurrently, resulted in other changes – not only dietary, but also shifts in mobility, demography, economy, social relationships, identities, potential belief systems, material culture, and site forms, for example (of which an overview is provided in Chapter 2).

This research aims to adopt a new biomolecular approach to this period of British prehistory, to provide new information on the lifeways of both the Mesolithic and Neolithic, and the transitional period. The term ‘lifeways’ can simply be defined as the manner in which people lived, and as such can incorporate exploration of the nature of subsistence of populations, mobility, demography, technology, and so forth. A lifeways approach ultimately aims to move toward an understanding of lived human experience. The lifeways approach adopted throughout this thesis specifically utilises archaeological materials which have often been overlooked, or considered unimportant or unusable: namely fragmentary ‘unidentifiable’ bone, disarticulated remains, and dental calculus. These materials have typically been seen to hold less interpretative value – but through the utilisation of biomolecular techniques can provide useful and exciting information on these periods, and as such, may allow us to investigate specific aspects of prehistoric lifeways in more detail. Through the utilisation of a number of different biomolecular techniques in tandem on these materials therefore, this thesis aims to focus on five main research areas:

- Identification
- Diet
- Mobility
- Chronology
- Health/ disease

It is these research themes which are present throughout this thesis, and aim to link the individual case studies presented in each chapter together. Within British prehistory there is now a need for a move away from traditional narratives and a heavy focus on specific aspects of change at the Mesolithic-Neolithic transition. Instead, we can now work towards broadening our focus of study on the Mesolithic and Neolithic of Britain – attempted within this research through combining scientific methods with more traditional modes of
study, on materials previously not utilised within these discussions – to view lifeways, and changes to these, across the Mesolithic and Neolithic of Britain.

1.2. Aims and Objectives

The main aim of this thesis is to investigate the biomolecular information which can be obtained from Mesolithic and Neolithic excavated, unstudied materials (i.e. fragmentary and disarticulated bone, and dental calculus) on prehistoric lifeways, specifically focusing on five main research areas: identification, diet, mobility, chronology, and health/disease. This study aims to adopt a multi-methodological approach to study, therefore making this the largest combined application of these techniques to British Mesolithic and Neolithic material to date. By comparing the data obtained with our current understanding of these periods, this research aims to contribute to existing discussions and debates – both within the two periods themselves, and on the transition between them.

In order to achieve the aims outlined above, a number of key objectives need to be met:

1. To assess the state of our current understanding of the Mesolithic-Neolithic transition in Britain
2. To evaluate the utilisation of ZooMS (Zooarchaeology by Mass Spectrometry) as a means via which to identify prehistoric bone fragments
3. To collect new dietary information from both Mesolithic and Neolithic human remains through the use of $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis
4. To produce new mobility data utilising strontium isotopic analysis ($^{86}Sr/^{87}Sr$)
5. To undertake new AMS dates where possible to aid our understanding of chronology (of individual sites, the transition, and/or changes perceived to have occurred)
6. To employ novel metagenomic and metaproteomic analyses of dental calculus, and assess if this may be a means via which to obtain new information on prehistoric health and disease
7. To develop and formulate a standpoint on the mechanisms behind the Mesolithic-Neolithic transition in Britain
8. To examine our current knowledge of Mesolithic and Neolithic mortuary practices, and propose new means via which this knowledge could be expanded

The process which this thesis will follow therefore can broadly be conceptualised as:
Overall, a total of 302 individual archaeological samples were successfully analysed within this research, from nine different Mesolithic and Neolithic British sites, utilising six different biomolecular methodologies (Figure 1).
1.3. Outline of Thesis

As stated, the focus of this thesis is on the five key research areas highlighted above (identification, diet, mobility, chronology, and health & disease), and in the obtaining of
information on these through the utilisation of a number of different biomolecular techniques. Each chapter of the thesis therefore focuses on a different facet of the Mesolithic–Neolithic transition and the surrounding period, and a different aspect of the five research areas.

Chapters 2, 3 and 4 all provide the archaeological, theoretical, and methodological basis of this work. It is hoped that Chapters 2 and 3 provide the context in which this research is set, and highlight the aspects of both the Mesolithic and Neolithic periods which are still unclear to us as archaeologists, and which require further study. These chapters also aim to indicate the lack of research currently focused upon Mesolithic and Neolithic human remains in Britain, and our poor understanding of both diet and disease within these periods still. These then lead into Chapter 4, which aims to suggest how biomolecular approaches and new scientific techniques may be used to help fill these knowledge gaps. The integrated scientific nature of this research is outlined here – providing justification for the methodologies used throughout this research, and how they can be used on prehistoric material to increase our knowledge and understanding on these distant time periods.

Crucially, whilst highlighting the advantages of biomolecular techniques and of a multi-methodological approach, Chapter 4 also indicates the nature of the material used within this research – which is excavated archaeological material previously overlooked: heavily fragmented and/or disarticulated skeletal material, and dental calculus. In doing so, this research as a whole aims to highlight how material previously considered as unimportant, un-useable, or as holding less interpretative value can actually provide relevant, useful, and exciting archaeological information, which can contribute to wider debates within the discipline.

Thematically, this thesis starts by looking at identification – and how we can obtain useful taxonomic information from previously ‘unidentifiable’ bone fragments. ‘Finding the Mesolithic’ (Chapter 6) highlights the potential of ZooMS for fragmentary and disarticulated British Mesolithic skeletal material, and builds upon the successes of ‘Rediscovering Oronsay’ (Chapter 5); which successfully utilises ZooMS to identify both human and faunal remains from fragmentary remains. The thesis then moves on towards a consideration of Mesolithic and Neolithic diets, and of the transition between the two. Whilst ‘Rediscovering Oronsay’ (Chapter 5) aims to provide new insights into Mesolithic diets and the potential timings of Mesolithic–Neolithic dietary change, both ‘Banbury Lane, Northampton: A Large Scale Multi-Isotopic Study on an Unusual Neolithic
Assemblage’ (Chapter 7) and ‘Biomolecular Analysis of Dental Calculus’ (Chapter 8), provide a new characterisation of diet within the Neolithic itself, beyond the transition. Following this, the thesis considers prehistoric mobility and movement, utilising strontium isotopic analysis in Chapter 7 to determine levels of mobility within the Banbury Lane assemblage, and PCA plots of endogenous (host) DNA obtained from dental calculus to determine genetic origins in Chapter 8, a ‘Biomolecular Analysis of Dental Calculus’. Finally, the last theme this thesis focuses on is prehistoric health and disease – with Chapter 8 providing the first ever application of metaproteomic and metagenomic analyses to Neolithic dental calculus, and highlighting the future research potential of analyses of this kind in providing novel disease information for prehistoric periods.

To conclude, Chapter 9 provides a detailed discussion and summary of the research undertaken within this thesis, aiming to bring together the different strands of research which have emerged throughout this work, the multi-methodological approach adopted here, and elucidate how the results obtained here feed into the broader context of Mesolithic and Neolithic studies, as outlined in Chapters 2 and 3. The Chapter aims to highlight the advantages of the multi-stranded and multi-methodological research focus adopted here, and how this could be adopted within further studies of both British Mesolithic and Neolithic material. Crucially, it also aims to pinpoint the potential avenues for future research, and judge how far biomolecular methods may assist in filling the knowledge gaps still deemed to present within Mesolithic and Neolithic studies.
Chapter 2 – The Mesolithic and The Neolithic Periods in Britain

“Successful farmers have social relations with one another, while hunter-gatherers have ecological relationships with hazelnuts”

(Bradley, 1984, 11)

The following chapter aims to outline the context in which this research is set: the time periods the work will focus on (i.e. the Mesolithic and the Neolithic), how these periods have been defined culturally and chronologically, the archaeological evidence available for both periods, and also how previous scholars have studied both the Mesolithic and the Neolithic in the past – looking at changes in scholarship due to factors such as theoretical movements and the adoption of new scientific or other techniques. The chapter will therefore firstly discuss the Mesolithic period, then the Neolithic, and will finally consider the transition between the two periods, and the degree of change and continuity visible. Finally, the chapter will also focus on the archaeological evidence for diet and dietary change in these periods, as this has been a key mode via which they have been defined in the past, and is a theme which will be considered throughout this research. It is hoped that by highlighting the broad themes, modes of study, archaeological evidence and theoretical positions which have been studied and adopted for both periods, that this will provide a platform from which the key research questions of this work can be based upon, and can be placed securely within the context of previous archaeological research undertaken on these time periods. In doing so, the chapter also aims to fulfil objective 1 of this thesis.

2.1. Categorisation of the Prehistoric Past

The initial idea of segregating prehistory into separate cultural epochs was first envisioned by C.J. Thomson in 1836, when he created a tripartite division of the prehistoric past by dividing the artefacts held at the National Museum of Copenhagen into separate collections of stone, bronze, and iron items, all with stylistic differences (Gamble 2007, 12). This therefore led to categorisation of the Stone Age, the Bronze Age, and the Iron Age – the ‘Three Age System’. Alongside subsequent work on stratigraphy by Jens J.A. Worsaae (1849), this formed the basis for relative chronologies (based upon seriation and stratification) to be constructed for the prehistoric past, based upon social evolutionary theories of the Enlightenment period (Trigger 2006, 84).
In terms of chronological definitions or categorisation however, it is always extremely complex to divide up time in the past along somewhat arbitrary categories based upon relatively sparse archaeological data. Placing nomenclature upon chronological periods, whilst advantageous in many ways, can also sometimes hinder interpretations as archaeologists are confined to prescribed chronological frameworks. Equally, never in any period – prehistoric or historic – has there been a conscious, concerted and active effort by peoples in the past to ‘change’ period; i.e. there was not a specific day when the Neolithic started! Boundaries between periods can thus never be truly tightly bound, as change does not happen overnight. It is ideas such as this which have prompted academics such as Steve Mrozowski (2012) to examine archaeological concepts of temporality, and Mrozowski has even called for “an end to prehistory” in North America – seeing the traditional nomenclature and chronological divisions of the past as boundaries to study.

Nonetheless, categorisation of prehistoric periods is important. Whilst it is a conceptual construct, considering the ways in which we can define and categorise the past is crucial – particularly as they will then affect or influence the interpretations we make from archaeological data. How we categorise the past is therefore a big intellectual question, but is something which is frequently left unaddressed in discussions and interpretations of the Mesolithic and Neolithic periods. The following sections aim to outline the numerous ways in which we can define and categorise the two periods, and the transition between them – and aim to consider which modes of definition may be most appropriate within archaeological discourse. Importantly however, whilst this chapter aims to present a theoretical position on the definition of both periods, and on the transition, it is crucial to note that this research as a whole generally attempts to look more broadly, encompassing the ‘transition’ period but attempting to address the research questions outlined in Chapter 1 without the constraints of traditional chronological boundaries.

2.2. Defining the Mesolithic

“Society...will always start with agriculture”

(Gamble 2007, 210)

The term ‘Mesolithic’ was first conceived by Westropp in 1872, in his ‘Pre-historic Phases’. In contrast, both the terms ‘Palaeolithic’ and ‘Neolithic’ were already widely in use within archaeology after being put forward by Lubbock in 1865. Lubbock proposed the division of early prehistory, building upon Thomson’s schema, and coined the terms
‘Palaeolithic’ and ‘Neolithic’ for subdivision of the Stone Age – thereby segregating prehistory into four time periods or cultural epochs: the Palaeolithic, the Neolithic, the Bronze Age and the Iron Age (Lubbock 1865). Westropp’s insertion of the ‘Mesolithic’ into this scheme unfortunately coincided with an adjacent publication by Evans in the same year (1872) deemed more comprehensive and authoritative, and which followed Lubbock’s system – ultimately resulting in the term ‘Mesolithic’ and its cultural associations being cast by the wayside (Rowley-Conwy 1996). In fact, it was not until the 1920’s that the term ‘Mesolithic’ really began to be adopted within archaeology again – and it was not until the work of Grahame Clark from the 1930’s onwards that the ‘Mesolithic’ as we know it today was properly defined (publications 1932 onwards).

The Mesolithic was traditionally viewed as a ‘hiatus’ period (both occupationally and culturally) between the cultures of the Palaeolithic and the Neolithic (Breuil 1921; Rowley-Conwy 1986), with suggestions that large areas of Europe (including Britain) were in fact devoid of population at this time – this idea being supported by the lack of distinct archaeological evidence for the period. This in part accounted for the lack of adoption of the term ‘Mesolithic’, particularly as the material which was available from the period challenged ideas of social evolution (both Victorian (Spencerian) and Marxist) (Dawkins 1894; MacCurdy 1924; Childe 1925; Zvelebil 1986(a)). However, the discovery of the site of Mas d’Azil by the French prehistorian Piette in the late 19th century, after which the Azilian culture was named, showed the presence of cultural horizons between the Palaeolithic and Neolithic deposits at the site, thereby proving the existence of a ‘Mesolithic’ period in Europe (Piette 1895). Despite this, however, ideas of the Mesolithic as a transitional or ‘prelude’ period of little significance appear to have persisted (Zvelebil 1986(b); Gaffney et al. 2009, 40).

The meaning of the term ‘Mesolithic’, and the period it represents, has varied somewhat significantly over the past 80 years however (Zvelebil 1986(a); Figure 2). In terms of chronological definitions, the Mesolithic period in Britain is today generally taken to refer to the period from c.10,000 (perhaps 9,600) to 4,000 cal. BC (12,000 to 6,000 BP) (Milner and Mithen 2009; Gaffney et al. 2009, 39), and it has been suggested by Spikins (2008) that this c.6,000 year period may have covered over 200 generations of peoples.
As the Mesolithic period sees the end of the Pleistocene, there are a range of climatic, floral, and faunal changes associated with this – and as such, many definitions of and research on the period have focused on environmental and climatic conditions. The Mesolithic period marks the start of the most recent post-glacial period, heralding the beginning of the geological epoch of the Holocene, and the Pre-Boreal climatic phase (Figure 3). From the start of the Holocene onwards, the climate of Europe gradually grew warmer, and the ice-sheets which had previously covered the landscape began to melt, causing sea-level rise throughout the Mesolithic. By 6,000 BC the physical connection between Britain and the mainland European continent no longer existed, with ‘Doggerland’ now being completely flooded, and the North Sea and Atlantic Ocean joined together (Weninger et al. 2008; Milner and Mithen 2009; Darvill 2010, 58; Figure 4). The late Mesolithic in particular has also been suggested to be a period of significant climatic instability, with floods, tsunamis (c.5840 BC), and reduced rainfall (7,000-5,500 BC) alongside rising sea levels (Weninger et al. 2008; Spikins 2008; Darvill 2010, 68). The
Mesolithic period is thus frequently painted as a period of cultural adaptations to climatic conditions, within a hunter-gatherer mode of subsistence (Zvelebil 1986(a); Tolan-Smith 2008).

<table>
<thead>
<tr>
<th>Geological epoch</th>
<th>Geochronological stage</th>
<th>Biostratigraphic division/diagnostic phase</th>
<th>Pollen Zone (Godwin Zone)</th>
<th>Date (calibrated radiocarbon age: BC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-glacial</td>
<td>Holocene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Handian (interglacial stage)</td>
<td>Sub-Atlantic</td>
<td>IX (VIII)</td>
<td>1000 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sub-Boreal</td>
<td>VIII (VIIb)</td>
<td>3000 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atlantic</td>
<td>VII (VIIb, VIIc, VIIa)</td>
<td>4000 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boreal</td>
<td>VI (VIA)</td>
<td>6000 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-Boreal</td>
<td>V</td>
<td>8500 BC</td>
</tr>
<tr>
<td>Late-glacial</td>
<td>Pleistocene</td>
<td>Younger Dryas (Loch Lomond)</td>
<td>III</td>
<td>10,000 BC</td>
</tr>
<tr>
<td></td>
<td>Devensian (glacial stage)</td>
<td>Altered Oscillation</td>
<td>II</td>
<td>10,500 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Older Dryas (Windermere)</td>
<td>Ic</td>
<td>12,600 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bomb Inception</td>
<td>Ib</td>
<td>12,500 BC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Older Dryas (Donington)</td>
<td>Ia</td>
<td>33,000 BC</td>
</tr>
</tbody>
</table>

Figure 3: Table depicting the periods and subdivision of the late glacial and post-glacial periods in Britain (Darvill 2010, xix)
The changes in climate in the Mesolithic resulted in soils maturing and the emergence of new flora and fauna. In particular, palynological analyses have revealed new woodland species colonised Britain, consisting of birch, pine and hazel in the early Mesolithic, and later also oak, elm, lime, ash and elder (French and Lewis 2005; Spikins 2008). However, it has been suggested that the traditional view of the landscape of the Mesolithic, which persists today, of open grasslands and tundra being replaced by a wooded landscape (Clark 1980, 44), may not necessarily have been as clear-cut as is often assumed. Vegetation cover is unlikely to have been uniform, and there may have been significant regional differences, due to differences in soil, climate and topography (French and Lewis 2005; Bradley 2007, 15). These new environments and landscapes were inhabited by new fauna, such as red and roe deer, wolf, fox, bear, auroch, wild pig, badger, pine marten, lynx, beaver and hare in Britain (Gaffney et al. 2009, 45; Darvill 2010, 58). However, whilst
landscape and environmental studies of the Mesolithic have been undertaken on a significant scale, little consideration is often given to how the landscape may have been articulated via the relationships between people and certain activities, animals, materials, or woodlands. Instead, focus of study – particularly of British material – has been heavily ecological, looking at pollen analysis, forest ecosystems and ecological productivity (Rowley-Conwy 1986; McFadyen 2008).

Associated with the climatic, floral and faunal changes seen within the Mesolithic, new styles of flint, stone and bone industries emerge (Clark 1932, 2) – and these have also often been used as a defining feature of the period. Stone and flint tools are the most common artefacts found within the Mesolithic record, with the characteristic flint tool type being the microlith, which has perhaps not always been in the period’s favour: “[it is] symbolic that the new age should be represented by microliths, whose diminutive size neatly suggested their historical insignificance” (Clark 1978, 3). Across the Mesolithic we also begin to see tools being made from bone and antler – uniserial and biserial barbed points, antler mattocks, bevel-ended tools, and so forth (Tolan-Smith 2008). The interpretation of all Mesolithic tool types has however predominately focused on their function in hunting and resource exploitation (i.e. their economic role).

Perhaps most importantly however, the Mesolithic has long been recognised as being a period characterised by peoples who were hunter-gatherers, or hunter-fisher-gatherers. This mode of foraging subsistence is seen as a distinguishing and defining aspect of the period, and clearly demarcates it from both the Palaeolithic and the Neolithic. The hunter-gatherer-fisher lifestyle, and the associated dietary implications of this, have therefore perhaps been seen as the key mode of definition of the period. However, because of this, the Mesolithic has been subject to archaeologists’ preconceptions of hunting and gathering societies, and a lack of academic focus on the period in the recent past may be due in part to the retention of prejudices toward hunter-gatherer populations, due to European colonial expansion (Zvelebil 1986(a)). Throughout the past 30 years however, ethnographic parallels have been drawn between Mesolithic populations and modern hunter-gatherer groups, and have attempted to acknowledge the possible complexity and diversity within these communities – in terms of social systems, economy, social or gender divisions, mobility, world views, systems of exchange, violence, ceremonial and social activities, site formation processes, and technology (Bender 1978; Binford 1980; Testart 1982; Bettinger 1991; Spikins 2000; Brody 2002; Grøn and Kuznetsov 2003; Jordan 2003(a); 2003(b);
Fewster 2005; Kelly 2007; Grøn et al. 2009; Zvelebil 2009; Johnson 2013). There have however been issues in the past with the stereotyping of specific gender roles within hunter-gatherer populations. The ‘traditional’ androcentric view, is that plants are more linked to female gender roles – i.e. men= hunters; women= gatherers. For example, Wing and Brown (1979, 93) note, “[gathering] is an activity that was primarily engaged in by women”. This gender dichotomy has persisted despite widespread ethnographic evidence that men do gather, women do hunt, food preparation can be undertaken by both sexes and all genders, and also that some aspects of both hunting and gathering can only be undertaken by specific people, regardless of gender – and may be linked instead, for example, to age or social standing. This complexity in gender roles, particularly those related to gathering and plant processing, has been recently highlighted by Taylor (2014).

However, the use of ethnographic analogy within archaeology is problematic in that there is known to be considerable diversity within hunter-gatherer populations (and thus we cannot create a hunter-gatherer ‘stereotype’), and also because the translation from ethnography to archaeology cannot be direct (Kelly 2007, 338-339). Ethnography has also in the past been used to depict hunter-gatherers as ‘savages’, and as less advanced populations, in-line with social evolutionary theoretical positions (for an overview see Bettinger 1991) – something which has in recent years been the subject of much critical attention (Warren 2010, 17). That the Mesolithic is so frequently defined by its modes of subsistence and economy has however meant that the period is still heavily linked with social evolutionary and Marxist theories (Milner and Woodman 2005).

In tandem with the subsistence models which have been posited for the British Mesolithic, the period has also often been defined by the levels of mobility perceived within populations at the time. The hunter-gatherer-fisher lifestyle seen in the Mesolithic is thought to have resulted in highly mobile populations, who moved across or within landscapes throughout the year, perhaps on a seasonal basis due to availability of different resources. As such, many Mesolithic ‘sites’ or evidence of activity from the period are in the form of flint scatters – which have been suggested to “mark an important axis of movement across the landscape” (Tilley 1994, 147). This idea of heavily mobile populations has prompted much work on seasonality of use of sites – in an attempt to determine how groups may have moved around a landscape throughout the year. Work on seasonality has perhaps most famously been undertaken at Oronsay, Inner Hebrides (Mellars 1978; 1979; Jardine and Jardine 1983; Richards and Mellars 1998; Wicks et al.
2014; see Chapter 5 also) and Star Carr (Pitts 1979; Legge and Rowley-Conwy 1988; Carter 1998; Schulting and Richards 2002(b)). Indeed, as Milner (2005(b), 56) comments, seasonality studies appear to have become “an essential part of the methodological framework used within Mesolithic studies”. However, there are limitations in using solely seasonality studies to determine levels of mobility, timings of site occupation and seasonal usage of a site to determine site function, as is frequently done (Milner 2005(b)).

Despite the traditional belief that there were no structures within the British Mesolithic due to high levels of population mobility, research within the last 30 years has revealed more than 25 structures dating to the period, with the majority being discovered within northern England and Scotland (Darvill 2010, 73). In particular, fairly substantial structures are now known at the sites of Mount Sandel, Howick, East Barns, and Star Carr (Milner and Mithen 2009). The discovery of Mesolithic structures or ‘houses’ has somewhat changed the way in which we both define and consider the period in Britain – and although populations are still considered to be highly mobile, the evidence of structures perhaps suggests a higher degree of sedentism (or differential use of sites) than perhaps previously thought. Nonetheless, the British Mesolithic as a whole is still perceived as a period of highly mobile populations utilising the landscape, possibly on a seasonal basis. A change in the degree of mobility seen throughout the period has however been repeatedly suggested – whereas early Mesolithic peoples are seen as highly mobile colonisers, living in small population groups, the late Mesolithic is seen to give rise to ‘complex hunter-gatherers’ with larger population sizes, increased sedentism, and larger site sizes (Conneller et al. 2012; see Chapter 9).

The British Mesolithic has also frequently been defined by a number of key sites – which are seen as ‘type sites’ for the period, despite little evidence of other similar sites across the country. Melting ice-sheets caused by climatic change (discussed above) resulted in the formation of new lakes which dominated the landscape of early Mesolithic Britain, and it is often in these locations which we see evidence of human activity (e.g. Star Carr, Thatcham, Deep Carr) (Tolan-Smith 2008). Some of the key UK Mesolithic sites are listed in the below (Table 1), but it is perhaps Star Carr which is now the most well-known of these – particularly due to significant fieldwork at the site (a number of periods of excavation, from the 1950’s onwards; see Table below) and media coverage at the site in recent years (e.g. Coughlan 2010; Bodmer 2010; Derbyshire 2010; Time Team 2013), alongside public outreach work (e.g. Yorkshire Museum 2013; Milner et al. 2013). Star
Carr is now often considered to be the defining ‘type site’ for the British Mesolithic, although no other site like it has yet been found in the UK. Much research on Star Carr has focused upon the seasonality of use of the site (Fraser and King 1954; Jacobi 1978; Caulfield 1978; Pitts 1979; Andresen et al. 1981; and so forth), and also the deposition and use of the antler frontlet ‘headdresses’ discovered there (Clark 1954; Legge and Rowley-Conwy 1988; Pollard 2000; Bevan 2003; Conneller 2003; 2004; Warren 2006).

Interestingly, despite the wealth of archaeological evidence from the site, no human remains have ever been detected within the bone material excavated. More recent work has however revealed potential structure(s), and has also focused on nearby Flixton Island, where large amounts of flint artefacts and preserved Mesolithic horse hoof-prints have been discovered.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Date</th>
<th>Main Archaeological Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star Carr</td>
<td>Vale of Pickering, North Yorkshire</td>
<td>Early Mesolithic (c.9,000 BC)</td>
<td>Faunal remains, in-situ preserved worked timber platforms, lithics, post holes (structures), bone and antler tools and artefacts, shale beads, antler frontlets</td>
<td>Clark 1954; Clark 1972; Pitts 1979; Andresen et al. 1981; Legge and Rowley-Conwy 1988; Roberts et al. 1998; Mellars and Dark 1998; Chatterton 2003; Conneller 2003; Milner et al. 2007; 2011(a); 2011(b); 2013; Conneller et al. 2012; Taylor 2012</td>
</tr>
<tr>
<td>Howick</td>
<td>Northumberland</td>
<td>Early Mesolithic (c.7800 BC)</td>
<td>Lithics, faunal remains, hazelnut shells, hearths, shell fragments, ‘hut’ structure</td>
<td>Waddington et al. 2003(a); Waddington et al. 2003(b); Boomer et al. 2007</td>
</tr>
</tbody>
</table>
Oronsay  Inner Hebrides  Late Mesolithic (c.5500-4000 BC)  Six shell middens (containing human bone, animal bone, lithics, antler harpoons)  Mellars 1978; Mellars 1987; Mellars and Wilkinson 1980; Richards and Mellars 1998

Severn Estuary  Mouth of Bristol Channel; boundary between England and Wales  Mid-later Mesolithic (c.5800 BC)  Mesolithic human and animal footprints  Aldhouse-Green et al. 1992; Bell et al. 2000; Scales 2002; Allen et al. 2004; Bell 2007

Table 1: Number of key UK Mesolithic sites

The British Mesolithic can therefore be seen to be broadly defined by mobile hunter-gatherer-fisher groups occupying an environmentally changing landscape. These populations developed a range of sophisticated technologies, shown through flint, stone, bone and antler industries, and the construction of structures. Yet despite this, the period has traditionally been viewed as being characterised by cultural adaptations to the environment, and populations being dominated by environmental and climatic pressures. As such, approaches to the British Mesolithic have been predominately functional or ecological in nature (Young 2000). This focus on ecological factors has however also led to a range of bioarchaeological approaches to study; with the term ‘bioarchaeology’ even being coined by Clark himself in his study of Star Carr (1972). Clark was in fact the first to apply such kinds of ecological theory to the prehistoric past, focusing on linking archaeological assemblages with their landscape and natural settings (Clark 1939; 1952; 1980). The functional approaches adopted by Clark however have persisted within Mesolithic archaeology in Britain, with much work undertaken on ideas of seasonality, site catchment analysis and optimal foraging theories (Roper 1979; Monks 1981; Alden Smith 1983; Mithen 1988; 1990; 1991; Layton et al. 1991; Russell et al. 1995; Rowley-Conwy 2004(b)). As a result, much less focus has traditionally been placed on ‘lifeways’ or social aspects of Mesolithic life (as highlighted by Young (2000) and Warren (2010)). Due to this, we have fewer academic ideas about how people may have actually lived in the Mesolithic period in Britain, as logistic organisation of sites and people has generally taken primacy over interpretations of social facets of the Mesolithic (Spikins 2008). The British Mesolithic is therefore seen to be ‘lagging behind’ theoretically, especially when compared to studies of the British Neolithic (Conneller and Warren 2006) – with the period often being considered as a “chronological period rather than a social epoch” (Milner and
Woodman 2005, 4). In recent years new theoretical perspectives have slowly been applied to the period nonetheless, in particular, ideas on ritual and ideology (Connelly 2000; 2004; 2011; Chatterton 2006; Milner and Mithen 2009), as well as work on gender (Gero 1991; Bevan 1997; Schmidt 2000; Sternke 2005; Pugsley 2005), and potential cognitive inferences of hunter-gatherers (Mithen 1990; 1991; Zvelebil 2009).

It can be seen however that the Mesolithic has traditionally been an understudied period of prehistory, particularly compared to its preceding Palaeolithic and succeeding Neolithic neighbours – lacking the artwork and new cognitive abilities seen in the Palaeolithic, and the advent of farming, monuments and ‘civilisation’ in the Neolithic. Indeed, the Mesolithic was traditionally viewed as a “transition period” between the Palaeolithic and Neolithic (Burkitt 1926, 8), with the alternative terms ‘Epi-Palaeolithic’ and ‘Proto-Neolithic’ being posited (Clark 1932, 1), which perhaps highlight contemporary views of the epoch. Indeed, even Clark’s 1980 publication on the Mesolithic was subtitled “The Palaeolithic-Neolithic Transition in Old World Prehistory”. The following quote perhaps sums up prevailing ideas on the Mesolithic which, for some reason, appear to have persisted almost right up to the present day:

“Mesolithic times as a whole are perhaps rather unprogressive and present scenes of primitive culture little relieved by either wealth of industries or beauty of art. But with the arrival of the Neolithic civilisation among these primitive people a sudden change took place and cultures containing the germs of many modern developments soon grew up and progressed rapidly” (Burkitt 1926, 47)

As Warren (2010, 16) rightly points out however, the problem with academic study of the Mesolithic is not the archaeological evidence for the period itself, but the preconceptions which archaeologists subconsciously attach to it – namely that all material is associated with mundane economic activity (i.e. the gathering of food). It can therefore be seen that there is perhaps a gap for bioarchaeological applications to early prehistoric material in the UK, and that by incorporating biomolecular approaches to the period, we may be able to reverse the longstanding view of the Mesolithic as a time of “cultural stagnation” (Fagan 2001, 27).
2.3. Defining the Neolithic

“The still dominant understanding of the Neolithic in Britain rests upon its identification as primarily economic phenomenon”

(Thomas 1999, 7)

The term ‘Neolithic’ was first coined by Lubbock in 1865, and was originally devised to describe technologies based on ground and polished stone artefacts, and pottery (Bradley 2007, 27). Therefore, whilst the initial use of the term ‘Neolithic’ was readily adopted, and represented a technological phenomenon and a stage in a general evolutionary scheme, the dominant understanding and definition of the period today is as “a primarily economic phenomenon” (Thomas 1999, 9), but with specific cultural expressions and the development of new ways of thinking (Edmonds 1999, 5-6; Rowley-Conwy 2004(a)).

In terms of chronological definitions, the Neolithic period in Britain is generally taken to refer to the period c.4000–2400 cal. BC (Whittle 2009). The period has long been characterised and defined by the adoption of agriculture and perceived associated sedentary lifestyle, alongside the emergence of new forms of material culture and the construction of a range of monument forms (Cummings 2008). This characterisation has thus led to the idea of a Neolithic ‘package’ (consisting of farming, domesticates, sedentism, pottery, and polished stone artefacts), which has permeated studies and definitions of the period, and is thought to be reflective of changes in ideology and the adoption of new world views (Darvill 2010, 77; Rowley-Conwy 2004(a)). In recent years there has however been a move toward ideas of more mobile Neolithic populations, with the recognition that agriculture or horticulturalism does not necessary equate to sedentism (Rafferty 1985; Kent 1989; Thomas 1991; Edmonds 1995; 1999; Whittle 2003; Milner 2005(a)), and that pastoralist economies may have been practiced (see discussion in Chapter 9).

The concept of a ‘Neolithic package’ – frequently utilised a key defining feature of the period – can however be seen to stem from the idea of a ‘Neolithic Revolution’, which was first conceived by Childe in 1935. This ‘revolution’ was seen as a functional-economic stage, involving changes in food production, evidence of domesticated species, new stone artefacts, and pottery (Gamble 2007, 12) – thus bearing many similarities to the ‘Neolithic package’ concept. The Neolithic Revolution was proposed to be akin to, or indeed perhaps the forerunner of, the Industrial Revolution of the 19th century (Clark and Piggott 1976, 148), particularly in terms of population growth, the establishment of large settlements,
and systems of self-government. However, Gamble (2007, 16) suggests that Childe’s ideas of ‘revolution’ were simply a mechanism for his own views – opposing nationalism and the inevitability of totalitarianism – and are a reflection of the political climate of the 1930’s and 1940’s in which he was writing. This idea of a ‘Neolithic Revolution’ did persist within archaeology for a significant period of time however – for example, Clark and Piggott in 1976 described the ‘Neolithic Revolution’ as “comparable in economic and social importance to the Industrial Revolution of modern western Europe, and as the inevitable progenitor of urban societies and civilisation” (1976, 148). Even in 2000, Cauvin proposed that the Neolithic Revolution was representative of a transformation of the mind, and was a “revolution of symbolism” (2000, 71), and in 2007 Tilley proposed that the Neolithic Revolution was actually a “sensory revolution” (2007, 329) via which other changes altered individual’s experiential conditions of existence, leading to new ways of thinking and new ideas. The actual use of the term ‘revolution’ is however problematic, as it suggests that populations have total control over the situation they are creating, and are actively adapting their behaviour for best future success (Gamble 2007, 15). Other criticisms levelled at the idea of a ‘Neolithic Revolution’ are that ‘revolutions’ are simply convenient historical concepts which we can use to explain our current state, political systems, global relations and so forth (Clark 2003, 42). The idea of a ‘Neolithic Revolution’ also has inherent implications that the Neolithic brought with it a wave of new, revolutionary aspects of life, and additionally, promotes ideas of ‘checklists’ of the supposed revolution – which ultimately negates the fact that change will never occur in the exactly the same way or in the same timeframe in any two places. It over-simplifies the activities, actions and changes occurring in this period, and ultimately does not address the reasons why we see differences in the archaeological records of the Mesolithic and the Neolithic. Determining the process through which the Neolithic period emerged in Britain is complex however, particularly as the evidence from the Mesolithic into the Neolithic does not appear to follow a linear relationship of increasing social complexity, increased sedentism and the introduction of small amounts of farming in the Mesolithic, as was originally posited by culture-historical modes of thought, and as has been proposed for Scandinavia (Thomas 1988). Indeed, the beginnings of the Neolithic period in Britain are still poorly understood, and heavily debated (as discussed below, section 2.4.2.).

In contrast to the British Mesolithic in some ways, there is however a wealth of archaeological evidence from the Neolithic in Britain, which perhaps in part reflects why it has been more heavily studied – and has also meant that the archaeological material
The Neolithic is well known as a period which heralds the advent of agriculture in Britain – and is viewed archaeologically through evidence of cereal cultivation, the faunal remains of domesticated animals, and primitive furrows. As such, the new subsistence practices, diet and economy seen within the British Neolithic have become one of the key modes of definition of the period. Indeed, Thomas (1999, 7) even notes that within British study, the term ‘Neolithic’ is “often used as being synonymous with ‘mixed farming economy’”. The earliest evidence for cereal pollen in the UK dates to 4050-3850 BC, with the earliest charred cereal grains in Britain dated to 3950-3630 cal. BC. The domesticated species thought to be grown include emmer wheat (Triticum dicoccum), einkorn wheat (Triticum monoccoccum), and barley (Hordeum vulgare), alongside emerging animal husbandry of domesticated species – predominately cattle, pig, sheep and goats (Thomas 1999, 8, Brown 2007; Bradley 2007, 32; Darvill 2010, 88). Evidence of cereal cultivation is however seen not only through pollen records and carbonised plant remains, but also via phytoliths, starch grains, impressions on pottery, and artefacts such as sickles and querns (Darvill 2010, 90; Langlie et al. 2014). Tied to this is the emergence of an agricultural landscape, and whilst field-systems are not widely known (apart from at sites such as Fengate and at Céide Fields, Ireland), furrow marks have been found at number of sites (e.g. Windmill Hill), suggesting that although it was traditionally assumed that hoe cultivation was practiced in the Neolithic, more advanced systems of husbandry may actually have been utilised (Smith 1974; Thomas 1999, 10).
A wide range of monument forms are seen to emerge in the Neolithic, and it has been suggested that the landscape of the Britain at this time “was framed by enduring built monuments” (Whittle 1996, 235). In the Early Neolithic (4000-3200 BC), monuments generally take the form of chambered tombs, wooden mortuary structures, cursus monuments, long barrows, and causewayed enclosures; whilst in the Late Neolithic (3200-2500 BC) henges, stone circles, timber circles, and palisades were predominately constructed (Cummings 2008). The idea of ‘monumentality’ as a defining feature of the British Neolithic was first suggested by Renfrew (1973), although the study of Neolithic monuments dates back to the antiquarians of the 18th and 19th centuries. Renfrew (1973) adopted a new processualist approach to the study of Neolithic monuments however, suggesting that their architecture was a direct reflection of social relations and organisation. By determining the effort required in the construction of different monuments, Renfrew (1973) proposed that the earlier Neolithic saw the emergence of chiefdoms, whilst larger late Neolithic monuments represented confederations of chiefdoms working together to construct them. Other interpretations of Neolithic monuments have instead suggested that they were linked to power relations, with only certain members of society being allowed to enter monuments (Barrett 1994), or that monumental architecture aimed to hide the ranked nature of Neolithic societies by giving the appearance that everyone in death was equal (Shanks and Tilley 1982). Monument construction has also been considered in terms of investment of time, risk, the nature of materials used, landscape setting, the revisiting or reworking of sites over time (Cummings 2008), and more recently, also patterns of movement which link sites across landscapes, and ideas of a ‘technology of memory’ (Edmonds 1999, 7). The scale of many monuments has also meant that their function has often come into question, and they have been variously interpreted as being used for feasting, gift exchange, ritual or ceremonial activities, as defensive positions, as cattle enclosures, in the establishment of power and social differentiation, and in the bringing together of different groups/communities (Whittle 1996, 274; Darvill 2010, 97). Monuments are also frequently associated with burial of the dead (discussed further in Chapter 3), but have primarily been interpreted as material representations of new ideologies and new ‘senses of being’ (Thomas 1991; Bradley 1998; Cummings 2007). It has also been suggested that monuments may in fact have had multiple meanings in the past, which related to how people understood the world around them – and that these meanings were constantly changing (Cummings 2008). Recent excavations at the site of Warren Field in Aberdeenshire have however pointed to
the idea that monumentality may have originated in the early Mesolithic instead (Hilts 2013).

Much research has also focused on evidence for Neolithic settlements and structures, in an attempt to determine levels of sedentism perceived to accompany agriculture. The most prominent evidence of Neolithic structures or ‘houses’ comes from Orkney, at sites such as Skara Brae, Barnhouse and Knap of Howar. Elsewhere in Britain, there is less evidence of structures, and the majority that are known are from southern England. The lack of structural evidence in England has been suggested to be due to modes of construction which did not leave subsoil traces (Whittle 1996, 233; Thomas 1999, 9; Bradley 2007, 44). Where present, Neolithic structures tend to be rectangular or square in plan, constructed of timber or occasionally stone, and often suggested to have been covered by wooden frames or skin tents (Bradley 2007, 41; Darvill 2010, 84; Whittle 2009). The traditional view of the Neolithic therefore, supported by agricultural and settlement evidence, is that communities were highly sedentary. However, more recent work has suggested that earlier Neolithic landscapes in particular may have been fragmented and dispersed, populated by small kinship groups, and based around patterns of structured movement or short term sedentism (Edmonds 1999, 16; Whittle 2009; Milner 2005(a); see also Chapters 7 and 9).

Finally, we also see new forms of material culture in the Neolithic – notably the emergence of pottery, and polished ground stone tools – which have also been seen as a defining characteristic of the period. Pottery takes a range of different forms and fabrics – from undecorated Carinated Bowls in the Early Neolithic to later, more decorative styles – and is seen as “one of the principal innovations of the period” (Thomas 1999, 96). The function of pottery is believed to have been for storage and serving of food, and also the warming/cooking of foodstuffs, indicated by residue analyses (discussed further below) (Bradley 2007, 29; Whittle 1996, 277). As such, pottery is generally considered to be related to cultural identity and new mediums of sharing or serving foods, and the emergence of new forms of cuisine; in that foods could be combined in new ways, linked to Lévi-Strauss’ ideas of endocuisine (Lévi-Strauss 1969; Jones 2007, 159). However, it is interesting that pottery is considered such a key aspect of the ‘Neolithic package’ given that there are numerous archaeological and ethnographic examples highlighting that ceramics are not restricted to either sedentary communities or agriculturalists (Brown 1989; Hoopes and Barnett 1995).
New stone and flint tools also emerge in the Neolithic – notably axes and leaf-shaped arrowheads – and flint mines are known at a range of sites (e.g. Grimes Graves, Church Hill, Blackpatch) (Smith 1974; Edmonds 1995). Neolithic axes in particular have been widely studied, and have also been considered to have a subjective significance beyond their utility, shown through their deposition in tombs and ‘ritual contexts’, along with symbolic chalk replicas (Hodder and Lane 1982; Hodder and Hutson 2003, 152; Whittle 2009). Other interpretations have suggested that axes were important due to their roles in exchange networks, forming a “mobile set of social relationships” (Thomas 1996, 159).

Unlike the British Mesolithic, there are so many Neolithic sites known in Britain at present that it is not possible to provide an overview of the ‘main’ or ‘key’ British Neolithic sites here – but the quantity and variety of Neolithic sites (particularly those which are monumental) are often cited as a defining factor of the period in Britain. A number of examples of sites are given below in Table 2 to highlight this variety seen within the period. Many of the sites chosen below have also been studied extensively for significant periods of time, and are well known outside of the archaeological discipline.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Date</th>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Sweet Track</td>
<td>Somerset Levels</td>
<td>3800 cal. BC</td>
<td>2km wooden (oak) trackway across wet fenland</td>
<td>Coles and Orme 1981; Coles and Coles 1986; Hogan and Maltby 1996; Brunning et al. 2000</td>
</tr>
<tr>
<td>Avebury</td>
<td>Wiltshire</td>
<td>2850 BC</td>
<td>Henge monument</td>
<td>Gillings and Pollard 2003; Brown et al. 2005; Gillings et al. 2008</td>
</tr>
</tbody>
</table>
The British Neolithic can therefore be seen to be defined not only by agriculture, but also increased and new forms of materiality – from new types of material culture to monuments and new settlements and structures. It has been suggested that this “increased constructed materiality of life provided a whole new arena for social manipulation and engagement” (Bailey and Whittle 2005, 5). It is however also interesting to note the broad distinctions made in studies between the Mesolithic and Neolithic periods in Britain, with the Neolithic predominately seen as a time of political, monumental landscapes with rich material culture, in comparison to the natural, highly romanticised and apolitical landscapes of the Mesolithic (Conneller 2010).

As a result, Neolithic research has been suggested to be at the forefront of theoretical studies for the past 25 years (Whittle 2009), and the period has long been considered in both economic and ideological terms (Bradley 1998, 13). In particular, from the 1980’s onwards the Neolithic has also been the subject of a range of post-processual and phenomenological approaches. For example, a number of scholars have focused on auditory scene analysis, looking at associations of acoustic information within Neolithic settlements or sites (Lawson et al. 1998; Watson and Keating 1999; 2000; Mills 2000; 2005). In contrast to Mesolithic studies, much focus has also been placed on phenomenology and looking to generate models of lived experience, aiming to see the Neolithic as a distinctive form of social existence. These kinds of approaches highlight how other epistemological positions lack adequate consideration of agency, and suggest
that people in the past may have related to the world around them via understanding gained through years of dwelling and mnemonic geographies (Tilley 1994; Edmonds 1999; Thomas 1999; Hodder 1999; Ingold 2000; Brück 2001; Hodder and Hutson 2003, 106; Tilley 2007; Harris 2010). However, whether phenomenological introspection and hyper-interpretive texts will ever truly determine the original nature of experience in the past is still debated (Gazzaniga 1998, 21; Hodder and Hutson 2003, 117; Fleming 2006).

Alongside these varying post-processual theoretical interpretations however, there has also been a significant amount of scientific work being undertaken, focusing predominately on stable isotope analysis (see Chapter 4) and organic residue analysis (e.g. Craig 2001; Agozzino et al. 2001; Copley et al. 2005(b); Mukherjee et al. 2008; Craig et al. 2015).

Even Neolithic interpretations have however traditionally been grounded in a meta-narrative of continuous, progressive development toward the present (Thomas 1999, 2). For example, the Late Neolithic is often seen as a period of population growth and economic intensification – despite little archaeological evidence to support this (Whittle 2009). Furthermore, the idea of the Neolithic as a time of social and economic change, sedentism, and agriculture, still forms the basis of nearly all study of the period – and there are still widespread generalisations surrounding mobility, sedentism, and farming which permeate study of the period (Rowley-Conwy 2004(a); Bailey and Whittle 2005). The idea of the Neolithic heralding the emergence of ‘civilised societies’ is also an idea which appears to have permeated studies of British prehistory – even very recent texts advocate these notions: “farming made civilization possible” (Lieberman 2013, 203).

2.4. Defining the Mesolithic–Neolithic Transition

The Mesolithic-Neolithic transition is an archaeological timeframe which has perhaps been studied more than any other, and which has been described as “the most important event in human prehistory” (Price 2000(a), 1). Our fascination with this early period of prehistory has gripped scholars since Darwin’s 1868 work ‘The Variation of Plants and Animals Under Domestication’. The ways in which the transition has been studied, defined and understood have differed somewhat over the last century however, mainly due to the emergence of new theoretical positions within the archaeological discipline. Differences in the study of the Mesolithic-Neolithic transition are also due in part to the nature in which we have defined the periods known as the ‘Mesolithic’ and the ‘Neolithic’ – traditionally either through changes in material culture, or changes in economy and subsistence, or
simply imposed chronological frameworks. However, unsurprisingly, all current methods of definition of the Mesolithic and Neolithic periods are problematic. Due to this, there are therefore also flaws in our determinations and definitions of how and when the transition between the two periods occurred in Britain.

Chronological definitions, as noted in section 2.1., are always extremely complex. Placing nomenclature upon chronological periods, whilst advantageous in many ways, can also sometimes hinder interpretations as archaeologists are confined to prescribed frameworks. In particular, trying to tightly define exactly when the Mesolithic-Neolithic transition occurred is problematic – not least because in order to define when the transition occurred, we need to have a clear consensus on what the transition was, how this may be reflected in the archaeological record, and how it occurred. In terms of material culture or technological definitions used in separating the Mesolithic and the Neolithic, boundaries are again blurred as we see elements of both continuity and change. For example, there is continuity in many of the modes of flint working; microliths are known to have continued use into the Neolithic, and the manufacture of stone axes is thought to have begun in some areas in the late Mesolithic (Edmonds 1995; Edmonds 1999, 19 & 42; Spikins 2002, 43; David and Walker 2004; Holgate 2004; Costa et al. 2005; Bradley 2007, 27 & 34; Hey and Barclay 2007). In other areas we also sometimes see ‘mixed’ assemblages containing both pottery and microliths (Schulting 2000). Definitions and delineations of the Mesolithic and Neolithic using economic or subsistence strategies are also problematic – primarily because they do not account for regional variability, and also because the terms used (e.g. ‘hunting’, ‘foraging’, ‘gathering’, ‘farming’) are rarely defined (Ingold 1991). The modes of subsistence occurring across the UK may have differed significantly, being defined instead by the landscape, local ecology, and natural resources available. Equally, there are also numerous examples of populations across the world who have mixed hunter-gatherer/cultivation economies (Lourandos 1980; Sandbuckt 1988; Solway and Lee 1990; Layton et al. 1991) – does farming therefore have to be the sole defining characteristic of the Neolithic?

These difficulties in definition are also based in part on a lack of archaeological evidence, and additionally on a desire to tightly define this period in some way (Woodman 2000) – thereby also serving to highlight a key question: why are we so fascinated by the Mesolithic-Neolithic transition? The previous sections provide a brief overview of the archaeological evidence for the Mesolithic and Neolithic periods in Britain respectively,
and expose the significant differences which we perceive to be present between the two ‘periods’. In part perhaps, the preoccupation with the Mesolithic-Neolithic transition stems from the unknown – despite years of study, we still do not fully understand the nature, speed, timings or mechanisms behind the adoption of agriculture in the UK, and the associated changes in lifestyle, living, technology and material culture which are believed to have accompanied this. The Neolithic has however classically been heralded as the beginning of ‘civilised’ societies – the idea of a transition from wild, savage, hunter-gatherer types to the sedentary, sophisticated civilisations which formed the basis of today’s modern day populations (Gamble 2007, 17) – “agriculture is not merely a necessary pendant to civilisation; it is its life force” (Massingham 1926, 207). As such, the Neolithic has traditionally been viewed as a period which “allowed the population of Britain for the first time to gain mastery of its environment, and so to rise from brute savagery to the higher levels of barbarism” (Atkinson 1956, 148). The idea therefore, proposed in more recent years, that there may not have been a smooth continuum of increasing complexity from the past to the present day, and even more so, that there may have been major horizons of cultural discontinuity at a fundamental level in the prehistoric past (Thomas 1991, 2) is also something which this research aims to attempt to examine.

Although current definitions of the transition are problematic and challenging in a number of ways, there is, as archaeologists, a need to find suitable means via which we can distinguish between ‘periods’, and categorise to an extent the material which we recover from specific chronological or cultural contexts. It is clear that there are distinct changes between the Mesolithic and Neolithic periods, and as such, current definitions of the periods have tended to focus on the very visible aspects which are different between the two periods – for example, the move from hunter-gatherer-fisher modes of life to the introduction of agriculture (i.e. a significant subsistence change); or changes in material culture, notably the introduction of pottery; or changes in mobility from a perception of highly mobile hunter-gatherer populations to more sedentary agricultural communities.

The following sections will explore various areas of change at the Mesolithic-Neolithic interface (diet, demography, health, and social structures) in more detail, and discuss how these may be useful in our studies of not only the transitional period, but also the Mesolithic and Neolithic periods in Britain respectively. All can be seen to be viable ways in which we can study and define the period, although some have received more scholarly attention than others – and some are more easily viewed in the archaeological record of
Britain. Nonetheless, these are themes which run throughout this thesis, and which are considered again in Chapter 9.

2.4.1. Dietary Change

Dietary change and the adoption of agriculture have traditionally been seen as one of the most fundamental aspects of the Mesolithic-Neolithic transition in Britain, marking a social, cultural and biological change, and the development of human control over the reproduction and evolution of animals and plants (Stock and Pinhasi 2011). Thus, the move from hunter-gatherer-fisher lifeways to agricultural modes of subsistence with domesticated plant and animal species has been extensively studied within the archaeological discipline. In recent years however, the application of stable isotope analyses has formed the basis of the majority of work on Mesolithic-Neolithic dietary change – with focus being placed specifically on the nature, timings and mechanisms of subsistence shift. It should however be noted that this focus on stable isotope analyses in the study of Mesolithic and Neolithic diets has in part stemmed from the relative scarcity of other dietary information available from the periods in terms of organic and faunal remains – “trying to work out the relative importance of plant and animal foods in the ancient diet on the basis of floral and faunal remains is probably impossible” (O’Connell et al. 2000, 203).

Mesolithic diet in Britain is typically seen as being characterised wholly by a dependence on wild resources (both plant and animal) and significant contributions of marine protein. Evidence of potential foodstuffs has predominately been found at shell midden sites, along with large faunal assemblages from sites such as Star Carr and Thatcham. Whilst significant archaeological focus has been placed on the high marine protein component of Mesolithic diets, an idea of the potential range of terrestrial resources populations may have exploited is suggested in Table 3 and in Bishop et al. (2014). In terms of floral remains, there is often little evidence beyond hazelnut shells at Mesolithic sites in Britain – the importance of which, in terms of dietary contribution, is still debated (Milner 2006). However, in general, study of Mesolithic subsistence and diet has predominately focused on economic models, availability of resources, and hunter-gatherer practices. As such, Milner (2006, 63) suggests that interpretations of Mesolithic subsistence “have barely changed over the last 50 years”.
<table>
<thead>
<tr>
<th>Star Carr Flora and Fauna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roe deer (<em>Capreolus capreolus</em>)</td>
</tr>
<tr>
<td>Red deer (<em>Cervus elaphus</em>)</td>
</tr>
<tr>
<td>Elk (<em>Alces alces</em>)</td>
</tr>
<tr>
<td>Pig (<em>Sus scrofa</em>)</td>
</tr>
<tr>
<td>Auroch (<em>Bos primigenius</em>)</td>
</tr>
<tr>
<td>Hare (<em>Lepus europaeus</em>)</td>
</tr>
<tr>
<td>Beaver (<em>Castor fiber</em>)</td>
</tr>
<tr>
<td>Bear (<em>Ursus sp.</em>)</td>
</tr>
<tr>
<td>Fox (<em>Vulpes vulpes</em>)</td>
</tr>
<tr>
<td>Dog (<em>Canis familiaris</em>)</td>
</tr>
<tr>
<td>Pine marten (<em>Martes martes</em>)</td>
</tr>
<tr>
<td>Badger (<em>Meles meles</em>)</td>
</tr>
<tr>
<td>Hedgehog (<em>Erinaceus europaeus</em>)</td>
</tr>
<tr>
<td>White stork (<em>Ciconia ciconia</em>)</td>
</tr>
<tr>
<td>Common crane (<em>Grus grus</em>)</td>
</tr>
<tr>
<td>Red-breasted merganser (<em>Mergus serrator</em>)</td>
</tr>
<tr>
<td>Re-throated diver (<em>Colymbus stellatus</em>)</td>
</tr>
<tr>
<td>Great crested grebe (<em>Podiceps cristatus</em>)</td>
</tr>
<tr>
<td>Little grebe (<em>Podiceps ruficollis</em>)</td>
</tr>
<tr>
<td>Lapwing (<em>Vanellus vanellus</em>)</td>
</tr>
<tr>
<td>Buzzard (<em>Buteo buteo</em>)</td>
</tr>
<tr>
<td>Duck (<em>similar to Anas acuta</em>)</td>
</tr>
</tbody>
</table>

Table 3: Range of Mesolithic foodstuffs available at Star Carr (adapted from Milner 2006, 72)

In sharp contrast, British Neolithic diets appear isotopically to be dominated by C3 plant resources – thought to be domesticated cereals – and terrestrial animals, with little or no marine protein input. Introduced domesticates in Britain are thought to have included emmer wheat (*Triticum dicoccum*), einkorn wheat (*Triticum momococcum*), and barley (*Hordeum vulgare*), alongside domesticated faunal species – predominately cattle, pig, sheep and goats (Thomas 1999, 8, Bradley 2007, 32; Darvill 2010, 88; see also section 2.3.). The contribution of crop-derived protein to Neolithic diets has been suggested however to typically be underestimated (Bogaard et al. 2013). The introduction of dairy (either raw milk or processed milk products) into the diet, obtained from domesticated fauna, is also variously debated to have occurred in the Neolithic in Britain (as discussed in greater detail in Chapter 8). Evidence for dairying has primarily come from organic residue analysis on Neolithic pottery (e.g. Craig 2001; Copley et al. 2003; Craig et al. 2005; Cramp et al. 2014(a); 2014(b); Smyth and Evershed 2015), and zooarchaeological analyses and mortality profiling (e.g. Legge 2005; Greenfield and Arnold 2015).
Stable isotope analysis of $\delta^{13}$C and $\delta^{15}$N has been most frequently used in the study of both Mesolithic and Neolithic diets, and in determining dietary change between the two periods. A scientific overview of how stable isotope analysis works can be found in Chapter 4, and measured isotopic compositions of Mesolithic and Neolithic diets in Britain can be found in Appendix B, as well as Chapters 5 and 7. In particular however, stable isotope analysis of Mesolithic and Neolithic human remains has resulted in the idea of a ‘rapid’ dietary shift at the transition. Whilst it was traditionally thought that dietary changes were introduced gradually over a significant period of time, more recent isotopic results have led to the idea that subsistence change may have been very significant, and occurred relatively quickly (Figure 5; see also Chapter 5). However, whilst the graph below shows a change in $\delta^{13}$C values at the onset of the Neolithic period in Britain, it is worth noting the number of points plotted for the Mesolithic and Neolithic periods respectively. As can be seen below, prior to c.5,500 years BP there are only a handful of human isotopic values known, and also very few from the period directly surrounding the Mesolithic-Neolithic transition, c.4,000 cal. BC (c.6,000 BP).

![Figure 5: Graph depicting a shift in $\delta^{13}$C values at the onset of the Neolithic period in Britain. Squares represent individuals less than10km away from the coast, and crosses are individuals from greater than 10km away from the coast (Richards et al. 2003)](image)

This idea of a rapid dietary change at the Mesolithic-Neolithic transition in Britain – from a dependence on marine resources to an entirely terrestrial diet – is based almost solely
upon isotopic analyses undertaken on late Mesolithic human remains from Oronsay, Inner Hebrides (Mellars 1987; Richards and Mellars 1998; Richards and Sheridan 2000; Richards and Schulting 2003; see also Chapter 5). Overall, the dominant narrative which has emerged is that dietary change at the transition was abrupt, and that a change in subsistence occurred in tandem with changes in material culture, the appearance of domesticated plant and animal species, and monumentality – thus suggesting a rapid and dramatic change c.4000 cal. BC (Richards 2003). It has also resulted in the idea that early Neolithic individuals were actively engaged in the avoidance of marine resources – prompting suggestions that fish and other marine foods may have been seen as ‘taboo’, which in turn could be a reflection of new world views or beliefs (Richards 2003; Thomas 2003). This idea of the apparent wholesale cessation of the consumption of marine foods in the Neolithic has, perhaps unsurprisingly, come under criticism from a range of authors – most notably Milner et al. (2004), who highlight the wealth of non-isotopic evidence suggesting continued consumption of aquatic resources into the Neolithic period; such as archaeological evidence for Neolithic fishing and continued use of shell midden sites, as well as the small sample sizes used in isotopic study and the flaws in using only isotopic analyses to determine information on diet. The dangers in making ideological interpretations (e.g. on beliefs or taboos) without fully understanding diet have also since been highlighted (Milner et al. 2006). The work undertaken on the Oronsay material has however also been critiqued in a variety of ways – “considering that Oronsay currently supports a population of five people, the wider applicability of these results remains to be seen” (Noble 2010, 126). Dietary change at the site of Cnoc Coig, Oronsay is however discussed in further detail in Chapter 5. Finally, Mesolithic-Neolithic isotopic work has also prompted questions about how dietary protein may be routed, and if there is some way in which marine foodstuffs are being under-represented in Neolithic bone collagen (Lee-Thorp 2008; see also Chapter 4). It can thus be seen that the issues surrounding the idea of a ‘rapid’ dietary shift are potentially manifold – and in particular that there are numerous issues with the sample sizes used in these studies – thus highlighting a need for the identification of more late Mesolithic and early Neolithic human skeletal material, and also more careful consideration of how we define a ‘rapid’ change (as discussed in more detail in section 2.4.5).

The issue of using stable isotopes in palaeodietary reconstruction are discussed in Chapter 4 (section 4.3.), but the problems in making bold interpretations from isotopic data for the Mesolithic and Neolithic periods in Britain are further compounded by additional issues,
such as the scarcity of faunal isotopic values available for the British Mesolithic. Due to this, a database of known UK Mesolithic and Neolithic faunal isotopes was created during this research (Appendix B), and faunal baselines were included within all isotopic studies undertaken here. The relative scarcity of known human remains from the later Mesolithic and early Neolithic (as discussed in Chapter 3) is an additional major limiting factor in determining dietary change. Due to the lack of Mesolithic human remains available for study in Britain, a number of authors have attempted to use dog isotopic values as a proxy for human diets (Clutton-Brock and Noe-Nygaard 1990; Schulting and Richards 2002(b); 2009) – resulting in various debate (Day 1996; Dark 2003). However, it is also worth noting that the majority of Neolithic skeletons used in dietary comparison analyses are from long barrows and causewayed enclosures dated to 200-300 years after the Mesolithic-Neolithic transition is thought to have occurred (Thomas 2008). In other words, most directly dated early Neolithic human remains fall after 3800 cal. BC, leaving a number of ‘unknown’ centuries (Schulting 2011).

Finally, smaller amounts of work have tried to determine Mesolithic and Neolithic diets via lithic microwear and organic residue analyses (Shafer and Holloway 1977; Piperno and Holst 1998; Dominguez-Rodrigo et al. 2001; Wadley et al. 2004; Borel et al. 2014), which along with providing information on what stone tools may have been used for, may also provide some insight into the food resources exploited in the Mesolithic and Neolithic, and thus may have potentially also been consuming. Work on Neolithic pottery has also used organic residue analysis (Dudd et al. 1999; Craig 2001; Agozzino et al. 2001; Copley et al. 2003; 2005(a); 2005(b); Craig et al. 2005; Mukherjee et al. 2007; 2008; Cramp et al. 2014(a); 2014(b); Smyth and Evershed 2015), analysing food residues on ceramics and potsherds derived from cooking or the storage of foodstuffs. A recent study on Neolithic ceramics from the British Isles also suggested that it provided evidence supporting the idea of a rapid dietary transition and the avoidance of marine foods in the Neolithic – as no marine lipid biomarkers were found within the pottery studied (Cramp et al. 2014(a)). The authors suggest their findings provide “unequivocal” evidence of the “rejection of marine resources by early farmers” (Cramp et al. 2014(a), 1). However, the extent to which this may be true is debatable – it is entirely possible that Neolithic peoples were still consuming marine foodstuffs, but that they simply did not process them within pottery (see also Chapter 5).
Understanding the dietary changes which occurred between the Mesolithic and Neolithic periods in Britain is therefore a crucial part of understanding the nature, timings and mechanisms behind the transition itself. The subsistence changes visible between the two periods represent not only a change in diet, but also would have also had an effect on a wide range of other aspects of everyday life. Hunting, gathering and fishing signify a very different way of life to farming and herding – they present different demands on time, labour, organisation, settlement size, group structure, surplus, storage, seasonality, mobility, and the possible emergence of social inequality (Schulting 2011). Stable isotope analysis has become the dominant methodology used in the study of dietary change at the Mesolithic-Neolithic transition, but it is crucial to remember that isotopic homogeneity does not necessarily equate to dietary homogeneity – something which is perhaps particularly important to consider when looking at Neolithic populations (Schulting 2011; see Chapter 7). Indeed, stable isotope analysis has been suggested to be “a rather blunt instrument when considered in the context of the likely complexity of diets” (Thomas 2008, 73). Nonetheless, stable isotope analysis provides a useful and direct tool in the study of past diet and dietary change as it delivers a critical and unique means via which to determine dietary change. As such, isotopic analysis is perhaps at present the best method via which to gain an insight into Mesolithic and Neolithic diets, and due to this, was utilised within this research.

2.4.2. Demographic and Population Change

Demographic change at the Mesolithic-Neolithic transition has long been a source of interest for archaeologists, and is primarily linked to the proposed mechanisms behind the transition, and has also been suggested to be related to the new modes of subsistence which are seen to occur within the Neolithic of Britain. As such, significant amounts of work have focused on the potential mechanisms behind the Mesolithic-Neolithic transition, and on potential population movement or replacement. Currently, there are two main theories: either that the transition was caused through indigenous acculturation (via contact with continent) or by the influx of new populations (Milner and Craig 2009) – i.e. the movement of ideas OR the movement of peoples. In recent years this debate has become increasingly polarised, with proponents of both theories publishing opposing research – a debate which has been described as being characterised recently by “unfruitful and heuristically dubious dichotomous conceptualisations” (Marciniak 2011).
The idea of the Mesolithic-Neolithic transition being caused or driven by incoming population(s) and an associated new economic system was proposed very early on – with Clark (1966, 176), for example, stating, “the whole complex of technology, practices and ideas that make up our Neolithic culture must have been introduced from overseas” – and was the predominant theory prior up until the 1970’s, although it has recently been more readily adopted again (e.g. Bogucki 2000; Sheridan 2000; 2004; 2007; 2010; Pinhasi et al. 2005; Collard et al. 2010), despite being critiqued by a number of authors (e.g. Thomas 2007; 2008; Cummings and Harris 2011). Proponents of this theory nonetheless suggest the introduction of ‘packages’ of new practices, resources, ideas and modes of subsistence via new incoming, colonising populations from the European continent (Sheridan 2010).

Authors such as Piggott (1954), and more recently, Sheridan (2000; 2004; 2007; 2010), have suggested that an externally introduced Neolithic may have been caused by a series of independent arrivals of small migrant groups, perhaps from different areas or following different routes (Figure 6), who brought with them knowledge of agricultural practices and new material cultures – something Sheridan (2010) has termed a ‘multi-stranded’ colonisation. Whilst early proponents of the colonisation theory (e.g. Piggott 1954; Childe 1940; Case 1969) did not consider where immigrant populations to the UK may have come from, most recent supporters of this theory have suggested that incoming populations would have arrived in the UK from Brittany, Normandy, north and central France, and the Low Countries (Woodman 2000; Sheridan 2010; Cummings and Harris 2011). Yet, some authors (e.g. Thomas 2007; 2008) have questioned how these small influxes of new populations would have brought about the sudden extinction of the British Mesolithic and the adoption of new ‘Neolithic’ aspects of life. There are however historical examples of demographic expansion caused by small population movement/colonisation – such as the French Canadians and Dutch farmers who established the Cape Colony via initial migration of just a few thousand individuals (Cavalli-Sforza 2003). However, it has been suggested that it is these historical examples of agricultural expansion in the 18th and 19th centuries which have often been used to create colonisation models and have resulted in ideas of an ‘agricultural frontier’, and that direct analogues between historical events and the prehistoric past should be made with caution (Zvelebil 1986(a)).
Figure 6: Proposed routes of Neolithic colonisation of Britain, indicating multiple strands of colonisation (Sheridan 2010, 93)

The similarities between continental European material assemblages and those found in Britain, particularly with regards to the appearance and distribution of carinated bowl pottery, have been used extensively as evidence to support models of colonisation for the Mesolithic-Neolithic transition (Sheridan 2004; 2007; 2010) – but as Noble (2010) comments, we must not prioritise one form of material culture over any other, and there is a danger in aiming to characterise a specific form of material culture as being representative of a particular ‘identity’ or origin. Thomas (2007; 2008) has also criticised this idea that similarities or differences between material culture assemblages provide an indication of the degree of contact between human groups, and has instead proposed that British Neolithic assemblages appear to have selective elements of those found on the continent – thus indicating that there was not a wholesale transfer of continental assemblages at the outset of the Neolithic. Bradley (2007, 37) also makes a very valid point in his comment that “it is always easier to document the movement of objects than the spread of ideas”.

38
In contrast to the demic diffusionist approaches of colonisation theories therefore, ideas of an indigenous acculturation (cultural diffusion) model for the mechanisms behind the Mesolithic-Neolithic transition first became popular in the 1990’s, with the main proponent for this theory in the academic literature being Julian Thomas (1988; 2003; 2004(b); 2008). Acculturation models propose that indigenous hunter-gatherer populations actively chose to adopt Neolithic traits and material cultures, but whilst retaining aspects of Mesolithic lifestyles (e.g. mobility). Change is thus seen as occurring solely from within existing communities, and is not based upon a large-scale external demographic change. These kinds of approaches have also been dubbed ‘continuity models’, and have a basis in post-processual modes of thought, placing much less focus on economic factors, and instead seeing the Neolithic as a new form of identity adopted by Mesolithic peoples (Cummings and Harris 2011). Proponents of the indigenous acculturation theory frequently cite the paucity of house (or house-like structures) in Neolithic Britain as evidence of continued non-sedentary modes of life, and cereal agriculture is not seen as a primary subsistence practice (Thomas 1999; 2007; 2008; Fairbairn 2000; Price 2000(b)). The speed and ready adoption of certain ‘Neolithic’ traits in Britain has also been suggested to be representative of the fact that the Neolithic ‘package’ had taken on a character which meant that it could be readily assimilated by a native population (Thomas 2007).

Whilst Thomas (2007, 427) has proposed that “indigenous populations had a dynamic role in the formation of the British Neolithic”, arguments for an acculturationist, gradualist position on the Mesolithic-Neolithic transition have however also been critiqued by a range of authors (e.g. Monk 2000; Rowley-Conwy 2004(a); Warren 2005; Sheridan 2004; 2007; 2010) – with the main flaws in the model often suggested to be that it relies on the assumption that Britain prior to the transition was in contact with continental farming groups (despite little evidence for this), and that it is simply the wholesale application of a model originally posited for changes seen in coastal north-west Europe, where there are no sea borders between populations. Many authors also question how the Neolithic would have emerged and spread across Britain without actual human expertise arriving alongside domesticated plants and animals and new forms of material culture (Cummings and Harris 2011).

A number of academics have thus since suggested that a combination of the two theories – i.e. partial indigenous acculturation alongside incoming new populations – may be the
most parsimonious explanation for the Mesolithic-Neolithic transition in Britain (Smith 1974; Price 2000(b); Gkiasta et al. 2003; Whittle 2007; Bayliss et al. 2008; Darvill 2010; Fort 2012; Von Cramon-Taubadel et al. 2013). The idea that monolithic or dichotomous patterns cannot be used to define the mechanisms behind the Mesolithic-Neolithic transition is thus being more widely recognised (Price 2000(b)), with authors instead suggesting that models involving a combination of limited migration, rapid acculturation and extensive interaction may be more appropriate (Zvelebil 1986(a); Darvill 2010, 83). Potential evidence to support theories of movement of people – be they exogamous or endogenous – has also more recently been reappraised, particularly with regards to evidence of possible boat technologies (Warren 2000; Bonsall et al. 2013). The recent discovery of domesticated wheat DNA from a soil sample off the coast of the Isle of Wight dating to 8000 BP (Smith et al. 2015) has also renewed discussions regarding relationships between hunter-gatherers and farmers, and British contact with the continent within the Mesolithic (Larsen 2015), despite claims that the DNA may not in fact be prehistoric in date (Weiβ et al. 2015).

A recent paper by Cummings and Harris (2011) also proposed that focus should be shifted away from this polarised debate of indigenous acculturation vs. colonisation, to one which seeks to understand traditions and continuities of practice, suggesting a model for the Mesolithic-Neolithic transition which incorporates both incoming peoples and autochthons. Primarily, Cummings and Harris (2011) present the practices of hunting and gathering and relationships with animals as evidence for continuity between the two periods – a point which has since been criticised heavily, both in principle, and in terms of oversimplifying the archaeological evidence and for providing no direct definition of ‘continuity’ (Sheridan 2011; Pollard 2011; Marciniak 2011; Thomas 2011). Furthermore, it can also be levelled that Cummings and Harris (2011) do not consider the context of practice changes, or the regional context of change (i.e. that the transition may have occurred in different ways in different places), and the ideas posited of ‘hunting peoples’ suggest uncomfortable links to cannibalism and seem to have little archaeological basis. Nonetheless, the publishing of papers which aim to move away from polarised ‘migration’ vs. ‘acculturation’ models (e.g. Robb and Miracle 2007; Cummings and Harris 2011) must be praised.

It can therefore be seen that there are criticisms of all present theories on the mechanisms behind the Mesolithic-Neolithic transition, and none of the current models proposed fit the
evidence perfectly. Perhaps one of the major problems when looking at the causes and means of demographic and/or population change at the transition lies in the fact that it is generally considered that there are “only two possible processes which patterns of transition must be matched to” (Robb and Miracle 2007, 103). Current approaches to the Mesolithic-Neolithic transition have also been further criticised for making broad generalisations across large geographical areas, and often applying data from specific regions to much wider locales (Noble 2010). Debates surrounding the transition are also made more complex due to the lack of material evidence and subsequent radiocarbon dates for the horizon between the Mesolithic and the Neolithic periods (Edmonds 1999, 5; Price 2000(b); Woodman 2000). Finally, the current proposed models of the transition frequently negate ideas of social action, and instead only look at culture and economy (Robb and Miracle 2007), and are based on the idea that farming is a superior mode of production, regardless of context, seeing it as a dynamic economy (Zvelebil 1986(a)). Indeed, it is also suggested that current study of the transition considers people as constructed, essentialist categories, rather than social identities (Robb and Miracle 2007), perhaps due to the fact interpretations of the transition in Britain have largely “been based upon the relative merits of the different theoretical perspectives, rather than on archaeological criteria” (Woodman 2000, 224).

It can therefore be seen that there is perhaps a need to move away from traditional static or simple explanations of demographic change – and in particular we need to stop reducing agricultural origins and the Mesolithic-Neolithic transition to “a simple issue of colonisation or acculturation” (Noble 2010, 130). Instead, it is distinctly possible that the transition to agriculture may have been a dynamic process, and that it was not a singular broad-scale event – but instead occurred at different times and perhaps in different ways at different places. Interestingly, it is taken that Romanisation in Britain was a varied and complex process (Millet 1995, 13-25; Millet 1996; Hanson 2010), and also resulted in diverse populations (e.g. Leach et al. 2009) – why can we not also adopt these modes of thinking to the Mesolithic-Neolithic transition and the adoption of agriculture? Indeed, Hanson (2010, 175) describes the transition between the Iron Age and the Roman periods has having “no clearly defined break, but elements of overlap and continuity”. In the same way, we cannot presume that Mesolithic populations suddenly and ubiquitously became ‘Neolithic’.
Following on from this, it can therefore be seen that archaeologists often neglect the ways in which we view the Mesolithic-Neolithic transition in terms of scale, context, and regional variability. There is often a tendency with archaeology to lean towards a desire to determine broad-scale models of explanation – for example, many studies of the transition to farming have held a national or European-wide focus, often looking at change over two millennia (as highlighted by Noble 2010; e.g. Linden et al. 2013). Whilst there are benefits to looking at the transition in broader terms, the danger in these kinds of approaches lies in the fact that they imply that change happened in a uniform way – thus negating the possibility of variation due to numerous variables (e.g. topography, landscape, population sizes, etc.). Therefore, there is now the suggestion that the current modes of thought which are applied to the study of the Mesolithic-Neolithic transition may be too simple, dichotomist, and essentialist – Whittle and Cummings (2007) even go as far as to suggest that we should consider the possibility that all post-glacial societies may have been in a current state of ‘transformation’, thus making the Mesolithic-Neolithic transition a much less significant event in time. However, as Thomas (2004(a)) has rightly commented, it is likely that there was not “a single Neolithic in north-western Europe, but many ways in which localised communities made use of new economic and symbolic resources”.

2.4.3. Health Change

Our knowledge of health and disease in both the Mesolithic and Neolithic is poor in comparison to other aspects of these prehistoric periods. Primarily, our lack of knowledge stems from the paucity of human remains dating to the periods in Britain (as discussed in Chapter 3). Alongside this, our understanding of prehistoric health and disease is hindered by the same problems faced in trying to ascertain levels of health and disease in any archaeological period (also discussed in more detail in Chapter 4, section 4.6.1.).

It has long been assumed that the large scale changes to lifeways believed to have occurred at the Mesolithic-Neolithic transition would have impacted upon the health of populations during and after this transition. Significant lifestyle changes as seen at the transition may have altered health in different ways however. For example, following domestication, increased and closer contact with animals may have led to the development and/or increase in zoonoses. Equally, the sedentism perceived to have occurred with the advent of agriculture would have resulted in larger group or population sizes and increased contact with people within one location. This may have allowed for easier spread and transmission
of diseases, and perhaps even the development or increased virulence of (new) diseases. In this respect, the health changes which may have occurred at the Mesolithic-Neolithic transition could perhaps be suggested to be akin to those seen in the 18th-19th centuries in England, when large segments of the population moved from smaller rural populations into slum-like conditions in new cities. The increased population density which occurred from this (in the 18th/19thC) resulted in the emergence and increased virulence of many diseases, such as TB or cholera (Stolley and Lasky 1995, 32; Lönnroth et al. 2009; Roberts and Manchester 2010, 18). Whilst this should not be used as a direct analogy for the health and disease states which may have resulted from lifestyle changes at the Mesolithic-Neolithic transition, it is perhaps a good parallel to draw upon – and highlights how changes in population density, lifestyle and living conditions can drastically alter health. Within epidemiology, it is widely recognised that changes such as these can alter the “relation of humankind with parasites and the microbial world and [introduce] new threats to human health” (Stolley and Lasky 1995, 21), and it has even been suggested that the transition to agriculture “represents the first epidemiological transition” (Armelagos et al. 2005, 756). Ideas such as this have resulted in the creation of theories such as the ‘mismatch hypothesis’ and ‘dysevolution’, which propose that the transition to agriculture resulted in the emergence of new diseases such as type II diabetes, osteoporosis, cardiovascular diseases, asthma, and cancers. Dysevolution works on the premise that the rate of cultural evolution has since outstripped that of biological evolution, meaning that ‘mismatches’ occur and natural selection cannot filter out new diseases (Lieberman 2013, 168-9; Wheelwright 2015). Lieberman (2013, 202) proposes that there “are probably one hundred infectious mismatch diseases that were caused or exacerbated by the origin of agriculture”; although how far this is true is unclear, particularly given the difficulties associated with studying infectious diseases in prehistory. Others, such as Zuk (2013), have however also questioned suggestions that biological evolution has occurred too slowly for our bodies to adapt genetically to agricultural lifestyles and diets – instead noting that increased population sizes may have allowed for evolution to occur at a quicker rate, and also that a number of well-characterised genetic adaptations are known to have occurred since the transition to agriculture (such as lactase persistence (see Chapter 8), and sickle cell alleles). On an evolutionary level therefore, it is still unclear as to whether agriculturalism resulted in positive or negative changes to our genomes and overall health states.

Many of the human remains which are known from the British Mesolithic and Neolithic have not been studied in great detail with regards to health (Roberts and Cox 2003, 45) –
instead much focus has been placed on diet (via isotopic analyses) and burial contexts. For example, whereas the 2006 volume ‘Mesolithic Britain and Ireland: New Approaches’ (Conneller and Warren 2006) contains a section on ‘Death’, this is focused upon mortuary treatments, depositional contexts and possible Mesolithic perceptions of death (Conneller 2006). The potential causes of the deaths discussed are not considered. Similarly, in Pinhasi and Stock’s 2011 volume, ‘Human Bioarchaeology of the Transition to Agriculture’, there are no sections or contributions dedicated to health or disease, surely a significant aspect of human bioarchaeology as a discipline.

Where data is available, the majority of osteological evidence for disease from the Mesolithic period appears in the form of joint disease, and also smaller frequencies of osteoarthritis and spinal degeneration – all of which are suggested to be linked to the active and mobile hunter-gatherer way of life. Where teeth are available for study, small numbers of caries and dental enamel hypoplasia (DEH) are seen in the dentition. Dental defects such as this have been suggested to have been caused in the Mesolithic by possible nutritional stress, perhaps linked to seasonal diets or food shortages (Roberts and Cox 2003, 49-51). In the Neolithic, it has been suggested that the emergence of density dependent diseases, such as infectious disease (although generally lesions indicative of non-specific infection), thought to be linked to increased population sizes, can be seen osteologically (Roberts and Cox 2003, 58). In particular, a number of papers have tried to assess the potential evidence of tuberculosis in Neolithic skeletons, predominantly through the use of lipid biomarkers (mycolic acid) and DNA (e.g. Hershkovitz et al. 2008; 2015; Masson et al. 2013; Borowska-Strugińska et al. 2014). There has also been the suggestion that the transition to agriculture also resulted in increased dental caries due to higher carbohydrate consumption, and a decrease in overall body size (Larsen 2011). A recent paper by Ruff et al. (2015) also suggested that increased sedentism in the Neolithic bought about a decline in limb bone robusticity, resulting in a more gracile skeleton.

It can therefore be seen that much further work is needed on Mesolithic and Neolithic health and disease in general, and that until we characterise the health and disease states which may have occurred in both these time periods, then it is problematic and challenging to accurately determine whether any change occurred between them. However, it can perhaps be reasonably postulated that the changes in lifestyle, diet, mobility, and living conditions which are thought to have occurred between the two periods must have resulted in a degree of change in terms of health and disease – although whether this was a positive
or negative change still remains to be seen. The timings and speed of any health changes also need to be taken into consideration – both in terms of the fact that changes to lifestyle, diet, mobility and so forth may have occurred over fairly significant time periods (as discussed throughout this chapter), and also the time period it may take for new diseases to emerge within a population. A closer examination of both British Mesolithic and Neolithic health and disease states is therefore now needed – and new and novel methods for determining these are perhaps required (as discussed further in Chapter 4, section 4.6., and Chapter 8).

2.4.4. Social Change

The changes in lifestyle perceived to have accompanied the Mesolithic-Neolithic transition have also been suggested to have resulted in social changes within British populations at this time. Indeed, Darvill (1987, 48) suggests that the transition from the Mesolithic to the Neolithic represents the “most significant social transition…ever to have taken place”. In particular, mobile hunter-gatherers and sedentary agriculturalists represent very different modes of life and social experiences. For example, increased sedentism and larger population sizes will have different social dynamics and structures compared to smaller hunter-gatherer groups.

The social changes perceived to have occurred at the advent of the Neolithic have frequently been suggested to be linked to the emergence of inequalities and new social hierarchies. Specifically, agricultural subsistence allows for the creation of surpluses – which in turn may have resulted in the formation of new social orders (Bender 1978; Price 2000(b)). In tandem with this, the new material culture forms which emerge in the Neolithic, combined with new agricultural production methods, could have resulted more easily in socio-economic inequalities and status displays (Price 2000(b)). New modes of food production seen in the Neolithic period are also thought to be linked to feasting activities, which again have a significant social and status display aspect (Hayden 1990; Jones 2007). However, others have suggested that the origins of social inequality and complex social structures lie earlier, in the Mesolithic or even the Palaeolithic, and have challenged the traditional notion that hunter-gatherer groups are purely egalitarian (e.g. Kelly 2007; Wengrow and Graeber 2015). Therefore, whilst new cultural aspects of the Neolithic may have had the propensity for increased social inequalities, to presume that all
Mesolithic groups were egalitarian is perhaps an oversimplification, and negates the complexities of hunter-gatherer-fisher populations.

The Neolithic has also been suggested by many to have heralded another social change however – the increased or total emergence of violence within populations. The increase of interpersonal violence, or emergence of warfare, may have been caused by greater economic complexity and social stratification (Hutton Estabrook 2014). Keeley (1997) has also suggested that on mainland Europe, violence emerged between late Mesolithic hunter-gatherers and the new LBK farmers as the farming communities colonised northern Europe. Evidence for increased violence in the Neolithic has been posited due to finds such as mass graves (at sites such as Talheim (Wahl and König 1987) and Schöneck-Kilianstädten (Meyer et al. 2015), both in Germany; see also Chapter 3), and the predominance of axes and other ‘weapons’ in Neolithic contexts. The presence of arrowheads within graves, sometimes embedded into skeletons – such as at Ascot-under-Wychwood (Oxfordshire), Hambledon Hill (Dorset), and the Cat’s Water site (Fengate, Peterborough) – has also been suggested to be evidence of conflict (Mercer 1999).

However, evidence of ‘violent’ deaths is also known from Mesolithic contexts – such as the Bavarian site of Ofnet, which is thought to represent a massacre of individuals, with selected body parts (notably crania believed to have been decapitated) buried in two mass graves (Frayer 1997; Chapter 3). Evidence of skeletal trauma to the cranium and forearms and perimortem projectile injuries are also seen at a range of other European Mesolithic sites, such as Schela Cladovei, Romania (Boroneant et al. 1999); Vasilyevka, Ukraine (Lillie 2004); Téviec, France (Newell et al. 1979); and Vlasac, Serbia-Romania (Roksandić et al. 2006). It has also been suggested that perimortem fractures to the cranium and maxilla seen in one individual from Gough’s Cave in Somerset may also be attributable to violence (Newell et al. 1979; Hutton Estabrook 2014). Indeed, Hutton Estabrook (2014) has suggested that the ‘roots’ of interpersonal violence may lie in the Mesolithic, or potentially even the Mid-Late Palaeolithic – where we also see evidence of skeletal trauma and embedded projectiles in human skeletons. There is also evidence of violence within a wide range of hunter-gatherer groups from around the world; both in Holocene hunter-gatherer populations from various geographical locations, and also in more recent, ethnographically documented hunter-gatherer groups (Gordón 2014; Darwent and Darwent 2014; Schmidt and Osterholt 2014; Sutton 2014; Gat 2015).
However, discussions of violence in the archaeological past have often been controversial — but Keeley (1997) believes that archaeologists need to be less reluctant to acknowledge that some Neolithic social interactions or environments may have involved violence, conflict and/or warfare. He suggests that to avoid discussions of violence and warfare results in the adoption of Neo-Rousseauian attitudes, and that these may “hide much from us and unnecessarily complicate our understanding of prehistoric life” (Keeley 1997, 317). Equally, however, these arguments can also be applied to Mesolithic studies. Nonetheless, actually defining violence is complex, as is then detecting it in the archaeological record. At present, we often tend to be biased towards modern-day understandings of violence and physical assault (Williams 1983, 330) — but projecting these onto the past is problematic in a variety of ways.

As discussed above, a significant body of work has focused upon the potential causes of the Mesolithic-Neolithic transition may have been, and a substantial number of these are bound to ideas of social change occurring. A number of different authors have therefore now proposed that social change may in fact have been a causative agent in the Mesolithic-Neolithic transition (e.g. Bender 1978; Hayden 1990; Mithen 2007). For example, Mithen (1996) has argued that there were four main changes which occurred in the mind, which were critical to the successful adoption of agriculture at the beginning of the Neolithic period. One of these key changes is defined as:

“the propensity to develop ‘social relationships’ with plants and animals, structurally similar to those developed with people. This is a further consequence of integration of social and natural history intelligence” (Mithen 1996, 256)

For Mithen (1996) therefore, the Neolithic sees greater development of social relationships, the basis of which is evidenced in the Mesolithic and Palaeolithic. This has led to ideas surrounding a ‘misapplication of social intelligence’ being the formative agent of the adoption of agriculture (Mithen 2007) — i.e. that social competition influenced the development of domesticated species, and cognitive fluidity enabled farming to be easily adopted. The idea of individuals only creating ‘relationships’ with plants and animals at the advent of the Neolithic however is perhaps unfair — and a growing corpus of research on Mesolithic populations’ relationships and interactions with both flora and fauna is now emerging within Britain (e.g. Overton 2014; Taylor 2014), and also on how hunter-gatherers can utilise, manage and/or manipulate plants without domestication (e.g. Politis 1999). Ethnographic studies have also highlighted the complex relationships that hunter-
gatherer groups may also have with plants and/or animals (e.g. Willerslev 2004; Kohn 2007; Nadasdy 2007; Hill 2011; Barton 2014). Archaeological evidence from sites across Europe has shown that Mesolithic populations had very sophisticated knowledge of plants, indicated through varied and highly diverse uses of plant materials. With this in mind therefore, perhaps the Neolithic heralds a change in the relationships between people and plants/animals, rather than increased development, as suggested by Mithen (1996).

A recent paper by Gowdy and Krall (2014) has instead however argued that change was economically and behaviourally driven – proposing that the transition to agriculture was related to a change in human behaviours, leading to the emergence of ‘ultrasociality’ – which is defined as a form of social organisation with fulltime division of labour, and both producers and non-producers of food. The drivers behind this change are seen to be solely economic, and are orientated towards the production of an agricultural surplus. Whilst this kind of research promotes new and novel ways in which to view the Mesolithic-Neolithic transition, the mechanisms behind it, and possible social changes, Gowdy and Krall’s (2014) study does not consider the archaeological evidence available for this period. Furthermore, it leans somewhat towards social evolutionary and social Darwinist explanations for the adoption of agriculture. However, the study does highlight the modern-day implications and parallels of understanding the transition to agriculture, particularly in terms of biophysical consequences.

Finally, if we do accept social changes as being a driving factor behind the Mesolithic-Neolithic transition (perhaps combined with economic changes), Price (2000, 314) rightly comments that we then must also face the question as to “what bought about the rise of status differentiation in the first place?”. Perhaps the most parsimonious interpretation of the current available archaeological data for the Mesolithic-Neolithic transition is the idea, put forward by Thomas (1999, 15), that the new forms of material culture and emerging lifeways perceived to have occurred at the interface between the two periods are likely to have been taken up and understood in different ways within individual populations or communities. In this respect, there may not have been a uniform social change across the country, or indeed Europe as a whole – but instead the transition may have resulted in various ‘social transformations’. These ideas therefore also link to the notion that the transition from hunter-gatherer to farmer should not be considered as an evolutionary or developmental stage. Nonetheless, it is interesting to note that a negative view of Mesolithic social complexity – as highlighted in the quotation at the start of this Chapter –
is something which has persisted within the academic literature, even within very recent texts: “the communities of the British Isles did reach levels of social complexity comparable to those found on the Pacific Northwest Coast, but during the Neolithic rather than the Mesolithic period” (Tolan-Smith 2008, 157). However, whilst social changes between the two periods may have occurred, due to different relationships with people, places, material culture, animals, and landscapes emerging, these changes are perhaps unlikely to have been the same across the whole of Britain.

2.4.5. Change or Continuity?

From the above discussions of the Mesolithic and Neolithic periods in Britain, and the ‘transition period’ between them, it is evident that significant changes can be seen to have occurred from the archaeological evidence currently available. There is clearly a dietary and subsistence change between the two periods if taken as a whole, but how this change occurred – and particularly whether farming was adopted ‘rapidly’ or not – is still unclear. Similarly, demographic change is still not fully understood, and although more recent aDNA demographic studies and profiles have aimed to shed light on the movement, origins and potential replacement of Mesolithic and Neolithic populations, there at present still appears to be discrepancies between the stories emerging from mtDNA versus nuclear DNA. Emerging from these other changes is also the idea and suggestion of social change between the Mesolithic and Neolithic periods, with changes in lifeways resulting in different social pressures, structures, competition, potential violence, and possible hierarchies. Health changes are perhaps the least understood aspect of change discussed within this chapter, but are an important archaeological consideration and question. Changes in health and disease are at present assumed to have accompanied the other changes perceived to have occurred at the interface between the Mesolithic and the Neolithic periods in Britain, but have held little academic focus – in part due to the difficulties in studying prehistoric health and disease, and also the lack of skeletal material available for these periods, particularly in Britain (see Chapter 3).

The degree of continuity vs. change between the two periods is therefore problematic to determine – but an awareness that different aspects of change may have occurred at different rates to others has now emerged. This idea was perhaps first suggested by Zvelebil and Rowley-Conwy (1984), but has since been widely commented upon (Woodman 2000; Milner and Craig 2009). For example, it is now often argued that
changes in material culture may have occurred very rapidly, reflecting changes in ideology, but that changes in subsistence strategy were taken up much more gradually (Thomas 1999, 16; 2008; Rowley-Conwy 2004(a); Milner et al. 2004; Gaffney et al. 2009, 42; Figure 7). Others however have argued that changes in subsistence economy did in fact occur very quickly, often citing isotopic evidence suggested to support this (Richards and Hedges 1999(b); Woodman 2000; Schulting and Richards 2002(c); Richards et al. 2003; Richards and Schulting 2006). Many aspects and archaeological evidence traditionally perceived to be ‘Neolithic’ in origin however also appear to have roots in the Mesolithic – for example, Cummings (2003) even suggests that monumentality first emerged in the British Mesolithic, and that Mesolithic peoples were using and inhabiting large and enduring places within the landscape (e.g. shell middens), much like large Neolithic monumental forms. Ideas of continuity in mortuary practices are also discussed in Chapter 3.

Figure 7: Economic and material culture changes between the Mesolithic and Neolithic periods (Rowley-Conwy 2004(a), S84)

One of the major problems with discussions of change vs. continuity however is that definitions of terms such as ‘rapid’ or ‘gradual’ are very rarely given – and instead the actual meanings behind these are left wide open to interpretation by the reader. For example, does ‘rapid’ refer to a period of only a number of years, or within an individual’s lifetime, or a generation, or a period of c.500 years? For example, Rowley-Conwy (2004(a)) states that economic changes happened “very rapidly”, then later going on to state that these changes took “at most a century or two” (ibid, S97). Does a 200 year period constitute a ‘rapid’ change? As Milner (2010) rightly points out, the term ‘rapid’ is often
taken, when discussing the Mesolithic-Neolithic transition, to refer to periods of time c.300-400 years long – the same length of time as the duration of Roman Britain.

The Mesolithic-Neolithic debate in Britain can thus be seen to be “one of the most contested periods in prehistory” (Cummings and Harris 2011, 392), and it has been suggested that it has remained to be so due to the general lack of very late Mesolithic and very early Neolithic material available in the UK (Zvelebil and Rowley-Conwy 1986). Due to this, our understanding of the degree of change and continuity between the two periods, and the chronologies over which this occurred, is still poor. We still also have very little understanding of why the transition occurred, as well as how. Previous suggestions for the catalysts behind the Mesolithic-Neolithic transition have ranged from ideas about staving off famine (resource degradation), to the progression of ‘civilisation’, to population growth, to a desire to secure food surpluses, to parallels to a religious conversion, to risk reduction strategies, to a ‘misapplication of social intelligence’ (i.e. social competition) (Humphrey 1984; Hayden 1995; Bradley 1998, 13; Price 2000(b); Robb and Miracle 2007; Mithen 2007; Kuijt 2009). A number of post-processualist schools of thought have also suggested that the transition may have been linked to an ideological change, thereby seeing the role of agriculture as a symbolic and cultural construct (Hodder 1990; Price 2000(b)). An interesting paper which aims to address why change may have happened however is Tipping’s (2010) suggestion that the Mesolithic-Neolithic transition coincided with a period of rapid and significant climate change – and that this climatic stress may have forced the adoption of agriculture in Britain.

In terms of the ‘change’ between the two periods themselves, research on the origins of the Mesolithic-Neolithic transition in Britain has therefore been suggested to have reached “a Kuhnian phase of collapse of a consensual model” (Arias 2004, S99). However, Arias (2004, S99) blames this on academics in this field “inability to integrate new data”, which is perhaps somewhat unfair – especially when considering the amount of new scientific data currently being generated from material from these periods (also see section 2.5. below). Nonetheless, the mechanisms, changes, and advent of the Mesolithic-Neolithic transition are still heavily debated within archaeology, to perhaps the point of some academic mudslinging in recent years in some cases (e.g. see comments on Cummings and Harris 2011).
Despite this, we still do not have a clear picture of the Mesolithic-Neolithic transition in Britain. For example, Whittle’s (1990) survey concluded four possibilities regarding the origins of the Mesolithic-Neolithic transition: early or late colonisation by farmers, and early or late indigenous acculturation. He concludes: “much further research is needed, and the outlook is unfortunately gloomy” (Whittle 1990) – and sadly it would appear that little has changed in the past 25 years. There is still much debate as to whether the transition was exogamous or endogenous, whether it was gradual or abrupt, uniform or regionalised (Thomas 2008); our interpretations of the Mesolithic-Neolithic transition have “remained static for at least the last 2 decades” (Robb and Miracle 2007, 99). Furthermore, the problems in studying the transition have been further compounded due to the differences in available evidence for the two periods in Britain, and the fact that they have been traditionally studied in very different ways (Edmonds 1999, 5). Generally however, the Mesolithic and Neolithic periods have been described in terms of long timescales, focused around the transitional change between the two periods – which is perhaps due in part to the fact that radiocarbon is still the dominant mode of dating used. Whilst looking at processes and change on larger timescales is sometimes useful, there is also now a need to consider issues such as timing and tempo of change between the two periods (Whittle 2013). Finally, although focus has traditionally been placed on the transition, Gamble (2007, 24-26) has questioned what we actually mean by ‘change’, and whether we can every truly determine if change has occurred, or if we are simply seeing a variation on an existing theme. Change can be studied in terms of discrete events, structures (frameworks of action), or processes (Dark 1998), but re-creating the past in the present will always be problematic; the study of change in the past even more so, as it suffers from becoming entrapped within the concerns of the present (Gamble 2007, 26). Determining the ‘character’ of change at the Mesolithic-Neolithic transition will perhaps always be challenging therefore, and may vary between sites or geographical locations, requiring more fine resolution timescales to be considered. Whatever the ‘changes’ which occurred at the transition however, Thomas (2008, 80) believes that they would have led to “the transformation of the everyday” – how far this is true still remains to be seen however. The Mesolithic-Neolithic transition nonetheless, is still viewed as “truly a radical change” (Wickham-Jones 2010, 16) within the cultures of Britain.
Chapter 3 – Human Remains in the British Mesolithic and Neolithic

“Acts involving the body, whether living or dead, possess a great capacity to engage us”
(Schulting 1998, 204)

“Funerary remains form one of the major datasets for reconstructing social beliefs and social organisation in prehistory”
(Pettitt 2006, 292)

Whereas the previous chapter aimed to provide a broad overview of the Mesolithic and Neolithic of Britain, this chapter aims to provide a more specific overview of the known human remains and mortuary practices adopted within these periods. In doing this, it is hoped that this chapter will provide a secure context and rationale for the basis of this research – primarily in highlighting the need to identify more human remains dating to the periods, and the information which this skeletal material may provide us with. As such, this chapter will firstly outline the known human remains and burial practices from the British Mesolithic, and aim to question why we do not have more human skeletal material from this period. Following on from this, an overview of Neolithic British human remains will also be given, and subsequently lead into how new approaches will be adopted within this research to study British Mesolithic and Neolithic skeletal material. Finally, as the material used in this study is of human origin, the ethics of dealing with human remains – and specifically those of a prehistoric date – will also briefly be considered. The main mortuary practices seen in the archaeological record for both the Mesolithic and Neolithic periods are therefore outlined below in an attempt to place the work undertaken on human remains within this thesis within a solid archaeological context. However, whilst burial evidence is incredibly important, it must be considered within the wider context of the periods themselves, as outlined in Chapter 2.

3.1. Human Remains From the British Mesolithic

“Human remains dating to the Mesolithic are notoriously rare in Britain”
(Schulting and Richards 2002(a), 1012)

Despite the now plentiful evidence that people were inhabiting and subsisting in Britain during the Mesolithic period (see Chapter 2), there are still very few known human
remains dating to this c.6000 year period of British prehistory. The lack of known, identified human skeletal material dating to the British Mesolithic, and the apparent difficulties in determining where more human remains from the period may be, has meant that skeletal material has often not been considered as a key research priority within Mesolithic archaeology. This has therefore limited the extent of our understanding of Mesolithic diet, health and lifeways. Study of the actual physical remains of past populations is crucial as it can provide unique information unobtainable from other sources. The lack of human remains from Britain at present could therefore be argued to be a limiting factor in advancing our understanding of Mesolithic lifeways. Where work has been undertaken on Mesolithic human remains, this has generally focused on contributing towards debates surrounding the Mesolithic-Neolithic transition. Due to this, less focus has traditionally placed on wider considerations of Mesolithic skeletal material, such as the context of deposition, osteological analyses, disease, and health, for example.

The following sections highlight that there is a “widespread perception of the poverty of burial evidence in the Mesolithic in Britain and Ireland” (Conneller and Warren 2006, 7). In wide-scale discussions of Mesolithic burials, mortuary practices and human remains, Britain is rarely mentioned “for the simple reason that there is very little bone preservation from the Mesolithic” (Schulting 1998, 219). As such, the British mortuary evidence is considered here in a broader European framework.

3.1.1. Mortuary Practices Adopted in Mesolithic Britain

“The various ways in which people deal with death and with their dead reveal other dimensions of that society”

(Nilsson Stutz 2009, 657)

The origins of burial and deliberate mortuary practice in the archaeological record emerge in the Palaeolithic, at sites such as Sungir in Russia and Dolní Věstonice in the Czech Republic – where we see formal inhumations with grave goods dating to the Upper Palaeolithic (c.20-30,000 years ago) (Formicola et al. 2001; Formicola and Buzhilova 2004; Pettit 2006; Trinkaus et al. 2010; Nalawade-Chavan et al. 2014). Nonetheless, on the whole, Palaeolithic burials are uncommon. With the advent of the Mesolithic period in Europe however, we begin to see the emergence of more frequent formal inhumations, cremations, and even the establishment of cemetery or multiple burial sites. Throughout the Mesolithic period as a whole, we can therefore see more varied forms of mortuary
practice emerging. As Schulting (1998, 203) notes, “the burial record of Mesolithic Europe exhibits remarkable diversity”. The following sections will therefore address the types of mortuary practice seen in the Mesolithic – with particular reference to the British record, and how this compares to the burial record seen across Europe and the rest of the world, and the number of remains known.

**Inhumation**

As mentioned above, the number of inhumations seen in the European Mesolithic sees a marked increase from the preceding Upper Palaeolithic record. Within the British Mesolithic burial record however, few definite inhumations are known – but this may be a consequence of the paucity of known human remains from the period as a whole. Perhaps the two most well-known British sites with formal Mesolithic inhumations are Gough’s Cave and Aveline’s Hole. At Gough’s Cave in Cheddar, Somerset, one intact, articulated Mesolithic skeleton was recovered, known as ‘Cheddar Man’ (Humphrey and Stringer 2002). ‘Cheddar Man’ comprises an inhumation of a young adult male, thought to date to the Early Mesolithic, c.9080 ±150 BP (Stringer 1985; Jacobi and Higham 2009). In Conneller’s (2006) table of dated Mesolithic human skeletal material from Britain and Ireland, Gough’s Cave is the only site listed to contain solely articulated remains. The site of Aveline’s Hole, in Burrington Combe, Somerset, is however perhaps the most unique Mesolithic site in Britain with human remains. Discovered in 1797, the site was excavated throughout the late 18th and early 19th centuries, and again in the early 20th century. It is dated to 10000-8500 BP, with the inhumation of human remains thought to date to a brief period between 8460-8140 cal. BC. Both single burials and one double inhumation were recovered, with c.50-100 individuals originally excavated (Schulting and Wysocki 2002; Schulting 2005; Boycott and Wilson 2010). Combinations of double and single inhumations have also been found at other European Mesolithic sites – such as Tågerup in Scania, Sweden (Ahlström 2003). However, it is important to note that in Britain, “it is possible that a few of the other sites now represented by disarticulated remains originally held intact bodies” (Conneller 2009, 691). The small number of inhumations currently known may therefore not be representative of the original number of burials present in the Mesolithic period.
Cremation

Cremation has traditionally been seen as a much rarer mortuary practice than others mentioned within this chapter in the Mesolithic period. However, examples of cremations are now known across Europe, at sites in Denmark, Sweden, Holland, France, Poland and the Iron Gates (Schulting 1998). It is possible that a significant number of other Mesolithic cremations have been missed however due to a lack of dating, and because of the assumption that cremation is associated with later periods. That both inhumation and cremation are present within the Mesolithic record however raises the question – why cremate some individuals but bury others? Cremation is a difficult process, with very high temperatures and significant periods of time needed in order to fully burn the body. In modern cremations for example, it takes between 1-1.5 hours at a temperature of 700-1000°C to fully cremate a human body (Roberts 2009, 52). The pyre structure itself also needs to be constructed, and requires the use of significant amounts of raw materials and labour. Parker Pearson (2009, 49) suggests that on average, around a tonne of dry timber is needed in order to successfully cremate an adult human body. In all, cremation is a high cost exercise. Additionally, as noted by McKinley (2006), cremation in Britain in the past would have always been more problematic or difficult than cremation in other parts of the world due to the climate – a pyre will not burn if it is raining or the wood used is wet. The timings of cremations would therefore have been important, and Mesolithic populations would have needed detailed knowledge in order to undertake successful cremations. Perhaps due to this, cremation has often been seen as reflecting some new form of belief or spiritualism set aside from inhumation – as a “heat-mediated transformation” of the body (Osetigaard 2000, 44). Ethnographically however, there is evidence of populations where both cremation and inhumation are used interchangeably, and is not reflective of beliefs (Ucko 1969), much akin to the change to cremation seen within British populations from the 19th century onwards (Parker Pearson 2009, 41-42).

The only known cremation from Britain dating to the Mesolithic was very recently discovered (April 2015) in Langford, Essex. The deposit was recovered during commercial excavations in advance of pipeline construction, and has been dated to 5,600 cal. BC. As such, it has been dubbed the ‘oldest human cremation in Britain’ (Oxford Archaeology 2015). The cremation was recovered from less than a metre-wide pit feature, and contained the incomplete remains of a single adult individual. The cremation deposit also contained large amounts of other burnt material and charcoal, prompting suggestions that it represented the partial remains of a pyre (Gilmour and Loe 2015).
The only other Mesolithic cremations known from the British Isles derive from the site of Hermitage in Co. Limerick, Ireland, where two early Mesolithic cremations were recovered from pit features. One, Pit A, was a smaller, sub-circular pit noted to have the remains of a post-hole in the base. The cremation deposit was arranged in a crescent shape around the post-hole, with a large adze polished stone axe placed on top of the cremation. The deposit is thought to represent the cremation of an entire single adult, possibly male, and was potentially ground following cremation (Collins and Coyne 2003; Collins 2009). The second cremation at the site, Pit B, again contained the remains of a single adult. The smaller nature of the cremated deposit however has been suggested to represent only part of the whole cremated individual, and is perhaps representative of a ‘token burial’ (Collins and Coyne 2003; Collins 2009). The two cremations at Hermitage, although very similar in date (7530-7320 cal. BC and 7090-7030 cal. BC respectively (Collins 2009)), highlight the potential variability within Mesolithic cremations. The discovery of a post-hole within cremation Pit A at Hermitage is also particularly interesting, as it raises potential ideas of a marker to the cremation – and from this, ideas of Mesolithic remembrance and grave markers.

Cremations are also known from other Mesolithic sites across Europe, but are typically less common than other types of mortuary treatment. Cremations are however known from the cemetery sites of Vlasac, Serbia (Borić et al. 2014), and Vedbæk, Denmark (Brinch Petersen and Meiklejohn 2003), as well as Franchthi Cave, Greece (Cullen 1995), and Skateholm I and II, Sweden (Larsson and Stutz 2014), for example.

**Disarticulation**

Disarticulation has traditionally been viewed as a Neolithic funerary practice, with Mesolithic evidence often being disregarded as disturbed burials, or even as evidence of cannibalism. However, it is now becoming more widely accepted that disarticulation is apparent throughout the Mesolithic record – albeit on a smaller scale to that seen in the Neolithic. The movement of disarticulated material has also been suggested to be a deliberate form of mortuary practice from the Upper Palaeolithic onwards throughout Western Europe (Schulting 1998) – such as the recently discovered non-adult remains discovered at Borsuka Cave in southern Poland (Wileżyński et al. 2016). There is also evidence of disarticulated remains from a number of British Upper Palaeolithic sites, such
as Gough’s Cave, Somerset (discussed above), and Sun Hole, Somerset (Richards et al.
2000; Stevens et al. 2010).

When considering the British material, the majority of human remains dating to the
Mesolithic “are composed of isolated, disarticulated material” (Conneller 2009, 690), and
is known from sites such as Cnoc Coig (Oronsay), Badger Hole (Somerset), and Totty Pot
(Somerset) (Conneller 2006). Whilst these have frequently been considered the result of
taphonomic or diagenetic processes, a number of authors now suggest that disarticulation
is likely to have been a deliberate mortuary practice (e.g. Cauwe 2001; Conneller 2006;
2009; Gray-Jones 2011; 2013). What has previously therefore been dismissed as ‘loose
bone’ may therefore actually be the result of deliberate acts, and was a part of, not separate
from, other types of Mesolithic mortuary practices. Gray-Jones (2013) has suggested that
disarticulated remains or loose bone have often been understudied as they have been seen
to hold less interpretative value than intact inhumations. This is primarily because it is
generally harder to determine on aspects such as sex, age and status from disarticulated
remains. Gray-Jones (2011) has therefore adopted techniques from zooarchaeology –
namely bone representation indices (Bello and Andrews 2006) – to best analyse ‘loose’
human bones from Mesolithic contexts, something which has also recently been applied to
commingled Neolithic human remains (Mack et al. 2015).

We can also consider how Mesolithic peoples may have disarticulated their dead, as there
are a range of different ways in which this can be undertaken, but all require knowledge of
the decomposition of the body and various taphonomic factors. Disarticulation can occur
through allowing the body to naturally decompose – possibly through burial – or by
allowing various animals to ‘strip’ and clean the body, or via deliberate human-induced
dismemberment and/or defleshing of the body. Discussions of excarnation or exposure of
the body are frequently found within the Mesolithic literature, particularly in relation to
shell-midden sites (as discussed below). The presence of cut-marks has been noted on a
variety of disarticulated Mesolithic human skeletal material across Europe, including
material recovered from Gough’s Cave, Somerset, and Kent’s Cavern, Devon (Schulting et
al. 2015), which may indicate evidence of defleshing. Whilst anthropophagy and
cannibalism are often commonly discussed in relation to these kinds of cut-marks however
(e.g. Cauwe 2001; Andrews and Fernández-Jalvo 2003; Chandler et al. 2009), we must be
wary of sensationalism and the desire to define these marks as evidence of some form of
cannibalistic activity, rather than as a part of a mortuary practice. We must also be aware
of equifinality in relation to cut marks – that multiple practices may result in the same pattern of changes to the skeleton (Gray Jones 2011). Additionally, when considering cannibalism, it is important to note both exocannibalism and endocannibalism – and the distinctions between the two. Endocannibalism is the “ritual consumption of the flesh of the deceased” (Oestigaard 2000, 43), and is most famously known ethnographically from specific populations in Papua New Guinea, where consumption of brain tissue from deceased relatives until the 1950’s is thought to have caused a neurodegenerative prion disease known as kuru (Huillard d’Aignaux et al. 2002; Collinge et al. 2006). Therefore whilst endocannibalism is funerary in nature, exocannibalism is not, and may be related to war or famine. Distinguishing between these two types of cannibalism, particularly simply from cut-marked bones, is incredibly complex, and very difficult to determine within the archaeological record however.

The reasons for disarticulation in Mesolithic mortuary practice are nonetheless likely to always remain unclear. However, as noted by Cauwe (2001, 161), “dismemberment, secondary inhumation and removal of bones are the opposite of immediate and definitive inhumation” – suggesting that disarticulation reflects not only a different mortuary practice in itself, but potentially also a different relationship between the living and the dead than that experienced through inhumation. Cauwe (2001, 161) suggests that disarticulation also represents the integration of human remains into “dynamic processes” by Mesolithic populations – retaining individuals within the realms of the living and allowing for the movement of individuals across landscapes or between sites or locales. Disarticulation has also been suggested to be related to phases of liminality and as a means of transition from a body or individual into bones – therefore being reflective of mortuary beliefs and potential rites of passage (Hellewell and Milner 2011). In this respect, disarticulation can therefore be seen as a spatially and temporally extended practice throughout the Mesolithic period, via the movement of human remains. Disarticulation allows for the separation of the body and renders it easily transportable – and as such also means that aspects of an individual can be retained within a population even after they are deceased. Due to this, disarticulation has often been considered as a mortuary practice associated with ancestral ties and belief systems, particularly when seen in the Mesolithic (Hellewell and Milner 2011).
Caves

Whilst the burial record for the British Mesolithic is relatively scarce, the majority of the currently known human skeletal evidence available for Britain has been found in caves (Collins 2009). Indeed, of the known sites with human remains in southwest England, all are cave sites – and four of these are in the Mendip Hills, north Somerset (Meiklejohn et al. 2011; Table below). Caves have also been used for the deposition of human remains across Europe – for example, at Spanish sites such as Poza L’Egua Cave, Colomba Cave, Los Azules and Los Canes Cave in Asturias and El Truchiro in Cantabria (Arias and Garralda 1996; Drak and Garralda 2009; Arias et al. 2009). However, it has been noted that nearly all caves used for the deposition of human remains in the Mesolithic in Britain were also previously used in some form in the Palaeolithic – either via evidence of occupation, tools, or the remains of Pleistocene mammals. One such example is Gough’s Cave, which was found to contain both Upper Palaeolithic and Mesolithic inhumations (Humphrey and Stringer 2002). Interestingly, however, cave sites in the Mesolithic appear to have only been used for burials, and not for other activities as they may have been in the Palaeolithic (Conneller 2006).

It has also been noted that the use of caves in mortuary practice is primarily seen in the early Mesolithic – “there was no deposition of human remains in caves during the last two millennia of the Mesolithic before the practice recommenced in the early Neolithic” (Conneller 2009, 691). This idea of a cessation of cave use for burials in the late Mesolithic has also been suggested by Chamberlain (1996), and due to this the use of caves in Neolithic mortuary practice is frequently seen as a new funerary rite, rather than a continuation of Mesolithic traditions (Hellewell and Milner 2011). However, when we consider the chronology of Mesolithic cave sites in Britain, it is apparent that they were used for the deposition of human remains throughout the period. Whilst in the later Mesolithic there are fewer cave sites used, there does not appear to be a complete cessation of caves as places for the burial of human remains (Table 4; Hellewell and Milner 2011).

<table>
<thead>
<tr>
<th>Site</th>
<th>MNI</th>
<th>Nature of remains</th>
<th>Elements Present</th>
<th>Date BP</th>
<th>Date cal. BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badger Hole, Somerset</td>
<td>2</td>
<td>Disarticulated</td>
<td>Cranium, 2 juvenile mandibles</td>
<td>9360 ±100</td>
<td>8850-7800</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9060 ±150</td>
<td></td>
</tr>
<tr>
<td>Gough’s Cave, Somerset</td>
<td>1</td>
<td>Articulated</td>
<td>All</td>
<td>9100 ±100</td>
<td>8700-7750</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9080 ±150</td>
<td></td>
</tr>
</tbody>
</table>
The use of caves for the deposition of human remains in the Mesolithic has frequently been linked to liminality – set aside from occupation areas, and seen as dark, mysterious places offering entry into the earth. Many of these ideas of caves being more liminal and potentially ritual places, with confined space and light, have come from the Neolithic literature – and caves are now often seen to possess many of the same features as monuments when considered as mortuary spaces (Barnatt and Edmonds 2002; Conneller 2006; 2009; Hellewell and Milner 2011).

<table>
<thead>
<tr>
<th>Site</th>
<th>Quantity</th>
<th>Articulation</th>
<th>Associated elements</th>
<th>Calibration range</th>
<th>Approximate dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aveline’s Hole, Somerset</td>
<td>50-100</td>
<td>Articulated and disarticulated</td>
<td>Various elements</td>
<td>9115 ±100 to 8740 ±100</td>
<td>8650-7550</td>
</tr>
<tr>
<td>Worm’s Head, Gower (Wales)</td>
<td>3</td>
<td>Disarticulated</td>
<td>Ulna, rib innominate, fibula, metacarpal, femur, 2 scapulae, cranial frags (child)</td>
<td>8800 ±90</td>
<td>8190-7580</td>
</tr>
<tr>
<td>Oreston (Third Bone Cave), Devon</td>
<td>1</td>
<td>Disarticulated</td>
<td>Clavicle</td>
<td>8615 ±75</td>
<td>7840-7520</td>
</tr>
<tr>
<td>Ogof-yr-Ychen, Caldey (Wales)</td>
<td>6</td>
<td>Disarticulated</td>
<td>Tibia, 2 innomimates, 2 mandible, 2 cranial fragments</td>
<td>8760 ±55 to 7020 ±100</td>
<td>7865-7170 to 5990-5640</td>
</tr>
<tr>
<td>Daylight Rock, Caldey (Wales)</td>
<td>1</td>
<td>Disarticulated</td>
<td>Mandible</td>
<td>8655 ±60</td>
<td>7800-7165</td>
</tr>
<tr>
<td>Potter’s Cave, Caldey (Wales)</td>
<td>2</td>
<td>Disarticulated</td>
<td>Ulna, metacarpal</td>
<td>8580 ±60 to 7880 ±55</td>
<td>7790-6455</td>
</tr>
<tr>
<td>Totty Pot, Somerset</td>
<td>4</td>
<td>Disarticulated</td>
<td>Humerus, tibia</td>
<td>8180 ±70</td>
<td>7380-7040</td>
</tr>
<tr>
<td>Kent’s Cavern, Devon</td>
<td>1</td>
<td>Disarticulated</td>
<td>Maxilla</td>
<td>8070 ±90</td>
<td>7350-6650</td>
</tr>
<tr>
<td>Paviland, Gower (Wales)</td>
<td>1</td>
<td>Disarticulated</td>
<td>Humerus</td>
<td>7190 ±90</td>
<td>6160-5790</td>
</tr>
<tr>
<td>Pontnewydd, Clwdy (Wales)</td>
<td>1</td>
<td>Disarticulated</td>
<td>Mandible (non-adult)</td>
<td>7420 ±90</td>
<td>5730-5560</td>
</tr>
<tr>
<td>Foxhole Cave, Gower (Wales)</td>
<td>1</td>
<td>Disarticulated</td>
<td>Teeth, 2 phalanx</td>
<td>6785 ±50 to 4625 ±40</td>
<td>5720-5650 to 3500-3360</td>
</tr>
<tr>
<td>Killuragh Cave, Co. Limerick (Ireland)</td>
<td>3</td>
<td>Disarticulated</td>
<td>Mandible</td>
<td>5455 ±50</td>
<td>4450-4220</td>
</tr>
<tr>
<td>Foxhole Cave, Derbyshire</td>
<td>1</td>
<td>Disarticulated</td>
<td>Humerus, tibia</td>
<td>5485 ±75 to 5185 ±60</td>
<td>4440-3830</td>
</tr>
</tbody>
</table>

Table 4: Dated British Mesolithic cave sites containing human remains (adapted from Conneller 2006, with additions)
Finally, interestingly, the deposition of human remains in caves in the Mesolithic encompasses both disarticulation and inhumation (Table above). Only one site, Aveline’s Hole, however contains both disarticulated and articulated inhumations within the same cave. From the table above, it does appear that the use of caves for inhumations is an earlier Mesolithic trend, and by the mid and late Mesolithic the deposition of disarticulated remains in caves was favoured. This also fits with evidence for the deposition of human remains in caves seen in Britain in the late Upper Palaeolithic, which again sees both inhumations and disarticulated material (e.g. articulated remains are known from Kendrick’s Cave, Conwy (Wales) dating to the late 12th millennium BC (Richards et al. 2005)). Whether this differentiation between inhumation and disarticulation is representative of Mesolithic populations making distinctions between people (as suggested by Conneller 2009), or not, is however unclear and needs further consideration in the future. The prevalence of disarticulation however suggests that this was more frequently seen as the normative funerary practice associated with caves.

**Middens**

The discovery of human remains within middens is also a mortuary practice which has been seen to widely characterise the Mesolithic period. At present, human remains from midden contexts in Britain appear to primarily only occur in the Late Mesolithic – but it is worth noting that few early Mesolithic middens in the UK survive due to sea-level rise, and thus the current picture of deposition is biased towards later surviving examples (Chatterton 2006). Within the British record, the most well-known midden sites with human remains are from Oronsay, Inner Hebrides. On the island, three of the known five Mesolithic shell middens have yielded human skeletal remains: Cnoc Coig, Caisteal nan Gillean II, and Priory Midden. Of these, Cnoc Coig is perhaps best known, due to the recovery of 49 pieces of human bone, thought to represent at least four individuals (Mellars 1987; Meiklejohn and Denston 1987; Meiklejohn et al. 2005; see Chapter 5). The remains from all three Oronsay middens however are disarticulated, and do not represent full skeletons or deposition of entire bodies. Additionally, the predominance of human hand and foot bones within the Cnoc Coig human skeletal assemblage has led to suggestions of the site being used for excarnation or the placing of bodies on scaffolds (Meiklejohn et al. 2005; but also see Chapter 5).
Evidence of deliberate graves and inhumations within Mesolithic shell middens is however known on the continent. For example, at the site of El Collado, Valencia, Spain, 15 human burials were recovered from an open-air shell midden, dating to c.6590-6250 cal. BC (Guixé et al. 2006). Similarly, a large number of burials are also known from the late Mesolithic shell-midden sites of Téviec and Hoëdic, in Brittany, France. A total of 10 graves containing 23 individuals were recovered from Téviec, and 9 graves were found at Hoëdic containing 14 individuals (Schulting 1996). In the Sado Valley, where 11 Mesolithic shell-middens are known, a total of 6 burials with an MNI of 120 have been discovered (Peyroteo-Stjerna 2015). Holocene hunter-gatherer burials in shell-middens are however also known beyond Europe – for example, in the sambaquis of Brazil (e.g. Colonese et al. 2014).

Deposition of human remains in middens has been suggested to reflect human and animal relationships, via the parallels between similar element distributions between human and animal skeletal material at British sites (Conneller 2009). By treating all skeletal material in the same way, no distinctions are made between the human and animal worlds. Chatterton (2006, 115) therefore suggests these “acts of intentional and regularised disposal can be seen as a process of regeneration”, and following Conneller’s (2009) suggestions, is perhaps telling about the way in which animals were viewed in relation to humans in the Mesolithic period. There is also some debate in the literature as to whether middens represent foci for feasting and large gatherings, and if the human skeletal material buried within them is linked to these rituals. If so, the deposition of human skeletal material within them may be tied to ideas of maintaining links to particular locations within a landscape (Thomas and Tilley 1993; Schulting 1996; Woodman 2001; Chatterton 2006).

Islands and Water

The association of Mesolithic human remains with water is a topic variously discussed within the British literature. As discussed in Chapter 2, the majority of currently known Mesolithic sites within Britain are in ‘watery’ locations – either in coastal areas or on the edges of lakes. In terms of deposition within water, a singular human bone was recovered from a flooding layer at Thatcham, and human bone from Staythorpe was from a river channel. However, Conneller (2006) suggests that the human remains at both these sites could represent either original deposition within water, or, as both contexts have
occupational activity nearby, could instead characterise deposition of human remains at occupational sites which have then subsequently been flooded. Chatterton (2006, 108) ascertains however that the intentional deposition of items (both human remains and other items) within water in the Mesolithic period was commonplace, and that water may have been both a “symbolic as well as economic resource”.

In terms of other British sites with human remains, a large number of these are also found at sites in coastal areas or near to water sources. For example, at the site of Greylake, Somerset, which comprises a small island on the floodplain of the Somerset Levels and Moors, five crania and additional long bones were recovered in 1928. However, only two of these skulls remain today, along with four tibiae fragments, a phalanx and half a metatarsal – but suggests that complete bodies may have been originally interred at the site. These remains have been dated to 8460-8275 cal. BC (Brunning and Firth 2012). The site of Greylake is unique within the British Mesolithic record, but suggests the potential of open-air cemeteries being utilised within the period, and also the burial of individuals on islands and within water associated contexts.

Looking more broadly, we also see sites across Europe where human remains are found associated with, or close to, water. For example, Oleneostrovski Mogilnik (Red Deer Island Cemetery; also known as Olenii Ostrov), in Karelia, is a cemetery site located on a small island in Lake Onega. A total of 170 inhumations were discovered at the site, including a number of double and triple burials (O’Shea and Zvelebil 1984). Deposition of human bone into water is also known at Strandvägen, Motala, in Sweden, where 96 fragments of disarticulated human bone were recovered from the riverbed (Hagberg and Gummesson 2015), and at Kanaljorden, Sweden, where the remains of 10 adult individuals and 1 infant were discovered on a stone platform at the base of the palaeolake at the site (Hallgren 2011; 2015).

The deposition of bones into watery contexts or water however may partially explain why we have so few known human remains in Britain dating to the Mesolithic – particularly given that water does not provide the best preservational environment for bones, and given that they are very difficult to excavate (Conneller 2006). The current bias in the British human skeletal record towards watery locations however can be seen, as discussed above, to be a product of the sites currently known in Britain, of which the majority are in locations close to water. The extent to which this is reflective of the original Mesolithic
record as a whole however is unclear, and problematic given preservational environments required and rising sea levels throughout the Mesolithic period. Chatterton (2006) has suggested that a dating programme for human remains found in riverine contexts is needed, as many other human remains recovered may in fact be Mesolithic.

Cemeteries
Mesolithic cemeteries have long been a source of controversy, and there is much debate as to when cemeteries first emerge, and whether they exist in the Mesolithic period. Mithen (1994) suggests that cemeteries first appear in the Late Mesolithic, at a mean age of c.6250 BP (c.5600-5300 cal. BC), with no evidence for cemeteries in the Early Mesolithic. However, more recent work has revealed that there are a number of possible early Mesolithic cemeteries within Europe, including Oleneostrovski Mogilinik (100+ individuals; 8300-8000 BC), Zvejnieki (300+ individuals; 7000-6800 BC), and Aveline’s Hole (50+ individuals; c.8000 BC; discussed below). In their study of European sites, Meiklejohn et al. (2009) suggested that cemeteries occur throughout the Mesolithic, extending back to the 11th millennium cal. BC. Therefore, at present, the Mesolithic period is generally taken to represent the earliest emergence of deliberate and formal cemeteries – although Pettitt (2011, 249) has suggested that cemeteries actually emerge at the end of the Palaeolithic.

Although Woodman (2015, 313) suggests that cemeteries are “a relatively rare phenomenon of the Mesolithic… a product of local, very special circumstances”, a large range of Mesolithic cemetery sites across Europe are in fact now known, such as at Skateholm (southern Sweden), Tybrind Vig (Demark), Téviec and Hoëdic (France), and Tågerup (Sweden) (Ahlström 2003), amongst others (Figure 8). At some cemetery sites, multiple funerary practices are also seen – for example, at Vlasac (Serbia), at least 133 individuals were recovered, and evidence of both primary and secondary inhumation, cremations, multiple burials, and the removal of skulls from inhumations were all found (Borić et al. 2014). Similarly, at the site of Vedbæk (Denmark), both single and double inhumations were recovered, along with a smaller number of cremations (Brinch Petersen and Meiklejohn 2003).
At a number of early Mesolithic European sites, collective burials also seen, for example at sites such as Abri des Autours and Grotte Margaux (both Belgium). These two sites are particularly interesting as they bear many similarities to Neolithic tombs, and clearly highlight that, contrary to traditional belief, collective burials are not restricted to Neolithic mortuary contexts in Europe. The apparent spatial distribution of the remains, selective retention of skeletal elements, disarticulation, and potential secondary burial of remains is remarkable, and highlights that complex burial rites were undertaken in the Mesolithic, and are therefore not related to Neolithic changes in ideology or belief systems, as is routinely described in the archaeological literature (Cauwe 2001). Whether these sites of collective burial within Europe represent ‘cemeteries’ however, is still something which perhaps needs to be given greater consideration (see discussion below).
At present, there are no real ‘cemeteries’ known in the British Mesolithic archaeological record, unlike the burial record on the continent. The only two potential cemetery sites in Britain are Aveline’s Hole and Greylake, Somerset (both discussed above). Whilst the original MNI of the Aveline’s Hole assemblage is believed to have been at least c.50, little recording was undertaken during the initial excavations and the majority of human skeletal material from the site has unfortunately since been lost through WWII bombing of the store the remains were held within. The remaining skeletal assemblage does however consist of 860 human skeletal elements, thought to represent an MNI of 15 individuals (Schulting and Wysocki 2002; Schulting 2005; Conneller 2006). Similarly, of the human remains from Greylake, Somerset, only two crania and a small number of assorted post-cranial elements remain, and as such the assemblage has only an MNI of two. However, the site has been dated to 8460-8275 cal. BC (Brunning and Firth 2012) – thereby also providing an early Mesolithic date for a potential British cemetery. The extent to which cemeteries within the British Mesolithic record are discussed and considered however is poor, and combined with the early excavation dates of the two currently known possible British cemetery sites, and subsequent loss of material, cemeteries are generally not often widely considered as contributing to the British Mesolithic funerary record.

In comparison to the British material, Mesolithic cemeteries throughout Europe have been heavily studied, although primarily through neo-evolutionary approaches in an attempt to gain potential information of rank, status, social structures and demography in Mesolithic populations (Conneller 2009). The study of Mesolithic cemeteries has also tended to dominate the European literature, and discussions of Mesolithic mortuary practices. Whilst there is variability within and across Mesolithic European cemeteries, Gray-Jones (2013) suggests that this has not been fully addressed – particularly given that we frequently see multiple mortuary practices occurring, such as secondary burials, cremations and disarticulation for example. Primarily, however, cemeteries have often been linked to ideas surrounding narratives of increasing complexity – with cemeteries often equated to sedentism.

It is also interesting to note that at a number of cemeteries very few burials appear to have been disturbed, thereby suggesting ideas of remembrance or of grave markers. Nilsson-Stutz (2009) suggests that the lack of post-depositional disturbance at sites such as Vedbæk and Skateholm is related to ideas of respect for the integrity of interred bodies by Mesolithic peoples. However, where disturbance has occurred via the construction of later
graves, no attempts appear to have been made to restore the original burials – therefore somewhat contradicting these ideas of a Mesolithic desire for intact burials. The presence of post holes in some graves, such as the cremation at Hermitage, Ireland (Collins 2009), and also the selected removal of elements such as skulls from inhumations at Vlasac, and their subsequent reburial elsewhere (Borić et al. 2014), suggests that there may have been elements of memory and/or markers associated with some Mesolithic mortuary sites. This raises interesting ideas of remembrance, and the marking or knowledge of locations of graves, which are more frequently discussed in relation to Neolithic human remains (see below).

However, the term ‘cemetery’ frequently not defined in discussions within the Mesolithic literature. For example, how many individuals are required at a site in order to constitute a cemetery? What form should the site take? Do inhumations need to be present? Simple questions such as these are crucial to elucidate discussions of both Mesolithic and Neolithic mortuary practices. By clearly defining what we mean by different types of mortuary treatment and funerary processes, discussions across different sites and different geographical areas are more easily undertaken, and comparative analyses more easily facilitated. Indeed, Meiklejohn et al. (2009) even question the use of the word ‘cemetery’ within Mesolithic archaeology, whereas Pettitt (2011, 249) suggests that the term simply refers to “a place given over in the main or entirely to the dead, with little or no evidence of settlement”.

Finally, there is also a growing body of literature on skull nests within European contexts, which is perhaps also worth highlighting in reference to the above discussion of cemeteries. ‘Skull nests’ are seen at a number of sites, for example at Ofnet, Germany – where two ‘nests’ containing 27 and six skulls respectively were recovered. The presence of cervical vertebrae and cut-marks has been interpreted as suggesting decapitation of flesheled remains (Schulting 1998; see Figure above for site location). Removing crania, either peri- or post-mortem, or separate selective burial of crania in alternate locations, is a reoccurring theme when we consider the European Mesolithic burial record – and raises interesting ideas about deliberate manipulation, disarticulation, and curation of human remains in the Mesolithic. It is also interesting to consider why it is frequently skulls which appear to have been curated and preferentially chosen for collective burial. Interestingly, this practice appears to continue throughout later prehistory – e.g. Iron Age ‘cult of the head’.
3.2. Why do we not have more human remains from the Mesolithic in Britain?

“The Mesolithic population of Britain did not bury their dead in a way that has left any archaeological trace”

(Wickham-Jones 2010, 62)

“Absence of burial in no way signifies absence of afterworld beliefs”

(Ucko 1969, 265)

It can be seen from the above discussion that there is currently plentiful evidence across Europe of Mesolithic mortuary practices and human remains. In particular, large Mesolithic cemeteries are known throughout Europe – but the burial evidence for Britain is still scarce in comparison. As discussed in Chapter 2, we know people were inhabiting Britain in the Mesolithic via significant archaeological evidence, but are yet to determine where the remains of these peoples are. Where and how were the Mesolithic populations of Britain disposing of their dead? Little focus has been placed on this within British archaeology. However, a number of more recent studies have attempted to address the multiple funerary processes which may have been utilised in the Mesolithic (e.g. Hellewell and Milner 2011; Gray Jones 2011).

Whilst in 1932 Clark commented that it was “impossible to agree with Sir Arthur Keith that we have any skeletal remains definitely attributable to the Mesolithic Age of Britain” (Clark 1932, 107), upon Keith’s (1931) suggestion that the remains from Kent’s Cavern, Aveline’s Hole, and Gough’s Cave may be Mesolithic in date; we now have a number of sites across Britain with human skeletal material dating to the period. What we need to perhaps address now is why do we not have more evidence for the people of the British Mesolithic?

With this in mind therefore, there are a number of questions and considerations to address:

- Are the methods of prospection we are using to locate human remains not working or are unsuitable?
- The majority of Mesolithic sites in Britain are known from commercial excavation – therefore their location is not chosen out of choice but instead from necessity
- Were people in the Mesolithic practicing different funerary customs to those of which we are currently aware?
- Were populations dealing with their dead in a way which is not visible archaeologically?
An additional problem lies in the fact that a significant number of British Mesolithic sites were excavated in the 18th, 19th and 20th centuries, and thus the recording methods and techniques used were very different to those utilised today. For example, of the sites discussed by Meiklejohn et al. (2011) in their consideration of Mesolithic human remains in Britain, the majority were excavated prior to 1900, and only two sites discussed had been excavated since 1980. Similarly, the majority of Mesolithic sites known in Ireland (including those with human remains) were excavated in the period 1920-1939 (Meiklejohn and Woodman 2012). Combined with this, excavated prehistoric material stored since these periods has frequently been moved around and often associated paperwork or contextual information has therefore subsequently been lost. For example, only a singular human clavicle is currently known from the site of Oreston Cave, as the majority of the human and faunal material from this site has been destroyed since its excavation in the 19th century (Conneller 2009). Additionally, as Conneller (2009, 691) notes, early excavations would have been “more likely to locate intact skeletons than isolated human bones” – and therefore additional human skeletal material may have been recovered in the past but either been discarded or not recognised. This research therefore aimed to develop strategies for future work as to how we can identify more human remains from the Mesolithic period in Britain, and also increase the amount of useful information we can obtain from the skeletal material we currently have (see Chapter 4).

3.3. Neolithic Human Remains

“The burial of the dead was an important ritual amongst the Neolithic communities of Britain, and it contrasted markedly with the Mesolithic peoples’ apparent lack of concern with their ancestors”

(Malone 2001, 103)

Across Britain we see significant numbers of human remains within the Neolithic – which is somewhat striking compared to the dearth of human remains known from British Mesolithic. However, although we have many more Neolithic human remains, they still represent only a small portion of the Neolithic dead in total – and therefore perhaps Neolithic communities were also sometimes disposing of their dead in ways which leave little archaeological trace, as in the Mesolithic (Scarre 2007, 24). However, Neolithic human remains and funerary practices have been heavily considered theoretically, and are covered well by Beckett and Robb (2006).
Nonetheless, much as in the Mesolithic, a range of diverse mortuary practices were utilised throughout the European Neolithic. However, whilst Mesolithic human remains appear to have often been placed in somewhat inconspicuous locations (e.g. caves, islands), upon entering the Neolithic the dead become much more visible. ‘Visibility’ of the dead is meant in terms of both the number and frequency of Neolithic skeletal remains recovered, but also their depositional locations. The dead are now more visible within the landscape, often being housed in large monumental structures in prominent locations. Therefore, whilst many of the mechanisms for dealing with the dead and mortuary practices adopted appear to show a significant degree of continuity between the two periods, the locations in which the dead were housed are very different. The location of the dead within the landscape also perhaps suggests a connection between places, personal identity, and genealogies – with mortuary practice creating these relationships to the land and to particular locations (Thomas 2000). In this way, it can be suggested that the dead become intrinsic within Neolithic populations, and mortuary practice an integral way in which both personal identities and communities were formed. If we adopt this view, this perhaps marks a shift in the relationship between the living and the dead between the Mesolithic and Neolithic periods.

3.3.1. Mortuary Practices Adopted in Neolithic Britain

“One of the features characterising burial rites is their speed of change and their relative instability”

(Ucko 1969, 273)

Approaches to Neolithic death and Neolithic funerary practices have in recent years been heavily post-processual, with a strong focus often on aspects such as ritual and social reproduction (Downes 1999). However, more recently, it has been suggested that many mortuary practices traditionally considered to be ‘Neolithic’ (or later) actually have a basis in the Mesolithic instead (Hellewell and Milner 2011), as discussed previously.

There are a huge range of different mortuary practices seen throughout Neolithic Britain however, with variations in location, orientation, content, context, materials, size, longevity of use, and shape seen across the period, to name but a few aspects (Malone 2001, 107). The variability in funerary practices seen in the Neolithic has been linked to changing views or beliefs, and changing ‘strategies of representation’ of the dead (Thomas 1991, 138) – i.e. a change in ideas on how individuals were viewed in death, perhaps
related to new technologies of power and ideas of ancestry. These kinds of ideas as to why mortuary practice changed through the Neolithic period stem initially from culture-historical approaches, by authors such as Childe (1940). More recently, however, innovation within funerary practices has been suggested by Schulting (1998) to generally occur amongst social groups with a vested interest in power and moving up social hierarchies.

The following sections will therefore address the types of mortuary practice seen in the Neolithic – with particular reference to the Britain, and how this compares to the burial record for the period seen across Europe and the rest of the world.

**Inhumation**

Inhumation is one of the most common mortuary practices seen within the British Neolithic, although Bradley (2007, 60) suggests that it was more frequent in the south of England than elsewhere in the British Isles. Inhumation is seen within the Neolithic both in the form of collective burials, as discussed below, and also individual inhumations. Inhumations are also known across the European continent, for example, in the lower Rhine area and also parts of Europe further east (Whittle 1988), at sites such as Jechtingen (Germany) (Mörseburg et al. 2015), Füzesabony-Gubakút (Hungary) (Whittle et al. 2013), Ząbie (Poland) (Pospieszny 2015), Çatalhöyük (Anatolia) (Larsen et al. 2015), Vaihingen an der Enz (Germany) (Fraser et al. 2013), and Swifterbant 2 and 3 (Netherlands) (Smits et al. 2010), to name but a few.

In Britain, inhumation is seen across a range of different contexts. For example, primary inhumation is seen in many tombs and monuments (as discussed below) – such as articulated burials at Ascott-under-Wychwood, a Cotswold Severn tomb in Oxfordshire (Bayliss et al. 2007); multiple inhumation burials in the barrow at Stockton, Wiltshire (Ashbee 1970); and three single articulated burials at the oval mound at Radley, in the Thames Valley (Scarre 2007, 86). Crouched inhumations are also seen at a number of British Neolithic sites, such as the barrow at Alfriston, East Sussex (Drewett 1975), Launceston Down, Cornwall (Piggott and Piggott 1944), Maiden Castle, Dorset (Wheeler 1943), and Offham Hill, East Sussex (Drewett et al. 1977). At Duggleby Howe, a round barrow in Yorkshire, a number of different primary inhumations are seen – three crouched inhumations, two inhumations each in shallow graves, and an additional six non-adult
inhumations (Loveday 2002). An initial single primary adult inhumation lies at the bottom of the grave shaft at Duggleby Howe, and all subsequent burials lie above this – both in the fill of the grave shaft and in the mound of the barrow. Whittle (1988, 191) suggests that the nature of the inhumations at Duggleby Howe, combined with the fact that both sexes are represented, along with both adults and non-adults, indicates that although all burials are individual at the site, they have a group nature and context. Edmonds (1999, 122) has commented on the potential patterning of individuals chosen for inhumation in the Neolithic – with more women and children being buried at a number of British sites, such as Maiden Castle and Hambledon Hill. Whether inhumation rites were more frequently reserved for females and non-adults is an interesting concept, and one which has not been given sufficient consideration within the literature, but surely deserves attention in future research both in Britain and Europe.

Interestingly, Edmonds (1999, 121) also suggests that some Neolithic inhumations may have only been temporary – i.e. one stage in a wider mortuary practice involving secondary rites – whereas other inhumations were clearly meant to be permanent, perhaps indicating a differentiation in their function. We also see much variation in inhumation types across Europe – from supine inhumations as seen at many sites (such as Swifterbant 2 and 3 (Smits et al. 2010)), to flexed inhumations at sites such as Çatalhöyük (Larsen et al. 2015) – to variations in the location of burials across European contexts. For example, inhumations at the site of Füzesabony-Gubakút (Hungary) were recovered from the corners of houses within the settlement (Whittle et al. 2013). Clearly, when we discuss inhumation within the Neolithic we must be aware of the wide variety of deviation from what we may perceive (with a modern, Westernised view) to be the ‘norm’ for inhumation burials – perhaps singular inhumations arranged in a cemetery-type location.

Formal inhumation appears to have less prevalent in the earlier Neolithic, with collective burials more common instead (Malone 2001, 103). This apparent change in mortuary practice from collective burials to individual inhumations as the period progresses has frequently been suggested to be linked to the arrival of new populations, namely the Beaker peoples (Thomas 2000). Other interpretations of the change from collective to individual graves have included changes in social structure, ideology or the relationships between the living and the dead, a greater preoccupation with individual prestige in the later Neolithic, a desire to distance the living community from the mortuary deposit, and new ways of marking descent (Renfrew 1973; Shennan 1982; Thomas 2000; Scarre 2007).
It has also been posited that selection for formal, individual burial was a sign of differentiation, and may also be related to ideas of kinship and group identity (Whittle 1992, 33). The idea that “particular individuals were being singled out for preferential treatment in death” (Thomas 1991, 125) also suggests that those buried in individual graves were of known identity – rather than the perceived anonymity of collective burials (Scarre 2007, 24). The emergence of single inhumations in the later Neolithic therefore is a funerary practice which has prompted much discussion within the academic literature, but the reasons behind it are still unclear.

**Burial Monuments**

As discussed in Chapter 2, the Neolithic sees the emergence of monumentality – but many monumental forms also have a mortuary function or association, and many Neolithic built structures were “principally concerned with the veneration of ancestors” (Thomas 2000, 656). Burial monuments are first seen to emerge in north-west Europe, particularly in coastal areas surrounding the Atlantic façade, from Sweden to Spain (Parker Pearson 2009, 135). Initially, Neolithic monumental funerary structures were postulated by archaeologists such as Childe (1940) to represent the emergence of a new religion in Britain, perhaps bought from the Mediterranean. More recently, the emergence of burial monuments has instead been suggested to be related to Neolithic beliefs surrounding ancestry – and were places in which people could both encounter the physical remains of their ancestors, and also meeting places for the veneration of them (Thomas 2000). However, we should not assume that burial within a large monument was a ‘normal’ mortuary rite Kinnes (1975). The numbers of human remains recovered from burial monuments do not equate to population sizes from the period – and therefore it is clear that not all individuals were buried in this way. Whilst we are aware that other varied mortuary rites were also being undertaken, this raises the question as to why certain individuals or members of a population were buried within monuments whilst others were not. Price (2000) has suggested that the burial of only certain individuals within monuments is related to dramatic changes in social organisation throughout northern and western Europe, and is evidence of social inequality.

Another consideration is the time, cost and labour investment in the creation of these burial structures – especially when compared to other forms of mortuary treatment. It is likely that significant numbers of people would have been involved in the construction of a burial
monument – and these ideas are also supported by the inclusion of non-local stone in some monuments, such as at West Kennet long barrow (Whittle 1988, 167). Additionally, we must also consider the siting of these monumental funerary structures. In Britain, many of them are placed within highly visible locations within the landscape (see Chapter 2). This has often led to ideas surrounding burial monuments representing territorial markers and ‘ownership of land’ (Thomas 2000; cf. Renfrew 1973). However, there is some variability in the location of these monumental funerary structures in both Britain and Europe. Tilley’s (1993; 1994) studies of the landscape settings of Neolithic tombs in Britain and Sweden highlights this – showing the regional variability which may exist between burial monuments and their landscapes.

There is also diversity in the range of forms of monuments used for funerary practice, some of which are discussed below. In particular, long mounds/barrows, long enclosures, and long cairns are frequently seen to be used as burial structures in northern Europe during the Neolithic – and the linear nature of these monuments has been suggested to be linked to the long houses of the Banderkeramik cultures, and new modes of organisation of space emerging (Thomas 1999, 131). Within these structures, burials were either placed directly onto the internal floor of the monument, on paved platforms, in burial pits, within cairns made of chalk, stone and flint, or in wooden mortuary chambers inside the monument (Malone 2001, 113). During the Neolithic we also see burial monuments as both built, man-made, primarily earthen and wooden structures, and also as monuments which incorporate natural features, living rock or megalithic slabs. At some sites, both were combined within one burial monument – such as at New Grange, Ireland, a megalithic passage and chamber, which was roofed by oversailing slabs of rock (Clark 1977, 135).

Some of the most widely studied stone burial monuments from Britain are the group of 120-130 Cotswold Severn tombs, found across the south-west of England and southern Wales, and the megalithic passage graves which are frequently found around the Irish Sea area (Malone 2001, 129; 139). Of all the Neolithic long barrows in Britain, West Kennet in Wiltshire is one of the most well-known – and is also the longest of the Cotswold Severn tombs. The barrow comprises predominately of a chalk, sarsen and flint mound, but also has stone-built chambers at its eastern end (Malone 2001, 129). It has recently been dated to 3670-3635 cal. BC, with mortuary activity thought to have taken place over 10-30 years; perhaps representative of the use by only one-two generations of people (Bayliss et al.
West Kennet was found to contain the primary inhumation of 36 individuals across all five chambers within the monument, with both sexes and all ages represented (Piggott 1962; Bayliss et al. 2007; Figure 9). Interestingly, access to the tomb appears to have been restricted during its use. Due to this, and the composition of the skeletal assemblage, Beckett and Robb (2006, 58) suggest it may have served as “an arena for ritual practices reproducing the structural components of the group and its solidarity”.

Belas Knap is another long barrow of the Cotswold Severn type, but is uniquely aligned north-south, rather than east-west. The monument has three side chambers, a smaller southern chamber, an open cist, and a false portal in a forecourt at one end. The remains of 38 inhumations were recovered from the monument (Berry 1929; Radford 1930). As well as being very visible within the landscape however, the site has also been noted to have unusual acoustic properties, which mean sound inside the monument is distorted, and is even believed to induce hallucinations (Graves and Proaj-Wilczynska 2009).

Deposition of human remains in megalithic tombs and monuments is also known across Europe, for example at Rioka Alavesa-Sonsierra and Cameros in northern Spain (Fernández-Crespo and de-la-Rúa 2015), Passy in France (Whittle 1996, 195), Le Varde and Les Fouillages on Guernsey (Kohring 2014), Mysinge (Resmo) and Rössberga
(Västergötland) in Sweden (Lidén 1995), as well as large numbers of monumental tombs in north-west France and Iberia (Bradley 1998, 61). A good overview of megalithic tombs in north-west Europe is given by Sherratt (1990) and also by Bradley (1998).

We can see therefore, that there is significant variation in the types of monumental structures used for mortuary practices within the Neolithic period, along with variability in the mortuary treatment afforded to individuals deposited inside these monuments. We also see varying numbers of individuals within monumental structures, and the length of time each monument was used for deposition also appears to have been wide-ranging (as shown in Whittle et al. 2007). Internal use of space within structures also differed between monuments – with some northern European tombs (e.g. the Totenhütten mortuary structures in Germany) showing very simple, un-subdivided interiors, compared with Maes Howe, Orkney, which shows both linear and grouped compartmentalisation of space inside (Whittle 1988, 169). The construction of burial monuments also stretches across the entire Neolithic – although with changes in form and depositional methods throughout the period.

**Disarticulation**

Disarticulation is more frequently associated with the earlier Neolithic period in Britain (Thomas 2000), and is also generally associated with burial monuments. As discussed above, however, disarticulation can also be seen as a continuation of mortuary practice from the Mesolithic. When considering Neolithic disarticulated human skeletal material, much academic discussion has focused around the loss of skeletal integrity caused by the practice, as individual skeletal elements were comingled with those from other individuals, creating ‘anonymity’ within the mortuary deposit. Because of this, disarticulated deposits in the Neolithic have been linked to ideas surrounding a community of ancestors, in which individual identity was unimportant (Scarre 2007, 24). It is interesting to note however the nature of theoretical interpretation applied to Neolithic disarticulated material considered to that from the Mesolithic period, despite deposits often being very similar in nature.

As mentioned above, many of the examples of disarticulated remains in the Neolithic are associated with funerary structures – with many monuments akin to Medieval ossuaries (Taylor 2001, 32). However, within these Neolithic monuments we frequently see disarticulated material being moved around “in complex and formal ways” (Thomas 2000, 659). At a number of sites disarticulated remains are grouped or placed in specific ways,
resulting in distinctive arrangements of material, and there is also evidence of the selective retention of specific body parts. Indeed, at nearly all early Neolithic cairns and barrows, “particular skeletal elements appear to be unevenly represented” (Thomas 2000, 659). For example, at the site of the Brochtorff Circle, at Xaghra in Malta (a mortuary complex used for burial from 4000-2500 BC), around 250,000 human bones were recovered in “distinctively packaged and compartmentalised modules” (Stoddart et al. 1999, 97), showing discrete ordering and patterning of the disarticulated remains. At the Cotswold Severn tomb of Hazleton North, Gloucestershire, the disarticulated remains of 21 adults and 19 non-adults were found, comprising 9,000 individual bones. The remains were split between the northern and southern chambered areas of the monument, and again appeared to show patterning in the distribution of specific elements and also the age of the individuals (Saville 1990; Hedges et al. 2008; see also Chapter 8). Similarly, at Fussell’s Lodge, a long barrow situated at the eastern end of Salisbury Plain, five main groups of disarticulated material were found within the primary mortuary structure, which show distinct patterning. There is an overall predominance of long bones and vertebrae, but few phalanges and other small bones, which has been suggested to represent decomposition elsewhere, before being transported to the tomb (Beckett and Robb 2006; Wysocki et al. 2007). Interestingly, the skeletal material present also appears to be stacked in a unique way, with some of the bone groups being very deliberately ordered. For example, in two groups, long bones and post-cranial fragments were stacked aligned with the long axis of the monument, with cranial remains concentrated around the edge of the stacks (Shanks and Tilley 1982; Wysocki et al. 2007). The movement of skeletal material between the two sides of the burial chamber, which is split in two by a medial post, has also been suggested.

The nature of disarticulated remains in Neolithic archaeology has also raised ideas about fragmentation within the literature – considering how elements can be fragmented and then recombined, perhaps with other materials such as faunal remains, pottery, or structures. Fragmentation can also “enchain or accumulate [bodies] in webs of identity” (Gamble 2007, 138), and as such, the acts of breaking items up, making them transportable, and potentially storing them together at a central place, can be seen to be linked to ideas of personhood. In this way, there may also be a social aspect to fragmentation. A greater consideration of fragmentation, and how this can be theoretically interpreted in prehistoric contexts, can be found in Chapman (2000; and Chapman and Gaydarska 2007 – although not specifically relating to human remains). It is important to note however that these ideas
can be equally applied to Mesolithic disarticulated remains – but have not yet been done so.

Thomas (1999, 137) suggests that the varying nature of disarticulated Neolithic deposits may be reflective of their transient nature. Rather than seeing disarticulated assemblages within monuments as final resting places, we should instead consider that their disarticulation would have facilitated their movement to other locations, and use in other practices. Given the varying nature of mortuary practices visible within the Neolithic period as a whole, as described throughout this Chapter, this idea does not seem inconceivable. It also links well to ideas surrounding Neolithic attitudes towards the dead, the relationships between the living and the dead, and possible Neolithic ideas on ancestry. However, Thomas (1999, 137) also postulates that the disarticulation of individuals within monuments also represented the transformation of individuals “into another kind of being or substance”. The transformative nature of disarticulation is evident – turning a complete body into multiple fragments – but perhaps the most we can speculate is that disarticulation would have rendered skeletal remains easily transportable, and bodies could be split between multiple locations (see section on Secondary Rites below). The importance and significance behind this for Neolithic communities however remains elusive, as does whether disarticulation was viewed in the same way or held the same meanings for both Mesolithic and Neolithic populations.

Middens

Whilst the burial or deposition of human remains in middens is often generally considered a Mesolithic funerary practice, we also see its continuation into the Neolithic. Often, however, Neolithic human remains are found within existing Mesolithic middens – raising interesting ideas about memory, ancestorship, particular links to a location, and commemoration. The fact that Neolithic deposits within these shell middens are also normally only individual or token burials is particularly interesting to note, and has prompted suggestions that they may have “served to unite the living with the ancestors past” (Whittle 1996, 250).

Across Europe, we see evidence of this practice. For example, at the late Mesolithic (Ertebølle) Danish køkkenmøddinger (shell-midden) site of Bjørnsholm, in north Jutland, an early Neolithic grave was found (Andersen and Johansen 1990). A middle Neolithic
burial has also been recovered from the shell-midden at Rolfsåker in Halland, Sweden (Lidén et al. 2004). Similarly, many other Scandinavian Mesolithic shell-middens have early Neolithic occupation, such as at Norsminde, Jutland, and at some sites this also includes the deposition of human remains (Whittle 1996, 229). Interestingly, the deposition of human remains in shell-middens within the early and mid-Holocene is also known beyond the European continent (e.g. Sri Lanka (Kulatilake et al. 2014)). In Britain, we see similar practices at the shell midden site of An Corran, on the Isle of Skye. The midden is located within a rock-shelter, and human remains recovered consist of 39 disarticulated bones and seven teeth, thought to represent at least 5 individuals (Bruce and Kerr 2012). However, whilst the main midden deposits are believed to be Mesolithic, radiocarbon dates from five of the human bones recovered from the site have all been found to be Neolithic in date, ranging from 3885-4650 BP (Saville and Hardy 2012).

Cremation

Cremation is also seen within the Neolithic period, and in Britain is most frequent at sites across eastern England. Cremation is also seen in parts of northern Britain however, and at many sites in Ireland (Bradley 2007, 60). Overall, however, cremation within the Neolithic period appears to remain a minority practice across Europe, with inhumation and disarticulation much more common. Scarre (2007, 87) highlights that cremation does appear to increase in frequency towards the end of the Neolithic however – which may again be a reflection of possible changes in attitude towards death within the period. Importantly, however, Beckett and Robb (2006, 69) suggest that cremation in the Neolithic did not constitute “an opposed rite to inhumation so much as a particular focus or moment in a complex burial programme”.

On the continent, cremations are known at a range of sites across Europe, and are often seen at cemeteries. For example, at sites such as Elsoo in the Netherlands, Niedermerz in the Rhineland, and Schwetzingen in southern Germany – which has several hundred graves (Bogucki 2000). In the British Neolithic, cremation burials comprise c.4% of all mortuary deposits, and are often only a few grams in size (McKinley 2006). The small size of the cremations recovered suggests they may be token burials – which again raises interesting questions as to why this mortuary rite was afforded to a small number of Neolithic individuals. In Britain, cremations are often found at sites which also have inhumation burials – for example at Duggleby Howe (Loveday 2002; discussed above), Streethouse,
West Yorkshire (Kinnes 1992), Llandegi in Gwynedd, Wales (Houlder 1968), and Stoney Littleton, Avon (Hoare 1821). Cremation cemeteries are also known, but are rarer in Britain. One example however is the cremation cemetery at Bellateare on the Isle of Man (Bersu 1947, cf. Thomas 1999). Thomas (1999, 155) has however noted that multiple cremations, or even cremation cemeteries, are frequently found within henges or other circular monuments (e.g. round barrows, pits, ring ditches) in Britain. Examples of this include cremations found at sites such as Stonehenge, Dorchester on Thames, West Stow, and Coneybury.

Due to the other available mortuary evidence for the Neolithic period, cremations appear, particularly in Britain, to have often been viewed somewhat as the ‘poor relation’ (McKinley 2006). However, it is now apparent that cremation would have not only been difficult to undertake (as discussed above in relation to Mesolithic cremation), but may have also provided a spectacle for those present. The transformative nature of fire, in particular, is something which should not be underestimated, and has frequently been linked to ideas of ‘ritual’ (Downes 1999). The significance of cremation as an alternative burial rite within the Neolithic however remains unknown. Thomas (1999, 155) has however suggested the presence of cremations at monuments which may have also been used for earlier burials represents a desire for continued relationships between the living and the dead, and a desire to re-use the same location for funerary practices – despite changes in the form of this funerary practice.

**Secondary Rites**

Secondary rites are an additional mortuary practice seen within the Neolithic burial record, generally thought to occur between 4200-3000 BC (Parker Pearson 2009, 50), and which are variously discussed within the academic literature. The term ‘secondary rites’ can in itself refer to a wide range of mortuary treatment, but is generally taken as a cover-all terminology for multiple mortuary practices being undertaken on the same remains. This may be as simple as secondary burial of human remains, but can also refer to a range of other funerary treatment – including all mortuary practices discussed elsewhere within this Chapter. Parker-Pearson (2009, 50) suggests there must be a “long intermediary period” between the initial funerary treatment or location of deposition and the secondary rite – but this could be argued to be a modern interpretation of the practice. For example, if defleshing a body using excarnation, a body can be quickly and cleanly stripped in a short
space of time, following which the remains may be removed and undergo further mortuary treatment. As such, there is no reason why there must be a long duration of time between primary and secondary funerary rites. Alternative ways to understand Neolithic secondary rites in funerary practices have come from ethnographic comparisons. In particular, mortuary practices in Madagascar have been used as a comparative population for the Neolithic, in particular with regards to processes surrounding secondary burial of individuals and deposition in collective tombs (Whittle 2003, 127).

Whilst secondary rites are widely commented on within Neolithic literature – although not always explicitly – in reality, they are often difficult to view archaeologically (Beckett and Robb 2006). Whilst some mortuary practices may leave traces evident on the remains themselves, many of these are non-diagnostic. For this reason, studies using techniques such as microscopy and bone histology are now becoming more important, as they may be able to give insights into the ‘histories’ of skeletal remains after death. For example, recent work on bone microstructure and histology has aimed to reveal if we can determine the taphonomic histories and burial environments of skeletal remains (e.g. Jans 2004; Turner-Walker and Jans 2008; Hollund et al. 2012; Booth 2013; White and Booth 2014), and may reveal if bones have been defleshed or excarnated by studying the levels of microbial attack to the bone structure. Bodies deposited whole will generally demonstrate higher levels of microbial attack than those defleshed, due to the presence of high levels of bacteria present within the internal organs, which after death attack the skeletal tissues.

In Britain, examples of secondary treatment are seen at Fussell’s Lodge, Wiltshire (discussed above), where human remains present in the monument are believed to have undergone secondary burial. Other skeletal material shows evidence of weathering, indicating previous exposure before being placed in the monument (Wysocki et al. 2007). Additionally, at Hambledon Hill there is evidence for a range of mortuary treatments being applied to the same remains. For example, some skeletal material at the site shows evidence of defleshing, exposure, weathering, and disarticulation before deposition within the ditches at the monument. Furthermore, other human remains at the site appear to have been charred prior to deposition, and some bones have been curated before being bought to the monument (Harris 2010).

An additional secondary treatment of remains variously discussed within the literature is the idea of the movement and circulation of human remains throughout the Neolithic. The
circulation of human bones is seen to be a particular feature of southern Britain, but also extended across the rest of the British Isles (Bradley 2007, 60). As such, the movement of skeletal material is suggested to have been akin to that of relics, and may also explain why we see incomplete disarticulated human remains across such a variety of contexts in the Neolithic period (Harris 2010). Thomas (2000) likens the circulation of human remains to that of objects in a gift economy – as a means of creating or consolidating relationships between living individuals, and also between the living and the dead. In this way, human remains may have been circulated throughout Neolithic landscapes for significant periods of time before being deposited at sites such as those discussed above – which may also indicate why many human remains are poorly preserved and show a ‘worn’ appearance. In this way therefore, through the movement of human remains, the dead are seen by Thomas (2000, 662) to have been “actively involved in the production of social relationships”, playing an important role in the identities and interactions of the living.

**Cemeteries and Collective Burials**

In the Neolithic we also see the presence of both cemeteries and collective/multiple burials within the archaeological record, as in the Mesolithic. Both of these types of mortuary practice in the Neolithic are thought to be related to the creation of a “spatial and temporal order amongst the dead” (Thomas 2000, 656). Cemeteries, although present across Europe in the Neolithic, are frequently fairly small in size (often <20 burials) (Whittle 1988, 150). One notable exception to this is the cemetery site of Tiszapolgár-Basatanya in Hungary, where 150 burials were recovered, of which the majority were individual inhumations. Both sexes and all age groups are represented, and it is thought that the graves may have been individually marked (Whittle 2003, 62). Similarly, the cemetery at Wandersleben in Germany has more than 200 scattered burials (Bogucki 2000). In Britain, in the later Neolithic we see the emergence of ‘barrow cemeteries’ – round mounds (or groups of) under which burials (often multiple) have been found. These round mounds are often deemed to be the precursor to the characteristic round barrows of the Early Bronze Age (Thomas 2000). One such example is the round mound at Orton Longueville near Peterborough, where over four phases a total of eight inhumations, along with multiple disarticulated remains, were recovered (Taylor 2001).

Collective burials are often found to be associated with burial monuments (as discussed above), and in Britain can vary greatly in size from only a few individuals to up to >100
people. Similarly, German Totenhütten can range from a couple to several tens of people (Whittle 1988, 171), and at Herxheim in southern Germany, c.80 deposits of collective burials have been recovered, thought to represent c.1000 individuals (Boulestin et al. 2009). A good analysis of collective burials in Neolithic Ireland is given by Beckett and Robb (2006). Collective and multiple burials are not solely found in monumental funerary structures however. At the site of Fengate in Peterborough, a collective grave pit was discovered, which held the remains four individuals – one articulated adult male, one infant (3-4 years), and two disarticulated individuals, an adult female and a child aged 8-12 years (Pryor 1976). Similarly, at Sumburgh in Shetland, an early Neolithic stone-lined cist with no associated monument was discovered, containing the remains of at least eighteen individuals (Hedges and Parry 1980). Similarly, the recently published mass grave at Schöneck-Kilianstädtten, Germany contained the remains of at least 26 co-mingled individuals, and was located within a large pit feature in a settlement site containing 18 LBK houses (Meyer et al. 2015). Equally, at Talheim, Germany, the remains of 34 individuals were found within a pit feature (Wahl and König 1987; Price et al. 2006).

The prominence of collective burials, particularly within monumental forms, within the British Neolithic has frequently been interpreted as a reflection of the beliefs, relationships, and social structure of Neolithic populations. The dominance of the collective over the individual has, therefore, been seen as a replication of ‘group solidarity’ and the balance of social power within a community (Whittle 1988, 142). In this vein, collective deposits have been seen as relating to kinship or even family groups (Whittle 2003, 130) – although this is not based on aDNA data. However, Whittle (1988, 170) rightly highlights that “it is very unlikely that collective burials represent anything other than a fraction of a total population”.

Caves

Much less frequently seen within the Neolithic, and often considered a more ‘Mesolithic’ mortuary practice, is the use of caves for the deposition of human remains. At present however, c.70 Neolithic cave sites across Britain are known, comprising 256 burials (Taylor 2001, 30). In the Peak District alone, there are believed to be 26 caves with Neolithic/EBA burials (Barnatt and Edmonds 2002). One example is Dowel Cave, near Earl Sterndale, which contained the remains of a minimum of eight individuals, including one partially intact crouched inhumation, a number of extended inhumations, and
disarticulated skeletal material. Associated with these remains were also a number of dog inhumations. The skeletal material within the cave appears to have been manipulated in a similar way to that seen within many monumental funerary structures, including the grouping of skulls (Barnatt and Edmonds 2002). Other caves with Neolithic human skeletal remains include Tom Tivey’s Hole, Somerset, which contained the disarticulated remains of one ?female individual (Barrett 1966).

Neolithic cave burials are also known elsewhere in Europe. Interestingly, hypogea are also known in some areas of Europe, such as the Paris Basin and south-western Portugal. These artificial caves were constructed in the late Neolithic through being carved into natural chalk, and then were subsequently utilised as collective tombs (Blin 2015; Waterman et al. 2016). The idea of artificially creating caves for use within funerary practices is fascinating, and perhaps highlights the perceived importance of caves within mortuary practices in the European Neolithic. In the Neolithic we can therefore see that burial deposits within caves were often very similar in nature to those found within monuments such as causewayed enclosures and barrows (Barnatt and Edmonds 2002).

3.4. Mesolithic and Neolithic Mortuary Practices – Similarities and Distinctions

Having addressed the currently available evidence for Mesolithic and Neolithic mortuary practices in Britain, and their European context, we can consider the degree of similarity and distinction between funerary processes in both periods. Whilst there are clearly some significant differences in mortuary treatment between the two periods, there are degrees of continuity too. Many of the locations of deposition of human remains in the Mesolithic – such as caves, and dark, liminal places – are also seen in the Neolithic. However, these degrees of liminality are perhaps more pronounced or ‘visible’ in the Neolithic, with the deposition of human remains within monumental forms. Whilst these are undoubtedly very clear markers within the landscape, and can be seen by all – perhaps much like middens in the Mesolithic – the interior of monuments may not have been accessible or visible to all, and upon entering them, they would undoubtedly have seemed dark, other places, over which a threshold had to be crossed. Selective access to the interior of monuments in the Neolithic has been widely discussed within the literature, but these ideas could equally be applied to caves, both in the Mesolithic and the Neolithic.
Similarly, the different forms of processing seen in the Mesolithic are generally also seen in the Neolithic too – disarticulation, cut-marks, single inhumations, multiple burials, cemeteries, and cremation. Whilst many of these mortuary treatments are more frequently and traditionally associated with the Neolithic in academic literature, the above discussions show that they can also be found – although perhaps not always as numerously – in the Mesolithic across Europe too. Within the British literature in particular, the paucity of Mesolithic human remains within the UK has meant that they have been afforded much less attention, and also much less theoretical consideration. This lack of scholarly and critical attention has therefore perhaps created a false dichotomy between Mesolithic and Neolithic funerary treatments. With this in mind therefore, the perceived change in belief systems or ideology in the Neolithic, reflected in funerary treatments, may not be so distinct. In order to truly define whether there is a distinction between Mesolithic and Neolithic mortuary treatments, and whether we see a change in the relationships between the living and dead between the two periods, much greater scholarly attention and theoretical consideration of the Mesolithic is needed. Although considered by authors such as Nilsson Stutz (2003), ideas surrounding Mesolithic mortality, treatment of the dead, and relationships with the dead, are still highly under-developed.

However, it is perhaps also important to note that of many of these mortuary practices described above in the Mesolithic and Neolithic may in fact have origins in the Palaeolithic. For example, Orschiedt (2013) provides a good overview of the evidence for primary burials, disarticulation, and the fragmentation and manipulation of human remains seen in the Magdalenian (Late Palaeolithic) in Europe. Similarly, Pettitt (2011, 249) provides a summary of the late Pleistocene evidence for formal cemeteries. Ideas of collectivity of the dead, and the curation of human remains, are also seen to have a basis in the Late Palaeolithic, extending into and throughout the Mesolithic (Pettitt 2011, 259). With this in mind therefore, degrees of continuity can be seen to run through from the Palaeolithic to the Neolithic – and therefore perhaps highlights the academic boundaries that categorisation of the prehistoric past and nomenclature can have (as discussed in Chapter 2, section 2.1.). Although some important distinctions can be made between the mortuary treatments afforded in these prehistoric periods, aspects of continuity can also be seen, as can the gradual emergence of practices over long periods. For example, middens in the Mesolithic could be seen to be precursors to Neolithic monuments – both being used for mortuary activity, food related activities, meeting places, being used on periodically or seasonally, and occupying significant or prominent locations within the landscape.
Similarly, manipulation of the body is seen throughout the Mesolithic and Neolithic (and indeed also in the Palaeolithic). The selective retention and patterning of disarticulated material seen in many Neolithic assemblages, particularly those associated with causewayed enclosures and other monuments such as long barrows, can also be seen to be mirrored in some Mesolithic assemblages – for example, remains associated with mounds, or the recovery of skull nests. Post-depositional manipulation of Mesolithic remains has even been seen in the form of selective removal of skeletal elements from graves, for example at the sites of Skateholm and Vedbæk-Bøgebakken (Nilsson Stutz 2003, 310).

3.5. Rest in Peace? The Ethics of Dealing With Prehistoric Human Remains

Although this research is predominately scientific in nature, it is important to consider it alongside current theoretical frameworks in archaeology, as highlighted both within this chapter, and the previous. One aspect of this study which is therefore perhaps pertinent to address is the use of prehistoric human remains. There is currently a wealth of literature on the topic of ethical consideration of human remains, their excavation, and subsequent treatment. Whilst archaeologists in Britain have not become subject to such restrictive legislation as in other countries (e.g. Australia, USA, New Zealand etc.) (Bowdler 1992; Bray 1996; Rose et al. 1996; Smith 2004) – mainly due to the fact that there are no ‘native’ or ‘aboriginal’ groups in the UK – there is still a growing consensus within British archaeology that ethical considerations and post-excavation treatment (e.g. storage, archiving) must be more fully considered.

Human remains of a prehistoric date are often considered slightly differently to later remains, due to the scarcity of human skeletal dating to these periods in Britain (as discussed above). Therefore, whilst these remains may often hold huge research potential, the issues associated with them are frequently manifold, and museums and institutions are sometimes reluctant for analyses to be undertaken on them due to their rare and unique nature. Furthermore, issues over analyses and ‘ownership’ are often more pertinent with prehistoric remains, and more commonly attract both media attention and become the focus for modern repatriation groups (e.g. Brothwell 2004; Swain 2007; Honouring the Ancient Dead (HAD) 2008; Council of British Druid Orders 2010) – which can result in very problematic circumstances for archaeologists. The proliferation of modern Druid and Pagan groups in the UK in recent years has been particularly notable, with groups such as Honouring the Ancient Dead (HAD), the Council of British Druid Orders, the Druid
Network, and the Pagan Federation all associating themselves with prehistoric human remains. A cursory internet search quickly reveals the multiple groups of Pagans and Druids who have in recent years pushed for claims to the ‘ancient dead’, with many arguments focusing around the desire for reburial of prehistoric human remains by these groups. This has perhaps been most notable in a number of high profile media cases, such as the retention and study of Neolithic remains uncovered at Stonehenge and Avebury (HAD 2009; Maughfling 2009; Morris 2011; Wallis and Blain 2011; Druid Song 2013; Stonehenge Druids, n.d.), and also the subsequent display of human remains at the Stonehenge visitor centre in 2013 (HAD 2013(c); Druid Song 2013). HAD has even recently published its own handbook on reburial, as well as guidance on ‘committal rites’ and respectful treatment for reburials (HAD 2013(a); 2013(b)). Indeed, HAD’s tagline reads “promoting respect for those who have gone before” (HAD 2014), implying that archaeologists or other non-Druid/Pagan groups do not promote respectful treatment of human remains. More recently, Druid and Pagan groups have also waded into the debate surrounding the proposed tunnel construction at Stonehenge, with the potential disturbance of possible additional human remains which may be recovered surrounding the site (Somers 2014). In tandem with this, journalists such as Liz Williams of The Guardian (2011(a); 2011(b); 2013(a); 2013(b)), and Emma Restall-Orr (2004) have further promoted Pagan and Druid beliefs in the media.

The proliferation of such groups in the UK in recent years is perhaps due in part to the successes of international groups elsewhere in the world in terms of repatriation and reburial of human remains – in particular native American, Australian and New Zealand groups. However, Neo-Pagan and Druid groups emerging in Britain also have parallels on the European continent, with similar large movements now also known in France, Germany and Poland for example. However, in a number of these European countries, calls for claims to human remains and appropriation of archaeological sites by these groups has also been in tandem with advancement of political issues or political groups. In Germany for example, a number of large prehistoric megalithic sites are now frequented not only by Neo-Pagan and Druid groups, but also Neo-Nazi groups – who are attempting to appropriate or use these sites to ‘strengthen’ or publicise their political views (Perschke 2013). In Britain however, the emergence of Neo-Pagan and Neo-Druid groups has been suggested to be linked to ideas of new forms of spirituality, due to disillusionment with the modern world, and an attempt to return to nostalgia and mythology (Pawleta 2013). Calls for claims to, or reburial of, prehistoric human remains however may be related to ideas of
identity (both individual and collective), aspirations of ‘deep’ ancestry, and a desire to undermine the dominant role of science in modern life via proliferation of alternative discourses.

The problem however lies in the opposing views held by archaeologists and heritage professionals compared to Pagan and Druid groups (see Text Box below), specifically when considering the analysis, retention, and reburial of human remains. Now included in consultations on prehistoric sites, for example at Stonehenge, it would appear that claims to the ‘ancient dead’ made by Pagan/Druid groups must now be considered in future work – as promoted by Wallis and Blain (2011).

When archaeologists desecrate a site through excavation and steal our ancestors and their guardians, they are killing me as well as our heritage. It is a theft. I am left wounded. My identity as a Druid is stolen and damaged beyond repair. My heart cries. We should assert our authority as the physical guardians of esoteric lore. We should reclaim our past.

(Davis 1997, 12-13; cf. Wallis and Blain 2011)

The often destructive nature of modern scientific methods is also an issue which needs careful consideration, as by obtaining this data we are ultimately destroying a small amount of a person’s skeletal remains in the process. Archaeologists and all those with a vested interested in the archaeological past must therefore consider carefully the information which may be obtained through these methods before their sampling. A combined biomolecular approach – using multiple methods in tandem on the same samples – adopted within this research (outlined in Chapter 4), meant however that ‘destruction’ of skeletal samples was kept to a minimum, whilst significant amounts of biomolecular data were obtained (as highlighted in Chapter 5; see also discussion in Chapter 9).
4.1. New Approaches to Studying the Mesolithic and Neolithic of Britain

This research focuses on five key themes or research areas – identification, diet, mobility, chronology, and health/disease – all of which are central to debates and discussions surrounding both the Mesolithic and Neolithic periods, and which are discussed in detail within Chapter 2. This chapter will therefore provide an overview of the methodologies used within this research via which we can address these five areas, and the research themes raised within Chapters 2 and 3; discussing previous applications of the methodologies applied, alongside how each technique will be utilised within this study.

One of the key aims of this research is to utilise a novel, integrated, and holistic scientific approach to obtaining more information on the British Mesolithic and Neolithic, and the transition period, focused predominately around the five key research areas listed. This approach is attained through the utilisation of materials which have not traditionally been used within these debates – notably skeletal fragments previously considered unidentifiable or unimportant, disarticulated bone, and dental calculus. Whilst these materials arguably have little osteological value, they hold, at a molecular level, key taxonomic, dietary, proteomic, and genetic information that can be placed within spatial, contextual, and temporal frameworks. Therefore, to contribute new information on this elusive period, this research provides the first application of a range of integrated scientific techniques to prehistoric material – notably collagen peptide mass fingerprinting (ZooMS), stable isotope analysis, archaeoproteomics, aDNA analysis, and AMS dating. The use of cutting-edge proteomic and genomic technologies alongside more traditional modes of scientific study aims to provide a new approach to studying the Mesolithic and Neolithic of Britain – and hopes to reveal new information on diet, subsistence, disease, lifeways, chronology, mobility, and burial practices in the period surrounding the British Mesolithic-Neolithic transition.
4.2. Identification

4.2.1. Why identification is important

“Identification is absolutely fundamental to all that will follow and must therefore be of the highest standard”

(Chaplin 1971, 41)

“Identification of the retrieved bones might seem the most straightforward and fundamental of processes, yet it is fraught with practical and theoretical difficulties”

(O’Connor 1996, 9)

Human and faunal skeletal identification aim to determine both the anatomic location and phylogeny of the species from which the material is believed to have derived. In this respect, identification may resemble a form of typological classification, and is thus seen as “the basic unit of zoological classification” (Driver 2011(a), 20) and binominal systems. By determining the species a bone belongs to, we can obtain primary data which can then be included in broader discussions and debates. Identification of skeletal material from archaeological contexts therefore allows for a greater degree of interpretation to be undertaken. For example, determination of faunal species can provide insights into the relationships between humans and animals throughout the archaeological past (Reitz and Wing 2008, 153).

Despite Binford and Bertram’s (1977, 125) ascertation that “all bones, even the smallest fragments, may be identified given sufficient training in osteology”, it is frequently the case, particularly in prehistoric assemblages, that skeletal material or bone fragments may be problematic or difficult to identify, or may indeed be ‘unidentifiable’ – often due to heavy fragmentation. The frequency with which we recover very small, heavily fragmented and/or disarticulated skeletal material within archaeological contexts has prompted the emergence of a range of techniques to aid identification – some of which are now becoming widely applied within bioarchaeological studies of human and faunal material. Some of the current methods of species identification are discussed below, in an attempt to highlight both the advantages and limitations of the available approaches, and to place the identification technique used within this study into a broader context.
4.2.2. Previous methods of species ID

Traditionally, skeletal identification of both human and faunal material has been undertaken using osteology and macro-scale analyses. Zooarchaeological identification of skeletal material aims to determine both the anatomical location of the bone in question, and the species from which it derived. Similarly, human osteological studies also aim to determine the location of the bone within the human body. However, particularly in faunal assemblages, identification frequently results in broader descriptions of skeletal material concerned with the possible element the bone fragment may derive from, and its dimensions, but not the species or genus (O’Connor 2000, 36). In order to correctly ascertain speciation of bone fragments osteologically, some form of diagnostic morphological indicator must be present on the bone. If missing, then it is often not possible to go beyond broad categorisation such as ‘large ungulate’ and so forth. Bone fragments which are not designated to a particular species are also often classified as ‘indeterminate’ or ‘unidentifiable’, and are not included in calculations or discussions of species prevalence (Chaplin 1971, 38).

Osteological determination of species also generally requires skeletal reference collections, and this will frequently necessitate specimens of varying ages and both sexes due to morphological differences caused by sexual dimorphism, development or maturation. Amassing and preparing a comparative reference collection of this kind however is “a time consuming and costly enterprise” (Wing and Brown 1979, 113). An appreciation of intertaxonomic vs. intrataxonomic variation at a species level is also crucial (Lyman 2002). Osteological species identification is also problematic as certain faunal species may look morphologically very similar to other, distinct (but perhaps closely related) species, thereby making it difficult to differentiate between the two. One common example of this is sheep and goat bones, which are morphologically very similar. Similar problems occur when attempting to distinguish between cattle and bison, species within the equids, camels, wild vs. domestic mice, and a range of fish remains, for example (Davis 1987, 33; Gobalet 2001; Bochenski 2008). Osteological determination of species can also be hindered by taphonomy and various diagenetic and biostratinomic processes, as these may obscure or remove diagnostic species markers on a bone. For example, processes such as burial, gnawing, fragmentation, exposure, fracturing, burning, abrasion, and weathering will all alter a bone (Lyman 1994, 3).
Due to the some of the issues surrounding osteological identification of species (of which Driver (2011(a)) provides a comprehensive and critical overview of), a number of scientific and bioarchaeological methods have also been developed. For example, aDNA analyses have been implemented in a number of studies as a method of species identification, but very few papers have utilised aDNA as means by which to independently confirm taxonomic identifications based upon traditional zooarchaeological/osteological analyses (Driver 2011(b)). The use of aDNA as a means of species determination is now starting to be recognised however (Wolverton 2013), and be incorporated into archaeological studies, particularly using co-amplification methods (e.g. Yang et al. 2004; 2005; Speller et al. 2005; Horsburgh 2008).

Microscopy and spectroscopy have also been utilised as alternative scientific techniques for species identification of bone (e.g. Cattaneo et al. 1999; Walter et al. 2004; Cuijpers 2006; Martiniaková et al. 2006; 2007; Hillier and Bell 2007; Cuijpers and Lauwerier 2008; Greenlee and Dunnell 2010; Brits et al. 2014; Sawada et al. 2014) and also other skeletal materials such as ivory (e.g. Edwards et al. 1997; 2006) and horn or shell (Edwards et al. 1998). These techniques use differential histological patterning of the bone micro-structure and osteonal canal dimensions to determine species (Cattaneo et al. 1999; Greenlee and Dunnell 2010). Whilst the benefit of this technique lies in that fact that it is relatively inexpensive (particularly compared to DNA analyses), and can be undertaken non-destructively, there are issues in that the post-depositional environment and various degradation processes – particularly microbiological attack – may alter or affect the collagenous component of the bone or its histological structure, thereby making it difficult to morphologically assign the bone structure unambiguously to species level (Edwards et al. 2006; Greenlee and Dunnell 2010).

Finally, a small number of studies have used metrical analyses – particularly cortical bone thickness – or more recently, morphometrical analyses, as an additional means of species identification (e.g. Croker et al. 2009; 2010; Saulsman et al. 2010; Rérolle et al. 2013). These studies are often forensically based, although sometimes do utilise archaeological material, but have often been found to be an ineffective method of species identification, particularly in distinguishing between human and non-human skeletal remains (e.g. Rérolle et al. 2013). Smaller numbers of studies have also attempted to use radiographic differences between human and non-human bones within species identification (Croker et al. 2013), micro-proton induced X-ray and gamma-ray emission (micro-PIXE/PIGE)
methodologies (Müller and Reiche 2011), and X-ray diffraction techniques (Piga et al. 2013) – which also appear to have had little success (particularly with regards to XRD).

4.2.3. Proteomic Approaches to Species Identification

“The systematic attribution of specimens to taxa is essential”

(O’Connor 1996, 10)

Proteomics can be defined as the study of both proteins and proteomes, which are the complete set or range of proteins produced by a cell (van Doorn 2012). Proteomics is fast becoming one of the newest applications of existing scientific technologies to archaeological material – thus far having been applied to materials such as archaeological eggshell, hair, feather, horn, parchment, glue, skin/hide, and dental calculus (e.g. Hollemeyer et al. 2002; 2007; 2008; Charlton 2012; Toniolo et al. 2012; Dallongeville et al. 2013; Kirby et al. 2013; Stewart et al. 2013; 2014; Warinner et al. 2014(a); 2014(b); O’Connor et al. 2015; Rao et al. 2015; Fiddyment et al. 2015). A growing body of recent research within palaeoproteomics has however also focused upon the sequencing of ancient proteins within bone (e.g. Buckley et al. 2009; 2010; Richter et al. 2011; van Doorn et al. 2011). This has most notably been done with collagen, the most abundant bone protein, and has subsequently allowed for the development of a technique known as Zooarchaeology by Mass Spectrometry (ZooMS). ZooMS is a novel mode of archaeological proteomic analysis, involving collagen peptide mass fingerprinting, which allows for species or genus level identification of collagenous materials. It has therefore predominately been used for taxonomic identification, and, as such, has significant applications and connotations for archaeological and zooarchaeological work.

ZooMS will be utilised within this research as a method of determining species or genus level identification of bone fragments. Discussion here will focus on how the technology and methodology work (both in terms of our understanding of bone and collagen structure and also the instrumentation used), and previous applications of the technique. An overview of bone structure will therefore firstly be given below, before a detailed discussion of protein sequencing in bone is provided. It is crucial to have detailed knowledge of bone microstructure and the constituents of bone in order to fully understand how ZooMS works, and how it can be applied to archaeological materials successfully. Furthermore, this discussion also has relevance for stable isotope analysis on bone, which is discussed below in section 4.3.2.
4.2.3.1. Collagen and bone structure

Archaeological studies of bone have traditionally been osteological in nature, sometimes utilising basic technologies such as X-ray. However, the advent of human bioarchaeology and biomolecular archaeology has attempted to apply biological, chemical and medical concepts and technologies to archaeological material. This has therefore led to the study of aDNA within bone, the analysis of stable isotopes in skeletal material, and more recently, the study of ancient proteins within bone.

Bone is a highly complex and ordered hierarchical mineral-organic composite material (Weiner and Traub 1992). It is a connective, structural, living tissue, and is unique in that it undergoes continuous renewal and reconstruction throughout life, and can respond to external stimuli (Davis 1987, 47). Bone is composed of c.70% mineral (by weight) and c.30% organics, thereby making it a composite material (Mays 2010, 1). Composite materials are known to have elastic properties, and are generally stronger than either of the materials from which they are formed (Currey 2002, 105). The organic constituents of bone allow it to resist tension and have a slight flexibility, whereas the mineral phase enables bone to resist compression and provide weight-bearing capabilities. The combination of resistance to both sheer and compressive forces therefore means that bone is actually very similar to reinforced concrete (Halstead and Middleton 1972, 16; Gunn 2002, 1; Eroschenko 2005, 74; Waldron 2009, 14). Due to this, bone is “one of the strongest biological materials in existence” (White and Folkens 2005, 33). A detailed discussion of the effects of bone as a composite material is given by Currey (1984; 2002).

Bone has three main components – a complex protein scaffold (organic phase), a mineral which hardens this scaffold (inorganic/mineral phase), and a ‘ground substance’ of organic compounds (organic phase) (O’Connor 2000, 5). The inorganic mineral aspect of bone is principally composed of calcium and phosphate ions, and a carbonated form of non-stoichiometric hydroxyapatite, known as dahlite [(Ca, X)_{10} (PO_4, CO_2)_6 (O, OH)_{26} – where X= Na, K, Sr, etc.] (Nielsen-Marsh et al. 2000; Pollard and Heron 2008, 273). The terminology used to describe the mineral aspect of bone is however often confusing, with the terms ‘bone apatite’, ‘hydroxyapatite’, and ‘bone mineral’ being used interchangeably – despite the fact it is a carbonate hydroxyapatite mineral. Nonetheless, the mineral aspect of bone takes the form of thin plates or crystals, which are c.2-3nm thick and have a hexagonal crystallographic symmetry. The small size and unique shape of these crystals gives the bone mineral a very large surface area (Weiner and Traub 1992; Nielsen-Marsh
et al. 2000; Pollard and Heron 2008, 273). Bone mineral is also isomorphous or ‘impure’, as it will readily incorporate ‘foreign’ ions such as minerals but without changing structure (as highlighted by the ‘X’ in dahllite’s chemical composition, seen above). Carbonate is the major impurity in bone mineral, with CO$_3$ content comprising 7.4% of the total mineral (Nielsen-Marsh et al. 2000; Tuross 2003).

The organic aspect of bone is predominately composed of the fibrous structural protein collagen (c.90% by weight), whilst the remainder of the organic fraction comprises other non-collagenous proteins and lipids (Pollard and Heron 2008, 273). Collagen is found in all metazoan animal phyla, but only in vertebrates does it form part of a mineralised skeletal structure (Currey 2002, 5). At present, 19 genetically distinct collagens are known throughout the human body, but it is type I collagen which is found within bone – although traces of type III, V and X collagens are also sometimes found during certain stages of bone formation to regulate collagen fibril diameter (Jee 2001). Type I collagen is the only collagen within the body however which “supports the deposition of hydroxyapatite in vitro” (Tuross 2003, 68).

At a molecular level, type I collagen is comprised of molecules known as tropocollagen, which are themselves composed of a repeated pattern of polypeptides. The polypeptide chains themselves contain triplet regions viewed as:


(Where Gly = glycine, and X and Y = any other amino acid)

Interestingly, the X and Y regions in the above schematic are most commonly proline or hydroxyproline, but can be composed of any other combination of 17 different amino acids. In general however, type I collagen has a composition of 33-35% glycine, 7-13% proline, and 9-13% hydroxyproline, with other amino acids comprising the remainder. Hydroxyproline in particular is a unique constituent, as it is a significant part of collagen but does not appear in any other human proteins (Pollard and Heron 2008, 274). Nonetheless, these triplet polypeptide chains which form tropocollagen molecules generally span a sequence of 1014 amino acids and account in part for the stability of tropocollagen’s structure, by forming uninterrupted helical domains which contain semi-flexible rods (Miller and Gay 1987).
Tropocollagen molecules, with these triplet polypeptide sequences, then aggregate to make chains known as microfibrils. The tropocollagen molecules form microfibrils by lining up and then forming cross-linking covalent bonds between molecules in neighbouring files. The inter-molecular cross-links these bonds between tropocollagen molecules form also provide stability to the microfibrils (Currey 2002, 5-6). Three microfibrils, which are macromolecules of collagen, are then arranged into a left-hand spiralled triple helix structure, which then itself spirals to the right about a central axis – in very much the same way as the structure of traditional Hawser-laid rope (O’Connor 2000, 5). Each triple helix within Type I collagen therefore contains three microfibril chains, of which two are the same [α1(I)] and one is different [α2(I)], and is therefore generally written as: α1(I)2 α2(I).

Both α1(I) and α2(I) microfibril chains are the same size however, at 95,000Mr in length (Miller and Gay 1987; Pollard and Heron 2008, 274). Individual triple helix structures (also commonly referred to as ‘ropes’ or ‘heterotrimeres’) are known as fibrils:

The relationship between the organic and inorganic constituents of bone is complex. As we know, the internal structure of bone is composed of the fibrous organic matrix, which is then surrounded by and contained within the finely crystalline mineral phase (Pollard and Heron 2008, 273). When bone is formed, the matrix of collagen and other organic components are believed to be laid down first (known as the osteoid), with the mineral then being deposited in the gaps between collagen fibrils and also along the length of the fibrils too. It is thought that the plate-like shape of the mineral crystals allows them to be aligned to the collagen fibrils in parallel layers (Currey 2002, 10). This idea of the mineral being
located within the small spaces of the collagen fibrils themselves, and forming a layered composite structure, is known as the ‘staggered array model’, and was first proposed by Hodge and Petruska (1963) (for a good overview see also Woodhead-Galloway 1980). Nonetheless, the microarchitecture and relationship between the collagen framework and mineral crystals is still an area of bone microstructure which is not yet fully understood (Weiner and Traub 1992).

Understanding bone structure and microstructure is important as it allows us to gain a greater understanding of the multiple constituents and components of bone tissue, and in tandem with this, why bone holds the characteristics it does. A detailed knowledge of bone structure also allows us to understand the mechanisms of mineralisation, and the interactions between the organic and inorganic aspects of bone (Weiner and Traub 1992). Bone is directly comparable between individuals (and species) and also provides direct evidence for cellular physiology, as bone cells and tissues are direct records of metabolic activity (Mishra 2009). Understanding all of the above is therefore vital in order to gain insights into pathological conditions, bone preservation in archaeological contexts, and for the biomolecular study of archaeological skeletal materials.

**4.2.3.2. Protein Mass Spectrometry and Peptide Mass Fingerprinting**

The discovery that amino acids could persist in fossils was first made by Hare and Abelson (1965), and sparked an interest amongst biologists, biochemists and archaeologists that proteins and other potentially informative biomolecules may survive in archaeological skeletal material. From this, in the past 15 years, has emerged the use of soft ionisation mass spectrometry (MS) as a means via which to detect peptide sequences within proteins from archaeological materials. The use of the term ‘soft’ indicates that the energy used to ionise the peptide is not sufficient to degrade the molecule, thereby allowing for ‘fingerprinting’ of the protein (van Doorn 2012). This was first successfully applied by Ostrom et al. (2000), and detected the bone protein osteocalcin in archaeological samples. The success of this work led to a large number of other subsequent protein fingerprinting studies, sequencing both osteocalcin from archaeological bones (e.g. Buckley et al. 2008(a)), and more recently, collagen (e.g. Buckley et al. 2008(b); 2009; 2010; 2014(a); Zhang et al. 2009; Richter et al. 2011; van Doorn et al. 2011; Welker et al. 2015(a); 2015(b)). Collagen has been preferentially chosen in most recent studies over other bone proteins as it is more readily isolated and detected within archaeological remains, and is
known to persist for significant periods of time within the archaeological record (Holmes et al. 2005; Collins et al. 2010).

All MS works using the basic sequence of ionisation, followed by separation of ions by mass to charge ratio (m/z), and then the detection of ions to obtain a mass spectrum. Before being run using MS however, proteins need to be digested using a specific protease, which, in the case of archaeological protein studies, is normally trypsin. Trypsin hydrolyses (cuts) peptide bonds after each lysine (K) and arginine (R) residue in the protein, therefore creating a predictable cleavage pattern. By ‘cutting’ the protein in a predictable manner, the individual sections form a ‘fingerprint’ of that protein, which can then be used to identify it (Henzel et al. 2003). As such, this technique is known as peptide mass fingerprinting (PMF).

The most common soft ionisation MS method used within protein fingerprinting studies has been matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS works by irradiating a given sample with a nitrogen laser, which then excites the molecules of an energy absorbing matrix solution placed over the top of the sample (usually α-cyano-4-hydroxycinnamic acid (CHCA)). The sample therefore becomes ionised, which in turn separates out individual peptides within the sample. The ions are accelerated towards a detector at the end of the MS machine through a field free drift region flight tube. As the ions pass through the detectors their mass is calculated – determined via the length of time it takes for them to move through the machine. Low mass ions will take less time to pass through the flight tube (i.e. a shorter time of flight) than heavier ions (Ostrom et al. 2000). The masses of individual peptides sequenced are then either compared to a database consisting of the theoretical peptide masses of proteins, such as NCBI or Swiss-Prot (Damodaran et al. 2007), or an individual database consisting of known protein sequences. For archaeological samples however, an extensive reference database may sometimes be needed, with details of species specific peptide markers. Nonetheless, once this is collated, it can be used repeatedly, and shared between researchers (Henzel et al. 2003; Richter et al. 2011). PMF using MALDI-TOF MS is therefore the most commonly utilised method in archaeological proteomic study currently. The technique is advantageous because it is fast, sensitive, accurate, and allows for correct protein identification even if the peptides contain post-translational modifications (Henzel et al. 2003; Thiede et al. 2005).
4.2.3.3. ZooMS – Zooarchaeology by Mass Spectrometry

It has long been recognised that collagen “from different sites has different amino acid compositions” (Currey 1984, 24). Although it may not have been Currey’s original intention, we should read ‘sites’ as not only referring to different areas within the human body (i.e. bone vs. soft tissues etc.), but also different species. ZooMS works by taking advantage of the fact that tropocollagen molecules within collagen microfibrils (as discussed above) are composed of chains of polypeptides and amino acids. The sequence of amino acids within type I collagen typically differs between species – therefore meaning that if the individual peptides can be separated out from the collagen in bone (through the use of MALDI-TOF MS), we can determine species (Figures 10 and 11). ZooMS is therefore a method of collagen peptide mass fingerprinting, and has been used as a qualitative analytical technique for taxonomic identification (e.g. Buckley et al. 2009; 2010; Richter et al. 2011; Sluis et al. 2014; O’Connor et al. 2015; Welker 2015(a)).

Figure 10: Image highlighting the ZooMS methodology and the principle of peptide mass fingerprints. Species identification is determined through amino acid differences in collagen sequences (reproduced with kind permission of Matthew Collins)
ZooMS offers a high-throughput method for the species identification of bone. There are however a range of different methodologies or variants on the ZooMS technique which have been successfully applied. The wide range of variations developed from the initial ZooMS methodologies proposed (Buckley et al. 2008(b); 2009; 2010) is due in part to the differing nature of the samples being studied (e.g. size, age, importance, worked materials, post-depositional context, etc.) – therefore meaning that the material being analysed may necessitate a deviation from the ‘standard’ methodology. There are however two predominant ZooMS methodologies which have been applied to archaeological material – a destructive and a non-destructive method. The destructive ZooMS methodology uses acid demineralisation (using 0.6M hydrochloric acid (HCl)) to release insoluble collagen from bone fragments, followed by gelatinisation via heating, and digestion of the peptides using the proteolytic enzyme trypsin (as in Buckley et al. 2009; 2010; Richter et al. 2011; Welker et al. 2015(a)), although an earlier study (Buckley et al. 2008(b)) did instead use the type III bacterial collagenase Clostridium histolyticum instead of trypsin as a peptide cleaving agent. Whilst the destructive method was utilised within this research, a new ZooMS methodology was however also developed and utilised, building upon the development of
a non-destructive ZooMS approach by van Doorn et al. (2011) (see Chapter 5 and Appendix A). The non-destructive ZooMS methodology uses an ammonium bicarbonate buffer soak to leach out the small fraction of soluble collagen present within archaeological bone samples (instead of using an acid demineralisation step), leaving the material visibly intact.

As a means of species identification, ZooMS has a range of advantages over other methods. Whilst DNA based identification methods provide phylogenetic information, aDNA is subject to contamination, very costly to analyse, requires the use of special aDNA clean labs, and is often highly degraded in archaeological samples. In contrast, protein sequences are much more robust, and are frequently found to survive intact within archaeological samples, even those of significant age. Furthermore, protein sequences do not require amplification (unlike DNA), nor are they subject to contamination issues. ZooMS methodologies can also be applied even after DNA within a sample has been destroyed or damaged (Buckley et al. 2009; 2010; Collins et al. 2010; see Chapter 5). Other major advantages of ZooMS are its cost – significantly less than aDNA or other methods – and its high-throughput nature, and that it requires only very small sample sizes, notably less than 1mg (Stewart et al. 2013). In archaeological contexts, ZooMS is particularly valuable on heavily fragmented or morphologically indistinct bone samples, which could not otherwise be identified using traditional osteological or morphological methods/indicators – something which this research aims to utilise.

However, ZooMS does have some disadvantages and limitations. One of the first major problems with ZooMS is the need for a complete reference database with which to compare the obtained collagen peptide sequences. At present, there is somewhat of a paucity of fully sequenced faunal species – particularly in terms of fish remains and also eggshell protein sequences (Richter et al. 2011; Stewart et al. 2013) – although it is possible to use a statistical approach based upon peptide masses to determine species identification when sequence information is unavailable (Richter et al. 2011). Genomic data has also been used in additional support for, or in tandem with, the peptide markers recovered too (Buckley et al. 2009; Welker et al. 2015(b)). Another key consideration is that ZooMS is aided by the fact that collagen is a slowly evolving protein, meaning that we can still determine and identify animal species from the prehistoric past. However, the slow evolution of collagen also has some disadvantages, mainly that ZooMS can often only identify collagen to a family or genus level, rather than species (Collins et al. 2010).
ZooMS therefore does not offer the complexity or resolution of information that DNA does (Buckley et al. 2010; van Doorn et al. 2011; Stewart et al. 2013). Furthermore, ZooMS does not provide much of the information which can be obtained from traditional morphological study of faunal remains such as sex, age, cut marks, size, or disease markers, and should therefore be considered as an additional zooarchaeological tool, rather than a wholesale replacement (Richter et al. 2011).

In all however, the long-term survival of collagen, coupled with the ease and low-cost nature of the ZooMS methodology, means that it is an incredibly useful identification method for archaeological bone fragments from all time periods. The use of mass spectrometry also means that the methodology does not rely solely on bulk collagen, is not affected by collagen quality, works well on poorly preserved bone, and that species identification does not require the identification of every single peptide sequence (Buckley et al. 2009; van Doorn et al. 2011). Due to this, there have been a significant range of applications of ZooMS already, to a variety of different materials; for example, to ovicaprid bones to aid distinctions between sheep and goat remains (Buckley et al. 2010); to fragmentary fish remains (Richter et al. 2011); marine mammal bones (Buckley et al. 2014(a)); Palaeolithic cave material (Welker et al. 2015(a)); to extinct Late Quaternary South American ungulates (Welker et al. 2015(b)); and to determine the taxonomic relationships between extinct and extant ovidovids (Campos et al. 2010). Due to the method’s effectiveness, it has since been termed the “barcode of death” (Hofreiter et al. 2012, 2).

This research aims to utilise ZooMS by applying it to fragmentary bone material dating to the Mesolithic period in Britain, with the primary intention of gaining useful biomolecular data from skeletal material which is currently overlooked. The fragmentary nature of the majority of prehistoric bone in Britain means that our interpretations of this material are often limited using traditional modes of study (see also Chapter 3). Using ZooMS to determine taxonomic identification of bone fragments however opens up the possibility of increasing our knowledge of this period of British prehistory. For example, determining the faunal species represented by these bone fragments will hopefully allow for a greater contribution to discussions of Mesolithic fauna, exploitation, and subsistence. Additionally, there is the distinct possibility that some of the bone fragments analysed within this research may in fact be human. To date, ZooMS has not been used specifically as a methodology by which to determine human remains from fragmentary skeletal
material – but has the capacity to do so due to the fact that human collagen sequences are very distinct from other species. The advantage of using the ZooMS method in this way lies in that it works well even on poorly preserved bone – as much of the Mesolithic bone in the UK is. This research therefore aims to be the first broad-scale application of ZooMS to prehistoric material which will actively aim to determine both faunal and human remains. Furthermore, by identifying human remains, further additional scientific methods can then also be applied to the same bone fragments (e.g. stable isotope analysis, AMS dating), which will provide broader information on diet, health, and lifeways in the British Mesolithic.

4.3. Diet

4.3.1. The Longstanding Interest in Mesolithic and Neolithic Diets

“Archaeological study of diet is a little like navigating in the vicinity of an iceberg: more than four-fifths of what is interesting is not visible”

(Isaac 1971, 280)

Dietary change and the adoption of agriculture have traditionally been seen as one of the most fundamental aspects of the Mesolithic-Neolithic transition in Britain, marking a social, cultural and biological change (see Chapter 2). Due to this, the move from hunter-gatherer-fisher lifeways to agricultural modes of subsistence with domesticated plant and animal species has been extensively studied within the archaeological discipline, in a wide range of different ways. In recent years however, the application of stable isotope analyses has formed the basis of the majority of work on Mesolithic-Neolithic dietary change – with focus being placed specifically on the nature, timings and mechanisms of subsistence shift. This focus on stable isotope analyses in the study of Mesolithic and Neolithic diets has in part stemmed from the relative scarcity of other dietary information available from the periods – “trying to work out the relative importance of plant and animal foods in the ancient diet on the basis of floral and faunal remains is probably impossible” (O’Connell et al. 2000, 203).

As discussed in Chapter 2, we can see dietary change, and an understanding of the nature and timings of this change, as being at the core of our understanding of the Mesolithic-Neolithic transition. This research therefore aims to attempt to attain new dietary
information on the British Mesolithic and Neolithic using stable isotopes, from samples not previously utilised within these discussions and debates.

4.3.2. Carbon and Nitrogen Isotopes

The advent of stable isotope methodologies, coupled with processual modes of thought, has resulted in huge amounts of stable isotopic analyses being undertaken on archaeological skeletal material over the past 30 years. Isotopes were first recognised by the radio-chemist Frederick Soddy, with the actual term ‘isotope’ being coined by Margaret Todd in 1913. The discovery of ‘stable isotopes’ (i.e. non-radioactive isotopes) was made by JJ Thomson in the same year (Thomson 1913). An isotope is an atom of the same element but with a different mass. All atoms consist of a nucleus containing positively-charged protons and neutral neutrons, surrounded by negatively charged electrons. The number of protons and neutrons combined in an atom is known as its mass number. Isotopes of elements have different numbers of neutrons (but the same number of protons and electrons) – thus meaning that they have a different atomic mass, or mass number (Schoeninger and Moore 1992; Lee-Thorp 2008). Stable isotopes, unlike radioactive isotopes, do not break down over time, and therefore their ratios reflect the environment in which they were formed.

The two stable isotopes most commonly used in palaeodietary reconstruction are those of carbon (C) and nitrogen (N). Carbon has two isotopic forms used within dietary reconstruction, $^{12}\text{C}$ and $^{13}\text{C}$, depicted as $\delta^{13}\text{C}$, reflecting the ratio of $^{12}\text{C}$ to $^{13}\text{C}$ in the sample, which is caused by kinetic fractionation. To ensure consistency, this ratio is always expressed relative to an international standard of PDB (a marine Cretaceous belemnite rock, Peedee belemnite). The majority of biological materials have lower $^{12}\text{C}/^{13}\text{C}$ ratios than PDB, and thus most $\delta^{13}\text{C}$ values are negative. Nitrogen has two stable isotopes, $^{14}\text{N}$ and $^{15}\text{N}$, and thus nitrogen values are noted as $\delta^{15}\text{N}$, reflecting the ratio of $^{14}\text{N}$ to $^{15}\text{N}$ in the sample expressed relative to atmospheric air. Most biological materials have a higher $^{15}\text{N}/^{14}\text{N}$ ratio than the atmosphere due to soil nitrogen levels, thereby resulting in a positive $\delta^{15}\text{N}$ value (Schoeninger and Moore 1992; Schoeninger 1995; Table 5).
<table>
<thead>
<tr>
<th>Element</th>
<th>Isotopes</th>
<th>Natural Abundance (%)</th>
<th>Isotope Ratio Measured</th>
<th>Notation</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>$^{12}\text{C}$</td>
<td>98.90</td>
<td>$^{13}\text{C}/^{12}\text{C}$</td>
<td>$\delta^{13}\text{C}$</td>
<td>V-PDB</td>
</tr>
<tr>
<td></td>
<td>$^{13}\text{C}$</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$^{14}\text{N}$</td>
<td>99.64</td>
<td>$^{15}\text{N}/^{14}\text{N}$</td>
<td>$\delta^{15}\text{N}$</td>
<td>AIR</td>
</tr>
<tr>
<td></td>
<td>$^{15}\text{N}$</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Stable isotopes used within this thesis, their natural abundance, ratios, notation and standards (adapted from Schoeninger 1995)

The isotope ratio of a sample is reported in parts per mille (‰) deviations ($\delta$) relative to the international standard using the formula of McKinney et al. (1950):

$$\delta \, (‰) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

(Where $R = \text{ratio of heavy to light isotope in the sample or standard}$)

Atmospheric CO$_2$ is the major carbon source of all terrestrial plants (which form the basis of all terrestrial foodchains), and thus floral $\delta^{13}\text{C}$ values are determined by the isotopic composition of atmospheric carbon and the plant’s photosynthetic pathway (Schoeninger and Moore 1992). Two main metabolic pathways are known amongst plants: C$_3$ pathways and C$_4$ pathways. The majority of plants will photosynthesise using the C$_3$ pathway, which results in $\delta^{13}\text{C}$ values of between $-19‰$ and $-34‰$ (van der Merwe and Medina 1991; Heaton 1999). Typical C$_3$ plants include trees, woody shrubs, herbs, and temperate grasses, along with cereals such as wheat, barley, oats and rice, and all root staples such as potatoes and yams, as well as beans and nuts (Pate 1994; Lee-Thorp 2008). C$_4$ pathway plants are less common, and typically include tropical grasses and sedges, along with maize, millet, sorghum and cane sugar. C$_4$ pathways are designed to help plants metabolise in higher light levels, higher temperatures, and with little water, resulting in $\delta^{13}\text{C}$ values of typically between $-12‰$ and $-16‰$ (Ehleringer and Monson 1993; Lee-Thorp 2008). Due to these differences, $\delta^{13}\text{C}$ values can be used to distinguish between individuals who are consuming C$_3$ plants vs. those who are consuming C$_4$ plants (Figure 12).
Other factors beyond photosynthetic pathways can also alter $\delta^{13}C$ values however. General baseline shifts in carbon isotopic ratios can also be caused, for example, by what is termed the ‘canopy effect’. This refers to the influence on $\delta^{13}C$ values in open vs. closed forest environments. In forested areas, there is a vertical gradient in $\delta^{13}C$ values – with lower $\delta^{13}C$ values at the bottom of the canopy, and higher $\delta^{13}C$ values at the top. This is due to depleted atmospheric $^{13}C$ in the canopy understory caused by CO$_2$ recycling by leaf litter, combined with lower light lights which alter photosynthetic activity (Tieszen 1991; Krigbaum 2003; Drucker et al. 2008). Similarly, temperature and climate can also affect $\delta^{13}C$ values. High temperatures and low humidity cause plants to conserve water, resulting in decreased discrimination against the heavier isotope and subsequently enriched $\delta^{13}C$ values (Tieszen 1991; Van Klinken et al. 2002; Stevens and Hedges 2004). Finally, $\delta^{13}C$ values can also be used to distinguish the degree of marine vs. terrestrial foods within the diet (Craig et al. 2013). This is because marine CO$_2$ derives from dissolved inorganic carbonate (predominately bicarbonate (HCO$_3^-$)), which is c.7‰ more enriched than atmospheric carbon. As such, marine plants typically have more enriched $\delta^{13}C$ values than C$_3$ terrestrial plants. This offset between marine and terrestrial environments is maintained through foodchains to consumers (Chisholm et al. 1982; Schoeninger and DeNiro 1984).

Nitrogen isotopic systematics are less well understood than those of carbon, but $\delta^{15}N$ values are commonly used to distinguish between terrestrial and marine ecosystems. Terrestrial plants acquire their nitrogen from soils, which is generated through bacterial degradation of organic materials (Schoeninger 1995). However, the level of nitrogen in
soils is highly variable and little understood, and can be affected by rainfall, temperature, soil pH, salinity and grazing intensity (Ambrose 1991; Pate and Anson 2008). Overall, however, terrestrial plants in temperate ecosystems often have mean δ¹⁵N values of 0-6‰, but can range from -5‰ to +20‰ (Ambrose 1991; Pate 1994). Marine plants generally have more enriched nitrogen levels than terrestrial plants, due to the fact that most of the available nitrogen in marine systems is produced by bacterial denitrification and taken up by phytoplankton, which are at the base of the food web (Schoeninger and Moore 1992; Pate 1994). As such, fauna feeding on marine plants will consequently also have higher ¹⁵N/¹⁴N ratios (White and Folkens 2005, 413). In long-chain marine foodwebs this effect is enhanced, thus resulting in distinctively high δ¹⁵N values in many marine foods. Freshwater ecosystems are also thought to behave in a similar way to marine systems, again resulting in higher δ¹⁵N values (although with δ¹³C values closer to terrestrial ecosystems) (Schoeninger and Moore 1992; Lee-Thorp 2008).

Nitrogen isotopes also vary with trophic level shifts, with traditionally a +3-5‰ shift in δ¹⁵N from plants to herbivores, and herbivores to carnivores (but see section 4.3.3.2. below). As such, terrestrial mammals tend to have an average bone collagen δ¹⁵N value of +5.9‰, whereas marine mammals have a mean value of +15.6‰ (Pollard and Heron 2008, 355). The trophic level shifts seen in δ¹⁵N values can also be used to determine weaning ages and timings, in both humans and fauna (Richards et al. 2002; Fuller et al. 2006; Burt and Garvie-Lok 2013; Tsutaya and Yoneda 2013). δ¹⁵N values can also be anthropogenically affected however, particularly through the use of manuring, which artificially enriches nitrogen values in soils and plants, sometimes by up to +9‰ (Bogaard et al. 2007; 2013; 2016; Treasure et al. 2015).
4.3.3. Dietary Routing and Fractionation

In palaeodietary reconstructions, the δ\(^{13}\)C and δ\(^{15}\)N isotopic values of bone collagen are assessed to determine diet of the consumer. However, whilst we know that the carbon and nitrogen within bone collagen derive from dietary inputs, our understanding of exactly which part of the diet these values reflect, and the process of how they are incorporated into bone protein, is still lacking. As such, one the major problems surrounding stable isotopic analysis is the degree of isotopic fractionation which occurs between diet and bone collagen values, and how dietary protein is actually routed.

4.3.3.1. Carbon routing models and fractionation

Isotopic analyses are widely assumed to predominately provide information on the protein component of the diet, as it is known that dietary protein is preferentially routed to collagen. In particular, δ\(^{13}\)C values have been suggested to preferentially reflect dietary protein (Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Lee-Thorp 2008), meaning that plants or other low-protein foodstuffs may be under-represented in collagen values. However, as highlighted above, collagen biosynthesis and the degree of isotopic
fractionation which occurs between diet and bone collagen values are still not fully understood. Therefore, the traditional idea of a linear relationship between dietary protein δ\(^{13}\)C and bone collagen δ\(^{13}\)C – with δ\(^{13}\)C collagen values becoming more enriched with increasing protein consumption – may not hold true. Instead, the relationship between dietary protein δ\(^{13}\)C and bone collagen δ\(^{13}\)C is likely to be much more complex, particularly as it is now suggested that δ\(^{13}\)C in collagen may be influenced not only by dietary protein, but also other dietary macronutrients such as lipids and carbohydrates (Pate 1994; Hedges 2004; Barberena and Borrero 2005; Jim et al. 2006; Craig et al. 2013). Due to this, a range of feeding experiments have been undertaken in an attempt to help better understand collagen biosynthesis and where collagen carbon comes from (DeNiro and Epstein 1978; Ambrose and Norr 1993; Cerling and Harris 1999; Howland et al. 2003; Passey et al. 2005; Jim et al. 2006; O’Connell et al. 2012).

Whether δ\(^{13}\)C values obtained are reflective of only dietary protein (the ‘protein routing’ model), or instead reflect all (total) dietary carbon (the ‘scrambling’ model) (Hedges 2004) is a problem yet to be resolved within palaeodietary studies. The ‘protein routing’ model presents an alternative to the traditional linear relationship between dietary protein δ\(^{13}\)C and bone collagen δ\(^{13}\)C, but assumes that bone collagen δ\(^{13}\)C values obtained are predominately reflective of dietary protein sources, rather than the total dietary intake of carbon (Schwarcz 1991; Schwarcz 2000). The ‘protein routing’ model considers the synthesis and preferential routing of exogenous amino acids (AAs) to support these assumptions. As discussed above in section 4.2.3.1., collagen is composed of amino acid sequences – both essential amino acids (eAAs), which cannot be synthesised by the body, and non-essential amino acids (neAAs), which can be synthesised in vivo. The ‘protein routing’ model proposes that exogenous AAs (derived from the diet) are preferentially routed into collagen, resulting in the suppression of endogenous synthesis of neAAs. As eAAs are not synthesised by the body, they retain their original δ\(^{13}\)C value when incorporated into bodily proteins. As such, bone collagen δ\(^{13}\)C isotopic composition will be reflective of dietary protein δ\(^{13}\)C (Schwarcz 1991; Schwarcz 2000).

More recently, however, a number of macronutrient ‘scrambling’ models have instead been proposed for palaeodietary study. The major macronutrient groups within any given diet are protein, lipids and carbohydrates (Krueger and Sullivan 1984). The ‘scrambling’ model suggests that whilst dietary protein acts as the major determining factor in bone collagen δ\(^{13}\)C values, other dietary macronutrients also have a significant effect on
collagen carbon (Froehle et al. 2010). Overall, it is now accepted that at least 50% of bone collagen carbon is obtained from dietary protein, with an even higher percentage in high protein (>20%) diets (Jim et al. 2006; Craig et al. 2013). However, different authors have suggested different percentages – with Froehle et al. (2010) proposing that 65% of collagen carbon derives from dietary protein, and Fernandez et al. (2012) instead proposing a figure of 74%. Nonetheless, the assertion that bone collagen δ¹³C values are affected not only by protein but also other dietary constituents is highly significant. As Craig et al. (2013) show, even a relatively small amount of ‘scrambling’ can have a significant effect on δ¹³C values and their interpretation (Figure 14).

![Figure 14: Predictions of collagen δ¹³C with increasing contributions of marine protein in the diet. (a) 100% routing of protein to collagen; (b) 100% scrambling, carbon derived equally from all dietary macronutrients; (c) combined model of mixed routing and scrambling (50:50) (Craig et al. 2013, 349)](image)

The matter of whether bone collagen δ¹³C values are reflective of only dietary protein (the ‘protein routing’ model), or instead reflect all (total) dietary carbon (the ‘scrambling’ model) is therefore still not fully understood. As highlighted above, this is a particular issue when considering levels of marine resource consumption however – a key factor within discussions of Mesolithic-Neolithic diet. Hedges (2004) neatly highlights the importance of this issue, indicating that in a low protein diet, where collagen values are synthesised via a scrambling model, an individual could be consuming up to 30% of their protein from a marine source and yet this would have little to no effect on their δ¹³C bone collagen isotopic values. In effect, the way in which dietary carbon is routed can result in
significantly different bone collagen $\delta^{13}C$ values – and as such, give rise to different dietary interpretations.

4.3.3.2. Nitrogen models and fractionation

In contrast to $\delta^{13}C$ in bone collagen, $\delta^{15}N$ values derive solely from dietary protein (Craig et al. 2013). As discussed above in section 4.3.2., $\delta^{15}N$ collagen values are often used to determine information on trophic levels within palaeodietary studies. The ‘standard model’ of interpretation of $\delta^{15}N$ values obtained from human remains relies on the assumption that individuals eating only plant protein would have the same $\delta^{15}N$ values as local herbivores; and that humans eating herbivores would have a $\delta^{15}N$ value enriched 3-5‰ from those fauna (Hedges and Reynard 2007). Indeed, the trophic level enrichment ($\Delta^{15}N_{\text{diet-body}}$) in nearly all archaeological palaeodietary studies is assumed to be +3-5‰.

However, a paper by Hedges and Reynard (2007) questions these traditional assumptions surrounding trophic levels in palaeodietary studies and the ‘standard model’. For example, the standard model assumes that animal forage and human cereal have the same $\delta^{15}N$ values, and that human and herbivore $\Delta^{15}N_{\text{diet-body}}$ are the same. Instead, they generate a model whereby different $\delta^{15}N$ bone collagen values as a function of trophic level are altered by the dietary animal protein fraction (Figure 15). A $\Delta^{15}N_{\text{diet-body}}$ value of 4-5‰ is used throughout. Through this model it can be seen that even a 1‰ difference in assumptions from the ‘standard model’ can significantly alter estimates of trophic level and average dietary animal protein fractions.
Figure 15: Variation of $\delta^{15}N$ with dietary animal protein fraction. (1) the ‘standard model’ (4‰); (2) cereal $\delta^{15}N$ is enriched to forage by 1‰; (3) in addition to (2), humans are enriched 1‰ to herbivores; (4) in addition to (3), humans on an all plant diet are enriched 1‰ than humans on an all meat diet (Hedges and Reynard 2007).

Similarly, a controlled dietary study on humans with isotopically known diets by O’Connell et al. (2012) revealed a $\Delta^{15}N_{\text{diet-collagen}}$ offset of +6‰, therefore surpassing the figure of +3-5‰ generally used in archaeological studies. If correct, this suggests that estimations of animal protein may have previously been overestimated in stable isotope analyses of prehistoric diet. It can therefore be seen that our knowledge of bone collagen $\delta^{15}N$ values, trophic levels and $\Delta^{15}N_{\text{diet-collagen}}$ offset is still limited, and additional data is needed to strengthen our understanding. Additional issues may also abound however if we consider the use of manuring in the archaeological past, which can significantly elevate $\delta^{15}N$ values of plant foods (Bogaard et al. 2007; 2013), and the use of freshwater fish resources, which can result in $\delta^{15}N$ values intermediate between marine and terrestrial systems (Schoeninger and DeNiro 1984).

### 4.3.4. Isotope Ratio Mass Spectrometry

Isotope ratios within any given sample are determined via the use of isotope ratio mass spectrometry (IRMS). The broad principles of mass spectrometry, as discussed above (section 4.2.3.2.), still hold true for IRMS, but the difference in this techniques lies in that samples are converted into a simple gas (H$_2$, CO$_2$, N$_2$ or CO$_1$) before being ionised.

Continuous flow elemental analyser isotope ratio mass spectrometry (EA-IRMS) was undertaken on all samples isotopically analysed for carbon and nitrogen in this research. During EA-IRMS, samples in tin capsules are individually combusted in a 1000°C furnace in an oxygen (O$_2$), chromium oxide (Cr$_2$O$_3$) and silvered copper oxide (CuO) atmosphere using an elemental analyser, causing the sample to convert to pure N$_2$ and CO$_2$. The gas products of this combustion are swept in a carrier helium (He) gas and passed through a reduction unit made of copper oxide to remove oxides. N$_2$ and CO$_2$ are then separated on a gas chromatography (GC) column before entering the mass spectrometer where they are ionised and separated by their $m/z$ ratios, then simultaneously measured by a Faraday cup universal collector array. The dual inlet gas system on the mass spectrometer means it is possible to simultaneously analyse both carbon and nitrogen ratios in the same sample (Brand 2004; Grassineau 2006; Sercon 2016). A measurement of the ratio of heavy to light
isotopes in a sample is produced, which can then converted to relative abundances (δ‰) by comparison with an international standard, as discussed above. Further details on the EA-IRMS analysis undertaken within this research can be found in Appendix A.

4.3.5. Isotopes as a Palaeodietary Tool in Archaeology

Although originating as a geochemical technique, isotopic analyses have also been widely applied within plant and animal physiology sciences, chemistry, biology, geology – and more recently, archaeology. The interdisciplinary nature of isotopic studies has meant that they have been utilised to obtain a range of different information from archaeological samples, such as evidence of mobility, diet, geochemical environments, and climatic zones (Ezzo 1994; Pate 1994). The first significant application of stable isotope analysis to archaeological material was undertaken by Vogel and van der Merwe (1977), and used carbon isotopes to determine early maize consumption in North America. From the late 1970’s onwards therefore, stable isotope analysis has been recognised as a potential means of studying past diet in a unique way – and has since been widely applied to archaeological material from a multitude of geographical locations, contexts, and time periods. Due to its wide-ranging application, it has even been suggested that isotopic analyses could be defined as a third ‘revolution’ in archaeological science, akin to the ‘radiocarbon revolutions’ suggested by Renfrew (1976; see section 4.5.) (Bogaard and Outram 2013).

Palaeodietary analysis using carbon and nitrogen stable isotopes predominately utilises the bone protein collagen, of which a detailed overview is provided above in section 4.2.3.1., but has also been applied to tooth dentine and enamel, and also hair. The broad principle behind these kinds of analyses is that different foodstuffs have different isotopic signatures, which, when consumed, leave an isotopic signature of that dietary component within the body (Figure 16). In this way, the old adage ‘you are what you eat’ is true – the isotopic signatures within the body are a reflection of the foodstuffs consumed during life. These isotopic signatures are useful in archaeology because they are preserved in skeletal remains. Importantly, even when a large proportion of bone collagen has been lost or degraded, the original isotopic composition of the collagen remains intact (Lee-Thorp 2008).
Stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ has a number of advantages for palaeodietary reconstruction. Primarily, stable isotope analysis provides direct evidence of diet, thus making it different from other methodological approaches which rely on indirect archaeological evidence (e.g. faunal remains, plant remains etc.) (Sillen et al. 1989). Understanding diet in the past is important in a huge variety of ways, not least because it provides a context for the study of other aspects of life and health, such as growth, stress, and disease (White and Folkens 2005, 413). Isotopic analyses may also reveal possible dietary differences or inequalities within a population (e.g. between individuals, sexes, age groups) – thus potentially giving an insight into possible social structure, status, economic strata, culture, and/or beliefs (e.g. Barrett and Richards 2004; Alexander et al. 2015; Fontanals-Coll et al. 2015; Waterman et al. 2015). As stable isotope analysis provides information on diet at an individual level, it also opens up opportunities for broad-scale inter-group, inter-population or even inter-species comparisons (Lee-Thorp 2008; Parker Pearson 2009). Finally, stable isotope analysis of human bone collagen provides information on diet over the last 10 years or so of life, but bone also contains a proportion of collagen synthesised during adolescence (Schulting and Richards 2001; Hedges et al. 2007), thereby providing an averaged approximation of general diet over a significant period – unlike other direct sources of dietary information (e.g. coprolites, stomach contents) which only give evidence of diet on a very short-term scale, and may not be
representative of everyday subsistence throughout life. However, a number of papers have suggested that the collagen turnover rates of different bones may vary – meaning that that there may be isotopic variation between skeletal elements within one individual as they could reflect shorter/longer timeframes of an individual’s diet (Schoeninger and Moore 1992; Hedges et al. 2007). This is important to note due to the disarticulated and fragmentary nature of the skeletal samples utilised within this research, meaning that in some instances different skeletal elements were sampled across a population (e.g. Chapter 5).

Other limitations of isotopic work are due to the gaps in our understanding of dietary routing and isotopic fractionation, as discussed above. As such, it has been questioned whether δ values can be converted accurately into dietary percentages (Sillen et al. 1989), and if dietary contributions of animal protein have been overestimated (O’Connell et al. 2012). An additional issue is that δ13C and δ15N isotopic analyses, whilst giving an idea of diet on a generalised scale, do not provide specific information on the exact constituents of the diet, or how they were consumed. Determining how foodstuffs were processed, consumed, or shared may have been as important as the actual foods chosen to eat. Furthermore, whilst stable isotopes can provide information on plant consumption, which is traditionally very difficult to determine in the archaeological record, all British flora follow C3 pathways – and therefore it is not possible to distinguish between a diet based upon wild indigenous plants and a diet based on domesticated plant species or cereals. There are also issues with determining freshwater resource consumption, as freshwater values can often fall into a ‘grey’ area, being insufficiently isotopically distinct from either terrestrial or marine values. Due to this, a recent paper by Naito et al. (2013) has suggested that δ15N analysis of individual collagen amino acids (particularly glutamic acid and phenylalanine) may be a more accurate indicator of aquatic consumption than bulk collagen δ15N values. Nonetheless, stable isotope analysis can be seen as a useful tool in the understanding of prehistoric diets – particularly given that it is the only technique currently available which allows for accurate individual scale resolution of dietary components, thereby permitting more complex and intricate understandings of past subsistence.
4.3.5.1. Previous Applications to Mesolithic and Neolithic Material

In 1981 stable isotope analysis was used for the first time to investigate Mesolithic-Neolithic dietary change in Europe (Tauber 1981), and has since become one of the dominant biomolecular methods of investigation of the period, as discussed within Chapter 2. A huge number of palaeodietary isotopic studies on both Mesolithic and Neolithic skeletal material have been undertaken, both on British and European sites.

In the UK, stable isotopic analyses on a range of sites has revealed high marine protein consumption in Mesolithic populations (e.g. Schulting and Richards 2000; 2002(a); 2002(c); Schulting 2005; 2009; see Appendix B), but due to the lack of human remains available (see Chapter 3), a number of studies have attempted to use dog isotopic values as proxies for humans (Clutton-Brock and Noe-Nygaard 1990; Day 1996; Schulting and Richards 2002(b); Dark 2003). However, from this isotopic work has emerged the idea of a rapid dietary change at the Mesolithic-Neolithic transition in Britain, from a dependence on marine resources to an entirely terrestrial diet (see Chapter 2, section 2.4.1.) (Richards et al. 2003(b); Richards 2003). Similar isotopic studies on Mesolithic human remains and the Mesolithic-Neolithic transition have also been on undertaken material from Denmark, Sweden, Portugal, France, Spain, Croatia, Serbia, and Ukraine, for example (e.g. Richards and Hedges 1999; Lillie and Richards 2000; Richards et al. 2003(a); Lidén et al. 2004; Bonsall et al. 2004; Fischer et al. 2007; Lightfoot et al. 2011; Salazar-García et al. 2014(a); Guiry et al. 2015). Studies of Neolithic human remains in Britain have also been undertaken, and broadly show the consumption of a terrestrial diet within a C3 plant component, with little to no marine input (e.g. Schulting and Richards 2002(c); Taylor et al. 2006; Hedges et al. 2006; 2008; Richards 2008; Stevens et al. 2012; Schulting 2013; Montgomery et al. 2013; see Appendix B). Again, similar kinds of isotopic studies have been undertaken on European populations from Germany, Portugal, Spain, and Turkey, for example (e.g. Fraser et al. 2013; Carvalho et al. 2015; Fontanals-Coll et al. 2015; Pearson et al. 2015; Waterman et al. 2015).

4.3.5. Mixing Models

Due the issues with using stable isotope analysis as a palaeodietary tool, as highlighted above, isotopic mixing models are now frequently utilised to assess the contributions of different potential dietary sources to bone collagen. There are currently a large range of
carbon and nitrogen isotopic mixing models published (e.g. Phillips and Koch 2002; Phillips and Gregg 2003; Phillips et al. 2005; Moore and Semmens 2008; Froehle et al. 2012; Arcini et al. 2014; Fernandes et al. 2012; Fernandes 2015(a)), but all broadly aim to allow for the resolution of dietary information from isotopic data. Here, an isotopic mixing model approach utilising the statistical software FRUITS (Food Reconstruction Using Isotopic Transferred Signals) (Fernandes et al. 2014) was applied to all δ¹³C and δ¹⁵N isotopic values obtained throughout this research. SOPs for the application of FRUITS within this study can be found in Appendix A. FRUITS uses Bayesian statistics to attempt dietary reconstruction, but importantly, has the capability to allow for dietary routing and the contribution of different macronutrients to bone collagen (as discussed above in section 4.3.3.). It has also previously been successfully applied to populations of a prehistoric date (Fernandes et al. 2015(b)).

The FRUITS model provides estimates on the contributions of multiple dietary sources or food groups to a consumer. Importantly, the model allows for multiple food fractions (e.g. proteins, carbohydrates, lipids, single amino acids) to be assigned to each dietary source or food group (e.g. plant, animal, fish) (Figure 17). The model can also account for any diet-to-tissue offset caused by isotopic fractionation. Dietary contribution is weighted by the concentration of food fractions (i.e. macronutrients) in each food group, and overall, the model determines the contribution of each food fraction towards the consumer signal, accounting for routing of each fraction (Fernandez et al. 2014). Model parameters (i.e. isotopic values for dietary sources) are predefined by the user, and using a Bayesian analysis, parameter posterior distributions are generated by combining the user-defined priors and a likelihood function based on a probability model (Fernandez et al. 2014).
Figure 17: Schematic of the mixing concept utilised within the FRUITS model (taken from FRUITS manual, available via http://sourceforge.net/projects/fruits/)

FRUITS therefore provides an alternative way to reconstruct past diet, and is advantageous in that it considers whole diet. However, the major limitation of FRUITS lies in the fact that it does not provide a posterior predictive $p$-value for the models created. A posterior predictive $p$-value is designed to reveal a lack of fit of a generated model to inputted data (Rubin 1984; Meng 1994). FRUITS will always generate an output, regardless of the data inputted – however, it does not reveal how well the model created fits the isotopic data. As such, this is something to consider when utilising and interpreting FRUITS models.

The FRUITS model was applied to isotopic data within this research due to the evidence currently available which suggests that macronutrients other than protein can contribute to bone collagen $\delta^{13}$C and $\delta^{15}$N isotopic values (e.g. Jim et al. 2006). However, the use of it within archaeological scenarios, particularly within prehistory, is compounded by the lack of data often available on food sources and their relevant fractions. This is particularly the case for plants, of which there are very few published prehistoric wild plant and cereal isotopic values. Furthermore, the creation of an isotopic baseline of food sources is generated from archaeological remains such as faunal bone collagen and charred grains, which do not always hold the same isotopic values as the edible food fractions (i.e. meat protein, plant carbohydrates) (Fernandez et al. 2014). As such, it is necessary to provide
conservative estimates of food fractions within the model – therefore meaning that the uncertainties on the generated data may be increased, and the model may not be as accurate. Nonetheless, at present, FRUITS remains the most advanced isotopic mixing model available for palaeodietary reconstruction – and importantly, allows us to begin to consider diet in more complex ways, and acknowledges that a range of dietary macronutrients may contribute to bone collagen isotopic values.

4.4. Mobility

4.4.1. Understanding Prehistoric Mobility and Demography

“The level of mobility in past populations remains a key archaeological question”

(Lewis et al. 2014, 174)

Archaeologists have long been interested in all aspects of prehistoric mobility – for example in determining multiple dietary catchment areas, the places people visited, movement around a landscape, movement throughout individual lifetimes, or areas where food was pastured and grown (Bentley et al. 2004). The study of mobility is particularly interesting when we consider British Mesolithic and Neolithic populations however. Traditionally, Mesolithic populations have been seen as highly mobile groups, moving seasonally around the landscape exploiting different resources. In stark contrast, Neolithic populations have been painted as being very sedentary – with small groups having very little mobility. In more recent years however, with the recognition that Neolithic populations may have practiced a more pastoralist subsistence, discussions surrounding degrees of Neolithic mobility have slowly emerged, but are generally still underdeveloped in large scale narratives of the period (see Chapter 2). Determining degrees of mobility and movement within the Mesolithic and Neolithic, and at the interface between the two periods, can however contribute to discussions of indigenous acculturation vs. colonisation. In particular, the detection of ‘non-local’ individuals in early Neolithic populations may reveal degrees of mobility and migration in the period surrounding the transition. In this way, Mesolithic-Neolithic mobility is also intrinsically linked to demography, and the two dominant theories surrounding the transition involve either no or very significant demographic and/or population change (see Chapter 2).

Creating palaeodemographic profiles for populations and trying to determine levels of mobility from any archaeological period can be problematic, but particularly so for
prehistoric populations in the UK due to the lack of skeletal material available and the lack of large-scale cemetery sites (as discussed in Chapter 3). This lack of prehistoric material further exacerbates the longstanding issue within palaeodemographic studies which is that of archaeologists only ever being able to locate a small proportion of the total past population (Parker Pearson 2009, 5). The following sections will discuss the methodologies via which we can determine prehistoric mobility, and the potential information which they may provide. The methodology used here – strontium isotope analysis – will then be discussed in detail, both in terms of how it has been previously applied to Mesolithic and Neolithic material, and its utilisation here.

4.4.2. Studying prehistoric mobility

There are a number of different ways in which archaeologists have attempted to determine past population mobility and migration. The most commonly used methodologies have however included lead (Pb) isotope analysis, strontium (Sr) isotope analysis, oxygen (O) isotopic analysis, and aDNA analyses – although other, less common methods, such as limb bone robusticity as a marker for sedentism (Ruff et al. 2015) for example, have also been posited. A large body of work has attempted to use aDNA to study population movement and/or replacement at the Mesolithic-Neolithic transition, the advent of the Neolithic in both Europe and Britain, and the genetic relationships between modern day Europeans and its Mesolithic and Neolithic inhabitants (e.g. Richards et al. 2000; Bramanti et al. 2009; Soares et al. 2010; Hervella et al. 2012; Deguilloux et al. 2012; Skoglund et al. 2012; Barbujani 2012; Brotherton et al. 2013; Rasteiro and Chikhi 2013). These kinds of studies all utilise aDNA to investigate mobility and demographic change in the period surrounding the Mesolithic-Neolithic transition – with some using faunal or plant species as proxies for human population dynamics (e.g. Ottoni et al. 2013; Colominas et al. 2015; Brown et al. 2015; Smith et al. 2015). As such, many also aim to help resolve the issues surrounding the long-standing acculturation vs. migration debate (as discussed in Chapter 2). Additional studies of Mesolithic and Neolithic mobility have instead utilised Pb (lead) isotopes in human teeth an attempt to determine population movement. Lead isotopic analysis emerged from geological studies, and is used to determine the relationship between isotope data obtained from samples and the isotopic composition of geological ores (Budd et al. 2004(b)). Whilst traditionally used within archaeology on metal artefacts to determine ore source (Pollard et al. 2007, 192), lead isotopes of human bones and teeth have also been used to determine past mobility and movement, with a number of studies
undertaken on European Neolithic populations (e.g. Montgomery et al. 2000; Chiaradia et al. 2003; Budd et al. 2004(b); Smits et al. 2010). Finally, a much smaller body of work has also used oxygen isotopes as a means via which to assess Neolithic mobility (e.g. Müller et al. 2003; Krigbaum 2003; Neil et al. 2016), but the technique is often not considered to be as informative as other methods as it can only differentiate between eastern and western Britain due to levels of rainfall. Instead, strontium has been the most widely used isotope in archaeological mobility studies (Brown and Brown 2011, 85).

4.4.3. Strontium Isotopes

Strontium isotopes are deemed to be “one of the most effective means to characterise mobility in past populations” (Lewis et al. 2014, 173), as they can be used to ‘provenance humans’ (or indeed animals) (Pollard and Heron 2008, 370) by acting as geochemical signatures which can be linked to specific geological areas (Bentley 2006). There are four naturally occurring isotopes of strontium – $^{84}\text{Sr}$, $^{86}\text{Sr}$, $^{87}\text{Sr}$ and $^{88}\text{Sr}$. Three of these are non-radiogenic ($^{84}\text{Sr}$, $^{86}\text{Sr}$, $^{88}\text{Sr}$), whereas the fourth ($^{87}\text{Sr}$) is radiogenic, and is formed through the decay of rubidium ($^{87}\text{Rb}$). Due to this, there are two potential sources of $^{87}\text{Sr}$ in any mineral: that which is naturally formed through primordial nucleosynthesis (along with the other isotopes of Sr), and that formed via the decay of $^{87}\text{Rb}$ (Bentley 2006; Pollard et al. 2007, 174). The Rb-Sr decay system has previously been used widely in geological and geochronology studies, as the $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio is seen as an important geochemical tracer (e.g. Depaolo and Ingram 1985; Elderfield 1986; Millet et al. 2009; de Souza et al. 2010; Négre et al. 2015). $^{87}\text{Sr}/^{86}\text{Sr}$ is known to vary substantially between different geological terrains, and this variability is linked both to rock type and geological age (Ericson 1985; Bentley 2006). As a result, $^{87}\text{Sr}/^{86}\text{Sr}$ values vary across Britain, ranging from values of c.0.7073 on Cretaceous chalk to 0.7115 on Triassic sandstone (Budd et al. 2004(a)).

Soil, plant, and animal $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratios are known to be related to those of the underlying geology in an area and the local hydrology (Budd et al. 2004(a)). This is due to geological weathering of rocks, which releases strontium into soils and water (Bentley et al. 2004). Strontium passes from eroding geological material into soils, and through the food-chain (via plants and animals) into humans. Strontium has an atomic radius similar to calcium (Ca), meaning that it “readily substitutes for Ca in minerals, including phosphates in bones and teeth” (Pollard et al. 2007, 174). Importantly, strontium ratios can pass
unmodified through the food-chain – this is because strontium is a high mass element, and so the difference between the two isotopes (\(^{87}\text{Sr}/^{86}\text{Sr}\)) is relatively small, therefore resulting in negligible fractionation (Ericson 1985; Bentley et al. 2004). However, it is important to note that Sr concentrations in the skeleton/teeth do not have a linear relationship with the amount of Sr ingested by an individual (Montgomery et al. 2007). Crucially for archaeology however, the \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio in skeletal tissues is a reflection of the foods consumed (and therefore also the underlying geology) at the time of tissue formation or remodelling (Bentley et al. 2004).

Depending on the skeletal tissue analysed, strontium ratios can reflect different periods of an individual’s life (Budd et al. 2000). Strontium isotope ratios in bone are reflective of Sr uptake over the last 10-20 years prior to death, due to bone turnover rates (Hedges et al. 2007; Lewis et al. 2014). However, bone is very subject to diagenetic and post-depositional change, and can become contaminated by groundwater strontium following deposition. This contaminating Sr can therefore obscure or replace the in vivo \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio within the mineral portion of a bone (Bentley 2006). In contrast to bone, neither dentine nor enamel remodel once fully mineralised, and therefore strontium ratios within them are reflective of childhood – and these ratios are retained throughout life. Both enamel and dentine should also have similar \(^{87}\text{Sr}/^{86}\text{Sr}\) isotopic ratios, as both tissues form in tandem (Budd et al. 2000; Lewis et al. 2014). However, studies have shown that whilst enamel does not appear to go undergo significant post-depositional alteration, dentine is highly subject to diagenetic attack (Budd et al. 2000). Due to the fact that the integrity of biogenic Sr in enamel appears to be greater, it is now favoured within archaeological studies.

Teeth mineralise sequentially from cusp to root, and this means that any changes in strontium intake throughout mineralisation of the tooth are preserved within the growth axis of the enamel (Lewis et al. 2014). The formation timings of different teeth have also previously been well characterised in humans (Moorrees et al. 1963; Van Beek 1983; Hillson 1996; Liversidge 2003; Ubelaker 2008), and therefore it is possible to tie this chronological information in with changes seen in \(^{87}\text{Sr}/^{86}\text{Sr}\). By comparing different teeth from the same individual, it may also be possible to see differences in Sr values between earlier and later childhood (Budd et al. 2004(a)). It is this potential to see changes in \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios during childhood which new laser ablation methods of analysis (as discussed below) aim to reveal.
4.4.4. Using Strontium to Determine Mobility – Methods and Approaches

As discussed above, biogenic strontium is taken up into the skeleton through dietary input, and is a reflection of the underlying geology of the region in which this food was produced. By determining the Sr isotopic values within an individual’s skeleton therefore, we can begin to characterise past mobility and movement across geological zones. In order to determine and interpret levels of mobility through Sr isotopes however, establishment of regional strontium signatures across the study area is crucial. For Britain, strontium isotope biosphere mapping has already been undertaken (Evans et al. 2010; Chenery et al. 2010), and from this a map has been created which can be used in conjunction with archaeological data obtained (Figure 18).

Figure 18: Biosphere $^{87}\text{Sr}/^{86}\text{Sr}$ isotope map of Britain (Evans et al. 2010)

It is important to note however that whilst we can create geological maps of $^{87}\text{Sr}/^{86}\text{Sr}$ across the UK, for example, the actual amounts and values of biogenically available Sr at any one point are variable, and part of a complex geological and ecological relationship. For example, minerals and rocks with different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios will weather at differential
rates, and therefore the $^{87}\text{Sr}/^{86}\text{Sr}$ entering the environmental cycle may vary through time (Bentley 2006). Although mixing equations can be created to calculate the available Sr within an area (considering factors such as weathering, water, biomass and fertilisers), generally within archaeology a more generalised Sr value for an entire region, based upon the underlying bedrock and geology of the area in question, is deemed suitable enough for interpretation. Additionally, due to the fact that Sr ratios change slowly over time (the half-life of $^{87}\text{Rb}$ is $4.88 \times 10^{10}$ years (Bentley 2006)), it is possible to use modern geological maps to interpret archaeological Sr data (as above). Due to this, the method is seen to have “a predictive capacity” within archaeology (Pollard and Heron 2008, 371).

A number of different methods have been utilised in the application of Sr isotope ratios in archaeology. The most common has been ‘bulk’ analysis using thermal ionisation mass spectrometry (TIMS). The TIMS methodology first emerged in the life sciences, and involves the chemical deposition of sample onto a refractory material (e.g. platinum wire), which is then heated via the passage of an electrical current through it. This then ionises the sample and allows it to be emitted into a vacuum and measured by a mass spectrometer (Pollard et al. 2007, 173). Whilst TIMS has been used extensively in archaeology however (e.g. Budd et al. 2000; Müller et al. 2003; Bentley et al. 2004; Montgomery et al. 2007; Haak et al. 2008; Chenery et al. 2010), the method, although precise and reliable, is also slow and expensive (Pollard et al. 2007, 189; Lewis et al. 2014). The time-intensive nature of the TIMS method (also known as a ‘solution method’) is also exacerbated by the labour-intensive elemental purification steps required prior to MS analysis, which can involve ion exchange chromatography (Simonetti et al. 2008). Additionally, the TIMS methodology is destructive, and therefore is not always suitable for use on rare or very small samples (Copeland et al. 2008). It also, crucially, gives a bulk averaged value for a tooth, and cannot detect changes in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios during mineralisation of the tooth unless sequential sampling is undertaken.

More recently, a number of archaeological strontium studies have instead utilised laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS). ICP-MS first became available commercially in 1983 and is used widely within trace-level elemental analysis. In recent years, LA-MC-ICP-MS has been heavily applied in inorganic analyses in the environmental and earth sciences, and in geochemical studies (Pollard et al. 2007, 197; Copeland et al. 2008). LA-MC-ICP-MS works through samples being ablated by an excimer laser (a form of ultraviolet laser), which evaporates a discrete area that is
then swept from the laser cell using a mixture of helium (He), argon (Ar), and nitrogen (N\textsubscript{2}) gases, before entering a plasma ion source (Lewis et al. 2014). The ionised sample then enters a high vacuum magnetic selector device, which separates ion streams according to their mass-to-charge ratio (\(m/z\)), and which are then detected by multiple ion beam collectors. The use of multiple detectors simultaneously measuring different masses allows for greater precision and accuracy of results (Pollard et al. 2007, 199). Recently, given that it has been detected that \(^{87}\text{Sr}^{86}\text{Sr}\) ratios obtained using LA-MC-ICP-MS can be altered due to molecular isobaric interference from Ca-P-O with a mass of 87 (which then overlaps the \(^{87}\text{Sr}\) signal) (Simonetti et al. 2008), a LA-MC-ICP-MS study by Lewis et al. (2014) also included the introduction of a customised plasma interface using a collision cell, which aimed to remove Ca-P-O molecules with a mass of 87 – again aiming to improve accuracy of the method.

Advances in MC-ICP-MS technologies in recent years, combined with the coupling of a laser ablation system, mean that LA-MC-ICP-MS can now provide comparable precision and accuracy data to that obtained using TIMS (Simonetti et al. 2008; Lewis et al. 2014). LA-MC-ICP-MS also offers a much quicker alternative – taking only c.15-30 mins per sample (dependent on the size of the tooth), compared to 1-2 hours per sample using TIMS (Simonetti et al. 2008). Additionally, the LA-MC-ICP-MS method can generate c.500+ individual measurements per tooth during this time, compared to the singular bulk value obtained through TIMS. The use of a laser in LA-MC-ICP-MS also allows for high resolution data and the ability to undertake spatially resolved sampling (using laser spots 100\(\mu\)m in diameter, for example), and means that small-scale changes in \(^{87}\text{Sr}^{86}\text{Sr}\) ratios can be detected, providing new information on movements and migration during childhood which could not be obtained using other methods (Prohaska et al. 2002; Richards et al. 2008; Moffat et al. 2012; Lewis et al. 2014). Furthermore, as the laser track is computer controlled, continuous profiles through the growth axis of the enamel can be measured (Lewis et al. 2014). Additionally, laser ablation is much less destructive than bulk solution Sr analyses, leaving only a small laser track along the enamel surface. Due to this, it has been proposed to be much more suitable for the measurement of \(^{87}\text{Sr}^{86}\text{Sr}\) ratios in small teeth (e.g. those of micromammals), and also very rare samples (Copeland et al. 2008). The use of the laser for analysis means that no chemical dissolution is needed, and has also therefore been suggested to result in a reduced risk of contamination in comparison with traditional TIMS analysis (Moffat et al. 2012). Due to these advancements it has been
suggested that LA-MC-ICP-MS may “revolutionise the manner in which migration studies of ancient civilisations are carried out in the future” (Simonetti et al. 2008, 372).

However, regardless of the methodology used, one the major problems with Sr analysis lies in the fact that there must be distinct differences in the geologies (and therefore the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios) an individual moved around in order for migration to be determined. If an individual moved across an area with the same underlying geology (as seen in large areas of the UK), then no movement or migration would be detected through the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Significant variation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios is therefore needed in order to make meaningful interpretations about past mobility and migration (Ericson 1985). Additionally, when determining Sr values from enamel, only information on childhood movement (when the teeth were mineralising) can be determined. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in dental enamel therefore only provide information on mobility during a small snapshot of an individual’s life. In addition to this, $^{87}\text{Sr}/^{86}\text{Sr}$ values do not simply reflect migration from one geological region to another, but instead represent a more complex signature of multiple areas an individual visited, as well as the consumption of any non-local foodstuffs (Bentley et al. 2004).

4.4.5. Previous Applications of Strontium Isotopes to Mesolithic and Neolithic Material

Strontium isotopes have been utilised within archaeology since the 1980s onwards (e.g. Ericson 1985; Nelson et al. 1986; Tuross et al. 1989; Sealy et al. 1991; Koch et al. 1992), and have previously been applied to archaeological samples from a range of different time periods (e.g. Price et al. 1994; Sillen et al. 1995; Budd et al. 2004(a); Richards et al. 2008; Simonetti et al. 2008; Chenery et al. 2010; Gerling et al. 2012; Park et al. 2015). Much like the application of Pb isotopes however, Sr isotopic analysis has been utilised in a number of studies looking at Neolithic mobility and migration, but has been employed far less on Mesolithic assemblages. In Britain, this is no doubt due to the lack of Mesolithic human remains known for the period (as discussed in Chapter 3), but even where more skeletal material is available across Europe, the method still appears to have had less application on pre-Neolithic assemblages. The desire to understand the emergence of the Neolithic period in Europe however means there are a number of strontium studies aiming to determine whether this ‘Neolithisation’ was bought about by a movement of people or by the movement of ideas (Pollard and Heron 2008, 371; Boric and Price 2013; see Chapter 2).
On UK material, a number of studies have revealed considerable movement of individuals throughout the Neolithic, and exploitation of resources beyond the immediate geological area (e.g. Montgomery et al. 2000; 2007; Budd et al. 2004(a)). A recent paper by Neil et al. (2016) on individuals from Hazleton North, Gloucestershire, also indicated residential mobility within the early Neolithic of Britain. More broadly, $^{87}\text{Sr}/^{86}\text{Sr}$ studies have been undertaken on Neolithic material from across Europe, for example on a number of sites across Germany, the Netherlands, Portugal and Italy, which have also indicated variable mobility within Neolithic populations (e.g. Chiaradia et al. 2003; Müller et al. 2003; Nehlich et al. 2009; Smits et al. 2010), and in some cases has been interpreted as representing itinerant pastoralism (e.g. Carvalho et al. 2015). Interestingly, in some populations it has also been shown that females were most likely to exhibit non-local Sr signatures, which may be indicative of exogamy and patrilocality (Bentley et al. 2004; Haak et al. 2008). Overall therefore, the current body of strontium isotopic work on Neolithic material suggests that there was considerable variability in the movement and migration of European populations, both within individual populations and throughout the period. This data is therefore interesting to compare against the traditional narrative of increasing sedentism throughout the Neolithic period (see Chapter 2).

Although more frequently applied to studies of humans, strontium isotope analysis can also be utilised to look at animal movement and migration too. Studies of animal ecology and movement can be applied both to look at the catchment areas for the hunting of wild species, and also to investigate the movement of domesticated species and livestock (Ericson 1985). In this way, the movement of domesticated species can perhaps also be considered as a proxy for human movement and migration too. One particularly effective use of $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis on fauna is the study of cattle from the late Neolithic site of Durrington Walls, Wiltshire (Viner et al. 2010). The analysis of teeth from thirteen cattle revealed that whilst two of the animals studied were raised in a geology akin to that at Durrington Walls, the remaining eleven animals appear to have been bought to the site from a range of other areas of the UK – some potentially up to 100km away (Viner et al. 2010). High mobility of domesticated fauna (particularly cattle) is also seen elsewhere in Neolithic Europe, such as, for example, at settlement sites in western Sweden (Sjögren and Price 2013). Other studies have also looked at both animal movement as well as human movement, and tried to compare the two through the use of strontium isotopes. For example, a study by Hoekman-Sites and Giblin (2012) analysed $^{87}\text{Sr}/^{86}\text{Sr}$ in both human and faunal (cattle, sheep, and goat) dental enamel from a number of late Neolithic sites on
the Great Hungarian Plain, in an attempt to determine both human and domesticated animal movement.

Strontium isotopic analysis of dental enamel will be applied within this research where possible to try to elucidate further information on prehistoric movement and mobility in Britain. Whilst it is broadly accepted that Mesolithic communities were highly mobile, and may have moved seasonally around the landscape, it is generally often assumed that Neolithic populations were, by contrast, highly sedentary. Whilst the adoption of domesticated fauna and flora will, by their nature, limit the extent of movement possible for populations, the actual degree of sedentism adopted within the British Neolithic is still little explored. Levels of movement within Neolithic populations may also be tied to the emerging debates within the literature surrounding sedentary agriculturalists vs. more mobile pastoralists (Chapter 2).

4.5. Chronology

4.5.1. Understanding prehistoric chronologies

“Without a reliable chronology the past is chaotic”

(Renfrew 1976, 21)

Obtaining secure knowledge of chronology is vital in gaining a more sophisticated understanding of prehistoric archaeology. The timings and emergence of, for example, the Mesolithic-Neolithic transition, the domestication of animals, or the adoption of agriculture are crucial in helping archaeologists to understand the processes and changes which occurred in the prehistoric past, and the potential alterations to lifestyle, diet, health, and demography, past populations may have undergone. It can therefore been seen that understanding when also can then lead to more detailed discussions of where, how, and why. If we use chronology as a basis or template, we can begin to form chronometric timescales and frameworks on which we can then layer other archaeological information. Adopting these kinds of approaches to the archaeological and prehistoric past should therefore hopefully allow for a deeper, more coherent and sophisticated understanding of the archaeological material currently available to us.
4.5.2. AMS dating and the radiocarbon revolution

The so-called ‘radiocarbon revolution’ began in the early 1970’s, with the emergence of calibrated radiocarbon dates, and prompted a re-focus within archaeology on chronology (Whittle 2007). The application of radiocarbon dating was hugely significant for British prehistory, with $^{14}$C dates providing “the central core around which late Pleistocene and Holocene prehistoric timescales have been built” (Taylor 1987, ix). In particular, the advent of radiocarbon dating was of huge importance for the study of the British Mesolithic as it allowed for re-classification and the establishment of more rigorous chronological boundaries within the period (Rowley-Conwy 1986).

Radiocarbon dating was first developed by WF Libby (1955), and utilises carbon-14 ($^{14}$C), a radioactive isotope of carbon. $^{14}$C is a cosmogenic nuclide, meaning that it is constantly formed in the atmosphere, where it can then be taken up into the biosphere (i.e. into all plants and animals) (Bowman 1990, 10). After the death of an organism, the carbon within its cells is no longer being replaced as a part of the carbon cycle, and thus starts to decay. $^{14}$C decays at an immutable rate, with the number of atoms decreasing by 1% every 83 years, resulting in a half-life of 5,730 years. This rate of decay is unaffected by climate or environment (Aitken 1990, 56-57; Buck et al. 1996, 44). By determining the level of decay of $^{14}$C atoms within a given material therefore, it is possible to define its age. Radiocarbon dating can be applied to all organic materials, but is most commonly used in archaeology on substances such as bone, shell, charcoal, plant material, antler, tooth, leather, textiles, hair, ivory, parchment, and sediments (Bowman 1990, 12-13). A major advantage of radiocarbon dating in this respect therefore is that it can provide comparable age estimates for organic materials worldwide (Taylor 1987, 33).

The dates provided by radiocarbon dating give a figure in ‘radiocarbon years’, rather than calendar years. Due to this, and also because levels of atmospheric $^{14}$C have not been constant throughout time, radiocarbon values must be calibrated in order to provide a calendar age which can then be used in archaeological interpretations. Calibrated values are determined via consultation of the relationships between $^{14}$C values and dendrochronological data (Taylor 1987, 5), resulting in a ‘calibration curve’ of data points. The calibration curve which is most frequently used is that which can be accessed via the free calibration software OxCal, developed by the Oxford Radiocarbon Accelerator Unit (ORAU; University of Oxford). This calibration curve is not completely smooth due to the fact that calibration is not a monotonic function (Bowman 1990, 46) (i.e. calendar age and
radiocarbon age do not necessarily have a linear relationship), and thus ‘wiggle matching’ is often undertaken – which involves trying to determine the exact match along the calibration curve between radiocarbon value and calendar age. However, because of these ‘wiggles’ in the calibration curve, multiple probabilistic calibrated dates can emerge from a singular $^{14}$C date (Pollard and Heron 2008, 287). Due to this, Bayesian statistics have been widely applied within the calibration process, in an attempt to provide the most accurate calendar age estimates possible. Bayesian modelling works by combining calibrated radiocarbon dates with archaeological information (e.g. stratigraphic interpretations). A good overview of how these statistics work when applied to radiocarbon dating is provided in Buck et al. (1996), and recent practical applications of them to Mesolithic and Neolithic material have been undertaken by Wicks et al. (2014) and Wysocki et al. (2013).

It should be noted however that calibration of $^{14}$C dates on human remains can be more complicated if the individual has consumed significant amounts of marine protein during life – something which is particularly pertinent for Mesolithic human remains. This is because there is an offset between atmospheric and oceanic carbon reservoirs, caused by a residence time of 1000 years for carbon in deep oceans, versus 10-20 years for atmospheric carbon. This therefore means that marine organisms will give dates that are often up to 400 radiocarbon years too old; a phenomenon known as the marine reservoir effect (MRE) (Schulting and Richards 2001; Ascough et al. 2004; 2007; see Chapter 5). This $^{14}$C offset between contemporaneous marine and terrestrial samples must therefore be taken into consideration when dating either marine organisms or humans with a marine diet.

AMS dating is the most common form of radiocarbon dating utilised within archaeology today. It was first developed in the late 1970’s, with the first published application of AMS dating on archaeological samples undertaken by Muller et al. (1978). AMS was subsequently heralded as a third ‘radiocarbon revolution’ (with the first being the initial application of radiocarbon to archaeology, and the second being the advent of calibration) (Taylor 1997). AMS is so called as it utilises an accelerator mass spectrometer which allows for a stream of carbon ions to be bent into separate streams of $^{12}$C, $^{13}$C and $^{14}$C and, as such, is a form of ‘direct counting’ high precision radiocarbon dating (Taylor 1987, 90; Aitken 1990, 82). AMS dating holds a number of advantages over traditional radiocarbon dating, notably in that it requires much smaller sample sizes, that measurements can be easily repeated, and that it has the ability to date different chemical fractions of a sample.
AMS is also a high throughput method, meaning that large number of samples can be easily run, and crucially, the method extends the chronological range of traditional radiocarbon methods. Finally, AMS is also beneficial in that it allows for the direct dating of objects and human remains, and the more rigorous chemical purification processes samples are subjected to means that contamination is reduced. The method also works well on sample types with low organic carbon content, such as bone, ceramics and seeds. In all therefore, AMS is thought to provide the most precise and efficient method of radiocarbon dating samples (Taylor 1987, 145; Aitken 1990, 85; Bowman 1990, 37; Taylor 1997; Pollard and Heron 2008, 271).

Radiocarbon can therefore be seen to have provided archaeology with a new absolute dating method, which ultimately has transformed our understanding of chronology in the archaeological past. It has also, importantly, provided archaeologists with a means by which to construct unified chronological frameworks and chronometric timescales globally, unhindered by local, regional, or continental boundaries (Renfrew 1976, 66; Taylor 1997). The application of radiocarbon dating within archaeology is thus “considered to be a watershed [moment] in the history of archaeology and prehistoric studies” (Taylor 1987, ix). Radiocarbon dating allows us to view the prehistoric past on scales previously not thought possible – it is now down to archaeologists to determine the appropriate scales of observation we should be using to interpret the past, and the potential of using these technologies to view different processes throughout prehistory (as highlighted by Bailey 2007). As this research is concerned with the period surrounding the Mesolithic-Neolithic transition, chronology is of upmost importance. AMS dating will be used within this research as a complementary tool to the other biomolecular methods being utilised where possible (see Chapter 5), and aims to allow for a deeper understanding of chronological frameworks within the period, in tandem with information obtained on diet, health, disease, and species identification.

4.6. Health and Disease

4.6.1. Understanding prehistoric health and disease

Obtaining an understanding of health and disease is vital in allowing us to gain an insight into the lives of prehistoric peoples. However, gaining knowledge on prehistoric health and disease in Britain – particularly from early prehistory – is difficult and problematic for a
number of reasons. Primarily, as discussed in Chapter 3, there is a distinct lack of human skeletal material dating to the Mesolithic and Neolithic of Britain, which ultimately hinders the amount of information on health and disease which we can obtain from these periods.

Where we do have human remains available for study, additional problems abound. For example, prehistoric human remains are frequently heavily fragmented and/or disarticulated, making interpretations more complex. Additionally, in order to study disease from human remains, we need either osteological or biomolecular evidence. Osteological evidence of disease is often hindered by small assemblage sizes of prehistoric human remains, which mean that making broader interpretations about overall population level health is difficult. Furthermore, the issues of small sample sizes are also compounded further by the longstanding problem of the osteological paradox. The term ‘osteological paradox’ was first coined by Wood et al. (1992), and promotes the idea that the skeletons we view are only the non-survivors of a population, and therefore should not be taken to represent the original, healthy living population. It also challenges the traditional osteological view that an absence of pathological lesions indicates that an individual was ‘healthy’. In order for pathological or stress indicators to be present on a skeleton, the disease episode must in fact be prolonged enough for lesions to be skeletally manifest, and crucially, the individual must also survive (Lewis 2007, 103). Therefore, as not all diseases are skeletally manifest, and some may be so acute that the individual dies before lesions have time to form, many conditions may actually be ‘invisible’ in the archaeological record.

In terms of biomolecular methods via which to determine information on past health and disease, skeletal samples are also needed. Most recently, diseases have been studied via the sequencing of pathogen DNA from human bones (e.g. Raoult et al. 2000; Bos et al. 2011; Hershkovitz et al. 2015), but there are issues with this as not all skeletal remains may contain pathogen DNA, and the methodologies used in sampling, sequencing and analysis have not been standardised across all studies (Roberts and Ingham 2008). Additionally, some of the marker sequences used to test for disease in archaeological skeletons, have, with the advent of new sequencing technologies, shown to be non-specific and unsuitable for disease identification (e.g. Müller et al. 2015).

The Mesolithic-Neolithic transition in Britain is thought to have bought about significant changes in health and disease, yet our current understanding of prehistoric health and
disease is incomplete (as discussed in Chapter 2, section 2.4.3.). Of the human remains which are known from the British Mesolithic and Neolithic, little study of them has focused on health (Roberts and Cox 2003, 45). Instead, much emphasis has been placed on diet (via isotopic analyses; see Chapter 2) and burial contexts (see Chapter 3). As such, there is now perhaps a need to refocus research on prehistoric health and disease, and in tandem with this, aim to determine new ways via which we can access disease information from the archaeological record, whilst dealing with the lack of human skeletal remains available from Britain from the Mesolithic and Neolithic periods. The emergence of dental calculus as a means via which we could potentially study past health and disease has emerged only very recently, but appears to show much potential in helping us to obtain a greater understanding of pathogens, immune response and health in the archaeological past. Again, issues lie in the scarcity of available skeletal material from the periods, but where it does persist, the potential information which we may be able to obtain appears to far surpass that which may be gained from other methods of study.

4.6.2. How dental calculus may help

“The average healthy person carries on the surface of their teeth nearly as many bacteria as there are humans on the Earth, and every day each of us swallows an average of 80 billion bacteria in our saliva”

(Warinner et al. 2015(a), 2)

Whilst it has long been recognised that dental calculus is repository for a range of macroscopically invisible compounds and elements – such as food debris, starch grains, and phytoliths (e.g. Henry and Piperno 2008; Hardy et al. 2009; 2015; Henry et al. 2011; Hendy et al. 2013; Buckley et al. 2014(b); Leonard et al. 2015) – there has, until very recently, been little investigation into the possibility that archaeological biomolecules, specifically proteins and aDNA, may also be found to survive within calculus too. Recent work on archaeological dental calculus from a number of different sites and time periods has however revealed that calculus is also a rich source of genomic and proteomic data; not only from the human individual, but also from commensal and pathogenic oral microbial species, and dietary inclusions (Charlton 2012; Adler et al. 2013; Warinner et al. 2014(a); 2014(b); 2015(a); 2015(b)). The application of high-throughput metagenomic and metaproteomic sequencing to human dental calculus has therefore revealed the possibility of calculus as new material through which we can study disease virulence and immune response in the past. It may also allow us to begin to reconstruct the interplay and
relationships between diet, infection, health and immunity in the archaeological past. Dental calculus therefore has the possibility of allowing archaeologists to determine previously archaeologically ‘invisible’ pathologies and conditions – and thereby gain a greater understanding of past health.

### 4.6.2.1. Human dental calculus

Dental calculus, Dobney and Brothwell (1988, 372) comment, is “one of the commonest types of ectopic concentration known to occur in man”, and is almost ubiquitous in past populations, occurring in archaeological skeletons from nearly all time periods and geographical locations. Calculus is mineralised dental plaque, which can form on any surface of the tooth aside from active attritional facets, but is most common the lingual and buccal surfaces (Jones 1972; White et al. 2012, 456). Following tooth eruption, salivary proteins and gingival crevice fluid rapidly adhere to the tooth surface, coating it to form an organic layer known as the enamel pellicle. Once this pellicle is formed, oral microorganisms then adhere to the surface of the tooth (Hillson 1996, 254; Jin and Yip 2002). There are thought to be over 700 different species and phylotypes of oral bacteria within the human mouth, which belong to nine distinct phyla (Zijnge et al. 2010). The tooth surface is an ideal place for these bacteria to adhere to, as whilst the remainder of the oral cavity (i.e. lips, cheeks, tongue, gums) are also colonised by micro-organisms, their ability to adhere is limited by constant shedding of the mucosa surface. By contrast, teeth have a uniquely non-shedding surface, thereby allowing communities of bacteria to easily build up (Hillson 1996, 254). This ‘build-up’ of bacteria on the tooth surface is known as dental plaque, and is a form of oral biofilm. Maturation of the biofilm results in co-aggregation of bacteria within the plaque, and bacterial growth. The nature of the bacterial flora within the plaque may however vary depending upon the location within the mouth, and on the tooth surface. Bacteria within plaque adhere not only to the tooth surface however, but also to one another via adhesive proteins present in the cell walls of many micro-organism species. Due to this, the structure of plaque forms a matrix with a well organised architecture (Freeth 2000; Hillson 2005, 287; Zijnge et al. 2010). The bacteria within the plaque survive by allowing nutrients to selectively diffuse into the matrix, including sugars either from the diet or the breakdown of starches and glycoproteins within the mouth by salivary enzymes. A range of proteins, peptides and amino acids also enter the plaque, and are then metabolised to produce products which balance the pH of the plaque fluid (Hillson 1996, 255).
Calculus is formed when the bacterial biofilm of plaque mineralises. The main mineral source for calcification is the salvia, which contains large amounts of calcium phosphate. Mineralisation begins with the deposition of precursor substances such as octacalcium phosphate and dicalcium phosphate dehydrate. This is then followed by the binding of calcium ions to the carbohydrate protein complexes of the organic bacterial layers of plaque, and the precipitation of crystalline calcium phosphate salts (Jin and Yip 2002; Jepsen et al. 2011). It is thought that the first parts of the plaque to be mineralised are the cell walls of the bacteria, followed by the plaque matrix (Hillson 2005, 288). Once calcified, calculus in life is as hard as bone (Hardy et al. 2009), and is more heavily mineralised than dentine and cement, but less so than enamel (Hillson 2005, 290). It is interesting to note that although the mineralisation agents of plaque are known, the actual mechanisms behind plaque calcification are still unknown (Hillson 1996, 256).

It can therefore be seen that calculus is formed when the organic matrix of plaque is mineralised. The resultant inorganic fraction of calculus consists of combinations of calcium and phosphate – predominately octacalcium phosphate, hydroxyapatite, and whitlockite (β-tricalcium phosphate) (Dobney and Brothwell 1988; Jin and Yip 2002; Jepsen et al. 2011). The organic matrix of calculus, following mineralisation, consists predominately of proteins, lipids and carbohydrates, and a range of micro-organisms (Jepsen et al. 2011). These micro-organisms are present as it is thought that whilst some bacteria may readily calcify, others will not, therefore resulting in non-mineralised bacteria being present within the calcified mass (Moolya et al. 2010). Throughout life, calculus is also always covered by a layer of plaque. This layer at the surface of the calculus continues to calcify and deposit more material throughout life, thereby leading to a thickening of the deposit over time (Middleton and Rovner 1994).

There are a wide range of factors which can affect the level and severity of calculus formation within the mouth, and due to this, the aetiology of calculus formation is suggested to be multi-causal. However, the aetiology of calculus is still not truly fully understood, as highlighted by the longstanding debate as to the effect diet has on facilitating calculus formation. For example, some researchers have suggested that diets high in carbohydrate may increase calculus formation (Hillson 1996; Lieverse 1999; Greene et al. 2005; Roberts and Manchester 2010, 71), whereas other authors have conversely proposed that calculus formation is greater in individuals with high protein
diets (Chamberlain 1994; Lillie 1996; Wesolowski et al. 2010; Scott and Poulson 2012). Diets high in fat have also previously been put forward as a causal factor in calculus formation (Smith et al. 1963; Baer and White 1966).

However, there are a range of other, non-dietary factors which are also thought to impact upon the level of calculus formation. Indeed, the dietary causes of calculus formation are in fact debated by some (e.g. Rugg-Gunn 1993). Other factors suggested include: salivary flow rate (indirectly related to fluid consumption), the mineral content of drinking water, salivary super-saturation with calcium phosphate salts, saliva pH, genetic factors, oral hygiene habits, age, ethnicity, the use of teeth as tools, gender, host response differences, disease, mental and physical handicaps, the smoking of tobacco, elevated calcium and phosphate levels in the blood, concentrations of oral bacteria, and prescribed medications (Hillson 1996; White 1997; Lieverse 1999; Jin and Yip 2002; Hardy et al. 2009; Wesolowski et al. 2010; Jepsen et al. 2011). However, not only are the aetiology and causal factors of calculus formation still not fully understood, neither are the actual formation timings of calculus, but are thought to be very variable both on an individual and population level (Wesolowski et al. 2010). Middleton and Rovner (1994) suggest that deposition of the enamel pellicle and subsequent bacteria (i.e. the precursor to plaque) can occur on teeth within just thirty minutes after cleaning, and Scott and Poulson (2012) indicate that plaque will harden and mineralise to form calculus after 10 days. However, whilst Piperno and Dillehay (2008) state that a calculus deposit will reflect at least several years’ worth of build-up and diet, Boyadjian et al. (2007) conversely propose that calculus is a short-term indicator, reflecting days to weeks before death, depending on the size of the deposit.

4.6.2.2. Previous studies of dental calculus

Calculus has been studied in a wide variety of ways in the past – but focus has mainly surrounded methodological developments in recording, and the potential dietary or plant use information which calculus may provide (via microscopy). Primarily, standardisation of the recording of dental calculus has been crucial, as much other work on calculus stems from or is affected by this. Whilst Waldron (2009, 241) states “it is probably not necessary to do more than simply record its [calculus’] presence or absence in the mouth”, recording the location and severity of calculus within the dentition allows for greater interpretations to be made about dental disease and its prevalence in the past. By using a standardised
method, comparisons of these prevalence rates can be made – between individuals, sites, and populations, in both temporal, geographical, and socio-economic terms – meaning we can gain an insight into the aetiology and causes of calculus formation, and also obtain information on past diet, health, and oral hygiene. One of the most influential papers on the recording of dental calculus has been that of Dobney and Brothwell (1987), which provides a method of recording allowing for the location, severity, and thickness of the calculus deposit to be assessed. Brothwell (1972, 150; Figure 19) also provides a good, commonly used method of recording calculus, but which does not take thickness of the deposit into consideration. It is important to note that in order to make these types of recording worthwhile and comparable, calculus should be reported in terms of the number of teeth affected (as a percentage of the total number of teeth observed), rather than simply in terms of the number of individuals affected. Sadly, many authors still only report the number of individuals affected by calculus, which means that prevalence rates of calculus in the past are often difficult to determine, and makes comparisons between sites complex (Roberts and Manchester 2010, 72).

Figure 19: Levels of calculus severity (Brothwell 1972, 150)

A large body of study in the past has focused upon the determination and detection of macroscopic remains within calculus – notably phytoliths and starch grains using scanning electron microscopy (SEM) – in order to obtain more information on past diets and items placed in the mouth. It is known that the formation processes of calculus often result in the trapping small amounts of food, plant particles and microfossils. These become present in the saliva via ingestion and may adhere to the tooth surface during mastication or oral manipulation, and thus become incorporated into dental plaque or calculus. As these
microfossils are well protected within the mineralised deposit, they are not subject to
diagenetic changes, and provide direct evidence of plants or materials placed directly into
the mouth. They also provide a useful record of the plants used by past populations,
particularly on sites with little or poor organic plant preservation (Middleton and Rovner
Phytoliths are discrete, microscopic silica cell bodies found in plants which are
recognisable at various taxonomic levels (Fox et al. 1996; Gobetz and Bozarth 2001;
Piperno 2006, 5), and have been recovered from both faunal and human calculus (e.g.
Middleton and Rovner 1994; Fox et al. 1996; Gobetz and Bozarth 2001; Henry and
Piperno 2008; Wesolowski et al. 2010; Leonard et al. 2015). Other work has focused upon
the detection of starch grains within dental calculus, and has provided information on past
plant use and plant domestication (Piperno and Dillehay 2008; Hardy 2009; Hardy et al.
2009; Buckley et al. 2014(b); Hardy et al. 2015; Leonard et al. 2015), as well as the
cooking of starches (Hardy et al. 2009; Henry et al. 2011). Other microscopic analyses of
dental calculus have detected artefacts such as cotton fibres (Blatt et al. 2011) and micro-
charcoal (Hardy et al. 2015).

Finally, two recent papers (Scott and Poulson 2012; Poulson et al. 2013) have attempted to
undertake carbon and nitrogen stable isotope analysis on dental calculus – the first studies
of this kind. However, the exact sources of this carbon and nitrogen are yet to be deduced,
meaning the isotopic values obtained may not be solely from dietary sources, and thus not
indicative of past diet. Indeed, it is likely that the isotopic values obtained may in fact be
representative of the carbon and nitrogen values of bacteria within the calculus, rather than
dietary indicators – thus making studies of this kind somewhat futile. A subsequent study
by Salazar-García et al. (2014(b)) has indeed shown that carbon and nitrogen isotopic
values obtained from dental calculus are not comparable to isotopic values from bone
collagen or dentine from the same individual.

4.6.2.3. Metaproteomic and metagenomic study of dental calculus – and moving
towards an understanding of the oral microbiome

As can be seen from the above discussion, previous work on dental calculus has
predominately focused microscopic techniques. A number of recent studies have however
now attempted to apply existing proteomic and genomic technologies, which have been
shown to be so successful on other archaeological materials and complex mixtures, to
dental calculus. It is now becoming clear that calculus, along with retaining a range of macroscopically invisible compounds and elements – such as food debris, starch grains, and phytoliths – may also preserve ancient biomolecules too.

4.6.2.3.1. Metagenomic approaches

The recognition that aDNA may persist within archaeological dental calculus has only occurred in recent years, and the potential wealth of genomic information which calculus may hold is being realised. An early paper by Kawano et al. (1995) managed to use DNA from dental calculus to determine the sex of individuals using PCR methods, but the first demonstration that bacterial DNA was preserved within calculus was undertaken using transmission electron microscopy (Preus et al. 2011). Subsequently, a number of papers have determined bacterial aDNA in calculus using both PCR methods (De La Fuente et al. 2013) and 16S rRNA amplicon (targeted) sequencing (Adler et al. 2013; Warinner et al. 2014(a)).

The successful recovery of ancient bacterial DNA from dental calculus marked the emergence of a new metagenomic approach to calculus studies. Metagenomics “applies a suite of genomic technologies and bioinformatic tools to directly assess the genetic content of entire communities of organisms” (Thomas et al. 2012), and as such, is the approach most frequently used to analyse microbial DNA. The initial metagenomic studies of dental calculus, as highlighted above, utilised 16S rRNA amplicon sequencing (Adler et al. 2013; Warinner et al. 2014(a)), a technique commonly applied in human microbiome studies. Amplicon sequencing (also known as targeted sequencing) focuses on one or more of nine variable regions (V1-V9) of the 16S ribosomal RNA gene, which is present in all bacteria and archaea (Warinner et al. 2015(b)). In archaeological studies, V3 and V6 are commonly targeted as the primer sets utilised for them are shorter in length (200bp) and therefore more suitable for degraded archaeological samples (Adler et al. 2013; Warinner et al. 2014(a); 2015(b)). Sequence divergence within 16S rRNA variable regions allows for taxonomic assignment, either to genus or species level (Weyrich et al. 2015; Warinner et al. 2015(b)). As such, the 16S rRNA gene is one of the most well-characterised genes in prokaryotes, and more than 100,000 full 16S rRNA sequences are publicly available (Ziesemer et al. 2015).
However, a recent paper by Ziesemer et al. (2015) suggests that 16S rRNA targeted sequencing is unsuitable for metagenomic analysis of dental calculus samples due to amplification and taxonomic biases. In particular, metagenomic approaches to ancient microbiomes which utilise the V3 region of the 16S rRNA gene show systematic taxonomic biases and do not conform to biological expectations. Ziesemer et al. (2015) also show that when used on archaeological calculus samples, 16S rRNA V3 sequencing results in differential PCR amplification – a problem emerging from DNA fragmentation. Indeed, they propose that the median DNA fragment lengths within archaeological calculus are less than half the required template length for amplification. Furthermore, it should be noted that 16S rRNA sequencing only targets bacteria – and therefore can only determine information on bacterial constituents of the oral microbiota, but cannot inform on, for example, viruses, fungi or dietary inclusions. Furthermore, a paper by Elof-Fadrosh et al. (2016) has shown that rRNA sequencing approaches routinely underestimate microbial diversity. Instead, shotgun metagenomic approaches should be utilised in the future study of archaeological dental calculus samples.

Shotgun metagenomics is a non-targeted sequencing approach, and as such, randomly amplifies and sequences a subset of the total DNA in a sample. Due to this, a shotgun approach can simultaneously sequence bacteria, viruses, archaea and eukarya (Weyrich et al. 2015; Warinner et al. 2015(b)). Furthermore, as it is non-target driven, it avoids primer bias and fragmentation driven amplification bias (Ziesemer et al. 2015). Shotgun sequencing can also provide functional metagenomic information (e.g. presence of antibiotic resistance), and can be combined with targeted enrichment using hybridisation capture, which may allow for the construction of whole genomes (Warinner et al. 2014(a); 2015(b); Weyrich et al. 2015). However, although huge amounts of data are generated through shotgun metagenomics, depth of coverage is low, and analysis of datasets is problematic due to their huge size and also the genomic variability of microbial species, meaning characterisation of species can be difficult (Weyrich et al. 2015; Warinner et al. 2015(b); Ziesemer et al. 2015). Despite this, a shotgun metagenomic approach was adopted within this research, and applied to archaeological dental calculus samples (Chapter 8).

Finally, it is important to note that the preservation of DNA within dental calculus is thought to be enhanced by its highly mineralised nature, and the presence of high levels of calcium phosphates (see section 4.6.2.1. above). Calcium phosphate is known to bind DNA effectively, and in calculus is found in more ordered aggregates than in bone – meaning
that it forms a matrix highly resistant to decay (Warinner et al. 2015(a)). Indeed, SEM and EDS imaging of archaeological dental calculus have shown that it is highly resistant to microbial attack and post-mortem alteration (Warinner et al. 2014(a)).

4.6.2.3. Metaproteomic approaches

As discussed above (section 4.2.3), proteomics is fast becoming one of the most novel applications of existing scientific technologies to archaeological material – and this is now also extending to dental calculus. Shotgun metaproteomics is a new tool in dental calculus analysis, and thus far, has had more limited application than metagenomic approaches. However, akin to shotgun metagenomics, shotgun metaproteomics sequences a subset of the total protein in a sample, thereby allowing for both microbial and host proteins to be characterised simultaneously (Warinner et al. 2015(b)). Additionally, metaproteomic approaches hold a number of advantages over metagenomics in that proteins survive longer in the archaeological record than DNA (Collins et al. 2010), and crucially, that shotgun metaproteomics can provide functional information as well as taxonomic identifications. This therefore means that a shotgun metaproteomic approach may provide an insight into pathogen-host interactions, bacterial virulence factors, and immune response (Warinner et al. 2015(a); 2015(b)) – things which have previously not been possible to study archaeologically.

Conversely, it is known that protein sequencing is more complex than DNA sequencing, and reference databases are often less complex and smaller in size than genomic databases. This is due in part to the fact that whereas genomic sequences are static, protein production can be altered by tissue or cell type, cell development, and physiological state. This therefore means that proteins deriving from a common DNA sequence can be present in alternate isoforms and exhibit different post-translational modifications, as each state will require different proteins (van Doorn 2012; Warinner et al. 2015(b)). The issues in protein assignment and function-based classification are well outlined by Kolmeder and de Vos (2014).

To date, all dental calculus shotgun metaproteomic approaches have utilised a filter-aided sample preparation (FASP) protocol, modified for degraded samples (Teoh 2011; Charlton 2012; Warinner et al. 2014(a); 2014(b)), with extracted proteins then being analysed by shotgun protein tandem mass spectrometry (MS/MS). The LC-MS/MS system used within
these studies comprises of the physical separation of samples (based on their chemical properties) using liquid chromatography (LC), followed by separation by m/z ratio using tandem mass spectrometry analysis. The use of LC is advantageous as it allows for the separation of isomers, which have the same mass and therefore cannot be detected by MS alone. Reverse-phase chromatography is typically used in LC-MS/MS as it removes salts, which are incompatible with the MS/MS system. The resulting fraction is then ionised through electrospray ionisation. MS/MS allows for individual peptides (ions of a particular m/z) to be selected and further fragmented, and the resulting fragments identified in a second mass spectrometer. Fragmentation normally occurs through neutral gas phase collisions (collision induced dissociation (CID)). The fragmentation patterns representing the amino acid sequences in each spectra form unique signatures for individual proteins, which can then be compared to existing protein databases (McCormack et al. 1997; Peng et al. 2003).

4.6.2.3.2. The Oral Microbiome, Health, and Disease

The basis for adopting metagenomic and metaproteomic approaches stemmed from the recognition that bacteria preserve in dental calculus, as discussed above. A preliminary proteomic study by Teoh (2011) managed to detect bacterial proteins from dental calculus indicating that the individual studied was suffering from bronchitis at the time of calculus formation, and subsequent small-scale preliminary investigations of archaeological dental calculus managed to detect c.100 different ancient proteins – comprising of oral bacteria, immune proteins, proteins indicative of diet (i.e. from plants and animals), upper respiratory tract bacteria, and digestive tract bacteria (Charlton 2012; Warinner 2012; Tina Warinner, pers. comm.). The first metagenomic NGS study of dental calculus was undertaken by Adler et al. (2013), and used 16S rRNA sequencing to study 34 calculus samples spanning from the Mesolithic (c.7550-5450 BP) to the present day. The study concluded that changes in bacterial community diversity had occurred through time, with particular shifts in Gram-positive bacteria corresponding to the transition to agriculture in the Neolithic and the Industrial Revolution (Adler et al. 2013). However, a more recent study has suggested that these apparent microbiome shifts and the lack of certain specific bacteria (e.g. *Streptococcus mutans*) in older samples may instead be the product of PCR-dropout caused by higher DNA fragmentation in samples of increased age (Ziesemer et al. 2015).
A study by Warinner et al. (2014(a)) comprised the first combined metagenomic and metaproteomic approach to archaeological dental calculus, and also was the first application of shotgun metagenomics to calculus (as outlined in section 4.6.2.3.1.). As such, the study generated a species-level taxonomic and protein functional characterisation of Medieval dental calculus, detecting 40 pathogenic species, >200 bacterial proteins, >40 human proteins, and DNA from dietary constituents. A subsequent study also revealed that the whey protein β-lactoglobulin (BLG) can also be preserved and recovered from archaeological dental calculus samples (Warinner et al. 2014(b)), and as such, may serve as a marker for past milk consumption.

The application of high-throughput metagenomic and metaproteomic sequencing to human dental calculus has therefore revealed calculus to be a novel and, as yet, comparatively unstudied reservoir of ancient biomolecular data from human individuals. The genomic and proteomic data obtained has revealed information not only from the human individual, but also from commensal and pathogenic oral microbial species and dietary inclusions. The level of information obtained therefore is unparalleled within archaeological material, as is the sheer volume of data generated within these studies. Studies of this kind on archaeological dental calculus may therefore now allow us to go beyond surface level interpretations and discussions of health and disease in the past – and move towards a deeper, more sophisticated understanding, including discussions of potential microbial and bacterial species. The genomic work in particular is also suggestive of allowing us to study disease and pathogen evolution through time, by providing an insight into changes in the genomes of pathological and bacterial agents. This may ultimately lead to permitting us to gain an understanding of the antiquity of certain diseases, and when they began to affect humans – thus providing a true appreciation of changes in health and disease, and the aetiological agents of these in the past. Excitingly, the proteomic data obtained appears to be able to provide functional information, therefore meaning that it may be possible to determine if a pathogen was active at the time of calculus formation. This level of disease information on the archaeological past is thus unprecedented.

The genomic and proteomic analyses already undertaken on human dental calculus however importantly also appear to provide us with an insight into past oral microbiomes. Historically, accessing ancient microbiomes is something which has been very difficult, due to the rarity of soft tissue preservation in archaeological samples. Coprolites and mummified remains have previously presented possible sources of archaeological
microbiome data, but are rarely recovered and therefore have not been extensively studied (Warinner et al. 2015(a)). Additionally, in the modern clinical literature, the importance and extent of human microbiomes has also only recently been realised, and it is only with the advent of high-throughput next generation DNA sequencing that we have been able to gain an idea of the sheer number and types of bacterial species living within the human body. The microbiome is now recognised to have four main functions: digestion, vitamin production, education of the immune system, and defence against pathogens (Warinner 2013(a)). However, the realisation that human microbiomes may also have functions beyond this, and may play a wide variety of roles within the human body – even to the extent of affecting our moods and feelings – is now beginning to be explored (Neufeld and Foster 2009; Heijtz et al. 2011; Cryan and Dinan 2012; Collins et al. 2012; Dinan and Cryan 2012; Clarke et al. 2013; Marques et al. 2013; Kumar et al. 2013; Schnorr et al. 2014; Rodakis 2015; Figure 20), suggesting that notions such as the idea of ‘gut feelings’ may actually be valid.
The number of microorganisms which inhabit the human microbiome as a whole is thought to be around 100 trillion, and outnumber the human cells in our bodies (10 trillion) by an order of magnitude (Ziesemer et al. 2015). The human oral microbiome specifically is known to comprise of over 2,000 taxa from 13 distinct phyla, and is the second largest...
microbial community in the human body (Dewhirst et al. 2010; Warinner et al. 2014(a); 2015(b); Figure 21). Whilst there are known to be a ‘core’ set of bacteria which represent the healthy oral microbiome (Aas et al. 2005; Zaura et al. 2009), clinical studies have additionally shown that many oral bacteria are linked to not only oral diseases (e.g. Philstrom et al. 2005; Costalonga and Herzberg 2014; Johansson et al. 2016), but also a range of systemic diseases such as pneumonia, cardiovascular disease, stroke and diabetes (e.g. Beck and Offenbacher 2005; Genco et al. 2005; Awano et al. 2008). Alongside this, the oral microbiome also helps to maintain host health (Warinner et al. 2015(a)).

Figure 21: Oral microbial diversity (Zijng et al. 2010, 2)

It can therefore be seen that microbiomes play a huge role in human health and disease, and equally, that there are still a huge number of unanswered questions surrounding them.
For example, we have very little understanding of how microbiomes may have evolved or changed over time, nor how microbiomes may be affected by factors such as diet or cultural practices. The complexity of host-microbe interactions within the human body is yet to be characterised. The opportunity to view microbiomes in the archaeological past has traditionally been limited, but calculus may now provide us with a way in which we can gain an insight into these oral communities, and may help us to start to address the plethora of questions surrounding microbiome evolution and composition. Through this, calculus may provide us with a new way to view health and disease through time.

Metagenomic and metaproteomic studies of dental calculus have therefore opened up the possibility of calculus as unique material through we which we can study bacterial communities, disease virulence, immune response, and diet in the archaeological past. Metagenomic and metaproteomic analyses of dental calculus will be applied within this research using samples from four Neolithic sites across the UK - meaning this will be the first combined genomic and proteomic study of prehistoric calculus ever undertaken (see Chapter 8), and furthermore, these will be the oldest samples ever analysed using this combined approach. As such, it is hoped that this study of human dental calculus may elucidate whether dental calculus of this age provides a robust biomolecular dataset, and additionally, whether the data obtained is indicative of an oral microbiome signal. If early prehistoric dental calculus can provide an insight into the oral microbiome of these early periods, then it may provide us with a new means via which to study prehistoric health and disease – something which has traditionally been so hard to study archaeologically (see section 4.6.1. above and Chapter 2). Furthermore, if this can be achieved, it will open up new research avenues for early prehistoric periods. This is particularly applicable to Neolithic calculus given the large scale changes in diet, lifestyle, and subsistence which are believed to be associated with period (as discussed in Chapter 2). Calculus may therefore provide a means via which to view potential diseases, bacteria or pathogens associated with this change – and an indication of the effect on which dietary changes may have on the composition and communities of the human oral microbiome.

4.7. Standard Operating Protocols (SOPs)

Full details of standard operating protocols for all methodologies used within this research can be found in Appendix A. The techniques used on all material presented will however also be clearly outlined in each Chapter.
4.8. The Advantages of a Multi-Methodological Approach – A New Means of Studying Prehistoric Archaeology?

The novel aspect of this research is that it aims to obtain useful and high quality biomolecular information addressing the five research areas (discussed above and in Chapter 2) from previously overlooked archaeological materials – notably bone fragments considered ‘unidentifiable’, disarticulated bone, and dental calculus. In order to obtain the most information from the bone and calculus used in this work, a range of integrated scientific techniques have been adopted, described in detail above (Figure 22).

Figure 22: Schematic of integrated scientific techniques utilised within this research

Comparing different scientific data obtained from the same sources highlights the advantages of using a multi-methodological approach such as is adopted in this research. The multi-disciplinary nature of this research is also beneficial, as by combining methods more traditionally used in chemistry, biology or the physical sciences and applying them to archaeological materials, a broader and novel perspective on the prehistoric past can be gained. The crucial aspect of this research therefore lies in the fact that a suite of biomolecular methods will be used in tandem, and applied to the same samples simultaneously – something which is not been done on a broad scale with prehistoric samples in Britain, but is important to develop given the importance and scarcity of skeletal material from early prehistory (see Chapter 3).
In this way, this research aims to provide a template via which to assess health, disease, diet, demography, mobility, and chronology within prehistoric archaeology. Crucially, in addition to this, this research aims to achieve this via the utilisation of skeletal materials which have traditionally been overlooked or understudied. In doing so, it is hoped that this research may highlight the biomolecular information currently dormant within excavated materials. The following chapters thus provide examples of application of the methodologies and themes outlined within this Chapter, and Chapters 2 and 3, to Mesolithic and Neolithic British material.
5.1. Introduction

This chapter comprises of a combined biomolecular study of ‘unidentifiable’ skeletal fragments from the Late Mesolithic site of Cnoc Coig, Oronsay (Inner Hebrides). The application of biomolecular methods to enhance our understanding of the transition from foraging, fishing and hunting to agricultural food production has, in Britain, previously been limited – due to the near absence of human remains dating to the period immediately preceding the arrival of farming c.4,000 cal. BC, i.e. the Late Mesolithic (as discussed within Chapter 3). Remarkably, the only directly dated sites from the whole of the 5th millennium BC with known human remains are from the small Inner Hebridean island of Oronsay (Meiklejohn et al. 2011), severely restricting meaningful comparisons with more abundant Neolithic remains found across Britain.

Despite the identification of only six individuals at the site of Cnoc Coig, Oronsay (Meiklejohn et al. 2005), it has become pivotal to the argument for a rapid dietary change with the arrival of agriculture in Britain (Schulting and Richards 2002(a)). Here, ZooMS was applied to 20 fragments of small, fragmentary ‘loose’ bone from the site, which had previously been determined as osteologically unidentifiable, to investigate whether additional human remains could be identified from the site. AMS dating and stable isotope analysis was then undertaken on any identified bone samples in the hope that this may enhance our understanding of the diet of Britain’s last forager groups and their chronological relationship to the earliest evidence for agriculture, and thereby also contribute to larger debates regarding the transition in Britain (Chapter 2). This study is therefore the first combined biomolecular study of its kind on British early prehistoric skeletal fragments.

The following sections provide a brief overview of the site and its skeletal remains, and the results of the ZooMS analysis and $\delta^{13}$C and $\delta^{15}$N isotopic analysis, as well as new AMS dates obtained for the site. Overall, it was hoped that the study may highlight the biomolecular information which can be obtained from skeletal material previously considered unidentifiable and/or unimportant. Furthermore, the study aims to indicate the worth of the bone protein collagen, in that it can be used to identify species, determine
date, and assess diet simultaneously – and that this information can be obtained even when aDNA does not survive. As such, the methodology presented here may provide a new means via which the hidden bone record of the British late 5th millennium BC may be bought to light.

5.2. Cnoc Coig

The site of Cnoc Coig is one of five Mesolithic shell middens on the island of Oronsay in the Inner Hebrides. The site was first excavated by Mungo Buchanan in 1911-1912 (Wickham-Jones et al. 1982), but is best known for excavations undertaken by Paul Mellars 1973-1979 (Mellars 1987). During Mellars’ excavations, c.70% of the midden was excavated (Meiklejohn et al. 2005), and a range of faunal remains, shellfish, flint artefacts, hazelnut shells, limpet scoops, hearths, and possible structural evidence, along with human remains, were recovered (Mellars 1978). Significant amounts of work have since focused predominately on determining seasonality of use of the site (Mellars 1978; 2004; Mellars and Wilkinson 1980; Mithen and Finlayson 1991; Meiklejohn et al. 2005), as well as on the human remains recovered.

During Mellars’ excavations, 49 pieces of human bone were recovered, predominately from the hands and feet, thought to represent at least four individuals (Meiklejohn and Denston 1987). Spatial analysis has suggested these human remains fall largely into seven circumscribed bone groups, although none are indicative of primary inhumation (Meiklejohn et al. 2005). This human bone was initially radiocarbon dated to c.4490-3840 cal. BC, but recalibration by Gordon Cook (see Milner and Craig 2009) instead suggested a date of 4250-3650 cal. BC (Table 4). Critically, the human remains recovered from Cnoc Coig represent one of the very few human skeletal assemblages dating to the end of the 5th millennium BC in Britain, immediately prior to the emergence of agriculture in Britain; although slightly earlier dates (4300 cal. BC) have been proposed for both Neolithic monuments and pottery on the West Coast of Scotland (Sheridan 2010; see also Chapter 2). Although small and fragmented, the Oronsay human remains have been subject to a range of analyses, including spatial analysis, taphonomic studies, discussions of burial practices, stable isotope analyses, and AMS dating (e.g. Jardine 1978; Mellars and Wilkinson 1980; Richards and Mellars 1998; Richards and Sheridan 2000; Meiklejohn et al. 2005; Wicks et al. 2014).
In particular, stable isotope analysis of the human bones from Cnoc Coig has shown a strongly marine isotopic signature, in contrast to the terrestrial signatures obtained from early 4th millennium sites along the west coast of Scotland and elsewhere in Britain (e.g. Hedges et al. 2008; Milner and Craig 2009). In the absence of other Late Mesolithic human remains, the Oronsay material has become pivotal to the argument for a rapid dietary change with the arrival of agriculture in Britain (Schulting and Richards 2002(a); Richards et al. 2003), despite being based on a very small number of individuals.

The lack of human skeletal material dating to the later Mesolithic and early Neolithic from Britain has compounded our interpretations of the nature and timings of the Mesolithic-Neolithic transition, and the dietary change perceived to accompany this (as discussed in Chapter 3). At present, broad scale interpretations of dietary change have been made from small sample sizes – and material from Oronsay has been widely implicated within this. As such, the rationale behind this study was primarily to identify additional human remains from the site of Cnoc Coig, but using excavated material currently overlooked. This was achieved via the use of ZooMS to determine the species of previously ‘unidentifiable’ bone fragments from Cnoc Coig, and alongside this, additional radiocarbon dating of material from the site was undertaken, in an attempt to clarify the dating and chronology of Cnoc Coig. It was hoped that through identification of further human remains for dating and dietary analysis, that new information may be obtained which could aid in our understanding of the Mesolithic-Neolithic transition in Western Scotland and also more generally across Britain as a whole.

5.2. Materials and Methods

5.2.1. Samples

Twenty fragments of disarticulated and heavily fragmented bone from the 1973-9 excavations, originally classified as ‘unidentifiable’ or ‘?human’, and which have therefore since remained unstudied, were utilised within this study (Figure 23). Although the trench number of the remains studied is known, little other contextual information is available. The majority of the bone fragments (n=15) studied derive not from the main midden structure, but instead lie just outside, in a singular outlying trench dug by Mellars (Figure 24). The five remaining ‘unidentifiable’ bones were selected from other areas within the main midden structure.
Figure 23: A selection of the twenty bone fragments obtained from the Cnoc Coig assemblage used within this research. The images highlight the range of sizes, element types, shapes and degrees of preservation condition seen within the bone fragment assemblage. From top, working left to right, these bone fragments were identified later using ZooMS as seal, pig, remainder human.
Figure 24: Plan of excavated areas of the midden 1973-1979 (adapted from Mellars 1987, 215), indicating the extent of the midden as defined by Mellars. The trench highlighted in red denotes Trench U, from which the human remains identified come from – this can clearly be seen to fall outside of the defined limits of the midden structure.
5.2.2. **A Combined Biomolecular Approach**

A multi-methodological, integrated scientific approach was adopted in the study of these bone fragments, combining ZooMS, stable isotopic analysis and AMS dating. Collagen was extracted and isotopically analysed using published protocols (Richards & Hedges 1999; Colonese et al. 2015), and ZooMS was undertaken on a sub-sample of the extracted collagen (<1mg), using a novel methodology. Protocols for each of the methodologies employed in this study are provided in Appendix A. Four samples with adequate collagen preservation were submitted for AMS dating at the NERC radiocarbon facility (Oxford) and calibrated using the procedure outlined in Appendix A (A.1.3).

5.3. **Results and Discussion**

5.3.1. **Identification**

Nineteen of the twenty bone samples analysed yielded identification information, including six that had insufficient collagen to undertake stable isotope analyses. Remarkably, fourteen of the twenty bone samples were identified to be human using ZooMS (Table 6). Identification was based upon peptide matching as outlined in Welker et al. (2015). An example spectrum is shown in Figure 25 and compared with known human bone. The detection of these bone fragments therefore increases the number of known human bone fragments from all five Oronsay middens from 55 (16) to 74 (including five fragments recently recovered at NMS) (Sheridan, pers. comm.). The remaining samples were identified as either as *Pinnipedia* (seal) or *Sus* (pig) (Table 6). The ZooMS data presented here therefore provides the first application of the method which has actively aimed to determine both human and faunal remains. The identification of fourteen bone fragments as human, however, highlights the potential of the method in obtaining more information on Mesolithic samples previously considered ‘unidentifiable’, and presents a means via which we may be able to expand the number of identified human remains from British Mesolithic contexts. The fragmentary nature of the majority of prehistoric bone in Britain means that our interpretations of skeletal material are often limited using traditional modes of study (see Chapter 3 and Chapter 4, section 4.2.). Using ZooMS to determine taxonomic identification of Mesolithic bone fragments however opens up the possibility of increasing our knowledge of this period of British prehistory, and of obtaining useful biomolecular data from skeletal material which is currently overlooked.
Figure 25: MALDI-TOF spectra of (a) known human bone and (b) sample identified as human from Cnoc Coig fragments

The location of the human remains identified within this study is also interesting – and could provide new insights into burial practices or deposition of human remains in the late 5th-early 4th millennium BC in Britain. The human remains identified here originate from a singular outlying trench (Figure 24), and may represent a different depositional event to human remains found within the midden itself. This raises interesting questions as to whether deposition in this location was purposive, or is a product of taphonomic processes. Given the ubiquitous nature of disarticulated human remains with the Mesolithic burial record, found in a wide range of depositional contexts, potential degrees of intentionality with regards to these kinds of deposits have previously been discussed. Gray Jones (2011), for example, has suggested that ‘loose bone’ or disarticulated remains may in fact be the result of deliberate acts, and thus a part of, rather than separate from, other types of mortuary practice.

Additionally, only one of the bone fragments identified here as human appear to originate from the hands or feet – which have previously been noted to be the dominant element types within human remains identified within the main midden structure deposits.
(Meiklejohn et al. 2005). The predominance of human hand and foot bones has led to suggestions of the site being used for excarnation, the placing of bodies on scaffolds, and the skeletal assemblage representing “a purposive cultural act” (Meiklejohn et al. 2005, 102). However, as can be seen in Table 6, the fragments identified as human here appear to come from a range of skeletal elements, including long bones, crania and vertebrae – therefore falling more in-line with the loose bone assemblages found in Scandinavian contexts (Denmark, Sweden and Norway) (Newell et al. 1979; Larsson et al. 1981; cf. Meiklejohn et al. 2005).

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

Table 6: ZooMS species ID and collagen stable isotope values obtained from newly identified human and faunal remains from Cnoc Coig. Estimated marine % of diet for humans calculated from isotopic data obtained after Schulting and Richards (2002(a)) (using marine and terrestrial carbon end-points of -12‰ and -21‰ respectively)
5.3.2. Isotopic Data and Dietary Inferences

Of the fourteen bone fragments identified here as human, nine yielded sufficient amounts of collagen of suitable quality for $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis (Table 6; Figure 26). Samples identified as human and seal were enriched in both $^{13}C$ and $^{15}N$ compared to those identified as pigs, implying a higher marine dietary component (Figure 26).

Figure 26: Plot of stable isotope values from Cnoc Coig and Caisteal nan Gillean human and fauna, against previously obtained human isotopic data (from Richards and Mellars 1998)

Isotopic data previously obtained from Cnoc Coig can be seen in Table 7, and was suggested to represent a diet where all protein was derived from marine sources (Richards and Mellars 1998). No faunal isotope values were previously available from Cnoc Coig however, and Richard and Mellars’ (1998) interpretations were based upon a singular
isotopic value from a seal bone from the midden site of Caisteal Nan Gillean II, also on
Oronsay. Isotopic data acquired in this study shows a degree of variability comparable to
the previously obtained isotopic values from human remains (Tables 6 and 7; Figure 26).
Variation in the isotopic data obtained however indicates that these bone fragments are
unlikely to be from individuals previously studied. At least two of the new human samples
are outside the error expected by replicate analysis of a single individual (Pestle et al.
2014), whereas the seal bones from Oronsay measured here and that analysed previously
(Richards and Mellars 1998) are within analytical error. Conservatively, if we use these
effects, combining this new human isotopic data with previous analysis (Richards and
Mellars 1998) suggests a potential minimum of seven human individuals are represented
isotopically, from thirteen pieces of bone (Figure 26).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Sample Type</th>
<th>Element</th>
<th>$\delta^{13}$C (‰)</th>
<th>$\delta^{15}$N (‰)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>18104</td>
<td>Human bone</td>
<td>Right clavicle</td>
<td>-13.2</td>
<td>14.5</td>
<td>3.1</td>
</tr>
<tr>
<td>17157</td>
<td>Human bone</td>
<td>Left clavicle</td>
<td>-12.3</td>
<td>16.0</td>
<td>3.1</td>
</tr>
<tr>
<td>17203</td>
<td>Human bone</td>
<td>3rd left metacarpal</td>
<td>-12.0</td>
<td>14.7</td>
<td>2.9</td>
</tr>
<tr>
<td>18284</td>
<td>Human bone</td>
<td>1st right metacarpal</td>
<td>-12.0</td>
<td>17.0</td>
<td>3.1</td>
</tr>
<tr>
<td>18089</td>
<td>Human bone</td>
<td>Frontal</td>
<td>-13.6</td>
<td>15.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 7: Previously obtained $\delta^{13}$C and $\delta^{15}$N isotopic results on human bone from Cnoc Coig (adapted from Richards and Mellars 1998)

The new isotopic data obtained here agrees with previous interpretations that the
individuals recovered from the site were consuming a high marine protein diet, and is now
supported by faunal baselines from the site (Figure 26). When comparing the isotopic data
obtained from Cnoc Coig to other sites on the West coast of Scotland of a comparable date,
it can be seen that the Cnoc Coig material clusters with the sole human isotopic value from
Caisteal nan Gillean II, another of the shell middens on Oransay. However, the Oronsay
samples are significantly enriched in both $\delta^{13}$C and $\delta^{15}$N in comparison to the Mesolithic-
Neolithic samples from both An Corran and Crarae (Figure 27).
Figure 27: Comparison of human isotopic data from Cnoc Coig plotted against data from other sites on the West coast of Scotland of a comparable date. Data obtained by the author (noted ‘SC’ in legend), and from Richards and Mellars 1998; Meiklejohn et al. 2005; 2011

In order to better characterise human diet, isotopic mixing models were created for the Cnoc Coig samples. However, the lack of known faunal and plant isotopic values for the site/island is problematic. Where actual values are unknown, δ\textsubscript{15}N values of Holocene plants are frequently assumed to be 3‰ (Bogaard et al. 2007; Fraser et al. 2013), or alternatively, are estimated from terrestrial herbivore bone collagen isotope values via the subtraction of an assumed trophic level change value, caused by collagen fractionation shift (c.4‰ for δ\textsubscript{15}N). However, as Fraser et al. (2013) note, both these approaches are problematic, particularly given that plant stable isotope values can be variable even within one geographical location or ecological zone. δ\textsubscript{13}C values of C\textsubscript{3} terrestrial plants are generally thought to be c.-27‰ (Kelly 2000; Dawson et al. 2002), and are potentially less variable than plant δ\textsubscript{15}N values due to the fact the δ\textsubscript{13}C values are determined by the photosynthetic pathway of the plant itself. However, Tieszen (1991) has suggested that
$\delta^{13}$C values of C$_3$ plants can range in some cases from -22‰ to -38‰, depending on water sources, nutrient availability, temperature and altitude. Due to the fact that the wild plant values for Cnoc Coig are unknown, $\delta^{13}$C and $\delta^{15}$N protein values were estimated for the FRUITS model, calculated from herbivore values using assumed dietary offsets. Energy values for plants were calculated using data from Tieszen (1991) ($\Delta^{13}$C$_{\text{protein-energy}} = +1\%$).

An additional problem in the use of an isotope mixing model for the Cnoc Coig data is the potential terrestrial animal dietary sources – which include both herbivores (e.g. red deer, aurochs) and omnivores (e.g. pigs). Due to this, two separate mixing models were created for the Cnoc Coig data, one where all terrestrial animal dietary sources (i.e. herbivores and omnivores) were considered together as a singular food group, and a second model wherein only terrestrial herbivores were considered as a food group. This second model was created as the currently available offsets for $\Delta^{13}$C$_{\text{protein-collagen}}$, $\Delta^{13}$C$_{\text{energy-collagen}}$, and $\Delta^{15}$N$_{\text{protein-collagen}}$ values in the literature are only for herbivores, not omnivores (Fernandes 2015), and therefore may not be suitable for application to pigs. However, it is likely that pigs were included within the diet of the inhabitants of Oronsay, and therefore their inclusion in the model is pertinent.

The $\delta^{13}$C and $\delta^{15}$N isotopic values utilised within the Cnoc Coig FRUITS model can be found in Table 8 below. Additional information on how these values were utilised and the assumptions made within the model can be found within Appendix A (section A.1.4.).

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Species within food group</th>
<th>No. of samples</th>
<th>$\delta^{13}$C (%o)</th>
<th>St. Dev.</th>
<th>$\delta^{15}$N (%o)</th>
<th>St. Dev.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial animals</td>
<td>Red deer, pig, cattle/aurochs, ruminant, ungulate</td>
<td>23</td>
<td>-21.8</td>
<td>0.9</td>
<td>3.9</td>
<td>2.2</td>
<td>This study; Schulting &amp; Richards 2002(a);(b); Montgomer y et al. 2013; Milner &amp; Craig 2012</td>
</tr>
<tr>
<td>Marine Foods</td>
<td>Seal, sea otter, cod, angler fish</td>
<td>11</td>
<td>-12.5</td>
<td>0.8</td>
<td>16.7</td>
<td>3.2</td>
<td>This study; Schulting &amp; Richards 2002(a);(b);</td>
</tr>
</tbody>
</table>
Table 8: $\delta^{13}$C and $\delta^{15}$N isotope values of food groups used within the FRUITS model generated for Cnoc Coig

<table>
<thead>
<tr>
<th>Terrestrial Plants</th>
<th>Unknown</th>
<th>N/A</th>
<th>-26.6</th>
<th>0.9</th>
<th>-1.6</th>
<th>2.2</th>
<th>Est. using Tieszen 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumer data</td>
<td>Human</td>
<td>14</td>
<td>-13.3</td>
<td>0.8</td>
<td>15.4</td>
<td>0.6</td>
<td>This study; Richards &amp; Mellars 1998</td>
</tr>
</tbody>
</table>

Figure 28: FRUITS model 1 for Cnoc Coig data, considering all terrestrial animals (herbivores and omnivores) as one food group
The isotopic mixing models (Figures 28 and 29) generated for the averaged human isotope values obtained in this study again support the idea of a high marine protein diet at Cnoc Coig, comprising at least 50% of the diet. However, it is interesting to note that this percentage of marine foods within the diet is somewhat lower than that suggested through calculations following Schulting and Richards (2002(a)), using marine and terrestrial carbon end-points of -12‰ and -21‰ respectively (Table 6). Both models also suggest that some degree of terrestrial protein was consumed (20-25% of the diet), but interestingly, this figure is slightly elevated in model 2, where only terrestrial herbivores were included. Finally, although the problems with estimating terrestrial plant consumption are discussed above, the results from the isotopic mixing models importantly indicate that terrestrial plant foods may have also constituted a fairly significant component of the diet of Mesolithic populations frequenting Cnoc Coig – circa 25% of the total diet. This is perhaps unexpected when we consider the foraging nature of hunter-gatherer subsistence, but is
something which is not frequently discussed within literature on Mesolithic diets – in part because it is so difficult to study. Instead, as discussed in Chapters 2 and 3, focus tends to be on the change from a high marine protein diet to a predominately terrestrial (domesticated) protein diet. However, the results presented here indicate that although the diets of individuals at Cnoc Coig appear to have been dominated by marine foods, fairly significant amounts of both plant foods and terrestrial animals were also consumed.

5.3.2.1. Mesolithic Pigs on Oronsay

The identification of three of the bone fragments as pig also raises some interesting dietary questions given the isotope values obtained from them (Table 1; Figure 4). Two samples (at least one individual) showed δ¹³C and δ¹⁵N values indicative of a terrestrial herbivorous diet, whilst the third had isotope values consistent with a more marine or omnivorous diet. It is known that pigs comprise a significant proportion of the faunal assemblage at Cnoc Coig – being only slightly less well represented than red deer (Grigson and Mellars 1987). From the original analysis, it was suggested that biometrically, the pig remains present at Cnoc Coig are likely to represent the wild, rather than domesticated, form of *Sus scrofa* (Grigson and Mellars 1987). The differential isotopic values obtained for the pig remains however (MNI=2) are intriguing. The presence of one pig with elevated δ¹⁵N values suggests some degree of marine consumption within the diet – obtained either through consumption of human refuse, foraging within the midden deposits, or being purposively fed marine protein by humans. The other two pig bones identified within this study appear to exhibit a solely terrestrial, herbivorous diet, compared to the apparent omnivorous diet of the other individual. It is well documented that pigs will happily consume marine protein sources, including marine vertebrates and invertebrates (Masseti 2007), but increased δ¹⁵N values in the Cnoc Coig pig sample may instead be due to the consumption of other marine foodstuffs, notably seaweed or similar. The use of seaweed as a purposive fodder for animals, including pigs, is widely noted, including on island locales (e.g. Balasse et al. 2006).

Grigson and Mellars (1987) suggested that pigs on Oronsay were likely to have been purposively bought to Oronsay by Mesolithic populations from the mainland or larger surrounding islands – which therefore suggests that differences seen isotopically between the pig remains here may be representative of differential origins; resulting in a differential diet before arriving on Oronsay. However, it should also be considered that a number of
authors have noted that *Sus scrofa* can swim significant distances, particularly between islands (Albarella et al. 2006; Masseti 2007). The idea of pigs coming from multiple locations/populations, or being brought to the island by humans, is mirrored in the original analysis of the Oronsay red deer populations. From analysis of the size of antlers, it was suggested that the red deer derived from two (geographically) distinct populations (Grigson and Mellars 1987). If red deer (or portions of) were bought to Oronsay from two different locations, then it is also possible that a similar process was occurring with pigs.

An additional consideration is that the isotopic values seen here may be reflective of the midden deposits potentially containing both wild boar and domesticated pigs. Previously, it has been suggested that wild boar and domesticated pigs look isotopically different (Hu et al. 2009). Wild boars are thought to be largely herbivorous, but domesticated pigs more carnivorous/omnivorous through increased human contact, feeding on human refuse, and/or a more controlled diet (Matsui et al. 2002; Bulliet 2005, 90; Albarella et al. 2006). This idea is also supported by isotopic data obtained from the Danish site of Västerbjer, where pigs were shown to have a terrestrial diet, despite the presence of marine waste at the site, and the isotopic values of dogs from the site being highly marine (Eriksson 2004). This has been suggested to represent dogs scavenging and feeding off human refuse, whilst pigs were not – thereby suggesting that the pigs were not domesticated (Rowley-Conwy et al. 2012). Distinguishing between wild boar and domesticated pigs is notoriously difficult however, as highlighted by a number of recent debates (Krause-Kyora et al. 2013; Rowley-Conwy and Zeder 2014(a); 2014(b); Evin et al. 2014) and is also more problematic when attempting to do so through the use of isotopes in solely C3 geographical locales, such as Britain. Nonetheless, the association of the consumption of pigs amongst Mesolithic populations with predominately marine dominated diets is seen across Europe – including sites in the Mediterranean, Baltic and across northern Europe (Masseti 2007).

In terms of comparative data, there are few Scottish Mesolithic and Neolithic sites with *Sus scrofa* isotopic data available. This is, in part, because a significant number of early prehistoric sites in Scotland were excavated in the 19th century, and in many cases the recovered faunal assemblages have since been lost (Smith 2000). Figure 30 displays the pig isotope data obtained here plotted against published Mesolithic and early-mid Neolithic *Sus scrofa* values from both Britain and mainland Europe. As can be seen, the anomalous Cnoc Coig pig value is significantly more enriched in δ15N in comparison to the other available published values for Mesolithic and Neolithic European pigs. The only other
value which is more enriched in δ¹⁵N is a singular wild boar sample from the Danube Gorges, taken from a juvenile scapula (Borić et al. 2004), and thus the enriched δ¹⁵N value may be a result of the weaning effect – as has been previously noted in human samples (Richards et al. 2002; Schurr and Powell 2005; Pearson et al. 2010; Oelze et al. 2011). However, differential δ¹³C values can also be seen between the Cnoc Coig samples analysed here. These may be caused by the canopy effect, and more depleted δ¹³C values (as seen in two of the pig samples here) may be the result of pigs feeding in areas with more dense vegetation (as suggested in Oelze et al. 2011).

Figure 30: Sus scrofa data from Mesolithic and early-mid Neolithic European sites (data compiled from Schulting and Richards 2002(a); 2009; Borić et al. 2004; Eriksson 2004; Craig et al. 2006; Bocherens et al. 2007; Fornader et al. 2008; Hedges et al. 2008; Lightfoot et al 2011; Oelze et al. 2011; Fontanals-Coll et al. 2014; the author)
The variability seen in the pig/wild boar isotopes in Figure 30 however also highlights the crucial importance of using faunal baselines when attempting to make interpretations from human isotopic data (as discussed in Chapter 4). It can be seen that there is a distinct need to better characterise the isotope variability of fauna from Cnoc Coig in order to accurately interpret the measurements on human bone. This raises interesting questions about how archaeologists generally interpret isotopic datasets, particularly given the low frequencies of faunal isotopic data often available for British prehistoric sites. If marine isotope signatures were a feature of significant numbers of terrestrial animals at this site, then the notion of heavy marine resource consumption by humans would potentially need to be revised.

5.3.3. Re-dating human remains at Cnoc Coig

A range of dating work has previously been undertaken on materials (including human remains) from Cnoc Coig (e.g. Jardine 1978; Switsur and Mellars 1987; Richards and Sheridan 2000; Schulting and Richards 2002(a); Meiklejohn et al. 2005; Wicks et al. 2014; Table 10). Whilst Mellars (2004) suggested that the site was occupied 4250-3450 cal. BC, others have utilised previous AMS dates on human remains to date the site to 4300-3800 cal. BC (Richards and Sheridan 2000; Milner and Craig 2009).

However, dating thus far has only been undertaken on shell samples and human remains with marine carbon isotope signatures – both of which are subject to uncertainties associated with the marine reservoir effect (MRE). Additional $^{14}$C dates on bulk charcoal are available (Switsur and Mellars 1987), although these may have derived from ‘old wood’ (Schiffer 1986), adding to the uncertainty regarding the dating of the site. An additional problem is that these dates were also obtained in the 1980’s, when errors on radiocarbon data are thought to have been underestimated (Table 10). New dates on humans and the first dates on short-lived terrestrial samples (e.g. faunal bone) using modern AMS technologies were therefore undertaken in this study (Table 9).
Solid dating and understanding of chronology is especially important for the site given that Cnoc Coig provides evidence of one of the very few late 5th-early 4th Millennium BC sites in Britain. Only four previous radiocarbon dates are available for the Cnoc Coig human remains, constituting the majority of dated individuals for the entire 5th millennium in Britain. As such, these additional dates on the newly identified skeletal material therefore contribute to the number of known, dated human remains from the British late Mesolithic (Table 9).

Table 9: AMS dates obtained within this study from newly identified human and fauna. A ΔR value of 47 ± 52 14C yr was used to calibrate the dates of samples 8257 and 8267 (Ascough, pers. comm.; Russell et al. 2015)

<table>
<thead>
<tr>
<th>Material Dated</th>
<th>Lab Ref. No.</th>
<th>14C Date BP</th>
<th>Original Published Date cal. BC</th>
<th>New Date cal. BC (with MRO) (95.4%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arctica islandica</em> shell</td>
<td>Birm-326Z</td>
<td>7240 ±200</td>
<td>6400-5100</td>
<td>6144-5354</td>
<td>Jardine 1978; Mellars 1987</td>
</tr>
<tr>
<td><em>Arctica islandica</em> shell</td>
<td>Birm-326Y</td>
<td>7290 ±120</td>
<td>6200-5450</td>
<td>6011-5527</td>
<td>Jardine 1978; Mellars 1987</td>
</tr>
<tr>
<td><em>Arctica islandica</em> shell</td>
<td>Birm-326X</td>
<td>7610 ±150</td>
<td>6500-5650</td>
<td>6399-5760</td>
<td>Jardine 1978; Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-1352</td>
<td>5430 ±130</td>
<td>4520-3970</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-1351</td>
<td>5495 ±75</td>
<td>4510-4070</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-1354</td>
<td>5535 ±140</td>
<td>4690-4040</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-1353</td>
<td>5645 ±80</td>
<td>4690-4340</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-3006</td>
<td>5675 ±60</td>
<td>4690-4360</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Bulk charcoal</td>
<td>Q-3005</td>
<td>5650 ±60</td>
<td>4660-4350</td>
<td>-</td>
<td>Switsur and Mellars 1987</td>
</tr>
<tr>
<td>Human bone (sample no. 17203)</td>
<td>OxA-8014</td>
<td>5495 ±55</td>
<td>4000-3800</td>
<td>4036-3686</td>
<td>Richards and Sheridan 2000</td>
</tr>
<tr>
<td>Human bone (sample no. 17157)</td>
<td>OxA-8019</td>
<td>5615 ±45</td>
<td>4200-4000</td>
<td>4232-3830</td>
<td>Richards and Sheridan 2000</td>
</tr>
</tbody>
</table>
Table 10: 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. Information on new calibration of dates can be found below

Calibration of dates was undertaken using OxCal v.4.2, using a mixed marine-terrestrial curve for the human samples in a proportion determined by marine/terrestrial carbon contribution to collagen (as in Barrett and Richards 2004; following best practice 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 (-12‰ and -21‰ respectively; Table 6). Calibration of AMS dates from Cnoc Coig using this approach has also previously been successfully undertaken by Gordon Cook (Milner and Craig 2009). The terrestrial herbivore samples were calibrated using only the terrestrial calibration curve however.

As noted above (Table 9), calibration of AMS dates from the human skeletal material in this study was also undertaken using appropriate ΔR values due to the marine reservoir effect (MRE) on samples containing marine-derived carbon. The MRE is a variable offset in 14C age between atmospheric (terrestrial) and oceanic carbon reservoirs caused by the mixing of ‘old’ and ‘new’ carbon within the marine atmosphere and CO2 transfer times within oceans (Ascough et al. 2005). A number of recent publications have however highlighted the variability of MRE and subsequent ΔR values both temporally and geographically, which are thought to be caused by palaeoclimatic, environmental and oceanographic changes (Ascough et al. 2004; 2005; 2007; 2009; Russell et al. 2015).

Initial dates on human remains from Cnoc Coig (Richards and Sheridan 2000) were not calibrated using ΔR values appropriate for the marine content of the samples, but subsequent re-calibration (Milner and Craig 2009) showed a significant alteration of the calendar dates obtained for these same samples. In the (2002(a)) Schulting and Richards paper, a ΔR value of -33 ±93 years was applied to skeletal material from Oronsay; taken from data from Reimer et al. (2002) on paired samples from sites around the North Atlantic Coast (Ireland, Scotland, Orkney Islands). In light of the isotopic data obtained, calibration of new AMS dates obtained here from human remains was undertaken with a marine reservoir correction using a mixed terrestrial/marine curve and appropriate ΔR offset. As
the MRE has not been assessed at Oronsay itself, a calculated mean ΔR value for Scotland was utilised (ΔR = 47 ± 52 $^{14}$C yr) (Ascough, pers. comm.; Russell et al. 2015) following best practices (Cook et al. 2015).

After calibration of both new dates generated here and those previously obtained, it is clear that all the humans overlap with the terrestrial fauna, and fall within the early part of the 4th millennium BC (Tables 9 and 10; Figure 31). This is a significant result as the Oronsay human dates, with marine isotope signatures, overlap with humans from other parts of Western Scotland with fully terrestrial isotope signatures (Figure 8) and with the earliest evidence for domesticated animals and plants in Britain. This suggests that there was considerable heterogeneity in human diets in the early part of the Neolithic reflecting specialisation in subsistence practices across the landscape and continuity of foraging, hunting and fishing into the period traditionally associated with agriculture and pastoralism. Sheridan (2010) has argued for the arrival of a ‘Breton Neolithic’ in this region from around 4300-4200 cal. BC, which would imply that both hunter-gatherer-fisher and farming lifestyles co-existed in the West Coast of Scotland for several hundred years. A marine diet may therefore have persisted in some areas of the UK after farming had been introduced.

Figure 31: Plot of $\delta^{13}$C values against recalibrated radiocarbon dates for humans from Scottish West Coast sites, c.4500-3500 cal. BC (data from this study; Richards and
5.4. Conclusions

This study adopted a novel, integrated, and holistic biomolecular approach to bone fragments from Cnoc Coig. By combining a variety of different scientific techniques and applying them to the same samples in tandem, a range of scientific data has been attained, providing a greater degree of resolution of archaeological information than perhaps has previously been obtained from the site, from very small samples. This study, importantly, highlights the research potential currently dormant within osteologically unidentifiable bone fragments from prehistoric contexts. It also shows that there is significant future potential application of the methodology used here method to other prehistoric sites with fragmentary or loose bone, such as caves and middens.

In total, fourteen new fragments of human bone dating to the British Late Mesolithic-Early Neolithic interface have been identified, increasing the number of known human bone fragments from the five Oronsay middens from 55 (Meiklejohn et al. 2005) to 73 (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 identification of additional faunal bone fragments has also allowed for suitable isotopic baselines to be created for human data from the site, and has meant that interpretations of diet at the site are more secure. The isotopic data also provides additional evidence of a high marine protein diet evident in Britain along the west coast of Scotland – but AMS dates obtained suggest that this marine diet may have extended into the 4th Millenium 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.

The data generated here therefore suggests the change in diet at the Mesolithic-Neolithic transition may be more complex than previously suggested - and that there may be an overlap between hunter-fisher-gatherer and agricultural lifeways at this early date. This is particularly pertinent given that the human remains from Cnoc Coig have previously been implicated in multiple debates about dietary change with the arrival of agriculture in Britain in the early 4th Millennium BC (e.g. Schulting and Richards 2002(a); Richards et al.
advocating a ‘rapid’ and total adoption of terrestrial domesticated diets, and the cessation of marine protein consumption. In terms of overall chronology, the newly identified fauna in this study have also allowed for new AMS dates to be undertaken on terrestrial samples without the issues of marine corrections or reservoir effects, or the possibility of dates being obtained from ‘old’ sources (e.g. as with charcoal dates potentially deriving from old wood; as discussed above). In all, the new dates obtained have allowed for a greater understanding of the chronology of the site, and the new AMS dates undertaken on the human remains shows that the data obtained from them is directly comparable to the human remains identified within the main midden structure previously.

The location of the bone fragments identified as human here however also raises interesting questions about the potential depositional locations of human remains in Mesolithic Britain and links to the discussions raised in Chapter 3. The success of the ZooMS methodology here also potentially highlights that more human remains may be present in British archaeological contexts. Perhaps in future greater consideration should be given to the perceived ‘peripheral’ areas around British Mesolithic sites – it would seem that Mesolithic peoples may not have simply just utilised middens themselves, but also the areas surrounding them too.

This study therefore perhaps highlights the research potential currently dormant within zooarchaeologically or osteologically unidentifiable bone fragments from British Mesolithic contexts. The work has shown that in some contexts, human skeletal remains are present within Mesolithic deposits already excavated, but lie as heavily fragmented and disarticulated remains. The advent of new biomolecular techniques in recent years which require small sample sizes and are high-throughput now means that we can obtain useful scientific data from these small bone fragments, which can contribute to our current understanding of Mesolithic lifeways in Britain.
Chapter 6 – Finding the Mesolithic: Using ZooMS to Identify Bone Fragments

6.1. Finding the British Mesolithic

As discussed in Chapters 2 and 3, human remains are sorely under-represented in British Mesolithic assemblages, but there is also a paucity of faunal remains from some areas of the UK however too. In all, our skeletal record for the period as a whole is lacking, and as such, our interpretations are limited. Where bone is present however, it is generally disarticulated and heavily fragmented – and due to this, is often osteologically ‘unidentifiable’. As discussed in Chapters 1 and 4, one of the key aims of this research is to utilise these fragments of bone which are morphologically indistinct and hold little osteological value. It is hoped that by using the biomolecular information entrapped within these small bone fragments, this research may be able to contribute useful information to discussions surrounding Mesolithic fauna, ecology, diet, and environments. Due to the successes of work undertaken on fragmentary, unidentifiable skeletal material from Cnoc Coig (Chapter 5), the same methodologies were also applied to skeletal material from other British Mesolithic contexts and sites. Additional fragmentary material was obtained from Cnoc Coig (Oronsay), as well as from the site of Blick Mead (Vespasian’s Camp) in Wiltshire, and three sites in the Western Isles of Scotland - Tràigh na Beirigh (Lewis), Northton (Harris), and Bágh an Teampuill (Temple Bay; Harris). It was hoped that similar information to that obtained from the initial study of fragments from Cnoc Coig may be attained, and that this taxonomic information could then be incorporated into wider discussions and may contribute to our understanding of the British Mesolithic.

In particular, it was hoped that additional human remains may be recovered from this fragmentary and ‘unidentifiable’ material. Based on our current knowledge of Mesolithic mortuary practices and deposits (as discussed in Chapter 3), the majority of skeletal material from Britain is fragmentary in nature, often taking the form of ‘loose bone’ deposits – potentially rendered unidentifiable by cultural practices. Therefore, within fragmentary skeletal assemblages, it could be hoped that some of the material may in fact be human – particularly given suggestions within the literature that human bone was often treated in a similar way to faunal bone in the Mesolithic (see Chapter 3). By using proteomics to identify the species of fragmentary bone, we may be able to go some way to addressing these theories.
6.2. Using ZooMS to Identify Prehistoric Skeletal Remains

ZooMS was applied here to five sites to try to determine the species identification of bone fragments classified as ‘unidentifiable’ following previous zooarchaeological analyses. Like many prehistoric skeletal assemblages, the sites used within this research all had a significant number of disarticulated, heavily fragmented and/or morphologically indistinct pieces of bone. These bone fragments however, whilst holding little osteological or zooarchaeological interest, do contain useful biomolecular information, as highlighted throughout this thesis. The importance of identification of archaeological bone is discussed in detail in Chapter 4 (section 4.2.1.).

Within archaeology, ZooMS has now been used widely as a qualitative analytical technique for taxonomic identification (e.g. Buckley et al. 2009; 2010; Richter et al. 2011; Sluis et al. 2014; O’Connor et al. 2015; Welker et al. 2015; Chapter 4), and as demonstrated here, the technique provides a suitable high-throughput method for the species identification of archaeological bone from prehistoric time periods. The sections below outline the application of the method to five British Mesolithic assemblages – and in doing so, highlight the research potential currently dormant within osteologically unidentifiable bone fragments from prehistoric contexts.

The five sites utilised here all date to the Mesolithic, but run right throughout the period – with Cnoc Coig and Tràigh na Beirigh dating to the Late Mesolithic, whereas Temple Bay dates to the earlier Mesolithic (7th millennium BC), for example. The rationale for the choice of sites and the sample sizes utilised here was simply governed the material available to the author however. Nonetheless, all five sites do have similarities. All are potentially settlement sites, or were occupied or utilised due to resource exploitation – although the form of the sites differs: with two being midden sites, one a ‘spring’ site, and two being open air sites. However, all are close to water – with Cnoc Coig and the three Western Isles sites all being in coastal, island locations, and Blick Mead being located next to a springhead and close by to rivers. Finally, and most importantly however, all five sites have considerable fragmentary skeletal assemblages – upon which the work outlined here is based upon. The following sections will therefore provide a brief overview and background to the sites utilised here, before presenting the results of the ZooMS analysis undertaken, and a discussion of these results. In all, the Chapter as a whole aims to provide a preliminary proof of concept study for the use of ZooMS as a high-throughput method to identify Mesolithic fragmentary bone in the UK from a range of different assemblages and
site types – and through doing this, assess the extent of information which studies of this kind may reveal.

6.3. Blick Mead, Wiltshire

The site of Blick Mead (also known as Vespasian’s Camp) has been known since the 18th century due to the presence of an Iron Age promontory hill fort and ramparts, but evidence of Mesolithic activity was only discovered in 2005. Preliminary radiocarbon dating of the site has revealed that it potentially presents the longest Mesolithic sequence of any British site, being utilised for c.3000 years – with AMS dates ranging from 7596-7542 cal. BC to 4846-4695 cal. BC (Jacques and Phillips 2014). The site sits within the ‘Stonehenge landscape’ in Amesbury, Wiltshire (Figure 32), at what would have been the largest of a complex of springheads in the area. British Mesolithic sites are often set within ‘watery’ contexts (as discussed within Chapter 2), but unusually at Blick Mead, the spring is still visible today, cut into the chalk bedrock (Hoare 2014).

Figure 32: Location of Blick Mead within the ‘Stonehenge landscape’ (Jacques and Phillips 2014, 9)

Excavations 2005 to present have revealed a significant flint assemblage representing tool use and knapping in situ (Bishop 2014); a faunal assemblage dominated by auroch (with
smaller amounts of red deer and pig) (Legge 2014); and, uniquely, the presence of a rare algae (*Hildenbrandia*) in the spring, which turns oxidised flint placed into the water a bright magenta pink once removed (Jacques 2014; Jacques and Phillips 2014). To date, no human remains have been recovered from the site. Due to the uniqueness of the site, its proximity to Stonehenge, and this long-scale Mesolithic occupation within what has always traditionally been seen as a Neolithic landscape, Blick Mead has received significant media and ‘popular press’ coverage in recent years (e.g. Jacques et al. 2013; BBC News 2013(a); 2013(b); 2014; Griffiths 2014; Horizon 2015; University of Buckingham nd.), which has increased again in 2014-2015 due to plans to construct a tunnel nearby Stonehenge, which would alter the water-table at Blick Mead dramatically (Jacques pers. comm.; Archaeology 2014; Guardian 2014; Buckingham Advertiser & Review 2014; Parry 2014; Stone 2014; Brown 2015).

The Blick Mead skeletal assemblage comprises a significant number of unidentified fragments, and therefore the ZooMS work undertaken here aimed to provide additional information on fauna from the site, and also to determine if the dominance of aurochs in the assemblage was a true archaeological depositional pattern, or instead the result of size or preservation bias, or due to sampling strategy.

6.4. Cnoc Coig, Oronsay

The site of Cnoc Coig is one of five Mesolithic shell middens on the island of Oronsay, Inner Hebrides, known to have a significant skeletal assemblage comprising a range of both faunal and human remains. A detailed overview and further information on the site can be found in Chapter 5. In terms of faunal remains at Cnoc Coig however, at least five distinct species are thought to be represented within the midden deposits (Grigson and Mellars 1987). Of these, grey seal (*Halichoerus grypus*) is the most abundant, followed by otter (*Lutra lutra*), red deer (*Cervus elaphus*), pig (*Sus scrofa*), and common seal (*Phoca vitulina*). It is also thought that nine fragments of bone may represent small cetaceans, possibly common porpoise (*Phocaena phocaena*) or common dolphin (*Delphinus delphis*) (Grigson and Mellars 1987).

Due to the success of ZooMS work undertaken on bone fragments from Cnoc Coig previously (Chapter 5), it was hoped that this additional analysis may reveal more detailed information on faunal species at the site, and potentially additional human remains.
6.5. Western Isles, Scotland

No Mesolithic presence in the Western Isles of Scotland (Outer Hebrides) was known until very recently, despite the wealth of Mesolithic archaeology known on islands in the Inner Hebrides. Here, three newly discovered Mesolithic sites from the Western Isles are presented – Northton, Tràigh na Beirigh, and Bágh an Teampaill (Figures 33 and 34). Although differing in date and site type, the three sites will be considered here together given the small sample sizes utilised here, overall similarities between the assemblages, and the fact that they currently collectively represent the first Mesolithic sites known in the Western Isles of Scotland.

In 2001, deposits at the open-air site of Northton, Harris, were dated to the 7th millennium BC. Two distinct phases of activity were identified at the site, the first dating to c.7060-6650 cal. BC, and the second to 6510-6090 cal. BC (Gregory et al. 2005) – making it the most north-westerly Mesolithic site identified in Europe to date (Bishop et al. 2011). Due to this, and the fact that the site is at risk of coastal erosion, additional excavation work was undertaken at Northton in 2010 to better characterise the deposits at the site and undertake detailed environmental (archaeobotanical and zooarchaeological) sampling (Bishop et al. 2011).

Following on from this work, a second Mesolithic Outer Hebridean site was found at Tràigh na Beirigh, Cnip, Lewis in 2010-2011, comprising an open-air shell midden, dated to the late Mesolithic (4400-4000 cal. BC) (Church et al. 2012; Blake et al. 2012(a)). In 2011, a third site was identified at Bágh an Teampaill (Temple Bay), also on Harris, and was dated to 5715-5368 cal. BC. The site was identified due to coastal erosion and the deposits uncovered were similar in character to those identified at Northton, representing an old ground surface and associated midden deposits (Church et al. 2011; Blake et al. 2012(b)). The deposits excavated contained a range of faunal remains, shells, hazelnuts, charcoal, a small number of flint and quartz worked lithics, and a red deer antler tine (Church et al. 2011; 2012).
Figure 33: Site location of Bágh an Teampuill (Temple Bay) and Northton, on the Isle of Harris (Blake et al. 2012(b))
Tràigh na Beirigh (TNB), being a shell midden, has slightly different deposits to the other two sites. The assemblage is dominated by molluscs and fish bones, but other faunal species such as hare are also represented (Blake et al. 2012(a)). The deposits at Northton and Temple Bay however differed to this somewhat, and both showed evidence of bioturbation. The faunal assemblage at Temple Bay however contained both fish and hare bones (Blake et al. 2012(b)), and at Northton shell fragments and fish bones were recovered (Bishop et al. 2011). Unfortunately, zooarchaeological reports or lists of fauna present were not available at the time of writing however. It is also important to note that
whilst Tràigh na Beirigh is a terminal Mesolithic open-air shell midden, and would have been a coastal site whilst in use, both Northton and Temple Bay would have been inland sites during the Mesolithic, in a woodland setting, potentially up to 50-100km away from the coast (despite being coastal today, due to sea level change) (M Church, pers. comm.).

Due to these three sites we now know that the Western Isles were occupied during the Mesolithic, and utilised at least 7051-4000 cal. BC (Piper 2014). However, very little else is known about the Mesolithic occupation of the Outer Hebrides. The analysis undertaken here therefore aimed to try to provide additional information on ecology, environment, and diet – and to also assess the viability of applying the ZooMS method to small, poorly preserved samples from these recently discovered sites.

6.6. Material Sampled and Methodology

6.6.1. Blick Mead, Vespasian’s Camp

Twenty bone fragments classified as ‘unidentifiable’ following zooarchaeological analysis were chosen for ZooMS analysis (Figure 35). The fragments were all small in size and morphologically indistinct. Unfortunately, many of the fragments were also visibly poorly preserved, and often very chalky in nature, due to the depositional contexts from which they were recovered, at the spring edge. The material analysed all derived from trench 19, but came from a range of different contexts within the trench. This was undertaken in order to assess if differential collagen preservation was evident between archaeological contexts.

Figure 35: Examples of bone fragments used in this study. (Left = sample 1004; right = sample 1008)

A novel ZooMS methodology was used on the twenty bone fragments, which is outlined in Appendix A (A.2.1.). The methodology applied was done so in the hope of combining both
ZooMS and isotopic analysis, in order to obtain greatest amount of biomolecular information from these samples as possible.

6.6.2. Cnoc Coig, Oronsay

Seventeen bone fragments classified as ‘unidentifiable’ were analysed using ZooMS, all of which were small in size (<200mg). Due to previous work undertaken on material from Cnoc Coig (see Chapter 5), a previously published acid ZooMS protocol (Welker et al. 2015) was utilised on these fragments (Appendix A, A.2.2.)

6.6.3. Western Isles

Seventeen bone fragments classified as ‘unidentifiable’ were utilised for ZooMS analysis – thirteen of these from Bágh an Teampuill (Temple Bay), three from Tràigh na Beirigh, and one from Northton. The fragments from all three sites however were small in size and morphologically indistinct. Due to the age of the samples and their depositional context, and following discussions with the excavators, it was decided to undertake an acid ZooMS protocol (as outlined in Welker et al. 2015; Appendix A, A.2.3.), in order to increase the likelihood of obtaining useful biomolecular data which would facilitate identification.

6.7. Species Identified

6.7.1. Blick Mead

From the ZooMS results (Table 11) it can be seen that the most widely identified species at Blick Mead was *Bos*. ZooMS analysis cannot distinguish between the wild and domestic forms of a species, but given the Mesolithic date of the material and the zooarchaeological information available, the identification of a wide number of fragments to *Bos* indicates that these samples are all auroch (*Bos primigenius*), rather than domesticated cow (*Bos taurus*). The identification of the majority of fragments studied here as auroch is therefore in line with initial zooarchaeological analysis undertaken on the skeletal material, which revealed 61% of the assemblage was auroch (Legge 2014) – making it the most well-represented species at the site. More recent zooarchaeological analysis also supports this interpretation of auroch as the dominant faunal species at Blick Mead (Rogers 2015; Rowley-Conwy pers. comm.). However, such a high proportion of auroch remains at a
Mesolithic site is somewhat unusual, and raises interesting questions regarding the hunting strategies and resource exploitation of Mesolithic peoples utilising the site and the surrounding landscape. Due to this high proportion of auroch remains, it has been suggested that the site may represent a specialised auroch hunting camp, with the animals being driven into the Blick Mead basin to facilitate easier hunting (Jacques 2014). However, smaller numbers of auroch are also known across other British Mesolithic sites – for example at Faraday Road (6.7% of faunal assemblage is auroch), Thatcham III (14.3% auroch), and Star Carr (16% auroch) (Overton 2015) – and therefore Mesolithic peoples clearly had the technology and knowledge via which to hunt and exploit these huge animals. Interestingly, however, Overton (2015) has noted that auroch tend to be more prevalent in northern British sites, particularly in the early Mesolithic, but are scarce/absent in the Kennet Valley and Colne Valley sites. The identification of such large numbers of aurochs at Blick Mead, a southern British Mesolithic site close to the River Kennet, is therefore interesting.

The high proportion of auroch at Blick Mead also raises interesting questions regarding the potential resource use of such large animals. Given the apparent ubiquity of auroch within early prehistoric faunal assemblages across Europe however, within the literature there is surprisingly little discussion of how populations may have actually utilised the carcasses of such large ungulates once hunted. Instead, much work has focused on the genetics of *Bos primigenius* (e.g. Edwards et al. 2007; Achilli et al. 2008; Scheu et al. 2008; Schibler et al. 2014) and biometric analyses (e.g. Grigson 1969; Rowley-Conwy 1995; Wright and Viner-Daniels 2015). However, previously, the lower proportion of auroch remains at European Mesolithic sites when compared to other faunal species has been attributed to the higher meat weight attained from these animals (Prummel and Niekus 2011). In this respect, the predominance of auroch at Blick Mead is all the more puzzling – but the presence of percussion fractures and cut-marks on a number of the auroch bones recovered from the site (Legge 2014; Rogers 2015) does suggest that they were being utilised for meat and marrow extraction.

One sample was also identified as elk/red deer via ZooMS. Again, from zooarchaeological assessment, 27% of the Blick Mead assemblage was identified to be red deer (*Cervus elaphus*) (Legge 2014), and so the identification of this bone fragment as elk/red deer is again unsurprising. However, it is interesting to note the much smaller representation of red deer remains at the site when compared to auroch (*Bos primigenius*). At many
Mesolithic assemblages red deer dominate instead (e.g. Oronsay (Grigson and Mellars 1987); Star Carr (Clark 1954); Three Ways Wharf (Grant et al. 2014)).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Context</th>
<th>Trench Number</th>
<th>ZooMS ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1001</td>
<td>76</td>
<td>19</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>1002</td>
<td>62</td>
<td>19</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>1003</td>
<td>62</td>
<td>19</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>1004</td>
<td>66</td>
<td>19</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>1005</td>
<td>66</td>
<td>19</td>
<td>Elk/red deer</td>
</tr>
<tr>
<td>1006</td>
<td>37</td>
<td>19</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>1007</td>
<td>76</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1008</td>
<td>76</td>
<td>19</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>1009</td>
<td>62</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1010</td>
<td>62</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1011</td>
<td>75</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1012</td>
<td>75</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1013</td>
<td>67</td>
<td>19</td>
<td>Wild boar/pig</td>
</tr>
<tr>
<td>1014</td>
<td>66</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1015</td>
<td>61</td>
<td>19</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>1016</td>
<td>63</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1017</td>
<td>63</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1018</td>
<td>67</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
<tr>
<td>1019</td>
<td>67</td>
<td>19</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>1020</td>
<td>67</td>
<td>19</td>
<td><em>Bos</em> (Auroch)</td>
</tr>
</tbody>
</table>

Table 1: ZooMS identifications of samples from Blick Mead

Finally, one bone sample was also identified as wild boar/pig (*Sus scrofa*). As mentioned above in relation to *Bos*, ZooMS cannot distinguish between the wild and domestic forms of a species, as the collagen sequences are not sufficiently different. As such, it is not possible to say whether the sample identified here is wild boar or domesticated pig – but again given the Mesolithic context in which the remains were found, it is likely that the sample is wild boar. It is widely noted however that is problematic to distinguish between wild and domesticated pigs zooarchaeologically too, and can generally only be broadly determined through size estimation of the animal and morphometrics (O’Connor 2000, 117; Matsui et al. 2005; Rowley-Conwy and Dobney 2007; Dobney et al. 2007; Rowley-Conwy et al. 2012; as also discussed in Chapter 5). Wild boar remains are common on many Mesolithic sites however (e.g. Faraday Road; Former Sanderson Site; Thatcham III (Overton 2015)). From zooarchaeological analysis, 8% of the faunal assemblage at Blick Mead was identified as *Sus scrofa* (Legge 2014), although five fragments of bone within this were interpreted to be domesticated pig, rather than wild boar (Rogers 2015).
As seen in Table 1, some bone samples could sadly only be identified to genus level – ZooMS indicates that they are terrestrial herbivores, but species level identification was not possible due to poor quality spectra. Additionally, some samples were ‘unidentifiable’ using ZooMS as the spectra generated were not distinct enough to allow species/genus identification – i.e. not enough species specific peptides were present. This is due to poor collagen preservation in the samples. The collagen preservation across the whole Blick Mead assemblage is discussed in more detail below in section 5.9.

Nonetheless, the ZooMS results obtained from Blick Mead support the zooarchaeological analysis already undertaken, and crucially, also highlight that auroch is truly the dominant species present at the site. Previously, there were concerns that the prevalence of auroch may not be a true reflection of the real species distribution, but rather a false picture caused by taphonomic processes and the bias created by the large size of auroch bone (Jacques, pers. comm.). The ZooMS results indicate however that the predominance of auroch is truly a distinct feature of the faunal assemblage at Blick Mead – and highlight the potential of the ZooMS method for cross-referencing or underpinning zooarchaeological interpretations from fragmentary prehistoric skeletal assemblages.

6.7.2. Cnoc Coig, Oronsay

It can be seen that the most commonly identified species from the bone fragment assemblage analysed from Cnoc Coig using ZooMS was wild boar/pig (Sus scrofa) (Table 12). Previous biometric analysis of the Sus remains from the site has suggested that they are likely to be wild boar, as opposed to domesticated pig (Grigson and Mellars 1987). Furthermore, original zooarchaeological analysis of the Cnoc Coig faunal assemblage suggested that Sus scrofa was the fourth most commonly represented species at the site, comprising 9% of the faunal assemblage (Grigson and Mellars 1987). The presence of pigs on Oronsay is a topic of interest in itself however, as the island itself is not believed to be large enough to support a population of pigs. It has therefore been suggested that pigs were purposively bought to Oronsay by Mesolithic populations from the mainland or larger surrounding islands (Grigson and Mellars 1987; Rowley-Conwy pers. comm.; see also Chapter 5). Sus has also been previously identified from bone fragments from Cnoc Coig using ZooMS (Chapter 5).
<table>
<thead>
<tr>
<th>Sample Number</th>
<th>ZooMS ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC01</td>
<td>Elk/red deer</td>
</tr>
<tr>
<td>CC02</td>
<td>Elk/red deer</td>
</tr>
<tr>
<td>CC03</td>
<td>Wild boar/pig</td>
</tr>
<tr>
<td>CC04</td>
<td>Cetacea</td>
</tr>
<tr>
<td>CC05</td>
<td>Seal</td>
</tr>
<tr>
<td>CC06</td>
<td>Terrestrial herbivore (?elk/red deer)</td>
</tr>
<tr>
<td>CC07</td>
<td>Seal</td>
</tr>
<tr>
<td>CC08</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>CC09</td>
<td>Terrestrial herbivore (?elk/red deer)</td>
</tr>
<tr>
<td>CC10</td>
<td>Wild boar/pig</td>
</tr>
<tr>
<td>CC11</td>
<td>Terrestrial herbivore</td>
</tr>
<tr>
<td>CC12</td>
<td>Terrestrial herbivore (?elk/red deer)</td>
</tr>
<tr>
<td>CC13</td>
<td>Wild boar/pig</td>
</tr>
<tr>
<td>CC14</td>
<td>Wild boar/pig</td>
</tr>
<tr>
<td>CC15</td>
<td>Terrestrial herbivore (?elk/red deer)</td>
</tr>
<tr>
<td>CC16</td>
<td>Terrestrial herbivore (?elk/red deer)</td>
</tr>
<tr>
<td>CC17</td>
<td>Seal</td>
</tr>
</tbody>
</table>

Table 12: ZooMS identifications of samples from Cnoc Coig

Two fragments of bone were also identified as red deer/elk using ZooMS, and a further five fragments were identified only as terrestrial herbivore, but potentially also represent red deer/elk. Unfortunately, these additional five fragments contained some species specific peptide markers, but not enough to securely identify them as red deer/elk. However, given that 11.3% of the faunal assemblage at Cnoc Coig was identified to be red deer (*Cervus elaphus*) (Grigson and Mellars 1987), this identification does not seem improbable. Red deer zooarchaeologically comprise the most frequently represented terrestrial mammal within the Oronsay assemblage – and interestingly, are also postulated to have been purposively bought to the island by visiting populations, like pigs (see Chapter 5; Grigson and Mellars 1987).

Three bone fragments from Cnoc Coig were also identified as seal, although unfortunately collagen preservation would not allow for this identification to be narrowed down to species (i.e. ribbon/spotted/ringed/grey/common/harp). Zooarchaeological analyses have revealed both grey (*Halichoerus grypus*) and common (*Phoca vitulina*) seal within the assemblage, however grey seal dominates the faunal assemblage (58%), whereas common seal comprises only 0.5% of the entire faunal assemblage (Grigson and Mellars 1987). Seal species are known to be difficult to differentiate between osteologically however, as morphologically they share many characteristics (Storå 2000). This problem is further compounded by the fact that seals are not widely studied within zooarchaeology, due to their infrequent inclusion within archaeological faunal assemblages. Nonetheless, seals are the most dominant mammalian species within the Cnoc Coig assemblage. Due to the
presence of more young pups and breeding age adults within the seal assemblage, it was suggested that seal exploitation was likely to have been undertaken in early autumn (September/October) by Mesolithic populations on the island (Grigson and Mellars 1987). This would have resulted in seals being hunted on dry-land, focusing on breeding colonies, rather than harpooning from boats within the water. However, we still know very little about how Mesolithic peoples may have hunted, exploited, and utilised seals (Milner 2009), but they are known to provide many products which are easily transportable and desirable. Ethnographically, whilst seals are known to provide useful food and energy sources (via meat and blubber), seal carcasses are also utilised for oil and skins. In ethnographic accounts of seal hunters from Greenland and Canada, no part of the animal is wasted, but precise rules govern the sharing and distribution of seal meat (Nuttall et al. 2005). Seal bones have also been utilised in prehistory for the production of bone implements; such as harpoons, fish hooks and beads (Lyman 1992), and also potentially as a fuel source (Vaneekhout et al. 2013). On Orkney, inhabitants frequently paid their rents in seal oil into the 20th century (Sherratt 1999), and historically, seal skins have even been used in the production of ropes for ships (Clark 1947). The presence of seals at Cnoc Coig has also previously been linked to human-animal relationships, and suggestions that human and seal element representations are very similar. Similar treatment of human and seal bones was noted by Nolan (1986; cited in Conneller 2009), where it was suggested that a cluster of human hand bones was deposited on top of a seal flipper. This has therefore been tied into discussions surrounding Mesolithic perceptions of animals, and of animal agency (Conneller 2009).

Finally, one bone was identified as a cetacean, but unfortunately could not be identified using ZooMS to either dolphin, orca or porpoise. Zooarchaeological analyses also identified cetacean, but similarly could not attribute these to species. However, Grigson and Mellars (1987) suggested that the small size of the fragments present indicated they may be common porpoise (*Phocaena phocaena*) or common dolphin (*Delphinus delphis*). These species were however only represented by nine bones, comprising 1.4% of the total faunal assemblage (Grigson and Mellars 1987). What is unclear however is whether these animals were actively hunted (perhaps from boats?) or opportunistically exploited, perhaps when beached on the island or at shallow water at the coastline.
6.7.3. Western Isles

From Bágh an Teampaill (Temple Bay), the most commonly identified species from the ZooMS analysis was whale (Table 13). Whilst this is notable in itself, as whale bones are not commonly found within British Mesolithic sites, what is interesting is that a number of different whale species are present within this assemblage. Whale bone is known at a number of Mesolithic sites in the Inner Hebrides (e.g. Priory Midden and Caisteal nan Gillean I (Grigson and Mellars 1987)), but is not attributed to a specific whale species, and is only present in very small quantities (<5 bones). In the Bágh an Teampaill assemblage, one sample was identified as a grey whale, two samples as either grey or humpback whale, and one sample as a Risso’s or pilot whale using ZooMS. Clark (1947) provides a comprehensive overview of different whale species identified and utilised historically on the Atlantic seaboard of Europe. It should however also be noted that whales were detected within three different contexts analysed from Bágh an Teampaill, including the oldest ground surface from the site (C.3), a mixed deposit of old ground surface and midden (C.5), and the fill of a pit (C.7) – although all are roughly of the same date (Blake et al. 2012(b)).

<table>
<thead>
<tr>
<th>Site Code</th>
<th>Context Number</th>
<th>Sample Number</th>
<th>ZooMS ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB11</td>
<td>C.1</td>
<td>S.1A</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TB11</td>
<td>C.1</td>
<td>S.1B</td>
<td>Deer</td>
</tr>
<tr>
<td>TB11</td>
<td>C.1</td>
<td>S.1C</td>
<td>Deer</td>
</tr>
<tr>
<td>TB11</td>
<td>C.1A</td>
<td>N/A</td>
<td>Seal</td>
</tr>
<tr>
<td>TB11</td>
<td>C.3</td>
<td>S.5A</td>
<td>Humpback/Grey whale</td>
</tr>
<tr>
<td>TB11</td>
<td>C.3</td>
<td>S.5B</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TB11</td>
<td>C.3</td>
<td>S.5C</td>
<td>Humpback/Grey whale</td>
</tr>
<tr>
<td>TB11</td>
<td>C.5</td>
<td>S.2A</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TB11</td>
<td>C.5</td>
<td>S.2B</td>
<td>Porpoise/Orca</td>
</tr>
<tr>
<td>TB11</td>
<td>C.5</td>
<td>S.2C</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TB11</td>
<td>C.5</td>
<td>S.2D</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TB11</td>
<td>C.7</td>
<td>S.3A</td>
<td>Grey whale</td>
</tr>
<tr>
<td>TB11</td>
<td>C.7</td>
<td>S.3B</td>
<td>Risso’s/Pilot whale</td>
</tr>
<tr>
<td>NT10</td>
<td>C.14A</td>
<td>S.20/S.17</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>NT10</td>
<td>C.14B</td>
<td>S.20/S.17</td>
<td>Porpoise/Orca</td>
</tr>
<tr>
<td>NT10</td>
<td>C.14C</td>
<td>S.20/S.17</td>
<td>Unidentifiable</td>
</tr>
<tr>
<td>TNB11</td>
<td>C.8</td>
<td>S.6A</td>
<td>Hare</td>
</tr>
</tbody>
</table>

Table 13: ZooMS identifications of samples from Western Isles sites (TB11 = Bágh an Teampaill (Temple Bay); NT10 = Northton; TNB11 = Tràigh na Beirigh)

Two other additional samples from Bágh an Teampaill were also identified as marine mammals using ZooMS – one as a seal, and one as porpoise/orca. One sample from Northton was also identified as porpoise/orca. Unfortunately, much like the Cnoc Coig
samples, the seal fragment from Bágh an Teampuill could not be assigned to a specific seal, and it should also be noted that the context from which it was recovered (C.1) is listed as a ‘cleaning context’ (Blake et al. 2012(b)), and therefore may not be Mesolithic. Whilst the presence of seals at the site is however perhaps unsurprising (see discussion above in section 5.8.2), the identification of two fragments to porpoise (*Phocaena phocaena*) or orca (*Orcinus orca*) is unusual. However, porpoise are known to be recovered from a significant number of Northern European Mesolithic sites (Price 1991), and both species are known to currently frequent waters surrounding the UK – predominately surrounding the north of England, Orkney, Shetland and down to the Isle of Man. Orca (*Orcinus orca*) are also known to occasionally partially beach themselves to catch seals, whereas porpoises are more shy and travel in small groups or alone. Both species are known to be found in shallow water and coastal areas however (ORCA 2015(a); 2015(b)). However, historically, porpoise (*Phocaena phocaena*) have also been known to become beached on British coastlines (Clark 1947).

Whale, porpoise, and dolphin bone has also been found at other coastal Mesolithic European sites, such as Tybrind Vig in Denmark (Trolle 2013), Dyrhølm, Jutland, in Denmark, Tévic in France, and Curran Point in Ireland (Clark 1947). It was also suggested that cetacean bone recovered from the Oronsay middens (Inner Hebrides) was likely to represent common porpoise (*Phocaena phocaena*) or common dolphin (*Delphinus delphis*) (Grigson and Mellers 1987). It is therefore clear that Mesolithic populations across Europe were utilising and procuring a range of marine mammals of different sizes. Again however, as discussed above (section 5.8.2), what is unclear is whether these large marine mammals were being intentionally and actively hunted and exploited, or whether they were opportunistically utilised when beached or similar on coastlines. Nonetheless, much like seals (as discussed above in section 5.8.2), cetaceans can also provide a range of resources beyond meat. For example, baleen has been known to be utilised for the manufacture of artefacts and in place of wood; whale bone has been used as fuel throughout prehistory; skins can be used for leather; and blubber can be used for oil and as a source of energy (Clark 1947). Blubber from marine mammals was also used in the Late Mesolithic (*Ertebølle*) in the Baltic in ‘blubber lamps’ – which were used as light sources and potentially also involved in night fishing (Heron et al. 2013). It is also interesting to note that at Northton, the context from which the porpoise/orca fragments derives is dated to the Mesolithic-Neolithic interface, and the deposit is thought to represent a buried land surface containing hearth deposits, fuel remnants and food waste (Bishop et al. 2011).
Additionally, two bones from Bágh an Teampuill (Temple Bay) were identified as deer using ZooMS. Unfortunately, the peptide markers present in these two samples were not specific enough to determine which species of deer these samples were (i.e. red/roe/reindeer). Deer are a common component of many British Mesolithic assemblages, as discussed elsewhere, but there has previously been much debate as to whether red deer would have been present in the Western Isles during the Mesolithic (Mike Church and Peter Rowley-Conwy, pers. comm.). Unfortunately however, the identification of these fragments as deer here cannot contribute to this debate as they are listed as deriving from a ‘cleaning context’ (Blake et al. 2012(b)), and therefore may not be Mesolithic. However, a red deer antler tine has previously been recovered from another context (C.5) at the site (Church et al. 2012; Blake et al. 2012(b)).

Finally, the one sample analysed from Tràigh na Beirigh was identified as hare (*Lepus europaeus*) using ZooMS. Whilst hare is known at a number of British Mesolithic sites (e.g. Star Carr (Milner 2009), it will be important to securely date either the sample itself, or determine that the context as a whole is undisturbed in order to not discount this sample as a modern inclusion. This is especially important given that the site has been subjected to large amount of coastal erosion (Blake et al. 2012(a); Bishop et al. 2013). The context from which this bone fragment derives however is within the main body of the midden structure, which lies on top of an old ground surface, and which has been dated to 4400-4000 cal. BC (Blake et al. 2012(a)). As such, if unbioturbated, it may reflect the presence of Mesolithic hare on the island.

Unfortunately, the remaining five samples from Bágh an Teampuill and two from Northton were unable to be identified to species or genus (Table 3), due to a lack of species specific peptide markers. This is perhaps unsurprising however, given the depositional context and age of the samples. The overall collagen preservation at the Western Isles sites is however discussed in more detail below in section 5.9.

**6.8. Collagen preservation**

As discussed in Chapter 4 (section 4.2.3.), collagen has been preferentially chosen now in most recent bone protein sequencing studies as it is more readily isolated and detected within archaeological remains, and is known to persist for significant periods of time within the archaeological record (Holmes et al. 2005; Collins et al. 2010). However,
although in temperate latitudes collagen in bone can persist from material dating to the Pleistocene (Hofreiter et al. 2012), the depositional context of skeletal assemblages is paramount to ensuring good collagen preservation, regardless of age of the bones in question.

Unfortunately, the collagen preservation in the samples analysed from Blick Mead was found to be poor. Collagen yields of less than 2% were obtained from all samples (from retentate only; following ultrafiltration), and in a number of samples, the collagen yields were less than 0.5% (see Appendix A; section A.2). The poor collagen preservation in the bone from Blick Mead also meant that not all samples analysed in this study were able to be assigned to genus or species level identification (as discussed above) – and additionally, could not be utilised for isotopic analysis as was originally hoped. This poor collagen preservation is likely to be due to the depositional context in which the skeletal remains were found. The site is located at a springhead, and excavations have shown that the Mesolithic material from the site lies within deposits indicative of slow-moving water, which are also believed to be alkaline (Jacques and Phillips 2014). Vespasian’s Camp is also underlain by chalk foundations (Hoare 2014). The Mesolithic deposits within trench 19, where the bone samples in this study were discovered, was also noted during excavation to be within an area of the site with a high water table currently (Jacques and Phillips 2014). Additionally, water appears periodically in the basin of the springhead itself – and thus water levels at the site are not constant throughout the year (Hoare 2014). This kind of water movement is extremely detrimental to bone collagen preservation, and the taphonomic actions of water can significantly decrease the amounts of collagen preserved, as the movement of water can effectively ‘flush’ the collagen out of the bone. Site hydrology, particularly on sites where water movement fluctuates, has been noted to be one of the largest factors affecting bone diagenesis and collagen preservation (Neil森-Marsh and Hedges 2000). O’Connor (2000, 23) notes that collagen preservation is likely to be poorest in “moist, slightly alkaline burial environments, and bones from chalk or limestone soils” – sadly the exact conditions present at Blick Mead.

Indeed, although radiocarbon dates have been obtained from some skeletal remains from the Blick Mead site (Jacques and Phillips 2014), other faunal remains provided insufficient collagen yields for AMS dates (David Jacques, pers. comm.). Sadly, the depositional context of the bones at Blick Mead, combined with the taphonomic processes bones have been subjected to at the site, has meant that collagen preservation overall is poor. However,
there is variability in both the water table levels and the depositional matrixes across the site – therefore meaning that collagen preservation may be better in other areas of the site.

Collagen preservation in the samples analysed here from Cnoc Coig and the Western Isles sites is slightly harder to determine, given that a full collagen extraction was not undertaken (unlike for the Blick Mead samples). However, the samples from all sites are at least 6,000 years old, and have similar depositional contexts – being predominately from midden sites off the west coast of Scotland. Overall collagen preservation in bone fragments from Cnoc Coig is discussed in greater detail in Chapter 5. Across the three Western Isles sites, collagen preservation appears to be somewhat variable. A number of samples from the three assemblages were not visibly well preserved – they visibly appeared very crumbly – which can sometimes also be indicative of poor collagen preservation. In particular, bone preservation at Northton seems to have been very poor, presumably not helped by the heavy levels of bioturbation present at the site. Indeed, previous attempts to get AMS dates from bone from Northton were unsuccessful due to poor collagen preservation (M Church, pers. comm.). This indicates why ZooMS analysis was unsuccessful in 2 of 3 bone fragments analysed here. Assessment of collagen preservation at Tràigh na Beirigh is not possible here, given that only one bone fragment was analysed using ZooMS. Finally, collagen preservation in bone fragments from Temple Bay appears to be better than that seen at Northton, with 8 of 13 fragments identifiable. However, in all samples, species level determinations were difficult to determine. Therefore, in order to fully assess collagen preservation levels at the three Western Isles sites, a much larger scale analysis would need to be undertaken in future.

6.9. Conclusions

This work has shown the initial application of the ZooMS methodology to bone fragments previously considered ‘unidentifiable’ from five Mesolithic sites in the UK – Blick Mead (Vespasian’s Camp), Cnoc Coig (Oronsay, Inner Hebrides), and three sites in the Western Isles (Outer Hebrides): Bágh an Teampaill (Temple Bay), Northton, and Tràigh na Beirigh. The findings from this analysis align well with zooarchaeological analyses of the material from all the sites, and of our current knowledge of Mesolithic fauna (Grigson and Mellars 1987; Legge 2014). However, the poor collagen preservation within some of the samples from the sites (particularly those from Blick Mead) has limited the degree of findings somewhat. Nonetheless, determination of the degree of collagen survival within bone from
the assemblages is a useful addition to our growing body of knowledge on the sites and of Mesolithic skeletal material from the UK as a whole. The determination of collagen survival within the assemblages is also useful for any future biomolecular analyses undertaken on skeletal material from the sites. For example, at all sites presented here (but particularly Blick Mead and Northton), it highlights the need to perhaps choose larger pieces of more visibly well-preserved bone for any future AMS dating or isotopic analysis wishing to be undertaken, in order to ensure adequate volumes of collagen.

Nonetheless, despite the recognition of the poor preservation of the bone at some of the sites analysed here (Blick Mead and Northton in particular), following this initial ZooMS analysis, there are a range of other avenues for future research now apparent. Firstly, additional analyses of more ‘unidentifiable’ bone could be undertaken following further zooarchaeological analysis, and as more skeletal material is recovered in future excavations at Blick Mead and the Western Isles sites. It would also be interesting at Blick Mead to analyse bone fragments from different contexts and/or different areas across the site – which may also reveal if there are any differences in collagen preservation within bone across the site. At Western Isles sites, there is now a significant amount more bone available, due to additional excavation work and the processing of previously recovered samples since the work for this thesis was undertaken. As such, there is huge potential for further work on these assemblages in future.

On well-preserved bone samples there is also the possibility of undertaking isotopic analyses of fauna from all five sites – such as $\delta^{13}$C and $\delta^{15}$N isotopic analyses to better understand the Mesolithic ecology of the sites for example. It may also be interesting to undertake other stable isotope analyses, such as $^{86}$Sr/$^{87}$Sr on the teeth from the faunal assemblages to gain information on origins and mobility (perhaps similarly to that undertaken in Craig et al. 2006; also see discussion in Chapter 4, section 4.4.5.). This would be particularly interesting to undertake on the auroch remains from Blick Mead, and the deer and pigs from Cnoc Coig, to help to determine if they are all locally sourced, or where the animals have moved from.

The detection of a range of different marine mammals at a number of the sites studied is also particularly interesting, and raises questions surrounding how Mesolithic populations both utilised these resources, and how they were obtained. As discussed above, many marine mammals can provide a wealth of different products beyond simply meat for
consumption. However, it is currently unclear whether these marine resources were actively fished/hunted or instead opportunistically scavenged. Active hunting, particularly of porpoise and whales, would require high levels of marine knowledge and technology – which these Mesolithic populations off the west coast of Scotland may have had, as the sites were only occupied periodically or seasonally through the year. At present however, there is still no evidence of boats or dug-out canoes in the UK – but examples have been found on the continent, for example at Tybrind Vig (Andersen 1985) and Møllegabet II (Grøn and Skaarup 1991) in Denmark, and Pesse and Hardinxveld-De Bruin, both in the Netherlands (Louwe Kooijmans and Verhart 2007). Paddles are also known at a range of European Mesolithic sites, such as Zamostje 2, Russia (Lozovski et al. 2014), Hardinxveld-Polderweg, Netherlands (Louwe Kooijmans and Verhart 2007), and Tybrind Vig, Denmark (Andersen 1985). Our knowledge of Mesolithic water/boating technologies in the UK however is still very underdeveloped.

Importantly, however, in conclusion, this research has utilised fragments of bone which traditionally would have remained unstudied and were considered osteologically uninteresting and of little value. Despite no additional human remains being identified within the analyses undertaken here, by unlocking the biomolecular information entrapped within these remains, this work has highlighted the importance of these bone fragments, and revealed the information they can provide – which can then be incorporated into broader debates and discussions of the period. The application of this technique to other ‘unidentifiable’ bone fragments from British Mesolithic assemblages could therefore be potentially transformative in allowing us to obtain useful information on Mesolithic ecologies and the types of faunal species which Mesolithic populations were targeting and utilising. This is particularly pertinent given that the British Mesolithic is a period which has long suffered from a lack of skeletal material – something which has traditionally hampered and limited our understandings of diet, ecology and environments. The method applied here can therefore be seen to be a useful additional tool to be utilised in future analyses of Mesolithic skeletal assemblages, alongside traditional zooarchaeological assessments.
Chapter 7 – Banbury Lane, Northampton: A Large Scale Multi-Isotopic Study on an Unusual Neolithic Assemblage

7.1. Introduction

This chapter comprises a large scale, multi-isotopic study of a Neolithic skeletal assemblage recently recovered from the site of Banbury Lane, Northampton (Figures 36 and 37). Stable isotopic analysis of $\delta^{13}$C and $\delta^{15}$N was undertaken on 165 discrete individuals, in an attempt to determine diet of individuals from the site, and the degree of dietary variability visible across such a large sample size. Additionally, strontium ($^{87}$Sr/$^{86}$Sr) isotope analysis was undertaken on 26 teeth from Banbury Lane to try to determine the origins and potential mobility of individuals from the site – something which was deemed to be of particular interest given that the assemblage appears to represent a singular depositional event. This study is therefore one of the largest isotopic analyses of British prehistoric human remains to date.

It was hoped that the combined multi-isotopic analysis of this disarticulated and unusual mortuary deposit may provide new or additional insight into the lifeways of populations from this period. In particular, the research aimed to contribute to discussions on the characterisation of diets within the Neolithic period, and the potential sources of foodstuffs populations were exploiting throughout the Neolithic – something often less considered academically, with focus instead often being on diet in the period surrounding the Mesolithic-Neolithic transition (as discussed in Chapter 2). Alongside this, it was hoped that strontium isotopic data could be tied into this dietary information – although the two sources of isotopic information cannot be linked to the same individuals due to the disarticulated nature of the deposit, on a broad level, it could be hypothesised that if differential diets were detected, that these may be the result of differential origins or mobility. Overall therefore, this research aimed to better characterise lifeways in the Middle Neolithic of Britain, and to utilise these findings to feed into larger discussions and debates surrounding the period, and how people may have lived and subsisted within it.

The following sections provide a brief overview of the site and its human remains, and place it within a regional context. The results of $\delta^{13}$C and $\delta^{15}$N isotopic analysis, as well as
*87Sr/86Sr* isotope analysis, will also be presented – in the hope that these may provide an insight into British Neolithic diet and mobility.

### 7.2. The Banbury Lane Site

The site at Banbury Lane, Northampton, comprises a triple-ditched monument dating to the middle Neolithic (Holmes 2012; Figure 38). The site was excavated in 2011 by Northamptonshire Archaeology in advance of the construction of residential housing on the land (Figures 36 and 37). In addition to the Neolithic monument, excavation also revealed a number of outlying pit features, believed to date from the Neolithic through to the Anglo-Saxon period, and a range of post-medieval field boundaries. An Early Bronze Age satellite inhumation was also discovered to the south of the monument (Holmes 2012).

![Figure 36: Site location map, indicating Northamptonshire within its East Midlands setting (Holmes 2012)](image)
The site is however unusual due to the presence of a large pit feature discovered within the monument, 2.1m x 1.4m wide, and 0.4m deep. The location of this pit suggests that it would have blocked the entrance to the inner ring ditch of the monument, which encloses 7.7-7.8m, and as such, the pit is not thought to be a primary feature (Chapman 2015; Figure 38). The pit has been dated to c.3370-3100 cal. BC (Table 14), and was found to contain the remains of at least 165 disarticulated individuals. At present, it is the only deposit of its kind known within the British Neolithic.
Radiocarbon dates have however revealed a complex chronology for the site (Table 14). As noted above, AMS dates from both human and animal bone from the pit (context 192)
have yielded dates of c.3370-3100 cal. BC; however, dating the cut of the pit has proved more problematic. Similarly, dating of the construction of the monument as a whole has also proven difficult given the lack of dateable material recovered from the ditch fills. Two pieces of red deer antler from the outer ditch have however been dated to 3020-2890 cal. BC, therefore making them later than the human remains within the pit in the centre of the monument. An additional burial in the outer ditch of the monument (burial 2, seen above in figure 38) has been dated to 3340-3020 cal. BC, meaning it is contemporary with the human remains in the pit feature. At present therefore, the chronology of the construction and use of the monument, and the deposition of the human remains within the pit, unfortunately still remains unclear.

<table>
<thead>
<tr>
<th>Laboratory &amp; Sample No.</th>
<th>Context</th>
<th>Sample details</th>
<th>$\delta^{13}$C ($%$)</th>
<th>$\delta^{15}$N ($%$)</th>
<th>Radiocarbon Age BP</th>
<th>Cal. BC Intercept</th>
<th>68% confidence</th>
<th>95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-410602 NBL11/191L10</td>
<td>Pit 192 Layer 10</td>
<td>Pig bone No 815 (17g)</td>
<td>-21.0</td>
<td>+7.0</td>
<td>4560+/-30</td>
<td>3350</td>
<td>3360-3340</td>
<td>3200-3195</td>
</tr>
<tr>
<td>Beta-410601 NBL11/191L01</td>
<td>Pit 192 Layer 1</td>
<td>Cattle bone (12g)</td>
<td>-22.5</td>
<td>+6.1</td>
<td>4550+/-30</td>
<td>3350</td>
<td>3360-3335/3210-3190/3150-3140</td>
<td>3365-3320/3235-3170/3160-3115</td>
</tr>
</tbody>
</table>
| Beta-308614 NBL/19201 | Pit 192 Layer 1 (Bone 18) | Human bone clavicle (10g) | -21.9 | +10.6 | 4530+/-30 | 3340/3200 | 3350-3330/3220-3120 | 3360-3260 | 3240-3100 (64%)
| Beta-308615 NBL/19216 | Pit 192 Layer 16 (frags) | Human bone (fibula) (14g) | -21.4 | | 4510+/-30 | 3330/3210/3190/3150/3130 | 3350-3260/3240-3100 (53%) | 3360-3090 |
| Beta-350766 NBL11/B2 | Burial 2 | Human bone infant burial outer ditch (3g) | -21.4 | +12.7 | 4470+/-30 | 3260/3240/3100 | 3330-3220 (48%) | 3180-3160/3120-3090 | 3340-3020 |
| Beta-363921 NBL11/108 | Fill 108 Pit 109 Base of OD | Red deer antler (160g) | -21.8 | | 4330+/-30 | 2910 | 2920-2900 | 3020-2890 |
Table 14: Radiocarbon values obtained for Banbury Lane, courtesy of Northamptonshire Archaeology (MOLA). Additional information on these dates can be found in Appendix A (OD = outer ditch; x% denotes date range with highest individual probability)

<table>
<thead>
<tr>
<th>Beta-360012 NBL11/100</th>
<th>Fill 100</th>
<th>Red deer antler (12.7g)</th>
<th>-21.6 +8.2</th>
<th>4310+/-30</th>
<th>2910</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta- 350767 NBL11/B3</td>
<td>Burial 3</td>
<td>Human bone Crouched burial (9g)</td>
<td>-22.0 +13.7</td>
<td>4100+/-30</td>
<td>2830/2820/2620</td>
</tr>
</tbody>
</table>

7.3. The Human Remains

A total of c.7,500 disarticulated human bones and c.9,400 bone fragments were recovered from the elongated pit cut into the entrance to the inner ditch of the monument (Holmes 2012). The human bone assemblage consisted of solely disarticulated material, and although most skeletal elements are present, long bone and skull fragments dominate. Significant numbers of pelvic and spinal elements, along with scapula fragments, were also recovered, but elements such as ribs, patella, clavicles and phalanges are rare (Caffell and Holst 2013). As is often seen within Neolithic disarticulated bone assemblages therefore, the smaller skeletal elements (particularly those from the hands and feet) are under-represented at Banbury Lane. The overall MNI of the assemblage has therefore been determined on the most frequent element present, the right femur, and through extensive refitting exercises (undertaken by the author, Anwen Caffell and Malin Holst), currently stands at 165 individuals.

Determination of the sex composition of the assemblage is complex, due to its disarticulated nature, and the lack of complete diagnostic pelvic or skull elements. Around 10% of the assemblage was assessed by York Osteoarchaeology Ltd., which determined from os coxae that the assemblage appeared to be predominantly male in composition (80.9%), with few females present (Caffell and Holst 2013). Alternative osteological sexing techniques using the dentition and other sexually dimorphic elements (such as the femur, tibia, ulna, radius and tarsals) have also revealed a much higher proportion of males than females within the assemblage (Nutbourne 2015). This osteological sex identification has been supported by preliminary Y-chromosome aDNA analysis, which has revealed
seven individuals randomly sampled thus far to be male (Martiniano 2015). Age determinations were similarly difficult to determine, but overall the assemblage appears to comprise predominately of younger adults (<35 years) and adolescents. Only 9.9% of the assessed assemblage comprised non-adult remains, and of these, nearly all were over the age of nine years (Caffell and Holst 2013).

Initial analysis of the trauma and fracture patterns has revealed a range of different types of breaks seen within the assemblage, across a variety of skeletal elements. The high number of mineralised breaks and fairly low levels of overall trauma however suggest that the assemblage is not the result of a violent attack or massacre, but may represent some degree of intentional manipulation post-mortem (Aitkin 2015; Caffell and Holst 2015).

The composition of the skeletal assemblage at Banbury Lane, combined with evidence suggesting that some bones appear to have undergone a period of exposure prior to inhumation (Caffell and Holst 2013; 2015), indicates that it represents a secondary burial, but appears to have been a single depositional event. It is also interesting to note the lack of artefacts within the pit, and the presence of only 15 rodentia bones, which are believed to be intrusive (Lichenstein 2012), and a total of five Bos and Sus fragments (Clegg 2014) within the feature. Why the human remains were deposited in such a manner is much more complex, and is discussed in more detail below – but could perhaps be seen to reflect a potential ‘closing’ act of the monument, or a means of cementing ancestral links to this location. The presence of such a large skeletal assemblage within the monument also links to ideas surrounding Neolithic visibility of the dead within the landscape (Chapters 2 and 3).

7.4. Banbury Lane – A Unique Human Bone Assemblage

Banbury Lane represents a unique British Neolithic site and human bone assemblage – both in terms of the quantity of human skeletal material present, and the nature of its deposition. A brief overview of the site and the main osteological findings are provided above, but it is also important to consider the Banbury Lane site and assemblage more broadly, setting it both in its regional and wider context.

There are known to be a range of Neolithic sites across Northamptonshire, including a number of other large monumental sites. For example, causewayed enclosures have been
found at the sites of Briar Hill, Dallington, and Southwick – although none of these sites have human remains present (Clay 2006; Deegan 2007). Other Neolithic monuments are known from the county in the form of Cotton Henge at Raunds, the long barrow at Redlands Farm, Stanwick, the long mound and long enclosure at West Cotton, and the potential mortuary enclosure/long enclosure (although with no associated human remains) at Grendon (Chapman 1999; Clay 2006). There is little evidence for Neolithic settlement sites however – aside from at the site of Ecton (Moore and Williams 1975) – although some pit features (e.g. Jackson 1978) are known.

Enclosures with funerary remains are known in the Northampton area from the sites of Aldwincle and Tansor Crossroads (Deegan 2007). Two adult male Neolithic burials were excavated from the Aldwincle mortuary enclosure (Jackson 1976), however the current location of these remains is unfortunately unknown (Andy Chapman, pers. comm.). Additionally, a number of middle Neolithic cremation burials were recovered from the site of Milton Ham, c.1km from the Banbury Lane site (Chapman 2015). A Neolithic cremation burial is also known from the quarry pit of the long mound at Raunds (Healey et al. 2007). Unfortunately, however, no biomolecular work has been undertaken on any of these remains.

It can therefore be seen that although there are other Neolithic sites across Northamptonshire, and some in close proximity to the Banbury Lane site itself, very few of these have yielded human remains. Of those where human remains are known, the assemblages appear to consist of very small numbers of individuals, and are often cremated. Other ring ditched monuments are known within Northampton, but again these do not appear to contain human remains. Interestingly though, aside from Banbury Lane, there are only four ring ditched monuments identified within Northamptonshire which have three circuits (Deegan 2007). The lack of other Neolithic sites with human remains within Northampton means there are few assemblages for comparison – and the lack of biomolecular work undertaken on those sites with human remains means there is no comparable isotopic data from the area.

The large number of human remains seen at Banbury Lane is unusual, but not totally unique within the British Neolithic. For example, significant numbers of human remains have been recovered from sites such as the Tomb of the Eagles, Orkney, and Quanterness; although neither sites exhibit deposition of human remains in a pit akin to that seen at
Banbury Lane. Whereas smaller numbers of disarticulated human remains are recovered from monumental sites across the UK, particularly long barrows and chambered tombs, it is crucial to remember that the remains we find only represent the final arrangements of human remains. Additional human remains may have been placed within and moved around sites, but transferred to elsewhere within the landscape, thereby leaving no archaeological traces at these monumental forms. Additionally, we must assume that not all individuals were deposited within monuments or large structures in the Neolithic, and therefore the presence of large numbers of human remains in other locations is perhaps unsurprising. However, as the assemblage represents a secondary burial, and was introduced into the pit in a single depositional event, some degree of curation of the remains must have occurred previously.

7.5. Materials and Methods

Due to the disarticulated nature of the skeletal assemblage, an MNI was calculated to ensure that any isotopic measurements undertaken were done so on discrete individuals. Femora were noted to be the most numerous element type within the assemblage, and therefore extensive refitting of femora was undertaken (by the author, Malin Holst and Anwen Caffell) to increase the MNI. The MNI for the assemblage (n=165) is based upon right femora, upon which stable isotopic analysis of $\delta^{13}$C and $\delta^{15}$N was undertaken. To provide faunal baselines for the human isotopic data obtained, $\delta^{13}$C and $\delta^{15}$N stable isotopic analysis was also undertaken on the small number of faunal fragments from the pit (n=5), which are also believed to be of Neolithic date. Collagen was extracted and isotopically analysed using published protocols using IRMS (Richards & Hedges 1999; Colonese et al. 2015). An inter-lab comparison was also undertaken on two of the Banbury Lane human bones, along with the standards used for IRMS analysis, to ensure accuracy and reliability of obtained isotopic data from this large dataset (see Appendix A, section A.3.2.).

Stable isotopic analysis of $^{87}$Sr/$^{86}$Sr ratios was undertaken on human teeth from the site using LA- MC-ICP-MS (Figure 39). As many of the teeth present within the assemblage had been lost post-mortem, or are loose teeth, a separate MNI derived from the dentition had to be determined to ensure that all teeth were from different individuals. As such, only teeth still ‘in-situ’ (i.e. still in the maxilla/mandible) were considered within MNI calculations. The MNI from the dentition (n=26) is based upon permanent left maxillary
first molars. \[^{87}\text{Sr}/^{86}\text{Sr}\] analysis was undertaken also using published protocols (Lewis et al. 2014).

Protocols for each of the methodologies employed in this study are provided in Appendix A.

![Figure 39: Selection of the Banbury Lane teeth once sectioned, mounted in epoxy resin, and polished, ready for LA- MC-ICP-MS analysis](image)

**7.6. Results and Discussion**

**7.6.1. Dietary Inferences**

The results of the stable isotopic analysis of \(\delta^{13}\text{C}\) and \(\delta^{15}\text{N}\) on 165 individuals are displayed below (Figure 40; see also Appendix A, Table S5). Collagen yields were calculated from retentate samples only, following ultrafiltration. Despite this, collagen yields of over 1.22% were obtained for all samples. Similarly, C:N ratios from all samples fell within the expected quality ranges (DeNiro 1985; van Klinken 1999). Of the five faunal fragments utilised, four provided sufficient collagen yields (from retentate samples following ultrafiltration) for \(\delta^{13}\text{C}\) and \(\delta^{15}\text{N}\) analysis (Appendix A, Table S6). Collagen quality again fell within prescribed quality ranges (DeNiro 1985; van Klinken 1999).
The δ\(^{13}\)C and δ\(^{15}\)N isotopic data obtained from the Banbury Lane human femora indicates a diet based on terrestrial protein and C\(_3\) plant sources (Figure 9; Appendix A, Table S5). Interestingly, however, the isotopic data generated shows a remarkable degree of homogeneity – with <2‰ difference in both δ\(^{13}\)C and δ\(^{15}\)N between all human samples. This degree of similarity is remarkable, and indicates that all individuals at the site were clearly consuming foodstuffs which resulted in very similar collagen isotopic values. However, it is crucial to note that isotopic homogeneity does not necessarily equate to dietary homogeneity (Schulting 2011) – and therefore similar collagen values could be generated via different diets, although all terrestrial protein and C\(_3\) plant based. In their study of the human remains from Hazleton North, Hedges et al. (2008) also note a lack of isotopic variability, and suggested that this homogeneity may be a reflection of a lack of ‘secondary effects’ on isotopic values – such as changes in physiology and bone remodelling rates.

![Figure 40] (Plot of isotope values obtained from Banbury Lane humans and fauna)
The only ‘outlier’ in the generated δ\textsuperscript{13}C and δ\textsuperscript{15}N dataset for Banbury Lane is sample 1262, from excavation layer 15 of the pit, which has a δ\textsuperscript{13}C value of -20.01‰, whereas most other samples appear to have δ\textsuperscript{13}C values below -20.60‰ (shown clearly in Figure 40). In an archaeological assemblage of this size, a δ\textsuperscript{13}C value difference of ~0.4‰ would not normally be considered as significant or noteworthy – but the tight clustering of the Banbury Lane humans means that this sample (1262) noticeably outlies the rest of the assemblage. The reasons behind this difference in δ\textsuperscript{13}C are not immediately clear, but may be the result of differential plant consumption, or through having a different proportion of plants and animals in the diet than the other individuals (as suggested in Stevens et al. 2012).

The homogeneity of the Banbury Lane samples is also seen in the distribution plots generated for δ\textsuperscript{13}C and δ\textsuperscript{15}N (Figures 41 and 42). As discussed above, sample 1262, with a δ\textsuperscript{13}C value of -20.01‰, can clearly be seen to be outlying the rest of the assemblage (Figure 41), but is not noticeable when we consider the δ\textsuperscript{15}N distributions of the dataset (Figure 42).

![δ\textsuperscript{13}C Frequencies](image)

Figure 41: Distribution plot of δ\textsuperscript{13}C values within the Banbury Lane human isotopic data
Unfortunately, there is no comparative Neolithic isotopic data available from Northamptonshire; however, a comparison of the data obtained from Banbury Lane with other Neolithic sites from the UK of a similar date is seen in Figure 43 below. On the whole, it would appear that the Banbury Lane assemblage has elevated δ¹⁵N values when compared to most other UK Middle Neolithic sites, but has similar δ¹³C values. However, samples from Quanterness (Schulting 2013) have similar δ¹⁵N values to those seen at Banbury Lane, but are less depleted in δ¹³C. This difference in δ¹³C values is likely due to marine protein consumption at Quanterness.
Figure 43: Comparison of Banbury Lane isotope data with $\delta^{13}$C and $\delta^{15}$N values from British Neolithic sites of a similar date (data from Schulting and Richards 2002; Hedges et al. 2008; Schulting 2013; Montgomery et al. 2013; the author)

The somewhat elevated $\delta^{15}$N values in the Banbury Lane humans however may be explained when considering the $\delta^{13}$C and $\delta^{15}$N isotopic values obtained from fauna from the site. When compared with other Neolithic sites from the UK, the faunal isotopic values from Banbury Lane appear to be slightly different (Figure 44). It can be seen that the cattle ($Bos$) values from Banbury Lane are more elevated in $\delta^{15}$N than other British Neolithic sites, but that they have similar $\delta^{13}$C values. Similarly, the singular pig ($Sus scrofa$) sample from Banbury Lane has a much higher $\delta^{15}$N value than all other sites – with only a singular value from Eton Rowing Lake (Stevens et al. 2012) having a more elevated value.
Figure 44: Cattle (blue) and pig (orange) values from British Neolithic sites (data from Richards 2000; Schulting and Richards 2002; Hedges et al. 2008; Milner and Craig 2009; Stevens et al. 2012; Montgomery et al. 2013; the author)

It can be seen that the Banbury Lane *Sus* sample is slightly elevated in both $\delta^{13}$C and $\delta^{15}$N when compared to the three *Bos* samples from the site (Figures 40 and 44; Table S6). This is to be expected, and reflects the differential diets of the two animals – with pigs known to be omnivorous, compared to the herbivorous diets of cattle. An overview of *Sus scrofa* stable isotope values by Hamilton et al. (2009) suggests that pig $\delta^{15}$N values are typically 0.1-0.7‰ higher than those of cattle or sheep at British Neolithic sites. When we consider the Banbury Lane fauna, it can be seen that the pig $\delta^{15}$N value obtained is 0.9‰ higher than the averaged cattle $\delta^{15}$N value however, thereby making the offset between the
animals closer to that seen at Iron Age sites, thought to be due to pigs foraging on human refuse (Hamilton et al. 2009). The elevated δ¹⁵N values seen in both the Banbury Lane cattle and pig samples (Figure 44) however may instead be due to manuring effects (Bogaard et al. 2007), a warmer annual mean temperature (Stevens et al. 2006), or differential nitrogen cycling in soils, affecting plant δ¹⁵N values (Handley et al. 1999). Nonetheless, as noted above, the δ¹⁵N enrichment seen in the Banbury Lane fauna can be used to explain the elevated δ¹⁵N values also seen in the humans from the site, when compared to other British Neolithic assemblages.

The differences in δ¹³C values between the two species (both at Banbury Lane and other British Neolithic sites; Figure 44) are likely to be the result of differential plant consumption, and/or animals being reared in different habitats or ecological niches. Less depleted δ¹³C values in fauna have previously been suggested to represent a diet derived from more open habitats, rather than from dense woodlands, representing a ‘canopy effect’ (Van der Merwe and Medina 1989; Bocherens et al. 1999; Krigbaum 2003; Noe-Nygaard et al. 2005; Hamilton et al. 2009). If correct, this may reflect the differential management of cattle and pigs in the Neolithic, with the two species occupying different habitats. However, a study by Stevens et al. (2006) has shown that a δ¹³C canopy effect is not always present in fauna inhabiting different environments, and as such, should perhaps be treated with caution. Less depleted δ¹³C values in pigs has also previously been suggested to represent woodland habitation and a fungi (or similar woodland resource) component to pig’s diets, particularly in the British Neolithic (Hamilton et al. 2009).

As noted above, there are currently no isotopic values for humans or fauna from Northampton dating to the Neolithic available. However, δ¹³C and δ¹⁵N values for cattle from the early Bronze Age sites of Irthlingborough (Northamptonshire), and Gayhurst (Buckinghamshire), both of which are <20 miles from Banbury Lane, are published (Towers et al. 2011). Both sites are round barrows dating to c.2000 cal. BC, and contained significant amounts of cattle bone – an MNI of 185 at Irthlingborough and 300 at Gayhurst – as well as single Beaker human burials. Stable isotope analysis of 10 cattle from Irthlingborough and 12 cattle from Gayhurst has previously been undertaken (Towers et al. 2011), and the results of these analyses are presented below with the cattle values obtained from Banbury Lane (Figure 45). It can be seen that the Banbury Lane data falls completely in-line with the early Bronze Age data available for the region. As the cattle from both Irthlingborough and Gayhurst have previously been determined to consist of a local
population using both strontium and sulphur isotopes (Towers et al. 2010; 2011), and this therefore perhaps suggests that the faunal $\delta^{13}$C and $\delta^{15}$N isotopic values are the result of local environmental conditions and/or local plant values.

Figure 45: Comparison of $\delta^{13}$C and $\delta^{15}$N stable isotope values of cattle from Banbury Lane with two early Bronze Age sites from the region

**7.6.2. Trophic Levels and Mixing Models**

The average human to herbivore offset ($\Delta^{15}$N$_{\text{fauna-human}}$) across the Banbury Lane assemblage is 4.86‰. A 4-4.5‰ offset has previously been seen between Neolithic humans and fauna at Eton Rowing Lake (Stevens et al. 2012), Hambledon Hill, Ascott, and Windmill Hill (Hedges and Reynard 2007). It can therefore be seen that the Banbury Lane
material shows a similar trend to other British Neolithic sites, despite the overall $\delta^{13}C$ and $\delta^{15}N$ values being somewhat different (Figure 43). However, in order to determine what these values mean in terms of human consumption of terrestrial animal protein, it is necessary to calculate the enrichment of human collagen over diet ($\Delta^{15}N_{\text{diet-body}}$). The standard model used within palaeodietary studies suggests this figure should be 3-5‰ (Bocherens and Drucker 2003; Hedges and Reynard 2007; see Chapter 4, section 4.3.).

Using this figure, a 0‰ enrichment of human collagen over terrestrial fauna would therefore indicate a fully herbivorous human diet, whereas a +4‰ enrichment would indicate a fully carnivorous diet. However, there are a number of assumptions inherent within this model (Model A below) which are problematic, as highlighted by Hedges and Reynard (2007) (see also Chapter 4, section 4.3.). Due to this, three other alternative models have been proposed:

[A] the ‘standard model’: forage and cereal have the same $\delta^{15}N$ value, and human and fauna enrichment has the same value (4‰)
[B] cereal $\delta^{15}N$ is 1‰ enriched over animal forage
[C] In addition to [B], the enrichment in humans is 1‰ greater than herbivores (5‰ for humans, 4‰ for herbivores)
[D] In addition to [C], the enrichment for humans eating an all meat diet is 1‰ less than for an all plant diet (4‰ for an all meat diet, 5‰ for an all plant diet)

These four dietary scenarios, as suggested by Hedges and Reynard (2007), and previously successfully applied to Neolithic material by Lelli et al. (2012), were therefore modelled using the Banbury Lane data (Table 15). Further details on the model can be found in Appendix A, section A.3.3.

<table>
<thead>
<tr>
<th>Dietary Components</th>
<th>Average Herbivore $\delta^{15}N$ Value (‰)</th>
<th>Average Human $\delta^{15}N$ Value (‰)</th>
<th>$\Delta^{15}N_{\text{fauna-human}}$ (‰)</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle &amp; pigs</td>
<td>6.08</td>
<td>11.3</td>
<td>5.22</td>
<td>131%</td>
<td>141%</td>
<td>106%</td>
<td>107%</td>
</tr>
<tr>
<td>Pigs only</td>
<td>7.1</td>
<td>11.3</td>
<td>4.2</td>
<td>105%</td>
<td>106%</td>
<td>80%</td>
<td>73%</td>
</tr>
</tbody>
</table>

Table 15: Determination of % animal protein in diet of individuals from Banbury Lane using the dietary models proposed by Hedges and Reynard (2007). Cattle and pig data from this study was combined with Towers et al. 2011 to increase sample size
The data clearly show that using the dietary models currently available for $\delta^{15}\text{N}$, the lowest possible percentage of animal protein in the diet of the humans from Banbury Lane is 73% (when we consider only the pig isotopic values from the site). However, if we consider both the cattle and pig isotopic data (with a lower average $\delta^{15}\text{N}$ value) then the model does not work, as the smallest percentage of animal protein in the diet is estimated to be 106%.

In modern-day developed countries the dietary animal protein fraction is 57%, and 30% for developing countries (Hedges and Reynard 2007), and it has been estimated that hunter-gatherer diets may have up to 80-90% dietary animal protein fraction (Cordain et al. 2000). It therefore seems unlikely that the values of $>70\%$ animal protein are correct for a British middle Neolithic diet. As such, this indicates that either there is either (a) an additional animal protein source (with higher $\delta^{15}\text{N}$ values) in the diet of the Banbury Lane individuals which is currently not being recognised – such as freshwater fish, (b) that these values may be evidence of significant consumption of milk and/or dairy products, as well as meat, or (c) that the level of nitrogen enrichment for humans ($\Delta^{15}\text{N}_{\text{diet-collagen}}$) is greater than 5‰ (as suggested by O’Connell et al. 2012).

Given the issues with the % dietary protein values generated using the Hedges and Reynard (2007) model for Banbury Lane, and because the model only considers the protein component of the diet, further quantitative diet reconstruction was attempted through the use of the Bayesian mixing model FRUITS (Fernandes et al. 2014; Fernandes 2015; see Chapter 4, section 4.3.5.), which has previously been successfully applied to populations of a Neolithic date (Fernandes et al. 2015). Two models were created for the Banbury Lane data. Both considered the macronutrient (protein, lipid, carbohydrate) contribution from terrestrial plants and terrestrial fauna to the averaged human diet at Banbury Lane; although in model 1, all terrestrial animals (cattle, pigs) were considered together as a singular food group, whereas in model 2, cattle and pigs were inputted as two separate food groups. The values used and assumptions made within the model can be found in Appendix A, section A.3.3., but the overall bulk isotope values this data is extrapolated from can be found in Table 16. Furthermore, given the results obtained from the Hedges and Reynard (2007) models, a higher $\delta^{15}\text{N}$ enrichment value ($\Delta^{15}\text{N}_{\text{diet-collagen}}$) was used (5.5‰ ±0.5) – therefore making the model in-line with the findings of O’Connell et al. (2012).

Due to the lack of faunal values available from the Northampton area of a similar date to Banbury Lane, the terrestrial fauna isotopic values obtained within this study were
combined with those from the early Bronze Age sites of Gayhurst and I... of Gayhurst and I... and 

Similarly, as there are no plant isotopic values available for Banbury Lane, δ¹³C and δ¹⁵N values of emmer wheat (*Triticum dicoccum*) from Hambledon Hill (Bogaard et al. 2013) were used as an estimation of plant values for the site. Due to the lack of freshwater fish values available for Neolithic Britain, freshwater resources were not included as a foodgroup within the model – although it is possible that they may have been consumed by the Banbury Lane population (see above).

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Species within food group</th>
<th>No. of samples</th>
<th>δ¹³C (‰) average</th>
<th>St. Dev.</th>
<th>δ¹⁵N (‰) average</th>
<th>St. Dev.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial animals</td>
<td>Cattle, pig</td>
<td>26</td>
<td>-22.89</td>
<td>0.5</td>
<td>6.08</td>
<td>0.4</td>
<td>This study; Towers et al. 2011</td>
</tr>
<tr>
<td>Terrestrial Plants</td>
<td>Emmer wheat (<em>Triticum dicoccum</em>)</td>
<td>3</td>
<td>-23.13</td>
<td>0.7</td>
<td>3.60</td>
<td>0.6</td>
<td>Bogaard et al. 2013</td>
</tr>
<tr>
<td>Consumer data</td>
<td>Human</td>
<td>155</td>
<td>-20.88</td>
<td>3.3</td>
<td>11.30</td>
<td>0.3</td>
<td>This study</td>
</tr>
</tbody>
</table>

Table 16: δ¹³C and δ¹⁵N isotope values of food groups used within the FRUITS model generated for Banbury Lane

The data generated from the two mixing models is presented below (Figures 46 and 47), although it can be seen that the separation of cattle and pigs in model 2 does not result in dramatically different results. However, model 2 does suggest that pigs may have comprised a slightly larger component of the human diet at Banbury Lane than cattle. Overall, what is interesting however is that both the models estimate that up to c.70% of the diet may have comprised terrestrial plants – something which is not visible in the dietary models proposed by Hedges and Reynard (2007) above, which only consider δ¹⁵N values. The inference that terrestrial plants may have comprised a larger overall proportion of the diet than terrestrial animals is particularly interesting – and is something which cannot be inferred from bulk isotopic values on human bone collagen alone. Due to the lack of available data on plant isotopic values, the relative importance of terrestrial plants or cereals in prehistoric diet is still an underexplored area of palaeodietary research (Chapter 2, section 2.4.1.) – but the data presented here implies that they may have formed a considerable dietary component during the British middle Neolithic. This therefore highlights the advantage of using FRUITS to interpret isotopic data, as the model considers
the whole diet – rather than just the protein component as in the models of Hedges and Reynard (2007).

Figure 46: FRUI TS model 1 for Banbury Lane data (considering both cattle and pigs as a combined food group)
7.6.3. Origins and Movement: $^{87}\text{Sr}/^{86}\text{Sr}$ ratios

The $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis undertaken here utilised permanent left maxillary first molars (M1) (n=26). The formation timings of the human dentition are well-characterised, and although there may be some slight intra-individual variability, it is believed that dental formation has less variation than both tooth eruption and skeletal development, and is also less affected by environmental factors or nutritional stress (Smith 1991; Cardoso 2007). Tooth formation timings have however been estimated for different global populations, and therefore Roberts (2009, 132) suggests utilising calculated timings from those with most ‘similarity’ to the archaeological samples in question. Whilst it has been suggested that not all modern dental data may be suitable to apply to archaeological populations, often due to
potential differences in the timings of dental formation (e.g. Mappes et al. 1992; Tompkins 1996; Halcrow et al. 2007), for European populations, tooth formation timings are generally broadly agreed upon and can thus be applied to archaeological material.

It is generally taken that initial formation of the first molar crowns begins shortly after birth (c.1-2 months for males, c.2-3 months for females), and that the crown is typically completed by 2.5 years in males and 2.4 years in females (Smith 1991), but has been suggested by others to take up 3 years to completely form (Hillson 1996, 123). The whole tooth is completely formed and the apex of the root complete by the age of 9.4 years in males and 8.7 years in females, with the tooth erupting through the gums (gingival emergence) around 6 years of age (±2 years). The M1 is the first of the permanent dentition to both begin formation and emerge in the dental arc (Smith 1991; Hillson 1996, 125; White and Folkens 2005, 365; Ubelaker 2008).

$^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis utilises the enamel of the tooth crown (see Chapter 4, section 4.4.3.), and is therefore indicative of childhood movement during tooth formation and mineralisation. By utilising M1 teeth, the data presented here refers to a short period, very early within a child’s life (from birth to before 3 years of age), at a time when children are highly likely to have been breastfed for part, if not all, of this period. $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis will therefore provide evidence of location within this short stretch of early childhood, providing information on Neolithic mobility. In light of the dietary isotopic data obtained from the Banbury Lane individuals, it could be hypothesised that this $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic homogeneity may be due to all individuals originating from or inhabiting the same area. The degree of dietary similarity within the assemblage, particularly when compared to other Neolithic assemblages (Figure 13), conjures ideas of similar lifeways across the Banbury Lane individuals – and within this, potentially similar origins. With bioavailable strontium isotope composition maps now available for Britain (e.g. Evans et al. 2010), it is possible to utilise the $^{87}\text{Sr}/^{86}\text{Sr}$ values of enamel as a provenance tool, and a proxy for palaeomobility in the archaeological past.

7.6.3.1. Characterising Biosphere $^{87}\text{Sr}/^{86}\text{Sr}$

In order to successfully interpret the $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from the dental enamel of the Banbury Lane individuals however, it was important to attain a detailed characterisation of the biosphere strontium in the Northamptonshire area. From the
currently available biosphere strontium map of the UK, it can be seen that the $^{87}\text{Sr}/^{86}\text{Sr}$ value for the Northampton geology is 0.709-0.710, representing a bedrock geology of mudstone, siltstone, limestone, and sandstone (BGS 2015; Figure 48). However, although the available map covers the whole of Britain and the major lithologies, it is naturally broad in scale and does not account for any smaller regional variations in underlying geology and/or $^{87}\text{Sr}/^{86}\text{Sr}$ values. Due to this, interpretations using only this map are also bound to very broad in nature. As such, a range of soil samples were taken from the Northampton area surrounding the Banbury Lane site, in order to determine the approximate range of bioavailable strontium isotope composition for the local area. Soil is a suitable substrate to determine biosphere $^{87}\text{Sr}/^{86}\text{Sr}$ values as strontium is released from the underlying geology into the soil, where it is passed through the food chain into plants and subsequently animals and humans (Figure 18; see also Chapter 4, section 4.4.3.).

Figure 48: Strontium transfer between ecosystem components (Szostek et al. 2015, 139)

Soil samples were taken from 12 different sample locations with different underlying geologies (Figure 50), which are listed in Table 17 and indicated in Figure 49. Strontium values for each soil sample were obtained using previously published methods using TIMS (Evans et al. 2010; Maurer et al. 2012; see Appendix A, section A.3.4.).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Grid Reference (OSGB36)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Closest Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SP 40351 62613</td>
<td>52.26021</td>
<td>-1.4102271</td>
<td>Welsh Rd, Southam, Warwickshire CV47 2BH, UK</td>
</tr>
<tr>
<td>1A</td>
<td>SP 40625 65359</td>
<td>52.28488</td>
<td>-1.4058831</td>
<td>26 Dale Cl, Long Itchington, Southam, Warwickshire CV47 9SE, UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>SP 48946 62519</td>
<td>52.25867</td>
<td>-1.2843228</td>
<td>Woodview Cottages, Park Ln, Daventry, Warwickshire NN11 6Du, UK</td>
</tr>
<tr>
<td>4</td>
<td>SP 64215 52789</td>
<td>52.16964</td>
<td>-1.0624914</td>
<td>Unnamed Road, Towcester, Northamptonshire NN12 8FL, UK</td>
</tr>
<tr>
<td>5</td>
<td>SP 66907 54694</td>
<td>52.18644</td>
<td>-1.022761</td>
<td>5 Fosters Booth Rd, Pattishall, Towcester, Northamptonshire NN12 8JU, UK</td>
</tr>
<tr>
<td>5B</td>
<td>SP 65905 53712</td>
<td>52.17774</td>
<td>-1.0376056</td>
<td>1 Church Ln, Towcester, Northamptonshire NN12, UK</td>
</tr>
<tr>
<td>7</td>
<td>SP 68663 54669</td>
<td>52.186</td>
<td>-0.99708501</td>
<td>Banbury Ln, Towcester, Northamptonshire NN12, UK</td>
</tr>
<tr>
<td>7B</td>
<td>SP 68428 54699</td>
<td>52.1863</td>
<td>-1.0005157</td>
<td>Banbury Ln, Towcester, Northamptonshire NN12, UK</td>
</tr>
<tr>
<td>8</td>
<td>SP 69143 55833</td>
<td>52.19641</td>
<td>-0.98982816</td>
<td>Unnamed Road, Northampton, Northamptonshire NN7 3JF, UK</td>
</tr>
<tr>
<td>9</td>
<td>SP 66328 59321</td>
<td>52.22811</td>
<td>-1.0303239</td>
<td>10 Main Rd, Northampton, Northamptonshire NN7 3LZ, UK</td>
</tr>
<tr>
<td>11</td>
<td>SP 67694 58617</td>
<td>52.22161</td>
<td>-1.0104684</td>
<td>Mill Ln, Northampton, Northamptonshire NN7, UK</td>
</tr>
<tr>
<td>20</td>
<td>SP 68209 58269</td>
<td>52.21842</td>
<td>-1.0030008</td>
<td>Mill Ln, Northampton, Northamptonshire NN7, UK</td>
</tr>
</tbody>
</table>

Table 17: Details of soil sample locations

Figure 49: Location of soil samples (indicated by blue pins) utilised within this research (Google Maps 2016)
The $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from the soil leachates from the 12 sample locations are provided in Table 18 below.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Grid Reference (OSGB36)</th>
<th>$^{87}\text{Sr}/^{86}\text{Sr}$</th>
<th>±2SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SP 40351 62613</td>
<td>0.709924</td>
<td>0.000016</td>
</tr>
<tr>
<td>1A</td>
<td>SP 40625 65359</td>
<td>0.709601</td>
<td>0.000014</td>
</tr>
<tr>
<td>2</td>
<td>SP 48946 62519</td>
<td>0.709826</td>
<td>0.000019</td>
</tr>
<tr>
<td>4</td>
<td>SP 64215 52789</td>
<td>0.710728</td>
<td>0.000013</td>
</tr>
<tr>
<td>5</td>
<td>SP 66907 54694</td>
<td>0.709636</td>
<td>0.000016</td>
</tr>
<tr>
<td>5B</td>
<td>SP 65905 53712</td>
<td>0.709969</td>
<td>0.000015</td>
</tr>
<tr>
<td>7</td>
<td>SP 68663 54669</td>
<td>0.708648</td>
<td>0.000015</td>
</tr>
<tr>
<td>7B</td>
<td>SP 68428 54699</td>
<td>0.708709</td>
<td>0.000017</td>
</tr>
<tr>
<td>8</td>
<td>SP 69143 55833</td>
<td>0.710402</td>
<td>0.000015</td>
</tr>
<tr>
<td>9</td>
<td>SP 66328 59321</td>
<td>0.709179</td>
<td>0.000016</td>
</tr>
<tr>
<td>11</td>
<td>SP 67694 58617</td>
<td>0.710126</td>
<td>0.000016</td>
</tr>
<tr>
<td>20</td>
<td>SP 68209 58269</td>
<td>0.709065</td>
<td>0.000024</td>
</tr>
</tbody>
</table>

Table 18: $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from soil samples analysed

It can be seen from Figure 51 that seven of the twelve soil values fall within the estimated biosphere strontium range for Northampton (0.709-0.710). However, the remaining five soil samples exhibit $^{87}\text{Sr}/^{86}\text{Sr}$ values beyond this range. Two samples (7 and 7B) show $^{87}\text{Sr}/^{86}\text{Sr}$ values of 0.786-7, which is particularly interesting given that these samples were taken closest to the Banbury Lane site (Table 17 and Figure 49). This therefore indicates that the immediate local geology of Banbury Lane may in fact fall beyond the expected
range for Northampton – something which is particularly pertinent to consider alongside the human values obtained for the site. The remaining three soil samples exhibit strontium values in excess of 0.710, with one sample reaching 0.7107. Overall, however, it can be seen that from the soil values obtained here, the biosphere strontium range for Banbury Lane and the surrounding area appears to be 0.786-0.7107. This therefore indicates that the local range for Northampton as indicated by the Evans et al. (2010) map (Figure 18) is not conservative enough, and there is more small scale regional strontium variability than this map indicates.

![Figure 51: $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from soil samples analysed. The green box indicates the biosphere value for Northampton (0.709-0.710) as calculated by Evans et al. (2010)](image)

7.6.3.2. $^{87}\text{Sr}/^{86}\text{Sr}$ values from Banbury Lane

As discussed within Chapter 4, the more recent utilisation of laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS) within archaeological strontium studies holds a range of benefits over the more traditional dissolution methods such as thermal ionisation mass spectrometry (TIMS). Notably, LA-MC-ICP-MS has a faster analysis time, can provide high spatial resolution results, has reduced risk of
contamination, and results in less damage to samples (Moffat et al. 2012; Lewis et al. 2014). Here, strontium isotopic analysis was undertaken on 26 permanent left maxillary first molars from Banbury Lane using LA-MC-ICP-MS (see Appendix A, section A.3.4.).

The benefits of the LA-MC-ICP-MS can clearly be seen. For example, if we consider only the ‘bulk’ averaged $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from each tooth (as would have been generated via traditional TIMS analysis), it can be seen that four geological zones are indicated across the individuals (Table 19), which can be seen to broadly reflect different geological zones across the UK (Figures 18 and 52). Of the 26 teeth analysed here, half (n=13) have bulk strontium signatures which are consistent with a childhood period spent in an area of underlying geology similar that found in the region of Northampton (0.709-0.710). The remaining thirteen teeth however exhibit bulk $^{87}\text{Sr}/^{86}\text{Sr}$ values which indicate that their early childhood was spent in areas of a differential geology to that of Northamptonshire – with three individuals showing strontium signatures akin to a underlying chalk geology (less radiogenic than Northamptonshire; highlighted by the light blue areas in Figure 52), six individuals having slightly more radiogenic values than the Northamptonshire geology (shown in light green in Figure 52), and the remaining four individuals indicating significantly more radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ values (shown by the yellow areas in Figure 52) (see Figure 18).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Age /Years</th>
<th>Sex</th>
<th>Averaged Enamel $^{87}\text{Sr}/^{86}\text{Sr}$ Value</th>
<th>Averaged Dentine $^{87}\text{Sr}/^{86}\text{Sr}$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.00</td>
<td>18-25</td>
<td>?Female</td>
<td>0.7092</td>
<td>N/A</td>
</tr>
<tr>
<td>128.010</td>
<td>13-14</td>
<td>Unknown</td>
<td>0.7112</td>
<td>0.7094</td>
</tr>
<tr>
<td>132.017</td>
<td>12-25</td>
<td>Unknown</td>
<td>0.7100</td>
<td>N/A</td>
</tr>
<tr>
<td>138.003</td>
<td>26-35</td>
<td>Unknown</td>
<td>0.7097</td>
<td>N/A</td>
</tr>
<tr>
<td>141.00</td>
<td>14-17</td>
<td>Unknown</td>
<td>0.7100</td>
<td>N/A</td>
</tr>
<tr>
<td>172.00</td>
<td>11-12</td>
<td>Unknown</td>
<td>0.7104</td>
<td>N/A</td>
</tr>
<tr>
<td>201.001</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.7096</td>
<td>N/A</td>
</tr>
<tr>
<td>201.002</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.7102</td>
<td>N/A</td>
</tr>
<tr>
<td>240.005</td>
<td>16-25</td>
<td>Unknown</td>
<td>0.7091</td>
<td>N/A</td>
</tr>
<tr>
<td>302.008</td>
<td>11-14</td>
<td>Unknown</td>
<td>0.7091</td>
<td>N/A</td>
</tr>
<tr>
<td>309.012</td>
<td>26-35</td>
<td>Unknown</td>
<td>0.7084</td>
<td>N/A</td>
</tr>
<tr>
<td>440.003</td>
<td>18-35</td>
<td>Unknown</td>
<td>0.7093</td>
<td>0.7091</td>
</tr>
<tr>
<td>440.004</td>
<td>10-17</td>
<td>Unknown</td>
<td>0.7111</td>
<td>0.7093</td>
</tr>
<tr>
<td>462.007</td>
<td>26-35</td>
<td>Unknown</td>
<td>0.7103</td>
<td>N/A</td>
</tr>
<tr>
<td>551.00</td>
<td>10-12</td>
<td>Unknown</td>
<td>0.7093</td>
<td>0.7092</td>
</tr>
<tr>
<td>838.003</td>
<td>17-25</td>
<td>Unknown</td>
<td>0.7094</td>
<td>N/A</td>
</tr>
<tr>
<td>838.004</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.7092</td>
<td>0.7093</td>
</tr>
<tr>
<td>901.00</td>
<td>11.5-12.5</td>
<td>Unknown</td>
<td>0.7092</td>
<td>0.7097</td>
</tr>
<tr>
<td>1076.005</td>
<td>15-17</td>
<td>Unknown</td>
<td>0.7092</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 19: Averaged Sr values of Banbury Lane individuals analysed. Osteological data from Caffell and Holst (2012). Dentine values were measured on a small number of individuals to provide an indication of the biosphere Sr value of the immediate burial environment.

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Sex</th>
<th>Average Sr</th>
<th>Reference Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1078.008</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>0.705</td>
</tr>
<tr>
<td>1079.00</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>0.705</td>
</tr>
<tr>
<td>1303.001</td>
<td>25-35</td>
<td>Unknown</td>
<td>0.706</td>
<td>N/A</td>
</tr>
<tr>
<td>1336.003</td>
<td>18-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>0.705</td>
</tr>
<tr>
<td>2003.039</td>
<td>14-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>N/A</td>
</tr>
<tr>
<td>2005.213</td>
<td>14-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>N/A</td>
</tr>
<tr>
<td>2013.002</td>
<td>17-25</td>
<td>Unknown</td>
<td>0.706</td>
<td>0.705</td>
</tr>
</tbody>
</table>

Figure 52: Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ values of Banbury Lane individuals. This figure has been colour-coded to correlate directly to the Sr map of the UK (Figure 18). Square icons represent enamel samples, and crosses represent dentine samples.

However, whilst this is useful, it provides only a very broad interpretation, and importantly, does not allow for any intra-tooth variability to be viewed. The major advantage of LA-MC-ICP-MS lies in the fact that it can provide spatially resolved strontium data, and can generate 500+ measurements per tooth, rather than a singular bulk value for the tooth as a whole. Due to the use of the laser, continuous profiles through the growth axis of the enamel can be measured, meaning that any mobility throughout the time of tooth formation can be viewed. As permanent first molars (M1) were used within this...
study, this therefore means that any mobility during early childhood (birth to three years; see section 7.6.3. above) will be visible using a LA-MC-ICP-MS approach.

Interestingly, the data generated from the 26 molars indicates variable degrees of movement across all individuals sampled – with some individuals exhibiting significant mobility, others with slight, potentially periodic movement, and finally some exhibiting little to no mobility during early childhood. The full $^{87}\text{Sr}/^{86}\text{Sr}$ plots for each individual can be found in Appendix A, section A.3.4.; Figures S2-S22). Presented below however are a number of individuals which highlight the variability of results obtained and subsequently, childhood mobility. All data plots represent incremental strontium values along the growth axis of the enamel, taken from crown to cervix, and green shaded areas indicate the expected biosphere strontium range for Northampton indicated by soil analyses (0.786-0.7107; section 7.6.3.1. above). Figure 53 shows the data generated from sample 201.001, which shows very little movement along the growth axis of the tooth, and all movement is within the strontium values expected for the Northampton area as indicated by soil analysis. Similarly, whilst sample 2003.039 showed much more movement and variability along the tooth, nearly all the values obtained again fall within the expected Northampton range (Figure 54). This variability, likely indicative of regional movement around the Northampton area in early childhood, is only visible using LA-MC-ICP-MS – and can provide much more potential information on Neolithic mobility than a singular bulk Sr value.
Figure 53: LA-MC-ICP-MS plot generated for sample 201.001

Figure 54: LA-MC-ICP-MS plot generated for sample 2003.039
Other individuals indicate very little movement, akin to that of sample 201.001, but beyond the expected strontium values of Northampton and the surrounding area (0.786-0.7107). For example, sample 309.012 (Figure 55) shows only slight movement across the enamel growth axis, but many of the values fall outside of the expected values for Northampton, suggesting that their childhood was not spent in the area in which they were buried. By comparing these results with the UK strontium map (Figure 16), it can be seen that less radiogenic chalk bedrock is found in fairly close proximity to Northampton however, to both the east and south, stretching down towards London and the south-east coast. Conversely, other individuals with slight movement along the enamel growth axis appear to have spent their early childhood in an area with significantly more radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ values than Northampton, as seen in sample 128.10 (Figure 56). The largest closest outcrops of more radiogenic rock are found to the north-west of Northampton and in Wales, but geologies with $^{87}\text{Sr}/^{86}\text{Sr}$ values of 0.710-0.712 are found less frequently across Britain, with the most significant outcrops being found in Devon, Cornwall, Cumbria and the north of England – implying substantial distances from Northampton.

Figure 55: LA-MC-ICP-MS plot generated for sample 309.012
Finally, other individuals appear to have undergone significant movement/mobility during early childhood, moving between both geologies akin to Northampton and those beyond the expected range for the region. An example of this is seen below in sample 400.004 (Figure 57) where the individual can be seen to have spent time during early childhood within areas of underlying geology within the expected range for Northampton (0.786-0.7107), but also moved across areas of more radiogenic strontium values too (up to 0.713).
Interestingly, however, all individuals sampled here showed strontium values within the range of 0.786-0.7107 (as indicated by the green shaded areas in the isotope plots; see also Appendix A, section A.3.4.1.) at some point across the growth axis of the enamel of the M1. However, whilst 12 individuals did not exhibit values beyond this range, another seven individuals showed at some point strontium values indicating movement to less radiogenic geologies, and a further seven to more radiogenic geologies. These differences could unfortunately not be correlated to sex given the lack of sex identifications for the individuals sampled here (Table 19). There also does not appear to be any close correlation of this movement to age at death.

Dentine samples were also analysed for a number of teeth (n=11) to characterise the ‘local’ Sr signature of the site and depositional context. As dentine, like bone, is diagenetically altered post-depositionally, it can be utilised to provide a characterisation of the \(^{87}\text{Sr} / ^{86}\text{Sr}\) values of the burial environment (see Chapter 4, section 4.4.3). All dentine samples analysed exhibited \(^{87}\text{Sr} / ^{86}\text{Sr}\) values akin to that of the Northampton geology, 0.709-0.710 (Figure 52).
It is important to note however, in relation to the data presented above, that although the strontium range of 0.786-0.7107 is taken here to be indicative of the underlying geology of Northampton and the surrounding area, whether these values are actually reflective of Northampton itself or not is unfortunately unclear due to the lack of variation in the underlying geology of the UK as a whole (Figure 18). Therefore, whilst it is possible that these individuals may have derived from the Northamptonshire area, and spent their childhood there, due to the lack of variation in geology and subsequently biosphere strontium across the UK, we can only conclude that these individuals originate from an area with a geology akin to that of the Northampton area, and sadly cannot interpret beyond that. Furthermore, given the lack of geological variability across large areas of Britain, it is important to note that it is equally possible that the movement within the strontium values of 0.786-0.7107 may represent mobility beyond Northamptonshire – but due to the geological homogeneity of large swathes of the UK (Figure 18), that this is not isotopically visible.

Traditionally, discussions of Neolithic mobility have focused around ideas of farming populations being highly sedentary, and in marked contrast to the extremely mobile hunter-gatherers of the Mesolithic (see Chapter 2). More recently however, the idea that some degree of Mesolithic mobility may have been retained into the Neolithic period, or that more mobile pastoralism may have been practiced, has been promoted (Edmonds 1999; Milner 2005(a)) – and is starting to change our perceptions of Neolithic mobility. Often, these ideas have been linked to demic diffusionist models posited for the Mesolithic-Neolithic transition (see Chapter 2, section 2.2.4.), promoting ideas of multi-directional movement and exogamous community movement (Sheridan 2010). However, even proponents of an indigenous acculturation (cultural diffusion) model for the Mesolithic-Neolithic transition have also suggested that patterns of mobility seen in the Mesolithic of Britain were maintained into the Neolithic (Thomas 2004(b)).

Alongside the idea that Neolithic mobility may also have been on a seasonal basis, much akin to that seen in the Mesolithic of Britain (Edmonds 1999, 17; see also Chapter 2), the suggestion that populations or communities moved around the landscape in patterns of temporality – linked both to resource procurement and production, but also social relationships – has also been posited by authors such as Pollard (1999). Notions of temporality can thus also be seen to be linked to ideas of biographies and personal or group identity. These concepts are supported by archaeological evidence at sites such as
causewayed enclosures, where communities may have come together at different times to feast and meet. Neolithic movement and temporality can also be seen to be intrinsically linked to broader ideas surrounding kinship networks within the British Neolithic. For example, when discussing the movement and meeting of Neolithic populations in Britain, Edmonds (1999, 17) suggests that “more often than not, those groups comprised close kin”. ‘Kinship’ however does not necessarily have to refer to close descent; groups may be matrilineal, patrilineal, nonunilineal, ambilineal, or work on a cognatic (bilateral) kinship basis – where ancestors can be traced back to commonality through any combination of female or male links (Whittle 2003, 129).

In terms of bioarchaeological evidence to support ideas of Neolithic movement, strontium has contributed significantly (as discussed in Chapter 4). For example, $^{87}$Sr/$^{86}$Sr values obtained from individuals at Cranbourne Chase, a henge monument in Dorset, showed considerable amounts of movement, in the range of 100km. Interestingly, in the children analysed differential strontium values were found in the deciduous vs. the permanent tooth enamel, indicating movement across geologies within early childhood as the teeth were mineralising and forming (Montgomery et al. 2000). A recent strontium isotope study on individuals from the Cotswold-Severn chambered tomb of Hazleton North, Gloucestershire, also indicated degrees of regional movement within individuals, and was suggested by the authors to be reflective of a model of ‘tethered mobility’, routine movement and/or cyclical transhumance (Neil et al. 2016). Movement of Neolithic individuals from a range of different European sites has also been detected using strontium isotopes (an overview of these kinds of study can be found in Chapter 4, section 4.4.5).

It is possible to therefore create a narrative, based upon ideas of Neolithic mobility, kinship networks and communication between groups, and the available bioarchaeological evidence, that there may have been more significant amounts of movement and mobility within British Neolithic populations than has previously been assumed. From the data generated here, it can be seen that some individuals show larger degrees of movement across the growth axis of the tooth than others, which is suggestive of differential mobility between individuals within a population. Given that the teeth analysed here are reflective of a period of early childhood from birth to three years however, the fact that we do not see similar patterns of movement across all individuals suggests that it is unlikely that these populations were practicing seasonal mobility. Instead, this movement must have been driven by other factors. Furthermore, whilst some individuals studied here appear to have
spent their early childhood within an area of underlying geology akin to Northampton, others appear to have moved across areas of both significantly more and significantly less radiogenic geologies – but interestingly, all were buried together at the Banbury Lane site. As such, this ties into ideas surrounding multiple but connected communities within Britain during the Neolithic, kinship networks, and pastoralist communities (as discussed in more detail in Chapter 9).

7.7. Conclusions

It can be seen that Banbury Lane represents a fairly unusual British Neolithic site, and skeletally, does not comprise a ‘typical’ British Neolithic assemblage (see Chapter 3 for a detailed discussion of Neolithic mortuary practices and skeletal evidence available). Stable δ\(^{13}\)C and δ\(^{15}\)N isotope analysis of 165 individuals has revealed remarkable isotopic homogeneity at the site, but strontium isotope analysis has indicated that some these individuals had childhood origins spanning different geological zones across the UK, some of which are significant distances from the site location, and that many experienced significant amounts of mobility during early childhood. However, despite different childhood origins and mobility, all experienced an isotopically similar diet as adults. Furthermore, the secondary deposition of all the remains at Banbury Lane in one episode suggests that these individuals were curated and specifically chosen for deposition at the site. The lack of faunal material from the site has however meant that dating of the monument has been more complex, as has interpretation of the human diets. Whilst the isotopic homogeneity of the human remains is fascinating, the elevated δ\(^{15}\)N values seen appear to be due to elevated δ\(^{15}\)N in the fauna. The reasons behind this remain unclear, but are discussed above in section 7.6.1. The δ\(^{13}\)C and δ\(^{15}\)N isotope values of the Banbury Lane therefore highlight that greater characterisation of British Neolithic fauna is still needed – much the same as for the Mesolithic (see Chapter 5) – and also emphasises the variability which can exist within terrestrial herbivore isotopic values. The use of FRUITS isotopic mixing models however has suggested that terrestrial plants may have comprised a more significant proportion of the diet amongst the Banbury Lane individuals than perhaps would be assumed. However, the FRUITS model should simply be taken as an additional means via which to reconstruct diet. The issues with isotopic mixing models are discussed within Chapter 4 (section 4.3.5.), but it is important to highlight here the lack of inclusion of freshwater fish resources into the Banbury Lane model, due to a lack of published data.
In future, further investigation of a potential freshwater fish component to the diet at Banbury Lane should be explored.

The lack of other very large Neolithic human skeletal assemblages within the UK means the scale of this dataset is currently unparalleled. However, due to this, and the lack of other available Neolithic human skeletal material from Northamptonshire, there is very little comparative data – both dietary $\delta^{13}$C and $\delta^{15}$N and mobility $^{87}$Sr/$^{86}$Sr. Despite this, Banbury Lane provides a unique insight into a large scale assemblage of Neolithic human remains – and the data generated from it can be tied into broader discussions of both Neolithic mobility and diet in Britain, as well as mortuary and funerary practices.

Finally, the $\delta^{13}$C and $\delta^{15}$N isotopic homogeneity seen at Banbury Lane is unusual, but it is interesting to note that given the nature of deposition of the skeletal samples, the assemblage may be socially or dietarily biased – and therefore may not be representative of the population as a whole; particularly given that skeletally the assemblage appears to be dominated by adult males. As Hedges and Reynard (2007) highlight however, there is a danger in suggesting that the differential $\delta^{13}$C and $\delta^{15}$N isotope values, and particularly the enriched $\delta^{15}$N values, seen at Banbury Lane are the result of a minority population with a different subsistence level to the majority – but it is important to recognise that the assemblage from Banbury Lane does not appear to be reflective of the local population as a whole. Furthermore, the dietary homogeneity seen within the population is contrasted by the variability seen in the strontium isotopic data obtained from M1 teeth from the assemblage. The strontium data indicate significant differences in both degrees of movement and the geologies over which this movement occurred. Crucially, it has only been possible to obtain this level of mobility information through the use of LA-MC-ICP-MS – and the resulting data links well to theoretical positions previously put forward for the period in Britain, such as residentially mobility, linked kinship networks, and pastoralist subsistence strategies.
Chapter 8 – Biomolecular Analysis of Dental Calculus: A New Way to Discover Neolithic Diet and Disease

8.1. Determining New Sources of Prehistoric Disease Information: Metaproteomic and Metagenomic Study of Dental Calculus

Whilst the Mesolithic-Neolithic transition is thought to have brought about huge changes in human health and the potential emergence of new diseases, our understanding of health within this period is still hugely limited (see Chapter 2, section 2.4.3.). As outlined in Chapter 4 (section 4.6.), the purpose of this research is to determine if dental calculus may provide a new source of archaeological information which may be able to contribute to our existing knowledge of early prehistoric health and disease (objective 6 of thesis, Chapter 1). As discussed in Chapter 4 (section 4.6.), the study of dental calculus in archaeology has traditionally focused on simply recording the presence/absence and severity of calculus, or on the microscopic analysis of starch grains, phytoliths and food debris entrapped within the calculus matrix. However, the past four years have seen the emergence of metaproteomic and metagenomic analyses of archaeological human dental calculus – which have now revealed that it is a rich source of dietary and disease information (Charlton 2012; Adler et al. 2013; Warinner et al. 2014(a); 2014(b); 2015(b)). Excitingly, initial studies have revealed that archaeological calculus preserves biomolecules relating not only to the human individual and their diet, but also from commensal and pathogenic oral microbial species (Charlton 2012; Warinner et al. 2014(a)). The preservation of these biomolecules is a reflection of the complexity and diversity of microbiota known to inhabit the mouth throughout life;

“the average healthy person carries on the surface of their teeth nearly as many bacteria as there are humans on the Earth, and every day each of us swallows an average of 80 billion bacteria in our saliva” (Warinner et al. 2015(b), 2).

The growing body of clinical and dentistry work on the oral microbiome, the expansion of palaeomicrobiology as a research field, and the recognition that archaeological dental calculus can provide a reservoir of biomolecular information, mean that calcified plaque is emerging as a new way in which to study ancient oral microbiomes, diet, and disease. Previous metaproteomic and metagenomic work on archaeological dental calculus has been undertaken on samples dating back to the Bronze Age (Warinner et al. 2014(a)), but
the viability of earlier prehistoric calculus for biomolecular research has not yet been fully explored. Presented here therefore is the first study of Neolithic dental calculus from the UK using a combined metagenomic and metaproteomic approach – of which the initial results from four British Neolithic sites are provided below. The analyses and data presented here are not intended to be in any way exhaustive – rather, they aim to present a broad overview of the potential of this method. The analysis undertaken aimed to provide a preliminary indication of the survival of biomolecules in dental calculus of this age, in order to gauge whether the data obtained formed a robust dataset, and to assess the degree of contamination apparent within samples of this age. Overall therefore, the work undertaken here provides a preliminary investigation into the potential information which we may be able to obtain from genomic and proteomic studies of this kind on early prehistoric dental calculus, and whether it may be a new and useful means of studying health and disease in the prehistoric past.

An overview of the four sites utilised here is therefore given first – detailing the nature of the site and the skeletal material recovered – followed by the results obtained from the metagenomic and metaproteomic analyses of the dental calculus from each site. A discussion of the potential of the method is provided throughout, and is also revisited within Chapter 9.

8.2. Sites

8.2.1. Hambledon Hill

Hambledon Hill is an early Neolithic monument complex in Dorset, comprising of two causewayed enclosures, two long barrows, and a range of other outer earthworks (Mercer and Healy 2008, xiii; Figure 58). The largest feature at the site is one of the two causewayed enclosures (known as the main causewayed enclosure), which is dated to 3680–3310 cal. BC (Mercer and Healy 2008, 405). The second causewayed enclosure is known as Stepleton enclosure, and is much smaller in size, encompassing about an eighth of the area of the main enclosure, but has a similar date, 3650–3370 cal. BC. The Stepleton spur consists of three causewayed outworks which lie outside and partially replaced the Stepleton enclosure, c.3650–3360 cal. BC, forming a continuous rampart 1km long around the hill the enclosure sits upon (Mercer and Healy 2008, 202-3). Finally, the Shroton spur outwork lies to the east of the main enclosure, and is also believed to be contemporary,
being dated to 3650-3310 cal. BC. The outwork is believed to have acted as a field boundary, and runs for nearly 300m in an arc shape (Mercer 1980, 44; Mercer and Healy 2008, 187 and 405). In between Shroton spur and the main causewayed enclosure lie a series of cross dykes, and the main enclosure is bordered by these cross-dykes on three sides (Figure 58). These cross-dykes have previously been suggested to be controls for herding animals (Malone 2001, 41). It can therefore be seen that Hambledon Hill represents a large and very varied monument complex, with different areas of the site potentially serving different functions, although many of the elements seen across the site appear to be contemporary or similar in date to one another (Mercer and Healy 2008, 405).

Figure 58: Plan of Hambledon Hill monument complex (Mercer and Healy 2008, 5)

Human remains were recovered at Hambledon Hill from sixteen different locations across the complex, but most numerosly from the ditch of the main causewayed enclosure. The
contents of the ditch of the central enclosure appear to differ from those of the subsidiary enclosure in being “more specialised and selected” (Whittle 1992, 220), and consisted of human bone, animal bone, flints and potsherds (Thomas 1999, 75). In total, however, across the site as a whole there are believed to be eleven early Neolithic articulated skeletons present, along with fifteen separate crania, and >1650 pieces of disarticulated bone, representing a potential 75 individuals (McKinley 2008). It has therefore previously been argued that the assemblage at Hambledon Hill represents the largest number of human remains recovered from any southern English Neolithic site (Whittle 1988, 147; although see Chapter 7). Interestingly, both non-adults (n= 24) and adult individuals (n= 25) are well-represented at the site (McKinley 2008), including two juvenile articulated burials whose appearance is believed to have been affected in life due to the premature fusing of their crania, which have subsequently been linked to ideas surrounding Neolithic emotion, phenomenology, and mnemonics (Harris 2010).

Human remains sampled in this study come from a number of locations across the monument complex, although all are Neolithic in date. The table below details the individuals sampled for dental calculus here, and their associated osteological information (from McKinley 2008; Table 20). Associated dates for all of these locations can be found in the text above.

<table>
<thead>
<tr>
<th>Finds Number</th>
<th>Sample Number</th>
<th>Location Type</th>
<th>Age</th>
<th>Sex</th>
<th>Pathology</th>
<th>Other Info.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH74 HB3</td>
<td>HH3</td>
<td>Main enclosure Frag.</td>
<td>Infant/Juvenile Unknown</td>
<td>Calculus</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>HH77 610</td>
<td>HH610</td>
<td>Main enclosure Frag.</td>
<td>Older adult Unknown</td>
<td>AMTL, caries, calculus, PD, OA, MV</td>
<td>Cut marks, ?sooting</td>
<td></td>
</tr>
<tr>
<td>HH76 1916</td>
<td>HH1916</td>
<td>Shroton spur outwork Frag.</td>
<td>Older adult Female</td>
<td>Calculus, PD, abscess</td>
<td>Cut marks</td>
<td></td>
</tr>
<tr>
<td>ST81 3181</td>
<td>HH3181</td>
<td>Stepleton spur 4B F712 Artic</td>
<td>Young adult Male</td>
<td>Calculus, OP, OA, MV, squatting facets</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>ST81 3188</td>
<td>HH3188</td>
<td>Stepleton enclosure Artic</td>
<td>Mature adult Female</td>
<td>Calculus, PD, OA, OP, MV</td>
<td>Sooting, rodent ganwing</td>
<td></td>
</tr>
</tbody>
</table>
Table 20: Individuals sampled for dental calculus within this study from the Hambledon Hill assemblage (adapted from McKinley 2008). (Pathology: AMTL = ante-mortem tooth loss; PD = periodontal disease; OA = osteoarthritis; OP = osteophytes; MV = morphological variation). Sample numbers detailed here were created by the author for ease of analysis

A range of previous study has been undertaken on the human remains from Hambledon Hill. Stable isotopic analysis of δ\(^{13}\)C and δ\(^{15}\)N on some of the individuals from the site has revealed a diet based on terrestrial protein and C\(_3\) plants, and as such, have been utilised within debates surrounding the abandonment of marine resources in the British Neolithic and a rapid dietary change from the Mesolithic (Richards and Hedges 1999; Richards 2008). Additional isotopic work on the human remains from Hambledon Hill has focused around trying to determine evidence of manuring and crop management in the British Neolithic (Bogaard et al. 2007; 2013).

Additional dietary information has been obtained from the faunal remains recovered from the two causewayed enclosures and the long barrow at the site. Domesticated cattle (\textit{Bos taurus}) are the most dominant species at Hambledon Hill, followed by smaller amounts of pig (\textit{Sus scrofa}) and ovicaprids (\textit{Ovis aries} and \textit{Capra hircus}). Much smaller numbers of bones from deer, domestic dogs and a number of wild species (e.g. wild boar, beaver, badger, hare, fox, pine marten) were also recovered (Legge 2008). The high number of meat-bearing bones, notably from cattle and pigs, has been interpreted as evidence of feasting at the site, and also as intentional offerings or wasting of meat (Thomas 1999, 27). Previously, the presence of mature cows at the site was suggested that the assemblage may be indicative of a dairying herd (Whittle 1992, 221), but analysis by Legge (2008) found that across the entire monument complex, cattle had a restricted age distribution and were predominately early adult animals, between 15 months to 6 years of age. Interestingly, the majority of cattle remains were also female, and the narrow age distribution of the remains suggests that the animals were unlikely to have derived from a resident herd. Alongside this, the high number of cattle skulls has also been interpreted as being representative of an association between the deposition of human and faunal remains (Thomas 1999, 28), and parallels between the treatment of human and cattle remains has been suggested to represent ‘respectful consumption’ of beef (Jones 2007, 164). The large scale deposition of cattle remains however, alongside large amounts of burnt wheat, barley and hazelnuts, has prompted suggestions of feasting at the site (Mercer 2008; Jones and Legge 2008).
Finally, potential information on diet and dairy consumption at Hambledon Hill has been obtained through organic residue analysis of pottery from the site (Copley et al. 2003; 2008). This has indicated the presence of both porcine and ruminant fats, and ruminant adipose and dairy fats. The presence of dairy fats in >25% of pot sherds analysed has been suggested to indicate that “dairying was a very important element of animal husbandry at Hambledon Hill” (Copley et al. 2008, 535).

8.2.2. Hazleton North

Hazleton North is a Cotswold-Severn chambered tomb near Cheltenham, Gloucestershire, dating to c.3800-3620 cal. BC (Saville et al. 1987; Meadows et al. 2007). The site is a trapezoidal lateral long cairn, and is one of a pair of monuments (the other being termed Hazleton South). Internally, the tomb has two lateral chambers, one northern and one southern, which each consist of an entrance, a passage and a chamber, and are both L-shaped. Each chambered area was accessed separately from opposite sides of the monument, with the entrances being roughly central to the cairn as a whole (Saville et al. 1987; Saville 1990; Meadows et al. 2007; Figure 59).
An MNI of 35 individuals were recovered from Hazleton North, comprising of adults (n=21), non-adults (n=13), and foetuses (n=2). A range of different interments were also recovered, with articulated inhumations, cremations and disarticulated remains all present within the tomb (Saville 1990, 80 and 250; Table 21). Overall, a total of >9,000 human bones were recovered from the site (Rogers 1990; Figure 60). The range of articulated inhumations, partially articulated elements, and disarticulated human remains across the site has resulted in the hypothesis that intact corpses were initially introduced into both chambered areas, and following decomposition were disarticulated and moved to elsewhere within the chamber to allow for the introduction of more remains and to allow access into the tomb (Saville 1990, 251).
<table>
<thead>
<tr>
<th>Area</th>
<th>Remains present</th>
</tr>
</thead>
</table>
| North entrance                     | 1 extended adult male inhumation  
1 crouched adult ?male inhumation (incomplete)  
2 non-adult inhumations  
Disarticulated remains of 1 adult male  
Cremated remains of one adult and one non-adult |
| North chamber                      | Disarticulated remains of 2 ?male adults  
Disarticulated remains of 2 ?female adults  
Disarticulated remains of 4-6 non-adults  
Remains of 1 foetus (4-5 months old) |
| South chambered area (entrance, passage and chamber combined) | Disarticulated remains of 14 adults  
Disarticulated remains of 6-11 non-adults  
Remains of 1 foetus (6-7 months old) |
| South entrance                     | 1 articulated group of 5 vertebrae  
1 articulated right femur, fibula and tibia  
1 articulated mandible and maxilla  
1 group of ribs in correct anatomical order  
4 adult and 1 non-adult crania |
| South passage                      | 1 articulated group of 6 vertebrae  
3 adult and 1 non-adult crania |
| South chamber                      | 1 group of ribs in correct anatomical order  
3 adult and 3 non-adult crania |

Table 21: Human remains present in each area of the chambered tomb at Hazleton North. Information taken from Saville (1990) and Rogers (1990)
Eight individuals were sampled here for dental calculus, and come from a number of different areas within the cairn itself (Table 22):

<table>
<thead>
<tr>
<th>Finds Number</th>
<th>Sample Number</th>
<th>Location</th>
<th>Type</th>
<th>Age</th>
<th>Sex</th>
<th>Pathology</th>
<th>Other Info.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN81 3793</td>
<td>HN3793</td>
<td>South entrance, SE quadrant</td>
<td>Frag.</td>
<td>25-35 years</td>
<td>Male</td>
<td>Calculus, caries, PD</td>
<td>Mandible glued back together</td>
</tr>
<tr>
<td>HN81 4786</td>
<td>HN4786</td>
<td>South passage, SE quadrant</td>
<td>Frag.</td>
<td>25-35 years</td>
<td></td>
<td>Calculus, ?PD</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 60: Plan of human bone from both the north and south chambered areas at Hazleton North (Saville 1990, 93 and 99)
<table>
<thead>
<tr>
<th>Number</th>
<th>Fragment</th>
<th>Site/Location</th>
<th>Sex</th>
<th>Age</th>
<th>Pathology</th>
<th>Other Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN81 5037-1 (Skeleton 1)</td>
<td>Artic. 33-45 years Male</td>
<td>Calculus, PD, caries, AMTL, abscesses</td>
<td>Extended inhumation; stature estimate of 169.8 cm; animal gnawing; some teeth glued back into mandible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN81 5880</td>
<td>Frag. 17-25 years Male</td>
<td>Calculus, PD, AMTL</td>
<td>Maxilla/ crania glued together at points</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN81 7387</td>
<td>Frag. Male</td>
<td>Calculus, bone resorption, PD</td>
<td>Mandible glued back together</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBG81 7656</td>
<td>Frag. 25-35 years</td>
<td>Calculus, PD</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN82 10213</td>
<td>Frag. 25-35 years Female</td>
<td>Calculus, PD, AMTL</td>
<td>Flecks of paint on teeth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN82 11456</td>
<td>Frag. 35-45 years</td>
<td>Calculus, bone resorption, PD, AMTL, caries, abscesses, OA</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22: Individuals sampled for dental calculus within this study from Hazleton North (osteological data from Rogers 1990). (Pathology: AMTL = ante-mortem tooth loss; PD = periodontal disease; OA = osteoarthritis). Sample numbers detailed here were created by the author for ease of analysis.

The human remains from Hazleton North have not been studied extensively since their excavation. Stable isotopic analysis of $\delta^{13}$C and $\delta^{15}$N on some of the individuals from the site has however revealed a diet high in meat and animal protein, supplemented by C$_3$ plants (Hedges et al. 2008). Interestingly, the human isotopic values from the site were seen to have little variability, indicating “an isotopically homogenous population” (Hedges et al. 2008, 122) – which is very similar to isotopic patterning seen at the site of Banbury Lane (discussed below and in Chapter 7). However, significant isotopic variation was seen in terrestrial fauna at Hazleton North, even within the same species. This was suggested to
indicate differential management of domesticated species, particularly sheep (Hedges et al. 2008).

Alongside the human remains within the chambered areas of the tomb, a smaller number of animal bones were also recovered, predominately larger mammals (domesticated cattle, sheep and pig; total of 24 fragments), but also four fragments of roe deer, and one dog scapula. Unusually, the partial remains of a perinatal ovicaprid were also recovered from the south chamber – bearing similarities to the recovery of new-born and foetal lambs, calves, and deer at Quanterness in Orkney – and thus being tentatively ascribed as a ‘ritual’ deposition, presumably in the spring (Levitan 1990).

8.2.3. Coldrum

Coldrum is a dolmen monument (a single chambered megalithic tomb) near Maidstone in Kent, which forms one of the Medway group of megalithic monuments. The Medway group have a number of shared architectural motifs, which are not seen elsewhere in Britain. They are all constructed from locally sourced sarsen stones, generally taking the form of rectangular chambers with a single entrance, at the end of mounds or long barrows. The internal chambers are often divided by medial slabs. Of the Medway megaliths however, Coldrum is the only directly dated site from the group, and therefore remains important for understanding these regional structures (Bennett 1913; Ashbee 1993; Wysocki et al. 2013).

Coldrum consists of a low rectangular mound, bounded by (a now partly ruined) revetment of sarsen stone slabs, sat atop the edge of a high slope, meaning that the monument has views over the Medway Valley. At the eastern end of the mound lies a dolmen – a rectangular chamber built from four large sarsens, divided internally into eastern and western chambers or compartments. Due to its form, Coldrum is often considered to be a cromlech – a dolmen surrounded by stones. However, the exact arrangement of stones at the site, their size, and their regularity is unique. The exact form the monument and its immediate surrounding environs may have originally undertaken is however still unclear, and will remain so, due to the fact the monument has been heavily damaged, and many stones appear to have been removed. A significant number of collapsed sarsen stones have been found at the foot of the slope below the monument however – but whether these represent part of the original Coldrum monument, or another monument entirely, is unclear
Recent dating of the site indicates that the monument at Coldrum was first utilised 3980-3800 cal. BC, and extended to 3730-3540 cal. BC (Wysocki et al. 2013) – thereby making it one of the few early Neolithic sites in the UK.

Figure 61: Plan of Coldrum monument, with dolmen indicated in the centre of the rectangular long barrow (Ashbee 1998, 12)

Human remains have been recovered from Coldrum throughout a series of excavations from 1910 onwards, although it was noted that two skulls were also recovered from the site
in 1804 and 1825 (Bennett 1913; Keith 1913; Ashbee 1993). A full record of the archaeological work undertaken at Coldrum, including early plans of the site, can be found in Ashbee (1993; 1998). An MNI of 17 individuals has most recently been suggested for Coldrum, consisting of nine adults (four female; five male), two older non-adults (c.16-20 years old), and six non-adults (Wysocki et al. 2013).

Three individuals from Coldrum with calculus were utilised here (Table 23). Calculus from these individuals was sampled by Sam Neil, as the teeth were being analysed for isotopic analysis (pers. comm.). Interestingly, however, the presence of calculus was not noted in the original osteological analysis of the remains (Keith 1913) – although this may be due to the very small amounts of calculus present on the teeth (c.5mg or less). It should also be noted that all three individuals were initially believed to be Neolithic in date, but that subsequent AMS dating revealed one individual to in fact date to the 5th-6th century AD (Table 23) – and so is included here as a comparative sample.

<table>
<thead>
<tr>
<th>Finds Number</th>
<th>Age</th>
<th>Pathology</th>
<th>AMS Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL EU.1.5.130</td>
<td>Adult</td>
<td>Calculus</td>
<td>5th-6th C AD</td>
</tr>
<tr>
<td>COL UN</td>
<td>Adult</td>
<td>Calculus</td>
<td>4000-3800 cal. BC</td>
</tr>
<tr>
<td>COL UN8</td>
<td>Non-adult, &gt;12 years</td>
<td>Calculus</td>
<td>4000-3800 cal. BC</td>
</tr>
</tbody>
</table>

Table 23: Individuals sampled for dental calculus within this study from the Coldrum assemblage. Age determination is based upon dental eruption/tooth formation following AlQahtani et al. (2010), undertaken by Sam Neil. AMS dates were provided by Sam Neil (pers. comm.)

Significant osteological re-analysis of the human skeletal assemblage from Coldrum has recently been undertaken by Wysocki et al. (2013), following previous analysis in the early 20th century, which focused heavily on craniometrics (Keith 1913). This recent analysis most notably focused on the significant amount of anthropogenic modification seen on the human remains at Coldrum however – which included cranial trauma indicative of interpersonal violence, and cut-marks on six post-cranial elements suggesting dismemberment/disarticulation using lithic tools, on already partially skeletonised remains (Wysokci and Whittle 2000; Wysocki et al. 2013). The Coldrum assemblage is therefore suggested to be the “largest [assemblage] exhibiting peri-mortem dismemberment so far reported from a southern British Neolithic long barrow” (Wysocki et al. 2013, 8).

Stable isotope analysis has also been undertaken on some of the human remains from the site, and revealed similar δ13C values to other British Neolithic sites, but more elevated
δ¹⁵N values. A lack of faunal remains from the site however meant that no faunal isotopic baselines could be created on which to base interpretations of the human data – and therefore understanding of diet at the site still remains somewhat limited. The elevated δ¹⁵N values, which also appear to increase through time, were suggested by the authors to potentially indicate a diet high in terrestrial animal protein, but with some reliance or inclusion of freshwater river resources (Wysocki et al. 2013) – although it is also surely feasible that the δ¹⁵N values may represent a manuring effect (as described in Bogaard et al. 2007).

8.2.4. Banbury Lane

Banbury Lane is a recently excavated middle Neolithic triple-ditched monument, located in Northampton. An overview of the site, its background, context, finds, and human skeletal assemblage can be found in Chapter 7. Five individuals were sampled within this study for dental calculus, and samples of bone from the maxilla were also taken from three of the five (Table 24). Bone samples were taken as a control for calculus samples.

<table>
<thead>
<tr>
<th>Finds Number</th>
<th>Layer</th>
<th>Type</th>
<th>Age</th>
<th>Sex</th>
<th>Pathology</th>
<th>Other Info.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 132.17</td>
<td>2</td>
<td>Frag.</td>
<td>Adolescent/ Young adult</td>
<td>Unknown</td>
<td>Calculus</td>
<td>Root marks</td>
</tr>
<tr>
<td>BL 201.1</td>
<td>3</td>
<td>Frag.</td>
<td>18-25 years</td>
<td>Unknown</td>
<td>Calculus</td>
<td>Root marks, sampled for both calculus and bone</td>
</tr>
<tr>
<td>BL 201.2</td>
<td>3</td>
<td>Frag.</td>
<td>18-25 years</td>
<td>Unknown</td>
<td>Calculus</td>
<td>Root marks, sampled for both calculus and bone</td>
</tr>
<tr>
<td>BL 309.12</td>
<td>4</td>
<td>Frag.</td>
<td>26-35 years</td>
<td>Unknown</td>
<td>Calculus, PD, rotation of both premolars</td>
<td>Root marks</td>
</tr>
<tr>
<td>BL 440.4</td>
<td>5</td>
<td>Frag.</td>
<td>10-17 years</td>
<td>Unknown</td>
<td>Calculus</td>
<td>Root marks, sampled for both calculus and bone</td>
</tr>
</tbody>
</table>

Table 24: Individuals sampled for calculus in this study from Banbury Lane. Age, sex, and root mark information taken from Caffell and Holst (2012)
The Banbury Lane skeletal assemblage has been subject to extensive $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis, but unfortunately, due to the disarticulated nature of the assemblage, specific $\delta^{13}C$ and $\delta^{15}N$ isotopic values cannot be linked to individuals utilised in this calculus study. However, it is worth noting that the Banbury Lane assemblage as a whole showed a remarkable degree of isotopic homogeneity, indicating that all individuals at the site had an isotopically similar diet (Chapter 7) – as was also noted to be seen at Hazleton North (see section 8.8.2. above). The Banbury Lane assemblage did however also exhibit relatively enriched human $\delta^{15}N$ values (much like those noted at Coldrum, see section 8.8.3. above), which may indicate a manuring effect too. $^{87}$Sr/$^{86}$Sr isotopic analysis was also undertaken, the results of which can be found in Chapter 7, and which indicated significant childhood movement in some individuals from the site.

8.3. Materials and Methods

Overall, a total of thirty-three individual calculus samples from the four sites were utilised in this research, along with three bone samples. All calculus samples were collected using a bleached-sterilised dental pick, and stored in individual eppendorf tubes per tooth. No distinction during collection was made between supragingival and subgingival calculus on the archaeological specimens (as in Warinner et al. 2014(a)).

Twenty-three samples (twenty calculus samples, and three bone samples) from the four sites were analysed for aDNA (Table 25). This comprised of five calculus samples from Hambledon Hill, eight calculus samples from Hazleton North, three calculus samples from Coldrum, and four calculus and three bone samples from Banbury Lane. Calculus samples were weighed to <50mg, and bone samples c.50-100mg for analysis. Additional information on all individuals sampled can be found in Tables 20 and 22-24. DNA was extracted and sequenced using previously published protocols (Meyer and Kircher 2010; Dabney et al. 2013; Warinner et al. 2014(a)), utilising an extraction methodology designed for the optimal recovery of short DNA fragments in archaeological samples, followed by Illumina shotgun metagenomic sequencing (see also Chapter 4, section 4.6.2.3.1.).
<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Number</th>
<th>Sample Type</th>
<th>Calculus Location</th>
<th>Weight Used /mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hambledon Hill</td>
<td>HH3</td>
<td>Calculus</td>
<td>Maxillary left M2</td>
<td>15.4</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH610</td>
<td>Calculus</td>
<td>Mandibular left I2</td>
<td>15.3</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH3188</td>
<td>Calculus</td>
<td>Mandibular left M3</td>
<td>17.3</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN4786</td>
<td>Calculus</td>
<td>Maxillary right I2</td>
<td>17.9</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN5037-1</td>
<td>Calculus</td>
<td>Mandibular right M3</td>
<td>21.6</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN7387</td>
<td>Calculus</td>
<td>Mandibular left I2</td>
<td>33.3</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN7656</td>
<td>Calculus</td>
<td>Mandibular left PM1 &amp; PM2 (combined)</td>
<td>18.0</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN11456</td>
<td>Calculus</td>
<td>Mandibular right M2</td>
<td>23.1</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL132.17</td>
<td>Calculus</td>
<td>Maxillary left M1</td>
<td>2.5</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL309.12</td>
<td>Calculus</td>
<td>Maxillary left M1</td>
<td>14.7</td>
</tr>
</tbody>
</table>

Table 25: Samples utilised for aDNA analysis

Ten calculus samples from three of the four sites (Table 26) were also analysed using a previously published shotgun metaproteomic approach utilising liquid chromatography-tandem mass spectrometry (Warinner et al. 2014(a); 2014(b); see also Chapter 4, section 4.6.2.3.2.). The ten samples comprised of three samples from Hambledon Hill, five samples from Hazleton North, and two samples from Banbury Lane. Wherever possible, the same individuals utilised for aDNA analysis were selected. Calculus samples were weighed to <50mg for proteomic analysis.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Number</th>
<th>Sample Type</th>
<th>Calculus Location</th>
<th>Weight Used /mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hambledon Hill</td>
<td>HH3</td>
<td>Calculus</td>
<td>Maxillary left M2</td>
<td>15.4</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH610</td>
<td>Calculus</td>
<td>Mandibular left I2</td>
<td>15.3</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH3188</td>
<td>Calculus</td>
<td>Mandibular left M3</td>
<td>17.3</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN4786</td>
<td>Calculus</td>
<td>Maxillary right I2</td>
<td>17.9</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN5037-1</td>
<td>Calculus</td>
<td>Mandibular right M3</td>
<td>21.6</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN7387</td>
<td>Calculus</td>
<td>Mandibular left I2</td>
<td>33.3</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN7656</td>
<td>Calculus</td>
<td>Mandibular left PM1 &amp; PM2 (combined)</td>
<td>18.0</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN11456</td>
<td>Calculus</td>
<td>Mandibular right M2</td>
<td>23.1</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL132.17</td>
<td>Calculus</td>
<td>Maxillary left M1</td>
<td>2.5</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL309.12</td>
<td>Calculus</td>
<td>Maxillary left M1</td>
<td>14.7</td>
</tr>
</tbody>
</table>

Table 26: Samples utilised for proteomic analysis
Details of the extraction protocols utilised in aDNA extraction, sequence generation and shotgun library building, protein extraction, and data analysis information can be found in Appendix A (section A.4).

8.5. Results and Discussion

8.5.1. Calculus as a reservoir for early prehistoric aDNA

As discussed above, the oldest calculus samples yet analysed using a combined metaproteomic and metagenomic approach date to the Bronze Age (Warinner et al. 2014(b)). The investigation of Neolithic calculus samples here, therefore, aimed to determine the potential time depth of survival of biomolecules within dental calculus. Through the use of shotgun DNA sequencing, as in Warinner et al. (2014(a)), DNA was successfully extracted from all calculus (and bone) samples analysed (see Table S13 and Figure S23), and DNA extracts from 18 of the 25 Neolithic samples were sent for sequencing (choosing only those samples with the highest ng/µl Qubit quantification). The results of this, including statistics of read generation and post-quality control filtering, along with reads mapped to the human genome, and % endogenous DNA for each sample, are provided in Table 27 below.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Index Sequence</th>
<th>No. of processed reads (Total Reads)</th>
<th>No. of trimmed reads (Final reads, post-QC)</th>
<th>Mean Insert Size</th>
<th>Reads mapped to human genome (without duplicates)</th>
<th>Total reads mapped (with duplicates)</th>
<th>% endogenous DNA</th>
<th>% coverage of human genome</th>
<th>Mean Depth Coverage (Human Genome)</th>
<th>Material Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL201.1C</td>
<td>CCTGGAT</td>
<td>52153 758</td>
<td>4174 8898</td>
<td>26.80</td>
<td>15532 1</td>
<td>15822 1</td>
<td>0.37</td>
<td>0.09%</td>
<td>1.74</td>
<td>Calculus</td>
</tr>
<tr>
<td>BL201.1B</td>
<td>GCCAGT</td>
<td>44870 410</td>
<td>3870 6426</td>
<td>23.36</td>
<td>13965 58</td>
<td>15393 48</td>
<td>3.60</td>
<td>0.52%</td>
<td>2.01</td>
<td>Bone</td>
</tr>
<tr>
<td>BL309.12C</td>
<td>CGATCGG</td>
<td>37896 412</td>
<td>3536 5300</td>
<td>25.33</td>
<td>69740 70130</td>
<td>19 0.19</td>
<td>0.03%</td>
<td>1.71 Calculus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL201.2C</td>
<td>AGTTGACC</td>
<td>43683 506</td>
<td>3691 9826</td>
<td>25.03</td>
<td>23813 7</td>
<td>24081 6</td>
<td>0.64</td>
<td>0.09%</td>
<td>2.05</td>
<td>Calculus</td>
</tr>
<tr>
<td>BL201.2B</td>
<td>ATTATCGA</td>
<td>39866 164</td>
<td>3744 2662</td>
<td>23.54</td>
<td>11165 59</td>
<td>14998 31</td>
<td>2.98</td>
<td>0.43%</td>
<td>2.04</td>
<td>Bone</td>
</tr>
<tr>
<td>BL440.4B</td>
<td>GATGATTA</td>
<td>32401 900</td>
<td>3076 9754</td>
<td>23.57</td>
<td>44467 4</td>
<td>74834 3</td>
<td>1.4</td>
<td>0.15%</td>
<td>2.22</td>
<td>Bone</td>
</tr>
<tr>
<td>COL1.1033</td>
<td>AGAACGAC</td>
<td>43308 462</td>
<td>3828 9690</td>
<td>23.55</td>
<td>88932 8</td>
<td>90859 5</td>
<td>2.32</td>
<td>0.40%</td>
<td>1.71</td>
<td>Calculus</td>
</tr>
<tr>
<td>COL1.1035</td>
<td>TCCGTCCG</td>
<td>47869 412</td>
<td>3522 7558</td>
<td>24.66</td>
<td>11045 4</td>
<td>11315 2</td>
<td>0.31</td>
<td>0.05%</td>
<td>1.67</td>
<td>Calculus</td>
</tr>
<tr>
<td>COL1.1038</td>
<td>TTGGCGAGA</td>
<td>47841 510</td>
<td>3852 5910</td>
<td>24.90</td>
<td>18859 9</td>
<td>19093 5</td>
<td>0.48</td>
<td>0.08%</td>
<td>1.85</td>
<td>Calculus</td>
</tr>
<tr>
<td>HH3C</td>
<td>ACGAATT</td>
<td>18578 132</td>
<td>1826 1706</td>
<td>23.87</td>
<td>56011 5</td>
<td>56281 2</td>
<td>3.06</td>
<td>0.26%</td>
<td>1.64</td>
<td>Calculus</td>
</tr>
<tr>
<td>HH191.6C</td>
<td>AACCAGAAC</td>
<td>37929 292</td>
<td>3089 7124</td>
<td>25.17</td>
<td>87766 8</td>
<td>88318 2</td>
<td>0.28</td>
<td>0.04%</td>
<td>1.73</td>
<td>Calcus</td>
</tr>
<tr>
<td>HH610</td>
<td>GAACGCATT</td>
<td>39627 342</td>
<td>3330 3144</td>
<td>26.93</td>
<td>85701 5</td>
<td>86145 2</td>
<td>0.25</td>
<td>0.05%</td>
<td>1.69</td>
<td>Calcus</td>
</tr>
<tr>
<td>HH318.1</td>
<td>CTGGAGA</td>
<td>53666 792</td>
<td>4174 4070</td>
<td>24.94</td>
<td>10202 5</td>
<td>10370 1</td>
<td>0.24</td>
<td>0.05%</td>
<td>1.70</td>
<td>Calcus</td>
</tr>
<tr>
<td>HN114.56C</td>
<td>ATAGTTA</td>
<td>35103 704</td>
<td>3395 6576</td>
<td>25.31</td>
<td>98965 9</td>
<td>99814 1</td>
<td>0.29</td>
<td>0.05%</td>
<td>1.78</td>
<td>Calcus</td>
</tr>
<tr>
<td>HN503.7</td>
<td>TGGAACGCA</td>
<td>35499 84</td>
<td>3255 1836</td>
<td>24.22</td>
<td>16570 9</td>
<td>16635 4</td>
<td>0.50</td>
<td>0.09%</td>
<td>1.59</td>
<td>Calcus</td>
</tr>
<tr>
<td>HN478.6</td>
<td>CAATTGAGG</td>
<td>89949 94</td>
<td>8038 484</td>
<td>25.68</td>
<td>28772 29032</td>
<td>0.35</td>
<td>0.02%</td>
<td>1.61 Calcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN765.6</td>
<td>CCAGAATTCT</td>
<td>34522 90</td>
<td>3144 1902</td>
<td>25.02</td>
<td>61591 6</td>
<td>62161 1</td>
<td>0.19</td>
<td>0.03%</td>
<td>1.63</td>
<td>Calcus</td>
</tr>
<tr>
<td>HN379.3</td>
<td>GCGTTAGAGC</td>
<td>39184 744</td>
<td>3264 2572</td>
<td>45.89</td>
<td>14473 6</td>
<td>14583 0</td>
<td>0.44</td>
<td>0.11%</td>
<td>1.76</td>
<td>Calcus</td>
</tr>
<tr>
<td>eBK1</td>
<td>TTATGAGTC</td>
<td>70566 92</td>
<td>2787 6</td>
<td>23.75</td>
<td>953 963</td>
<td>N/A</td>
<td>0.00%</td>
<td>N/A</td>
<td>ExtraCTION blank</td>
<td></td>
</tr>
<tr>
<td>eBK2</td>
<td>GTTCGGCGC</td>
<td>38138 42</td>
<td>2347 864</td>
<td>24.10</td>
<td>62322 78899</td>
<td>N/A</td>
<td>0.03%</td>
<td>N/A</td>
<td>ExtraCTION blank</td>
<td></td>
</tr>
<tr>
<td>LBL1</td>
<td>TGGTCTCTG</td>
<td>16605 8</td>
<td>1112 --</td>
<td>--</td>
<td>28 28</td>
<td>N/A</td>
<td>0.00%</td>
<td>N/A</td>
<td>Library blank</td>
<td></td>
</tr>
<tr>
<td>LBL2</td>
<td>TGGCGAGTTA</td>
<td>93707 4</td>
<td>5800 8</td>
<td>22.9</td>
<td>7573 10109</td>
<td>N/A</td>
<td>0.00%</td>
<td>N/A</td>
<td>Library blank</td>
<td></td>
</tr>
</tbody>
</table>

Table 27: DNA data obtained from all Neolithic samples
The total number of reads and the number of post-QC reads for the calculus samples analysed here (Table 27) can be seen to be significantly higher than those reported by Warinner et al. (2014(a)) from shotgun analysis. Shotgun data generated from Medieval dental calculus samples by Warinner et al. (2014(a)) averaged total reads of >10 million (average = 10,809,817 reads), whereas the average number of total reads of the calculus samples analysed here was >34 million (average = 34,189,350 reads). Similarly, the average number of post-QC reads in the Warinner et al. (2014(a)) study was 10,408,616, but 32,594,306 reads within this research. It is worth noting however, that the shotgun analysis by Warinner et al. (2014(a)) was only undertaken on two individuals (but multiple calculus samples from each individual), compared to the eighteen different individuals analysed here. The increased number of reads from the calculus samples analysed here may also be the result of altered extraction protocols (detailed in Appendix A, section A.4.1.). It is also worth noting that 16S rRNA analysis of Neolithic calculus previously undertaken by Adler et al. (2013) unfortunately did not provide the number of reads per sample, but the number of sequences reported is in the order of thousands, rather than millions.

Table 27 also highlights the number of reads within each calculus sample mapping to the human genome. To date, the endogenous human DNA content of dental calculus has not yet been fully investigated, and therefore there is no comparative data currently available. Warinner et al. (2015(a)) suggest that host DNA may comprise only c.0.5% of DNA in archaeological dental calculus, and the data obtained here appears to support this, with endogenous human DNA comprising only a small fraction of DNA within the calculus sample, and bacterial DNA instead dominating the DNA extract. It can be seen that the % endogenous DNA content of the calculus samples is very low (averaging only 0.67%; Table 27), whereas, for example, aDNA extractions from bone of a Neolithic date from Europe (i.e. temperate climate and northern latitude), in a recent publication by Skoglund et al. (2012) ranged from >2% to over 6% endogenous content. Nonetheless, the endogenous DNA content of dental calculus may still be such that it can be utilised to gain genetic information about the host, and could be utilised in a similar way to aDNA obtained from skeletal (bone or tooth) samples. The idea of calculus as new source of human or host DNA, which can be utilised alongside osteological data, although previously unexplored, may hold great potential, as discussed below (sections 8.5.3. and 8.5.4.).
Finally, in order to accurately authenticate that the DNA (both bacterial and endogenous) obtained from the calculus samples here was indeed ancient, the mapDamage2.0 package (Jónsson et al. 2013) was utilised to assess damage patterns in the sequencing reads. aDNA sequences should typically show an increase in C-T (cytosine to thymine) substitutions toward the sequencing start (3’), and corresponding G-A (guanine to adenine) transitions toward read ends (Jónsson et al. 2013). All calculus and bone samples analysed here broadly showed these characteristic misincorporation patterns, whereas blank samples did not (see Appendix A, section A.4.4.1.2.; Figures S26-S46). The presence of these C-T and G-A substitutions is highly important, as they are widely considered an authentication criterion in ancient DNA studies (e.g. Krause et al. 2010; Prüfer and Meyer 2015) – as very recently highlighted by Weiβ et al. (2015) – thus proving that the DNA obtained is truly ancient, and not the result of exogenous contamination.

8.5.2. Bacterial Composition and Pathogens

As discussed within Chapter 2 (section 2.4.3) and Chapter 4 (section 4.6), the Neolithic period is of key importance when assessing prehistoric health and disease, as it is believed that the huge changes in lifeways and diet occurring at this time may have resulted in the emergence and development of new diseases and health states. In particular, it has been suggested that the transition from hunter-gatherer-fisher lifeways to agriculturalists may have resulted in the emergence of many ‘modern-day’ diseases – such as diabetes, for example. Previously, however, prehistoric health and disease has been notoriously difficult to study, owing to the lack of skeletal remains available and the fact that few diseases will leave physical traces on the skeleton. Dental calculus therefore offers a new way in which we can assess and explore health and disease at this important period within prehistory.

All trimmed, quality filtered, and merged fastq files (generated through Cutadapt (Martin 2011) and PEAR (Zhang et al. 2013)) from the DNA analysis were uploaded to OneCodex (https://beta.onecodex.com) for sample comparisons at the phyla level (see Appendix A, section A.4.4.1.). From this broad level comparison, it could be seen that all calculus samples had predominately oral bacteria present, and aligned with what we may expect to see from an oral sample – displaying a number of the same bacteria as reported by Warinner et al. (2014(a)) and Adler et al. (2013); discussed in more detail below. Conversely, the bone samples analysed alongside the calculus samples (as a control for the depositional environment), did not display any oral bacteria, and at the phyla level showed
a completely different bacterial composition. Similarly, the negative controls also run (both
two extraction blanks and two library building blanks) also showed a very different
composition to the calculus samples. This is particularly reassuring, as it indicates that the
bacterial species present within the calculus samples are indeed ancient, and are not the
result of modern contamination, nor are they products of the depositional context.

Figure 62 highlights these differences, showing a phylum level comparison of one calculus
sample from each site analysed here, two bone samples (from Banbury Lane), an
extraction blank, and a library blank. It can be seen that the dominant phylum within the
bone samples is Actinobacteria, which comprises a bacterial group amongst one of the
largest taxonomic units of the Bacteria domain. As such, the Actinobacteria phylum
comprises a range of different kinds of bacteria, including pathogens, plant commensals,
soil inhabitants, and gastrointestinal inhabitants (Ventura et al. 2007). The presence of
Actinobacteria within bone samples is therefore unsurprising, and likely indicates that they
represent soil bacteria, such as Streptomyces, for example. However, Actinobacteria can
also be seen to be present in varying quantities within the calculus samples depicted, which
again may represent soil bacteria, incorporated into the sample through adhering soil on the
calculus surface, rather than inclusion within the matrix itself (see SEM images in
Warinner et al. 2014(a) for an indication of the lack of post-depositional microbial attack
to calculus samples, vs. bone or dentine). Alternatively, a proportion of the Actinobacteria
present within the calculus samples may also represent (oral) pathogens, such as
Corynebacterium, Mycobacterium, or Propionibacterium, and/or gastrointestinal
inhabitants. Indeed, in a study by Warinner et al. 2014(a), a range of Actinobacteria were
detected within human dental calculus, including Actinomyces odontolyticus (a causative
agent in dental infections (Cone et al. 2003)), Corynebacterium matruchotii (associated
with the pathogenesis of dental plaque, caries and periodontitis (Barrett et al. 2001)), and
Rothia mucilaginosa (a bacteria commonly associated with respiratory tract infections
(Cho et al. 2013)).
Figure 62: Absolute abundance of bacteria (phyla level assignment) present within samples analysed within this research. From left, calculus samples BL201.1C (BA1; Banbury Lane), COL11038 (COL3; Coldrum), HH3181 (HH81; Hambledon Hill), and HN3793 (HN93; Hazleton North); bone samples BL201.1B (BA2) and BL201.2B (BA6) from Banbury Lane; and an extraction blank (EBK1) and library blank (LBL1).

In contrast, the two blank samples, ran as negative controls alongside the calculus and bone samples, can be seen to be dominated solely by Proteobacteria (Figure 62). Proteobacteria are known to comprise the vast majority of Gram-negative bacteria, and include a large number of human, animal, and plant pathogens (Gupta 2000). Looking briefly at a genus level (considered as a mixed sample against the OneCodex database), all blank extracts ran within this analysis can be seen to be composed mainly of Mycobacterium and Bradyrhizobium. Bradyrhizobium, a bacteria often associated with soil and plants, has previously been noted to be a common contaminant within DNA extraction kits and other lab reagents (Salter et al. 2014). Mycobacterium is a genus of bacteria containing many well-characterised and pathogenic species, such as Mycobacterium tuberculosis, the causative agent of TB. However, the majority of Mycobacterium are not pathogenic, and
are commonly found within the environment, in dust, soil, and even water (Gangadharam et al. 1976; Vaerewijck et al. 2005). Additionally, *Proteobacteria* are known to be commonly detected within clean-room environments (La Duc et al. 2004).

We can consider the calculus samples at a phyla level on a site by site basis (Figures 63-66). The figures below however highlight that the same dominant phyla are present within the calculus samples across all sites: *Actinobacteria, Proteobacteria, Firmicutes*, and *Chloroflexi*. In a smaller number of the samples from Hambledon Hill, Hazleton North and Banbury Lane, *Bacteroidetes* were also present, and in one sample from both Hazleton North and Banbury Lane *Spirochaetes* can also be seen. *Fusobacteria* were also present in one sample from Hambledon Hill. All of these bacteria have previously been found to be within the most abundant phyla detected in calculus samples (Adler et al. 2013; Warinner et al. 2014(a)), and these bacterial phyla are also known to be dominant within the oral microbiome today (Zaura et al. 2009; Dewhirst et al. 2010; Duran-Pinedo and Frias-Lopez 2015). We can therefore infer that the phyla present within the calculus samples here are indicative of an oral environment, and provide an insight into a British Neolithic oral microbiome. Importantly, as in Warinner et al. (2014(a)), *Acidobacteria*, a ubiquitous soil bacteria phylum, was not found to be present within the Neolithic calculus here.
Figure 63: Absolute abundance of bacteria (phyla level assignment) present within Hambledon Hill samples (generated from OneCodex analysis)

Figure 64: Absolute abundance of bacteria (phyla level assignment) present within Hazleton North samples (generated from OneCodex analysis)
Figure 65: Absolute abundance of bacteria (phyla level assignment) present within Banbury Lane samples (generated from OneCodex analysis)

Figure 66: Absolute abundance of bacteria (phyla level assignment) present within Coldrum samples (generated from OneCodex analysis)
Actinobacteria and Proteobacteria have been discussed above, and so will not be repeated here. Firmicutes is a large phylum of bacteria, which contains some notable pathogens, and is a well-known component of both the oral and gut microbiomes – with the presence of Streptococcus, Veillonellaceae, Lactobacillales, and Granulicatella specifically being common within the oral microbiota (Paster et al. 2006; Zaura et al. 2009). In a study by Warinner et al. (2014(a)), Firmicutes was the most abundant phyla observed within archaeological dental calculus samples. Indeed, Firmicutes bacteria such as Clostridium difficile, Gemella morbillorum and Veillonella parvula were detected within the study, along with a range of Streptococcus bacteria, including the causative agent of caries, Streptococcus mutans (Warinner et al. 2014(a)). Similarly, in a 16S study of LBK calculus, Firmicutes was seen in abundance (Adler et al. 2013).

Bacteroidetes is known to constitute a large component of the oral microbiota, with 107 taxa identified in the oral microbiome, in the genera Prevotella, Bacteroides, Porphyromonas, Tannerella, Bergeyella, and Capnocytophaga (Dewhirst et al. 2010). DNA from the periodontal pathogens Porphyromonas gingivalis and Tannerella forsythia (both in the Bacteroidetes phylum) has previously been sequenced from archaeological dental calculus (Warinner et al. 2014(a)). The periodontal pathogen Treponema denticola (Spirochaetes phylum) has also previously been noted to be present within calculus samples (Warinner et al. 2014(a)), and is the third of the ‘red complex’ bacteria thought to cause periodontitis (Socransky and Haffajee 2005; Holt and Ebersole 2005). Other bacteria from the Spirochaetes phylum are also known to be commonly found within the oral microbiome (Huttenhower et al. 2012).

Fusobacteria are a phylum of bacteria commonly associated with the oral cavity, and are known to be a constituent of the modern oral microbiome (Bennett and Eley 1993; Paster et al. 2006). Fusobacteria such as Leptotrichia buccalis and Streptobacillus moniliformis have previously been detected within archaeological dental calculus (Warinner et al. 2014(a)), but interesting, Fusobacteria were not seen in Neolithic age samples analysed via 16S rRNA in a previous study (Adler et al. 2013). Finally, Chloroflexi, found within calculus samples from all sites analysed here, is a smaller component of the oral microbiome, with just one taxa from the phylum present (Dewhirst et al. 2010). This genus has also previously been found within metagenomic studies of archaeological dental calculus (Warinner et al. 2014(a)).
Although all the above phyla are bacterial however, and many contain pathogenic species, it has been noted in clinical studies that the oral microbiome is dominated by these bacteria even within healthy individuals. As such, these bacterial phyla are seen to constitute a ‘core microbiome’ within the oral cavity which is characteristic for health (Zaura et al. 2009). However, interestingly, it has been noted that oral microbial diversity tends to be higher in diseased individuals, with *Spirochaetes* and *Bacteroidetes* specifically being more abundant during disease (Griffen et al. 2012). Contrastingly, *Proteobacteria* and *Fusobacteria* have been suggested to be found at higher levels in healthy individuals (Duran-Pinedo and Frias-Lopez 2015). The presence of *Spirochaetes* and *Bacteroidetes* in a small number of samples from Hambledon Hill, Hazleton North, and Banbury Lane therefore may suggest that these individuals were suffering from some kind of disease state during the time of calculus formation. In contrast, the presence of *Fusobacteria* in one sample from Hambledon Hill may indicate something about the health state of this individual, and indicate that they were not suffering from active disease at the time of calculus formation.

Overall, however, simply by looking at a phyla level at the bacterial composition of the calculus samples analysed here, it can be seen that they accurately reflect the oral microbiome, and are in accordance with the findings of previous metagenomic studies of archaeological dental calculus. Dental calculus of a Neolithic date can therefore be seen to provide a robust biomolecular dataset, and serves as a reservoir for early prehistoric aDNA – both human and bacterial. Due to this, we can see that metagenomic analysis of dental calculus can provide a new and viable means via which to investigate past oral microbiomes and health and disease states in the British Neolithic.

### 8.5.3. Sex Identification

The endogenous DNA content detected within the dental calculus samples (as discussed above) means that it is possible to utilise this data in other ways – as with traditional aDNA studies on bone. One such application is using human DNA within calculus samples to determine biological sex identification. This is particularly useful given the often fragmentary and disarticulated nature of British Neolithic assemblages (see Chapter 3), including those analysed here (as discussed above), which mean that sex identification is not always straightforward or easily determined.
In order to accurately sex human skeletons osteologically, one or more sexually dimorphic elements need to be present. There are a broad range of differential osteological sexing techniques which can be applied, but the most common are the utilisation of the skull and the pelvis, as these are known to be the two most sexually dimorphic elements within the human body. Of the two, the pelvis is sometimes preferentially utilised, due to clear differences between males and females due to childbirth (Mays and Cox 2000; Roberts 2009, 124). However, sex identification has been attempted using most of the bones within the skeleton, including the long bones (e.g. Black 1978(a); Dittrick and Suchey 1986; Brown et al. 2007), the patella (Introna et al. 1998), the dentition (e.g. Black 1978(b); Beyer-Olsen and Alexandersen 1995; Schwartz and Dean 2005), the metacarpals (Stojanowski 1999), and the metatarsals (Robling and Ubelaker 1997; Mountrakis et al. 2010), to name but a few, with varying degrees of success and reliability. Nonetheless, regardless of the skeletal element utilised, sex can often be difficult to determine osteologically. For example, it is not possible to undertake sex estimation on non-adults, as sexually dimorphic changes to the skeleton will not have yet occurred; although it may sometimes be possible to assign a ‘probable’ sex to older non-adults or adolescents. Additionally, there are also other issues in that young adult males may often show more ‘female’ osteological traits, and older females may often look osteologically more ‘male’ (Mays and Cox 2000; Roberts 2009, 122-3; Roberts and Manchester 2010, 32).

Determining sex is important primarily because it can allow us to reconstruct demographic profiles, but also determine biological sex related patterning (Roberts 2009, 121; Daskalaki et al. 2011) – for example, differences in disease frequency between males and females, differences in diet or behaviour, or in this case, potential differences in oral microbiota or oral pathogens. Using DNA to determine sex identification of archaeological skeletons has previously been undertaken in a significant number of studies, but generally only using PCR and focusing on marker loci such as the amelogenin gene (e.g. Faerman et al. 1995; 1998; Stone et al. 1996; Waldron et al. 1999; Cunha et al. 2000; Mays and Faerman 2001; Schmidt et al. 2003; Gibbon et al. 2009; Daskalaki et al. 2011; Quincey et al. 2013). Here, a Python script by Skoglund et al. (2013) for sex identification using shotgun DNA data was instead utilised. This script has been shown to be successful even on samples with low aDNA content, and is believed to be more accurate than previous methods as it utilises both X and Y chromosomal data to determine sex (Skoglund et al. 2013). Unfortunately, due to the low endogenous DNA content and short fragment length of the samples
analysed here however (see Table above), using the standard script as outlined by Skoglund et al. (2013) sex identification was unsuccessful in the majority of samples (12 out of 18 samples). It was thought that this may be due to the endogenous DNA fragment length and damage, meaning that not all reads may be properly paired. Due to this, a short script was run on all samples before attempting sex identification (see Appendix A, section A.4.4.1.), which ensured that only forward and reverse reads on the same chromosome were included within further analysis. By adapting the Skoglund et al. (2013) script slightly therefore, sex was able to be identified in all samples analysed.

Osteological sex identifications of the individuals analysed here, and the results of aDNA sex identification from calculus, are given in Table 28. It can be seen that in a significant number of individuals, sex could not be determined osteologically, most likely due to heavy fragmentation, disarticulation, or a lack of sexually dimorphic skeletal elements being present – particularly given that very few of the individuals analysed here were represented by complete or articulated skeletons.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Number</th>
<th>Sample Type</th>
<th>Osteological Sex Identification</th>
<th>aDNA Sex Identification</th>
<th>Ry 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hambledon Hill</td>
<td>HH610</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0618-0.0816</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH3</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.091-0.1003</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH3181</td>
<td>Calculus</td>
<td>Male</td>
<td>XY</td>
<td>0.0749-0.0959</td>
</tr>
<tr>
<td>Hambledon Hill</td>
<td>HH1916</td>
<td>Calculus</td>
<td>Female</td>
<td>XY</td>
<td>0.0606-0.0816</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN3793</td>
<td>Calculus</td>
<td>Male</td>
<td>XY</td>
<td>0.2117-0.2364</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN5037-1</td>
<td>Calculus</td>
<td>Male</td>
<td>XY</td>
<td>0.1078-0.1253</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN4786</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0741-0.1151</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN7656</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0886-0.1175</td>
</tr>
<tr>
<td>Hazleton North</td>
<td>HN11456</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0826-0.1059</td>
</tr>
<tr>
<td>Coldrum</td>
<td>COL EU.1.5.130</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0924-0.0999</td>
</tr>
<tr>
<td>Coldrum</td>
<td>COL /UN</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0677-0.087</td>
</tr>
<tr>
<td>Coldrum</td>
<td>COL UN8</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0741-0.0906</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL201.1C</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.1102-0.1407</td>
</tr>
</tbody>
</table>
Table 28: Comparison of osteologically determined sex identification (data from Rogers 1990; McKinley 2008; Caffell and Holst 2012) with aDNA sex identifications from endogenous DNA within the dental calculus (XX = female; XY = male). \( R_Y \) 95% CI represents the fraction of sequences aligned to the Y-chromosome expressed as a ratio of the total number of sequences aligned to either sex chromosome, at a 95% confidence interval, as generated by the Skoglund et al. (2013) script.

<table>
<thead>
<tr>
<th>Banbury Lane</th>
<th>BL201.1B</th>
<th>Bone</th>
<th>Unknown</th>
<th>XY</th>
<th>0.1014-0.1079</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banbury Lane</td>
<td>BL201.2C</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0644-0.078</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL201.2B</td>
<td>Bone</td>
<td>Unknown</td>
<td>XY</td>
<td>0.1285-0.1364</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL309.12C</td>
<td>Calculus</td>
<td>Unknown</td>
<td>XY</td>
<td>0.0707-0.0971</td>
</tr>
<tr>
<td>Banbury Lane</td>
<td>BL440.4B</td>
<td>Bone</td>
<td>Unknown</td>
<td>XY</td>
<td>0.1139-0.1262</td>
</tr>
</tbody>
</table>

Interestingly, as can be seen from the above table, all samples analysed here were identified as male (XY). In the majority of cases, it had previously not been possible to determine osteological sex, but in samples where this was noted (Rogers 1990; McKinley 2008; Caffell and Holst 2012), the calculus sex identification agrees with this in all but one of four cases. Furthermore, where both bone and calculus samples from the same individual were analysed, the sex identification obtained was in agreement in all instances. The identification of all samples analysed as male however is remarkable, and raises interesting questions regarding the deposition of human remains along gender lines in the British Neolithic. A higher proportion of males than females has however previously been noted at a range British Neolithic sites (e.g. Brothwell 1973; McKinley 2008). However, equally, larger numbers of females than males have been identified at other British Neolithic sites, such as at West Kennet (Piggott 1962) and Ascott-under-Wychwood (Chesterman 1977, cf. McKinley 2008). Due to this, the DNA results need to be considered with caution, as it must be remembered that only a small percentage of the individuals from each site were analysed here. As McKinley (2008) noted in her analysis of the Hambledon Hill skeletal assemblage, the apparent sexual imbalance currently apparent may not reflect a true cultural bias.

In only one individual analysed here, the sex identification determined via the calculus DNA analysis did not match the osteological sex ID (as recorded in McKinley 2008). This individual (HH1916), from Hambledon Hill, is represented by only one mandibular fragment (with associated teeth; Figure 67) – and therefore sex appears to have been derived from the mandibular shape, using the traits outlined in Bass (1987) and Brothwell.
(1972). The reliability of using mandibular shape as a means of sex identification is variously discussed within the osteological literature, and it is generally taken that post-puberty, males display an accentuation of the chin, whereas females retain a more gracile, juvenile form (Mays and Cox 2000). Mandibular ramus flexure has previously been suggested to be an accurate means of determining sex (Loth and Henneberg 1996), as has gonial eversion (Loth and Henneberg 2000). However, in the sex identification of HH76 1916 (HH1916) (McKinley 2008), it was not noted that these sexing techniques were specifically utilised. Moreover, more recent publications have shown that both these methods can be highly subject to intra- and inter-observer error (Donnelly et al. 1998; Hill 2000; Kemkes-Grottenthaler et al. 2002), and that there may be considerable overlap between the features seen in both sexes (Oettl é et al. 2009), and are therefore not accurate sexing techniques. The conflicting osteological and calculus DNA sex identifications for this individual (HH1916) therefore suggest that one of the two methods has incorrectly sexed this skeletal fragment. In order to ascertain the true sex of the individual therefore, additional sex identification on DNA extracted from the mandible bone – which would have a higher endogenous DNA content than calculus – would need to be undertaken in future.

Figure 67: Image of mandibular fragment of HH76 1916 (HH1916), osteologically identified as female (McKinley 2008). Photograph by the author
On the whole however, it can be seen that the aDNA results match the osteological sex IDs. However, excitingly, endogenous DNA within calculus samples also managed identify the sex of individuals where this could not be done osteologically – highlighting huge future potential within the method. Indeed, this is the first application of utilising endogenous DNA content from archaeological calculus samples generated through shotgun sequencing to determine host sex.

8.5.4. Genetic Population Affinities

Due to the successes of sex identification from the dental calculus, it was postulated that the endogenous DNA content within the calculus samples might also be utilised to determine information on genetic affinities or ancestry of the populations studied. This was attempted here utilising the software package bammds, which allows for visualisation (in a PCA style format using the first two principal components; Figure 68) of the samples analysed against an existing reference panel of genotypic data, using multidimensional scaling based on genetic differences (Malaspinas et al. 2014). In effect, it highlights the genetic affinities of samples analysed to genotypically known groups or populations. This kind of investigation of population structure is particularly pertinent for samples of a Neolithic date, given discussions within the academic literature regarding population movement and influx both at the start of the Neolithic in Britain, and throughout the period (as discussed in Chapters 2, 4 and 9).

![Figure 68: Example of generated bammds plot, highlighting genetic affinities of a reference sequence ‘G’ to known populations](image-url)
Due to the very low endogenous DNA content in the calculus samples (as discussed above; Table 27), it was unclear however whether this population affinity analysis using bammds would be successful or not. Malaspinas et al. (2014) suggest that ancestry information can be obtained from samples with 30,000 or more mapped reads, and/or 1% endogenous content. The number of reads from each sample here, following re-mapping to the human genome for bammds analysis, and removal of all forward and reverse reads not mapped to the same chromosome, is given in the Table below. Although all samples analysed here appear to have over 30,000 reads mapped to the human genome (Table 29), all have <1% endogenous DNA content (Table 27).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>No. of reads mapped to human genome</th>
<th>Total No. of paired reads</th>
<th>No. of reads with itself and mate mapped</th>
<th>No. of single reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA1</td>
<td>124534</td>
<td>41715972</td>
<td>116344</td>
<td>8190</td>
</tr>
<tr>
<td>BA2</td>
<td>981487</td>
<td>38149908</td>
<td>879004</td>
<td>102483</td>
</tr>
<tr>
<td>BA3</td>
<td>56961</td>
<td>35352186</td>
<td>51966</td>
<td>4995</td>
</tr>
<tr>
<td>BA5</td>
<td>187348</td>
<td>36866599</td>
<td>173777</td>
<td>13571</td>
</tr>
<tr>
<td>BA6</td>
<td>759227</td>
<td>36703941</td>
<td>676353</td>
<td>82874</td>
</tr>
<tr>
<td>BA7</td>
<td>222714</td>
<td>20678194</td>
<td>192434</td>
<td>30280</td>
</tr>
<tr>
<td>COL1</td>
<td>663264</td>
<td>38045241</td>
<td>616093</td>
<td>47171</td>
</tr>
<tr>
<td>COL2</td>
<td>85094</td>
<td>35199618</td>
<td>77878</td>
<td>7216</td>
</tr>
<tr>
<td>COL3</td>
<td>148097</td>
<td>38483288</td>
<td>137172</td>
<td>10925</td>
</tr>
<tr>
<td>HH19</td>
<td>69645</td>
<td>30878566</td>
<td>64682</td>
<td>4963</td>
</tr>
<tr>
<td>HH3</td>
<td>423996</td>
<td>18123458</td>
<td>396872</td>
<td>27124</td>
</tr>
<tr>
<td>HH61</td>
<td>70622</td>
<td>33287864</td>
<td>66306</td>
<td>4316</td>
</tr>
<tr>
<td>HH81</td>
<td>80733</td>
<td>41721274</td>
<td>74488</td>
<td>6245</td>
</tr>
<tr>
<td>HN11</td>
<td>80728</td>
<td>33937634</td>
<td>74588</td>
<td>6140</td>
</tr>
<tr>
<td>HN37</td>
<td>130611</td>
<td>32516351</td>
<td>121779</td>
<td>8832</td>
</tr>
<tr>
<td>HN47</td>
<td>22835</td>
<td>8032358</td>
<td>21402</td>
<td>1433</td>
</tr>
<tr>
<td>HN76</td>
<td>48465</td>
<td>31428289</td>
<td>44155</td>
<td>4310</td>
</tr>
<tr>
<td>HN93</td>
<td>128353</td>
<td>32626242</td>
<td>123284</td>
<td>5069</td>
</tr>
</tbody>
</table>

Table 29: Number of mapped and paired reads for bammds analysis

Unfortunately, none of the samples analysed here provided a successful bammds analysis – which is likely due to their low overall endogenous DNA content. Although the samples had more mapped reads than the recommended minimum (Malaspinas et al. 2014), the low overall endogenous DNA content suggests that insufficient reads from the samples here were mapped onto the SNPs which bammds utilises to discern genetic/population affinity. This highlights the problems of working with aDNA, and it has previously been noted that determining genotypes for low-depth genetic data (much like the data generated here, and in many aDNA studies) is challenging and problematic (Nielsen et al. 2011). With improvements in SNP calling and genotype determination through the development of new
software, we may in future be able to more easily determine genotypes – and by association genetic/population affinity – within low-depth coverage NGS data. At present, however, the low endogenous DNA content of calculus samples, combined with the low coverage of the human genome this data provides, means that it currently does not appear that calculus can provide genetic information on population affinity. Whilst disappointing, particularly for Neolithic samples given the interest in population movement and/or replacement during the period (see Chapters 2, 7 and 9), the analysis undertaken here does serve to highlight the limitations of calculus aDNA data, particularly that of endogenous origin, which has not previously been explored.

8.5.5. Protein Concentration and Function

Across all 10 calculus samples analysed here, a total of 377 proteins were recovered, comprising of 4,595 assigned peptides, deriving from a total of 104,712 spectra (Table 30). Assignment of proteins and peptides was achieved via the use of Mascot, at a target FDR (false discovery rate) of 5% (above identity or homology threshold) (as in Warinner et al. 2014(a)) and an ion cut-off score of 25 and significance threshold of \( p < 0.05 \) (as in Warinner et al. 2014(b); see Appendix A, section A.4.4.2.). In Warinner et al. (2014(a)), the total number of assigned peptides in three pooled samples was 754 (Table 31), therefore meaning an average of c.251 peptides were detected per calculus sample. Here, across all 10 samples, an average of c.460 peptides were detected within each calculus sample – nearly double that of Warinner et al. (2014(a)). Similarly, the average number of spectra (queries) per calculus sample reported in Warinner et al. (2014(a)) was 5434, whereas in the samples studied here an average of 10471 spectra per calculus extract were detected. The reason for this increased spectra and peptide yield is likely due to the altered and improved extraction protocols utilised within this study, which are outlined in Appendix A (section A.4.3.). It can however be clearly seen that the number of peptides identified here from the Neolithic calculus samples is still significantly lower than that obtained from modern dental calculus (Table 31).
Table 30: Spectrum assignment for proteomic data obtained from dental calculus samples and an extraction blank (eBK)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>No. of Spectra (Queries)</th>
<th>No. of Assigned Peptides</th>
<th>No. of Assigned Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH3</td>
<td>7,612</td>
<td>495</td>
<td>48</td>
</tr>
<tr>
<td>HH610</td>
<td>17,494</td>
<td>807</td>
<td>68</td>
</tr>
<tr>
<td>HH3188</td>
<td>12,928</td>
<td>498</td>
<td>49</td>
</tr>
<tr>
<td>HN4786</td>
<td>7,367</td>
<td>515</td>
<td>25</td>
</tr>
<tr>
<td>HN5037-1</td>
<td>8,755</td>
<td>337</td>
<td>41</td>
</tr>
<tr>
<td>HN7387</td>
<td>19,762</td>
<td>693</td>
<td>77</td>
</tr>
<tr>
<td>HN7656</td>
<td>3,104</td>
<td>213</td>
<td>23</td>
</tr>
<tr>
<td>HN11456</td>
<td>10,388</td>
<td>134</td>
<td>10</td>
</tr>
<tr>
<td>BL132.17</td>
<td>8,379</td>
<td>657</td>
<td>25</td>
</tr>
<tr>
<td>BL309.12</td>
<td>8,923</td>
<td>246</td>
<td>11</td>
</tr>
<tr>
<td>eBK (Blank)</td>
<td>748</td>
<td>77</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 31: Spectrum assignment for ancient and modern dental calculus (using ProteinPilot v.4) in Warinner et al. 2014(a)

<table>
<thead>
<tr>
<th>Basic statistics</th>
<th>Ancient Dental Calculus</th>
<th>Modern Dental Calculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total spectra</td>
<td>16,304</td>
<td>20,928</td>
</tr>
<tr>
<td>Peptide assignment conf. threshold (%)</td>
<td>95.5</td>
<td>95.9</td>
</tr>
<tr>
<td>Assigned peptides</td>
<td>754</td>
<td>3,447</td>
</tr>
<tr>
<td>Proportion of assigned peptides (%)</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

Notes:

aIncludes pooled spectra from B17 (Z27), B61 (Z46), and B78 (Z28).
bIncludes pooled spectra from P1 (Z5, Z6) and P2 (Z7, Z8).
cConfidence level determined by distinct peptide level 5% local FDR.

To allow for a broad level comparison of protein composition and function between calculus samples, all Mascot assigned peptides were uploaded to Unipept (http://unipept.ugent.be/), a metaproteomic data analysis pipeline tool (Mesuere et al. 2015). Unipept computes peptides generated from shotgun MS/MS analysis, and compares them against the NCBI Taxonomy Database to determine the taxonomic lineage of each peptide. These lineages are then analysed using a scanning algorithm to determine the lowest common ancestor. This data is then visualised both as a table of all matched peptides, and as a tree-view and ‘sunburst’ which bundle all taxonomic lineages (Mesuere et al. 2015). This Unipept analysis provides a fast and easily to visualise method of determining the dominant phyla within the calculus samples – but should be taken as an initial means of viewing the data generated through the metaproteomic analyses undertaken here, rather than a fully robust analysis, particularly given that the data is only compared against one protein database. In this way therefore, the Unipept analysis is very...
similar to that of the OneCodex analysis undertaken on the metagenomic data here (see above).

All calculus samples analysed here were seen to contain both proteins from eukaryota and metazoa, but also, importantly, from bacteria. ‘Sunburst’ diagrams, detailing all taxonomic lineages within a sample, generated using Unipept, are provided below (Figures 69-79). From these diagrams, it can be seen that there are a range of broad similarities within the phyla present across all calculus samples analysed, and the bacteria present are also in accordance with findings by Warinner et al. (2014(a)).

Of the bacteria present, Actinobacteria was detected in all calculus samples analysed. As discussed above in section 8.5.2., Actinobacteria comprises a bacterial group amongst one of the largest taxonomic units of the Bacteria domain, and as such contains range of different kinds of bacteria, including pathogens, plant commensals, soil inhabitants, and gastrointestinal inhabitants (Ventura et al. 2007). In a study by Warinner et al. (2014(a)), proteins from a number of Actinobacteria were detected within human dental calculus, including Actinomyces odontolyticus (a causative agent in dental infections (Cone et al. 2003)), Corynebacterium matruchotii (associated with the pathogenesis of dental plaque, caries and periodontitis (Barrett et al. 2001)), and Rothia mucilaginosa (a bacteria commonly associated with respiratory tract infections (Cho et al. 2013)). Firmicutes can also be seen to be present in all calculus samples here, and much like Actinobacteria, was also detected in abundance in metagenomic analyses of calculus samples here (section 8.5.2.). Firmicutes is also known to be a common component within the oral microbiome, and proteins from Streptococcus bacteria have previously been identified in ancient dental calculus (Warinner et al. 2014(a)).

Additionally, proteins relating to both Proteobacteria and Synergistetes were found to be present within eight of the ten calculus samples analysed here. Proteobacteria are known to comprise the vast majority of gram-negative bacteria, and include a large number of human, animal, and plant pathogens (Gupta 2000). Previously, Proteobacteria such as Campylobacter rectus (a putative periodontal pathogen (Rams et al. 1993)), Eikenella corrodens (a common inhabitant of the oral cavity, which can be implicated in infections and/or periodontitis (Chen et al. 1989; Chen and Wilson 1992)), and Neisseria species (a common oral component, with numerous pathogenic species (Aas et al. 2005)) have been detected within proteomic extracts from dental calculus, and were also noted to be one of
the dominant bacterial phyla present within DNA extracts from dental calculus samples here (section 8.5.2.). *Synergistetes* is a phylum composed of anaerobic, gram-negative bacteria, which have previously been detected within humans, animals and terrestrial environments (Jumas-Bilak et al. 2009). *Synergistetes* are known to be commonly detected within the oral cavity, and are particularly found to be present at disease sites. Additionally, some *Synergistetes* bacteria have been identified as markers for periodontitis (Vartoukian et al. 2009; Marchesan et al. 2015). Although known to be detected within modern plaque samples and the human oral microbiome (Paster et al. 2001; Dewhirst et al. 2010), *Synergistetes* have not previously been detected proteomically within archaeological dental calculus samples.

Proteins from *Bacteroidetes* were also detected within seven of the ten calculus samples analysed here. *Bacteroidetes* is known to constitute a large component of the oral microbiota, with 107 taxa identified in the oral microbiome (Dewhirst et al. 2010). Proteins relating to *Bacteroidetes* bacteria have previously been identified from archaeological dental calculus – for example, the periodontal pathogens *Porphyromonas gingivalis* and *Tannerella forsythia* (Warinner et al. 2014(a)). *Fusobacteria* proteins were also detected within two calculus samples here (both from Hazleton North). *Fusobacteria* are a phylum of bacteria commonly associated with the oral cavity, and are known to be a constituent of the modern oral microbiome (Bennett and Eley 1993; Paster et al. 2006). *Fusobacteria* such as *Fusobacterium nucleatum* and various *Streptococcus* species have previously been detected within metaproteomic analyses of archaeological dental calculus (Warinner et al. 2014(a)). Finally, *Cyanobacteria* was also identified in one sample from Banbury Lane (BL309.12). *Cyanobacteria* are one of the most diverse bacterial phyla (Shih et al. 2013), but have previously been noted to be a small component within the normal oral microbiome (Dewhirst et al. 2010). Much like *Synergistetes* discussed above however, it has also not previously been detected proteomically within archaeological dental calculus samples.

Aside from bacterial proteins, it can also be seen that a range of proteins from other sources were also detected. *Thaumarchaeota* proteins were detected in half (n=5) the calculus samples analysed here (two samples from Hazleton North, two from Banbury Lane, and one from Hambledon Hill). *Thaumarchaeota* are one of the most abundant archaeal phylum, and are often associated with soils and other environmental samples, but have been significantly under-studied (Brochier-Armanet et al. 2008; Stieglmeier et al.
As such, their potential presence within calculus deserves further investigation. Similarly, fungi were interestingly detected in three calculus samples here (two from Hambledon Hill, and one from Banbury Lane). Fungi are known to be ubiquitous constituents of the oral microbiota, and whilst they can be associated with pathologies, they are also known to play a role in healthy oral ecology (Ghannoum et al. 2010; Krom et al. 2014). However, previously, fungal spores have been detected in dental calculus using microscopy, and have been interpreted as deriving from fungal spores on stored grain (Afonso-Vargas et al. 2015), or the consumption of mushrooms (Power et al. 2015). Finally, interestingly, Streptophyta – a broad clade of green plants – was identified in one sample (HH3), and Mollusca was also identified in one sample (HN7387). Whilst dietary proteins have previously been detected within archaeological dental calculus (Warinner et al. 2014(a)), further investigation of these peptide sequences is needed before they can be confirmed.

Finally, importantly, the extraction blank analysed alongside all calculus samples here was shown to have no bacterial proteins present (Figure 79).
Figure 69: Sunburst diagram detailing phyla present within BL132.17
Figure 70: Sunburst diagram detailing phyla present within BL309.12
Figure 71: Sunburst diagram detailing phyla present within HH3
Figure 72: Sunburst diagram detailing phyla present within HH610
Figure 73: Sunburst diagram detailing phyla present within HH3188
Figure 74: Sunburst diagram detailing phyla present within HN4786
Figure 75: Sunburst diagram detailing phyla present within HN5037-1
Figure 76: Sunburst diagram detailing phyla present within HN7387
Figure 77: Sunburst diagram detailing phyla present within HN7656
Figure 78: Sunburst diagram detailing phyla present within HN11456
The proteomic data obtained here can therefore be seen to be a reflection of the human oral microbiome, and bears many similarities to a previous metaproteomic study of archaeological dental calculus (Warinner et al. 2014(a)), and modern studies of the oral microbiome (e.g. Dewhirst et al. 2010). The dominant phyla detected within the protein data also match the dominant phyla identified from the metagenomic analysis (see section 8.5.2.). Neolithic dental calculus can therefore be seen to provide a reservoir for early prehistoric proteins. Due to this, metaproteomic analysis of dental calculus (much like metagenomic analyses) may provide a new means via which to investigate past oral microbiomes and potential dietary inclusions in the British Neolithic.
8.5.6. Neolithic Milk Drinking & The Archaeological Evidence

Within the archaeological literature, there has long been a debate over the origins of milk drinking and dairy product consumption (see Chapter 2). Milk is a significant nutritional resource, containing fats, sugars and proteins, as well as vitamins, minerals and essential amino acids. Even today, milk has the highest production value of any foodstuff globally (Bovenhuis et al. 2013; FAO 2015). Lactose is the main nutrient in milk, comprising 3.8-5.3% of total content, and is a type of disaccharide sugar. In order for to humans to digest lactose however, it must be broken down by the enzyme lactase-phlorizin hydrolase (more commonly known simply as ‘lactase’; EC 3.2.1.108), which allows it to be absorbed by the body in the intestine. As infants, all humans have the ability to digest raw milk and lactose. However, after weaning, the body naturally stops producing lactase, unless the individual has a genetic adaptation which allows for the continued production of it – known as lactase persistence (LP). LP therefore allows for the continued consumption of milk into adulthood, and is a polymorphic trait (Luinge et al. 1993; Wang et al. 1998; Vesa et al. 2000; Ingram et al. 2007; Leonardi et al. 2012; Gerbault et al. 2013). Indeed, LP is thought to be the clearest example of gene-culture coevolution – the idea that cultural practices can alter the genome – that we currently have (Bersaglieri et al. 2004; Nielsen et al. 2007; Sabeti et al. 2007; Ingram et al. 2009).

Previously, it has been suggested that the ability to consume raw milk must have provided a selective advantage, due to its nutritional qualities – and therefore that LP was positively selected for following the advent of agriculture, resulting in increased frequency (Cavalli-Sforza 1973; Berja-Pereira 2003; Bersaglieri et al. 2004; Nielsen et al. 2007). It has generally been assumed that Neolithic populations began dairying – and therefore also milk drinking – from early on in the period. These ideas have been traditionally supported by a number of different types of archaeological evidence, particularly organic residue analysis of Neolithic pottery showing the presence of milk lipids (e.g. Craig 2001; Copley et al. 2003; 2005(a); 2005(b); Craig et al. 2005; Cramp et al. 2014(a); 2014(b); Salque et al. 2012; 2013; Smyth and Evershed 2015), and zooarchaeological analyses and mortality profiling of domesticated fauna (e.g. Legge 2005; Mulville et al. 2005; Vigne 2008; Greenfield and Arnold 2015).

These kinds of analyses have resulted in the hypothesis that dairying emerged in the Neolithic alongside agriculture, utilising newly domesticated fauna. It was therefore commonly assumed that these Neolithic individuals had LP, or that LP emerged
throughout the period, increasing through time – and was supported by initial investigations of the LCT gene (C-13910 > T; -13910*T allele) responsible for LP (although not on archaeological materials) (e.g. Bersaglieri et al. 2004; Coelho et al. 2005; Myles et al. 2005). However, with advances in next-generation sequencing technologies and the emergence of full genome characterisation of archaeological samples, new aDNA data suggests that LP may not have emerged in the Neolithic, or if present, would have been in very low frequencies across a population. The absence of LP in European Neolithic populations has now been noted in a number of studies (e.g. Burger et al. 2007; Itan et al. 2009; Gamba et al. 2014; Witas et al. 2015), and a recent paper by Allentoft et al. (2015) suggested that LP had a very low frequency (5-10%) in Bronze Age European populations, indicating that LP actually emerged in the Late Bronze Age or perhaps even later. Similarly, a large-scale study by Mathieson et al. (2015) suggests that LP in Europe only emerged in the last 4,000 years. The current genetic evidence therefore suggests that European Neolithic populations would not have had LP, and as such, could not have digested unprocessed dairy products or raw milk, as previously thought. This indicates that whilst people were practicing dairying in the Neolithic, as evidenced through lipid residues on pottery and faunal assemblages, that these dairy products must have been either consumed in very small quantities, or processed in such a way as to remove the lactose (e.g. through the production of hard cheeses).

8.5.6.1. β-lactoglobulin (BLG) and Dental Calculus

The recent discovery of the milk protein β-lactoglobulin (BLG) in human dental calculus (Warinner et al. 2014(b)) has indicated a new way in which we can detect milk consumption in the archaeological past. BLG has been well-studied, due to it being one of the major allergens within ruminant milk (Restani et al. 2009; Bu et al. 2013). Composed of 162 amino acid residues, it is the dominant protein within the whey fraction of milk, belonging to the lipocalin family of proteins (Kontopidis et al. 2004; Wal 2004; Monaci et al. 2006). It is a useful milk biomarker as it is found within nearly all mammalian species, but is crucially not found within human milk (Crittenden and Bennett 2005; Restani et al. 2009), thereby meaning that its presence within dental calculus cannot have a host origin. Additionally, the amino acid sequence of BLG differs between species, meaning it can be used as a species specific indicator (Kontopidis et al. 2004). Finally, BLG is only found in milk, making it a specific biomarker, and it is more resistant to microbial attack and enzymatic degradation than other milk proteins (Warinner et al. 2014(b)).
To date, the oldest calculus samples BLG has been detected in date to the Bronze Age (Warinner et al. 2014(b)), but applications of metaproteomic analyses to earlier prehistoric calculus have not yet been explored. Whether BLG survives in older samples is therefore currently unknown. It was proposed previously however that BLG could be used as a proxy for lactose, given that they partition together in the whey fraction of milk during processing (Warinner et al. 2014(b)). If correct, the presence of BLG in Neolithic calculus may provide an additional source of information on Neolithic dairy consumption and the origins of raw milk consumption.

Of the ten Neolithic calculus samples analysed for proteins here, over half (n=6) were found to test positive for BLG peptides (Table 32). Samples with identified BLG peptides derived from both the Hambledon Hill (n=3) and Hazleton North (n=3) assemblages. No BLG peptides were identified within the calculus samples from Banbury Lane, the remaining two samples from Hazleton North, or from the extraction blank. In total, 125 spectra (comprising 45 unique peptides) were assigned to BLG. For each of the samples which tested positive for BLG, a consensus BLG sequence could be assigned to ruminants of the Pecora infraorder of Artiodactyla, with five samples containing bovid-specific (Bovidae) peptides, and one containing caprid-specific (Caprinae) peptides (see Appendix A for further information).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Total spectra assigned to BLG</th>
<th>Identified peptide sequences</th>
<th>Modifications</th>
<th>Mascot Ion Score</th>
<th>Peptide Taxonomic Assignment</th>
<th>Protein Taxonomic Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH3</td>
<td>2</td>
<td>R.VYVEE LKPTPEG DLEILL (2)*</td>
<td></td>
<td>42, 30</td>
<td>Bovidae</td>
<td>Bovidae</td>
</tr>
<tr>
<td>HH610</td>
<td>68</td>
<td>L.VLDTD YK.K</td>
<td></td>
<td>38</td>
<td>Mammalia</td>
<td>Caprinae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K.ALPMH IR.L</td>
<td>Oxidation (M)</td>
<td>37</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L.DAQSA PLR.V</td>
<td>Deamidated (NQ)</td>
<td>35</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>K.IDALNE NK.V (2)</td>
<td>2 Deamidated (NQ)</td>
<td>49, 38</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F.KIDALN ENK.V*</td>
<td>Deamidated (NQ)</td>
<td>71</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F.KIDALN ENK.V (4)*</td>
<td>2 Deamidated (NQ)</td>
<td>61, 40, 54, 51</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>K.VL.VLD TDYK.K</td>
<td></td>
<td>69, 68</td>
<td>Pecora</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.VLVLD TDYKK.Y (5)*</td>
<td>49, 45, 36, 37, 29</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.TPEVD NEALEK. F (7)*</td>
<td>Deamidated (NQ)</td>
<td>77, 49, 50, 58, 38, 44, 30</td>
<td>Bovidae*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q.KWENG ECAQK.K * 2</td>
<td>Deamidated (NQ)</td>
<td>47</td>
<td>Bovidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.TPEVD KEALEK. F*</td>
<td>Deamidated (NQ)</td>
<td>51</td>
<td>Caprinae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.VYVTEE LKPTPEG. N (2)*</td>
<td>52, 45</td>
<td></td>
<td>Bovidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.TPEVD NEALEKF .D (8)*</td>
<td>Deamidated (NQ)</td>
<td>58, 57, 54, 53, 39, 57, 55, 48</td>
<td>Bovidae*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.DISLLD AQSAAPLR .V (2)*</td>
<td>Deamidated (NQ)</td>
<td>62, 59</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.DISLLD AQSAAPLR .V*</td>
<td>Deamidated (NQ)</td>
<td>45</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.SDISLL DAQSAAPLR R.V (8)*</td>
<td>Deamidated (NQ)</td>
<td>60, 50, 46, 45, 56, 59, 49, 51</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.AASDI SLLDAQS APLR.V (3)*</td>
<td>Deamidated (NQ)</td>
<td>73, 30, 71</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.AASDI SLLDAQS APLR.V*</td>
<td>Deamidated (NQ)</td>
<td>42</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.TPEVD NEALEKF DK.A (6)*</td>
<td>Deamidated (NQ)</td>
<td>55, 44, 39, 35, 75, 41</td>
<td>Bovidae*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.MA ASD ISLLDAQS APLR.V (4)*</td>
<td>Deamidated (NQ), Oxidation (M)</td>
<td>71, 51, 51, 53</td>
<td>Pecora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.VYVTEE LKPTPEG NLEIL.L (6)*</td>
<td>Deamidated (NQ)</td>
<td>52, 33, 39, 45, 43, 38</td>
<td>Bovidae</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HH4786** 12 | R.TPEVD NEALEK. F (6)* | Deamidated (NQ) | 61, 41, 60, 52, 51, 45 | Bovidae* | Bovidae |
<p>| R.TPEVD NEALEKF .D (2)* | Deamidated (NQ) | 41, 40 | Bovidae* |
| A.SDISLL DAQSAAPLR R.V (2)* | Deamidated (NQ) | 35, 33 | Pecora |
| R.TPEVD | Deamidated | 70, 44 | Bovidae* |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HN7387</td>
<td>33</td>
<td>K.IDALNE NK.V</td>
<td>2 Deamidated</td>
<td>31</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ)</td>
<td></td>
<td></td>
<td>Bovini</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F.KIDALN ENK.V*</td>
<td>Deamidated</td>
<td>57</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F.KIDALN ENK.V*</td>
<td>2 Deamidated</td>
<td>62</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>K.VLVLD TDYKK.Y (4)*</td>
<td></td>
<td>43, 36, 32, 62</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.TPEVD DEALEKF .D (2)*</td>
<td></td>
<td>61, 40, 40</td>
<td></td>
<td>Bovini</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.VYVVEE LKPTPEG.G *</td>
<td></td>
<td>37</td>
<td></td>
<td>Bovidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.VYVVEE LKPTPEG. D*</td>
<td></td>
<td>30</td>
<td></td>
<td>Bovidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.TPEVD DEALEKF .D (2)*</td>
<td></td>
<td>35, 52</td>
<td></td>
<td>Bovini</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.DISLLD AQSAQLR V*</td>
<td></td>
<td>63</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.DISLLD AQSAQLR V*</td>
<td>Deamidated</td>
<td>60</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A.SDISLL DAQSAPL R.V (2)</td>
<td></td>
<td>65, 49</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A.SDISLL DAQSAPL R.V (3)*</td>
<td>Deamidated</td>
<td>60, 42, 44</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.TPEVD DEALEKF .D (2)*</td>
<td></td>
<td>43, 38, 70, 38</td>
<td></td>
<td>Bovini</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A.MAAASD ISLLDAQ SAPLR.V (2)*</td>
<td>Deamidated</td>
<td>92, 59</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ), Oxidation (M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A.MAAASD ISLLDAQ SAPLR.V*</td>
<td>Oxidation (M)</td>
<td>50</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.VYVVEE LKPTPEG DLEIL.L (4)*</td>
<td></td>
<td>39, 37, 39, 27</td>
<td></td>
<td>Bovidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R.VYVVEE LKPTPEG DLEILLQ. K*</td>
<td></td>
<td>34</td>
<td></td>
<td>Bovidae</td>
</tr>
<tr>
<td>HN7656</td>
<td>3</td>
<td>K.IDALNE NK.V</td>
<td>Deamidated</td>
<td>36</td>
<td></td>
<td>Pecora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(NQ)</td>
<td></td>
<td></td>
<td>Bovini</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K.IDALNE</td>
<td>2 Deamidated</td>
<td>46</td>
<td></td>
<td>Pecora</td>
</tr>
</tbody>
</table>

290
Table 32: BLG peptides identified within dental calculus samples. All sequences have been verified for specificity by conducting a protein BLAST (blastp) search against the NCBI nr database, and sequences that uniquely match BLG are marked with an asterisk (*). Only samples with at least one spectrum uniquely matching BLG are considered BLG+.

Excludes spectra with a Mascot ion score <25. Peptides identified more than once are followed by parentheses indicating the total number of observations. Species identification indicated with (a) is done so as among Bovidae, Bovinae (cattle, yak and buffalo) are distinguished from Caprinae (sheep and goats) by N→D at residue 71. However, because N deamidation to D is a common post-mortem modification, it is uncertain if the D at this residue is authentic or a damage artefact, and therefore species identification can only be to Bovidae

Of the three individuals from Hambledon Hill in which BLG peptides were detected here, two indicated BLG deriving from Bovidae (HH3; HH4786), whilst the other contained ovicaprid specific (Caprinae) peptides (HH610). Bovidae are a family of ruminants which includes cattle, buffalo, bison, antelopes, sheep, goats, and gazelles (Gentry 1992), and therefore the presence of Bovidae BLG within the calculus of two individuals at Hambledon Hill means it may derive from cattle, sheep, or goat. Caprinae, a subfamily of Bovidae, comprises of domesticated sheep (Ovis) and goats (Capra), and related genera such as chamois (Rupicapra), mountain goat (Oreamnos), and muskox (Ovibos) (Hassanin et al. 1998). This therefore indicates that the BLG detected within the calculus of individual HH610 derived from either sheep or goat. As noted in section 8.2.1. above, domesticated cattle (Bos taurus) were the most dominant species present within the Hambledon Hill faunal assemblage, followed by smaller numbers of ovicaprids (Ovis aries and Capra hircus) (Legge 2008). Additionally, the cattle remains at Hambledon Hill have
previously been suggested to be indicative of a dairy herd (Whittle 1992, 221; Copley et al. 2003; but see section 8.2.1.).

Of the three individuals from Hazleton North in which BLG peptides were detected, all were specific to Bovini. The Bovini tribe of the Bovidae family includes cattle (Bos), buffalo (Bubalus) and bison (Bison) (Gentry 1992), and therefore the identification of BLG peptides from three samples from Hazleton North as Bovini is likely to represent BLG from cattle. As noted in section 8.2.2. above, the remains of domesticated cattle were recovered within the chambered areas of the tomb at the site, and therefore the utilisation of cattle milk by the individuals at Hazleton North is perhaps unsurprising.

Overall, however, it is interesting to note that the BLG results obtained from dental calculus from two different British sites here indicate that Neolithic populations were utilising milk and/or dairy products from both cattle and ovicaprids. The results obtained here also may indicate differential levels of milk or dairy consumption between individuals, based upon the varying number of BLG spectra detected (Figure 80). For example, one individual from Hambledon Hill (HH3) exhibited a weak signal of milk consumption, evidenced by only two spectra. Contrastingly, individual HH610 from Hambledon Hill showed a strong indication of dairy consumption, with a total of 68 spectra matching BLG peptides (14 unique peptides). Previously, Warinner et al. (2014(b)) suggested that two individuals with a combined total of 38 spectra matching BLG peptides (12 unique peptides) indicated “strong evidence of dairy consumption”. Indeed, in the Warinner et al. (2014(b)) study, the highest number of spectra identified within any one calculus sample was 48. The detection of 68 spectra matching BLG peptides within a Neolithic individual from Hambledon Hill is therefore very exciting, and may in part be a product of improved proteomic extraction techniques (see Appendix A). Why some samples have a significantly different number of BLG spectra than others however is still unclear, but is something which certainly warrants further study in future. Additional study of the abundance of BLG spectra within different dental calculus samples may reveal if this is truly linked to the amount of dairy consumed by an individual, or instead if it is the result of preservational biases or environments, or the timings and nature of calculus formation. In order to accurately assess this however, a much broader scale study, with the likely inclusion of modern dental calculus samples from individuals with known diets, would be needed. Further study should however reveal if individuals with no evidence of
BLG peptides within their calculus (as seen here, and within Warinner et al. (2014(b))) were truly not consuming dairy products.

Figure 80: Total number of spectra matching BLG peptides within each dental calculus sample analysed here proteomically

It is important to note, however, that due to recent discoveries of LP prevalence in the European Neolithic (see section 8.5.1. above), it is unlikely that the British Neolithic individuals studied here would have had lactase persistence. The presence of BLG within the dental calculus however indicates the use and consumption of dairy products, and is supported by other archaeological evidence for dairying from the period, as discussed above and in Chapter 2. The likely absence of LP, but presence of BLG, therefore suggests that Neolithic populations must have been processing milk to remove the lactose, but in such a way that the BLG was retained.
Lactose can be removed from or decreased in milk products through a range of different processing methods. For example, cheese contains little or no lactose, as it is removed during processing with the whey fraction of the milk (Salque et al. 2013). Indeed, 98% of lactose is removed in the whey during most cheese production (Izco et al. 2002). The production of cheese within prehistory has however previously been suggested to have been beneficial for past populations not only due to the reduced lactose content, thereby making it more readily digestible and suitable for non-LP individuals, but also because it allowed for “the preservation of milk products in a non-perishable and transportable form” (Salque et al. 2013, 522).

Lactose content is also known to be much decreased in fermented milk products, such as yoghurt, kefir, and buttermilk (Alm 1982; O’Brien 1999). Indeed, fermented milk products have been shown to be suitable for consumption by lactose intolerant individuals (Alm 1982). Yoghurt is produced through the incubation of whole milk with bacteria, commonly Lactobacillus bulgaricus and Streptococcus thermophilus. These bacteria ferment the milk, reducing both its pH and its lactose content, in some cases by up to 30% (Somkuti and Holsinger 1997; Savaiano 2014). A study by O’Brien (1999) documented the amounts of lactose present in cultured or fermented dairy products, showing that the fermentation process reduces lactose content, with yoghurts (made from cow’s milk) typically containing 3.29-3.67% lactose, compared to whole milk, which typically has a lactose content of ~4.8%. Sheep’s milk yoghurt was seen to contain only 1.61-2.21% lactose, and a fermented milk drink 1.8% (O’Brien 1999). Therefore, although yoghurt does contain small amounts of lactose, it is believed that this lactose is more easily digested than that found within whole milk, due to hydrolysis and autodigestion of lactose by the yoghurt bacteria – thus improving its absorption and creating a ‘lactase activity’ in the gastrointestinal tract (Kolars et al. 1984; Savaiano 2014).

The idea of people processing milk within the Neolithic in order to consume it has previously been proposed – e.g. in the form of cheese making (Salque et al. 2013) – and is unsurprising given the new genetic evidence emerging, which suggests that Neolithic populations did not have LP (see discussion above). Of the three sites analysed here, only the pottery from Hambledon Hill has previously had organic residue analysis undertaken upon it. As discussed in section 8.2.1., analysis of 72 sherds from the site revealed that 58% of these yielded appreciable lipid residues. Of these, 26% exhibited δ13C values indicative of dairy fats (Copley et al. 2003). Overall, therefore, we can suggest that British
Neolithic populations, whilst utilising and consuming dairy (as evidenced through BLG peptides within calculus detected here, and lipid residue analyses on pottery), would not have had LP, and thus appear to either have been processing raw milk in order to decrease lactose content, or alternatively were only consuming very small amounts of milk. However, given the abundance of BLG peptides present within some of the calculus samples analysed here, the hypothesis of milk processing prior to consumption appears to be the most parsimonious explanation. This therefore raises a host of interesting new questions about the ways in which Neolithic populations may have processed milk, the technologies needed to do this, the kinds of products which may have been created, and links to cuisine – which are discussed in greater detail in Chapter 9 (section 9.2.2.).

An alternative hypothesis however is that some British Neolithic individuals may indeed have had LP, but that the levels of LP at this time are too low to be detected within large scale aDNA studies (Mark Thomas, pers. comm.). This hypothesis is valid given that LP must have been present in low levels prior to the Bronze Age to enable positive selection for the LCT gene to have occurred. Positive selection is the principle that beneficial traits will become more frequent over time. However, the study of the processes of selection is difficult (Sabeti et al. 2006), and is further compounded by the limited amount of human skeletal material available for prehistoric periods. However, even if LP was present at low levels within Neolithic populations, it seems unlikely that all individuals sampled here would have had LP, and thus some degree of processing of raw milk must still have been undertaken to allow those without LP to consume dairy products. In order to fully assess the degree to which milk may have been processed due to a lack of LP however, it would now be needed to undertake aDNA analysis on bone samples from all individuals studied here to determine if they were lactase persistent or not.

8.6. Conclusions

Overall, this Chapter has aimed to highlight the potential that calculus may hold for prehistoric study. The above analyses and discussion are not exhaustive, but instead aim to provide an initial analysis of the data generated from calculus within this study, and an indication of the multiple sources of information which metagenomic and metaproteomic analyses may be able to provide. In tandem with this, it is hoped that the research presented here also highlights how this generated data may contribute to broader archaeological discussions – particularly those surrounding prehistoric diet and disease. It is also
important to note that although all the material analysed here is Neolithic in date, the methodologies utilised could equally be applied to Mesolithic materials too. Due to the lack of identified skeletal remains dating to the Mesolithic in Britain, and the lack of calculus on those known however, sadly no Mesolithic material was included here. Importantly however, the metagenomic and metaproteomic analysis undertaken here on Neolithic samples have indicated that dental calculus of this date can provide robust genomic and proteomic datasets, and that contamination is not an issue. This therefore indicates that it may in future be possible to extend the time depth of analyses of this kind on human dental calculus even further back – to Mesolithic or potentially Upper Palaeolithic materials.

Metagenomic analyses undertaken here have highlighted calculus as a new source of aDNA for early prehistoric periods. As anticipated, the genomic data obtained from samples here was dominated by bacterial DNA, but very small amounts of endogenous human DNA were also recovered too. A broad level analysis of the bacterial DNA sequenced from the calculus samples has shown that it is indicative of oral microbiota, and is similar in nature and composition to that obtained from other metagenomic analyses of human dental calculus on later material (Adler et al. 2013; Warinner et al. 2014(a)). The data obtained here therefore suggest that analysis of human dental calculus may provide the first insight into Neolithic oral microbiomes, and as such, also Neolithic health and disease states.

The realisation that dental calculus may also preserve small, but useable amounts of host (human) DNA is also exciting, particularly given the scarcity of human skeletal remains from these early periods (see Chapter 3), and as seen here, this endogenous DNA can be utilised for the investigation of sex identification, for example. As discussed above and in Chapter 3, being able to determine sex from heavily fragmented human remains, such as those commonly found within Neolithic contexts, is often problematic – and highlights the use of metagenomic analyses beyond bacterial DNA investigation, as has been undertaken previously (Adler et al. 2013; Warinner et al. 2014(a)). The consistency of sex identification between calculus and bone samples from the same individual analysed here also indicates the success of the method. However, the low endogenous DNA content of the calculus samples analysed here was unfortunately insufficient to provide information on population or genetic affinity (section 8.5.4.). Nonetheless, the investigation of the small amounts of endogenous DNA recovered here provides the first analysis of host DNA
from dental calculus samples of any date through shotgun sequencing. Future further investigation of this endogenous content may allow for the investigation of the relationship between host genotype and microbiome composition (Tims et al. 2011; Warinner et al. 2015(a)), particularly given that it is now recognised that the bacteria present within the oral microbiome may be ethnicity-specific (Mason et al. 2013).

Additionally, metaproteomic analyses undertaken here also revealed a range of proteins indicative of the oral microbiome – and akin to the bacterial species identified through metagenomic investigation. Moreover, the bacteria represented within the metaproteomic analysis are also in accordance with previous proteomic findings by Warinner et al. (2014(a)). Excitingly, the proteomic data generated also revealed the presence of the milk protein β-lactoglobulin (BLG) in over half the calculus samples analysed here – thereby making this the earliest identification of BLG in human dental calculus to date. The presence of both bovid-specific (Bovidae) and caprid-specific (Caprinae) peptides within the calculus samples indicates the potential information which analyses of this kind may be able to provide in future on early prehistoric patterns of milk consumption – and could also be tied into larger discussions regarding dietary variables driving natural selection in humans and gene-culture co-evolution. Additionally, the indication that the individuals studied here may not have had LP (see section 8.5.6.1. above and Chapter 2), but were consuming and utilising dairy products, indicates a range of exciting new research avenues exploring how Neolithic populations may have been processing raw milk, and the potential variability which may have existed within these processes in the past.

Overall, however, the recovery of bacterial DNA and proteins, with damage patterns characteristic of ancient material, within Neolithic human dental calculus analysed here importantly indicates that it is a robust reservoir of prehistoric ancient biomolecular information. The detection of the same bacterial phyla within both the genomic and proteomic data generated further strengthens this assertion, and indicates that calculus may allow us to now gain an insight into Neolithic oral microbiomes. In this way, calculus may provide a new means via which we can investigate potential changes in health and/or disease associated with the transition to agriculture, and new ways of living in Britain.
Chapter 9 – Discussion: Discovering Mesolithic and Neolithic Lifeways

As stated in Chapter 1, this research aimed to adopt a combined bioarchaeological approach to examine the period surrounding the Mesolithic-Neolithic transition in Britain, focusing on five main research areas: identification, diet, mobility, chronology, and health/disease. It was hoped that this approach would elucidate more information on lifeways during these two periods, and indicate how these may have changed through time. As explored in Chapter 2, whilst academic attention within both periods has often focused on other aspects – such as flint technologies, ecology, or monumentality, for example – it could be argued that in order to understand the periods themselves, we need to better understand the people who created them.

Objective 1 of this thesis aimed to assess the current state of understanding of the Mesolithic-Neolithic transition in Britain, and was achieved through discussions within Chapter 2, which highlighted that study of both periods has often tended to be on broad scales, with research often having a national or European-wide focus. This thesis instead adopted a finer-grained, population level approach. The lack of consideration of lifeways of individuals within prehistory is not a new observation – for example, it has been commented that we currently have few academic ideas about how people may have actually lived in the Mesolithic period in Britain, as logistic organisation of sites and people has generally taken primacy over interpretations of social facets of the Mesolithic (Spikins 2008). The direct, multi-methodological biomolecular approach adopted here aimed to determine more detailed information about Mesolithic and Neolithic lifeways. Alongside determining what life may have been like on an individual or population level, and by targeting already excavated material, this research also aimed to highlight that it may be possible to obtain valuable biomolecular information from material excavated but currently unstudied, housed in universities and museum or curatorial stores.

The following sections provide an overview of how the biomolecular approaches used here have addressed five main research areas, if the original objectives of the thesis have been met, and considers how the techniques/approaches may potentially be improved upon in future studies. Whether this approach has contributed useful information to broader discussions of the period surrounding the Mesolithic-Neolithic transition in Britain will also be evaluated.
9.1. Identification: Revealing the Mesolithic

As discussed throughout this thesis, and most notably in Chapter 3, one of the major problems limiting our understanding of the Mesolithic of Britain is, arguably, the lack of skeletal material available – particularly that of human origin. Not all British Mesolithic sites thus far excavated have yielded bone, but of those which have, the skeletal assemblages are frequently heavily fragmented and/or disarticulated, often meaning that not all bone can be identified to species (see Chapters 2 and 3). Additionally, where skeletal material is present, this is generally, at British sites, faunal in nature. The lack of human skeletal material dating to the British Mesolithic remains a significant challenge in our understanding of the period as a whole.

As the large quantities of disarticulated and/or heavily fragmented skeletal material previously excavated from British Mesolithic sites may often not be attributable to species, they have traditionally been seen to have little to no osteological, zooarchaeological, or interpretative value. However, whilst morphologically indistinct, these bone samples do in fact contain useful biomolecular data, such as taxonomic information. This biomolecular data can then be placed within contextual, temporal, and spatial frameworks, and incorporated into larger debates. As such, ZooMS was utilised within this research to taxonomically identify fragmentary bone previously classified zooarchaeologically or osteoarchaeologically as ‘unidentifiable’ from a number of Mesolithic sites across Britain. The ZooMS work undertaken within this research is presented within Chapters 5 and 6, and consisted of the analysis of 74 ‘unidentifiable’ fragments of bone from five different Mesolithic sites. Importantly, this work was successful in taxonomic assignment in all fragmentary Mesolithic skeletal assemblages analysed – the identification of faunal species at all five sites, and within one assemblage, Cnoc Coig, (Chapter 5), ZooMS analysis also managed to identify previously unknown additional human remains. As such, objective 2 of this PhD – the successful application of ZooMS to bone fragments – was met. The following sections will discuss the information which this successful application of the method provided.

9.1.1. Detecting Faunal Species

The identification of faunal species from unknown fragments of bone within a skeletal assemblage holds a number of beneficial aspects. Primarily, it can be utilised in tandem
with zooarchaeological reports and assessments already undertaken – and can either lend additional weight to species presence/absence and frequencies previously established (as was seen at Vespasian’s Camp and Cnoc Coig for example (Chapters 5 and 6)), or can alternatively provide additional zooarchaeological information on species morphologically unidentifiable within the assemblage (as was seen at the Western Isles sites (Chapter 6)). In this way, ZooMS provides a complementary zooarchaeological tool for archaeologists, to be used alongside macroscopic level analyses.

Identifying fauna at sites using ZooMS also provides a better idea of the kinds of species populations were exploiting within the Mesolithic of Britain – shown most clearly here through the results obtained from Bágh an Teampaill (Chapter 6). This information can then feed into larger discussions on human-animal relationships within the Mesolithic of Britain, and how animals may have been utilised. For example, the identification of a range of marine mammal species and cetaceans at the Western Isles sites (Chapter 6) raises interesting questions about opportunistic exploitation of stranded or beached marine species within the Mesolithic, and of hunting strategies. Given the historical and ethnographic evidence for the multiplicity of uses of large marine mammals – including not only meat, but also secondary products such as skins, furs, baleen, fat, oils, and so forth (e.g. Denniston 1974; Hovelsrud-Broda 1999; Nuttall et al. 2005; Hovelsrud et al. 2008) – it also presents interesting ideas surrounding how Mesolithic populations may have utilised different animal species. The number of different marine species identified at the coastal sites studied in Chapter 6 also indicates the complexities of Mesolithic lifeways, particularly in island or coastal locations. Significantly, the data presented within Chapter 6 could potentially suggest previous underestimation of early prehistoric resource exploitation.

The identification of additional faunal remains via ZooMS can also potentially provide other ideas about Mesolithic diet – as the animals identified may have been exploited as foodstuffs. This kind of further exploration of Mesolithic diet is important as we still have relatively few ideas about the exact constituents of diet in Britain at this time, and most research on Mesolithic diet has taken the form of isotopic analyses (as discussed in Chapters 2 and 4), which cannot always easily distinguish the proportions of different dietary constituents. Additionally, further information on fauna at Mesolithic sites can also contribute to discussions of environment, climate, and ecology.
Traditionally, Mesolithic fauna have been considered in economic terms (e.g. meat yields, calorific content, energy expenditure; Figure 81), something perhaps enhanced by the typically ecological approaches adopted towards the period as a whole (Chapter 2), but which forces the past into modernist categories and classifications (Thomas 1999, 5).

However, whilst human-animal relationships and interactions are now starting to be more broadly considered for the period (e.g. Bevan 2003; Conneller 2004; Overton 2014; Živaljević 2015), the multiple uses that animals may have had has not yet been given significant attention, and therefore is surely something worth greater consideration in future studies. Animals can provide many differential products, have multiple different uses, and be viewed in any number of ways. Ethnographic accounts often discuss the importance of hunted and marine species as not only being economic, but also cultural and social, and the detailed knowledge of animal behaviour, locations, weather conditions and timings needed for hunting which these populations possess (Nuttall et al. 2005). This is particularly pertinent not only in terms of the identification of marine mammals at coastal Mesolithic sites, but also when we consider the identification of auroch at Blick Mead, which raises interesting questions about the complexities of Mesolithic hunting strategies (Chapter 6). Whilst untangling and determining these aspects of hunting and human-animal interactions for Mesolithic populations will always be difficult and complex, it is this kind of theoretical approach that the field of Mesolithic studies is surely lacking – particularly given that these types of theorisation regarding human-animal interactions have long been established for both the Palaeolithic and the Neolithic, predominantly perhaps due to faunal and anthropomorphic depictions in Palaeolithic art, and contrastingly, domestication being seen as a new form of human-animal relationship and interaction in the Neolithic (e.g. Mithen 1996, 186; 1998; Renfrew 1998; 2007, 145; Marciniak 1999; Gamble 2007; deFrance 2009; Russell 2012).

Figure 81: Example of interpretation of resource representation at Meilgaard shell midden, Denmark (Bailey 1978, 47)
Finally, the application of ZooMS to fragmentary skeletal material can also be used to refute potential biases in zooarchaeological assessments. For example, at Blick Mead the dominant fauna was determined to be auroch from identifiable bones (Chapter 6). However, there was a concern that this dominance may actually be the result of taphonomic bias, caused by the significant size difference of auroch remains in comparison to pig skeletal remains, for example (Jacques, pers. comm.), or equally, could be seen to be result of sampling strategy bias. The identification of smaller fragments within the assemblage to auroch too however indicated that the species prevalence rates calculated were correct, and supported conclusions made in the zooarchaeological assessment.

Overall, our understanding of British Mesolithic fauna is still broadly based upon Star Carr (Legge and Rowley-Conwy 1988) and Thatcham (Ellis et al. 2003), and shell midden sites such as Oronsay (Mellars 1978; 1987), Carding Mill Bay (Connock et al. 1992; Schulting and Richards 2002(c)) and An Corran (Saville and Miket 1994) – therefore meaning we have a limited understanding of the dominant Mesolithic fauna and their uses, and if this may have changed throughout the period. The utilisation of ZooMS therefore can provide new and additional zooarchaeological information on Mesolithic assemblages. Previously, the role of red deer within Mesolithic economies has often been emphasised (Woodman 2000; Milner 2006), but the small number of ZooMS case studies undertaken here indicate that other faunal species could have been of equal importance to Mesolithic populations at some sites. However, in order to fully ascertain this, much a much larger survey of bone fragments would be needed in future. In this way, ZooMS may help to reveal the relative importance of different faunal species in Mesolithic Britain. A final important issue to note however is the sampling strategy implemented in future ZooMS studies of this kind. Within this research, the bone samples analysed were those made available to the author – with the exception of the Cnoc Coig samples in Chapter 5, which were predominately targeted as they appeared most anthropomorphic in nature. In future studies, an awareness of taphonomic factors and inter-species bone fragmentation variability may help to provide a more representative faunal sample. Nonetheless, the issue still remains that in taking a subsample of bone fragments from any given assemblage it is unclear how representative of the faunal assemblage as a whole this is.
9.1.2. Detecting Human Remains

The application of ZooMS to fragmentary ‘unidentifiable’ skeletal material from Cnoc Coig, Oronsay (Chapter 5) also detected human remains, thereby contributing to the number of known human remains dating to the late 5th–early 4th millennium BC in Britain. This case study therefore provided the first application of ZooMS as means via which to identify both faunal and human remains within fragmentary skeletal assemblages. As such, it highlights the future potential of this technique to extend our current knowledge of Mesolithic mortuary practices (objective 8).

The successes of the Cnoc Coig research (Chapter 5) therefore highlight the future potential of ZooMS as a new means through which we can detect human remains within the British Mesolithic. The potential future applications of the method are therefore manifold – and due to the high-throughput nature of the technique and its low cost (see Chapter 4, section 4.2.3.), it could easily and rapidly be applied to other British Mesolithic fragmentary bone assemblages. This may then reveal additional human skeletal material, and present a new step towards increasing the number of known human remains from the period in Britain, and addressing the issues we currently face with the British Mesolithic record discussed in Chapters 2 and 3. For example, the detection of additional human remains from British sites would allow for the greater exploration of numerous aspects of Mesolithic lifeways. Alongside this, identification of additional human remains may contribute towards current discussions of deposition of human remains in the Mesolithic (Chapter 3). The potential of the ZooMS method in providing new information on Mesolithic mortuary practices is highlighted in Chapter 5, as the human remains identified were located outside the main midden structure, whereas all previous human skeletal material from the site was recovered from the midden itself. Whilst the deposition of human remains in shell middens is known at a large number of sites both within Britain and across Europe in the Mesolithic (see Chapter 3), the identification of human remains in an outlying trench at Cnoc Coig raises interesting ideas about deposition on the peripheries of Mesolithic sites – something which has not to date been explored in academic study of Mesolithic funerary practices. In this way therefore, the ZooMS technique may also be seen as a new way of detecting Mesolithic British mortuary contexts (objective 8; Chapter 1), and go some way towards addressing the questions raised in Chapter 3 regarding why we do not have more human remains available from the period in Britain. However, the fact that human remains were only detected in one of the five assemblages analysed here using ZooMS does perhaps suggest that human bone may not be a common component of
‘loose bone’ assemblages in the British Mesolithic, and as such, raises interesting questions about the nature of how Mesolithic communities in Britain were dealing with and disposing of their dead.

9.1.3. Utilising ZooMS as a Mesolithic Identification Tool

To conclude therefore, whilst these analyses were driven by the excavated material currently available from British Mesolithic sites, they have provided useful information from skeletal fragments previously considered to hold little value. The taxonomic information obtained from the bone fragments can be fed into larger discussions of Mesolithic Britain, particularly with regards to environment, ecology, the uses of animals, and diet. As discussed above (and in Chapter 5), the identification of some fragments as human can also contribute new information on Mesolithic mortuary practices and contexts of deposition. The methodology is however not without limitations, of which many are discussed in detail in Chapter 4. Of these limitations, the need for good collagen preservation within samples was most problematic within this research – as seen in Chapter 6. The age of the samples in question, combined with the depositional contexts present at some sites studied here, meant that collagen preservation was poor in some samples analysed – preventing in some cases either taxonomic identification or greater interpretation. Additionally, the taxonomic information generated via ZooMS is significantly less detailed than that provided by aDNA analyses, which can therefore also limit interpretations.

Nonetheless, the novel aspect of this ZooMS approach to Mesolithic material lies in the fact that it can provide this useful information from material already excavated, but currently unstudied – and therefore can contribute additional understanding to sites already known and analysed. In particular, as shown in the methodology utilised in Chapter 5, the technique can also provide taxonomic information from ‘empty’ tubes previously used within the collagen extraction process – and so could have greater application in future on samples analysed for AMS dates or isotopic results where taxonomy is unclear. The analyses provided here aimed to provide a proof of concept study that ZooMS research of this kind could be successfully applied to assemblages of a Mesolithic date, and could positively and actively contribute to larger discussions of the period (as above). The high-throughput nature of the ZooMS methodology, combined with its low cost, partially non-destructive nature (see Chapter 4, section 4.2.), and successes on Mesolithic material as
outlined here, means that it could be simply applied to other early prehistoric assemblages with relative ease in future, and is surely a good direction for future bioarchaeological study.

9.2. Chronology: The Mesolithic, the Neolithic, and the Transition

Objective 5 of this thesis (Chapter 1) was to investigate Mesolithic and Neolithic chronology through the use of new AMS dates. In reality, chronology is ultimately a broad theme which runs throughout all case studies presented here within the thesis however. Although the relative merits and also the limitations of a chronological focus are discussed in detail in Chapter 4, and also run throughout discussions within Chapter 2, it is difficult and complex to consider this period of British prehistory without a chronological focus. New AMS dates were however obtained during this research, both from skeletal material from Cnoc Coig, Oronsay (Chapter 5), and also from Banbury Lane, Northampton (Chapter 7).

Four new AMS dates were obtained here from the Late Mesolithic site of Cnoc Coig, in an attempt to date newly identified human remains from the site, and also to gain a clearer overall understanding of chronology at the site, and are detailed in Chapter 5. Previous dating of the site had been undertaken on samples with a marine component (shells and human remains with a marine diet (Jardine 1978; Richards and Sheridan 2000)), and on bulk charcoal (Switsur and Mellars 1987). This had proved somewhat problematic for providing an overall date for the site as a whole, as samples with a marine component are subject to uncertainties due to the marine reservoir effect (MRE) (Ascough et al. 2005), and charcoal may suffer from an old wood effect (Schiffer 1986). Two new dates on short lived terrestrial samples (identified as Sus using ZooMS) were therefore undertaken. The four new AMS dates from Cnoc Coig, combined with recalibration of existing dates utilising a new ΔR value appropriate for the marine content of the samples, indicated that all the humans overlap with the terrestrial fauna, and fall within the early part of the 4th millennium BC, rather than the late 5th millennium as previously proposed (see Chapter 5). The implications of this, given the marine isotope signatures of the human remains from the site, are discussed in more detail elsewhere in this Chapter (section 9.3.) – but present new information pertinent to discussions of the Mesolithic-Neolithic transition and associated dietary change in Britain.
The new AMS dates presented here, and the associated discussions of chronology, highlight the manifold issues of determining prehistoric chronologies and undertaking radiocarbon dating on prehistoric sites, as initially highlighted in Chapter 4. For example, the $^{14}$C dating undertaken at Cnoc Coig highlights the need to consider the nature of the archaeological materials utilised for dating prehistoric sites, and the importance of this when determining the overall chronology of a site. In tandem with this, the Cnoc Coig dating work also highlights the issues we still face in the UK (and elsewhere in the world) with MREs, and the crucial importance of using appropriate $\Delta R$ values to calibrate samples with a marine component. The lack of $\Delta R$ values currently available across the UK is surely one of the largest problems we currently face in radiocarbon dating, particularly on Mesolithic material, where human remains typically show isotopic signatures indicative of a marine (or partially marine) diet. As marine reservoir offsets are known to vary both temporally and geographically (Ascough et al. 2004; 2005; 2007; 2009; Russell et al. 2015), there is now a real need to generate more $\Delta R$ values for prehistoric Britain – even if these are more conservative, or are averaged values across a geographical area, as in a recent paper by Russell et al. (2015). Additionally, the Banbury Lane dating presented here highlights the issues which can arise if there is not sufficient material at a site for AMS dating to be undertaken. Whilst AMS remains the dominant dating method within archaeology (for both prehistory and historical archaeology; as discussed in Chapter 4), on prehistoric sites where dating is complex or there are few materials suitable for AMS, there is perhaps now a need to look for newer or alternative dating methods which may help to elucidate chronology. One such technique may be the recently developed chronometric use of earthworm calcite, which suggests the potential of being able to date archaeological contexts (Canti et al. 2015).

Overall however, issues still abound regarding the ways in which we can study the transition period, particularly with reference to chronology – as highlighted by Thomas (2013, 215), accounts of the Mesolithic-Neolithic transition have previously “been restricted to generalisation by the coarse grain of the chronologies available: both the lack of precision with which individual events can be ‘pegged’, and the comparative paucity of the determinations so far acquired”. The AMS data obtained from the Cnoc Coig samples also importantly highlights that our current assumption that the Mesolithic-Neolithic transition, and the entire simultaneous adoption of the ‘Neolithic package’, occurred c.4000 cal. BC in Britain may not hold true. Instead, different aspects of the transition may have occurred at different times – and therefore positing a singular calendar date for the
entire transition in Britain may in fact not be either useful or correct. As such, a greater re-focus on chronology, combined with large scale dating programmes, is therefore an avenue of future research for Mesolithic and Neolithic archaeology in Britain which holds great potential.

9.3. Mesolithic and Neolithic Diets and Dietary Change

9.3.1. The Isotopic Evidence

As discussed in Chapter 2, our understanding of British Mesolithic and Neolithic diets, and of the dietary change between the two periods, is still limited. At present, the models proposed for Mesolithic and Neolithic diets in Britain, and for the transition between them, have hardly changed in the past 15 years. As demonstrated in Chapters 5 and 7, investigation of both Mesolithic and Neolithic diet was undertaken here using carbon and nitrogen stable isotopic analysis (as outlined in Chapter 4), in an attempt to reveal further information on diet and dietary change within and between these two prehistoric periods – therefore fulfilling objective 3 of this thesis, and contributing toward objective 7 (Chapter 1).

Biomolecular investigation of Mesolithic diet was undertaken utilising skeletal material from Cnoc Coig, Oronsay, Inner Hebrides (Chapter 5). Stable isotope analysis (δ¹³C and δ¹⁵N) revealed a high marine protein diet, in accordance with that seen in previously obtained isotopic values from human remains from Oronsay (Richards and Mellars 1998). Interestingly, however, as discussed above, new AMS dates undertaken on skeletal material from the site, combined with recalibration of existing dates (see Chapter 5), suggest that the human remains from Cnoc Coig may date to the early 4th millennium BC, and are therefore coeval with the earliest evidence for domestic crops and animals in Scotland and other parts of Britain (Rowley-Conwy 2004; Brown 2007). This indicates that a marine diet may have extended into the 4th millennium BC – and in what is traditionally thought of as the ‘Neolithic’ period in Britain – and overlaps with dated human remains with terrestrial diets elsewhere in the UK. This therefore suggests that a marine diet may have persisted in some areas of Britain after farming had been introduced, and that there may have considerable heterogeneity in human diets in the early part of the Neolithic, potentially reflecting specialisation in subsistence practices across the landscape, and the continuity of foraging, hunting and fishing into the period traditionally associated
with agriculture and pastoralism. This idea of a continued marine diet, or significant marine component within the diet, may have been particularly pertinent for populations living in coastal or island locations, where marine resources were abundant and easily acquired. The idea of co-existence of both foraging and farming modes of subsistence in the early Neolithic has also previously been posited for sites in the Danube Gorges (Boric and Price 2013).

Contrastingly, Neolithic diets were primarily investigated within this research through $\delta^{13}$C and $\delta^{15}$N isotopic analysis of skeletal material from the site of Banbury Lane, Northamptonshire (Chapter 7). This case study aimed to provide a large-scale isotopic study of British Middle Neolithic diet, for which there has traditionally been less academic focus, utilising a recently excavated, unusually large disarticulated human skeletal assemblage. The $\delta^{13}$C and $\delta^{15}$N isotopic data obtained from Banbury Lane highlights remarkable isotopic homogeneity in the adult diet of this Neolithic assemblage. Whilst noted in Chapter 7 that isotopic homogeneity does not necessarily equate to dietary homogeneity (Schulting 2011), the lack of variability seen across all 165 individuals is consistent with the consumption a diet based on terrestrial protein and C$_3$ plants in similar quantities, which resulted in very similar collagen isotope values. Interestingly however, when compared to other British Neolithic assemblages, the Banbury Lane individuals appear to be more elevated in $\delta^{15}$N than perhaps would be expected (Figure 40), which seems to be due to both the cattle ($Bos$) and pig ($Sus$ $scrofa$) values from Banbury Lane also being more elevated in $\delta^{15}$N than other British Neolithic sites. This may be due to a range of factors, from manuring effects (Bogaard et al. 2007), a warmer annual mean temperature (Stevens et al. 2006), or differential nitrogen cycling in soils, affecting plant $\delta^{15}$N values (Handley et al. 1999). It however does highlight the need for clear characterisation of faunal baselines when analysing human $\delta^{13}$C and $\delta^{15}$N isotopic data and determining information on prehistoric diets using isotopic analysis.

Neither of the above sites completely conform to what is traditionally assumed surrounding Mesolithic and Neolithic diets and the transition between them (as discussed in detail in Chapters 2 and 4). Previous stable isotope studies of British material have resulted in the idea of a ‘rapid’ dietary shift at the Mesolithic-Neolithic transition (e.g. Richards et al. 2003; Richards and Schulting 2003), with an abrupt change c.4000 cal. BC from a diet heavily dominated by marine resources, to one based solely upon terrestrial (domesticated) plants and animals. As previously discussed in Chapter 2 (section 2.4.1.), this model of
dietary change has prompted ideas surrounding the active avoidance of marine resources in the British Neolithic, meaning that fish and other marine foods may have been seen as a ‘taboo’ foodstuff, which in turn could be a reflection of new world views or beliefs (Richards 2003; Thomas 2003). However, the data obtained here from Cnoc Coig suggests that the transition from a marine to terrestrial diet may not have been as rapid as has been previously suggested, and that the consumption of marine resources may have continued into the 4th millennium BC in Britain. As such, the idea of a rapid, deliberate and wholesale cessation of marine food consumption from 4,000 cal. BC across the entirety of Britain seems perhaps unlikely. Arguments for a transition this kind are also hindered by the lack of definition provided as to what constitutes a ‘rapid’ timeframe. As previously discussed (Chapter 2 section 2.4.5), the definition of terms such as ‘rapid’ is frequently very subjective, and often related to the timescales in which we are used to viewing the archaeological past. The rapidity at which dietary change at the Mesolithic-Neolithic transition occurred may have actually taken a period of up to 400 years – ‘rapid’ when looking at the whole duration of British prehistory, but certainly not ‘rapid’ when considered in terms of individual lived human experience (Milner 2010). Discussions of how quickly dietary change occurred between the Mesolithic and Neolithic periods in Britain therefore raise some interesting questions regarding subsistence and diet. Can dietary change ever truly be ‘rapid’?

Nonetheless, the Banbury Lane isotopic data generated here would suggest that once a domesticated, terrestrial diet was adopted within Britain, it became considerably homogenised. Middle Neolithic diets have typically received less academic focus, with study often concentrated on dietary change at the Mesolithic-Neolithic transition, rather than dietary or isotopic variability seen within the period itself. Nevertheless, the δ¹⁵N values obtained from the Banbury Lane assemblage do not entirely conform to what we would traditionally expect within a British Neolithic population. Neolithic diets, as discussed in Chapter 2, are assumed to be based upon domesticated faunal species, predominately cattle, pig, sheep and goats, alongside domesticated crops (namely emmer wheat (*Triticum dicoccum*), einkorn wheat (*Triticum monococcum*), and barley (*Hordeum vulgare*) (Thomas 1999, 8, Bradley 2007, 32; Darvill 2010, 88) – although see Stevens and Fuller (2012). If these dietary assumptions are correct however, the δ¹⁵N values (of both humans and fauna) from Banbury Lane are slightly higher than what may be expected, as discussed above (also see data in Appendix B). Alongside this, the remarkable homogeneity across all human individuals at the site raises interesting questions about
Neolithic dietary variability, the distribution of different foodstuffs, and degrees of access to foodstuffs by members of a population. The degree of isotopic similarity seen across all individuals suggests that differential access to foods – as posited by numerous authors for Neolithic populations, and linked to ideas of status differentiation (see Chapter 2) – if present, was not significant enough to be viewed isotopically within the Banbury Lane assemblage. However, this also then raises issues as to whether the Banbury Lane assemblage looks to be demographically reflective of a living population or a ‘standard’ death assemblage – which, as discussed in Chapter 7, it does not appear to be. This therefore presents the possibility that these individuals, selected to be buried within the Banbury Lane monument, represent a designated proportion of the living assemblage – and that these individuals, within this section of society or the population, were afforded a diet which was isotopically indistinguishable from one another. Alongside this however, the homogeneous isotopic results obtained from Banbury Lane also highlight a major issue with isotopic analysis, as discussed in Chapter 4, in that the method cannot distinguish to the level of a specific foodstuff, and therefore interpretations of diet can only be broad in nature.

Additionally, as touched on above, in the isotopic studies of both the Mesolithic (Cnoc Coig) and Neolithic (Banbury Lane) material here, variability in the faunal isotopic data was seen. Large scale isotopic studies of British prehistoric fauna have, to date, not been commonly undertaken, but the importance of creating faunal baselines to interpret human isotopic data should not be underestimated (as discussed in Chapter 4). At Cnoc Coig, the variability seen within the isotopic values obtained from the pig (Sus scrofa) skeletal samples indicated that whilst some pigs from the site may have consumed a terrestrial herbivorous diet, others may have had a more marine or omnivorous diet (Chapter 5). The variability of δ¹⁵N values seen within the pigs from the site is intriguing as it may indicate some degree of marine consumption by some of the animals, and as such, is something which warrants further future study. Whilst pigs are well-documented to be omnivorous, and will consume human refuse and marine protein sources (Albarella et al. 2006; Masseti 2007), in-depth isotopic investigations of prehistoric pig diets are yet to have been undertaken – but could reveal not only more information on the animals’ diets, but also about pig management in the past, and human-animal interactions. However, it is also important to note that if marine isotope signatures were a feature of significant numbers of pigs or other terrestrial animals at Cnoc Coig, then the notion of heavy marine resource consumption by humans would potentially need to be revised.
At Banbury Lane, isotopic analysis of the small number of faunal fragments from the site revealed elevated $\delta^{15}$N values compared to fauna at other British Neolithic sites, as discussed above (and in Chapter 7). Furthermore, differences in the $\delta^{13}$C and $\delta^{15}$N values between species at the site may indicate potential differential management of cattle and pigs in the Neolithic, and/or the two species occupying different habitats. Looking at the isotopic data obtained from both Cnoc Coig and Banbury Lane therefore, it can be seen that there is now a need for larger scale analyses of $\delta^{13}$C and $\delta^{15}$N values in both Mesolithic and Neolithic fauna in Britain. Additional stable isotope studies of fauna would potentially result in a clearer understanding of prehistoric ecology and environment, isotopic variability within prehistoric animals of the same species, past animal management strategies, and clearer faunal baselines from which to interpret human isotopic values.

9.3.2. The Proteomic Evidence

A new way in which to determine additional information on past diets has recently emerged in the form of metagenomic and metaproteomic analyses of human dental calculus. These analyses were applied here to Neolithic dental calculus (Chapter 8), in an attempt to reveal more information on diet and build upon both the existing isotopic data available for the period (see Appendix B), and that generated within this thesis (see section 9.2.1.).

Owing to the vast amounts of data generated via metagenomic and metaproteomic analyses, exploration of this data within Chapter 8 predominately took a broad-scale approach, aiming to determine if the results obtained represented a robust biomolecular dataset, particularly given that calculus of this age had never previously been studied. As such, it may be that DNA and/or proteins deriving from dietary sources are present within the calculus samples studied here, but a finer-scaled and more detailed analysis of the data generated is needed in order to elucidate these and provide additional dietary information. Nonetheless however, the milk protein $\beta$-lactoglobulin (BLG) was clearly detected in over half the calculus samples analysed here, from two of the three Neolithic sites analysed using a metaproteomic approach. The results, presented in Chapter 8, therefore indicate that BLG preserves within human dental calculus dating back to the Neolithic period in Britain, and builds upon previous work by Warinner et al. (2014(b)). As such, this is the
earliest identification of BLG in human dental calculus to date. The detection of BLG is also particularly pertinent given that Neolithic diets have traditionally only been studied through stable isotope analysis (Chapters 2 and 4), which cannot distinguish between protein obtained from meat vs. milk.

As discussed in Chapters 2 and 8, dairying in the British Neolithic has traditionally been studied through organic residue analysis on Neolithic pottery (e.g. Craig 2001; Copley et al. 2003; Craig et al. 2005; Cramp et al. 2014(a); 2014(b); Smyth and Evershed 2015), and zooarchaeological analyses and mortality profiling (e.g. Legge 2005; Greenfield and Arnold 2015), resulting in the hypothesis that dairying emerged in the Neolithic alongside agriculture, utilising newly domesticated fauna. However, more recent aDNA studies have suggested that lactase persistence (LP; needed for the digestion of raw milk in adulthood) would not have emerged in European populations until the Bronze Age (Allentoft et al. 2015; Mathieson et al. 2015), and that LP would therefore have been absent in Neolithic populations (Burger et al. 2007; Gamba et al. 2014; Witas et al. 2015). This therefore indicates that whilst people were practicing dairying in the Neolithic, that milk must have been either consumed in very small quantities, or processed in such a way as to remove the lactose.

Importantly, BLG is known to be present in processed milk products, but generally in significantly lower levels the more the whole milk is processed. BLG is known to be absent or at very low levels in cheese, for example, due to the removal of the whey fraction of the milk during processing (as discussed in Chapter 8, section 8.5.6.1). BLG is known however to be present in yoghurts and other fermented milk products, and it has been noted that it is not markedly subject to hydrolysis or proteolysis during the fermentation process (Bertrand-Harb et al. 2003; Tzvetkova et al. 2007). A study by Czerwenka et al. (2007) showed that whereas the recovery rate of BLG in whole milk is 100%, this decreases to only 50% in strongly processed (heat-treated) yoghurt-based products. An alternative study by Tzvetkova et al. (2007) however showed that bacterial strains used in yoghurt production were not able to reduce the level of BLG in the product by more than 30%. Additionally, Chen et al. (2005) suggest that heating milk over 80°C causes denaturation and results in the significant loss of BLG within milk as a whole. Overall, therefore, it can be seen that the heating or fermentation of milk will decrease the levels of BLG within it, but to varying degrees dependent upon the type and intensity of processing utilised (Czerwenka et al. 2007; Bu et al. 2013).
The indication that the individuals studied here would not have had LP (see Chapter 8, section 8.5.6.1., and Chapter 2), but were consuming and utilising dairy products, as evidenced through lipid residue analysis and the presence of BLG within Neolithic human dental calculus, is indicative of the processing of raw milk in an attempt to remove or reduce the lactose content. As such, this suggests a range of exciting new research avenues exploring how Neolithic populations may have been processing raw milk, ideas surrounding the production of new forms of dairy products, and the potential variability which may have existed within these processes in the past. We can then begin to link this to ideas surrounding the emergence of new technologies within the Neolithic period, and also, importantly, notions of cuisine – as discussed below in section 9.3.3. It is conceivable that past populations chose to utilise the milk of different animals purposively, and processed this in different ways, due to cultural reasons or even taste. At present, there are a huge number of regional cheeses found all across the UK for example – varying in terms of the types of milk used, the processing methods, if the finished product is a soft or hard cheese, and how long the cheese is left to mature for – but all still have strong regional associations, and some even have Protected Designation of Origin or Protected Geographical Indication status (British Cheese Board 2015). Modern examples of strongly regional cheeses include, for example, Cornish Yarg, Red Leicester, Exmoor Blue Cheese, Sussex Slipcote (a soft ewe’s milk cheese), and Harbourne Blue (a hard goat’s milk cheese made in Devon). Considering this modern dietary variability indicates that similar regional differences may have also existed in the prehistoric past in dairy processing and production.

Additionally, the presence of both bovid-specific (Bovidae) and caprid-specific (Caprinae) peptides within the calculus samples analysed here indicates that British Neolithic populations were exploiting multiple species for dairy products, and may indicate potential patterns of milk consumption. Further future work with an increased sample size may therefore indicate distinctions between the utilisation of different ruminant species for milk exploitation, and whether this varied on a population level (i.e. between sites), or was more closely aligned to social constructs. The idea that we may be able to distinguish if certain members of a society consumed differential amounts of dairy products, or dairy from different animals – for example along the lines of sex, gender, age or social standing – is truly fascinating, and would provide a unique new insight into British Neolithic culture and social structure.
Previously, the consideration of which domesticates Neolithic populations may have
exploited for dairying has not always been widely considered. Lipid residue analysis can
determine the presence or absence of dairy lipid biomarkers, but unfortunately these are
not species specific – and therefore interpretation of the nature of dairying occurring is
limited. The identification of dairying herds has frequently been attempted however
through zooarchaeological analysis, considering the species, age, and sex of fauna present
(e.g. Greenfield 2005). Typically, a dairying herd will always consist predominately of
adult female animals, with a few adult males for breeding. As animals can be utilised for
milking to an advanced age however, there will often be a significant number of older
females present in a dairying assemblage. Additionally, due to breeding, there will always
be a surplus of juvenile males generated, which are not needed themselves for breeding
and cannot be used for milking, and as such are culled (O’Connor 2000, 90-91; Legge
2005). Zooarchaeological determination of dairying is made more complex however as a
mixed population of the same species may be utilised for meat, milk and wool
simultaneously, therefore making the economic aims of the herd more difficult to
determine (O’Connor 2000, 89). There has subsequently been much discussion regarding
which species were initially utilised for dairying indicated by the zooarchaeological and
lipid residue data, with some authors suggesting only cattle were used for milk production
(Salque et al. 2013), some proposing both sheep and cattle were utilised (Bogucki 1986),
whilst others have suggested goats were preferentially exploited (Greenfield and Arnold
2015). The species specificity of BLG therefore allows for a more detailed and specific
consideration of Neolithic dairying (in Britain and beyond), and may help towards a clearer
understanding of the animals utilised within dairying through time, and between
populations or sites.

Overall therefore, the research presented within Chapter 8 highlights the potential of
metaproteomic study dental calculus as a method for understanding more about dairy
consumption and use across Neolithic Europe. Furthermore, the hypothesis that Neolithic
populations without LP were consuming dairy can also be tied into larger discussions
regarding dietary variables driving natural selection in humans and gene-culture co-
evolution. The successful application of the method here therefore warrants a broader
application, on a larger sample size of Neolithic material not only from Britain, but also
other European assemblages. Through doing this, a greater understanding of the emergence
of dairying, differential fauna/resource use, development of cuisine, the emergence of LP,
and gene-culture co-evolution may be obtained – thereby leading to a more sophisticated and nuanced understanding of both Neolithic diet, subsistence and economy.

9.3.3. How far do we understand Mesolithic and Neolithic diets in Britain?

Overall therefore, bringing all the evidence together, it is clear that dietary change has traditionally been seen as one of the most fundamental aspects of the Mesolithic-Neolithic transition, and that subsistence has often been one of the key means of definition of the two periods independently. Despite this, our understanding of diet and dietary change across the Mesolithic and Neolithic of Britain is still limited, and generally follows simplistic narratives, which tend to focus on a homogenous Mesolithic diet quickly followed by a homogenous Neolithic diet. Additionally, academic discussion of the timings and nature of the dietary change thought to have accompanied the Mesolithic-Neolithic transition, whilst active throughout the early 2000’s (e.g. Richards and Schulting 2003; 2006; Richards 2003; Milner et al. 2004; 2006), has stagnated in the past 10 years, and the inclusion of new data or interpretations to these dialogues has slowed somewhat. There is now, therefore, a need to reignite discussions of diet within the British Mesolithic and Neolithic, and explore new avenues via which it may be possible to expand our current knowledge. This thesis aimed to achieve this through the incorporation of new biomolecular data, as outlined in Chapters 1 and 4, and shown through discussion above and in the case studies presented in Chapters 5, 7, and 8. Whilst the data generated within this thesis has not resolved the issues surrounding our understanding (or lack of) of Mesolithic and Neolithic diets (as discussed in Chapter 2), it has hopefully presented results which challenge current narratives of diet and dietary change, and will encourage the start of a new body of research utilising biomolecular archaeology techniques to investigate the transition from hunter-gatherer modes of subsistence to agriculture, and the changes seen in diet across this time period.

Additionally, the data obtained from the Cnoc Coig assemblage (Chapter 5) prompts questions surrounding the nature and timings of the Mesolithic-Neolithic transition, which would benefit from additional biomolecular investigation in future. The use of ZooMS to identify additional human remains, combined with stable isotopic analysis to garner information on diet, and the recalibration of AMS dates using newly defined MREs, highlights how a combined biomolecular approach, even on a very small amount of skeletal material, and only using bone collagen, can provide new insights into the dietary
transition at the Mesolithic-Neolithic interface. In particular however, the work on Cnoc Coig also raises questions surrounding when we define the ‘Neolithic’ as starting – something discussed in Chapter 2, when discussing categorisation of the prehistoric past, and how we delineate the Mesolithic and Neolithic periods in Britain, and also discussed below in section 9.6. The Cnoc Coig data suggests that, at least in some areas of the UK, that an immediate dietary transition did not occur c.4000 cal. BC – and that some degree of marine consumption may have continued in some populations into the 4th millennium BC. The collation of all published isotopic data on Mesolithic and Neolithic human remains in Britain also highlighted that the delineation between Mesolithic and Neolithic diets may not always be as clear cut as has previously been assumed (Appendix B; Figure 82). It can be seen that whilst there are clearly some major differences between the diets of certain Mesolithic and Neolithic populations, there is also some isotopic overlap between the two periods. Furthermore, the Figure below also highlights the significant variability within Mesolithic diets, in stark contrast to the apparent isotopic homogeneity of British Neolithic diets. This therefore highlights that particularly for Mesolithic populations, there does not appear to have been a homogenous subsistence pattern across the whole of the UK – and to assume there was negates the variability which can be seen even in the small number of human isotopic values available for the period. Similarly, the idea of the a singular subsistence pattern for mainland European populations has also recently been contested (Fontanals-Coll et al. 2014).
Figure 82: Published $\delta^{13}$C and $\delta^{15}$N values on Mesolithic and Neolithic human remains from Britain. Data collated from Richards and Mellars 1998; Richards and Hedges 1999; Schulting and Richards 2000; 2002(b); Schulting 2005; 2009; 2013; Taylor et al. 2006; Hedges et al. 2006; 2008; Richards 2000; 2008; Schulting et al. 2008; 2010; 2015; Lightfoot et al. 2009; Meiklejohn et al. 2011; Brunning and Firth 2012; Milner and Craig 2009; 2012; Stevens et al. 2012; Montgomery et al. 2013

Crucially, however, alongside this biomolecular work, it is important within the academic study of prehistoric periods to expand both the narratives which we apply to discussions of diet and subsistence change in the past, and the range of theoretical thought applied to diet and the foodstuffs populations consume. One key aspect relevant to discussions of perceived dietary change between the Mesolithic and Neolithic is the notion that that diet is not static – and this is a concept which needs greater consideration within all discussions of perceived dietary change in the past. Diet changes through time, and between places, populations, and individuals. Although two current European populations would both be generally deemed as having similar Westernised diets, based predominately around domesticated (farmed) fauna (e.g. cow, sheep, pig) and C$_3$ crops (e.g. wheat, barley); our
current detailed knowledge of present day cuisine can inform us of the significant and culturally distinct differences between the diets predominant, for example, in France vs. Spain, or Britain, or Germany. In the archaeological past, our knowledge of diet is sadly not so detailed. However, to assume a blanket diet, comprising of the same foodstuffs, in the same quantities, existed for large swathes of the past or entire ‘periods’ negates the complexities which likely existed in past diets. Moreover, even if the same species were consumed within diets across multiple populations or broad geographical areas, and in identical quantities, the way in which foodstuffs were prepared, cooked, and consumed is undoubtedly to have differed – something which may be particularly pertinent when we consider isotopically homogeneous diets in the past (e.g. as seen at Banbury Lane). Therefore, much like the variety seen within European cuisines today, differences may also have existed in the past.

On an even smaller scale, regionally we can still see significant differences in certain foodstuffs across the UK today. Despite our largely homogenous, processed, C₃ and farmed meat dominated diets, certain foodstuffs are still only found in certain areas of the UK, or still bear strong associations to a particular place or geographical area – such as Staffordshire oatcakes (still only found within the county, and distinct from Derbyshire oatcakes), Yorkshire puddings (although consumed across the UK today, still strongly associated with the north of England), laver bread (still predominately only found within Wales), haggis (considered a strongly Scottish dish), a huge range of regional cheeses (as discussed above in section 9.2.2.), and a range of regional stews such as scouse (Liverpool), lobby (Stoke-on-Trent), Lancashire hotpot, and Irish stew, which are variously only found within certain areas of the UK or still bear strong geographical connections, for example. Considering this modern dietary variability alludes to ideas that similar regional differences may have also existed in the prehistoric past, and that separate populations may have had differences in the way in which they prepared, consumed and cooked the same foodstuffs. Whilst isotopic work cannot provide information on cuisine (as commented on above in section 9.2.2.), some recent work on Mesolithic and Neolithic material (although not British) is attempting to change the common perception that prehistoric peoples only consumed food for energy requirements, rather than taste (e.g. Saul et al. 2013). The foodstuffs which people choose to consume, and the ways in which they prepare, process and/or cook these have wider implications than simply representing a calorific input. Food can be imbued with social implications, cultural associations, religious or other beliefs, social stratification issues, or a whole host of other meanings – in other words, Lévi-
Strauss’ (1969) idea that food is ‘good to think’; that cuisine is representative of fundamental human values and beliefs.

Similarly, modern ‘hunter-gatherer’ diets – such as the ‘Paleo diet’ – nutritional discussions aside, fall down on a fundamental level simply because they assume dietary homogeneity in the past amongst all ‘hunter-gatherers’. Whilst issues abound plentifully with these kinds of modern diets in terms of their lack of chronological consideration of hunter-gatherers and of the variety of hunter-gatherer groups worldwide, and their inclusion of domesticated species (e.g. chicken, beef, pork) within what is claimed to be an ‘authentic’ hunter-gatherer diet (Cordain 2015; Wolf 2015) – their lack of archaeological basis and insistency of a homogenous ‘hunter-gatherer’ diet populate incorrect ideas of past diet into mainstream media and amongst the general public. Yet, whilst we know that current diets can vary significantly even within a small geographical area, and as archaeologists, becry the inaccuracies and oversimplification of hunter-gatherers and past diets within modern diet plans (e.g. Warinner 2013(b)), we still adhere to simplistic and under-theorised narratives surrounding both Mesolithic and Neolithic diets ourselves, both in Britain and Europe as a whole. Diet is not stable, static, or simple at present – and nor should we assume that it may have been in the past. To continue to adhere to narratives of a homogeneous Mesolithic diet ended by a rapid transition to a very different, but equally homogenous Neolithic diet, over a period of c.8,000 years, is surely a great oversimplification, and does a gross misjustice to the diets of those past populations. Whilst there was clearly a significant change in diet overall between the two periods, this transition needs to be considered in less binary terms – and is something which cannot be achieved through the analysis of bulk isotopic values alone.

Due to this, and to negate some of the issues with isotopic study of palaeodiet, the use of the statistical software FRUITS (Fernandes et al. 2014; 2015) was adopted to try to gain more detailed understanding of the complexities of Mesolithic and Neolithic diets in the UK, particularly the degree of plant consumption (Chapters 5 and 7). The use of FRUITS models aimed to enhance interpretation of δ¹³C and δ¹⁵N isotopic results obtained, and provided particularly interesting results regarding the consumption of plant foods in both the British Mesolithic and Neolithic. In both populations studied, it could be seen that terrestrial plant foods appeared to constitute a significant component of human diets, and suggests that the role of plant foods may have previously been underestimated in studies of early prehistoric diet in the UK. Whilst the FRUITS models are therefore useful in greater
interpretation of isotopic data obtained, and can provide an estimation of the relative proportions of inclusion of different foodgroups within a diet, they are often discrepancies in the isotopic data available for different sites, areas, or time periods. There are also potential issues in that the measured bone collagen isotopic values of foodstuffs may not reflect the isotopic values of the edible food fraction (Fernandes et al. 2014), and additionally, that few plant isotopic values are available for either the British Mesolithic or Neolithic. The overall problems with using stable isotope values to determine palaeodiets are discussed in Chapter 4 (section 4.3.2.), but in relation to the above discussion, they lack the specificity and detail required to provide more in-depth understanding of the complexities of past prehistoric diets. An additional isotopic approach which could be useful in future studies, and may provide more detail regarding the marine component of both Mesolithic and Neolithic diets however is the use of $\delta^{15}N$ analysis of single amino acids within bone collagen. Both the $\delta^{15}N$ values of glutamic acid and phenylalanine have recently been shown to be markers of aquatic resource consumption in humans, and may also be able to distinguish between marine and freshwater foods (Naito et al. 2015).

Therefore, whilst the use of mixing models (such as FRUITS) can potentially provide more accurate reconstructions of past diet, the model generated is still based upon assumptions made about isotopic values, routing, fractionation and offsets. Furthermore, the dietary reconstruction provided by the model is based upon the foodgroups inputted. Within the Banbury Lane FRUITS model, for example, freshwater fish were not included, as it was initially assumed they did not contribute to human diet at the site, and the lack of currently published freshwater fish isotopic values (Chapter 7). Therefore, whilst the use of isotopic mixing models can be useful, in order to obtain a clearer idea of dietary complexity stable isotope analysis should be used in tandem with other biomolecular methods and means of determining past diet. Here, stable isotope analysis was utilised as the main method for determining information on Mesolithic and Neolithic diets. Whilst this remains a useful tool, and within the chapters presented here has hopefully contributed to our understanding of both Mesolithic and Neolithic diet, in future studies there is perhaps a need to move towards greater theorisation (as discussed above) to allow for more complex narratives to be explored, and a greater overall integration of a range of biomolecular methods to investigate past diet. This thesis has aimed to highlight the possibilities available, and the information which can be obtained through a combined biomolecular approach – and this is something which should, in the author’s opinion, be adopted more widely in the future within other studies of Mesolithic and Neolithic diets in Britain. Only through a re-
focusing of academic research and discussion onto the Mesolithic-Neolithic dietary change can we begin to move forward our interpretations and understanding of this period and the subsistence changes which occurred. In tandem with this, as discussed above, greater theoretical thought and narrative construction is needed within future discussions of the Mesolithic-Neolithic interface. Through this combined approach, we may then begin to prompt an academic reconsideration of the change from hunter-gatherers to agriculturalists in Britain, and in doing so, increase our knowledge and understanding of this period within prehistory.

Tying all this information together, it can be seen that much more future work is needed in order to advance our understanding of the Mesolithic-Neolithic dietary transition – not only in terms of when it occurred, but also how, and why. At present, stable isotopic analysis of δ^{13}C and δ^{15}N remains one of the few ways in which we can determine direct information about individual prehistoric diets (as discussed in Chapter 4), although metaproteomic analysis of dental calculus has also been shown to provide new avenues of dietary information (Chapter 8). Without additional human remains for study however, our knowledge of diet will remain limited. As demonstrated within this research (Chapters 5 and 6), ZooMS may provide a novel way in which we can detect more human and faunal remains within prehistoric contexts, or from sites where human remains are not previously known – and through this, we may be able to attain further information on past diet and subsistence. Therefore, whilst determining a more detailed and specific knowledge of Mesolithic and Neolithic diet currently remains a challenging and complex task, instead of aligning to simplified narratives of past diet, we should instead face this challenge as an opportunity. The utilisation of broad suites of biomolecular techniques to increase our understanding of Mesolithic and Neolithic diets, is, as previously discussed in Chapter 2, an as yet underdeveloped avenue of archaeological research. This thesis has however aimed to show that it is also a research avenue which holds much promise – and that the potential new biomolecular information which we may be able to obtain may hopefully aid in our greater understanding of the complexities of past diets. Combined with greater theorisation and the broadening of the narratives we apply to diet in the prehistoric past, we can then hopefully begin to move towards a more nuanced and detailed understanding of past subsistence.
9.4. Prehistoric Mobility

When considering the mobility levels of prehistoric peoples, Mesolithic populations in Britain are depicted as being highly mobile bands of hunter-gatherers, moving seasonally around the landscape to exploit different resources. In stark contrast however, the traditional view of the British Neolithic, supported by agricultural and settlement evidence, is that communities were highly sedentary (see Chapter 2). Through adopting agricultural modes of subsistence, Neolithic populations are thought to have become increasingly sedentary throughout the period, with agricultural processes facilitating “the emergence of dense, residentially stable communities” (Thomas 2013, 190). These ideas are supported by concepts of ‘complex’ hunter-gatherers in the preceding Mesolithic, who may have practiced degrees of sedentism, thus providing a ‘pre-adaptation to agriculture’ (Arnold 1996; Thomas 2013, 190). In this way, sedentism is seen as increasing through time from the mid-late Mesolithic onwards, into the Neolithic. This narrative has permeated Mesolithic and Neolithic studies since Childe’s (1925) ‘Neolithic Revolution’ was first proposed – and is still seen across the literature today (as discussed in Chapter 2).

However, a growing body of work has now instead started to suggest that earlier Neolithic landscapes in particular may have been fragmented and dispersed, populated by small kinship groups, and based around patterns of structured movement, variable mobility, or short term sedentism (Edmonds 1999, 16; Whittle 1997; 2009; Milner 2005(a)). Equally, the idea that agriculture, sedentism, pottery, and social complexity are all interrelated, and occurred simultaneously (i.e. the Neolithic ‘package’), has started to be challenged – with the realisation that agriculture and sedentism are not always mutually exclusive, and that populations can utilise domesticates whilst remaining mobile (Kelly 1992; Marshall 2006; Whittle 2003, 40).

The mobility data obtained here was from the Neolithic site of Banbury Lane, Northampton (Chapter 7), and fulfilled objective 4 of this thesis (Chapter 1). Whilst the individuals analysed here from Banbury Lane showed remarkable dietary homogeneity (as discussed above, section 9.2.), strontium isotopic analysis indicated a greater degree of movement than would perhaps be expected from a British Neolithic population, particularly given the dominant narratives of sedentism for the period within the literature. This data is perhaps all the more interesting due to the fact that the Sr values obtained represent a short period of early childhood, from birth to before 3 years of age. Although nearly half the individuals studied (n=12) exhibited $^{87}\text{Sr}/^{86}\text{Sr}$ values which indicated that their early childhood was spent in an area of underlying geology similar that found in the
region of Northampton (based upon Sr values obtained from soil samples); the remaining individuals displayed strontium signatures at some point along the growth axis of the enamel which were consistent with areas of a differential geology to that of Northamptonshire. Within those individuals with $^{87}\text{Sr}/^{86}\text{Sr}$ values representative of what appears to be a ‘non-local’ geology, both more and less radiogenic geologies are apparent. When comparing these results with the strontium map created for the UK (Evans et al. 2010; see Chapter 7), some appear to indicate geological areas of the UK substantial distances from Northampton.

The Banbury Lane strontium data presented within Chapter 7 however raises some interesting points. Firstly, the characterisation of biosphere strontium values for Banbury Lane through soil analyses clearly highlights the importance of determining local strontium ranges for any given study area, rather than relying on the broad scale strontium maps currently available. The broad range of values attained for the Northampton area from the soil samples indicates that the local ranges as indicated by Evans et al. (2010) are not conservative enough, and that there is more small scale regional strontium variability across the UK than this map indicates. Understanding biosphere strontium values is of crucial importance in interpreting enamel mobility data. Additionally, the use of LA-MC-ICP-MS within the Banbury Lane study can be seen to be pivotal in determining and viewing movement within early childhood. Although incremental or serial sampling and analysis using TIMS has previously been applied (e.g. Balasse and Ambrose 2002; Montgomery et al. 2010; Viner et al. 2010) the level of resolution these kinds of analyses can provide is still significantly less than LA-MC-ICP-MS, and the time taken for analysis is considerably longer using TIMS. The use of LA-MC-ICP-MS to determine childhood movement therefore presents the most efficient and detailed means via which to obtain mobility data at present – and the resolution of data obtained is currently unparalleled. Through this method therefore, we can begin to expand our knowledge of early prehistoric mobility and movement. The results obtained via LA-MC-ICP-MS may indicate that we have previously underestimated the degree of human mobility within the British Neolithic.

Indeed, the $^{87}\text{Sr}/^{86}\text{Sr}$ values obtained from the Banbury Lane material suggest that British Neolithic populations may not have been as sedentary as traditionally assumed – and align more with theories surrounding Neolithic variable or structured movement and mobility, as discussed above. The Banbury Lane data is also in accordance with previous $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic studies on both British and European human skeletal remains (discussed in
Chapter 4, section 4.4.5.), which also indicate significant mobility within the Neolithic populations studied. However, whilst there is now a move towards seeing the Neolithic instead in terms of fluidity of movement, as having variation in mobility, and/or as semi-sedentary (Bailey and Whittle 2005), comparatively little investigation of this has actually taken place, particularly using biomolecular methods, as discussed below. Nonetheless, the Banbury Lane data indicates that individuals within the British Neolithic were mobile within childhood, and additionally, as discussed in Chapter 7 (section 7.5.2.), these results can also be linked to ideas surrounding Neolithic subsistence, temporality, biographies, and personal or group identity. From this, therefore, we can postulate two things – firstly, that the Banbury Lane data is perhaps indicative of a more pastoralist and/or mobile farming economy within the British Neolithic, rather than simply sedentary agriculturists, and secondly, that it provokes ideas of linked kinship networks and structured movement across Britain throughout the Neolithic period. These notions therefore link to ideas previously put forward by Whittle (2003), obtained from settlement data, suggesting degrees of mobility within the British Neolithic, and routine movements to different areas or sites by populations.

Ideas of Neolithic pastoralism or transhumance are sometimes found within the British literature, but are often not discussed, with traditional narratives of sedentary agriculturalism more frequently favoured (see Chapter 2). Definitions of both terms are also rarely found, and whilst pastoralism is often considered as referring to animal husbandry and the rearing of livestock, it is also found to sometimes be used interchangeably with ‘agriculture’ within the literature. Pastoralism here is used to refer to populations which utilise domesticated species, but maintain some degree of mobility, which allows for the movement of animals when needed, to find new pastures, water sources, or other resources. Transhumance is instead seen as the seasonal movement around a landscape, generally with domesticated species. Transhumance does not, however, have to be exclusively associated with the movement between lowland and upland areas, as it is frequently described to be, nor does it have to exclusively involve animals (Bradley 1978, 55). Additionally, confusingly, transhumance is occasionally referred to as ‘semi-nomadic pastoralism’, and the term ‘transhumant pastoralism’ is also sometimes found within the literature, seen as “the seasonal movement of domestic herds between altitudinally differentiated and complementary pastures” (Arnold and Greenfield 2004, 96). Both pastoralism and transhumance are however frequently seen in economic terms, or as a means of negating environmental risk (Arnold and Greenfield 2004). These
alternative models of subsistence do however highlight that the utilisation of domesticated species does not mean that populations must also be sedentary. Greater consideration of the potential movement of Neolithic communities in Britain, and what this may have meant for subsistence strategies, is therefore now needed. Despite Bradley’s (1978, 70) assertion that “at no time in British prehistory was an exclusive emphasis on pastoralism either likely or feasible”, the Banbury Lane data presented in Chapter 7 does suggest that Neolithic individuals were moving around the landscape – and that pastoralism may account for why this was.

Additional information on Neolithic mobility can be seen through the ‘Elm Decline’, c.3940 BC, which sees a rapid decline in elm pollen in the archaeological record due to disease spread, suggested to be facilitated by the movement of people (Robinson 2000; Parker et al. 2002). Furthermore, whilst Neolithic sites are frequently considered to be occupied year-round, it is often not considered whether the evidence for activity at a site for each ‘season’ comes from the same individual year (Bailey and Whittle 2005). A major problem however still lies in the fact there has been a lack of Sr studies on human remains dating to the British Neolithic (as discussed in Chapter 4 (section 4.4.5.) and Chapter 7), particularly using LA-MC-ICP-MS, therefore meaning that there is little comparative data available for study, and also that we cannot corroborate or refute theories and hypotheses surrounding prehistoric mobility. Additionally, this in turn also serves to highlight how little we still understand about both mobility and the nature of subsistence within the British Neolithic. The lack of incorporation of biomolecular methods to investigate Neolithic (and Mesolithic) mobility in the archaeological record however means that it serves to be an exciting avenue for future research. In undertaking more biomolecular studies of prehistoric mobility, this will hopefully allow us to increase our knowledge of how people lived in Britain in the archaeological past, and the ways in which they moved around or utilised the landscape in different areas of the country. Understanding levels of prehistoric sedentism and mobility is therefore important as it can provide a key insight into the lifeways of the populations in question. Seasonal or high mobility will represent a very different way of life to sedentism – and the degree of mobility within a population will affect many aspects of everyday life, including social relations, interactions between individuals, kinship, population density, competition, cooperation, gender roles, territoriality, and demography (Kelly 1992; Marshall 2006), to name but a few. However, the traditional idea that hunter-gatherer or mobile societies are more egalitarian, less competitive, or have a less ranked social structure than sedentary communities, due to their
movement patterns, has been contested by numerous authors (e.g. Binford 1980; Myers 1988; Flanagan 1989; Kelly 1992).

Despite this, it is important to note that it is well recognised that mobility and sedentism are difficult to study archaeologically – particularly when concerning variable levels and different forms of mobility (Bradley 1972; Kelly 1992). This is particularly pertinent when we consider the scales at which we wish to study mobility. As Kelly (1992) highlights, some individuals will move more than others, some individuals will move in different ways to others, and some movement will happen on a daily basis, whereas other movement may be seasonal, annual, or dictated by events or other forces. Equally, some movement may be behavioural, but other mobility may be cultural, social, or due to resources. Whilst strontium isotopic analysis, as used here to investigate prehistoric mobility, has a number of advantages over other methods of study (as discussed in Chapter 4, section 4.4.), it cannot provide information on the reasons behind movement, nor can it determine movement on the scales discussed above. Instead, strontium isotope analysis of dental enamel, as was undertaken here, will only provide mobility information pertaining to the period during which the tooth in question was mineralising (see Chapter 4, section 4.4.) – i.e. the method only provides a record of short periods of childhood mobility. An additional problem with the utilisation of $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic analysis lies in the fact that there must be distinct differences in the geologies (and therefore the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios) an individual moved around in order for migration to be determined. As seen in Chapter 7, this is particularly problematic for the UK, as large areas of the country have the same strontium signature. As such, it can therefore be problematic to determine small-scale movements using strontium isotopes, and movement across multiple geological zones will be averaged out within the tooth enamel – even if using LA-ICP-MS-MS. An overview of the advantages and disadvantages of utilising Sr isotopes and other methodologies to investigate mobility and movement in the archaeological record can be found in Chapter 4 (section 4.4.). Nonetheless, strontium isotopes still have great utility within archaeological studies, and are deemed to be “one of the most effective means to characterise mobility in past populations” (Lewis et al. 2014, 173). The utility of the method is hopefully highlighted within Chapter 7, and has revealed here that mobility was present within the British middle Neolithic.

Overall, however, we must remember when studying prehistoric mobility and movement that “mobility is universal, variable, and multi-dimensional” (Kelly 1992, 43). Whilst some
recent attempts have been made to question the traditional narratives of immediate sedentism following the Mesolithic-Neolithic transition (e.g. a recent paper by Ruff et al. (2015) suggested that the transition to sedentism was gradual, but increased due to agricultural intensification), greater consideration – both theoretical and bioarchaeological – now needs to be given to this area of study. In reality, within both the Mesolithic and Neolithic of Britain, there is likely to have been variability in mobility and movement – perhaps on a local, population, or regional level – ranging from highly mobile groups, to ‘embedded’ or ‘tethered’ mobility (Whittle 1997; 2003, 40), to short-term sedentism, and longer term or permanent sedentism. This variability may have differed chronologically through time, but also temporally within groups – with differential levels of movement at different times, related perhaps to external circumstances or resource availability – and also between individuals within a population, as seen at Banbury Lane. It is this variability however which is often neglected in archaeological discussions of prehistoric mobility – but which needs to be addressed in future studies and dialogue. Additionally, however, as Whittle (1997) rightly highlights, even within discussions which recognise that there may have been varying degrees of mobility within the British Neolithic, these need to be more clearly defined and outlined. A recent paper by Neil et al. (2016) suggested that communities in the Early Neolithic in Britain were not sedentary, but instead were residentially mobile. Importantly, however, the strontium isotope data presented within Chapter 7 suggests that this degree of mobility extended into the middle Neolithic too. This is particularly interesting when we compare it against arguments made by authors such as Stevens and Fuller (2012; 2015) who suggest that sedentary agriculture as a dominant subsistence strategy persisted throughout the Early Neolithic until c.3650-3600 cal. BC, at which point there occurred a shift from cereal-focused farmers to a more mobile and pastoralist society. In Britain, Stevens and Fuller (2012) see the transition from arable farming to pastoralism occurring c.3350 cal. BC, which is particularly pertinent when we consider that the human remains from Banbury Lane are dated to c.3370-3100 cal. BC. Could it be that the individuals from Banbury Lane represent a population whose movement signifies the adoption a new pastoralist lifeway, forced by population collapse and/or the decline in cereal cultivation? This interpretation would indeed fit with the stable isotope data obtained for the population.

The traditional dichotomy of highly mobile Mesolithic groups vs. highly sedentary Neolithic agriculturalist populations can therefore be seen to be an outdated notion – instead, greater consideration and investigation now needs to be given to the potential
types and degrees of movement which may have been present in both periods. Within future discussions of prehistoric mobility, there must now be an awareness that mobility and movements are likely to have differed in a whole manner of different ways, as discussed above. In opening up these new discussions however, we must also aim to distance ourselves from outdated notions that sedentism represents a higher or more advanced evolutionary stage than non-sedentary settlement and mobility patterns (Rafferty 1985; Arnold 1996). Whilst sedentism has benefits in allowing more easily for a larger population size and the creation of surpluses, it may also result in increased social stratification, and higher population densities and a potentially less diverse diet may result in health issues and the emergence of new diseases (as discussed in Chapter 2). As such, sedentism does not necessarily have a selective advantage (as previously promoted by Rafferty (1985)) over mobility or hunter-gatherer modes of subsistence. Additionally, as proposed by Bailey and Whittle (2005, 3), it can be suggested that “sedentism as a concept is restrictive as it sets up a binary opposition to mobility”, and thereby also limits our interpretations of the Neolithic.

Neolithic mobility can therefore be seen to still be underexplored within the archaeological literature – including the potential reasons why populations may have been moving. From the discussion here, movement due to subsistence strategies appears to represent a parsimonious explanation (i.e. pastoralism), but ideas of movement due to shared kinship networks and social reasons should however not be ignored. A lack of focus and bioarchaeological investigation into the reasons behind movement, or how movement may be linked to either subsistence strategies, or social reasons, or both, is surely a direction for future study. At present, small amounts of work have been undertaken using carbon and oxygen isotopes to investigate nomadism and pastoralism/transhumance in populations, but generally not on British material (e.g. Mashkour et al. 2005). Furthermore, in studies utilising $^{87}$Sr/$^{86}$Sr isotopic analysis, detailed consideration is not often given to why some individuals appear to be ‘non-local’ (examples provided in Chapter 4, section 4.4.5.). Concepts such as pastoralism or transhumance are sometimes mentioned, but often only as a brief comment (e.g. Carvalho et al. 2015; Neil et al. 2016). One way in which to investigate ideas of pastoralism or transhumance further would be through strontium isotopic analysis (using LA-MC-ICP-MS) of the teeth of cattle or other domesticated species. Nonetheless, an awareness in future studies and discussions of the possibility that Neolithic populations may have been mobile, and that movement may have varied between
populations, regions, or individuals, can serve only to increase our understanding of prehistoric movement and lifeways.

Finally, greater consideration of Mesolithic mobility is also now needed. As discussed within Chapter 4 (section 4.4.5.) there is a distinct lack of strontium isotope studies or other science-based mobility analyses on Mesolithic material. Instead, the traditional view of Mesolithic populations being highly mobile, nomadic, or undertaking seasonal movement has persisted, but in reality, is based upon sparse archaeological data. Additionally, as Wickham-Jones (2005) has highlighted, clear definition of what is meant by ‘mobile’ is rare, and the means via which British Mesolithic archaeologists have aimed to interpret or gauge movement during the period have been limited. As with discussion of Neolithic mobility, greater consideration of how we can study past population movement is now needed for the British Mesolithic, as is a reconsideration of why populations may have been moving, and when this movement may have taken place. It is only through a greater understanding of both Mesolithic and Neolithic mobility therefore that we can begin to determine whether a change in mobility truly occurred between the two periods, and how the types and levels of movement in the prehistoric past may have affected or altered the lifeways of British populations.

9.4.1. Neolithic Genetic Population Affinity

Although not one of the original objectives of this thesis, additional information on Neolithic population movement and/or replacement was attempted through genetic analysis of endogenous DNA from calculus samples analysed in Chapter 8, through the use of the software package bammds (Malaspina et al. 2014; see Chapter 8, section 8.5.4.). The use of ancient DNA in determining demographic change and the mapping of the spread of agriculture is well established, with large numbers of studies aiming to gain a clearer idea of population movement and/or replacement. Previously, researchers have often focused on generating ‘maps’ of genetic traits which aim to determine the genetic origins of European farmers, or attempts to determine differences in mtDNA diversity (via haplotypes and haplogroups) between hunter-gatherers and farmers (e.g. Ammerman and Cavalli-Sforza 1984; Sokal et al. 1991; Semino et al. 1996; 2000; Price 2000(b); Sykes 2001; 2003; Richards et al. 2002; Cavalli-Sforza 2003; Bentley et al. 2003; Currat and Excoffier 2005; Haak et al. 2005; Rasteiro et al. 2012; Hervella et al. 2012; Skoglund et al. 2012; Rasteiro and Chikhi 2013; Brotherton et al. 2013; Schroeder 2013; Hofman 2015;
Malström et al. 2015). Some studies have also looked at animal aDNA to determine domestication timings and movement – using this as a proxy for Neolithisation (e.g. Larson et al. 2007; Ottoni et al. 2013; Schubert et al. 2014; Blaustein 2015).

The analysis undertaken here aimed to link to these kinds of previous studies, and also to longstanding debates on Neolithic population movement and replacement, as discussed above and in Chapters 2 and 7. Unfortunately, however, determination of genetic population affinity from endogenous DNA content within the calculus samples analysed within Chapter 8 was unsuccessful – due to the low depth of coverage of the samples, a cause of their low overall endogenous content. This therefore highlights that calculus aDNA may not, in future studies, be a useful source of genotypic data for archaeological samples. Nonetheless, however, if human dental calculus samples in future analyses were found to have higher endogenous content or higher mean depth of coverage of the human genome, then running genetic population affinity analyses (using software such as bammds) may be worthwhile, and may indeed yield useful genotypic or population affinity information. Overall however, the aDNA analysis undertaken in Chapter 8 also raises interesting questions regarding why the endogenous DNA content is so low in the calculus samples. As suggested within the Chapter, the endogenous content within calculus may in fact be higher than reported here, but is simply swamped by the huge volumes of bacterial DNA present during sequencing. This therefore raises interesting ideas regarding whether in future analyses the methodologies currently utilised could be altered somewhat to allow for a higher proportion of the endogenous (human) DNA within the calculus samples to be sequenced. If possible, then it may indeed be possible to obtain genetic population affinity from endogenous DNA content within archaeological human dental calculus samples – and therefore contribute additional information to debates surrounding Neolithic population movement and replacement, from samples where traditional bone DNA analyses had not been undertaken.

9.5. Early Prehistoric Health and Disease

At present, we still currently have very little knowledge of Mesolithic and Neolithic health, as discussed in Chapters 2 (section 2.4.3.) and 4 (section 4.6.1.). Indeed, of the areas of ‘change’ perceived to have occurred at the Mesolithic-Neolithic transition covered within Chapter 2, the potential health changes at the transition to agriculture are arguably one of the least well understood. It has traditionally been proposed that the changes occurring at
the Mesolithic-Neolithic interface – particularly in diet, mobility, and population size – would have resulted in altered diseases states, and potentially also the emergence of new diseases or the increased virulence of existing conditions. Whether or not this is true, however, is difficult to ascertain given that our knowledge of health and disease in the two periods themselves is scarce. Traditionally, prehistoric health and disease has been investigated, where possible, through the analysis of human skeletal material – although the problems arising from this are variously discussed in Chapter 4 (section 4.6.) and Chapter 3. Here, an assessment of human dental calculus as a new source of health and disease information was undertaken. This involved the metaproteomic and metagenomic analysis of Neolithic human dental calculus (Chapter 8), building upon published work by Warinner et al. (2014(a)), and fulfilling objective 6 of this thesis (Chapter 1).

Metaproteomic and metagenomic analysis of human dental calculus has recently been shown to be a new source of health information on past populations (e.g. Adler et al. 2013; Warinner et al. 2014(a)). The realisation that archaeological calculus may hold useful biomolecular information has occurred in tandem with recent advances in microbiological research resulting in the recognition that the relationship between humans and microbial communities is complex, and that human microbiomes are a symbiotic community. As Warinner and Lewis (2015, 1) state, “no study of human health or evolution is complete without consideration of our microbial self”. In particular, study of the human oral microbiome is increasingly becoming an expanding area of both microbiological and medical research, as discussed in Chapter 4 (section 4.6.). The oral microbiome consists of a range of fungi, viruses, protozoa, and bacteria; but it is bacterial phyla which dominate the oral microbiome’s composition. Around 50% of oral bacteria are unculturable, but recent advances in NGS technologies have revealed the diversity of the oral microbiome. At present, over 700 bacterial species level taxa are currently known within the oral microbiome (Aas et al. 2005) – making it one of the most well characterised microbiomes within the human body. As discussed within Chapter 4 (section 4.6.) however, it is important to note that standard 16S rRNA analyses (such as those used by Adler et al. (2013)), significantly underestimate the richness of the oral microbiota and are subject to amplification bias (Wade 2015; Ziesemer et al. 2015), and therefore a shotgun metagenomic approach, as utilised within Chapter 8, should instead be applied to all future studies of dental calculus.
Given the difficulties in studying prehistoric health and disease, an initial pilot analysis of Neolithic dental calculus from numerous sites across Britain was undertaken here (Chapter 8) to determine if calculus of this date may provide a robust biomolecular dataset, and provide a reflection of the oral microbiota, as seen in previous studies (e.g. Adler et al. 2013; Warinner et al 2014(a)). This represents the first ever shotgun metagenomic analysis and metaproteomic analysis of Neolithic dental calculus. All generated genomic and proteomic data revealed bacterial species relating to or deriving from the oral microbiome - Actinobacteria, Proteobacteria, Firmicutes, Chloroflexi, Bacteroidetes, Spirochaetes, Fusobacteria, Synergistetes. Additionally, proteins deriving from Thaumarchaeota and Fungi were also detected in a small number of samples analysed (Chapter 8, section 8.5.5.). All of these phyla are recognised as core oral archaea, and ubiquitous constituents of the oral microbiome (Krom et al. 2014; Huynh et al. 2015; Duran-Pinedo and Frias-Lopez 2015). It can therefore be seen that dental calculus represents a viable new substrate for early prehistoric study, and with more detailed analysis, may be able to contribute to our understanding of Neolithic (or prehistoric as a whole) health & disease.

Nonetheless, there are however a number of limitations of the approach – which should be explored within this discussion of the application of the method to prehistoric calculus samples. Firstly, and perhaps most obviously, significant amounts of dental calculus will be needed for study in future in order to make meaningful interpretations about prehistoric health and disease. Whilst this may seem a simplistic concern, it is in fact crucially important to consider. Although dental calculus is often present on prehistoric dentitions, particularly those from the Neolithic, it is generally thought to be less prevalent in Mesolithic assemblages, potentially due to lower carbohydrate intake. Additionally, given that calculus was frequently cleaned from dentitions in the past in order to clearly view the teeth and their morphology, it may not be ubiquitous in all Mesolithic and Neolithic skeletal collections. The lack of excavated human remains from the Mesolithic and early Neolithic in Britain further compounds this problem. Calculus is, in effect, therefore, a finite resource – and is obviously not as abundant as bone or teeth. However, in order to truly assess prehistoric health and disease states, statistically significant sample sizes are needed. Alongside this, is also the cost aspect – both financial and the time taken in order to undertake analysis and interpretation – which is high (further information on this can be found in Kolmeder and de Vos 2014; Pible and Armengaud 2015; and Warinner et al. 2015).
Further issues abound regarding the bioinformatics pipelines used to analyse both microbiome metagenomic and metaproteomic data. The methodologies for data analysis used within this research are all provided in Appendix A, but as stated in Chapter 8, the analyses undertaken here were purposively broad in nature, with the primary aim of identifying the dominant phyla present within all samples, and whether calculus of a Neolithic date presented a robust biomolecular dataset. In order to fully assess the proteomic and genomic data obtained, and more detailed information on Neolithic health and disease, a more in-depth analysis will need to be undertaken. The way in which the data is analysed further however is something which needs careful consideration. As highlighted by Pible and Armengaud (2015, 3418), “microbiome meta-omics is a true challenge because of the levels of complexity and heterogeneity”. Additionally, an overview of the problems with current reference or sequences databases and analysis pipelines utilised within both proteomic and genomic work are clearly outlined in Kolmeder and de Vos (2014) and Pible and Armengaud (2015).

Finally, it must be acknowledged that analyses of kind may also be limited in the types of disease information they can provide. The bacteria and proteins recovered will all originate from the oral cavity, and as such, will likely be biased towards oral or respiratory tract disease information – although this is something which needs further study. Additionally, the availability of reference genomes, combined with strain genomic variability in microbiome constituents, means that characterisation of species present (both genomically and proteomically) is complex – and this is further compounded by the antiquity of the samples being studied, meaning that DNA or proteins sequenced may in fact reflect extinct microbial species for which no reference genomes exist (as discussed in Warinner et al. 2015). Furthermore, characterisation of proteins is made more challenging by post-translational modifications and damage patterns, and the limited number of reference spectra available (Warinner et al. 2015).

Nonetheless, the detection of bacteria in dental calculus is still a huge step forwards in our understanding of prehistoric health. Calculus studies of this kind however also have current-day applications or relevance, particularly given that modern dental diseases have a huge global economic impact. It has been estimated that in 2010, direct treatment and indirect costs of dental diseases worldwide amounted to US$442 billion (c. £293 billion) (Listl et al. 2015). However, it is also now recognised that many oral diseases may also be linked to systemic chronic diseases, such as strokes, diabetes, pneumonia, and
cardiovascular disease, as well as cancer (Seymour et al. 2007; Whitmore and Lamont 2014; Atanasova and Yilmaz 2014; 2015; Duran-Pinedo and Frias-Lopez 2015). This therefore suggests that the detection of oral microbiota within dental calculus may in fact be able to determine information about diseases other than those affecting the oral cavity – and, importantly, about conditions or diseases which are otherwise nearly impossible to study in the archaeological record (e.g. stroke, heart conditions), as discussed in Chapter 4 (section 4.6.). Furthermore, as more calculus studies are undertaken, the data generated may even allow us to discern if and how bacteria and/or pathogens have evolved through time. The detection and genome reconstruction of *Tannerella forsythia* in a study by Warinner et al. (2014(a)) highlights the potential of this – showing a <50,000bp gap in the genome reconstruction corresponding to tetracycline resistance genes.

In terms of further future analysis therefore, more detailed analysis of the data generated in Chapter 8 is firstly needed, as discussed above. Beyond this however, there are a huge number of potential future research avenues and applications of the method. One exciting future application would be to extend this research into looking at Mesolithic calculus. Through this, there may be the potential to see changes in health, disease, or the oral microbiome with the Mesolithic-Neolithic transition, and the adoption of agriculture. It has previously been suggested that the dietary change between the two periods may have resulted in an increase in cariogenic (e.g. *Strep. mutans*) and periodontal (e.g. *P. gingivalis*) bacteria in Neolithic populations (Huynh et al. 2015). This has previously been explored by Adler et al. (2013), who analysed six Polish Mesolithic calculus samples and six German Neolithic samples, and concluded that the “oral microbiota underwent a distinct shift with the introduction of farming in the early Neolithic period” (Adler et al. 2013, 453). However, this study only utilised targeted 16S rRNA DNA analysis (rather than shotgun metagenomics), which has since been shown to be problematic for dental calculus samples (Ziesemer et al. 2015; see also Chapter 4, section 4.6.). The constituents of the oral microbiome and symbiosis of the microbiota do however appear to be affected by the diet of the host, and a range of studies have shown differential oral microbial communities between different populations (Costalonga and Herzberg 2014). Indeed, the microbiome is now recognised to be affected by a range of complex factors, which results in complex host-microbiota interactions (Figure 83). Due to this, there is now a need therefore to undertake a larger study of both Mesolithic and Neolithic dental calculus, and truly determine if any changes in oral microbiota can be detected, and what the potential causes of these may in fact be.
However, it is important to note that we cannot simply consider changes in the constituents of the oral microbiome or their distribution. If differences in the types of bacteria present within the oral cavity are apparent between the Mesolithic and Neolithic periods, then there must also be a greater focus on the changes in community interactions which this would bring about – be these positive interactions, dependencies, or negative interactions. This kind of metacommunity approach, as outlined by Boon et al. (2014), may therefore help us to understand in more detail disease processes and health states, as it goes beyond simply noting the presence/absence of bacterial species, and instead considers microbial response, and how this may affect a host. Through this therefore, it may be possible to determine if the health change perceived to have accompanied the Mesolithic-Neolithic transition truly occurred.

Overall, the work undertaken on dental calculus within this research (Chapter 8) has highlighted that calculus is a new and viable means via which to gain new insights into prehistoric health and disease. As discussed throughout the thesis, expanding our knowledge of early prehistoric health is important as it can provide clearer insights into past lifeways – and at the moment, is an aspect of early prehistoric life about which we currently have little understanding of (see Chapters 2 and 4). The recovery of aDNA and proteins from Neolithic samples within this work has shown that biomolecular information
remains entrapped within dental calculus of this date, and the data obtained shows that both DNA and proteins recovered are reflective of the oral microbiome. Further analysis of the samples presented here is now needed in order to accurately assess the health and disease states of the individuals sampled, and this can then be compared with osteological analyses already undertaken on the remains. In future, it is hoped that this approach can be expanded to Mesolithic dental calculus samples too, as discussed above. In doing so, the method may provide the clearest means via to assess if there truly was a health and/or disease change between the Mesolithic and the Neolithic periods. Furthermore, the assertion that the oral microbiome has become less biodiverse through time (Adler et al. 2013; Costalonga and Herzberg 2014) is something which also warrants further investigation and consideration, particularly considering the incredibly small sample sizes of archaeological dental calculus which have so far been analysed. There has also been the suggestion that the bacteria present within the oral microbiome may be ethnicity-specific (Mason et al. 2013), and this therefore may be something which would also be interesting to investigate in future analyses of archaeological calculus. Through more future study therefore, the study of dental calculus has the potential to elucidate new information about prehistoric health and disease, and provides a unique opportunity via which to explore the links between human health, diet, environments, and microbial diversity.
10.1. Conclusions

This thesis has provided a new biomolecular examination of lifeways in the British Mesolithic and Neolithic, focusing on five main research areas: identification, diet, mobility, chronology, and health/disease. The thesis has hopefully highlighted the advantages of a multi-stranded and multi-methodological research focus, and indicated how this could be adopted within further studies of both British Mesolithic and Neolithic material. In doing so, it has aimed to assess how far biomolecular methods may assist in filling the knowledge gaps still deemed to present within Mesolithic and Neolithic studies. It is however important to note that the samples utilised within this PhD were led by the material which was available for study at the time of analysis. As the material spans both the Mesolithic and Neolithic periods, and comes from numerous different locations across the UK, this has meant that each chapter has aimed to provide a case study: highlighting how we can utilise different bioarchaeological techniques on already excavated archaeological material, and what kinds of information this can contribute to discussions of the Mesolithic-Neolithic transition in Britain. As such, the PhD has been successful in demonstrating that excavated but overlooked material (namely fragmentary and disarticulated bone, and dental calculus) can be effectively utilised within biomolecular analyses, and can provide novel information which can then be incorporated into broader discussions of the two periods. From this we can therefore start to create a biographical approach to prehistoric skeletal material, building from people to populations. A greater focus on individual ‘osteobiographies’ generated through biomolecular approaches is therefore something which should be considered in future analyses.

At the start of this thesis, Chapters 2 and 3 attempted to define both the Mesolithic and Neolithic in Britain – but concluded that assessing the degree of change vs. continuity between the two periods is problematic and challenging. The data generated during this research however highlights that the traditional narratives for the periods do not always appear to hold true, and that notions and assumptions of how people may have lived can be challenged through the generation of new biomolecular data. In particular, the dietary and chronological data obtained in Chapter 5 indicates that our understanding of the origins of the Neolithic period in Britain are still unclear, and the dietary and mobility data presented within Chapter 7 demonstrates that our knowledge of Neolithic lifeways is also currently limited. Furthermore, that human remains were only identified in one of the five
fragmentary Mesolithic skeletal assemblages analysed (Chapters 5 and 6) raises new questions surrounding early prehistoric mortuary practices and contexts of deposition. However, Chapter 8 highlights how the incorporation of new biomolecular methodologies and the study of different archaeological materials can enhance our understanding of past lifeways.

Overall, therefore, it can be seen that the main outcomes of this thesis are:

i) that additional human remains may be present within early prehistoric ‘unidentifiable’ fragmented bone assemblages

ii) dietary complexity in both the Mesolithic and Neolithic of Britain may be greater than previously thought

iii) that Neolithic mobility may be more complex than previously assumed

iv) a reconsideration of the approach to chronology at the Mesolithic-Neolithic transition, particularly with reference to the timings of dietary change

v) that dental calculus may provide a suitable and useful new medium via which to study early prehistoric health and disease in future studies

10.2. Future work

Alongside the aims and objectives of the thesis as a whole, this research also intended to pinpoint the potential avenues for future research arising from the work undertaken. As such, the following final section will broadly outline areas of potential future work which could be undertaken in each of the five main research areas of this thesis: identification, diet, mobility, chronology, and health/disease.

The technique used within this research for identification was ZooMS (Chapter 4, section 4.2.3.), and as shown in Chapters 5 and 6, the method can be successfully applied to material of a Mesolithic date, providing useful taxonomic information which can then be incorporated into larger debates or discussions, both on a site-level and beyond. The technique’s success, combined with its low cost and high-throughput nature, therefore means there is huge further future potential for its widespread application to other British Mesolithic assemblages, which, as discussed within Chapter 3, are often dominated by fragmentary and disarticulated skeletal remains.
There are also a range of potential avenues for future research on Mesolithic and Neolithic diets. Primarily, stable isotopic analysis was utilised in this thesis for palaeodietary reconstruction, combined with the use of isotopic mixing models to determine the relative importance of dietary contributions. Our continued lack of understanding of the degree of marine consumption within both Mesolithic and Neolithic populations however suggests that the investigation of $\delta^{15}$N analysis of amino acids within bone collagen (Naito et al. 2015) may be a useful addition to bulk isotopic analyses. Furthermore, issues with a lack of available data on plant isotopic values in both Mesolithic and Neolithic Britain (discussed in Chapter 9) indicate that this is also an area of research which requires greater scholarly attention. However, dietary information was also obtained in this research from proteomic study of dental calculus (Chapter 8) – and this is clearly an avenue for future research which may hold great potential, particularly with regards to expanding our knowledge of Neolithic dairy consumption.

In order to increase our understanding of prehistoric mobility, and to more closely investigate demographic change at the Mesolithic-Neolithic transition (as discussed in Chapter 2), it would be necessary to undertake additional aDNA analysis, focusing on bone samples to determine population affinity and define information on genotypes and SNPs. Although determination of genetic/population affinity was attempted in Chapter 8 through analysis of the small amounts of host DNA within the calculus samples analysed here, the low endogenous content of the samples meant that unfortunately this was not possible. However, investigation of this kind, utilising petrous bones (which have the highest aDNA yield of any skeletal element (Pinhasi et al. 2015)), would be an exciting avenue of future research, particularly given that to date, no British Mesolithic individuals have been successfully genetically sequenced. A recent study which sequenced the genome of a Mesolithic individual from Georgia (Jones et al. 2015) highlights the potential of studies of this kind however. Alongside this, the undertaking of additional Sr isotope analysis on other prehistoric assemblages would also be welcomed, particularly given that the current comparative dataset is very small, and no strontium values for British Mesolithic individuals are currently available. The use of LA-MC-ICP-MS, as undertaken here (Chapter 7), indicates the resolution of information it is now possible to obtain in studies of this kind.

Additionally, to be able to gain a tighter understanding of chronology within the British Mesolithic and Neolithic, there simply needs to be more AMS dating undertaken. It is
highly possible, for example, that additional human remains dating to both the Mesolithic and Neolithic have been excavated, but due to a lack of dating, are presumed to be much later in date (as discussed within Chapter 3). The recent discovery of the first Mesolithic cremation in Britain is a clear example of this – as it was not presumed to be Mesolithic (or even to be early prehistoric) until radiocarbon dating revealed a date of 5,600 cal. BC (Gilmour and Loe 2015). Alongside this, as discussed within Chapter 9, the lack of ΔR values currently available across the UK remains a significant problem, particularly in the dating of Mesolithic material, where human remains typically show isotopic signatures indicative of a marine (or partially marine) diet. As marine reservoir offsets are known to vary both temporally and geographically (Ascough et al. 2004; 2005; 2007; 2009; Russell et al. 2015), there is now a real need to generate more ΔR values for prehistoric Britain – even if these are more conservative, or are averaged values across a geographical area, as in a recent paper by Russell et al. (2015). Finally, where material suitable for AMS dating is not available, there is perhaps now a need to look for newer or alternative dating methods which may help to elucidate chronology, such as the chronometric use of earthworm calcite, which suggests the potential of being able to date archaeological contexts (Canti et al. 2015).

Finally, a greater focus on Mesolithic and Neolithic health and disease appears to be a much needed avenue of future research for the discipline. As discussed within Chapters 2, 4, 8 and 9, our knowledge of health and disease states in both periods is still severely limited. Dental calculus, as shown in this research, appears to provide a new potential source of disease information for prehistoric periods, and therefore more detailed analysis of dental calculus samples metagenomically and metaproteomically would appear to hold much promise. Larger sample sizes will however be needed in order to draw significant conclusions on early prehistoric health and disease states. Additionally, the analysis of Mesolithic dental calculus may be able to finally reveal if the changes in lifeways perceived to have occurred at the Mesolithic-Neolithic transition (e.g. in diet, mobility, population size) truly did have a significant effect on health, or bought about the emergence of new or ‘modern’ diseases.

However, as a final note, as Thomas (2008) rightly points out that despite an increase in the amount of available information on the Mesolithic and Neolithic in recent years (e.g. via radiocarbon dates, excavations, stable isotopes etc.), none of these sources have provided a definitive answer to the nature or mechanisms behind the transition, or to the
changes which are perceived to accompany this. As such, there is a need now to develop
greater theoretical discourse surrounding both periods, and surrounding the transition itself –
and the use of biomolecular data may help us to begin to challenge traditional narratives
and develop new theoretical models.
Appendix A – Standard Operating Protocols

The methodologies utilised within this research are outlined throughout this thesis, and a broad overview is also provided within Chapter 4. The following sections provide the standard operating protocols for all methodologies used, as are given on a chapter by chapter basis.

A.1. Chapter 5 – Rediscovering Oronsay

A.1.1. ZooMS Analysis

ZooMS is a qualitative analytical technique for taxonomic identification of archaeological materials (predominately bone) (e.g. Buckley et al. 2009; 2010; Richter et al. 2011; Van Doorn et al. 2011). ZooMS is a method of collagen peptide mass fingerprinting, and was therefore used within this research to determine if the bone fragments obtained were of faunal or human origin, and to attempt to determine their species. The methodology utilised involved a standard collagen extraction from c.500mg of bone (see below), followed by ZooMS analysis (as in Welker et al. 2015(a)) being undertaken on the ‘empty’ tubes used for lyophilisation of the 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 – therefore highlighting the method’s potential value in clarification of taxonomy in samples where identification or isotopic results appear ambiguous or unclear. To date, ZooMS has not been previously used extensively as a methodology by which to determine human remains from fragmentary skeletal material – but has the capacity to do so due to the fact that human collagen sequences are very distinct from other species.

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\textsubscript{18} ZipTips (Agilent), which were eluted using 50µl 50% ACN in 0.5% TFA. MALDI-TOF-MS
analysis using 1µl sample solution and 1µl matrix solution (Buckley et al. 2009; Welker et al. 2015(a)) was undertaken in triplicate for each sample on a Bruker Ultraflex III MALDI-TOF/TOF at the University of York. Replicates were averaged for each sample and manually analysed for peptide markers following the protocol detailed in Welker et al. (2015(a)).

A.1.2. Isotopic Analysis

Isotopic analyses of δ¹³C and δ¹⁵N were undertaken on all bone fragments identified as both human and faunal, following a modified Longin collagen extraction protocol using ultrafiltration on c.500mg of bone (Brown et al. 1988; Richards & Hedges 1999; Colonese et al. 2015). Isotopic analysis was undertaken in an attempt to contribute to the existing isotopic data for the site, which has been implicated in multiple debates regarding the nature of the dietary transition at the Mesolithic-Neolithic interface (Figure 31).

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 δ¹³C and δ¹⁵N. Stable isotope values are presented here relative to the internationally defined standards of VPDB for δ¹³C and AIR for δ¹⁵N.

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 1). 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 δ¹³C and δ¹⁵N values in this work have atomic C:N ratios of between 3.3-3.6 (Table 6).
A.1.3. AMS Dating

AMS dating of four bone fragments (two human samples and two terrestrial herbivores) identified using ZooMS and with associated isotopic information was also undertaken in an attempt to elucidate information about the chronology and contexts 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 (Tables 9 and 10). There have previously been no dates (or isotopic values) for terrestrial fauna from Cnoc Coig. The isotopic data obtained from faunal samples identified was also used as a baseline for interpreting the human isotopic data from the site, and thus it was important to identify if the fauna being studied are contemporaneous to the human remains.

As many of the bone fragments utilised in this study had high marine isotopic values however (Table 6), this also 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 were used in a proportion determined by marine/terrestrial carbon contribution to collagen (as in Barrett and Richards 2004; following best practice outlined in Cook et al. 2015). The latter was estimated for each individual from their δ\textsuperscript{13}C values following linear interpolation from the observed marine and terrestrial endpoints (Table 6). Calibration of AMS dates from Cnoc Coig using this approach has also previously been successfully undertaken by Gordon Cook (Milner and Craig 2009). The terrestrial herbivore samples were calibrated using only the terrestrial calibration curve however. All AMS data was generated by the NERC radiocarbon facility based in the Oxford Radiocarbon Acceleration Unit.

Calibration of dates was undertaken using OxCal v.4.2, using a ΔR value of 47 ±52 for human samples with marine isotopic signatures (Tables 9 and 10). This value is a mean ΔR value calculated for the entirety of Scotland (Ascough, pers. comm.; Russell et al. 2015).

A.1.4. Isotopic Mixing Model Creation (FRUITS)

The Bayesian mixing model FRUITS (Fernandes et al. 2014) was utilised in the analysis of the Cnoc Coig human δ\textsuperscript{13}C and δ\textsuperscript{15}N isotopic data. The model requires the imputation of a range of prior information in order to predict dietary intake:
- **Dietary proxies**: the isotopic proxies measured within an individual (e.g. δ¹³C and δ¹⁵N in bone collagen, δ¹³C in bone mineral, etc.)
- **Food groups**: the food groups to be considered for analysis (e.g. terrestrial mammals, cereals, fish, etc.)
- **Food fractions**: the macronutrient fractions within each food group (e.g. protein, lipid, carbohydrates)
- **Offsets**: the diet to tissue offset for each dietary proxy, caused by isotopic fractionation
- **Weights**: the weight contribution of different food fractions towards a dietary proxy signal
- **Concentrations**: the concentration of each food fraction within each food group

The data used within the Cnoc Coig models is displayed in the Tables below. Model 1 refers to the model where all terrestrial animal dietary sources (i.e. herbivores and omnivores) were considered together as a food group, whereas Model 2 includes only terrestrial herbivores, and no omnivores (i.e. no pigs). The offsets, weight contributions and concentrations of each food group or fraction are the same however across both models. Two food fractions were considered within each model – protein and energy. Energy refers to both lipids and carbohydrates, but were combined into a singular group given that terrestrial animal sources and marine foods do not contain any carbohydrates, whereas terrestrial C₃ plant sources contain significant amounts of carbohydrate (c.80%), but generally negligible amounts of lipids.

<table>
<thead>
<tr>
<th></th>
<th>Offset (%)</th>
<th>Weight Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>δ¹³C</td>
<td>4.8 (0.5)</td>
<td>74 (4)</td>
</tr>
<tr>
<td>δ¹⁵N</td>
<td>5.5 (0.5)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table S1: The diet to tissue offset and weight contribution of macronutrients towards dietary proxy signals. Values in parentheses represent uncertainty. Based upon data from Fernandes et al. (2014; 2015)

<table>
<thead>
<tr>
<th></th>
<th>Protein (%)</th>
<th>Energy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial Animals</td>
<td>30 (2.5)</td>
<td>70 (2.5)</td>
</tr>
<tr>
<td>Terrestrial Plants</td>
<td>15 (5)</td>
<td>85 (5)</td>
</tr>
<tr>
<td>Marine Foods</td>
<td>65 (5)</td>
<td>35 (5)</td>
</tr>
</tbody>
</table>

Table S2: Concentration values for each food group (dry weight composition of macronutrients). Values in parentheses represent uncertainty (based on data in Fernandes 2015)
Table S3: Food values used within FRUITS model 1. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%o$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%o$; and marine values using $\Delta^{13}C_{\text{protein-collagen}} = -1\%o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%o$ and $\Delta^{13}C_{\text{energy-collagen}} = -7\%o$. Plant values were estimated using the offsets shown in Table S5 and terrestrial animal values, and $\Delta^{13}C_{\text{protein-energy}} = +1\%o$ (Tieszen 1991; Fernandes 2015)

Table S4: Food values used within FRUITS model 2. Herbivore macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%o$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%o$; and marine values using $\Delta^{13}C_{\text{protein-collagen}} = -1\%o$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%o$ and $\Delta^{13}C_{\text{energy-collagen}} = -7\%o$. Plant values were estimated using the offsets shown in Table S5 and herbivore values, and $\Delta^{13}C_{\text{protein-energy}} = +1\%o$ (Tieszen 1991; Fernandes 2015)

A.2. Chapter 6 – Finding the Mesolithic

A.2.1. Blick Mead, Vespasian’s Camp

Twenty bone fragments classified as ‘unidentifiable’ was analysed using ZooMS. A full collagen extraction was undertaken on all samples initially (as is common for isotopic analysis of bone). c.400mg of bone from each sample was firstly demineralised in 0.6M HCl at 4°C, before being rinsed with ultrapure water and then gelatinised in pH3 HCl at 80°C for 48 hours. The supernatant from the samples (containing the collagen) was then ultrafiltered (Amicon 30kDa Ultra-4 Centrifugal Units, Millipore), frozen, and freeze-
dried. Any collagen extracted was then utilised for $\delta^{13}$C and $\delta^{15}$N isotopic analysis where possible (collagen yields and quality permitting). The falcon tubes used for the freeze-drying of collagen were then utilised for ZooMS analysis. Any collagen within the falcon tubes was firstly removed and transferred to new eppendorf tubes instead. 75µl 50mM AmBIC solution (ammonium bicarbonate buffer, pH 8.0) was added to each empty falcon tube used for freeze-drying, followed by 1µl trypsin (Promega) solution (50µl buffer used to re-suspend). Samples were then incubated overnight at 37°C (12-18 hours), and then 1µl 5% TFA was added. All peptides were then extracted using C$_{18}$ ZipTips (Agilent), using 50µl of 50% ACN in 0.5% TFA to elute, before being spotted onto a MALDI plate ready for analysis by MALDI-TOF MS. MALDI-TOF-MS analysis was undertaken at the Technology Facility at the University of York and followed the protocol outlined in Welker et al. (2015).

In effect, ZooMS analysis was undertaken on ‘empty’ falcon tubes, which in normal analyses would have been discarded. Macroscopically invisible amounts of collagen remain within the falcon tubes following removal of all ‘visible’ collagen however. These tiny amounts of collagen adhering to the inside of the tubes present sufficient volumes for ZooMS analysis.

A.2.2. Cnoc Coig, Oronsay

Seventeen bone fragments classified as ‘unidentifiable’ were utilised for ZooMS analysis, all of which were small in size (<200mg). Briefly, samples were demineralised in 250µl 0.6M HCl at 4°C, before being rinsed with ultrapure water, and then gelatinised in 100µl AmBIC solution (ammonium bicarbonate buffer, pH 8.0) for 1 hour at 65°C. Samples were centrifuged for 1 min at 13,000 RPM, after which 50µl of the supernatant was transferred to new eppendorf tubes to which 1µl trypsin (Promega) solution (40µl buffer used to re-suspend) was added. Samples were then incubated overnight at 37°C (12-18 hours), and then 1µl 5% TFA was added. All peptides were then extracted using C$_{18}$ ZipTips (Agilent), using 50µl of 50% ACN in 0.5% TFA to elute, before being spotted onto a MALDI plate ready for analysis. MALDI-TOF-MS analysis was undertaken at the Technology Facility at the University of York and followed the protocol outlined in Welker et al. (2015).
A.2.3. Western Isles

Seventeen bone fragments classified as ‘unidentifiable’ were utilised for ZooMS analysis – thirteen of these from Bágh an Teampaill (Temple Bay), three from Tràigh na Beirigh, and one from Northton. The fragments from all three sites however were small in size and morphologically indistinct. However, owing the very small amounts of collagen needed for ZooMS analysis, not all of the bone fragments were needed for analysis – and so a small fragment of bone (~10-30mg) was taken from each sample for ZooMS using pliers.

The samples had not been cleaned or washed, and therefore were cleaned briefly to remove contamination before ZooMS analysis was undertaken. Cleaning involved adding 250µl of 50mM AmBIC solution (ammonium bicarbonate buffer, pH 8.0) to each fragment and placing them on a rocker-roller at 4°C to remove all adhering soil. This supernatant was then discarded. Following this, an acid ZooMS methodology was applied, following previously published protocols (Welker et al. 2015). Briefly, samples were demineralised in 250µl 0.6M HCl at 4°C, before being rinsed with ultrapure water, and then gelatinised in 100µl AmBIC solution (ammonium bicarbonate buffer, pH 8.0) for 1 hour at 65°C. Samples were centrifuged for 1 min at 13,000 RPM, after which 50µl of the supernatant was transferred to new eppendorf tubes to which 1µl trypsin (Promega) solution (40µl buffer used to re-suspend) was added. Samples were then incubated overnight at 37°C (12-18 hours), and then 1µl 5% TFA was added. All peptides were then extracted using C18 ZipTips (Agilent), using 50µl of 50% ACN in 0.5% TFA to elute, before being spotted onto a MALDI plate ready for analysis. MALDI-TOF-MS analysis was undertaken at the Technology Facility at the University of York and followed the protocol outlined in Welker et al. (2015).

A.3. Chapter 7 – Banbury Lane, Northampton

A.3.1. Stable Isotope Analysis of $\delta^{13}C$ and $\delta^{15}N$

Stable isotopic analysis of $\delta^{13}C$ and $\delta^{15}N$ was undertaken on all identified human right femoral fragments (based on MNI calculations) and associated fauna, following a modified Longin collagen extraction protocol using ultrafiltration on c.300mg of bone (Brown et al. 1988; Richards & Hedges 1999; Colonese et al. 2015). Bone samples were drilled out from femoral midshafts using a dremmel drill under clean conditions, and cleaned manually using a scalpel to remove any contamination prior to collagen extraction. Briefly, samples were demineralised in 0.6M aq. HCl solution at 4°C, and the resulting insoluble fraction...
gelatinised for 48h at 80°C. The supernatant was then ultrafiltered (30kDa MWCO, Amicon) to isolate the high molecular weight fraction, which was then lyophilised.

Purified collagen samples (1mg) were analysed in duplicate 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 δ¹³C and δ¹⁵N. Stable isotope values are presented here relative to the internationally defined standards of VPDB for δ¹³C and AIR for δ¹⁵N.

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 c.6%. However, it should be 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 δ¹³C and δ¹⁵N values in this work have atomic C:N ratios of between 3.1-3.3 (Table S5 and S6).

<table>
<thead>
<tr>
<th>Bone Number</th>
<th>Layer</th>
<th>% Collagen Yield</th>
<th>δ¹⁵N (%)</th>
<th>δ¹³C (%)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>4</td>
<td>4.56%</td>
<td>11.43</td>
<td>-21.08</td>
<td>3.17</td>
</tr>
<tr>
<td>1094</td>
<td>13</td>
<td>1.77%</td>
<td>11.15</td>
<td>-21.34</td>
<td>3.20</td>
</tr>
<tr>
<td>85</td>
<td>2</td>
<td>2.20%</td>
<td>10.73</td>
<td>-21.02</td>
<td>3.20</td>
</tr>
<tr>
<td>822</td>
<td>10</td>
<td>3.13%</td>
<td>11.52</td>
<td>-21.21</td>
<td>3.20</td>
</tr>
<tr>
<td>339</td>
<td>4</td>
<td>2.86%</td>
<td>11.32</td>
<td>-21.00</td>
<td>3.19</td>
</tr>
<tr>
<td>1281</td>
<td>15</td>
<td>3.00%</td>
<td>11.41</td>
<td>-20.80</td>
<td>3.15</td>
</tr>
<tr>
<td>94</td>
<td>2</td>
<td>2.08%</td>
<td>11.61</td>
<td>-21.36</td>
<td>3.19</td>
</tr>
<tr>
<td>513</td>
<td>6</td>
<td>2.36%</td>
<td>11.18</td>
<td>-21.23</td>
<td>3.21</td>
</tr>
<tr>
<td>1213</td>
<td>14</td>
<td>2.56%</td>
<td>10.96</td>
<td>-20.89</td>
<td>3.17</td>
</tr>
<tr>
<td>363</td>
<td>4</td>
<td>1.77%</td>
<td>11.26</td>
<td>-21.71</td>
<td>3.21</td>
</tr>
<tr>
<td>1262</td>
<td>15</td>
<td>1.27%</td>
<td>11.84</td>
<td>-20.01</td>
<td>3.22</td>
</tr>
<tr>
<td>1316</td>
<td>16</td>
<td>2.93%</td>
<td>11.36</td>
<td>-20.91</td>
<td>3.20</td>
</tr>
<tr>
<td>1210</td>
<td>14</td>
<td>1.78%</td>
<td>11.63</td>
<td>-21.07</td>
<td>3.23</td>
</tr>
<tr>
<td>802</td>
<td>10</td>
<td>4.42%</td>
<td>11.41</td>
<td>-21.19</td>
<td>3.18</td>
</tr>
<tr>
<td>1278</td>
<td>15</td>
<td>3.20%</td>
<td>11.11</td>
<td>-20.69</td>
<td>3.20</td>
</tr>
<tr>
<td>433</td>
<td>5</td>
<td>3.12%</td>
<td>11.28</td>
<td>-21.16</td>
<td>3.21</td>
</tr>
<tr>
<td>106</td>
<td>2</td>
<td>2.49%</td>
<td>11.09</td>
<td>-21.23</td>
<td>3.23</td>
</tr>
<tr>
<td>928</td>
<td>11</td>
<td>6.40%</td>
<td>11.22</td>
<td>-21.11</td>
<td>3.18</td>
</tr>
<tr>
<td>1243</td>
<td>14</td>
<td>2.99%</td>
<td>11.35</td>
<td>-20.98</td>
<td>3.20</td>
</tr>
<tr>
<td>735</td>
<td>9</td>
<td>3.64%</td>
<td>11.73</td>
<td>-21.20</td>
<td>3.19</td>
</tr>
<tr>
<td>117</td>
<td>2</td>
<td>2.12%</td>
<td>11.43</td>
<td>-21.02</td>
<td>3.23</td>
</tr>
<tr>
<td>273</td>
<td>4</td>
<td>4.58%</td>
<td>10.94</td>
<td>-21.37</td>
<td>3.20</td>
</tr>
<tr>
<td>118</td>
<td>2</td>
<td>3.55%</td>
<td>11.00</td>
<td>-21.44</td>
<td>3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>3</td>
<td>2.36%</td>
<td>11.27</td>
<td>-21.24</td>
<td>3.21</td>
</tr>
<tr>
<td>1145</td>
<td>13</td>
<td>3.19%</td>
<td>11.49</td>
<td>-21.20</td>
<td>3.21</td>
</tr>
<tr>
<td>677</td>
<td>8</td>
<td>2.36%</td>
<td>11.11</td>
<td>-21.29</td>
<td>3.24</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>3.75%</td>
<td>11.31</td>
<td>-21.09</td>
<td>3.21</td>
</tr>
<tr>
<td>93</td>
<td>2</td>
<td>2.14%</td>
<td>11.61</td>
<td>-21.21</td>
<td>3.23</td>
</tr>
<tr>
<td>1082</td>
<td>13</td>
<td>2.90%</td>
<td>11.46</td>
<td>-21.22</td>
<td>3.22</td>
</tr>
<tr>
<td>974</td>
<td>11</td>
<td>1.22%</td>
<td>11.93</td>
<td>-21.10</td>
<td>3.26</td>
</tr>
<tr>
<td>761</td>
<td>9</td>
<td>3.23%</td>
<td>11.13</td>
<td>-21.77</td>
<td>3.21</td>
</tr>
<tr>
<td>1326</td>
<td>16</td>
<td>2.37%</td>
<td>11.29</td>
<td>-21.25</td>
<td>3.19</td>
</tr>
<tr>
<td>1232</td>
<td>14</td>
<td>1.63%</td>
<td>11.17</td>
<td>-21.28</td>
<td>3.23</td>
</tr>
<tr>
<td>100.0</td>
<td>2</td>
<td>2.49%</td>
<td>11.44</td>
<td>-21.08</td>
<td>3.25</td>
</tr>
<tr>
<td>450</td>
<td>6</td>
<td>3.28%</td>
<td>11.32</td>
<td>-21.41</td>
<td>3.21</td>
</tr>
<tr>
<td>1245</td>
<td>14</td>
<td>2.13%</td>
<td>11.61</td>
<td>-21.34</td>
<td>3.23</td>
</tr>
<tr>
<td>195</td>
<td>3</td>
<td>2.82%</td>
<td>10.50</td>
<td>-21.34</td>
<td>3.21</td>
</tr>
<tr>
<td>972</td>
<td>11</td>
<td>3.37%</td>
<td>10.89</td>
<td>-21.31</td>
<td>3.20</td>
</tr>
<tr>
<td>641</td>
<td>8</td>
<td>1.54%</td>
<td>10.98</td>
<td>-21.71</td>
<td>3.23</td>
</tr>
<tr>
<td>2014.27</td>
<td>14</td>
<td>1.51%</td>
<td>11.16</td>
<td>-20.84</td>
<td>3.20</td>
</tr>
<tr>
<td>908</td>
<td>11</td>
<td>2.42%</td>
<td>11.36</td>
<td>-21.14</td>
<td>3.22</td>
</tr>
<tr>
<td>864.0</td>
<td>10</td>
<td>2.24%</td>
<td>10.93</td>
<td>-21.35</td>
<td>3.25</td>
</tr>
<tr>
<td>984</td>
<td>11</td>
<td>5.92%</td>
<td>11.61</td>
<td>-21.29</td>
<td>3.19</td>
</tr>
<tr>
<td>71</td>
<td>1</td>
<td>1.50%</td>
<td>11.16</td>
<td>-21.13</td>
<td>3.24</td>
</tr>
<tr>
<td>660</td>
<td>8</td>
<td>1.44%</td>
<td>10.80</td>
<td>20.99</td>
<td>3.21</td>
</tr>
<tr>
<td>84</td>
<td>2</td>
<td>3.66%</td>
<td>11.17</td>
<td>-20.89</td>
<td>3.22</td>
</tr>
<tr>
<td>1251</td>
<td>15</td>
<td>2.84%</td>
<td>11.20</td>
<td>-21.30</td>
<td>3.20</td>
</tr>
<tr>
<td>567</td>
<td>7</td>
<td>2.37%</td>
<td>10.35</td>
<td>-21.45</td>
<td>3.22</td>
</tr>
<tr>
<td>291</td>
<td>4</td>
<td>3.38%</td>
<td>11.00</td>
<td>-21.26</td>
<td>3.18</td>
</tr>
<tr>
<td>354</td>
<td>4</td>
<td>3.31%</td>
<td>11.05</td>
<td>-21.17</td>
<td>3.21</td>
</tr>
<tr>
<td>279</td>
<td>4</td>
<td>2.43%</td>
<td>11.44</td>
<td>-21.08</td>
<td>3.25</td>
</tr>
<tr>
<td>830</td>
<td>10</td>
<td>3.83%</td>
<td>10.84</td>
<td>-21.16</td>
<td>3.17</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>1.81%</td>
<td>11.46</td>
<td>-21.04</td>
<td>3.23</td>
</tr>
<tr>
<td>110</td>
<td>2</td>
<td>1.52%</td>
<td>11.16</td>
<td>-21.19</td>
<td>3.30</td>
</tr>
<tr>
<td>1028</td>
<td>12</td>
<td>1.23%</td>
<td>11.30</td>
<td>-21.29</td>
<td>3.19</td>
</tr>
<tr>
<td>1328</td>
<td>16</td>
<td>1.87%</td>
<td>11.73</td>
<td>-20.87</td>
<td>3.16</td>
</tr>
<tr>
<td>394</td>
<td>5</td>
<td>3.37%</td>
<td>11.85</td>
<td>-21.42</td>
<td>3.16</td>
</tr>
<tr>
<td>1029</td>
<td>12</td>
<td>3.74%</td>
<td>11.02</td>
<td>-21.35</td>
<td>3.19</td>
</tr>
<tr>
<td>824</td>
<td>10</td>
<td>3.24%</td>
<td>10.67</td>
<td>-20.93</td>
<td>3.20</td>
</tr>
<tr>
<td>1126</td>
<td>13</td>
<td>1.46%</td>
<td>11.86</td>
<td>-21.78</td>
<td>3.25</td>
</tr>
<tr>
<td>1005</td>
<td>12</td>
<td>4.66%</td>
<td>11.52</td>
<td>-21.18</td>
<td>3.15</td>
</tr>
<tr>
<td>204</td>
<td>3</td>
<td>3.52%</td>
<td>11.60</td>
<td>-20.98</td>
<td>3.22</td>
</tr>
<tr>
<td>255</td>
<td>3</td>
<td>3.84%</td>
<td>11.44</td>
<td>-20.74</td>
<td>3.21</td>
</tr>
<tr>
<td>1296</td>
<td>15</td>
<td>3.78%</td>
<td>11.67</td>
<td>-21.27</td>
<td>3.20</td>
</tr>
<tr>
<td>674</td>
<td>8</td>
<td>1.72%</td>
<td>12.04</td>
<td>-20.83</td>
<td>3.25</td>
</tr>
<tr>
<td>801</td>
<td>10</td>
<td>2.65%</td>
<td>11.47</td>
<td>-21.49</td>
<td>3.19</td>
</tr>
<tr>
<td>1089</td>
<td>13</td>
<td>3.34%</td>
<td>11.50</td>
<td>-21.32</td>
<td>3.20</td>
</tr>
<tr>
<td>165</td>
<td>3</td>
<td>1.60%</td>
<td>11.77</td>
<td>-21.39</td>
<td>3.23</td>
</tr>
<tr>
<td>557</td>
<td>7</td>
<td>3.55%</td>
<td>11.58</td>
<td>-21.07</td>
<td>3.17</td>
</tr>
<tr>
<td>718</td>
<td>8</td>
<td>2.92%</td>
<td>11.27</td>
<td>-20.85</td>
<td>3.19</td>
</tr>
<tr>
<td>968</td>
<td>11</td>
<td>3.67%</td>
<td>10.77</td>
<td>-21.18</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>---</td>
<td>----------</td>
<td>------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>989</td>
<td>11</td>
<td>2.09%</td>
<td>11.11</td>
<td>-21.08</td>
<td>3.22</td>
</tr>
<tr>
<td>954</td>
<td>11</td>
<td>0.58%</td>
<td>11.35</td>
<td>-20.93</td>
<td>3.25</td>
</tr>
<tr>
<td>578</td>
<td>7</td>
<td>2.13%</td>
<td>11.55</td>
<td>-20.97</td>
<td>3.26</td>
</tr>
<tr>
<td>837</td>
<td>10</td>
<td>3.61%</td>
<td>11.85</td>
<td>-20.89</td>
<td>3.19</td>
</tr>
<tr>
<td>937</td>
<td>?</td>
<td>1.46%</td>
<td>11.82</td>
<td>-21.27</td>
<td>3.24</td>
</tr>
<tr>
<td>1097</td>
<td>13</td>
<td>3.37%</td>
<td>11.38</td>
<td>-20.96</td>
<td>3.23</td>
</tr>
<tr>
<td>407</td>
<td>5</td>
<td>2.39%</td>
<td>10.82</td>
<td>-21.31</td>
<td>3.24</td>
</tr>
<tr>
<td>1189</td>
<td>14</td>
<td>3.93%</td>
<td>10.81</td>
<td>-21.41</td>
<td>3.22</td>
</tr>
<tr>
<td>1320</td>
<td>16</td>
<td>3.21%</td>
<td>10.94</td>
<td>-21.05</td>
<td>3.19</td>
</tr>
<tr>
<td>1167</td>
<td>13</td>
<td>3.91%</td>
<td>11.03</td>
<td>-21.31</td>
<td>3.15</td>
</tr>
<tr>
<td>422</td>
<td>5</td>
<td>2.55%</td>
<td>11.40</td>
<td>-21.16</td>
<td>3.24</td>
</tr>
<tr>
<td>807</td>
<td>10</td>
<td>1.31%</td>
<td>11.27</td>
<td>-20.91</td>
<td>3.24</td>
</tr>
<tr>
<td>408</td>
<td>5</td>
<td>2.37%</td>
<td>10.96</td>
<td>-21.07</td>
<td>3.20</td>
</tr>
<tr>
<td>244</td>
<td>3</td>
<td>1.37%</td>
<td>11.60</td>
<td>-21.64</td>
<td>3.26</td>
</tr>
<tr>
<td>88</td>
<td>2</td>
<td>2.47%</td>
<td>11.63</td>
<td>-21.11</td>
<td>3.21</td>
</tr>
<tr>
<td>825</td>
<td>10</td>
<td>1.68%</td>
<td>11.27</td>
<td>-21.32</td>
<td>3.20</td>
</tr>
<tr>
<td>336</td>
<td>4</td>
<td>2.58%</td>
<td>10.99</td>
<td>-20.90</td>
<td>3.23</td>
</tr>
<tr>
<td>804</td>
<td>10</td>
<td>3.34%</td>
<td>11.20</td>
<td>-21.21</td>
<td>3.20</td>
</tr>
<tr>
<td>1010</td>
<td>12</td>
<td>1.62%</td>
<td>11.60</td>
<td>-21.09</td>
<td>3.23</td>
</tr>
<tr>
<td>412</td>
<td>5</td>
<td>3.91%</td>
<td>11.38</td>
<td>-21.07</td>
<td>3.18</td>
</tr>
<tr>
<td>800.1</td>
<td>10</td>
<td>2.13%</td>
<td>11.46</td>
<td>-21.24</td>
<td>3.23</td>
</tr>
<tr>
<td>101</td>
<td>2</td>
<td>1.67%</td>
<td>11.10</td>
<td>-21.43</td>
<td>3.24</td>
</tr>
<tr>
<td>1270</td>
<td>15</td>
<td>4.28%</td>
<td>11.12</td>
<td>-21.12</td>
<td>3.16</td>
</tr>
<tr>
<td>385</td>
<td>5</td>
<td>2.52%</td>
<td>10.77</td>
<td>-21.13</td>
<td>3.25</td>
</tr>
<tr>
<td>1216</td>
<td>14</td>
<td>2.03%</td>
<td>11.61</td>
<td>-21.07</td>
<td>3.22</td>
</tr>
<tr>
<td>907</td>
<td>11</td>
<td>1.94%</td>
<td>11.46</td>
<td>-21.27</td>
<td>3.21</td>
</tr>
<tr>
<td>1169</td>
<td>13</td>
<td>3.40%</td>
<td>11.37</td>
<td>-21.11</td>
<td>3.21</td>
</tr>
<tr>
<td>1150</td>
<td>13</td>
<td>3.51%</td>
<td>11.30</td>
<td>-21.25</td>
<td>3.18</td>
</tr>
<tr>
<td>1267</td>
<td>15</td>
<td>3.07%</td>
<td>11.12</td>
<td>-21.42</td>
<td>3.21</td>
</tr>
<tr>
<td>945</td>
<td>11</td>
<td>2.17%</td>
<td>11.12</td>
<td>-21.52</td>
<td>3.19</td>
</tr>
<tr>
<td>886</td>
<td>10</td>
<td>2.23%</td>
<td>11.36</td>
<td>-20.99</td>
<td>3.23</td>
</tr>
<tr>
<td>609</td>
<td>7</td>
<td>1.98%</td>
<td>10.88</td>
<td>-21.03</td>
<td>3.22</td>
</tr>
<tr>
<td>71</td>
<td>1</td>
<td>1.50%</td>
<td>11.16</td>
<td>-21.13</td>
<td>3.24</td>
</tr>
<tr>
<td>972</td>
<td>11</td>
<td>3.37%</td>
<td>10.89</td>
<td>-21.31</td>
<td>3.20</td>
</tr>
<tr>
<td>864.0</td>
<td>10</td>
<td>2.24%</td>
<td>10.93</td>
<td>-21.35</td>
<td>3.25</td>
</tr>
<tr>
<td>1288</td>
<td>15</td>
<td>2.98%</td>
<td>11.41</td>
<td>-21.37</td>
<td>3.20</td>
</tr>
<tr>
<td>1000</td>
<td>11</td>
<td>2.08%</td>
<td>11.77</td>
<td>-20.80</td>
<td>3.25</td>
</tr>
<tr>
<td>1233</td>
<td>14</td>
<td>3.00%</td>
<td>11.80</td>
<td>-20.60</td>
<td>3.20</td>
</tr>
<tr>
<td>1254</td>
<td>15</td>
<td>3.23%</td>
<td>11.43</td>
<td>-21.15</td>
<td>3.17</td>
</tr>
<tr>
<td>949</td>
<td>11</td>
<td>2.43%</td>
<td>10.81</td>
<td>-21.20</td>
<td>3.21</td>
</tr>
<tr>
<td>706</td>
<td>8</td>
<td>3.59%</td>
<td>11.28</td>
<td>-21.18</td>
<td>3.20</td>
</tr>
<tr>
<td>401</td>
<td>5</td>
<td>1.50%</td>
<td>11.63</td>
<td>-20.61</td>
<td>3.27</td>
</tr>
<tr>
<td>295</td>
<td>4</td>
<td>2.13%</td>
<td>10.93</td>
<td>-21.46</td>
<td>3.26</td>
</tr>
<tr>
<td>944</td>
<td>11</td>
<td>2.66%</td>
<td>11.08</td>
<td>-21.19</td>
<td>3.19</td>
</tr>
<tr>
<td>1035</td>
<td>15</td>
<td>2.10%</td>
<td>11.49</td>
<td>-20.73</td>
<td>3.21</td>
</tr>
<tr>
<td>546</td>
<td>4</td>
<td>1.43%</td>
<td>11.15</td>
<td>-21.41</td>
<td>3.26</td>
</tr>
<tr>
<td>1237</td>
<td>14</td>
<td>2.01%</td>
<td>11.90</td>
<td>-21.30</td>
<td>3.22</td>
</tr>
<tr>
<td>2000.10</td>
<td>0</td>
<td>3.62%</td>
<td>11.08</td>
<td>-21.44</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>1177</td>
<td>14</td>
<td>1.75%</td>
<td>11.03</td>
<td>-21.37</td>
<td>3.21</td>
</tr>
<tr>
<td>1256</td>
<td>15</td>
<td>3.33%</td>
<td>11.39</td>
<td>-21.14</td>
<td>3.19</td>
</tr>
<tr>
<td>1266</td>
<td>15</td>
<td>1.72%</td>
<td>11.13</td>
<td>-21.55</td>
<td>3.21</td>
</tr>
<tr>
<td>1066</td>
<td>12</td>
<td>1.81%</td>
<td>11.99</td>
<td>-21.09</td>
<td>3.21</td>
</tr>
<tr>
<td>153</td>
<td>3</td>
<td>3.24%</td>
<td>11.30</td>
<td>-20.76</td>
<td>3.18</td>
</tr>
<tr>
<td>1209</td>
<td>14</td>
<td>1.79%</td>
<td>10.84</td>
<td>-20.85</td>
<td>3.22</td>
</tr>
<tr>
<td>1293</td>
<td>15</td>
<td>2.10%</td>
<td>11.48</td>
<td>-21.31</td>
<td>3.19</td>
</tr>
<tr>
<td>967</td>
<td>11</td>
<td>3.28%</td>
<td>11.33</td>
<td>-21.02</td>
<td>3.21</td>
</tr>
<tr>
<td>556</td>
<td>7</td>
<td>1.50%</td>
<td>11.40</td>
<td>-20.92</td>
<td>3.24</td>
</tr>
<tr>
<td>1219</td>
<td>14</td>
<td>2.68%</td>
<td>11.42</td>
<td>-21.08</td>
<td>3.18</td>
</tr>
<tr>
<td>441</td>
<td>5</td>
<td>2.79%</td>
<td>11.54</td>
<td>-21.34</td>
<td>3.20</td>
</tr>
<tr>
<td>687</td>
<td>8</td>
<td>3.26%</td>
<td>11.49</td>
<td>-21.24</td>
<td>3.23</td>
</tr>
<tr>
<td>1015</td>
<td>12</td>
<td>2.21%</td>
<td>11.59</td>
<td>-21.28</td>
<td>3.22</td>
</tr>
<tr>
<td>226</td>
<td>3</td>
<td>2.73%</td>
<td>11.57</td>
<td>-21.35</td>
<td>3.21</td>
</tr>
<tr>
<td>1165</td>
<td>13</td>
<td>3.55%</td>
<td>11.64</td>
<td>-21.16</td>
<td>3.19</td>
</tr>
<tr>
<td>139</td>
<td>2</td>
<td>3.64%</td>
<td>11.09</td>
<td>-21.24</td>
<td>3.20</td>
</tr>
<tr>
<td>1098</td>
<td>13</td>
<td>4.75%</td>
<td>10.70</td>
<td>-21.34</td>
<td>3.18</td>
</tr>
<tr>
<td>613</td>
<td>7</td>
<td>2.09%</td>
<td>11.96</td>
<td>-21.51</td>
<td>3.23</td>
</tr>
<tr>
<td>245</td>
<td>3</td>
<td>5.23%</td>
<td>10.50</td>
<td>-21.19</td>
<td>3.18</td>
</tr>
<tr>
<td>64</td>
<td>1</td>
<td>2.15%</td>
<td>11.29</td>
<td>-21.25</td>
<td>3.23</td>
</tr>
<tr>
<td>290</td>
<td>4</td>
<td>3.53%</td>
<td>11.36</td>
<td>-20.97</td>
<td>3.22</td>
</tr>
<tr>
<td>1117</td>
<td>13</td>
<td>3.15%</td>
<td>11.93</td>
<td>-20.75</td>
<td>3.19</td>
</tr>
<tr>
<td>1253</td>
<td>15</td>
<td>3.46%</td>
<td>11.64</td>
<td>-21.32</td>
<td>3.19</td>
</tr>
<tr>
<td>1241</td>
<td>14</td>
<td>2.28%</td>
<td>11.14</td>
<td>-21.22</td>
<td>3.20</td>
</tr>
<tr>
<td>1132</td>
<td>13</td>
<td>2.21%</td>
<td>12.20</td>
<td>-21.02</td>
<td>3.23</td>
</tr>
<tr>
<td>1283</td>
<td>15</td>
<td>2.56%</td>
<td>11.64</td>
<td>-21.25</td>
<td>3.20</td>
</tr>
<tr>
<td>770</td>
<td>9</td>
<td>2.47%</td>
<td>10.58</td>
<td>-21.36</td>
<td>3.21</td>
</tr>
<tr>
<td>1092</td>
<td>13</td>
<td>2.96%</td>
<td>11.43</td>
<td>-21.12</td>
<td>3.21</td>
</tr>
<tr>
<td>779</td>
<td>9</td>
<td>3.01%</td>
<td>11.18</td>
<td>-21.23</td>
<td>3.20</td>
</tr>
<tr>
<td>1291</td>
<td>15</td>
<td>2.25%</td>
<td>11.02</td>
<td>-20.88</td>
<td>3.19</td>
</tr>
<tr>
<td>1333</td>
<td>16</td>
<td>3.02%</td>
<td>11.71</td>
<td>-20.69</td>
<td>3.18</td>
</tr>
<tr>
<td>341</td>
<td>4</td>
<td>3.75%</td>
<td>11.46</td>
<td>-20.80</td>
<td>3.19</td>
</tr>
<tr>
<td>606.0</td>
<td>7</td>
<td>1.77%</td>
<td>10.98</td>
<td>-21.22</td>
<td>3.25</td>
</tr>
<tr>
<td>916</td>
<td>11</td>
<td>2.43%</td>
<td>11.12</td>
<td>-21.11</td>
<td>3.18</td>
</tr>
<tr>
<td>222</td>
<td>3</td>
<td>2.10%</td>
<td>11.07</td>
<td>-20.99</td>
<td>3.18</td>
</tr>
<tr>
<td>1307</td>
<td>16</td>
<td>2.20%</td>
<td>10.62</td>
<td>-21.11</td>
<td>3.18</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>2.44%</td>
<td>11.03</td>
<td>-21.06</td>
<td>3.23</td>
</tr>
<tr>
<td>395</td>
<td>5</td>
<td>2.48%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>848</td>
<td>10</td>
<td>3.39%</td>
<td>11.24</td>
<td>-21.15</td>
<td>3.19</td>
</tr>
<tr>
<td>966</td>
<td>11</td>
<td>3.38%</td>
<td>11.11</td>
<td>-20.86</td>
<td>3.20</td>
</tr>
<tr>
<td>260</td>
<td>3</td>
<td>1.58%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1231</td>
<td>14</td>
<td>2.26%</td>
<td>11.37</td>
<td>-21.12</td>
<td>3.17</td>
</tr>
<tr>
<td>626</td>
<td>7</td>
<td>2.86%</td>
<td>11.34</td>
<td>-21.27</td>
<td>3.24</td>
</tr>
<tr>
<td>1172</td>
<td>14</td>
<td>1.58%</td>
<td>11.61</td>
<td>-21.40</td>
<td>3.22</td>
</tr>
</tbody>
</table>

Table S5: Collagen stable isotope values obtained from human remains sampled from the Banbury Lane assemblage. All above values were taken from right femora. Layers referred to correspond to the original excavation layers – which were arbitrary spits taken through the pit, and were determined by the excavators – not archaeological or contextual layers.
Table S6: Collagen stable isotope values obtained from fauna from Banbury Lane

<table>
<thead>
<tr>
<th>Bone Number</th>
<th>Layer</th>
<th>Species ID</th>
<th>Element</th>
<th>% Collagen Yield</th>
<th>δ&lt;sup&gt;15&lt;/sup&gt;N (‰)</th>
<th>δ&lt;sup&gt;13&lt;/sup&gt;C (‰)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>727</td>
<td>9</td>
<td>Bos taurus</td>
<td>Vertebra</td>
<td>3.01%</td>
<td>6.16</td>
<td>-22.61</td>
<td>3.23</td>
</tr>
<tr>
<td>815</td>
<td>10</td>
<td>Sus scrofa</td>
<td>Parietal</td>
<td>0.94%</td>
<td>7.11</td>
<td>-21.64</td>
<td>3.34</td>
</tr>
<tr>
<td>2001.25</td>
<td>1</td>
<td>Bos taurus</td>
<td>Vertebra</td>
<td>3.88%</td>
<td>6.09</td>
<td>-22.63</td>
<td>3.22</td>
</tr>
<tr>
<td>2005.312</td>
<td>5</td>
<td>Bos taurus</td>
<td>Vertebra</td>
<td>4.92%</td>
<td>6.38</td>
<td>-22.74</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(unfused)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A.3.2. Inter-Laboratory Comparison

Due to the number of samples analysed using stable isotope from Banbury Lane, and the significant homogeneity apparent within the human δ<sup>13</sup>C and δ<sup>15</sup>N values obtained, inter-laboratory tests were undertaken to ensure the accuracy and reliability of the data generated at York. As such, two human bone samples from the Banbury Lane assemblage, along with a number of modern reference samples were extracted and run at the Analytical Centre in the School of Archaeological Sciences at the University of Bradford (by Andy Gledhill). Alongside this, collagen from these same samples extracted at York was also run on the IRMS at Bradford. Overall, this data showed that values obtained between the two labs were within 0.1‰ for δ<sup>13</sup>C and 0.4‰ for δ<sup>15</sup>N on the standard using during IRMS analysis, and >0.2‰ for both δ<sup>13</sup>C and δ<sup>15</sup>N on two human bone samples from Banbury Lane (Tables S7 and S8).

<table>
<thead>
<tr>
<th></th>
<th>Bradford extract run at York</th>
<th>Bradford extract run at Bradford</th>
<th>York extract run at York</th>
<th>York extract run at Bradford</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.14</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Bov2</td>
<td>6.65</td>
<td>-22.99</td>
<td>6.61</td>
<td>-23.02</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.09</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>C/N Ratio</td>
<td>δ(^{15})N</td>
<td>δ(^{13})C</td>
<td>δ(^{15})N</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Bov3</td>
<td>3.19</td>
<td>3.24</td>
<td>3.13</td>
<td>3.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>δ(^{15})N</th>
<th>δ(^{13})C</th>
<th>δ(^{15})N</th>
<th>δ(^{13})C</th>
<th>δ(^{15})N</th>
<th>δ(^{13})C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.07</td>
<td>0.10</td>
<td>0.14</td>
<td>0.23</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.14</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.27</td>
<td>0.09</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C/N Ratio</th>
<th>3.36</th>
<th>3.50</th>
<th>3.40</th>
<th>3.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on all samples run</td>
<td>Based on all samples run</td>
<td>Based on all samples run</td>
<td>Based on all samples run</td>
<td></td>
</tr>
<tr>
<td>δ(^{15})N</td>
<td>δ(^{13})C</td>
<td>δ(^{15})N</td>
<td>δ(^{13})C</td>
<td>δ(^{15})N</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.12</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.14</td>
<td>0.14</td>
<td>0.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ(^{15})N</td>
</tr>
<tr>
<td>Bovine</td>
</tr>
<tr>
<td>Cod</td>
</tr>
<tr>
<td>Total st dev</td>
</tr>
</tbody>
</table>

Table S7: Results from Bradford-York interlab comparison on bovine and cod standards used during IRMS analysis
<table>
<thead>
<tr>
<th></th>
<th>York Extract run at York</th>
<th>York Extract run at Bradford</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ^{15}N</td>
<td>δ^{13}C</td>
</tr>
<tr>
<td>BAN273</td>
<td>10.95</td>
<td>-21.38</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Overall Standard deviation**

<table>
<thead>
<tr>
<th></th>
<th>δ^{15}N</th>
<th>δ^{13}C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAN273</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>BAN928</td>
<td>0.08</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table S8: Results from Bradford-York interlab comparison on two human bone samples from the Banbury Lane assemblage

### A.3.3. Estimation of Animal Protein in Diet & Creation of Mixing Models

Estimation of the fraction of animal protein in the diet from δ^{15}N was undertaken on the Banbury Lane assemblage using the models outlined in Hedges and Reynard (2007) and Lelli et al. (2012):

[A] The ‘standard model’: forage and cereal have the same δ^{15}N value, and human and fauna enrichment has the same value (4‰)

[B] Cereal δ^{15}N is 1‰ enriched over animal forage

[C] In addition to [B], the enrichment in humans is 1‰ greater than herbivores (5‰ for humans, 4‰ for herbivores)

[D] In addition to [C], the enrichment for humans eating an all meat diet is 1‰ less than for an all plant diet (4‰ for an all meat diet, 5‰ for an all plant diet)

The fraction of animal protein in total dietary protein (% Pro_{animal}) was estimated for each model using the following equation:

\[
\% \text{ Pro}_{\text{animal}} = \frac{x-p}{a-p}
\]
where,
\[ x = \text{mean } \delta^{15}\text{N} \text{ of human values}; \]
\[ \delta^{15}\text{N}_{\text{fauna}} = \text{mean } \delta^{15}\text{N} \text{ of terrestrial fauna} \]

Model A: \[ p = \delta^{15}\text{N}_{\text{fauna}}, \ a = ^{15}\text{N}_{\text{fauna}} + 4 \]
Model B: \[ p = \delta^{15}\text{N}_{\text{fauna}} + 1, \ a = ^{15}\text{N}_{\text{fauna}} + 4 \]
Model C: \[ p = \delta^{15}\text{N}_{\text{fauna}} + 1, \ a = ^{15}\text{N}_{\text{fauna}} + 5 \]
Model D: \[ p = \delta^{15}\text{N}_{\text{fauna}} + 2, \ a = ^{15}\text{N}_{\text{fauna}} + 5 \]

Cattle and pig data used within the model was taken from this study and Towers et al. (2011).

The Bayesian mixing model FRUITS (Fernandes et al. 2014) was also utilised in the analysis of the Banbury Lane human \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) isotopic data. The model requires the imputation of a range of prior information, as discussed above in section A.1.4. The data used within the two models created for Banbury Lane is displayed in Tables S9-S12 below.

<table>
<thead>
<tr>
<th>Weight Contribution (%)</th>
<th>Offset (‰)</th>
<th>Protein</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta^{13}\text{C} )</td>
<td>4.8 (0.5)</td>
<td>74 (4)</td>
<td>26 (1)</td>
</tr>
<tr>
<td>( \delta^{15}\text{N} )</td>
<td>5.5 (0.5)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table S9: The diet to tissue offset and weight contribution of macronutrients towards dietary proxy signals. Values in parentheses represent uncertainty. Based upon data from Fernandes et al. (2014; 2015)

<table>
<thead>
<tr>
<th>Protein (%)</th>
<th>Energy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial Animals</td>
<td>30 (2.5)</td>
</tr>
<tr>
<td>Terrestrial Plants</td>
<td>15 (5)</td>
</tr>
</tbody>
</table>

Table S10: Concentration values for each food group (dry weight composition of macronutrients). Values in parentheses represent uncertainty

<table>
<thead>
<tr>
<th>Model 1</th>
<th>( \delta^{13}\text{C} ) (‰)</th>
<th>Unc.</th>
<th>( \delta^{15}\text{N} ) (‰)</th>
<th>Unc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant protein</td>
<td>-23.13</td>
<td>0.7</td>
<td>3.60</td>
<td>0.6</td>
</tr>
<tr>
<td>Plant energy</td>
<td>-22.13</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terrestrial animal protein</td>
<td>-24.89</td>
<td>0.5</td>
<td>8.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Terrestrial animal energy</td>
<td>-30.89</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table S11: Food values used within the Banbury Lane FRUITS model 1. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%$, and plant energy values using $\Delta^{13}C_{\text{protein-energy}} = +1\%$ Tieszen 1991; Fernandes 2015)

<table>
<thead>
<tr>
<th>Model 2</th>
<th>$\delta^{13}C$ (‰)</th>
<th>Unc.</th>
<th>$\delta^{15}N$ (‰)</th>
<th>Unc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant protein</td>
<td>-23.13</td>
<td>0.7</td>
<td>3.60</td>
<td>0.6</td>
</tr>
<tr>
<td>Plant energy</td>
<td>-22.13</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cattle protein</td>
<td>-24.94</td>
<td>0.4</td>
<td>8.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Cattle energy</td>
<td>-30.94</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pig protein</td>
<td>-22.47</td>
<td>0.6</td>
<td>8.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Pig energy</td>
<td>-28.47</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table S12: Food values used within the Banbury Lane FRUITS model 2. Terrestrial animal macronutrient values calculated using $\Delta^{13}C_{\text{protein-collagen}} = -2\%$, $\Delta^{15}N_{\text{protein-collagen}} = +2\%$ and $\Delta^{13}C_{\text{energy-collagen}} = -8\%$, and plant energy values using $\Delta^{13}C_{\text{protein-energy}} = +1\%$ Tieszen 1991; Fernandes 2015)

A.3.4. Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) Analysis

Stable isotopic analysis of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios was undertaken on 26 permanent left maxillary first molars (M1) from the burial pit at the site. Strontium isotope analysis was undertaken using a laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS) method (as outlined in Lewis et al. 2014). Although this methodology has had relatively limited application to archaeological samples to date, it has been shown to provide a significantly higher degree of resolution of Sr data than previously obtained using thermal ionisation mass spectrometry (TIMS) (e.g. Richards et al. 2008; see also Chapter 4). Briefly, teeth were sectioned using a partially coated diamond dental cutting disk (0.15mm; Komet Dental, Germany; Figure S1), and then mounted in epoxy resin and polished (Figure 39). Laser ablation MC-ICP-MS analysis was undertaken at the National Oceanography Centre (Southampton), and utilised a NewWave 193nm Ar-F excimer laser ablation system coupled to a Thermo-Finnigan Neptune. Laser settings were a 150µm spot size and 15Hz repetition rate. The ablated sample was swept from the laser cell using 1.2 L/min He gas, which was subsequently mixed with 0.6–0.7 L/min and 0.05–0.08 L/min of Ar and N2 gas respectively, before entering the plasma ion source. The analytical error, calculated from internal standards, was 22ppm. $^{84}\text{Sr}/^{86}\text{Sr}$ values were measured to monitor for Ar-Ar and Ca-Ca dimer formation. If there is significant dimer formation, a reliable $^{87}\text{Sr}/^{86}\text{Sr}$ value cannot be calculated. However if $^{84}\text{Sr}/^{86}\text{Sr}$ is <0.064 then the contribution to
the $^{87}\text{Sr}/^{86}\text{Sr}$ of the dimers is $<50$ ppm (A. Pike, pers. comm.). $^{84}\text{Sr}/^{86}\text{Sr}$ was seen to be $<0.064$ in all data obtained within this study.

![Figure S1: Example of sectioned M1 tooth from the Banbury Lane assemblage](image)

Stable isotopic analysis of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios was also undertaken on 12 soil samples collected from the Northampton area. A few grams of soil were taken from each sample location from the uppermost 15cm of soil deposit, after removing the top humus layer. Briefly, soil leachates were obtained by placing c.1g of soil into a centrifuge tube with 10ml de-ionised water and agitating for 24 hours. Following centrifugation, the supernatant was removed. Samples were converted to chloride by adding 2ml 6M HCl to the supernatant solution, and then drying down overnight. The resulting fraction was then taken up in titrated 2.5M HCl and pipetted onto ion-exchange chromatography columns. Strontium was separated with Dowex (AG50-X8) resin (200-400 mesh). Samples were loaded onto filaments and Sr isotopic composition was measured via thermal ionisation mass spectrometry using a Triton (Thermo) and VG Sector 54 TIMS instrument at the National Oceanography Centre (Southampton). A mass-fractionation correction was applied to all obtained strontium ratio measurements (as in Kootker et al. 2016 and Neil et al. 2016).

**A.3.4.1. LA-MC-ICP-MS $^{87}\text{Sr}/^{86}\text{Sr}$ enamel data**

The data generated from the dental enamel of 26 permanent left maxillary first molars (M1) from Banbury Lane is displayed in Figures S2-S22. In all plots, the data are smoothed as a 20 point moving mean of the raw laser integrations with every 20th mean plotted, and run along the growth axis of the enamel from tooth crown towards cervix. The green shaded areas on all data plots indicate the expected biosphere strontium range for Northampton indicated by soil analyses (0.786-0.7107; see Chapter 7).
Figure S2: LA-MC-ICP-MS plot generated for sample 99.0

Figure S3: LA-MC-ICP-MS plot generated for sample 132.17
Figure S4: LA-MC-ICP-MS plot generated for sample 138.003

Figure S5: LA-MC-ICP-MS plot generated for sample 141.00
Figure S6: LA-MC-ICP-MS plot generated for sample 172.00

Figure S7: LA-MC-ICP-MS plot generated for sample 201.002
Figure S8: LA-MC-ICP-MS plot generated for sample 240.005

Figure S9: LA-MC-ICP-MS plot generated for sample 302.8
Figure S10: LA-MC-ICP-MS plot generated for sample 440.003

Figure S11: LA-MC-ICP-MS plot generated for sample 462.7
Figure S12: LA-MC-ICP-MS plot generated for sample 551.00

Figure S13: LA-MC-ICP-MS plot generated for sample 838.3
Figure S14: LA-MC-ICP-MS plot generated for sample 838.4

Figure S15: LA-MC-ICP-MS plot generated for sample 901.00
Figure S16: LA-MC-ICP-MS plot generated for sample 1076.5

Figure S17: LA-MC-ICP-MS plot generated for sample 1078.008
Figure S18: LA-MC-ICP-MS plot generated for sample 1079.002

Figure S19: LA-MC-ICP-MS plot generated for sample 1303.01
Figure S20: LA-MC-ICP-MS plot generated for sample 1336.3

Figure S21: LA-MC-ICP-MS plot generated for sample 2005.213
A.3.5. AMS Dating

AMS dates utilised within Chapter 7 were provided by Northamptonshire Archaeology (MOLA; Andy Chapman, Mark Holmes, Adam Yates), and data was generated by the Beta Analytic laboratory (Miami, Florida, USA). Calibration of dates was undertaken using INTCAL09 and INTCAL13 Radiocarbon Age Calibration.

A.4. Chapter 8 – Biomolecular Analysis of Dental Calculus

A.4.1. DNA Extractions

All DNA extractions were performed in the ancient DNA laboratory facility at the University of York. The aDNA Laboratory is comprised of two self-contained rooms with a positive-pressure air system, and is dedicated solely to ancient DNA research. The laboratory follows established contamination control workflows and clean-lab conditions, including the use of full-body suits, masks and gloves by all researchers, the cleaning of all work-surfaces in the lab with bleach and UV lighting, physical separation from all
laboratories where PCR work is undertaken, and one-directional workflows to avoid contamination with PCR products.

All aDNA extractions utilised a modified version of previously published protocols aimed to optimise the recovery of short DNA fragments in ancient samples, binding samples to a silica membrane (Dabney et al. 2013; Warinner et al. 2014(a)) – recently shown to be the most advantageous ancient DNA extraction technique (Gamba et al. 2015).

An initial extraction undertaken on three Hazleton North calculus samples utilised a different primary decontamination and demineralisation protocol however. Briefly, samples were weighed out and placed into low-bind eppendorf tubes with 1.5ml HPLC water, vortexed for c.15 seconds, and the supernatant discarded. Samples were then UV’d for 20 mins, placed into 2ml low-bind eppendorf tubes, and ground using a micropestle. 1ml extraction buffer (1mg/ml Proteinase K, 0.45M EDTA solution) added to each sample, and incubated overnight on rotator at 55°C.

All other samples (HH3181, HH1916, COL/UN, COL UN8, HN3793, HN4786, HN7656, HN10213, HN11456, BL201.1C, BL201.2C, BL309.12C, BL440.4C, BL201.1B, BL201.2B, BL440.4B) were extracted using slightly modified protocol in an attempt to minimise contamination and optimise demineralisation. Briefly, 500µl 6% bleach was added to each sample and vortexed for 1 min, before being centrifuged and the supernatant discarded. Samples were then rinsed three times with HPLC water, vortexing each time before removing the supernatant. Samples were then UV’d for 20 mins, then placed into 2ml low-bind eppendorf tubes and ground using a micropestle. 1ml of 0.45M EDTA was added to each sample and left to incubate for 24-96 hours on rotation at room temperature until fully demineralised. 50µl PK (20mg/ml) was then added to all samples and incubated on rotation at 50°C for 7 hours. Bone samples were then removed and stored at -20°C overnight. An additional 50µl PK (20mg/ml) was added to all calculus samples, heated at 80°C for 10 mins, and then incubated on rotation overnight at room temperature. All samples (calculus and bone) were then centrifuged at 13,300 RPM for 20 mins, then the supernatant from each sample was added to 13ml binding buffer (5M guanidine hydrochloride, 40% isopropanol, 0.05% Tween-20, 90mM sodium acetate solution, dH₂O) and passed through Zymo-spin V column extension reservoirs attached to a Qiagen MinElute silica spin column. Samples in the columns were centrifuged for 3 mins at 3,000 RPM, rotated 180° and then span for a further 3 mins at 3,000 RPM. All MinElute columns
were then transferred to collection tubes and dry spun for 1 min at 6,000 RPM. 650µl PE buffer was then added to the silica membrane of each sample column, and centrifuged for 1 min at 3,300 RPM, discarding all flow-through. This step was then repeated, adding an additional 650µl PE buffer to all samples. MinElute columns were then transferred to new collection tubes, 27.5µl EB buffer added to the silica membranes, incubated for 10 minutes, and then centrifuged at 13,300 RPM for 30 seconds. This step was then repeated, adding an additional 27.5µl EB buffer. All flow-through (55µl) was then transferred to new 1.5ml low-retention tubes and stored at -20°C. A 1µl aliquot of each sample was taken for Qubit analysis to determine DNA concentrations, the results of which are shown below in Table S13 and Figure S23.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Weight/ mg</th>
<th>Qubit Reading 1 (ng/µl)</th>
<th>Qubit Reading 2 (ng/µl)</th>
<th>Average concentration (ng/µl)</th>
<th>Elution Volume /µl</th>
<th>DNA/mg of calculus (or bone) (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH1916</td>
<td>21.3</td>
<td>5.54</td>
<td>5.5</td>
<td>5.52</td>
<td>55</td>
<td>14.25</td>
</tr>
<tr>
<td>HH3181</td>
<td>7.1</td>
<td>0.916</td>
<td>0.89</td>
<td>0.903</td>
<td>55</td>
<td>7.00</td>
</tr>
<tr>
<td>HH3188</td>
<td>28.9</td>
<td>24.7</td>
<td>25.9</td>
<td>25.3</td>
<td>50</td>
<td>43.77</td>
</tr>
<tr>
<td>HH3</td>
<td>18.6</td>
<td>21.1</td>
<td>20</td>
<td>20.55</td>
<td>50</td>
<td>55.24</td>
</tr>
<tr>
<td>HH610</td>
<td>9.4</td>
<td>2.58</td>
<td>2.56</td>
<td>2.57</td>
<td>55</td>
<td>15.04</td>
</tr>
<tr>
<td>HN10213</td>
<td>20.1</td>
<td>14.2</td>
<td>14.3</td>
<td>14.25</td>
<td>55</td>
<td>38.99</td>
</tr>
<tr>
<td>HN11456</td>
<td>48.7</td>
<td>32.6</td>
<td>32.6</td>
<td>32.6</td>
<td>55</td>
<td>36.82</td>
</tr>
<tr>
<td>HN3793</td>
<td>9.6</td>
<td>2.72</td>
<td>2.78</td>
<td>2.75</td>
<td>55</td>
<td>15.76</td>
</tr>
<tr>
<td>HN4786</td>
<td>22.7</td>
<td>5.72</td>
<td>5.76</td>
<td>5.74</td>
<td>55</td>
<td>13.91</td>
</tr>
<tr>
<td>HN7656</td>
<td>20.8</td>
<td>13.6</td>
<td>12.4</td>
<td>13.0</td>
<td>55</td>
<td>34.38</td>
</tr>
<tr>
<td>COL EU.1.5.13 0</td>
<td>5.1</td>
<td>0.531</td>
<td>0.464</td>
<td>0.4975</td>
<td>50</td>
<td>4.88</td>
</tr>
<tr>
<td>COL/UN</td>
<td>4.2</td>
<td>0.440</td>
<td>0.442</td>
<td>0.441</td>
<td>55</td>
<td>5.78</td>
</tr>
<tr>
<td>COL UN8</td>
<td>2.5</td>
<td>0.772</td>
<td>0.770</td>
<td>0.771</td>
<td>55</td>
<td>16.96</td>
</tr>
<tr>
<td>BL201.1C</td>
<td>2.4</td>
<td>0.436</td>
<td>0.436</td>
<td>0.436</td>
<td>55</td>
<td>9.99</td>
</tr>
<tr>
<td>BL201.2C</td>
<td>4.0</td>
<td>0.838</td>
<td>0.834</td>
<td>0.836</td>
<td>55</td>
<td>11.50</td>
</tr>
<tr>
<td>BL309.12C C</td>
<td>23.4</td>
<td>13.0</td>
<td>12.8</td>
<td>12.9</td>
<td>55</td>
<td>9.99</td>
</tr>
<tr>
<td>BL440.4C</td>
<td>3.6</td>
<td>0.116</td>
<td>0.112</td>
<td>0.114</td>
<td>55</td>
<td>1.74</td>
</tr>
<tr>
<td>BL201.1B</td>
<td>67.0</td>
<td>1.51</td>
<td>1.48</td>
<td>1.495</td>
<td>55</td>
<td>1.23</td>
</tr>
<tr>
<td>BL202.2B</td>
<td>38.6</td>
<td>0.256</td>
<td>0.276</td>
<td>0.266</td>
<td>55</td>
<td>0.38</td>
</tr>
<tr>
<td>BL440.4B</td>
<td>54.0</td>
<td>0.78</td>
<td>0.718</td>
<td>0.749</td>
<td>55</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Table S13: Post-extraction Qubit results for bone and calculus samples
Figure S23: DNA concentrations in dental calculus post-extraction (ng of DNA/mg of dental calculus)

A.4.2. DNA Sequence Generation and Shotgun Library Building

Shotgun sequencing is a non-targeted, random sequencing approach, which aims to sequence all DNA present within an extract (unlike 16S sequencing). Due to this, it has frequently been used in whole genome studies – initially sequencing the human genome (Weber and Myers 1997; Venter et al. 1998).

DNA extracts from all samples were converted into Illumina sequencing libraries for shotgun sequencing using previously published protocols (Meyer and Kircher 2010). Shotgun library building involves firstly repairing the ends of damaged DNA fragments, ligation of an adaptor, and the fill-in of spaces created through adapter ligation by Bst polymerase. Fragments are then indexed using Illumina primers and amplified using PCR (Figure S24; Text Box 1; Table S14).
Due to the number of DNA extracts, Illumina sequencing libraries were created in two separate batches (with half the samples in each). The methodology utilised is outlined in Text Box 1 below. Negative controls were processed along with all samples and monitored for contamination.
**Illumina Shotgun Library Building**

**Blunt End Repair**
25 µl DNA extract was added to 10 µl Blunt End Repair Mastermix (100 µM dNTPs; 1 mM ATP; 0.5 U/µl T4 Polynucleotide kinase; 0.5 U/µl T4 DNA polymerase; Buffer Tango (1x); dH2O) in PCR tubes on ice. Samples were then incubated in a thermocycler for 20 mins at 25°C, followed by 12°C ∞. Reaction clean-up utilised Qiagen MinElute columns, adding 175 µl PB buffer to samples in each column and centrifuging for 1 min at 3,000 RPM. 750 µl PE buffer was then added to each sample, and again spun for 1 min at 3,000 RPM. All flow-through was discarded. Samples were then dry-spun for 1 min at 13,300 RPM and the MinElute columns transferred to new collection tubes. To elute, 15 µl EB was added to columns, incubated for 5 minutes and then centrifuged for 1 min at 13,300 RPM. This final step was then repeated, and all flow-through transferred to new PCR tubes.

**Adapter Ligation**
The 30 µl DNA extract was added to 19 µl Adapter Ligation Mastermix (T4 Ligase Buffer (1x); 5% PEG-4000, 0.125 U/µl T4 Ligase) and 0.4 µM P5 adapter mix in PCR tubes on ice. The P5/P7 adapters used for each sample are indicated in Table 3 below. Samples were then incubated in a thermocycler for 30 mins at 22°C, followed by 12°C ∞. Samples were then purified again using Qiagen MinElute columns as described above, but using 250 µl PB buffer, and 20 µl EB buffer to elute instead.

**Adapter Fill-In**
The 20 µl DNA extract was added to 20 µl Adapter Fill-In Mastermix (Thermopol Buffer (1x); 250 µM dNTPs; 0.3 U/µl Bst Polymerase LF; dH2O) in PCR tubes, and incubated in a thermocycler for 20 mins at 37°C, followed by 20 mins at 80°C, and then 12°C ∞.

**qPCR (For optimising library amplification)**
An aliquot of 1 µl DNA extract was added to 9 µl EB buffer and 19 µl qPCR Mastermix (SYBR Mastermix (1x); 0.2 µM IS7 Forward Primer; 0.2 µM IS8 Reverse Primer; dH2O) into a 96-well qPCR plate.

**Indexing PCR**
3 µl DNA extract was added to 21.5 µl iPCR Mastermix (Accuprime Pfx Supermix (1x); 0.2 µM IS4 Forward Primer) and 0.2 µM P7 Indexing Primer in PCR tubes. Samples were then incubated in a thermocycler for 5 mins at 95°C, then 15 seconds at 95°C, 30 seconds at 60°C, 30 seconds at 68°C, 5 mins at 68°C, and then 10°C ∞. The number of PCR samples each sample was subjected to is outlined in Table 3 below. Samples were then purified again using Qiagen MinElute columns as described above, but using 125 µl PB buffer, and 20 µl EB buffer to elute instead. DNA concentrations were then quantified using a Qubit (Table S14).

Remaining DNA extracts were stored in 1.5 ml tubes at -20°C.
Table S14: Sample adapter numbers, number of PCR cycles and post-iPCR Qubit quantification

<table>
<thead>
<tr>
<th>Sample</th>
<th>Adapter Number</th>
<th>Adapter Sequence</th>
<th>Qubit ng/µl (post iPCR &amp; purification)</th>
<th>Number of PCR cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH1916C</td>
<td>index_8nt_4</td>
<td>AACCGAAC</td>
<td>13.35</td>
<td>11</td>
</tr>
<tr>
<td>HH3C</td>
<td>index_8nt_89</td>
<td>ACGAACTT</td>
<td>4.22</td>
<td>13</td>
</tr>
<tr>
<td>COL EU.1.5.130</td>
<td>index_8nt_129</td>
<td>AGAACGAC</td>
<td>5.84</td>
<td>13</td>
</tr>
<tr>
<td>HN1145</td>
<td>index_8nt_200</td>
<td>ATAGGTAT</td>
<td>7.14</td>
<td>13</td>
</tr>
<tr>
<td>HN4786</td>
<td>index_8nt_254</td>
<td>CAATTGAG</td>
<td>3.54</td>
<td>11</td>
</tr>
<tr>
<td>HN7656</td>
<td>index_8nt_309</td>
<td>CCGATCCT</td>
<td>4.85</td>
<td>13</td>
</tr>
<tr>
<td>BL309.12C</td>
<td>index_8nt_347</td>
<td>CGATCGGA</td>
<td>6.91</td>
<td>11</td>
</tr>
<tr>
<td>BL201.1B</td>
<td>index_8nt_497</td>
<td>GCCAGGGTT</td>
<td>7.68</td>
<td>16</td>
</tr>
<tr>
<td>LBL</td>
<td>index_8nt_686</td>
<td>TGGTCCTG</td>
<td>1.17</td>
<td>11</td>
</tr>
<tr>
<td>eBK</td>
<td>index_8nt_695</td>
<td>TTATCGTC</td>
<td>1.77</td>
<td>11</td>
</tr>
<tr>
<td>HH3188</td>
<td>index_8nt_398</td>
<td>CTATTCAT</td>
<td>0.148</td>
<td>14</td>
</tr>
<tr>
<td>HH3181</td>
<td>index_8nt_427</td>
<td>CTGGATAA</td>
<td>10.85</td>
<td>14</td>
</tr>
<tr>
<td>HH610</td>
<td>index_8nt_439</td>
<td>GAACGCTG</td>
<td>9.55</td>
<td>11</td>
</tr>
<tr>
<td>HN3793</td>
<td>index_8nt_517</td>
<td>GCGTTAGC</td>
<td>9.45</td>
<td>11</td>
</tr>
<tr>
<td>HN10213</td>
<td>index_8nt_582</td>
<td>GTCTTGGC</td>
<td>2.30</td>
<td>17</td>
</tr>
<tr>
<td>HN7387</td>
<td>index_8nt_589</td>
<td>GTTGCAAC</td>
<td>2.17</td>
<td>11</td>
</tr>
<tr>
<td>HN5880</td>
<td>index_8nt_610</td>
<td>TAGTTAGG</td>
<td>1.66</td>
<td>11</td>
</tr>
<tr>
<td>HN5037</td>
<td>index_8nt_678</td>
<td>TGGACGCA</td>
<td>6.28</td>
<td>11</td>
</tr>
<tr>
<td>COL /UN</td>
<td>index_8nt_702</td>
<td>TTTGCTGG</td>
<td>7.57</td>
<td>14</td>
</tr>
<tr>
<td>COL UN8</td>
<td>index_8nt_710</td>
<td>TTGGCGAG</td>
<td>15.15</td>
<td>13</td>
</tr>
<tr>
<td>BL201.1C</td>
<td>index_8nt_335</td>
<td>CCTTGAAAT</td>
<td>13.4</td>
<td>14</td>
</tr>
<tr>
<td>BL440.4C</td>
<td>index_8nt_301</td>
<td>CCATAGTC</td>
<td>1.34</td>
<td>17</td>
</tr>
<tr>
<td>BL201.2C</td>
<td>index_8nt_184</td>
<td>AGTTGAAC</td>
<td>14.80</td>
<td>11</td>
</tr>
<tr>
<td>BL201.2B</td>
<td>index_8nt_229</td>
<td>ATTATCGA</td>
<td>4.47</td>
<td>17</td>
</tr>
<tr>
<td>BL440.4B</td>
<td>index_8nt_475</td>
<td>GATGATAA</td>
<td>1.98</td>
<td>17</td>
</tr>
<tr>
<td>LBL</td>
<td>index_8nt_683</td>
<td>TGGCGTTA</td>
<td>1.175</td>
<td>17</td>
</tr>
<tr>
<td>eBK</td>
<td>index_8nt_559</td>
<td>GGTCGGCG</td>
<td>1.31</td>
<td>17</td>
</tr>
</tbody>
</table>

All Illumina libraries were pooled at equimolar concentrations and sequenced on two lanes of an Illumina HiSeq2000, using paired-end 100 bp chemistry, at the University of Copenhagen’s Centre for Geogenetics.

A.4.3. Protein Extractions

Proteomic analyses were undertaken using a modified version of the methodology previously published by Warinner et al. (2014(a)), which utilises a filter-aided sample preparation (FASP) protocol. Total protein extraction was undertaken on a total of 10
calculus samples from three of the four sites. Negative controls were also processed along with all samples and monitored for contamination. Protein extraction was undertaken in a laboratory at the University of York dedicated to proteomics work and where no bacterial culturing takes place.

Calculus samples were weighed out and placed into 2ml eppendorf tubes, where they were then ground to a fine powder using a micropestle. Samples were then suspended in 1ml 0.5M EDTA and incubated at room temperature on rotation until demineralised. Following centrifugation at 13,000 RPM for 15 mins, the supernatant (EDTA fraction) was transferred to sterile 15ml falcon tubes containing 9ml UA solution (8M urea; 0.1M (pH8.0) Tris/HCl), which was then passed through 10k Da Amicon Ultra-4 centrifugal units in 4ml increments, centrifuging at 4,000 RPM. 4ml UA solution was then passed through each Amicon ultrafilter, and all flow-through was discarded.

Pellets obtained after centrifugation of demineralised samples were also analysed, and were suspended in 300µl lysis buffer (0.5% SDS; 0.1M DTT; 0.1M Tris/HCl) and then incubated at 80°C for 10 mins and then agitated at room temperature for 30 mins. Samples were then centrifuged at 13,000 RPM for 20 minutes to pellet insoluble minerals and cellular debris.

Following this, 2ml UA solution (8M urea; 0.1M (pH8.0) Tris/HCl) was then added to the Amicon ultrafilter units previously used for the EDTA fraction of the samples, along with the supernatant (SDS fraction) from the lysed calculus pellet. The Amicon ultrafilter units were then centrifuged at 4,000 RPM until c.250µl liquid was retained within the ultrafilter. An additional 2ml UA solution was then added to the ultrafilters, and again centrifuged at 4,000 RPM until only c.250µl liquid was retained. All flow-through was discarded. Resuspension in 500µl CAA solution (0.05M 2-Chloracetamide; 8M urea; 0.1M (pH8.0) Tris/HCl) was then undertaken, and all ultrafilter units were incubated without light or motion for 20 mins at room temperature. Samples were then centrifuged at 4,000 RPM for 10 mins, and all flow-through was discarded. CAA was then removed by washing each ultrafilter with 2ml UA solution and centrifuging at 4,000 RPM for 15 mins, and discarding all flow-through. The urea was then removed from the ultrafilters by washing with 2ml ABC solution (0.05M ammonium bicarbonate (pH 7.5-8.0); dH2O), centrifuging at 4,000 RPM for 20 mins, and discarding all flow-through. The fraction retained within the ultrafilter was then re-suspended in an additional 300µl ABC solution, and a 1µl
aliquot was taken for protein quantification using a Qubit fluorometer (Invitrogen)(Table S15).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Weight/mg</th>
<th>Qubit Reading 1 (ng/ml)</th>
<th>Qubit Reading 2 (ng/ml)</th>
<th>Average concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH3188</td>
<td>17.29</td>
<td>1.69</td>
<td>1.69</td>
<td>1.69</td>
</tr>
<tr>
<td>HH3</td>
<td>15.41</td>
<td>1.63</td>
<td>1.80</td>
<td>1.72</td>
</tr>
<tr>
<td>HH610</td>
<td>15.29</td>
<td>3.05</td>
<td>3.18</td>
<td>3.12</td>
</tr>
<tr>
<td>HN11456</td>
<td>23.08</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>HN7387</td>
<td>33.25</td>
<td>3.40</td>
<td>3.42</td>
<td>3.41</td>
</tr>
<tr>
<td>HN4786</td>
<td>17.86</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>HN7656</td>
<td>18.00</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>HN5037-1</td>
<td>21.58</td>
<td>1.82</td>
<td>1.91</td>
<td>1.87</td>
</tr>
<tr>
<td>BL309.12</td>
<td>14.70</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>BL132.17</td>
<td>2.52</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>eBK</td>
<td>N/A</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

Table S15: Post-extraction (pre-trypsin digest) Qubit results for calculus samples

Protein digestion was undertaken by adding 6µl 0.5µg/µl sequencing grade trypsin solution (Promega) to each sample within the ultrafilter. Ultrafilter units were then transferred to new, sterile 15ml collection tubes, the lids parafilmed, and incubated overnight at 37°C. The following morning, the parafilm was removed, and samples were centrifuged at 4,000 RPM for 10 mins, with all flow-through being retained. 500µl ABC solution was then added to each ultrafilter and centrifuged at 4,000 RPM to elute any remaining digested peptides within the ultrafilter. All filtrate (containing the digested peptides) was then transferred to new, sterile 1.5ml eppendorf tubes and acidified with 10% TFA (trifluoroacetic acid) to reach a final concentration of 0.2-0.8% and a pH < 2. C-18 Empore (3M) solid phase extraction (SPE) Stage Tips were prepared in-house and sequentially conditioned with 150µl methanol, 150µl EB80 solution (80% acetonitrile; 0.5% acetic acid; dH2O), and 150µl AA solution (0.5% acetic acid; 99.5% dH2O), centrifuging each time at 5,000 RPM for 3 mins, and discarding all flow-through. The acidified peptides were then loaded in 150µl increments onto the Stage Tips and immobilised onto the C-18 membrane by centrifugation at 4,000 RPM. The C-18 membrane was then washed with 150µl AA solution (0.5% acetic acid; 99.5% dH2O), centrifuging at 3,000 RPM for 4 mins, and then dry-span at 5,000 RPM for 5 mins. All stage-tips were stored at -20°C until ready for MS/MS analysis.
On the day of MS/MS analysis, the Stage Tips were removed from storage at -20°C and placed into new, sterile 2ml collection tubes. Peptides were eluted sequentially three times with 40µl acetonitrile solution in increasing concentrations (40%, 60% and 80% acetonitrile; 0.5% acetic acid; dH2O), centrifuging each time at 5,000 RPM for 2 mins. Eluted peptides (flow-through) were then transferred to new, sterile 1.5ml eppendorf tubes and concentrated by centrifugal evaporation to a volume of < 4µl. Samples were then analysed by tandem mass spectrometry at the Target Discovery Institute, University of Oxford, using a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). Q-Exactive analysis was performed after UPLC separation on an EASY-Spray column (50 cm × 75 μm ID, PepMap RSLC C18, 2 μm) connected to a Dionex Ultimate 3000 nUPLC (all Thermo Scientific) using a gradient of 2–40% Acetonitrile in 0.1% Formic Acid and a flow rate of 250 nl/min @40°C. MS spectra were acquired at a resolution of 70000 at 200 m/z using an ion target of 3E6 between 380 and 1800 m/z. MS/MS spectra of up to f15 precursor masses at a signal threshold of 1E5 counts and a dynamic exclusion for 7 seconds were acquired at a resolution of 17500 using an ion target of 1E5 and a maximal injection time of 50 ms. Precursor masses were isolated with an isolation window of 1.6 Da and fragmented with 28% normalized collision energy.

A.4.4. Data Analysis

A.4.4.1. DNA Data Analysis

Raw Illumina sequencing data were initially prepared for analysis in UNIX by concatenating all generated fastq files of the same read direction, and then removing adapter sequences and barcodes using Cutadapt (Martin 2011). Quality filtering was also undertaken using Cutadapt, and all reads <20bp were discarded. Forward and reverse reads for each sample were then merged using PEAR (Zhang et al. 2013).

Merged fastq files were then uploaded to OneCodex (https://beta.onecodex.com) for sample comparisons at the phyla level. Samples were uploaded to OneCodex as mixed/metagenomic samples, and were compared against the OneCodex (July 2015) database. This database contains 30,825 bacterial genomes, 5,163 viral genomes, 633 fungal genomes, 504 archaeal genomes, and 57 protozoan genomes.
Alignment to the human genome was undertaken using BWA (Burrows-Wheeler Aligner; Li and Durbin 2009) and SAMtools (Li et al. 2009), mapping to a full reference human genome from NCBI in karyotypical order. Mean depth of coverage and fragment length were estimated using BAMStats (http://bamstats.sourceforge.net/). Sex identification was undertaken using endogenous DNA content, using a Python sexing script (Skoglund et al. 2013) following a short script using SAMtools to ensure both forward and reverse reads were mapped to the same chromosome:

```
samtools view datafile_sort_rd.bam | awk '($3==$7 || $7=="=")' > datafile_chrome.bam
```

**A.4.4.1.2. DNA Authentication**

Determining that the DNA which has been sequenced and analysed is indeed ancient and endogenous, and not the product of contamination, is of upmost importance within aDNA studies. This is also of particular importance in the analysis of dental calculus, where bacteria, rather than host DNA, dominate the sample and are the primary source of interest (Warinner et al. 2015(b)). Due to this, steps are needed to assess authenticity of the sequences. Here, authentication of DNA sequences was undertaken through the assessment of damage artefacts and patterns. Characteristic misincorporation patterns, particularly the deamidation of cytosine (C-T) and guanine (G-A), can be utilised to authenticate the antiquity of sequences (Jónsson et al. 2013; Weiβ et al. 2015).

Damage patterns in sequencing reads were assessed through the utilisation of the mapDamage2.0 package (Jónsson et al. 2013). All calculus samples analysed here broadly showed characteristic misincorporation patterns, whereas blank samples did not (Figures S25-S46), therefore indicating that the DNA is in fact ancient, and not the result of contamination.
Figure S25: MapDamage plot for sample BL201.1C

Figure S26: MapDamage plot for sample BL201.1B (bone sample)
Figure S27: MapDamage plot for sample BL201.2C

Figure S28: MapDamage plot for sample BL201.2B (bone sample)
Figure S29: MapDamage plot for sample BL309.12C

Figure S30: MapDamage plot for sample BL440.4B
Figure S31: MapDamage plot for sample HH3

Figure S32: MapDamage plot for sample HH1916
Figure S33: MapDamage plot for sample HH610

Figure S34: MapDamage plot for sample HH3181
Figure S35: MapDamage plot for sample HN11456

Figure S36: MapDamage plot for sample HN5037
Figure S37: MapDamage plot for sample HN4786

Figure S38: MapDamage plot for sample HN7656
Figure S39: MapDamage plot for sample HN3793

Figure S40: MapDamage plot for sample COL11033
Figure S41: MapDamage plot for sample COL11035

Figure S42: MapDamage plot for sample COL11038
Figure S43: MapDamage plot for sample EBK1 (extraction blank 1)

Figure S44: MapDamage plot for sample EBK2 (extraction blank 2)
Figure S45: MapDamage plot for sample LBL1 (library building blank 1)

Figure S46: MapDamage plot for sample LBL2 (library building blank 2)
A.4.4.2. Proteomic Data Analysis

Raw MS/MS spectra were converted to searchable mgf files (Mascot generic format) using Proteowizard version 3.0.7518, using the 200 most intense peaks in each MS/MS spectra. MS/MS ion database searching was undertaken using Mascot (Matrix Science, version 2.5.1 (Perkins et al. 1999)) against all available sequences in both the UniProt database and the Human Oral Microbiome Database (HOMD) (Chen et al. 2010). Searches were also performed against a decoy database to generate false discovery rates. Searches were run as semi-tryptic, with up to two missed cleavages, and with a peptide tolerance of 10ppm. MS/MS ion tolerance was set to 0.07 Da, and due to the archaeological age of the specimens used, post-translational modifications were set to carboxamidomethylation (fixed modification), acetyl (protein N-term), deamidated (NQ), glutamine to pyroglutamate (N-term Q), methionine oxidation, and hydroxylation of proline (variable modifications).

Protein and peptide recovery rates and concentrations were generated from Mascot data, at a target FDR of 5% (above identity or homology threshold) (as in Warinner et al. 2014(a)) and an ion cut-off score of 25 and significance threshold of \( p < 0.05 \) (as in Warinner et al. 2014(b)). Spectrum assignment for proteomic data without these quality controls is provided in Table S16.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>No. of Spectra (Queries)</th>
<th>No. of Assigned Peptides</th>
<th>No. of Assigned Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH3</td>
<td>7,612</td>
<td>595</td>
<td>85</td>
</tr>
<tr>
<td>HH610</td>
<td>17,494</td>
<td>998</td>
<td>159</td>
</tr>
<tr>
<td>HH3188</td>
<td>12,928</td>
<td>720</td>
<td>173</td>
</tr>
<tr>
<td>HN4786</td>
<td>7,367</td>
<td>640</td>
<td>59</td>
</tr>
<tr>
<td>HN5037-1</td>
<td>8,755</td>
<td>503</td>
<td>103</td>
</tr>
<tr>
<td>HN7387</td>
<td>19,762</td>
<td>1051</td>
<td>239</td>
</tr>
<tr>
<td>HN7656</td>
<td>3,104</td>
<td>283</td>
<td>52</td>
</tr>
<tr>
<td>HN11456</td>
<td>10,388</td>
<td>280</td>
<td>88</td>
</tr>
<tr>
<td>BL132.17</td>
<td>8,379</td>
<td>816</td>
<td>75</td>
</tr>
<tr>
<td>BL309.12</td>
<td>8,923</td>
<td>396</td>
<td>74</td>
</tr>
<tr>
<td>eBK (Blank)</td>
<td>748</td>
<td>83</td>
<td>10</td>
</tr>
</tbody>
</table>

Table S16: Spectrum assignment for raw proteomic data obtained from dental calculus samples and an extraction blank (eBK) without quality controls

All Mascot assigned peptides were uploaded to Unipept (http://unipept.ugent.be/), a metaproteomic data analysis pipeline tool (Mesuere et al. 2015), to allow for sample comparisons at the phyla level.
A.4.4.3. BLG Identification and Alignment

Mascot search results were filtered at a target FDR of 5% (above identity or homology threshold) (as in Warinner et al. 2014(a)), using an ion cut-off score of 25 and significance threshold of $p < 0.05$ (as in Warinner et al. 2014(b)). BLAST was used to verify matches to β-lactoglobulin (BLG), with each individual peptide identified in Mascot being analysed.

Using Bioedit version 7.2.5, consensus sequences were created for each individual, utilising all peptides identified using Mascot and verified using BLAST. These were then aligned to a range of different reference sequences of BLG in domesticated fauna, obtained from the UniProt database (Figure S47).
Figure S47: Sequence alignment of BLG in a range of fauna. Consensus sequences for each individual studied here are provided below the protein alignments. All BLG peptides identified within human dental calculus within this study are specific to the Pecora infraorder of Artiodactyla, with individual samples containing diagnostic polymorphisms for Bovidae and Caprinae (see Chapter 8).
Appendix B – Isotopic Database

Appendix B consists of a database of all currently published isotopic data on Mesolithic and Neolithic human remains and fauna in the UK. For each site included, the details provided, where possible, are:

- Burial/sample numbers
- AMS ref. number
- Date BP
- Date cal. BP
- Date cal. BC
- $\delta^{13}$C (‰)
- $\delta^{15}$N (‰)
- Reference the above data is taken from

Appendix B is attached in electronic format as an Excel file.


Afonso-Vargas, J, La Serna-Ramos, I and Arnay-de-la-Rosa, M (2015) ‘Fungal spores located in 18th century human dental calculi in the church “La Concepción” (Tenerife, Canary Islands)’, *Journal of Archaeological Science: Reports*, 2: 106-113


403


Brothwell, DR (1972) Digging Up Bones. London: British Museum (Natural History)


Buck, CE, Cavanagh, WG and Litten, CD (1996) Bayesian Approach to Interpreting Archaeological Data. Chichester: John Wiley and Sons


Budd, P, Montgomery, J, Evans, J and Trickett, M (2004(b)) ‘Human lead exposure in England from approximately 5500 BP to the 16th century AD’, Science of The Total Environment, 318: 45-58


Burkitt, MC (1926) Our Early Ancestors: An Introductory Study of Mesolithic, Neolithic and Copper Age Cultures in Europe and Adjacent Regions. Cambridge: Cambridge University Press


Childe, VG (1940) *Prehistoric Communities of the British Isles*. London: Chambers
Clark, JGD (1939) *Archaeology and Society*. London: Methuen

414


Darwin, C (1868) *The Variation of Plants and Animals Under Domestication*. London: J Murray and Sons


Driver, JC (2011(b)) ‘Twenty years after “Identification, Classification and Zooarchaeology”’, Ethnobiology Letters, 2: 36-39


Evens, J (1872) The ancient stone implements, weapons, and ornaments, of Great Britain. London: Longmans, Green, Reader and Dyke


428


Halcrow, SE, Tayles, N and Buckley, HR (2007) ‘Age estimation of children from prehistoric Southeast Asia: are the dental formation methods used appropriate?’, *Journal of Archaeological Science*, 34: 1158-1168


Halstead, B and Middleton, J (1972) *Bare Bones. An exploration in art and science*. Edinburgh: Oliver and Boyd


Hellewell, E and Milner, N (2011) ‘Burial practices at the Mesolithic-Neolithic transition in Britain: change or continuity?’, Documenta Praehistorica, 38: 61-68


Horizon (2015) *First Britons*, TV, BBC 2, 19th August, 8.00pm


Jackson, DA (1978) ‘Neolithic and Bronze Age Activity in the Harringworth Area’, *Northamptonshire Archaeology Report 13*


Lubbock, J (1865) Pre-historic Times as Illustrated by Ancient Remains, and the Manners and Customs of Modern Savages. Cambridge: Cambridge University Press


Massingham, HJ (1926) Downland Man. London: Jonathan Cape


Milner, N (2005(a)) ‘Can seasonality studies be used to identify sedentism in the past?’ in D Bailey, A Whittle and V Cummings (eds) (Un)Settling the Neolithic, 32-37. Oxford: Oxbow


Moore, WRG and Williams, JH (1975) ‘A Later Neolithic site at Ecton, Northampton’, Northamptonshire Archaeology, 10: 3-30


454


Perschke, R (2013) ‘Winter solstice at the sun observatory – The gap between inviting neopagans to archaeological sites and disliking their presence’, paper given at 19th Annual Meeting of the European Association of Archaeologists (EAA; Pilsen, Czech Republic), ‘Archaeological Sites as Space for Modern Spiritual Practice’ session, 6th September 2013


Piper, S (2014) ‘Mesolithic occupation in the Western Isles of Scotland: Island-hopping to the edge of Europe’, paper presented at Where the Wild Things Are 2.0 Conference (Durham University), 9th January 2014


458
ICP-MS and ICP-MS after Rb/Sr separation’, *Journal of Analytical Spectrometry*, 17: 887-891


Renfrew, AC (1973) ‘Monuments, mobilisation and social organisation in Neolithic Wessex’ in AC


Renfrew, C (1998) ‘All the King’s Horses. Assessing cognitive maps in later prehistoric Europe’ in

corticomedullary index valid to distinguish human from non-human bones: A multislice


milk allergens and their role in clinical events’, *Analytical and Bioanalytical Chemistry*, 395
(1): 47-56

Reynier, MJ (2000) ‘Thatcham Revisited: Spatial and stratigraphical analyses of two sub-
assemblages from site III and its implications for early Mesolithic typo-chronology in
Britain’ in R Young (ed) *Mesolithic Lifeways: Current Research from Britain and Ireland*,
33-46. Leicester: University of Leicester


Richards, M, Harvati, K, Grimes, V, Smith, C, Smith, T, Hublin, J-J, Karkanas, P and
Panagopoulou, E (2008) ‘Strontium isotopes evidence of Neanderthal mobility at the site of
Lakonis, Greece using laser-ablation PIMMS’, *Journal of Archaeological Science*, 35: 1251-
1256

Richards, M, Macaulay, V, Hickey, E, Vega, E, Sykes, B, Guida, V, Rengo, C, Sellitto, D,
Cruciani, F, Kivisild, T, Villems, R, Thomas, M, Rychkov, S, Rychkov, O, Rychkov, Y,
Gölge, M, Dimitrov, D, Hill, E, Bradley, D, Romano, V, Cali, F, Vona, G, Demaine, A,
Papiha, S, Triantaphyllidis, C, Stefanescu, G, Hatina, J, Belledi, M, Di Rienzo, A,
Novelletto, A, Oppenheim, A, Norby, S, Al-Zaheri, N, Santachiara-Benerecetti, S, Scozzari,

*Hambledon Hill, Dorset, England. Excavation and survey of a Neolithic monument complex
and its surrounding landscape*, 522-527. Swindon: English Heritage

Richards, MP (2003) ‘Explaining the dietary isotope evidence for the rapid adoption of the
Neolithic in Britain’ in M Parker Pearson (ed) *Food, Culture and Identity in the Neolithic
and Early Bronze Age*, 31-36. Oxford: Archaeopress

460

Richards, MP and Hedges, REM (1999(a)) ‘Stable isotope Evidence for Similarities in the Types of Marine Foods Used by Late Mesolithic Humans at Sites Along the Atlantic Coast of Europe’, Journal of Archaeological Science, 26: 717-722


Richards, MP, Schulting, RJ and Hedges, REM (2003(b)) ‘Sharp shift in diet at the onset of Neolithic’, Nature, 425: 366


Rodakis, J (2015) ‘An n=1 case report of a child with autism improving on antibiotics and a father’s quest to understand what it may mean’, Microbial Ecology in Health and Disease, 26: 26382


Rowley-Conwy, P (2004(a)) ‘How the West Was Lost: A Reconsideration of Agricultural Origins in Britain, Ireland, and Southern Scandinavia’, *Current Anthropology, 45*: S83-S113


Salazar-García, DC, Richards, MP, Nehlich, O and Henry, AG (2014) ‘Dental calculus is not equivalent to bone collagen for isotope analysis: a comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante, Spain)’, Journal of Archaeological Science, 47: 70-77


Schlueter, J, Elsner, J and Schlumbaum, A (2014) ‘Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding?’, *Scientific Reports*, 4: 5798


Schroeder, H (2013) ‘Recent advances in ancient DNA studies shed light on the population history of prehistoric Europe’, paper given at 19th Annual Meeting of the European Association of Archaeologists (EAA; Pilsen, Czech Republic) ‘Isotopes and aDNA’ session, 6th September 2013


Schulting, RJ and Richards, MP (2002(a)) ‘Finding the coastal Mesolithic in southwest Britain: AMS dates and stable isotope results on human remains from Caldey Island, South Wales’, *Antiquity*, 76: 1011-1025


468


Smith, LW, Baer, PN, King, CT and White, CL (1963) ‘Studies on experimental calculus formation in the rat III: Calculus formation as influenced by high fat, high carbohydrate diets, and sucrose in the drinking water’, Journal of Periodontology, 34: 327-329


471


473


Thomas, J (2004(a)) ‘Comments on Rowley-Conwy: Reconsideration of Agricultural Origins’, Current Anthropology, 45 (supplement): S105-S106

Thomas, J (2004(b)) ‘Current debates on the Mesolithic-Neolithic transition in Britain and Ireland’, Documenta Praehistorica, 31: 113-130


Time Team (2013) ‘Britain’s Stone Age Tsunami: A Time Team Special’, TV programme, Channel 4, 30th May 2013, 8pm


477


Westropp, HM (1872) *Pre-historic Phases; or, Introductory Essays on Pre-historic Archaeology*. London: Bell and Daldy


Williams, R (1983) *Keywords: A vocabulary of culture and society*. London: Fontana Press


483