

Sex Determination and Stable Isotope Analysis of the Nivåfjord Mesolithic Burials, Zealand, Denmark

Kurt J. Gron¹, Harry K. Robson^{1,2,7}, Nicolas A. Stewart³, T. Rowan McLaughlin⁴, Chris Meiklejohn⁵, Peter Rowley-Conwy¹, Ole Lass Jensen⁶ and Janet Montgomery¹

¹ Department of Archaeology, Durham University, South Road, Durham, DH1 3LE, United Kingdom

² BioArCh, Department of Archaeology, University of York, York YO10 5DD, United Kingdom

³ School of Applied Sciences, University of Brighton, Brighton, BN2 4AT, United Kingdom

⁴ Hamilton Institute, Maynooth University, Maynooth, Co. Kildare, Ireland

⁵ University of Winnipeg, Winnipeg, MB, R3B 2E9 Canada

⁶ Museum Nordsjælland, Frederiksgade 9, 3400 Hillerød, Denmark

⁷ Corresponding author (hkrobson@hotmail.co.uk) ORCID: 0000-0002-4850-692X

ABSTRACT

Since 1992 the prehistoric Nivåfjord in northeast Zealand, Denmark, has yielded an appreciable number of inhumation burials and cremations dating to the Mesolithic, especially the sites of Nivå 10 and Nivågård. Unfortunately, the micro-region is characterised by poor organic preservation, restricting the successful application of biomolecular techniques to human remains, including large-scale radiocarbon dating programmes as well as both stable isotope and ancient DNA analyses. Here, we apply an alternative technique, an acid etch peptide-based method, to determine the sex of eight individuals from Nivå 10 as well as the Nivågård child. Moreover, we revisit the utility of stable carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and sulfur ($\delta^{34}\text{S}$) isotope analysis of human tissues to reconstruct the life histories and diets of 10 individuals from Nivå 10 as well as the Nivågård child. To contextualise further, we sampled 14 *Capreolus capreolus* and three *Sus scrofa* from the Nivågård site for stable isotope analysis. We demonstrate that sex can successfully be determined from contexts susceptible to poor organic preservation, and show that the Nivågård child spent a proportion of its life outside a sea spray-influenced environment, and consumed significant quantities of marine protein as demonstrated by its $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values. By applying novel analytical methods, a wealth of information can both be gleaned from older collections as well as from sites with poorer conditions for organic preservation.

ARTICLE HISTORY

Received

25 April 2023;

Accepted

15 December 2023

KEYWORDS

Denmark; Middle Mesolithic; Late Mesolithic; Proteomic analysis; Stable isotope analysis; Human remains

Introduction

The prehistoric Nivåfjord is located *c.*30 km north of present-day Copenhagen (Figure 1). Here *c.*24 archaeological sites dating to the Stone Age have been recorded in a now-infilled inlet (see Lass Jensen 2001, 2003, 2009, 2016). Of these, two are central to this study, Nivå 10 and Nivågård. These date from the Middle Mesolithic Kongemose (*c.*6400–5400 cal BC) to the Late Mesolithic Ertebølle (*c.*5400–4000 cal BC) cultures, and were likely used for short-term occupations sporadically over this period (see Absolute Dating). Less than 10 km north of the world-famous Vedbæk complex of sites with their multiple Mesolithic burials (see Brinch Petersen 2015), the Nivåfjord sites have also yielded an appreciable

number of inhumation burials and cremations (Lass Jensen 2016).

At Nivå 10, a total of 15 individuals have been discovered, represented by nine inhumation burials and three cremations. Added to this are ‘a few loose human bones from two or three individuals’ (Lass Jensen 2016, 98). All are characterised by poor organic preservation or were cremated, and previous attempts utilising biomolecular techniques, such as radiocarbon dating, stable isotope and ancient DNA (hereafter aDNA) analyses, have largely been unsuccessful (though see Rasmussen et al. 2009; Table 1). In comparison, the single Nivågård burial has much better organic preservation. Since the two sites are separated by *c.*100 m and broadly date to the same period,



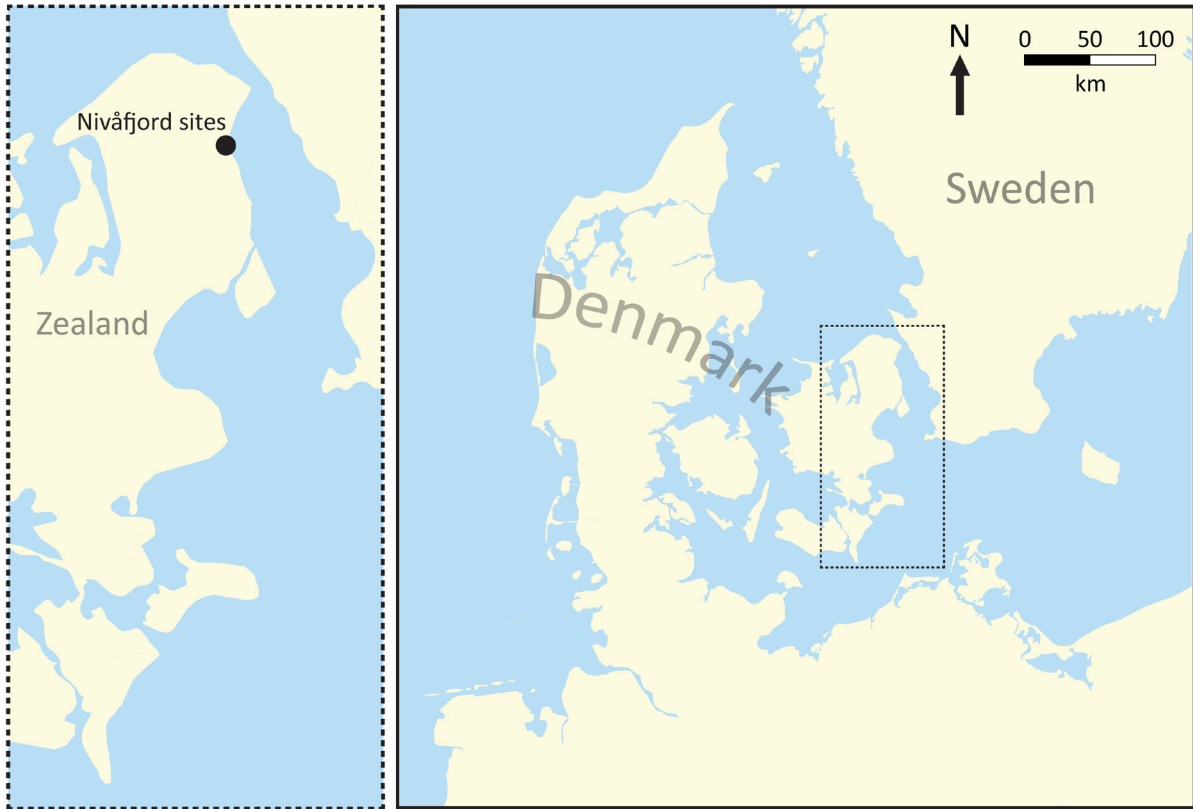


Figure 1. The locations of the Nivåfjord sites (Nivå 10 and Nivågård) on Zealand, Denmark (made with Natural Earth, provider of free vector and raster map data at naturalearthdata.com).

a combined analytical approach was undertaken to reconstruct Mesolithic life and death around prehistoric Nivåfjord.

We undertook a biomolecular study, including stable isotope and proteomic analyses of the human remains from Nivå 10 as well as human and faunal remains from Nivågård. The purpose of these analyses was multi-fold. Firstly, we aimed to determine the sex of as many of the individuals as possible, and in particular the non-adult individuals for which morphology-based sex determination is seldom reliable. Secondly, we aimed to obtain both dentine and bone collagen stable isotope data from as many of the human burials as possible in order to investigate the individuals' life histories as well as diet and other aspects of their lives. Finally, we aimed to contextualise the above data through the bone collagen stable isotope analysis of faunal remains in order to investigate the local environment, mobility, and other factors.

The Sites

Nivå 10 and Nivågård are just two of *c.*24 Stone Age sites located around what was formerly a multi-branched inlet on the northeast coast of Zealand, Denmark (Lass Jensen 2003), *c.*30 km north of present-day central Copenhagen. The inlet was formed by rising sea levels during the Atlantic chronozone (e.g. Christensen 1995), and would have been an economically productive environment for coastal hunter-gatherer-fishers (e.g. Paludan-Müller 1978). Due to the heightened sea level during the Stone Age, settlements were placed on higher ground within the landscape, a landscape that is and has since been threatened by modern agricultural practices (Lass Jensen 2003). Consequently, excavations were initiated in the 1990s, continuing into the 2000s, to investigate the character of the Stone Age settlements along the palaeoinlet before evidence of prehistoric activity was lost (Lass Jensen 2016).

Nivå 10 appears to have been located on a small island on the southernmost margin of the mouth



Figure 2. Several of the individuals sampled in this study. A: The Nivågård child (Photo: by Povl Merløe). B: The Nivågård child at exhibition (Photo: Museum Nordsjælland). C: Inhumation burial (Grave No. A161) at Nivå 10, including the remains of an adolescent/young adult female (Individual M) (Photo: Museum Nordsjælland). D: Inhumation burial (Grave No. A122) at Nivå 10, including the remains of a young middle/old middle adult (Individual L) (Photo: Museum Nordsjælland). E: Inhumation burial (Grave No. A124) at Nivå 10, including the remains of a young/young middle adult male (Individual K) (Photo: Museum Nordsjælland). F: Double inhumation burial (Grave No. A129) at Nivå 10, including the remains of a young/young middle adult male (Individual H) and a child (Individual O) (Photo: Arnold Mikkelsen). G: Double inhumation burial (Grave No. A151) at Nivå 10, including the remains of a young/young middle adult male (Individual F) and a young middle/old middle adult female (Individual G) (Photo: Arnold Mikkelsen). H: Inhumation burial (Grave No. A162) at Nivå 10, including the remains of an old middle/mature adult (Individual N) (Photo: Museum Nordsjælland).

of the inlet (Lass Jensen 2016). The site was in use from 6000 until *c.*4800 cal BC (see Absolute Dating), corresponding to the earliest (Blak phase) Kongemose to the early (Trylleskov phase) Ertebølle (Sørensen 2017; Vang Petersen 1984). Nivå 10 was a settlement, evidenced by four dwellings, two dated to the Kongemose and two to the Ertebølle (Lass Jensen 2003, 2009, 2016). Excavations have yielded one of the more convincing examples of an Ertebølle dwelling throughout southern Scandinavia, a sunken feature with clearly defined activity areas (Lass Jensen 2001). With-

in the settlement area, 12 burials containing 15 individuals were discovered, including three cremations (Lass Jensen 2016). Since the burials were associated with the dwellings, and were located within the settlement, they probably do not represent a formal burial ground, very similar to the pattern at Vedbæk-Bøgebakken (see e.g. Meiklejohn et al. 1998). Their placement also likely indicates that they are not contemporaneous, but instead reflects the duration of occupation represented by the dwellings. Moreover, there is some evidence for the deliberate disturbance of

one of the inhumation burials in prehistory, while the placement of human remains within one dwelling potentially served a ritual purpose (Lass Jensen 2009). Three of the inhumation burials contained more than one individual (e.g. Figure 2). A summary of the inhumation burials and cremations is provided in Table 1 (see Supplementary Material).

The second site, Nivågård, is *c.*100 m to the west of Nivå 10 (Lass Jensen 2001; Rasmussen et al. 2009), and is one of the very few Mesolithic sites from eastern Denmark with deposits of marine shells. Despite this, it is not regarded as a kitchen midden *sensu stricto* (Lass Jensen and Møller Hansen 1998). The site is considerably larger than Nivå 10 (Rasmussen et al. 2009). The preservation of organic remains on sites with depositions of shells is typically excellent, likely because the shells act as a buffer against acidic soils (e.g. Gron et al. 2015). During the course of excavations, an inhumation burial containing a single well-preserved skeleton of a 5 to 8 year-old child was discovered (Alexandersen et al. 1998; Lass Jensen and Møller Hansen 1998).

Comment is needed on the Nivågård child and its identification, especially related to the two initial descriptions of the find (Alexandersen et al. 1998; Lass Jensen and Møller Hansen 1998). The second of these provides an overview of the cultural context and associated archaeology, the first a limited description of the find, focusing most clearly on the teeth and sex of the child. Lass Jensen and Møller Hansen (1998) briefly mention a 5-year-old male child with a stature of *c.*95 cm, and imply that both dental and postcranial data support this conclusion, though with the caveat that the gender is difficult to determine. Alexandersen et al. (1998) focus more tightly on the skeletal material, though primary focus is on the teeth. Identification of the child as male is described as possible. Determination of the age as 5 years old is clearly framed in understanding that ‘teeth are generally more sensitive age indicators than bones’ (Alexandersen et al. 1998, 27; ‘tænder generelt er mere følsomme aldersindikatorer end knogler’). However, it is also made clear that osteological markers involving fusion of the skull, the vertebrae and the ischial and pubic bones of the pelvis give an age range

of 6 to 7, while unpublished analysis undertaken since identified an ossified navicular bone of the left hand, with full ossification seldom occurring before the age of 8. The mix therefore suggests an age range of 5 to 8 years, with closer accuracy being problematic. It also needs to be noted that the sex suggested by Lass Jensen and Møller Hansen (1998) assumes the accuracy of the age determination of 5. If the older assessment is correct, it becomes quite possible that the child is small for its age. There is no simple solution for this conundrum.

Additional comment is required on identifying the child’s remains as male, and the broader issue of the accuracy of determining the sex of sub-adult individuals from the analysis of skeletal remains alone. The answer seems to be that though differences can be detected if large sample sizes are studied, the accuracy of assessment for individual skeletons remains low, with the probability of a successful assessment generally <70%, consistently less than half the probability of accurate assessment in adult material (Byers 2008). In addition, there are differences in accuracy dependent on the population under study. Applied to the issue of assessing sex in Mesolithic Danish children, the low number of well-preserved remains makes any comparisons of minimal value. Current opinion suggests that the only markers of value are those involving pelvic details, the auricular surface of the ilium and depth of the sciatic notch (Byers 2008). In the case of the Nivågård child the initial assessment as male was based on more general features, not accepted under present standards. Though not considered in the initial 1998 publications, the final identification of the sex of the child (see below) affirms the degree that osteological identification of sub-adult age is problematic.

Finally, we wish to clarify use of the term child in identifying the Nivågård sub-adult. We do so since the terms juvenile and adolescent have also been used by some for individuals in the age range of the sub-adult, 5 to 8 years of age. In this study we follow a slightly modified version of the standards of Buikstra and Ubelaker (1994), with children identified as aged 1 to 12 years, older than infants (0-1 year) and younger than adolescents

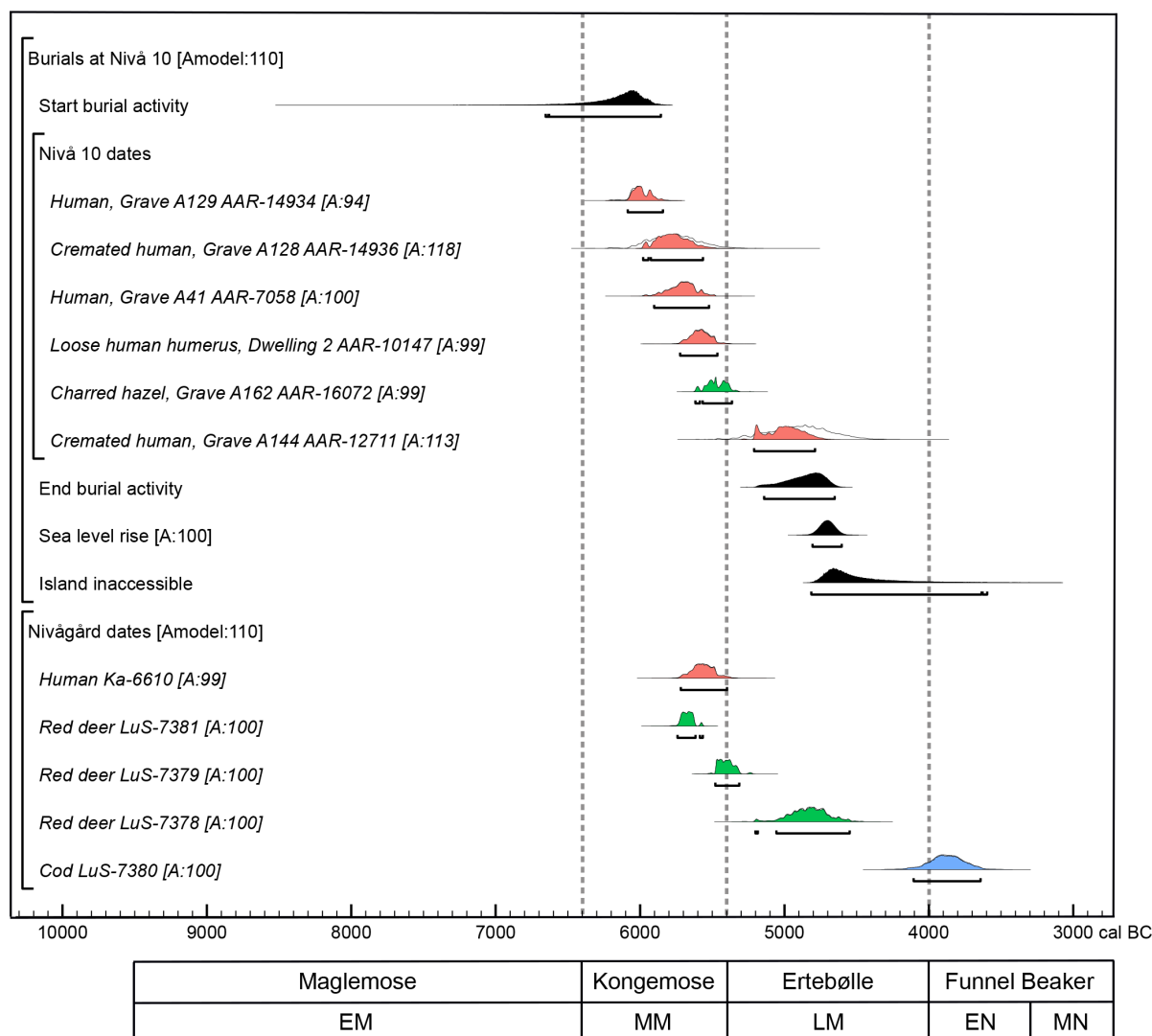


Figure 3. OxCal model output of ^{14}C dates from both Nivå 10 and Nivågård, as shown in Table 2. The chronological order of individual samples is sorted by estimated calendar date (full OxCal CQL code is provided in the Supplementary Material). Legend: red – use of a mixed calibration curve, including a ΔR value of -234 ± 61 ^{14}C years (Fischer and Olsen 2021; see text); green – use of the IntCal20.14c calibration curve (Reimer et al. 2020); blue – use of the Marine20.14c curve (Heaton et al. 2020), including a ΔR value of -234 ± 61 ^{14}C years (Fischer and Olsen 2021); EM – Early Mesolithic; MM – Middle Mesolithic; LM – Late Mesolithic; EN – Early Neolithic; MN – Middle Neolithic.

(12 to 18 years); the term juvenile is not used by Buikstra and Ubelaker (1994). These ranges and descriptive terms are also similar to that given by Lewis (2011) and were also used by Gron et al. (2022) in identifying children's remains at the Late Mesolithic mass burial site of Strøby Egede, Zealand, Denmark.

Absolute Dating

To refine the sites' chronologies, a total of 11 legacy radiocarbon ^{14}C dates (Enghoff 2011; Lass Jensen 2009, 2016; Rasmussen 1998) were modelled in

OxCal (Figure 3). The six dates from Nivå 10 were obtained from five human remains and a piece of charred wood (*Corylus* sp.), which was recovered from between the femurs of Individual N in Grave No. A162, while the five from Nivågård were obtained from the inhumation burial as well as the remains of three red deer (*Cervus elaphus*) and one Atlantic cod (*Gadus morhua*) from settlement layers (see Table 2 in the Supplementary Material for further information).

OxCal v.4.4.4. was used to implement the model (Bronk Ramsey 2009). The ^{14}C ages of the human remains were calibrated using OxCal's Mix_Curves

function (i.e. IntCal20.14c and Marine20.14c (Heaton et al. 2020; Reimer et al. 2020)), including a ΔR value of -234 ± 61 ^{14}C years (Fischer and Olsen 2021), taking into consideration the measured $\delta^{13}\text{C}$ values (when present) for specifying the proportion of each curve. The $\delta^{13}\text{C}$ values were used to determine the proportion of marine protein in the diets of the humans by applying the linear $\delta^{13}\text{C}$ model described by Arneborg et al. (1999) and applied by Fischer et al. (2007). We used a $\delta^{13}\text{C}$ value of -10.1‰ for a 100% marine diet and -21.7‰ for an entirely terrestrial diet. Cremations were calibrated using a mixture of the IntCal20.14c and Marine20.14c curves in an unknown ratio. In contrast, the ^{14}C ages of the charred wood and red deer were calibrated using the Northern Hemisphere atmospheric calibration curve, IntCal20.14c (Reimer et al. 2020), while the ^{14}C age of the Atlantic cod bone was calibrated using the same function and ΔR value as given above for the human remains. All reported calibrated date ranges have been rounded up or down to the nearest 10; for details of the OxCal model definition, see the Supplementary Material.

The modelled data are all reported at 95.4% confidence. The earliest inhumation burial at Nivå 10 dates from the Blak to Villingebæk phases of the Kongemose (Grave No. A129; Individual H; AAR-14934; 7265 ± 38 BP; 6080-5840 cal BC). Then, the cremation burial (Grave No. A128; AAR-14936; 7035 ± 35 BP; 5980-5560 cal BC) as well as deposition of Individual A (Grave No. A41; AAR-7058; 6900 ± 60 BP; 5900-5520 cal BC) and the loose humerus from within Dwelling 2 (AAR-10147; 6868 ± 46 BP; 5720-5460 cal BC) took place during the Villingebæk to Vedbæk phases of the Kongemose. A charred piece of hazel wood recovered from Grave No. A162 dates Individual N to the boundary between the Vedbæk phase of the Kongemose and the Trylleskov phase of the Ertebølle (AAR-16072; 6518 ± 60 BP; 5620-5360 cal BC). After an apparent hiatus of at least a century, a further cremation took place during the Trylleskov phase of the Ertebølle (Grave No. A144; AAR-12711; 6154 ± 45 BP; 5210-4790 cal BC). The calibrated date range for this sample has been constrained in our model by sea level rise, which would have made the island inac-

cessible from *c.*4700 cal BC onwards. Corroborating this is a lack of archaeological evidence, for instance flake axes or ceramics, demonstrating occupation during the Stationsvej or Ålekistebro phases of the Ertebølle, and it seems likely the settlement was abandoned prior to *c.*4700 cal BC.

At Nivågård, the child (Ka-6610; 6845 ± 65 BP) was buried between 5720-5400 cal BC, overlapping with several of the Nivå 10 inhumation and cremation burials on the boundary between the Villingebæk and Vedbæk phases of the Kongemose. The site, however, appears to have been sporadically used, probably for short-term occupations, as demonstrated by three red deer bones recovered from settlement layers that date to 5740-5570 cal BC (LuS-7381; 6770 ± 50 BP), 5480-5310 cal BC (LuS-7379; 6435 ± 50 BP) and 5200-4550 cal BC (LuS-7378; 5940 ± 100 BP), the Villingebæk-Vedbæk boundary of the Kongemose, the Kongemose-Ertebølle boundary and the Trylleskov-Stationsvej boundary of the Ertebølle respectively. Based on the date obtained from an Atlantic cod bone (LuS-7380; 5400 ± 50 BP; 4100-3640 cal BC), Nivågård was visited around the time of the Neolithic transition.

Sex Identification from Skeletal Remains – some comments

Before proceeding to peptide sex determination, comment is needed on why and whether such approaches can take precedence over direct observation of skeletal remains. We focus here on determining sex in adults and on southern Scandinavian Mesolithic human remains. Determination of sex in sub-adults from simple observation is much less accurate. Determining sex in adults is primarily based on growth patterns in later adolescence and early adulthood, features that are not clearly developed in sub-adults, especially children, and especially in the pelvis, the most accurate region of the skeleton for sex identification.

Determining sex in reasonably complete adult skeletal material is often seen as straightforward, and in many cases is just that. However, this cannot always be assumed, as patterns seen in

material from one region do not always apply equally to other regions, including sexual dimorphism. As well, some individuals show non-diagnostic patterns and identifying individuals falling into such categories is not always obvious. Errors in sex identification in reasonably complete skeletons range between five and 15 percent in samples of significant size, in part reflecting sexual dimorphism or its absence. Presence of reasonably complete pelvises is critical, as differences in this region are largely sex dependent, whereas differences in other regions reflect both sex and size. An example is seen in Figures 2A and 2B, of the Nivågård child, which show no features that are sex as opposed to age related. Figures 2D through 2H are of Nivå adults, with Figures 2D and 2E (Grave Nos. A122 and A124) showing individuals with little to no visible morphological features. Figures 2F, 2G and 2H (Grave Nos. A129, A151 and A162) are in various states of completion and preservation and all three show pelvic remains, though in poor (Figure 2H) and medium condition (Figures 2F and 2G). As shown in Figure 2, none are sufficiently preserved to allow accurate sex determination with strong confidence. That this is not site specific can be seen in two cases from the Vedbæk-Bøgebakken Mesolithic series, with 16 individuals that are fully adult. Of these, four or a quarter of the total number of adults, have sex identifications marked with a question. The type of problems that occur can be seen in two of the better-known burials, 19 and 22. The simpler is burial 22, one of the best preserved at Bøgebakken, a 40- to 60-year-old female. Diagnosis from the pelvis is clear, with all regular features scoreable and providing an overall score of -1.94 out of a maximum possible of -2.0. However, if the pelvis was absent the other clearest diagnostic would be cranial, with a score of +0.47/+2.0, almost fully in the expected male range. A more complex case is the triple burial 19, with two adults, 19A and 19C, and a child, 19B. Widely described in the literature as a male, a female and their child, the original description (Albrethsen and Brinch Petersen 1976, 14) states that ‘... sex of the skeletons ... cannot be established on anthropological criteria with any certainty’, though both adults are reasonably complete. However, both adults have incomplete pelvises, with sex primarily based on secondary

features, 19A, largely identified as male, in part due to a bone point lodged in the thoracic vertebrae, while 19C, identified as female has associated grave goods compatible with such a diagnosis. However, closer examination shows several discrepancies. Besides being younger, 19A is at the lower end of stature for males at the site, and the cranial composite score is -1.11/2.0 with all markers in the female range. The composite score for 19C is -0.45 with one feature at +3, a robust glabella, usually seen as a male marker. 19C, despite the associated grave goods, is the more robust, and 19A is apparently female, despite possibly being murdered. As suggested by Meiklejohn et al. (2000, 228; see also Tilley 1996, 39) it is ‘... conceivable that the burial consists of two females, if both the gendered identification and the normative pattern of robusticity are correct’. Clearly, identification of sex using simple observation and association with non-biological features has potential for error.

Peptide Sex Determination

Determination of sex of human remains can be performed on the basis of morphology, metrics, and aDNA. However, it is not always possible to apply these methods, and the latter relies on sufficient organic preservation, is destructive, and can be time consuming and expensive (Stewart et al. 2017). Fortunately, an acid etch peptide-based method for determination of sex on the basis of sexually-dimorphic chromosomally-linked amelogenin peptides in tooth enamel can accurately determine sex in a less-destructive way and one that works despite poor organic preservation and on sub-adult remains (Gowland et al. 2021; Stewart et al. 2017).

In order to determine the sex of the individuals from Nivå 10 we applied the method described by Stewart et al. (2017). Given the poor preservation, including the presence of cremations, it was not possible to sample every individual, while some were not available for analysis as they were on museum display. In total, eight individuals from Nivå 10 were sampled (Table 1, see Supplementary Material). Furthermore, we sampled the permanent mandibular central (R) incisor of the

Nivågård child. Upon inspection under a microscope it was discovered that a consolidant had been applied to the tooth (unbeknown to the curator), which was subsequently removed using an acetone-soaked cotton swab.

Stable Isotope Analysis

Nivå 10

Not all skeletal remains from Nivå 10 were available, accessible, and appropriate for stable isotope analysis. In total, nine bone and dentine samples were chosen, deriving from six individuals (see Supplementary Material, Table 3). For three individuals, both a tooth and a bone sample were selected in order to determine life-history differences between early life and the period preceding their deaths. Although radiocarbon dating had previously demonstrated that collagen preservation was poor (i.e. insufficient quantities of collagen for measurement, see Table 1; Lass Jensen 2009), the analyses were undertaken since tooth dentine is denser than bone collagen. Elsewhere, collagen has been successfully extracted from tooth dentine when bone collagen extraction has failed. The initial goal was to sample tooth dentine sequentially for the determination of a sequence through a restricted period in several individuals' lives (e.g. Beaumont et al. 2013). However, during demineralisation (see Results) the dentine samples lost their structural integrity and essentially 'melted' into sludge. The decision was therefore made to treat this sludge as a sample.

The bone and tooth samples were first cleaned of obvious surface contamination using a high-speed tungsten-tipped dental drill. For the teeth, where practicable, the remaining enamel was removed from the crown using a diamond-tipped dental saw. Peptide etching took place prior to stable isotope analysis and the results were confirmed before demineralisation. Samples were prepared using a modified Longin (1971) method (Ambrose and DeNiro 1986; O'Connell and Hedges 1999; DeNiro 1985) in the Stable Isotope Laboratory within the Department of Archaeology at Durham University. Once extracted, dissolved collagen was freeze-dried and measured by Iso-Analytical Ltd.

(Crewe, Cheshire, UK) for their carbon, nitrogen and sulfur isotope ratios. The samples were analysed using a Europa Scientific EA coupled to a Europa Scientific 20-20 IRMS. Due to the generally larger quantities of collagen required for sulfur measurements, only samples with sufficient collagen yields and acceptable atomic C:N ratios were measured.

Nivågård

To contextualise further, 17 faunal remains from Nivågård were selected for stable isotope analysis. These are listed in Table 4 (see Supplementary Material) and included 14 roe deer (*Capreolus capreolus*) and three wild boar (*Sus scrofa*) bones. Each specimen derived from a different individual. An MNI (Minimum Number of Individuals) based sampling strategy was undertaken using a manual overlap method. A total of 14 roe deer left distal humeri, and three wild boar right calcanei were selected. The samples were prepared and analysed in the same manner as the human samples from Nivå 10.

Results: Nivå 10

Tooth Etch Sex Determination

The teeth from Nivå 10 were very poorly preserved and, in addition, in some cases only a very thin sliver (<1 mm) of crown enamel remained with the majority of the occlusal chewing surface worn down through attrition. In comparison, the tooth from the Nivågård child was in excellent condition. In total, nine individuals from the two sites were etched to determine their sex. Of these, two etches produced no signal but clear interpretable spectra were obtained for seven: three females and four males were identified (Table 5, see Supplementary Material).

Stable Isotope Analysis

In total, nine bone collagen and dentine samples from Nivå 10 were subjected to stable carbon and nitrogen isotope analysis. Sample and collagen weights, collagen yields, and stable isotope data, including atomic C:N ratios are provided in Table 6

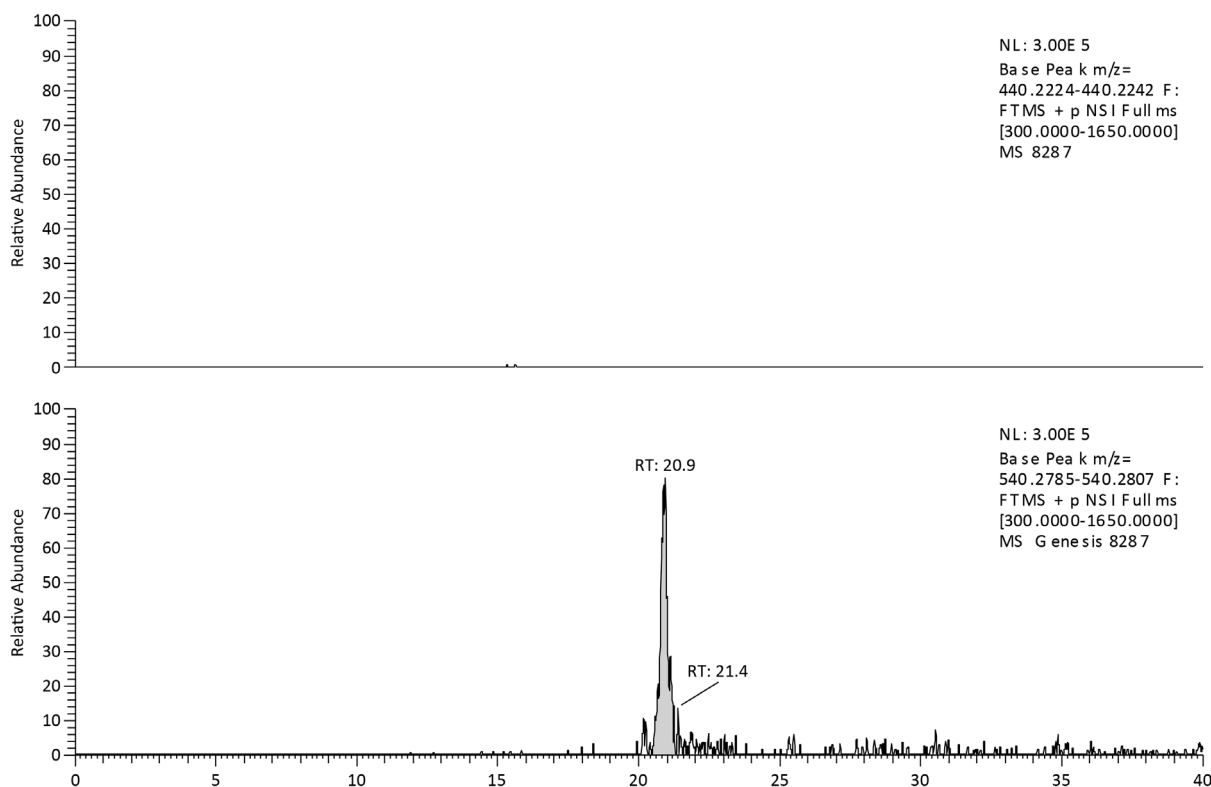


Figure 4. Reconstructed ion chromatograms at 10 ppm mass accuracy for the m/z of 440.2224 and 540.2785 for the peptides SM(ox)IRPPY ($[M+2H]^{+2}$, AmelY) (top) and SIRPPYPSY ($[M+2H]^{+2}$, AmelX) (bottom) respectively from the Nivågård child (Sample No. 8287). Since the AmelX isoform was only present, it is deduced that the individual's sex was female.

(see Supplementary Material). The collagen proved to be of low quality, indicating a higher likelihood of diagenesis. Collagen yields were either too low for measurement or the atomic C:N ratios were unacceptable according to the criteria set out by DeNiro (1985), i.e. outside of the range of 2.9-3.6. With one exception (Sample 1160/22), the samples also had insufficient quantities of nitrogen (%N) to permit nitrogen measurements. For these reasons, no acceptable stable isotope data was obtained from the samples from Nivå 10. Consequently, these results will not be discussed further.

Results: The Nivågård Child and Fauna

Tooth Etch Sex Determination

Previous assessments of the Nivågård child's sex, discussed above, assigned it as male (in Danish *dreng*, boy) (Alexandersen et al. 1998). Although peptide recovery was problematic for this sample, on the fifth attempt sufficient peptides were recovered for sex identification of female (Figure 4).

Stable Isotope Analysis

Stable carbon, nitrogen and sulfur isotope analysis was undertaken on a small fragment of cranium from the Nivågård child in addition to the faunal samples from the site. Sample information and stable isotope data are provided in Table 7 (see Supplementary Material). Analytical error for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was less than $\pm 0.2\text{‰}$ and less than or equal to $\pm 0.2\text{‰}$ respectively. For $\delta^{34}\text{S}$, analytical error was less than $\pm 0.3\text{‰}$. The explicit goal of the analysis of the Nivågård child was to obtain a $\delta^{34}\text{S}$ value, which can yield information concerning residency when compared with faunal $\delta^{34}\text{S}$ compositions. A single 480.72 mg sample produced 40.13 mg of collagen, and a collagen yield of 8.3%. Atomic C:N, C:S, and N:S ratios were all within the acceptable ranges of 2.9-3.6 (atomic C:N ratio), 600 ± 300 (atomic C:S ratio) and 200 ± 100 (atomic N:S ratio) proposed by DeNiro (1985) and Nehlich and Richards (2009), indicating no sign of diagenesis. Eleven of the roe deer specimens yielded sufficient quantities of collagen for stable carbon and nitrogen isotope analysis (Table 7, see Supple-

mentary Material) and of those, four were analysed for their $\delta^{34}\text{S}$ ratios. Two of the three wild boar specimens had unacceptable atomic C:N ratios (DeNiro 1985; Table 7) and are not considered further, while the remaining wild boar sample yielded enough collagen to measure all three isotope ratios. The 11 roe deer specimens had mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of -22.7‰ (SD = 0.89‰) and 4.0‰ (SD = 0.92‰) respectively, while the four roe deer specimens had a mean $\delta^{34}\text{S}$ value of 9.0‰ (SD = 2.48‰).

Discussion

The sex of seven individuals from Nivå 10 and Nivågård was determined. In all cases with previous determinations on a morphological basis, these agreed with the enamel peptide determination (Table 5). Two individuals that previously did not have securely assigned sex, Individual L (Grave No. A122) from Nivå 10 and the Nivågård child, were both identified as female based on enamel peptides.

The $\delta^{13}\text{C}$ values of the Nivågård herbivores indicated residency in both open and closed environments (e.g. Gron and Rowley-Conwy 2017; Figures 5A and 5B), and were likely hunted from a range of habitats. The wild boar has the highest $\delta^{13}\text{C}$ value among the terrestrial fauna, probably representing an individual living in an open environment with a partly omnivorous diet (Masseti 2007). For all taxa, the $\delta^{15}\text{N}$ values are typical for southern Scandinavian Mesolithic fauna (Gron and Rowley-Conwy 2017).

In comparison, fewer $\delta^{34}\text{S}$ values were obtained (Figure 5B; Table 7). Despite the broad range ($+5.8\text{‰}$ to $+11.7\text{‰}$), the $\delta^{34}\text{S}$ values reflect terrestrial diets (below $c.+12\text{‰}$) according to Nehlich (2015). However, the roe deer $\delta^{34}\text{S}$ values ($+5.8\text{‰}$ to $+11.7\text{‰}$) likely indicate that they resided in a number of locations, ranging from areas likely affected by sea spray and marine precipitation (marine sulfates) to saltmarshes (sulfide-derived) (Guiry et al. 2021a; Lamb et al. 2023; McArdle et al. 1998). Similarly, the wild boar probably used saltmarshes based on its $\delta^{34}\text{S}$ value of $+7.2\text{‰}$. In comparison, the Nivågård child is likely to have consumed animals that were sulfide-derived

(Guiry et al. 2021a), such as fish that live in seagrass meadows (Guiry et al. 2021b).

Given the slow collagen turnover rates in cranial bones (Fahy et al. 2017), the isotope values of the Nivågård girl should encompass the entirety of her life. Her $\delta^{13}\text{C}$ value of -13.8‰ (Figure 5A) indicates consumption of marine-derived protein, reflecting the absence of C_4 plants in Mesolithic Northern Europe (see Fischer et al. 2007). Moreover, the $\delta^{13}\text{C}$ value is similar to one obtained previously (-13.5‰ , Rasmussen 1998). Indeed, the consumption of large quantities of marine protein was commonplace during the Late Mesolithic of southern Scandinavia. When compared with contemporary individuals from the region (Fischer et al. 2007), the Nivågård girl seems to have enjoyed a similar diet.

Although the $\delta^{34}\text{S}$ value of 6.9‰ of the Nivågård child (Figure 5B) is somewhat at odds with the high marine protein diet demonstrated by the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, it likely indicates the consumption of sulfide-derived resources (Guiry et al. 2021a; Guiry et al. 2021b). Further stable sulfur isotope analysis of human and faunal remains from throughout the region is required to identify the drivers behind higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and lower $\delta^{34}\text{S}$ values.

Conclusions

A primary goal of archaeological research is to investigate past life-ways. The development of new methods allows even poorly preserved archaeological remains the potential to yield new lines of evidence, enriching our understanding of ancient economic and social systems. Nivå 10 falls into this category. Despite the lack of sufficiently well preserved bone collagen, our proteomic investigations of the human remains have confirmed the sex of several of the individuals and assigned to the same to a further individual, which had not previously been assigned a sex.

At nearby Nivågård, better-preserved bone collagen of both the inhumation burial of the child and the faunal remains has yielded crucial stable isotopic data. The presence of fauna, probably

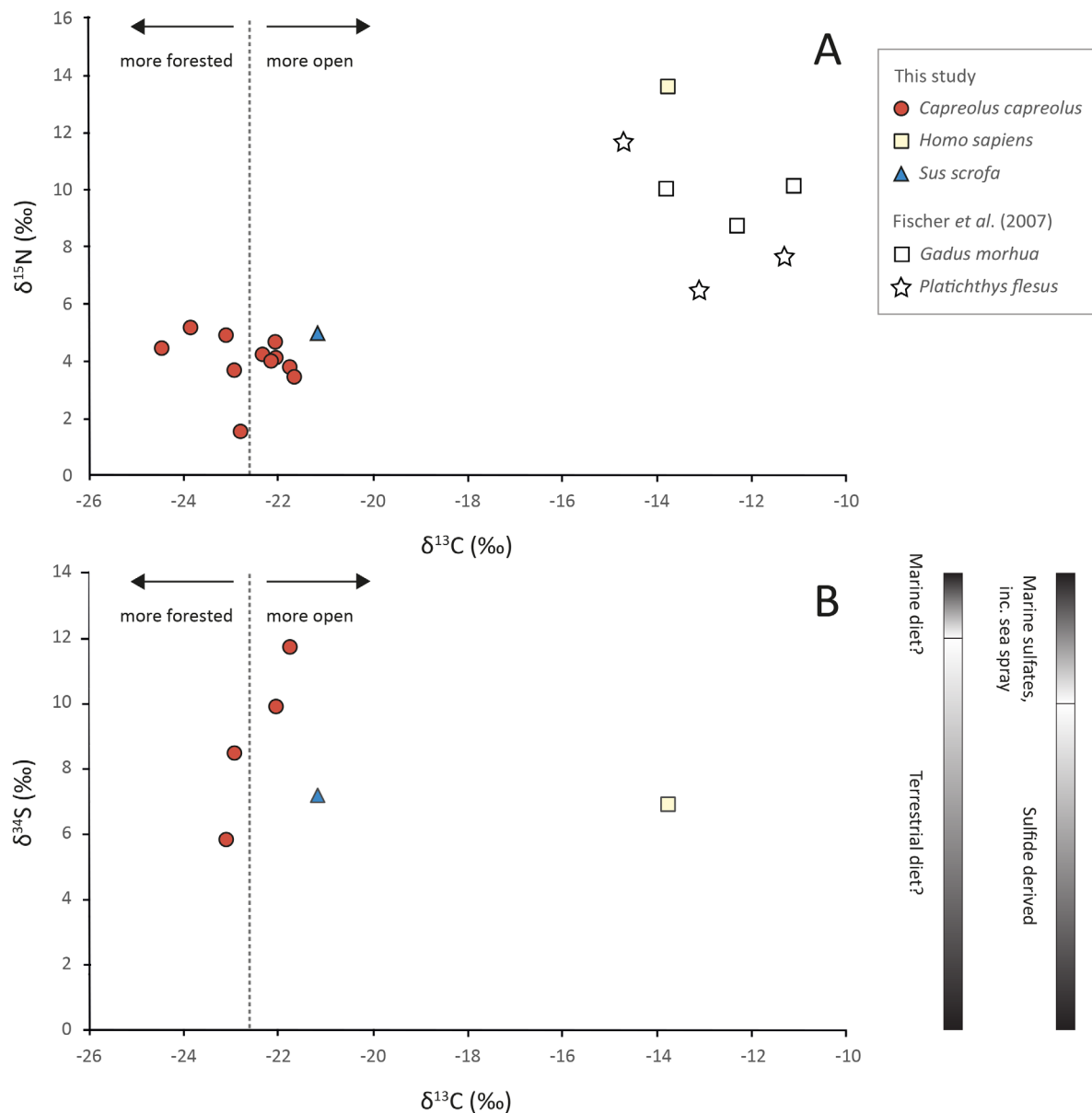


Figure 5. Bone collagen isotope data from Nivågård. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data obtained from Atlantic cod and European flounder bone collagen are also included for comparison (see Fischer et al. 2007; Table 7, Supplementary Material). A: Plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. B: Plot of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values.

originating from several places and a range of environments, speaks to the richness of the local resource base, while the $\delta^{34}\text{S}$ values illustrate that they were exploited from both sea spray-affected areas and saltmarshes. The disconnect between a high marine protein diet, demonstrated by the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, but absence of a marine $\delta^{34}\text{S}$ value, often cited as being derived from consumed protein, warrants further investigation. In determining the sex of the Nivågård child we hope to have returned even a small part of her identity to them and demonstrated the utility and value of revisiting older collections with novel analytical methods.

Acknowledgements

We thank Kristen Hopper for assistance with Figure 1. H.K.R. thanks Malin Holst for help with age classes and Eric Guiry regarding interpretation of $\delta^{34}\text{S}$ values. We also thank Pernille Bangsgaard, Gitte Gotfredsen, Darren Gröcke, and Lasse Sørensen. Lastly, we thank the two anonymous reviewers whose comments greatly improved the quality of the article.

Funding Details

This work was supported by the Leverhulme Trust under Grant RPG-2019-253. H.K.R. acknowledges the funding from the European Research Council (ERC) under the European Union's

Horizon 2020 research and innovation programme (grant agreement No 856488- ERC Synergy project "SEACHANGE: Quantifying the impact of major cultural transitions on marine ecosystem functioning and biodiversity") during the finalisation of this manuscript.

References

- Albrethsen, S.-E. and Brinch Petersen, E., 1976. Excavation of a Mesolithic Cemetery at Vedbæk, Denmark. *Acta Archaeologica* 47, 1-28.
- Alexandersen, V., Meiklejohn, C. and Brinch Petersen, E., 1998. Nivågård-barnet. *Hørsholm Egns Museum Årbog* 1998, 22-28.
- Ambrose, S.H. and DeNiro, M.J., 1986. Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios. *Nature* 319, 321-324. <https://doi.org/10.1038/319321a0>
- Arneborg, J., Heinemeier, J., Lynnerup, N., Nielsen, H.L., Rud, N. and Sveinbjörnsdóttir, Á.E., 1999. Change of diet of the Greenland Vikings determined from stable carbon isotope analysis and ^{14}C dating of their bones. *Radiocarbon* 41(2), 157-168. <https://doi.org/10.1017/S0033822200019512>
- Beaumont, J., Gledhill, A., Lee-Thorp, J. and Montgomery, J., 2013. Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. *Archaeometry* 55(2), 277-295. <https://doi.org/10.1111/j.1475-4754.2012.00682.x>
- Brinch Petersen, E., 2015. Diversity of Mesolithic Vedbæk. *Acta Archaeologica* 86, 1-202. <https://doi.org/10.1111/j.1600-0390.2015.12048.x>
- Bronk Ramsey, C., 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon* 51(1), 337-360. <https://doi.org/10.1017/S0033822200033865>
- Buikstra, J.E. and Ubelaker, D.H., 1994. *Standards for data collection from human skeletal remains*. Fayetteville: Arkansas Archaeological Survey (*Arkansas Archeological Survey Research Series* 44).
- Byers, S.N., 2008. *Introduction to forensic anthropology, 3rd edition*. Boston: Pearson Education.
- Chrisensen, C., 1995. The Littorina transgressions in Denmark. In: A. Fischer, ed. *Man and sea in the Mesolithic*. Oxford: Oxbow Books, 15-22.
- DeNiro, M.J., 1985. Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317, 806-809. <https://doi.org/10.1038/317806a0>
- Enghoff, I.B., 2011. Regionality and biotope exploitation in Danish Ertebølle and adjoining periods. *Scientia Danica. Series B. Biologica*. Vol. 1. København: Det Kongelige Danske Videnskabernes Selskab.

- Fahy, G.E., Deter, C., Pitfield, R., Miszkiewicz, J.J. and Mahoney, P., 2017. Bone deep: variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. *Journal of Archaeological Science* 87, 10-16. <https://doi.org/10.1016/j.jas.2017.09.009>
- Fischer, A. and Olsen, J., 2021. The Nekselø fish weir and marine reservoir effect in Neolithization period Denmark. *Radiocarbon* 63(3), 805-820. <https://doi.org/10.1017/RDC.2021.14>
- Fischer, A., Olsen, J., Richards, M., Heinemeier, J., Sveinbjörnsdóttir, Á.E. and Bennike, P., 2007. Coast-inland mobility and diet in the Danish Mesolithic and Neolithic: evidence from stable isotope values of humans and dogs. *Journal of Archaeological Science* 34(12), 2125-2150. <https://doi.org/10.1016/j.jas.2007.02.028>
- Gowland, R., Stewart, N.A., Crowder, K.D., Hodson, C., Shaw, H., Gron, K.J. and Montgomery, J., 2021. Sex estimation of teeth at different developmental stages using dimorphic enamel peptide analysis. *American Journal of Physical Anthropology* 174(4), 859-869. <https://doi.org/10.1002/ajpa.24231>
- Gron, K.J. and Rowley-Conwy, P., 2017. Herbivore diets and the anthropogenic environment of early farming in southern Scandinavia. *The Holocene* 27(1), 98-109. <https://doi.org/10.1177/0959683616652705>
- Gron, K., Andersen, S.H. and Robson, H.K., 2015. Bone fragmentation as a tool for quantification and identification of taphonomic processes and their effects: the case study from Havnø, a stratified Danish “Køkkenmødding.” In: N. Bicho, C. Detry, T.D. Price and E. Cunha (eds). *Muge 150th: The 150th anniversary of the discovery of Mesolithic shellmiddens Volume 2*. Newcastle upon Tyne: Cambridge Scholars Publishing, 189-206.
- Gron, K.J., Meiklejohn, C., Pedersen, K.B., Stewart, N.A., Alexandersen, V., Sørensen, L. and Montgomery, J., 2022. Sex and gender in the Mesolithic: adults and children from the Strøby Egede burial, Køge Bugt, Denmark. *Proceedings of the Prehistoric Society* 88, 1-23. <https://doi.org/10.1017/ppr.2022.7>
- Guiry, E., Noël, S. and Fowler, J., 2021a. Archaeological herbivore $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ provide a marker for saltmarsh use and new insights into the process of ^{15}N -enrichment in coastal plants. *Journal of Archaeological Science* 125, 105295. <https://doi.org/10.1016/j.jas.2020.105295>
- Guiry, E.J., Kennedy, J.R., O’Connell, M.T., Gray, D.R., Grant, C. and Szpak, P., 2021b. Early evidence for historical overfishing in the Gulf of Mexico. *Science Advances* 7(32), eabh252. <https://doi.org/10.1126/sciadv.abh2525>
- Heaton, T.J., Köhler, P., Butzin, M., Bard, E., Reimer, R.W., Austin, W.E.N., Bronk Ramsey, C., Grotes, P.M., Hughen, K.A., Kromer, B., Reimer, P.J., Adkins, J., Burke, A., Cook, M.S., Olsen, J. and Skinner, L.C., 2020. Marine20 – The marine radiocarbon age calibration curve (0–55,000 cal BP). *Radiocarbon* 62(4), 779-820. <https://doi.org/10.1017/RDC.2020.68>
- Lamb, A.L., Chenery, C.A., Madgwick, R. and Evans, J.A., 2023. Wet feet: developing sulfur isotope provenance methods to identify wetland inhabitants. *Royal Society Open Science* 10, 230391. <https://doi.org/10.1098/rsos.230391>

- Lass Jensen, O., 2001. Kongemose- og Ertebøllekultur ved den fossile Nivåfjord. *In: O. Lass Jensen, S.A. Sørensen and K.M. Hansen, eds. Danmarks Jægerstenalder – status og perspektiver.* Hørsholm: Hørsholm Egnsmuseum, 115-129.
- Lass Jensen, O., 2003. A sunken dwelling from the Ertebølle site Nivå 10, eastern Denmark. *In: L. Larsson, H. Kindgren, K. Knutsson, D. Loeffler and A. Åkerlund, eds. Mesolithic on the move.* Oxford: Oxbow Books, 230-238.
- Lass Jensen, O., 2009. Dwellings and graves from the Late Mesolithic site of Nivå 10, eastern Denmark. *In: S. McCartan, R. Schulting, G. Warren and P. Woodman, eds. Mesolithic Horizons.* Oxford: Oxbow Books, 465-472.
- Lass Jensen, O.L., 2016. Double burials and cremations from the Late Mesolithic site of Nivå 10, eastern Denmark. *In: J.M. Grünberg, B. Gramsch, L. Larsson, J. Orschiedt and H. Meller, eds. Mesolithic burials – rites, symbols and social organisation of early postglacial communities. International Conference Halle (Saale), Germany, 18th-21st September 2013.* Halle: Tagungen des Landesmuseums für Vorgeschichte Halle, 95-107.
- Lass Jensen, O. and Møller Hansen, K., 1998. Barnegraven fra Nivågård. *Hørsholm Egnsmuseum Årbog* 1998, 7-21.
- Lewis, M., 2011. The osteology of infancy and childhood: misconceptions and potential. *In: M. Lally and A. Moore, eds. (Re)Thinking the little ancestor: new perspectives on the archaeology of infancy and childhood.* Oxford: Archaeopress (BAR International Series 2271), 1-13
- Longin, R., 1971. New method of collagen extraction for radiocarbon dating. *Nature* 230, 241-242. <https://doi.org/10.1038/230241a0>
- Masetti, M., 2007. The economic role of Sus in early human fishing communities. *In: U. Albarella, K. Dobney, A. Ervynck and P. Rowley-Conwy, eds. Pigs and humans. 10,000 years of interaction.* Oxford: Oxford University Press, 156-170. <https://doi.org/10.1093/oso/9780199207046.003.0017>
- McArdle, N., Liss, P. and Dennis, P., 1998. An isotopic study of atmospheric sulfur at three sites in Wales and at Mace Head, Eire. *Journal of Geophysical Research Atmospheres* 103(D23), 31079-31094. <https://doi.org/10.1029/98JD01664>
- Meiklejohn, C., Brinch Petersen, E. and Alexandersen, V., 1998. The later Mesolithic population of Sjælland, Denmark, and the Neolithic transition. *In: M. Zvelebil, L. Domanska and R. Dennell, eds. Harvesting the sea, farming the forest: the emergence of Neolithic societies in the Baltic Region.* Sheffield: Sheffield Academic Press, 203-212.
- Meiklejohn, C., Brinch Petersen, E. and Alexandersen, V., 2000. The anthropology and archaeology of Mesolithic gender in the Western Baltic. *In: M. Donald and L. Hurcombe, eds. Gender and material culture in archaeological perspective.* Basingstoke: Macmillan Press, 222-237.
- Nehlich, O., 2015. The application of sulphur isotope analyses in archaeological research: a review. *Earth-Science Reviews* 142, 1-17. <https://doi.org/10.1016/j.earscirev.2014.12.002>

- Nehlich, O. and Richards, M.P., 2009. Establishing collagen quality criteria for sulphur isotope analysis of archaeological bone collagen. *Archaeological and Anthropological Sciences* 1, 59-75. <https://doi.org/10.1007/s12520-009-0003-6>
- O'Connell, T.C. and Hedges, R.E., 1999. Isotopic comparison of hair and bone: archaeological analyses. *Journal of Archaeological Science* 26 (6), 661-665. <https://doi.org/10.1006/jasc.1998.0383>
- Paludan-Müller, C., 1978. High Atlantic food gathering in northwestern Zealand, ecological conditions and spatial representation. In: K. Kristiansen and C. Paludan-Müller, eds. *New Directions in Scandinavian Archaeology*. Aarhus: Aarhus Universitetsforlag, 120-157.
- Rasmussen, K.L., 1998. Kulstof-14 dateringen af Nivågård-barnet. *Hørsholm Egns Museum Årbog* 1998, 36-40.
- Rasmussen, K.L., Bjerregaard, P., Gommesen, P.H. and Lass Jensen, O., 2009. Arsenic in Danish and Swedish Mesolithic and Neolithic human bones – diet or diagenesis? *Journal of Archaeological Science* 36(12), 2826-2834. <https://doi.org/10.1016/j.jas.2009.09.019>
- Reimer, P.J., Austin, W.E.N., Bard, E., Bayliss, A., Blackwell, P.G., Bronk Ramsey, C., Butzin, M., Cheng, H., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Hajdas, I., Heaton, T.J., Hogg, A.G., Hughen, K.A., Kromer, B., Manning, S.W., Muscheler, R., Palmer, J.G., Pearson, C., van der Plicht, J., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Turney, C.S.M., Wacker, L., Adolphi, F., Büntgen, U., Capano, M., Fahrni, S.M., Fogtmann-Schulz, A., Friedrich, R., Köhler, P., Kudsk, S., Miyake, F., Olsen, J., Reinig, F., Sakamoto, M., Sookdeo, A. and Talamo, S., 2020. The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0-55 cal kBP). *Radiocarbon* 62, 725-757. <https://doi.org/10.1017/RDC.2020.41>
- Stewart, N.A., Gerlach, R.F., Gowland, R.L., Gron, K.J. and Montgomery, J., 2017. Sex determination of human remains from peptides in tooth enamel. *Proceedings of the National Academy of Sciences* 114(52), 13649-13654. <https://doi.org/10.1073/pnas.1714926115>
- Sørensen, S.A., 2017. *The Kongemose culture (Nordiske Fortidsminder Serie B)*. Copenhagen: University Press of Southern Denmark.
- Tilley, C., 1996. *An ethnography of the Neolithic. Early prehistoric societies in southern Scandinavia*. Cambridge: Cambridge University Press.
- Vang Petersen, P., 1984. Chronological and regional variation in the Late Mesolithic of Eastern Denmark. *Journal of Danish Archaeology* 3(1), 7-18. <https://doi.org/10.1080/0108464X.1984.10589909>

Supplementary Material

Supplementary Material see also .xlsx- and .doc-attachment

Table captions:

Table 1. The Nivå 10 and Nivågård inhumation burials and cremations. Note that the loose human bones are not listed, though see Table 2. Those assigned an 'Etch Lab #' were subjected to an acid etch peptide-based method for determination of biological sex, while those assigned a 'Collagen Lab #' were subjected to stable isotope analysis. Note that when 'Age at Death' data were provided, the 'Age Classes' were standardised following Buikstra and Ubelaker (1994) and Lewis (2011) with alterations (Malin Holst personal communication).

Table 2. Radiocarbon dates from Nivå 10 and Nivågård, sorted by estimated calendar date. The calibrated dates of samples were obtained by the OxCal v.4.4.4. model provided in the Supplementary Material, as described in the text. The estimated % marine diet indicated was based on a linear $\delta^{13}\text{C}$ model (see Arneborg et al. 1999; Fischer et al. 2007), while their Bayesian updated values used in calculating the calibrated age range are in parentheses.

Table 3. Sample information for the Nivå 10 inhumation burials.

Table 4. Sample information for the Nivågård fauna.

Table 5. Nivå 10 and Nivågård tooth etch-based peptide sex determination results. Note that the osteological sex determinations are given for comparison. UND denotes undetermined due to a lack of peptide recovery.

Table 6. Nivå 10 stable isotope data. Key: struck through – sample yielding an atomic C:N ratio outside the acceptable range of 2.9-3.6 (DeNiro 1985).

Table 7. Stable isotope data from the Nivågård child and fauna, including previously published data obtained from Atlantic cod (*Gadus morhua*) and European flounder (*Platichthys flesus*) bone collagen (Fischer et al. 2007). Note that sulfur was not run in duplicate due to the amount of collagen required for analysis. Key: struck through – sample yielding an atomic C:N ratio outside the acceptable range of 2.9-3.6 (DeNiro 1985).