



Life histories at stone age Zvejnieki based on stable isotope profiles of tooth dentine

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ABSTRACT

The timing of infant weaning in the past is important for its implications for birth-spacing and infant survival, and hence for population maintenance or growth under different socio-economic regimes. Prior to the adoption of agriculture, breastfeeding is believed to have been more prolonged amongst hunter-gatherers due, at least partly, to the lack of suitable weaning foods that are available to agriculturalists. The introduction of pottery possibly also changed weaning patterns due to shifts in food preparation even prior to the adoption of domesticated foods. Here we apply stable carbon and nitrogen isotope sequential samples on dentine to explore differences in diet relating to weaning age, social roles and food sharing between children and adults in a well-preserved Mesolithic/Neolithic population from the cemetery of Zvejnieki, Latvia. We address whether there are differences in diet between the Mesolithic and the Neolithic periods, defined here by the appearance of pottery rather than the adoption of agriculture. Considerable variability in weaning patterns was observed, but in general individuals tended to be breastfed from birth, with the contribution of breast milk declining after the age of 6–12 months, and completely withdrawn by the age of 3 years. We note a difference in $\delta^{15}\text{N}$ dentine profiles between the Mesolithic and Neolithic, which may be linked to the introduction of pottery. We also assess differences in diets in relation to identities marked in death, specifically the presence or absence of animal tooth pendants. The carbon and nitrogen isotope profiles for sequentially sampled first molars show that adults who were buried without animal tooth pendants as grave goods consumed more freshwater resources during their childhoods than those buried with animal tooth pendants. We conclude that infant and childhood diet reflected different societal roles or identities within the population that continued into adulthood.

1. Introduction

Sequential stable carbon and nitrogen isotope analysis of tooth dentine is increasingly used for the investigation of individual 'life histories' related both to diet and to stress episodes during early development (e.g., Beaumont and Montgomery, 2016; Eerkens et al., 2011; Fernández-Crespo et al., 2018; Henderson et al., 2014; Lillie et al., 2016; Montgomery et al., 2013; Sandberg et al., 2014; Scharlotta et al., 2018; Tsutaya et al., 2015). In particular, weaning ages have been explored based on detailed isotopic analysis of the increments in the dentine of 1st permanent molars, which develop shortly after birth and for several subsequent years. Using this approach, episodes of dietary change or starvation have been detected in Neolithic Shetland (Montgomery et al.,

2013) and in mid-nineteenth century Ireland (Beaumont and Montgomery, 2016). In two Late Neolithic groups practicing different burial traditions in northern Spain, subtle long-term dietary differences in children and adults were detected using this method (Fernández-Crespo et al., 2020). So far, opportunities to explore the weaning practices of more mobile forager populations, which may have been distinct from those of more recent sedentary agricultural populations, have been limited (Eerkens et al., 2011; Scharlotta et al., 2018), at least partly because their inhumations are often infrequent (Fernández-Crespo et al., 2020).

The cemetery of Zvejnieki, northern Latvia, is unusual in that it contains a large number of well-preserved skeletons from the Mesolithic and succeeding 'Pottery Neolithic' periods, defined by the use of pottery

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rather than by the appearance of domesticates (Liiva and Loze, 1993; Piličiauskas et al., 2017). The ‘Pottery Neolithic’ has been observed as a widespread phenomenon across the high northern latitudes of Europe and Asia that has been frequently shown to be associated with the preparation of aquatic foods (e.g. Isaksson and Gibbs, 2018; Lucquin et al., 2016). A final phase, the ‘Late Neolithic’, sees the presence of domestic animals and thus fits more securely into conventional definitions of ‘Neolithic’ as indicating evidence for a degree of reliance on agriculture. The site thus offers a rare opportunity to compare changes in childhood diets and weaning practices in a population with a forager economy before and after the adoption of pottery from the same location. This is important since one of the assumed drivers for the use of pottery is the expansion of dietary breadth that it facilitates, including the preparation of soft foods suitable for weaning. Post-weaning, we are also able to investigate possible movements between the site’s inland location and the coast ca. 50 km to the west, suggested by the presence of dog tooth pendants with isotopic values characteristic of marine resources, as well as one adult bone collagen value exhibiting moderately enriched ^{13}C (Eriksson and Zagorska, 2003). Finally, we explore possible social differences within the population, since grave goods are associated with many of the burials (Schulting et al., 2020).

1.1. Site background

Zvejnieki is located on the shores of Lake Burtnieki in northern Latvia (Zagorskis, 1987) (Fig. 1). It has both an occupation and a cemetery site, the latter containing over 300 inhumations making it one of the largest known hunter-gatherer cemeteries in Europe. The cemetery was used throughout an extended period of time from the Mesolithic and Neolithic, into even later periods (c. 7000–3000 cal BC) (Meadows et al., 2018). A substantial number of radiocarbon determinations have shown that the cemetery was first established in the Middle Mesolithic, with the earliest directly dated burial at 8240 ± 70 cal BC (Ua-3634) (Zagorska, 1997). The majority are from the Late Mesolithic (c. 7000–5200 cal BC), Early Neolithic (c. 5200–4200 cal BC) and Middle Neolithic (c. 4200–3200 cal BC); again, with the Early Neolithic defined by the regional appearance of pottery c. 5200 cal BC. Definition of these periods at Zvejnieki is based almost entirely on direct radiocarbon dating of human remains, which for all periods – excepting the Late Neolithic Corded Ware – is complicated by the consumption of aquatic foods imparting significance amounts of ‘old carbon’, leading to a freshwater reservoir effect (Meadows et al., 2016; 2018). Dates are likely to be ‘too old’ by two to three centuries on average in individuals relying on freshwater foods (Meadows et al., 2018). We have not corrected radiocarbon dates here as not all individuals analysed have been directly dated, and we are only interested in broad period attributions.

The ‘Early Neolithic’ Narva culture in the Baltic region is defined by the presence of pottery, rather than of domesticates (Liiva and Loze, 1993; Piličiauskas et al., 2017). Thus, there is considerable continuity between the Mesolithic and Neolithic, as shown by the continued use of the cemetery, similar burial practices, the continued use of symbolism such as red ochre and animal tooth pendants within the graves and a sustained reliance on hunting, gathering and fishing (Zagorskis, 2004; Eriksson, 2006). The exception is a small shift in the Middle Neolithic to pendants made from the teeth of dogs and seals, versus the earlier predominance of red deer and wild boar (Larsson, 2006). The situation changes in the Late Neolithic when domestic animals first appear in the region in association with Corded Ware groups (Kriiska, 2003).

1.2. Previous isotopic Research at Zvejnieki

The first dedicated stable carbon and nitrogen isotopic studies on human and faunal remains from Zvejnieki were undertaken by Eriksson and colleagues (Eriksson and Zagorska, 2003; Eriksson, 2006). The $\delta^{15}\text{N}$ results for human bone and tooth collagen range from 8.9‰¹ to 17.7‰, and $\delta^{13}\text{C}$ from −25.0‰ to −18.8‰, suggesting highly diverse diets during the Late Mesolithic and Early/Middle Neolithic, with a strong reliance on freshwater resources for some individuals. The isotopic indication for a freshwater aquatic food diet at Lake Burtnieki is characterised by low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values, while marine foods in contrast are characterised by relatively high $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$. Additional isotope data for bone collagen since obtained by Meadows and colleagues (Meadows et al., 2016; Meadows et al., 2018) suggests no major dietary shift with the introduction of pottery. There is, however, a hint of a minor trend away from high-trophic-level foods in the 6th millennium BC, a more diverse diet in the 5th millennium BC and a possible narrowing of the diet in the mid-4th millennium BC. The small number of Late Neolithic Corded Ware individuals analysed to date tend to have lower $\delta^{15}\text{N}$ values on average, indicating a shift in diet consistent with the appearance of a farming economy (Eriksson, 2006).

The published faunal and human isotope data are summarised in Fig. 2. Animals with $\delta^{13}\text{C}$ values indicative of a marine diet include a mallard (*Anas platyrhynchos*) and seals (*Halichoerus grypus*), while the most negative $\delta^{13}\text{C}$ values came from freshwater fish and pond turtle (*Emys orbicularis*). The most elevated $\delta^{15}\text{N}$ values came from the aquatic animals including otters (*Lutra lutra*), pond turtles (*Emys orbicularis*), pike (*Esox Lucius*), and from badgers (*Meles meles*), which are terrestrial omnivores (Eriksson, 2006). Isotopic data for dog tooth pendants associated with burials clearly show that some dogs consumed food from marine resources, indicating the exchange of either live dogs or dog tooth pendants (Eriksson and Zagorska, 2003). Human adults tend to have elevated $\delta^{15}\text{N}$ values, comparable to those of otters, unidentified fish species and mallard.

An early ‘life history’ pilot study was undertaken by Eriksson (2007) for a single individual, a male in Grave 165, with a slightly higher bone collagen $\delta^{13}\text{C}$ value compared to other individuals at the site. It was therefore hypothesised that he may have consumed more marine foods and perhaps originated on the coast. It should be noted that $\delta^{15}\text{N}$ values do not show the typical trend of increasing alongside $\delta^{13}\text{C}$ values because freshwater fish are equally enriched in ^{15}N . Single samples of tooth dentine from the first, second and third molars (M1, M2, and M3, respectively) representing the age brackets of 2–4, 5–10 and 10–16 years were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The highest $\delta^{15}\text{N}$ value occurred in the youngest age period of 2–4 years (13.5‰), which was interpreted as reflecting at least in part a nursing signal. Modest variation in $\delta^{13}\text{C}$ was noted throughout the individual’s life (2–4 years −22.0‰, 5–10 years −20.9‰, 10–16 years −20.6‰, 20–35 years −18.8‰ and 30–35 years −21.2‰²), leading the author to suggest increasing marine input into the diet from childhood through to adolescence and early adulthood, declining again in later adulthood. Whether the individual made short regular trips to the coast or longer trips could not be ascertained due to the low temporal resolution of this sampling method (Eriksson, 2007).

2. Aims

The aim of this study was to apply incremental dentine carbon and nitrogen isotope analysis to better understand the childhood diets and weaning practices of individuals who survived into adulthood in a diachronic sample from Zvejnieki. Hunting and gathering societies are

¹ The lowest $\delta^{15}\text{N}$ value of 6.2‰ for a worn incisor pendant identified as human is very unusual compared to all other human data and may be a mis-identified faunal tooth.

² Obtained from bone collagen taken from different bone elements.



Fig. 1. Map showing the location of Zvejnieki adjacent to the large freshwater Lake Burtnieks. Map data © OpenStreetMap contributors, Microsoft, Esri Community Maps contributors, Map layer by Esri, Sources: Esri, USGS, National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp., Esri, HERE, Garmin, FAO, NOAA, USGS, Esri, USGS.

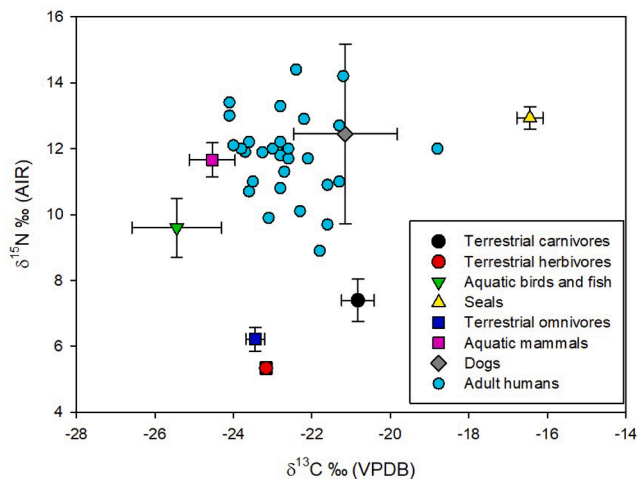


Fig. 2. A comparison of the average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in humans, compared to broad faunal trophic groups. Error bars show ± 1 standard error. Sample sizes are: 11 terrestrial carnivores, 37 terrestrial herbivores, 9 aquatic birds and fish, 7 seals, 11 terrestrial omnivores, 5 aquatic mammals, 9 dogs, and 31 adult humans. Data from Eriksson, 2006, Eriksson and Zagorska, 2003 and Meadows et al., 2018.

often thought to prolong breastfeeding, with consequences for infant health and survival, and lower birth rates (Anderson et al., 1986). This stands in contrast to generally earlier weaning in agricultural-based societies (e.g. Bocquet-Appel, 2011), leading to significant anthropological interest in weaning ages because of its influence on infant

survival and birth-spacing, and consequently on population structure and demography. Prolonged nursing, however, may apply primarily to highly mobile foragers, rather than to the more sedentary hunter-fisher-gatherers that better characterise southern Scandinavia and the eastern Baltic. But it is also not clear that sedentism alone is the major influence rather than availability of energy-rich foods, since reductions in birth interval are linked to maternal energy balance (Bocquet-Appel, 2011). In Zvejnieki, the appearance of pottery in the sequence allows us to investigate whether or not its adoption led to any isotopically detectable changes in weaning practices by making novel supplementary foods available (cf. Orlas et al., 2017).

Here we investigate the life histories of selected Mesolithic and Neolithic individuals from Zvejnieki as expressed in their isotopic intra-tooth sequences, to assess the following: 1) changes in weaning age and the nature of supplementary foods before and after the appearance of pottery; 2) whether higher resolution sampling can identify excursions to the coast as suggested by isotopic values for dog tooth pendants and by hints of enriched ^{13}C in one human (Grave 165); and 3) variability in childhood and adult diets in relation to the provision of animal tooth pendants as grave goods. We earlier noted differences in adult diets between those with and without pendants (Henderson, 2015; Schulting et al., 2020). The aim here is to investigate the age at which these material culture-related dietary differences can be detected, and whether they may have influenced other aspects of early life history (that are amenable to stable isotope investigation). Although a variety of grave goods were recovered, tooth pendants are the most common artefact class in both male and female graves. They may have represented status or identity that may have been ascribed from birth due to a familial relationship or affiliation with a particular group. It might thus be possible to explore issues of status and/or identity as well as any intra-

population variability or diachronic trends as noted above.

3. Materials and methods

The left mandibular first molars of 18 individuals were sampled (Supplementary Data, Table 4), in order to reflect approximately the first 0–8 years of development. Some of the teeth exhibited advanced attrition, which is often a feature of hunter-gatherers consuming coarse, gritty foods (Smith, 1984). Thus, the full life histories spanning 0–8 years old were not available in these cases. Where a loss of crown dentine had occurred, the number of missing segments was estimated.

The approach uses incremental samples of dentine, following methods developed earlier by Eerkens et al. (2011) and Beaumont et al. (2013). Teeth were first cleaned using aluminium oxide air abrasive to remove adhering sediments allowing for closer inspection of the enamel surface for the presence of small carious lesions which were avoided. Each tooth was sectioned using a Buehler low speed saw with a diamond wafer blade. One half of the tooth was conserved for future analysis or curation, and the remaining half was stripped of enamel using a hand drill. This half was placed in 0.5 M HCl at 4 °C in order to remove the mineral phase (Honch, 2008). The acid was changed every two days and the tooth remained submerged until it felt soft when probed with a pipette. The demineralisation time was highly variable between samples, depending on preservation. All tooth halves retained their shape after demineralisation.

The demineralised tooth halves were sectioned into ten horizontal incremental samples from the crown to the root using a scalpel, according to Henderson et al. (2014). Following this procedure, five samples were evenly spaced throughout the crown zone, while the remaining five were evenly spaced from the cemento-enamel junction to the root tip. Age assignments are based on the London Atlas of Tooth Formation (AlQahtani et al., 2010), as follows. Each samples' location was compared to the stage of dental development of the M1 and assigned to a putative age by using anatomical locations as temporal markers for growth rates and the data are plotted as presented in Czermak et al. (2020). Anatomical areas are defined according to The London Atlas as *crown*, *prefurcation*, *middle root*, and *root tip*, and are used as temporal markers for growth rates (Fig. 3, lower right). The median age of completion of each developmental stage was used: crown 3 years, prefurcation 5 years, middle root 6.5 years, root tip 7.5 years. As dentine formation occurs to a certain stage by a specific age, any sample from before this stage represents dietary intake by this age. Sample timespans are calculated by dividing the time of completion of an anatomical area with the number of samples taken within an anatomical area. For example, the crown formation of the first permanent molar starts around birth and ceases at approximately three years of age (AlQahtani et al., 2010). Each of the five samples taken from this area covers approximately a fifth of that timespan. Then the mid-point of each time-span was calculated. A midpoint of 0.25 years of age was used for the first sample, reflecting the average diet consumed between 0 and 0.5 years. Similarly, the second sample was assigned to a midpoint of 0.75 years of age reflecting the average consumed diet between 6 and 12 months. Thus samples from the crown are representative of a shorter period of time (~3 years overall) than those from the root representing ~3–8 years, meaning that crown segments provide a higher resolution sequence.

The demineralized slices were rinsed three times in distilled water, placed in test tubes in pH3 water and heated at 70 °C for two days in order to denature the collagen. Thereafter, they were cooled, filtered using Eze filters (Elkay Laboratory Products), and freeze-dried. Approximately 1 mg of the resulting dentine 'collagen' (i.e., including a small proportion of non-collagenous proteins) was weighed into tin capsules.

Samples were analyzed on a SERCON 20/22 continuous flow isotope ratio mass spectrometer coupled to a SERCON GSL elemental analyzer in the Research Laboratory for Archaeology and the History of Art,

University of Oxford. Analytical reproducibility is $\pm 0.2\%$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, based on multiple replicates of the internal laboratory standard, alanine, used to calculate instrumental drift. International standards IAEA6 and USGS40 and two well-characterised laboratory standards, COW and SEAL, were intermittently spaced between the unknowns, in order to further assess the quality of the run and to calibrate the results. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements were calibrated using a two-point calibration curve using the internal cow and seal bone collagen standards (following Coplen et al., 2006).

All statistical analyses were undertaken using the R platform (R Core Development Team, 2013). Generalized Linear Models (GLMs) were used to test for the effects of age and pendant presence on carbon and nitrogen stable isotope ratios. Statistical analysis of the effect of age and presence of pendants on the stable isotopes requires a nested analysis as multiple observations were collected from each individual tooth. The nlme library (Pinheiro et al., 2018) was used to fit a mixed effects model including nested random effects. A term was included for the correlation between measurements representing the different ages across a single tooth. The basic model for $\delta^{15}\text{N}$ is:

$$\delta^{15}\text{N} = \text{Pendant} + \text{Age} + \text{Pendant} * \text{Age}, \text{random} = \sim 1 | \text{Individual}$$

with the correlation between measurements for an individual tooth between ages given by a first order autoregressive model with a correlation = 0.78.

The basic model for $\delta^{13}\text{C}$ is similar to that for $\delta^{15}\text{N}$:

$$\delta^{13}\text{C} = \text{Pendant} + \text{Age} + \text{Pendant} * \text{Age}, \text{random} = \sim 1 | \text{Individual},$$

with the correlation between measurements for an individual between ages given by a first order autoregressive model with a correlation = 0.81. Statistical analysis of isotopes on adults was performed using analysis of variance using the aov R function.

Further to the age assignment methods outlined above, the term 'young infant' is used to refer to individuals up to 1 year of age, 'older infant' from 1 to 3 years, 'children' from 3 to 8.5 years. To estimate the maximum duration of the weaning process, two marker points that represent the last point before and the first point after weaning are used (Eerkens et al., 2011; Nitsch et al., 2011; Sandberg et al., 2014). The sample with the earliest highest $\delta^{15}\text{N}$ value before the typical decrease in $\delta^{15}\text{N}$, suggesting the introduction of complementary food, was used to estimate the onset of weaning. The sample with the lowest $\delta^{15}\text{N}$ value after the sustained drop represents the end of the transition and the point at which the child can be considered to be weaned.

The duration of weaning was estimated by subtracting lowest $\delta^{15}\text{N}$ value observed at the 'trough' of a decline from the highest value. Note that this need not always be the lowest value, since in some cases a slower decline continued into the mid-childhood 'dip' in $\delta^{15}\text{N}$ values seen in some individuals. In such cases there was usually still a marked change in the slope that was used to infer the completion of weaning (e.g., Grave 121). The difference between the two values is termed $\Delta^{15}\text{N}$. The first sequential sample – representing age 0–6 months – was given the value of 0.6 yr, since this slice is likely to be biased towards the later part of the range. The result of this is that weaning is assumed to begin only after six months, at least in a way that is detectable, which will depend in part on the difference in the isotopic values between breast-milk and the complementary foods. For subsequent increments, the midpoint of the age ranges was used.

4. Results

Comparison between dentine collagen carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope profiles of M1s from the three time periods is shown in Fig. 3. Sequential $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data for all individuals are shown in Fig. 4 and Supplementary Table S1. Averaged data for each period are summarised in Table 1, while all data and quality control indicators such as C/N ratios are provided in the Supplementary Material Table S1. Late Neolithic individuals also differ compared to those from the Early-/Mid Neolithic period, being significantly lower in $\delta^{15}\text{N}$ and higher in $\delta^{13}\text{C}$

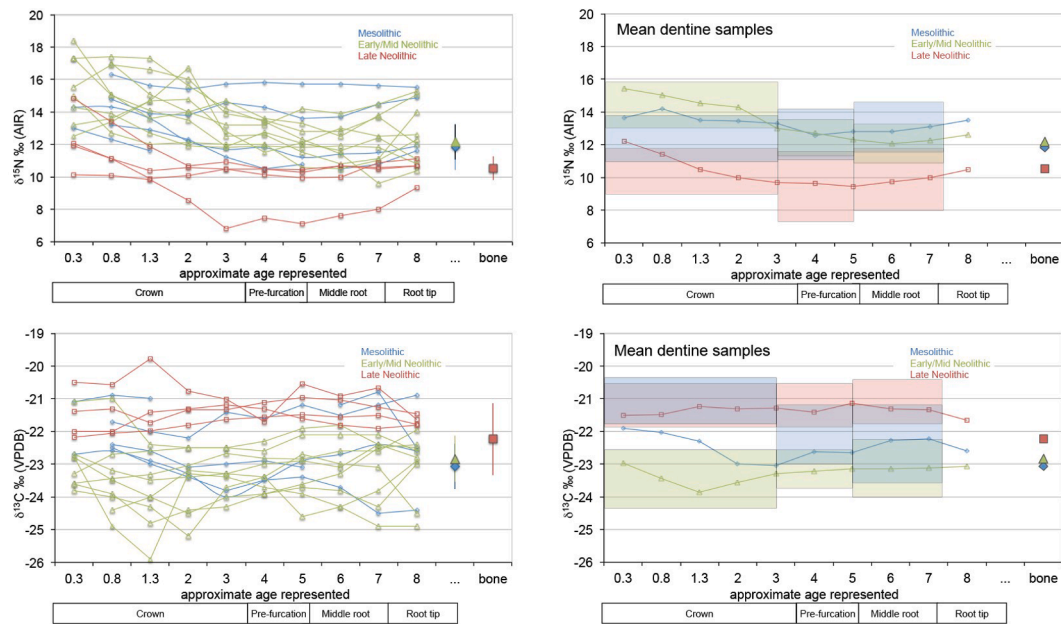


Fig. 3. Comparison between dentine collagen nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope profiles of M1s from the three time periods (Mesolithic: blue, Early-/Mid Neolithic: green, Late Neolithic: red). Mean values obtained from bone collagen representing individuals at adulthood are shown for reference on the edge of the plot. The schematic graph illustrates the correspondence between tooth developmental ages and anatomy used to assign age to each dentine sample. Data shown for each individual (left) and mean values for each period (right). Shaded areas represent the mean values ± 1 pooled SDs at different age periods (0–3, 3–5, 5–7 and 7–8 years). For corresponding data see Supplementary data Table S2 and S3.

values during the first 7 years. The difference in $\delta^{15}\text{N}$ results between Mesolithic and Late Neolithic is significant in the first 3 years (Fig. 3; statistical data see Supplementary Table S2 and S3). It is notable that the most elevated $\delta^{15}\text{N}$ in infants and children are seen in Middle Neolithic individuals. A further observation is that while the data for the two earlier periods vary between individuals, the life-history pathway for each individual seems to continue along the same trajectory, with elevated $\delta^{15}\text{N}$ in infancy, declining into childhood to below adult values, before rising again into adulthood.

The data for both isotopes are highly variable especially through the Mesolithic and Early/Middle (Pottery) Neolithic (Table 1). The $\delta^{13}\text{C}$ values for many individuals are low (ca. -23 to -25‰) suggesting strong reliance on aquatic foodstuffs. However, this is not always the case, for instance the individuals in Graves 92 and 170 do not follow this pattern as their values are closer to -21‰ , which one might expect for an individual living in a C_3 terrestrial environment at this latitude. More of the Late Neolithic individuals, on the other hand, have conventional $\delta^{13}\text{C}$ values close to -21‰ (mean value -21.4‰ SD 0.391). In general, $\delta^{15}\text{N}$ values declined steadily from those early segments representing the youngest infants (up to 1 year of age) (mean $14.05 \pm 0.38\text{‰}$), to the older infants ($12.80 \pm 0.28\text{‰}$) (aged 1–3 years) and children ($11.80 \pm 0.19\text{‰}$) (aged 3–8.5 years), and finally the adults ($11.8 \pm 0.2\text{‰}$) established from Eriksson (2006). Mean $\delta^{13}\text{C}$ values on the other hand do not show any clear corresponding age-trends. There is no difference between the children and adults (Student's t -test, $t = 0.788$, $p = 0.433$), and no statistically significant trend in $\delta^{13}\text{C}$ in relation to age ($F = 1.345$, $p = 0.261$). Two Early/Middle Neolithic adult males, from Graves 124 and 179 (the former, incidentally, with pendants, and the latter without), show similar early life histories to one another, distinct from those of all the other individuals (Fig. 4.). Both exhibit increasing $\delta^{15}\text{N}$ values from 0 to 6 months to a peak at ca. 2 years of age, following which they decline sharply. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ mirror each other in these two individuals, a pattern which is also seen most clearly in the Mesolithic individual 154 and the Neolithic individuals 173 and 207. Six individuals also have adult bone isotope values available from previous analysis (Meadows et al., 2018, Eriksson, 2006), comprising individuals 123, 170, 124, 313, 154 and 307. Excluding 313, which did not produce

data for the childhood period, these individuals had mean childhood isotope values of $12.79 \pm 0.45\text{‰}$ and $-22.43 \pm 0.26\text{‰}$ ($n = 5$). The same individuals had mean adult isotope values of $11.83 \pm 0.63\text{‰}$ and $-23.31 \pm 0.33\text{‰}$ ($n = 6$).

5. Diachronic trends in weaning practices through time

The shape of the curves shown in Fig. 3 show that the sequences for both isotopes are quite variable within each 'period', and one can observe that there is a striking difference in the profiles of both isotopes for the (pottery) Neolithic period compared to the preceding periods (which look similar).

The results of individuals' weaning pattern are shown in Table 1. The overall minimum duration of exclusive breastfeeding was 0.5 ± 0.3 years and is fairly consistent with 0.7 ± 0.2 years in Mesolithic ($n = 5$), 0.5 ± 0.4 years in Early-/Mid Neolithic ($n = 9$) and 0.4 ± 0.3 years in Late Neolithic ($n = 4$) individuals. While there is individual variability, the average maximum duration of the weaning process was 2.7 ± 1.4 years, with 2.5 ± 1.2 years in the Mesolithic, 2.7 ± 1.3 years in the Early-/Mid Neolithic and 2.0 ± 1.5 years in the Late Neolithic. The average age at weaning cessation was 3.0 ± 1.4 years, with 3.1 ± 1.1 years in the Mesolithic, 3.2 ± 1.6 years in Early-/Mid Neolithic, and 2.3 ± 1.5 years in Late Neolithic individuals (see Supplementary Figure S1). There are no significant differences in either duration of breastfeeding, weaning process or age at weaning cessation between the time periods, though sample sizes are small and all these findings should be taken as provisional.

Grave 206 (child, 7–14 years, Early-/Mid Neolithic) showed the longest estimated weaning period (5.2 years), the longest duration of exclusive breastfeeding (1.3 years) and the oldest age at weaning cessation 6.5 years. Late Neolithic Grave 137a (4.3 years) and Early-/Mid Neolithic Grave 207 (3.8 years) also showed longer weaning duration than the average (2.7 years). The overall shortest weaning duration was detected in Late Neolithic individuals 137 and 186 (1.1 years). The shortest weaning period during Mesolithic (1.3 years) was detected in individuals 154 and 170. While the duration of exclusive breastfeeding was average, the age at weaning cessation was at least 1.4

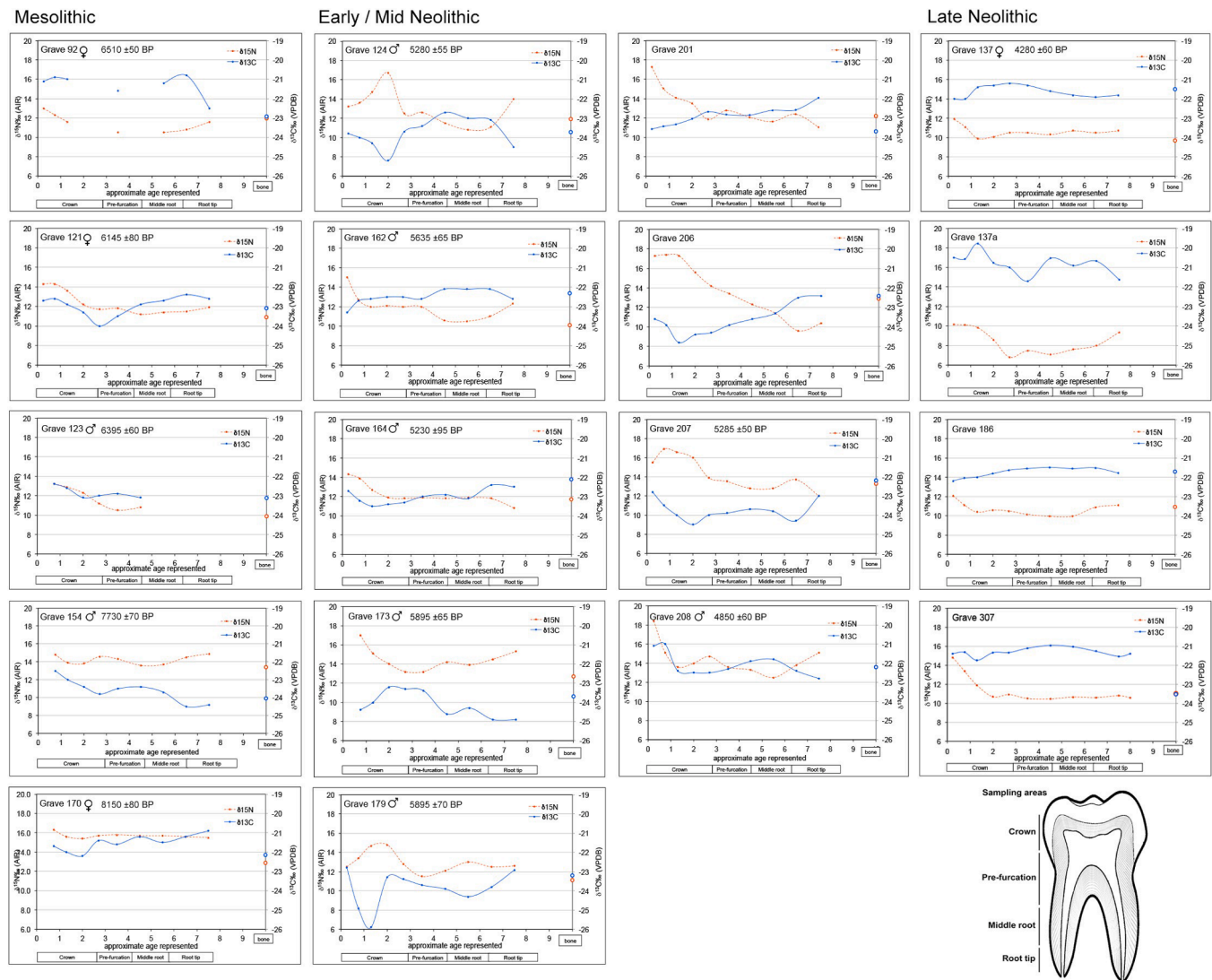


Fig. 4. Sequential $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data for all individuals analysed in this study, showing the former as red dotted lines and the latter as blue. Where available the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data for the adult is shown on the edge of the plot (from Eriksson, 2006; Meadows et al., 2018). The data are shown in columns according to period - Mesolithic, Early/Mid or Pottery Neolithic (across two columns), and Late Neolithic to the right. Each plot gives the grave number, the sex and the radiocarbon age where known. The x-axis provides an estimation of approximate age in years and the panel immediately below each plot shows the divisions. This is illustrated by the tooth section in the lower right of the figure. The radiocarbon dates are from Meadows et al. (2016) and Meadows et al. (2014), and the grave numbers and sex from Zagorskis (2004).

years earlier than those of the other three Mesolithic individuals and $\delta^{15}\text{N}$ values, both pre- and postweaning, were higher. However, no significant decrease in $\delta^{15}\text{N}$ during early life and thus no strong weaning signal can be seen.

The overall mean preweaning and postweaning $\delta^{15}\text{N}$ was $14.8 \pm 2.3\text{‰}$ and $11.5 \pm 1.9\text{‰}$, with $14.3 \pm 1.3\text{‰}$ and $12.3 \pm 2.2\text{‰}$ during the Mesolithic, $16.6 \pm 1.4\text{‰}$ and $11.9 \pm 1.2\text{‰}$ in the Early-/Mid Neolithic, and $12.2 \pm 1.9\text{‰}$ and $9.5 \pm 1.7\text{‰}$ in Late Neolithic individuals. The strongest decline in $\delta^{15}\text{N}$ can be seen in individuals 206 (7.7‰) and 201 (5.5‰), both Early-/Mid Neolithic, the lowest decline was detected in Mesolithic individual 170 (0.9‰). There is a significant difference in the decline in $\delta^{15}\text{N}$ during the weaning process between the Mesolithic and the Early-/Mid Neolithic ($p = 0.02$, t -test). The average decline is $3.3 \pm 1.7\text{‰}$, with $2.0 \pm 1.0\text{‰}$ in Mesolithic, $4.5 \pm 1.7\text{‰}$ in Early-/Mid Neolithic and $2.7 \pm 1.1\text{‰}$ in Late Neolithic individuals (Fig. 4). The lowest postweaning $\delta^{15}\text{N}$ showed Early-/Mid Neolithic individual 206 (9.6‰), and Late Neolithic individuals 137 (9.9‰) and 137a (7.1‰). However, while the decline was below the average of 3.3‰ in 137 and 137a (2.0 and 3.0‰), it was strongest in individual 206 (7.7‰). A strong

decline can also be seen in Early-/Mid Neolithic individuals 201 (5.5‰) and 208 (4.8‰), whereas the lowest decline can be seen in Mesolithic individuals 154 (1.0‰) and 170 (0.9‰) (Fig. 5).

5.1. Presence or absence of animal tooth pendants

Statistical analysis of pendants and age versus $\delta^{15}\text{N}$ in children showed that both pendant presence and age were significant predictors of $\delta^{15}\text{N}$ ($p = 0.032$ for pendants and $p < 0.001$ for age). Late Neolithic individuals were excluded from this analysis. There was however no significant pendant-age interaction term, allowing a simplified model to be used to eliminate the interaction term. This leads to the adoption of the simpler model: $\delta^{15}\text{N} = \text{Pendant} + \text{Age}$, random = ~ 1 /Individual. There is a decrease in $\delta^{15}\text{N}$ from infancy to childhood and variability in diet based on the presence or absence of tooth pendants in individuals between the ages of 3–8.5 years was found to be significant ($p = 0.03$). No statistically significant difference was found in $\delta^{13}\text{C}$ between young infants (0–1 year) buried with or without pendants ($p = 0.24$). There is a suggestion of a pendant effect on $\delta^{15}\text{N}$ values, with children buried with

Table 1

Duration of exclusive breastfeeding and weaning process for the 18 individuals with dentine microsampling results. The ‘**’ denotes graves containing animal tooth pendants. ‘#’ denotes that onset of weaning was determined by change in $\delta^{13}\text{C}$ as marker for the introduction of weaning food. Data from bone are taken from Eriksson (2006) and Meadows et al. (2018).

Grave	Period	Age at death	Sex	Estimated min. duration of exclusive breastfeeding (years)	$\delta^{15}\text{N}_{(\text{‰ AIR})}$ preweaning (dentine)	Estimated max. duration of weaning process (years)	Age at weaning cessation (years)	$\delta^{15}\text{N}_{(\text{‰ AIR})}$ postweaning (dentine)	$\delta^{15}\text{N}$ decline during weaning $\delta^{15}\text{N}_{(\text{preweaning})} - \delta^{15}\text{N}_{(\text{postweaning})}$	$\delta^{15}\text{N}_{(\text{‰ AIR})}$ adulthood (bone)
92*	Mesolithic	Adult	Male	0.25	13.0	≤ 3.3	≤ 3.5	10.5	2.5	12.0
121*	Mesolithic	Adult	Male	0.75	14.3	3.8	4.5	11.2	3.1	10.9
123*	Mesolithic	Child	Male	≤ 0.75	13.2	≥ 2.8	3.5	10.5	2.7	9.9
154	Mesolithic	Adult (40–45)	Male	≤ 0.75	14.8	≥ 1.3	2.0	13.8	1.0	13.4
170*	Mesolithic	Adult	Male	≤ 0.75	16.3	≥ 1.3	2.0	15.4	0.9	12.9
124*	Early-/Mid Neolithic	Adult	Male	n.d.	n.d.	n.d.	3.6	10.8	–	11.9
162	Early-/Mid Neolithic	Adult (30–35)	Male	0.25	15.0	1.5	1.3	12.0	3.0	10.1
164*	Early-/Mid Neolithic	Adult (25–35)	Male	0.25	14.3	2.5	2.7	11.8	2.5	11.7
173	Early-/Mid Neolithic	Adult	Male	≤ 0.75	≥ 17.0	≥ 2.0	2.7	13.2	3.8	12.7
179	Early-/Mid Neolithic	Adult	Male	n.d.	n.d.	n.d.	3.5	11.5	–	11.1
201*	Mid Neolithic	Child	Unknown	0.25	17.3	2.5	2.7	11.8	5.5	12.2
206	Mid Neolithic	Child (7–14)	Unknown	1.3	17.3	5.2	6.5	9.6	7.7	12.9
207	Mid Neolithic	Child (12–16)	Unknown	0.75	16.9	3.8	4.5	12.8	4.1	13.3
208	Mid Neolithic	Adult	Male	0.25	18.4	1.5	1.3	13.6	4.8	13.6
137	Late Neolithic	Adult	Male	0.25	11.9	1.1	1.3	9.9	2.0	9.7
137a	Late Neolithic	Unknown	Female	0.75	10.1	4.3	4.5	7.1	3.0	n.d.
186	Late Neolithic	Child	Unknown	0.25	12.1	1.1	1.3	10.4	1.7	10.9
307	Late Neolithic	Juvenile	Unknown	0.25	14.8	1.8	2.0	10.7	4.1	11.0
Mean (all)				≤ 0.4	≈ 14.8	≥ 2.7	3.0	11.5	3.3	11.8
σ				0.3	2.3	1.3	1.4	1.9	1.7	1.3
Mean Mesolithic (n = 5)				≤ 0.7	14.3	≥ 2.5	3.1	13.2	2.0	11.8
σ				0.2	1.3	1.2	1.1	2.2	1.0	1.4
Mean Early-/Mid Neolithic (n = 9)				0.5	16.6	2.7	3.2	11.9	4.5	12.2
σ				0.4	1.4	1.3	1.6	1.2	1.7	1.1
Mean Late Neolithic (n = 4)				0.4	12.2	2.0	3.0	11.5	2.7	11.8
σ				0.3	1.9	1.5	1.4	1.9	1.1	1.3

pendants having less elevated $\delta^{15}\text{N}$, though it narrowly fails to attain statistical significance ($p = 0.053$).

These analyses show that individuals without tooth pendants had, on average, higher $\delta^{15}\text{N}$ values. Individual 170 is a clear outlier from the group with pendants, with an average $\delta^{15}\text{N}$ of 15.7‰ during childhood. This male burial dates to the Middle Mesolithic (8159 ± 80 ^{14}C yr BP) and is notable for its impressive array of grave goods, including 167 animal teeth pendants from elk, wild boar and aurochs, ornaments and a headdress, suggesting this person may have been an important member of the community with high status (Zagorskis, 2004). (Note that, based on the early date, this individual was excluded from the analysis presented in Schulting et al. (2020)).

The $\delta^{13}\text{C}$ values in the group with pendants cluster into two distinct sets, with individuals 170 and 92 having less negative $\delta^{13}\text{C}$ values than the remaining individuals. As noted, individual 170 is an anomaly with elevated $\delta^{15}\text{N}$, whereas there is no distinguishing feature of individual 92 which marks it out from the other graves.

The mean adult $\delta^{15}\text{N}$ values, based on published data from Eriksson (2006) and Meadows et al. (2016), were found to be significantly different between individuals buried with and without tooth pendants, again excluding the Late Neolithic individuals (analysis of variance, $n = 30$, $F = 4.6$, $p = 0.041$). No significant difference in $\delta^{13}\text{C}$ was identified

(analysis of variance, $n = 30$, $F = 1.76$, $p = 0.195$), though the difference is in the direction expected given the negative relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in freshwater resources in this region (Fig. 2), i.e., those interred with pendants are higher in $\delta^{13}\text{C}$.

6. Discussion

6.1. Diet variability and life histories

There is a clear breastfeeding signal in infancy in most individuals, with elevated $\delta^{15}\text{N}$ in the first year (mean $\delta^{15}\text{N} = 14.6 \pm 0.4\text{‰}$). The small drop in $\delta^{15}\text{N}$ between the 0–6 month and 6–12 month age brackets suggests other foods began to be introduced to some individuals, but in general there was a consistent inclusion of breast milk between these two age brackets. The greater decline in $\delta^{15}\text{N}$ occurs between the 6–12 month and 12–20 month age brackets, although this is variable. This more substantial change indicates this was a period when breast milk began to be withdrawn, and continued to be withdrawn until completion of weaning around age 20–28 months. There is a large degree of variability between individuals in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values during infancy. This is either a reflection of different infant rearing strategies or a reflection of the mother/carer’s diet. Individuals 124 and 179

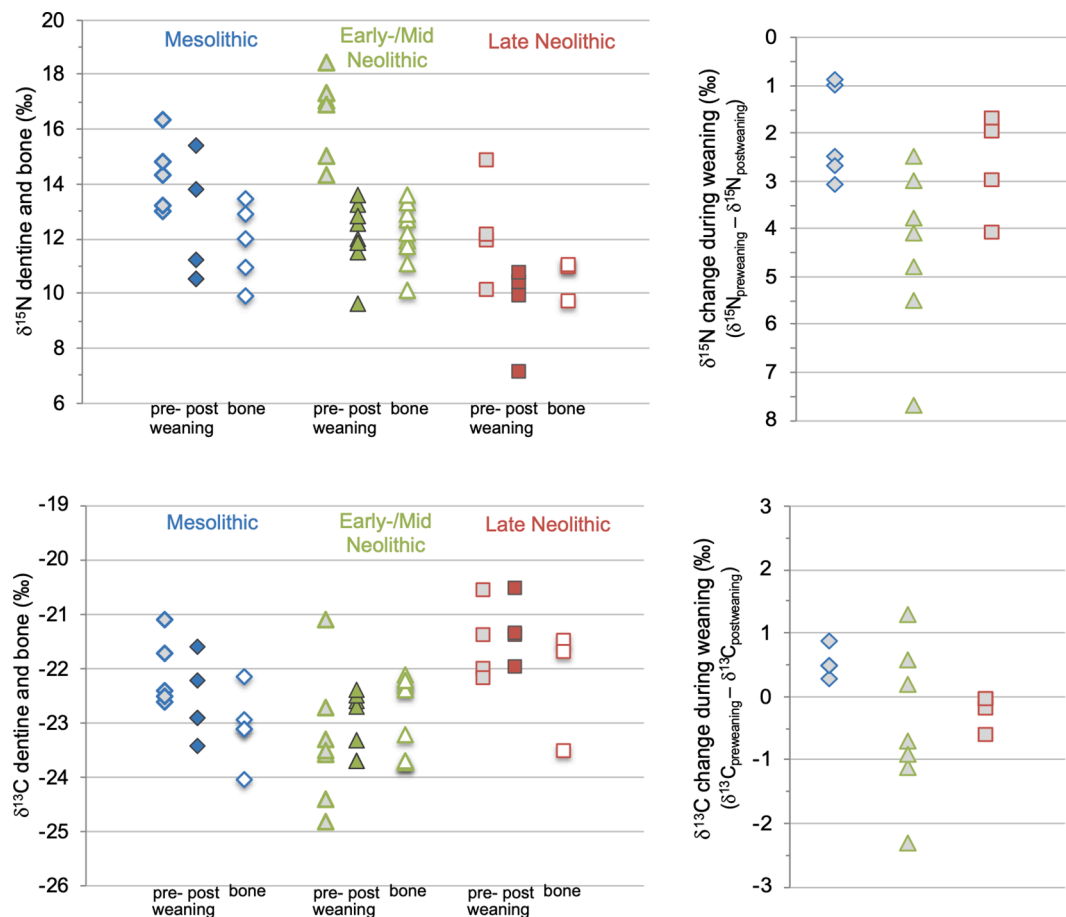


Fig. 5. Diachronic comparison of pre- and postweaning $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data: Pre- and postweaning dentine and bone $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of Mesolithic (blue), Early-/Mid Neolithic (green) and Late Neolithic (red) individuals (left). Change in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ during weaning (in ‰) ($\delta^{15}\text{N}_{\text{preweaning}} - \delta^{15}\text{N}_{\text{postweaning}}$) (right).

conversely show an elevation in $\delta^{15}\text{N}$ at this age, possibly implying the changes in these individuals had less to do with breastfeeding and more to do with switching to a diet much richer in aquatic foods. Other than breastmilk, aquatic foods are the most likely source of ^{15}N enriched values at this site.

A large degree of variability within groups has been observed across a series of weaning studies, although the methods used also vary and might contribute to some of the differences. For instance methods using bone from aged babies and children suffers from the dual problems of aging and that individuals who died early may not be representative of those who survived to adulthood (Sandberg et al., 2014). Waters-Rist et al. (2011) analysed long bone samples from non-adult individuals of various ages amongst Neolithic and Early Bronze Age hunter-gatherers at Lake Baikal. In many ways Neolithic Baikal presents a comparable population to that of Zvejnieki, with both sharing a strong focus on freshwater aquatic resources (though the isotope ecology of Lake Baikal differs). For the Neolithic group, it was found that isotopically visible weaning started late (1.0–1.5 yr) and was not complete until the age of 3.5–4.0 years. However, this is subject to the dual problems mentioned above. Using bone collagen of adults and bone and dentine collagen of children, Howcroft et al. (2014) found in their study of infant feeding practices at the Neolithic Pitted Ware Culture site of Ajvide on the island of Gotland a large variability in supplementary foods and the timing of their introduction, possibly related to season. Infants appear to have been weaned late at Ajvide, in the 3rd or 4th year of life (Howcroft et al., 2014). Based on isotopic composition of infants with approximately known age of death, Clayton et al. (2006) calculated breastfeeding for at least 1.5 years and weaning between 2 and 4 years in hunter-gatherer individuals with ample food sources at the Matjes River Rock Shelter

in South Africa.

Large variability in weaning practices was observed though sequential dentine sampling in a Central Californian hunter-gatherer population (ca. 3000–4000 years old), with some individuals weaned between 1 and 2, while others were not weaned until 5 or 6 years old (Eerkens and Bartelink, 2013). Scharlotta et al.'s (2018) results using sequential tooth dentine from Neolithic Baikal (including one of the same sites analysed by Waters-Rist et al. (2011)) found that the majority of individuals (60%, 18 of 28) showed what they termed 'abrupt' weaning, completed by age 2.5 yr. The remaining 10 individuals, however, showed a 'gradual' weaning pattern extending to age 4–5 yr, with a subset of these showing a more complicated pattern of an initial rapid drop followed by a slower decline in $\delta^{15}\text{N}$ values. As discussed by Scharlotta et al. (2018: 588), the interpretation of this latter group – comparable to that seen in some individuals at Zvejnieki – is not straightforward.

The pattern of increasing $\delta^{15}\text{N}$ from 0 to 6 months to 2 years in individuals 124 and 179 at Zvejnieki does not appear to present the usual 'weaning signal' and suggests instead the early introduction of high-trophic-level supplementary foods. The $\delta^{13}\text{C}$ values show an inverse 'mirroring' trend, which is entirely consistent with the consumption of freshwater aquatic resources. In these two cases, there may be a link between pottery and aquatic resources as a weaning food. This negative correlation between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, also shown clearly in individuals 154, 173 and 207, is presumably tracking variable consumption of freshwater fish.

Relatively high $\delta^{15}\text{N}$ values for individuals dating from the Mesolithic to Middle Neolithic suggest a variable, but overall important contribution of aquatic foods. This is further confirmed by the presence

of a freshwater reservoir offset in the radiocarbon dates on humans (Meadows et al., 2016). The results from previously analysed individual 165 indicate that after weaning the highest $\delta^{15}\text{N}$ was from the M3 (representing the 10–16 year age bracket, 13.4‰), becoming less elevated in the adult skull bone (representing 20–35 years, 12‰) (Eriksson, 2007). This individual, however, is a known outlier so that this pattern is unlikely to be typical of the population.

One other observation is the general pattern of elevated $\delta^{15}\text{N}$ in early infancy, dropping to below adult values in childhood, before once again rising. This trend has been seen across multiple populations from different time periods and varying socio-economic backgrounds (Beaumont et al., 2012; Eerkens et al., 2011; Fernández-Crespo et al., 2018; Henderson et al., 2014; Trueman et al., 2006), suggesting a physiological cause. The decrease in $\delta^{15}\text{N}$ during childhood may reflect the higher nutrient requirements of growing children compared to adults, that may in some circumstances lead to a nitrogen deficit and thus reduced isotope fractionation (Schurr, 1998). If nitrogen (protein) is limiting, fractionation of $^{15}\text{N}/^{14}\text{N}$ is less expressed, and thus the body's nitrogen pool has lower $\delta^{15}\text{N}$. This also raises a question about how weaning is interpreted, if the lowest point of this decline in $\delta^{15}\text{N}$ is interpreted as a sign of late weaning rather than physiological causes.

6.2. Dietary change over time

The results show no difference in the age of the onset of weaning or its cessation before and after the introduction of pottery. What variation there is, does not appear to be related to time or cultural period (Table 1). Hence there is no evidence that the introduction of pottery in the Early Neolithic led to a shortening of the weaning period, nor indeed is there any clear impact from the introduction of domestic plants and animals in the Late Neolithic in this regard. The limited difference in $\delta^{15}\text{N}$ between the Mesolithic and Early/Middle Neolithic, suggests little change in the kinds of supplementary foods used following the introduction of pottery. This is particularly interesting in that it runs contrary to the idea that the use of ceramics in hunter-gatherer contexts was primarily related to the more efficient exploitation of aquatic resources (Gibbs et al., 2017; Lucquin et al., 2016; Craig et al., 2013) and facilitated the preparation of weaning foods (Buikstra et al., 1986; Molleson, 1994). An ethnographic study of preindustrial populations that explored the hypothesis that weaning occurred earlier in agricultural and pastoral populations suggested that breastfeeding was longer in hunting and gathering groups, but that this was not explained by a lack of appropriate weaning foods (Sellen and Smay, 2001).

Considering childhood after the weaning period, the results indicate that, excluding the Middle Mesolithic individual, the post-weaning mean $\delta^{15}\text{N}$ for the Mesolithic is lower than for the Early/Mid Neolithic. The high $\delta^{15}\text{N}$ indicates they may have been consuming higher trophic level foods, likely to reflect a diet with a greater contribution of fish. This elevation is also reflected in the infants, which given they were consuming breastmilk is likely to reflect the ^{15}N -enriched foods consumed by their mothers or carers, typical of the community as a whole. By comparison the Late Neolithic children have the least elevated $\delta^{15}\text{N}$, reflecting a shift towards subsistence practices based on agricultural production rather than hunting and gathering. They also show less variability post-weaning, consistent with an isotopically more homogeneous diet.

6.2.1. Grave goods and diet

Statistical analysis of the data show that there is a link between the distribution of $\delta^{15}\text{N}$, which differ significantly between those with and without pendants in their graves. Individuals without tooth pendants consumed diets with a greater contribution of freshwater foods during their childhoods than those who were buried with pendants. The predominance of freshwater animals in the associated settlement, including 8427 fish remains from species such as perch, bream, pike and tench (Lõugas, 2006), indicates these foods were readily available and

freshwater resources might have been consumed by those with a lower, or at least different, social status. This suggests children were born into a family group, or perhaps a stratum of society, which influenced what foods were available to them. Whatever factor caused this dietary difference stayed with the individual throughout his or her life, influencing the artefacts with which they were interred.

One possibility is that children had different diets depending on the role their parents had in the group. For example, children whose parents were skilled fishermen might be more likely to consume freshwater fish, while children whose parents relied strongly on hunting would be more likely to consume larger quantities of elk, game and foraged foods. The different roles of adults within the community would therefore have affected childhood experiences by association. The difference between infants buried with and without pendants could be explained by the contribution of milk from their mothers. The mothers in those families that emphasised hunting and those that emphasised fishing could have produced milk which reflected their diets, which in turn would have influenced their infants' diets. Hunting and fishing are being emphasised here as they would have contributed far more protein than the plant foods that no doubt were gathered by all families, and hence dominate the isotopic signals. That said, the fact that the differences are seen mainly in $\delta^{15}\text{N}$ rather than $\delta^{13}\text{C}$ could suggest that a contribution from plant foods is blurring the freshwater signal, though the isotopic difference between aquatic and terrestrial foods is also greater for the former than the latter (Fig. 2).

The presence or absence of pendants, and the observations that their isotopic distributions differ significantly, may reflect different roles within the population, as hunters may have worn the trophies of their hunts as symbols of their hunting prowess. In this scenario, individuals from hunting families were buried with tooth and bone pendants, while families of individuals whose main skill or role was in fishing were buried without such pendants. While freshwater fish remains were not recovered frequently in the cemetery, they were present in vast numbers in the settlement area. Faunal remains recovered from the settlement are thought to represent species consumed, while those from the cemetery had a symbolic meaning (Lõugas, 2006). This suggests terrestrial animals and hunting were more highly valued, perhaps suggesting people who hunted also had a different standing within the community (Schulting et al., 2020). It cannot be argued that those lacking animal tooth pendants had low status, as other grave goods have been found in some of their graves, including spearheads, arrowheads, tools, amber, pottery and lithic flakes. Most graves also contained red ochre.

A further consideration is that children may have influenced their own diets from an early age. Children in hunter-gatherer groups often collect food, help adults prepare foods, or take a role in adult subsistence activities (Bird and Bliege Bird, 2000). It also seems clear from the care taken in their graves that children were valued by their communities, as they were buried alongside adults in the cemetery, laid out in their fine clothes, with their own grave goods (Larsson, 2006).

7. Conclusion

We analysed carbon and nitrogen stable isotopes in first molar dentine sequences from individuals recovered at the Mesolithic/Neolithic cemetery of Zvejnieki, Latvia in order to investigate childhood diet and weaning age, population variability, changes over time and whether there was a correlation between grave provisions and diet. We found that in general, individuals were 'exclusively' breastfed from birth until about 6–12 months (i.e., lacking isotopic evidence of weaning), whereafter breastmilk was completely withdrawn by the age of 3 years. There was a large degree of variability in weaning patterns within the population, suggesting either different weaning strategies or differences in the mothers' diets. Childhood diet, in the earlier phases of life at least, was derived from high-trophic-level food sources which had a terrestrial and freshwater component. We found no difference across the cultural periods in the onset or cessation of weaning, either with the introduction

of pottery or of domestic animals, though the high degree of inter-individual variability means that a larger sample size would be required to assess this more robustly. The limited differences in $\delta^{15}\text{N}$ between the Mesolithic and Early/Middle Neolithic do not provide strong support for the idea that the introduction of freshwater aquatic resources as a weaning food in the latter periods depended on the introduction of pottery.

There was a difference in the childhood and adult diet of those who were buried, as adults, with and without bone and tooth pendants. While all individuals at Zvejnieki consumed freshwater resources, those buried with animal tooth pendants had lower $\delta^{15}\text{N}$ values on average, suggesting that they consumed proportionally more terrestrial game compared to those interred without such pendants. The status or identity of people at Zvejnieki appears to have been ascribed from birth and particular economic roles within society, emphasising fishing or hunting, might have been filled by people born into families specialising in these activities.

CRedit authorship contribution statement

Rowena C. Henderson: Writing – original draft, Investigation. **Gunita Zariņa:** Resources. **Andrea Czermak:** Investigation, Writing – review & editing. **Rick J. Schulting:** Writing – review & editing. **Peter A. Henderson:** Formal analysis. **Dardega Legzdina:** Investigation. **Ilga Zagorska:** Resources. **Julia Lee-Thorp:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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