Crowder Kayla (Orcid ID: 0000-0002-0861-6489)

Childhood "stress" and stable isotope life-histories in Transylvania

Kayla D. Crowder [1], Janet Montgomery [1], Darren R. Gröcke [2], Kori L. Filipek[1,3]

[1] Department of Archaeology, Durham University, South Road, Durham, DH13LE, UK

k.d.crowder@durham.ac.uk, janet.montgomery@durham.ac.uk,

[2] Stable Isotope Biogeochemistry Laboratory, Durham University, South Road, Durham, DH13LE, UK

d.r.grocke@durham.ac.uk

[3] Transylvania Bioarchaeology, Durham, UK

k.l.filipek-ogden@durham.ac.uk

Corresponding Author: Kayla Diane Crowder, Department of Archaeology, Durham University, South Road, Durham, DH13LE, United Kingdom

Email: k.d.crowder@durham.ac.uk Phone: +44(0)79 56 646698

Key Words: carbon, nitrogen, incremental dentine, Gepid Kingdom, Romania, Hânsuri

Abstract

Aims and Objectives: Macroscopic skeletal analysis and stable carbon and nitrogen isotope analyses were employed to examine the relationship between skeletal "stress" lesions and changes in the isotopic life-history profiles of six non-adults from the Gepid population buried at the Archiud "Hânsuri" Cemetery (4th–7th Cent AD).

Materials and Methods: The Gepids were a migratory barbarian population in the Carpathian Mountain basin of Transylvania, Romania. Macroscopic skeletal analysis was conducted on 32 individuals and of those, six non-adults were selected for stable carbon and nitrogen isotope analysis of incrementally sampled dentine.

Results: Macroscopic skeletal analyses revealed 47% of the analysed population displayed evidence of childhood stress. Stable carbon and nitrogen isotope ranges were -17.7 to -11.8‰ for $\delta^{13}C$ and 9.4 to 15.1‰ for $\delta^{15}N$.

Discussion: The overall dietary profile indicates a mixed terrestrial diet (C_3/C_4) with increased consumption of C_4 plants during adolescence. The six non-adults appear to have been breastfed from one to six months and weaned by three years of age. High $\delta^{15}N$ values seen in pre- and post-natal increments may suggest a level of nutritional/physiological stress during gestation, and during the transitions from umbilical nutrients, breastmilk, and weaning foods. Although limited by the small sample size, this study supports the link between elevated $\delta^{15}N$ values and nutritional stress, the relationship and timing of skeletal lesions with changes in the isotope profiles and was among the first to combine palaeopathological analyses and incremental stable isotope analyses on the Transylvania Gepids.

1. Introduction

The application of stable isotope analyses has proven to be an effective tool to reconstruct the diets and behaviour of past populations (Schoeninger, DeNiro and Tauber, 1983). Stable isotope analysis of various body tissues (e.g., bone, enamel, dentine, hair, nails) has shown that diet and nutritional status are recorded while the tissue forms (Ambrose, 1990; Balasse, Bocherens and Mariotti, 1999; D'Ortenzio *et al.*, 2015). One of the most reliable mediums for archaeological isotope analysis are teeth because they preserve well in the archaeological record and are less susceptible to alteration from the burial environment (Hillson, 1996; Waldron, 2009). Primary dentine, the inner portion of a tooth, is secreted sequentially, at a rate of approximately 3–5 µm per day, is fully mineralised within 5–8 days (Schour and Poncher, 1937; Kawasaki, Tanaka and Ishikawa, 1980; Dean and Scandrett, 1995), and records changes in the carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values. With this, defined dentine increments correlate to periods of life (Beaumont *et al.*, 2013; Burt and Garvie-Lok, 2013). Advances in micro-sampling techniques now allow for variations in δ^{15}N and δ^{13}C to be seen sub-annually during development.

Certain variations within an isotopic life-history profile can be attributed to changes that person underwent during life. Fluctuations in micro-sampling profiles have been linked to dietary changes, migration, physiological stress, metabolic disorders and/or nutritional deficiency. A theoretical illustration showing the possible changes one might see in an isotopic life-history profile is shown in Figure 1. The shift from umbilical nutrients, breastmilk, and the introduction and cessation of weaning foods, can be tracked by the characteristic curve (Fuller *et al.*, 2006; Beaumont *et al.*, 2015; Reynard and Tuross, 2015; King *et al.*, 2018). The values of the infant are expected to be similar to the mother's at the time of birth (in-utero increment reflecting the mother's diet), increase a trophic level (2–5‰ for δ^{15}N and ~1‰ for δ^{13}C) above the mother's values during breastfeeding, and gradually drop down as weaning foods were introduced, until the infant's values finally fall within range of the adult values when weaning is complete (Fuller *et. al.*, 2006; Beaumont *et al.*, 2015; Reynard and Tuross, 2015; King *et al.*, 2018). The increase in δ^{15}N values during breastfeeding reflects the consumption of the mother's milk, making the infant appear to be in a higher order than the mother. Although profiles can follow a population-level trajectory, the details of each profile can provide a glimpse into a person's unique experience.

Carbon isotope ratios provide an insight to different types of food and can distinguish between marine, terrestrial or mixed dietary resources (Schoeninger, DeNiro and Tauber, 1983; Schoeninger and DeNiro, 1984; Privat, O'Connell and Hedges, 2007) (Appendix S1). There is a ~1‰ increase in δ^{13}C values between the diet and skeletal tissue values (Fuller *et al.*, 2006). Plants use two pathways to photosynthesize, C_3 (Calvin) and C_4 (Hatch-Slack), which result in differential δ^{13}C values (Schwarcz and Schoeninger, 1991; Fogel and Cifuentes, 1993). Carbon isotope ratios (δ^{13}C) provide information as the type of resources being consumed. Arid-adapted and tropical plants (e.g., sorghum, maize, sugar cane, millet) typically use a C_4 photosynthetic cycle, and exhibit a δ^{13}C range between -16 ‰ to -8 ‰ (Schoeninger and DeNiro, 1984; Mekota *et al.*, 2006; Sharp, 2017). Most other temperate plants (e.g., oats, fruit, vegetables, legumes, barley) use a C_3 photosynthetic cycle and exhibit a δ^{13}C range between -33 ‰ to -22 ‰ (Schoeninger and DeNiro, 1984; Sharp, 2017). Marine plant values fall between C_3 and C_4 plants and have a δ^{13}C range from -22 to -16‰ (Schwarcz and Schoeninger, 1991; Sharp, 2017). Stable nitrogen isotopes from archaeological remains provide information with respect to the protein component of an individual's diet, and reflect the individual's position within the food chain (Hedges and Reynard, 2007). A change in diet and/or move to a higher order results in a trophic level shift (2–5‰ increase) in $\delta^{15}N$ values (Hedges and Reynard, 2007). Combining the $\delta^{13}C$ and $\delta^{15}N$ values allows for specific types of food to be distinguished (i.e., fresh water vs. sea water plants) (Sealy *et al.*, 1987).

In addition to providing insight into breastfeeding and weaning practices and dietary patterns, stable carbon and nitrogen isotope analysis of incremental dentine can also reveal periods of physiological stress (Fuller *et al.*, 2005; Mekota *et al.*, 2006; Beaumont and Montgomery, 2016). The human body is considered to be at a metabolic equilibrium when there is a balance between anabolic processes (tissue building) and catabolic processes (tissue breakdown) (Fuller *et al.*, 2005; Hatch *et al.*, 2006; Mekota *et al.*, 2006; D'Ortenzio *et al.*, 2015). In an anabolic state, the body builds biomolecules through metabolic pathways and the individual is in a positive nitrogen balance (Fuller *et al.*, 2005; Mekota *et al.*, 2006; Little, Ramachandran and Schindeler, 2007). This typically occurs during periods of growth (Katzenberg and Lovell, 1999) and may also represent recovery or catch-up growth when sufficient nutrients were being introduced as the body recovers from a nutritional imbalance (Hatch *et al.*, 2006; Mekota *et al.*, 2006). When an individual is experiencing physiological stress (metabolic disorders, malnutrition, starvation, fasting), the body consumes stored protein (negative nitrogen balance), which presents as a trophic level increase (Hatch *et al.*, 2006; Mekota *et al.*, 2006; Little, Ramachandran and Schindeler, 2007). The body begins to breaks down stored biomolecules for energy, causing the remaining tissues to be enriched in ^{15}N, resulting in increased $\delta^{15}N$ values (Fuller *et al.*, 2005; Reitsema and McIlvaine, 2014; D'Ortenzio *et al.*, 2015).

In clinical studies, a catabolic event manifests in bodily tissues approximately 6–12 days after the metabolic balance was disrupted (Beisel, 1975, 1977; D'Ortenzio *et al.*, 2015). Within bioarchaeology, a prolonged catabolic event ("stress") can manifest as lesions in bone and dentition. In this paper, "stress" lesions, were defined as skeletal changes commonly associated with metabolic disorders, under- and malnourishment, and non-specific stress (see Appendix S1 for lesion descriptions). Metabolic deficiencies like scurvy and rickets can be identified macroscopically in skeletal tissues with a degree of confidence (Brickley and Ives, 2010). Cribra orbitalia (CO), porotic hyperostosis (PH) and enamel hypoplasia (EH) can be caused by multiple aetiologies, including those associated with nutritional imbalance (Zukerman, Turner and Armelagos, 2012). The position of EH (disruption in the formation of enamel striae) corresponds to an approximate age during life, which allows research to estimate when the stress event (arrested growth/tissue formation) occurred (Goodman, Armelagos and Rose, 1980, 1984). With the exception of EH, the other lesions can only be classified as active (new woven bone formation), healing (mixed woven and lamellar bone), or healed (organised lamellar bone) at the time of death. It is not yet possible to determine when the insult took place or how long it lasted with macroscopic analysis alone. Non-adults who died while their dentition was still forming are more likely to have isotopic changes relating to healing or active stress lesions.

There are methodological limitations that must be considered when conducting macroscopic analysis. New woven bone on non-adult skeletons can be due to normal growth or pathology, and is still very difficult to distinguish on a macroscopic level (Mann and Murphy, 1990; Ribot and Roberts, 1996). Wood and colleagues (1992) emphasized that those individuals without skeletal lesions aren't necessarily the healthy individuals, rather they may have died before lesions could manifest, and those with lesions survived long enough to exhibit skeletal changes.

Do individuals with catabolic signals in the isotope profile also have skeletal evidence of stress? Will the catabolic change in the profile correspond to hypoplastic enamel defects? To answer these questions, non-adults from a population with a high prevalence of skeletal stress lesions were analysed to examine the possible relationship between stress lesions and changes in the isotope life–history profiles.

The fall of the Roman Empire and the onset of the Migration Period (4th–6th Centuries AD) is characterized by invasions and settlements over large areas by various 'barbarian' groups, with very few examples of the kind of monumental burial structures typical of the Roman landscape in Europe (Cunliffe, 2001; Bogucki and Crabtree, 2003; Curta, 2010; Alcock and Austin, 2013). The vast majority of data from these migratory populations is derived primarily from small cemeteries, with burial goods slightly differing in style between Central and Eastern European regions. Sources detailing the cultural particulars of independent and interdependent migratory groups (e.g., Visigoths, Ostragoths, Huns, Avars, Gepids, Lombards) are often secondary, being written after the fact to detail the chronicles of the victors (e.g., Avars, Hungarians) or the impacts these groups had on the Roman Empire (Christie, 1998; Cunliffe, 2001; Bogucki and Crabtree, 2003; Pop and Nagler, 2010).

Despite accounts of Gepid occupation in the Transylvania region, hardly any settlements are known in the archaeological record. Resources that are available tend to focus on the spatial elements of human remains and certain types of burial goods; i.e., jewellery (Harhoiu, 2010; Kharalambieva, 2010; Pop and Nagler, 2010). Initially, the Gepids were thought to have migrated from the Baltic region, along with other barbarian groups such as the Visigoths and Ostrogoths and settled around and within Daco-Roman settlements. Originally the Gepids offered mercenary services (foederati) to the Roman Empire, and then occupied its fringes (Bogucki and Crabtree, 2004; Pop and Nagler, 2010; Djuvara, 2014). As the Roman Empire began to fall, the Gepids encroached on notable Roman buildings falling into disrepair. Because the Gepids reused Daco-Roman structures, obscuring their presence in the archaeological record, their cemeteries are used to gather biocultural data.

2. Materials and Methods

2.1. The Archiud "Hânsuri" *cemetery*

The site of Archiud "Hânsuri" is located in the north-eastern part of the Transylvania region of Romania (Figure 2). Excavations at Archiud first began in 1964 after the discovery of a Celtic bracelet during agricultural works. Excavations continued for approximately 30 years uncovering an expansive multi-period complex with associated necropoli ranging from the Eneolithic (2500 BC) through to the 6th–7th Centuries AD (Gaiu, 1999; Marinescu, 2003). The Gepid cemetery at Archiud revealed 61 discrete burial pits, with single or multiple inhumations. The cemetery was dated stylistically through burial goods and the inclusion of a coin from Constantius II (337–361 AD), giving a *terminus post quem* date of the 4th Century AD. Other burial goods such as pottery, weapons, and jewellery further differentiate the burials into two discrete assemblages from the 4th–5th Centuries AD and the 6th–7th Centuries AD (Gaiu, 1999; Marinescu, 2003). There were documented periods of starvation and famine in Central and Eastern Europe during this time (Todd, 1995). Given the historically documented period of unrest during the Gepid occupation, it was hypothesized that this population will have a high prevalence of skeletal lesions associated with stress. The aim of this study was to integrate incremental dentine stable isotope analysis with macroscopic skeletal analysis of human skeletal remains to ascertain whether stress lesions on the skeleton are associated with changes in the isotopic life-history profiles. The aim was delivered through the following objectives: determine overall dietary patterns; detect the duration of the breastfeeding and weaning; and to establish isotopically the onset and duration of catabolic events.

Skeletal analyses were conducted in conjunction with the Transylvania Bioarchaeology field school in Cluj-Napoca, Romania between 2013–2015 (Filipek, 2017). Due to agricultural works on site previous to excavation, the preservation of the remains was suboptimal, preventing a comprehensive examination of all the Gepidic individuals. All analyses and sampling were conducted per the BABAO Code of Ethics and Conduct (BABAO Working Group for Ethics and Practice, 2010). Pathological skeletal analyses follow the BABAO standards (Roberts and Connell, 2004) (Appendix S1). As non-adults are largely considered a sensitive barometer of cultural stress (Mays *et al.*, 2017; Inglis and Halcrow, 2018), this study focused on the six non-adult individuals to examine early-life stress of the "non–survivors." Tooth samples from the six non-adults were selected for stable isotope analysis to evaluate possible links to the non-specific indicators of childhood 'stress' (Table 1). Within this study, the category of non-adult was defined as individuals estimated to be ≤16 years at the time of death, based on dental formation (AlQahtani, Hector and Liversidge, 2010). Several individuals died before tooth formation was completed and their teeth are therefore more likely to capture the catabolic changes that may correlate to active stress lesions in the skeleton. The limited sample size is acknowledged and will be considered when interpreting the results of this research.

2.2. Carbon and Nitrogen Stable Isotope Analysis

Two teeth were sampled from each of the six non-adult individuals to produce a longer life-history. Deciduous teeth begin forming in utero (0.3 years prior to birth) and can be used to determine foetal values (Hillson, 1996; Beaumont and Montgomery, 2015). The foetal values act as a proxy for the mother's values during pregnancy (Beaumont and Montgomery, 2015). The mean of the foetal values acted as a proxy for the pregnant female mean (PFM). Deciduous dentition and the first permanent molar will capture the transition from umbilical nutrients, breastmilk (if consumed), and the introduction of weaning foods (Moorrees, Fanning and Edward E. Hunt, 1963; AlQahtani, Hector and Liversidge, 2010). The second permanent molar is completed at approximately 15–16 years of age, which enabled increments to be taken up to the time of death, based on the non-adult age limit within this study (Hillson, 1996; AlQahtani, Hector and Liversidge, 2010).

Sample preparation followed Method 2 from Beaumont et al. (2013) (see Appendix S1 for complete methodology). Stable carbon and nitrogen isotope analyses were conducted on consecutive sections of human dentine at the Stable Isotope Biogeochemistry Laboratory at the Department of Archaeology (Durham University). The samples were analysed on a Thermo Delta V Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS). This machine measures isotope ratios with an uncertainty of 0.2‰ (1sd) (Meier-Augenstein and Kemp, 2009).

3. Results

3.1. Macroscopic Skeletal Analysis

Of the individuals that were suitable for osteological analysis, approximately 47% (15/ 32), showed one or more childhood non-specific indicators of "stress", including CO, non-adult endocranial lesions and EH. Additionally, 28% (9/32) showed either active or residual lesions consistent with metabolic deficiencies (e.g., scurvy, rickets). Non-adults have the highest prevalence of enamel hypoplasia, porotic hyperostosis, and nutritional deficiencies (Filipek, 2017). The six non-adult individuals selected for isotopic analysis were estimated to be approximately 16 years or younger at the time of death, and have skeletal lesions associated with “stress” (see Table 1).

3.2. *Carbon and Nitrogen Stable Isotope Analysis*

The results of the carbon and nitrogen stable isotope analyses are plotted in Figure 3. Collagen should meet accepted quality parameters to verify adequate preservation, i.e. carbon to nitrogen ratios (C:N) are expected to be between 2.9–3.6 (Schoeninger and DeNiro, 1984), and collagen yields greater than 1% (Van Klinken, 1999). All samples met the parameters for well-preserved collagen. The δ^{15}N values range from 9.4 to 15.1‰ with a mean value of 11.4 ± 1.5 ‰ (1σ, n = 145). The δ^{13}C values range from -17.7 to -11.8‰ with a mean value of -15.2 ± 1.2 ‰ (1σ, n = 145). The complete data set can be found in the supplementary data file.

4. Discussion

4.1. *Subsistence*

The plotted data revealed two linear trajectories (Figure 3). The first, rising diagonally from left to right, showing positive correlation between δ^{15}N and δ^{13}C, and the second (horizontal) trajectory, indicating a variation in δ^{13}C only. Isotope data points from the same individual appear in both trajectories. A portion of group 1 appears to be a trophic level above group 2. This could be due to increased δ^{15}N values during breastfeeding (Jay *et al.*, 2008) and/or the relationship between increases in the δ^{15}N values and physiological stress (Hatch *et al.*, 2006; Mekota *et al.*, 2006). Group 2 (horizontal trajectory) appears to have a wider range of dietary δ^{13}C, with increased consumption of terrestrial C_4 plants, which is consistent with supplementary “famine foods” such as millet, that were documented in this region (Thompson, 1982). Although some isotope data points fall within the marine isotope range, this pattern could be the result of C_4 plant consumption and elevated δ^{15}N values (possibly as a result of breastfeeding or stress). Therefore, the overall subsistence was presumably a diet of mixed C_3 and C_4 terrestrial resources. The sub-annual life-history profiles will aid in determining the differential causes for this case.

To examine the overall dietary patterns across the life-course, each isotope data point was assigned the approximate age of formation during life (Beaumont and Montgomery, 2015). Figure 4 demonstrates patterns in age-related changes of the isotope profiles. The δ^{13}C profile (Figure 4b) supports the interpretation of a mixed terrestrial diet, with increased consumption of C_4 plants during the time the adolescent age increments were forming. As previously discussed, the PFM reflects the average values of the in-utero increments (13.9‰ for δ^{15}N, -15.6‰ for δ^{13}C). The δ^{15}N profile (Figure 4a) does not track the standard breastfeeding/weaning curve. Rather than starting at the PFM and increasing a trophic level during breastfeeding, the infant values match that of the PFM. One explanation for the variation in the δ^{15}N profile may be due to the inherent difference between the adult female mean and the PFM (see Appendix S1). Pregnancy takes a large toll on the body even when the mother is not experiencing additional physiological or nutritional stress (Fuller *et al.*, 2006). Because incremental dentine allows for a more defined temporal resolution, it is possible that the high PFM (δ^{15}N) is normal, but usually not seen in bone collagen due to the slow turnover rate, resulting in an average value over many years (Meier-Augenstein, 2011). However, in both cases, whether the mother was under additional physiological stress or not, it is expected that an infant’s values would increase a trophic level above the mother’s during breastfeeding. It is possible the variation of each individual was obscured when the data was combined (Figure 4), which would be revealed in the individual life-history profiles.

4.2. Early Life-history Profiles

When the isotopic profiles of deciduous and permanent dentition overlap incremental age categories, the profiles of the incomplete dentition (still forming at the time of death) show elevated values (δ^{15}N up to 3.5‰ and δ^{13}C up to 1.6‰) compared to dentition closer to being complete, from the same time of life. Dentine mineralisation rates vary across teeth (crown vs root) and between tooth types (i.e., incisors vs molars, deciduous vs permanent) (Schour and Poncher, 1937; Dean and Scandrett, 1995). The varying number of depositional layers (of dentine) in each horizontal incremental section appears to result in varying isotopic values for the same period of life. The more complete dentition will have more depositional layers than incomplete dentition, resulting in differential values between teeth. Both profiles reflect dietary isotope values during life however, the values of the complete dentition will reflect more depositional layers compared to incomplete dentition.

Six isotopic life-history profiles were analysed to establish possible breastfeeding and weaning patterns (Figure 5). All six profiles differ from the typical breastfeeding and weaning curve (Figure 1). The variations between the profiles can be attributed to each person's unique experience (different stressors) and their ability to survive (hidden heterogeneity) (Wood *et al.*, 1992). If we assume all six individuals were breastfed, the shaded regions on each plot denote the probable duration of breastfeeding and weaning. Although the profiles do not increase a trophic level, as would be expected with the introduction of breastmilk, the slight increase after birth may reflect a breastfeeding signal being obscured by early life stress. The profiles suggest the individuals in this study were breastfed for the first ~1–6 months of life and the weaning process was completed between 2–3 years of age, which follows the historical medical recommendations during this period (Fulminante, 2015). Further discussion can be found in Appendix S1.

ArchM1 and ArchM73 both had healing and active skeletal lesions and survived into adolescence. They also had anabolic phases (possible skeletal growth) after weaning and consumed a moderate amount of C_4 resources during adolescence. Increased C_4 consumption during this period suggests the need for increased the caloric intake, possible due to increased stress and/or the adolescent growth spurt. It appears these individuals were able to survive the initial stress event during childhood however, early-life stress may have resulted in increased frailty, making them more susceptible to illness (Barker, 1998; Yaussy, DeWitte and Redfern, 2016; Roberts and Steckel, 2018). ArchM1, the only non-adult in this study from the earlier burial phase (4th–5th Centuries AD), was buried with a single-blade iron knife (pelvis region), in a triple inhumation (male, female, non-adult) with a charcoal lined grave floor, and animal remains in the grave, suggesting differential status compared to the others in this study. Strontium and oxygen isotopes were also measured, and while ArchM1 did fall within the proposed local range for the Transylvania Basin, they may have been from a slightly higher latitude and/or altitude than the Archiud site (Crowder, 2015), further supporting this possibility ArchM1 was of different socio-economic standing within the population.

When the occurrence of EH was plotted on the isotopic life-history profiles (Figure 5), the approximate age of the visible defect seems to occur when weaning was nearly complete or completed (ArchM41, ArchM69, ArchM73). The hypoplastic events could be the result of nutritional/physiological stress (Goodman and Rose, 1990) and/or the compounded stress of the weaning process (McElroy and Townsend, 1989). These data may also suggest a delay between the onset of the stress event in the isotope profile (increasing δ^{15}N values) and the estimated age of the visible defect in the enamel striae. The observed offset between the initial increase in the δ^{15}N profiles (onset of stress event) and the timing of EH (peak/end of stress event) was between 0.5–2 years. The variation in formation rates of dentition is reported to be between ±0.5–6 months (AlQahtani, Hector and Liversidge, 2010; Beaumont and Montgomery, 2015) and the approximate uncertainty for the timing of EH can be between ±1–6 months (Goodman and Song, 1999). This would suggest the perceived offset was not due to variation in formation rates alone. Due to the small sample size, additional research is required to confirm this observation. ArchM55, M69 and M84 all had active and/or healing skeletal lesions and periods of prolonged elevated δ^{15}N. The active skeletal lesions for ArchM55 and M69 correspond to elevated δ^{15}N values prior to death. If the same stress event caused the increased δ^{15}N values and the skeletal lesions, it would appear the onset of the stress event began around 1.5–2.5 years for ArchM55 and around 2.5 years for ArchM69 and continued until death. Although the sample size was small, this research offers promising insight into the relation between skeletal lesions and isotope life-history profiles.

5. Conclusions

The hypothesis, the Gepid population buried at the Archiud Cemetery will have a high prevalence of metabolic disorders and nutritional stress, was confirmed. These individuals were breastfed for ~1–6 months, completed the weaning process between 2–3 years and subsisted on a mixed terrestrial diet (C_3/C_4) supplemented with increased C_4 resources during adolescence to increase caloric intake during growth and/or stress. Active and healing skeletal stress lesions corresponded to elevated δ^{15}N profiles for four of the six individuals. The three individuals with EH all had increased δ^{15}N values around the time of the stress event. The perceived offset between the appearance and timing of the enamel defects from the changes in the isotopic profiles may suggest a delay between the onset of the stressor and the visible enamel defect. This ongoing research offers promising insights into deciphering anomalies in isotopic life-history profiles and the relationship between early life stress and the ability to thrive later in life.

Acknowledgements

The authors would like to thank Corneliu Gaiu (Complexul Muzeal Bisţria-Năsăud) and Transylvania Bioarchaeology for granting access to the collection. Thank you to Transylvania Bioarchaeology and the Stable Isotope Biogeochemistry Laboratory at Durham University for funding the carbon and nitrogen stable isotope analysis. We would also like to thank the two external reviewers for their insightful comments.

References

Alcock, L. and Austin, D. (2013) *From the Baltic to the Black Sea: studies in medieval archaeology*. Abingdon: Routledge.

AlQahtani, S. J., Hector, M. P. and Liversidge, H. M. (2010) ‘Brief communication: The London atlas of human tooth development and eruption’, *American Journal of Physical Anthropology*, 142(3), pp. 481–490. doi: 10.1002/ajpa.21258.

Ambrose, S. H. (1990) ‘Preparation and characterization of bone and tooth collagen for isotopic analysis’, *Journal of Archaeological Science*, 17(4), pp. 431–451. doi: 10.1016/0305-4403(90)90007-R.

BABAO Working Group for Ethics and Practice (2010) *British Association of Biological Anthropology and Osteoarchaeology Code of Ethics*. Available at: http://www.babao.org.uk/assets/Uploads-to-Web/code-of-ethics.pdf (Accessed: 16 February 2016).

Balasse, M., Bocherens, H. and Mariotti, A. (1999) ‘Composition Used as Evidence of a Change of Diet’, *Journal of Archaeological Science*, 26, pp. 593–598. doi: 10.1006/jasc.1998.0376.

Barker, D. J. P. (1998) ‘In utero programming of chronic disease’, *Clinical Science*, 95(2), pp. 115–128. doi: 10.1042/cs0950115.

Beaumont, J. *et al.* (2013) ‘Childhood diet: A closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine*’, *Archaeometry*, 55(2), pp. 277–295. doi: 10.1111/j.1475-4754.2012.00682.x.

Beaumont, J. *et al.* (2015) ‘Infant mortality and isotopic complexity: New approaches to stress, maternal health, and weaning’, *American Journal of Physical Anthropology*, 157(3), pp. 441–457. doi: 10.1002/ajpa.22736.

Beaumont, J. and Montgomery, J. (2015) ‘Oral histories: a simple method of assigning chronological age to isotopic values from human dentine collagen’, *Annals of Human Biology*, 4460(August), pp. 1–8. doi: 10.3109/03014460.2015.1045027.

Beaumont, J. and Montgomery, J. (2016) ‘The great irish famine: Identifying starvation in the tissues of victims using stable isotope analysis of bone and incremental dentine collagen’, *PLoS ONE*, 11(8), pp. 1–21. doi: 10.1371/journal.pone.0160065.

Beisel, W. R. (1975) ‘Metabolic Response to Infection’, *Annual Review of Medicine*, 26(1), pp. 9–20. doi: 10.1146/annurev.me.26.020175.000301.

Beisel, W. R. (1977) ‘Magnitude of the host nutritional responses to infection’, *The American Journal of Clinical Nutrition*, 30(8), pp. 1236–1247. doi: 10.1093/ajcn/30.8.1236.

Bogucki, P. and Crabtree, P. J. (2004) *Ancient Europe: Encyclopedia of the barbarian world (8000CB-AD1000) Vol 2*. Edited by P. Bogucki and P. J. Crabtree. New York: Charles Scribner’s Sons.

Bogucki, P. I. and Crabtree, P. J. (2003) *Ancient Europe 8000BC to AD 1000: encylopedia of the barbarian world*. Edited by P. I. Bogucki and P. J. Crabtree. New York: Charles Scribner’s Sons Publishing.

Brickley, M. and Ives, R. (2010) *The Bioarchaeology of Metabolic Bone Disease*. Edited by

M. Brickley and R. Ives. Oxford: Academic Press is an imprint of Elsevier Lt.

Burt, N. M. and Garvie-Lok, S. (2013) ‘A new method of dentine microsampling of deciduous teeth for stable isotope ratio analysis’, *Journal of Archaeological Science*. Elsevier Ltd, 40(11), pp. 3854–3864. doi: 10.1016/j.jas.2013.05.022.

Christie, N. (1998) *The Lombards*. Oxford: Blackwell Publishing, Inc.

Crowder, K. D. (2015) *Isotopic profiling of diet, health, and mobility amongst the non-adult Gepid population buried at the Archiud Cemetery in Transylvania, Romania (4th – 7th centuries AD)*. (unpublished master thesis, Durham University).

Cunliffe, B. (2001) *The Oxford Illustrated History of Prehistoric Europe*. Oxford: Oxford University Press.

Curta, F. (ed.) (2010) *Neglected Barbarians*. Turnhout: Brepols Publishing.

D’Ortenzio, L. *et al.* (2015) ‘You are not what you eat during physiological stress: Isotopic evaluation of human hair’, *American Journal of Physical Anthropology*, 157(3), pp. 374–388. doi: 10.1002/ajpa.22722.

Dean, M. C. and Scandrett, A. E. (1995) ‘Rates of dentin mineralization in permanent human teeth’, *International Journal of Osteoarchaeology*, 5(4), pp. 349–358.

Djuvara, N. (2014) *A brief illustrated history of Romanians*. Bucharest: Humanitas.

Filipek, K. (2017) ‘Osteological analyses of the Archiud Hânsuri cemetery’. Cluj-Napoca.

Fogel, M. L. and Cifuentes, L. A. (1993) ‘Isotope Fractionation during Primary Production’, in Engel, M. H. and Macko, S. A. (eds) *Organic Geochemistry: Principles and Applications*. 11th edn. Boston, MA: Springer US, pp. 73–98. doi: 10.1007/978-1-4615-2890-6_3.

Fuller, B. T. *et al.* (2005) ‘Nitrogen balance andδ15N: why you’re not what you eat during nutritional stress’, *Rapid Communications in Mass Spectrometry*, 19(18), pp. 2497–2506. doi: 10.1002/rcm.2090.

Fuller, B. T. *et al.* (2006) ‘Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios’, *American Journal of Physical Anthropology*, 129(2), pp. 279–293. doi: 10.1002/ajpa.20249.

Fuller, B. T. *et al.* (2006) ‘Isotopic evidence for breastfeeding and possible adult dietary differences from Late/Sub-Roman Britain’, *American Journal of Physical Anthropology*, 129(1), pp. 45–54. doi: 10.1002/ajpa.20244.

Fulminante, F. (2015) ‘Infant Feeding Practices in Europe and the Mediterranean from Prehistory to the Middle Ages: A Comparison between the Historical Sources and Bioarchaeology’, *Childhood in the Past*, 8(1), pp. 24–47. doi: 10.1179/1758571615Z.00000000026.

Gaiu, C. (1999) ‘Habitat si manifestari rituale în secolul IV p. Chr. la Archiud’, *Revista Bistritei*, 12/13, pp. 267–316.

Goodman, A. H., Armelagos, G. J. and Rose, J. C. (1980) ‘Enamel Hypoplasias as Indicators of Stress in Three Prehistoric Populations from Illinois’, *Human Biology*, 52(3), pp. 515–528.

Goodman, A. H., Armelagos, G. J. and Rose, J. C. (1984) ‘The chronological distribution of enamel hypoplasias from prehistoric dickson mounds populations’, *American Journal of*

Physical Anthropology, 65(3), pp. 259–266. doi: 10.1002/ajpa.1330650305.

Goodman, A. H. and Rose, J. C. (1990) ‘Assessment of systematic physiological perturbations from dental enamel hypoplasias and associated histological structures’, *American Journal of Physical Anthropology*, 33(S11), pp. 59–110.

Goodman, A. H. and Song, R.-J. (1999) ‘Sources of variation in estimated ages at formation of linear enamel hypoplasias’, *Cambridge Studies in Biological and Evolutionary Anthropology*, pp. 210–240.

Harhoiu, R. (2010) ‘Where did all the Gepids go? A Sixth-to Seventh-Century Cemetery in Bratei (Romania)’, in Curta, F. (ed.) *Neglected Barbarians*. Bratei, RO: Brepols, pp. 209–244.

Hatch, K. *et al.* (2006) ‘An objective means of diagnosing anorexia nervosa and bulimia nervosa using 15N/14N and 13C/12C ratios in hair’, *Rapid communications in mass spectrometry : RCM*, 20, pp. 3367–3373. doi: 10.1002/rcm.2740 An.

Hedges, R. E. M. and Reynard, L. M. (2007) ‘Nitrogen isotopes and the trophic level of humans in archaeology’, *Journal of Archaeological Science*, 34(8), pp. 1240–1251. doi: 10.1016/j.jas.2006.10.015.

Hillson, S. (1996) *Dental Anthropology*. Cambridge: Cambridge University Press.

Inglis, R. M. and Halcrow, S. E. (2018) ‘The Bioarchaeology of Childhood: Theoretical Development in the Field’, in Beauchesne, P. and Agarwal, S. C. (eds) *Children and Childhood in the Bioarchaeology*. Project MU. Gainsville: University Press of Florida, pp. 33–60.

Jay, M. *et al.* (2008) ‘Iron age breastfeeding practices in Britain: Isotopic evidence from wetwang slack, east yorkshire’, *American Journal of Physical Anthropology*, 136(3), pp. 327–337. doi: 10.1002/ajpa.20815.

Katzenberg, M. and Lovell, N. (1999) ‘Stable Isotope Variation in Pathological Bone’, *International Journal of Osteoarchaeology*, 9(May), pp. 316–324. doi: 10.1002/(SICI)1099-1212(199909/10)9:5<316::AID-OA500>3.0.CO;2-D.

Kawasaki, K., Tanaka, S. and Ishikawa, T. (1980) ‘On the daily incremental lines in human dentine’, *Archives of Oral Biology*, 24, pp. 939–943.

Kharalambieva, A. (2010) ‘Gepids in the Balkans: A survey of the archaeological evidence’, in Curta, F. (ed.) *Neglected Barbarians*. Turnhout: Brepols Publishing, pp. 245–261.

King, C. L. *et al.* (2018) ‘Let’s talk about stress, baby! Infant-feeding practices and stress in the ancient Atacama desert, Northern Chile’, *American Journal of Physical Anthropology*, (November 2017), pp. 1–17. doi: 10.1002/ajpa.23411.

Van Klinken, G. J. (1999) ‘Bone Collagen Quality Indicators for Palaeodietary and Radiocarbon Measurements’, *Journal of Archaeological Science*, 26, pp. 687–695.

Little, D. G., Ramachandran, M. and Schindeler, A. (2007) ‘The anabolic and catabolic responses in bone repair’, *J Bone Joint Surg Br*, 89–B(4), pp. 425–433. doi: 10.1302/0301-620x.89b4.18301.

Mann, R. and Murphy, S. (1990) *Regional Atlas of Bone Disease. A Guide to Pathological and Abnormal Variation in the Skeleton*. Springfield Illinois: Charles Thomas.

Marinescu, G. (2003) ‘Sapaturile de la Archiud “Hansuri” Comuna Teaca, Judetul Bistrita-Nasaud’, in Cosbuc, G. (ed.) *Cecetari Arheologice in Judetul Bistrita-Nasaud vol 1*. Bistrita: Complexul Muzeal Judetean Bistrita-Nasaud, pp. 251–256.

Mays, S. *et al.* (2017) ‘Child Bioarchaeology: Perspectives on the Past 10 Years’, *Childhood in the Past*. Taylor & Francis, 10(1), pp. 38–56. doi: 10.1080/17585716.2017.1301066.

McElroy, A. and Townsend, P. (1989) *Medical anthropology in ecological perspective*. Boulder: Westview Press.

Meier-Augenstein, W. (2011) *Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis*. Wiley (Developments in Forensic Science).

Meier-Augenstein, W. and Kemp, H. (2009) ‘Stable Isotope Analysis : General Principles and’, *Wiley Encyclopedia of Forensic Science*, 13, p. 3104. doi: 10.1002/9780470061589.fsa1041.

Mekota, A.-M. *et al.* (2006) ‘Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa’, *Rapid Communications in Mass Spectrometry*, 20(10), pp. 1604–1610. doi: 10.1002/rcm.2477.

Moorrees, C. F. A., Fanning, E. A. and Edward E. Hunt, jr (1963) ‘Age Variation of Formation Stages for Ten Permanent Teeth’, *Journal of Dental Research*. SAGE Publications Inc, 42(6), pp. 1490–1502. doi: 10.1177/00220345630420062701.

Pop, I. and Nagler, T. (2010) *The history of Transylvania: Vol I (until 1541)*. Cluj-Napoca: Romanian Academy: Center for Transylvanian Studies.

Privat, K. L., O’Connell, T. C. and Hedges, R. E. M. (2007) ‘The distinction between freshwater- and terrestrial-based diets: methodological concerns and archaeological applications of sulphur stable isotope analysis’, *Journal of Archaeological Science*, 34(8), pp. 1197–1204. doi: 10.1016/j.jas.2006.10.008.

Reitsema, L. J. and McIlvaine, B. K. (2014) ‘Reconciling “stress” and “health” in physical anthropology: What can bioarchaeologists learn from the other subdisciplines?’, *American Journal of Physical Anthropology*, 155(2), pp. 181–185. doi: 10.1002/ajpa.22596.

Reynard, L. M. and Tuross, N. (2015) ‘The known, the unknown and the unknowable: Weaning times fromarchaeological bones using nitrogen isotope ratios’, *Journal of Archaeological Science*. Elsevier Ltd, 53, pp. 618–625. doi: 10.1016/j.jas.2014.11.018.

Ribot, I. and Roberts, C. A. (1996) ‘A Study of Non-specific Stress Indicators and Skeletal Growth in Two Mediaeval Subadult Populations’, *Journal of Archaeological Science*, 23(1), pp. 67–79. doi: 10.1006/jasc.1996.0006.

Roberts, C. A. and Connell, B. (2004) ‘Guidance on recording palaeopathology’, in Brickley, M. and McKinley, J. I. (eds) *Guidelines to the standards for recording human remains*. Southampton: British Association for Biological Anthropology and Osteoarchaeology, pp. 34–40.

Roberts, C. A. and Steckel, R. H. (2018) ‘The Developmental Origins of Health and Disease: Early Life Health Conditions and Adult Age at Death in Europe’, in Steckel, R. et al. (eds) *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia*. Cambridge: Cambridge University Press, pp. 325–351.

Schoeninger, M. J. and DeNiro, M. J. (1984) ‘Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals’, *Geochimica et Cosmochimica Acta*, 48(4), pp. 625–639. doi: 10.1016/0016-7037(84)90091-7.

Schoeninger, M. J., DeNiro, M. J. and Tauber, H. (1983) ‘Stable Nitrogen Isotope Ratios of Bone Collagen Reflect Marine and Terrestrial Components of Prehistoric Human Diet’, *Science*, 220(4604), pp. 1381–1383.

Schour, I. and Poncher, H. G. (1937) ‘Rate of apposition of enamel and dentin, measured by the effect of acute fluorosis’, *American Journal of Diseases of Children*, 54, pp. 757–776.

Schwarcz, H. P. and Schoeninger, M. J. (1991) ‘Stable isotope analyses in human nutritional ecology’, *American Journal of Physical Anthropology*, 34(13 S), pp. 283–321. doi: 10.1002/ajpa.1330340613.

Sealy, J. *et al.* (1987) ‘Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing’, *Geochimica et Cosmochimica Acta*, 51, pp. 2707–2718.

Sharp, Z. (2017) ‘Principles of stable isotope geochemistry’, *Delta*, p. 344. doi: 10.1016/S0037-0738(97)00056-0.

Thompson, E. (1982) *Romans and Barbarians: The declind of the Western Empire*. 2nd edn. London: The University of Wisconsin Press, Ltd.

Todd, M. (1995) *The Early Germans*. 2nd edn. Oxford: Blackwell Publishing, Inc.

Tudor, G. (2012) *Geological map of Romania, Geological Institue of Romania*. Available at: http://www.igr.ro/images/georom1000.jpg (Accessed: 12 May 2015).

Waldron, T. (2009) *Palaeopathology*. New York: Cambridge University Press.

Wood, J. W. *et al.* (1992) ‘The Osteological Paradox’, *Current Anthropology*, 33(4), pp. 343–370. doi: 10.1086/204084.

Yaussy, S. L., DeWitte, S. N. and Redfern, R. C. (2016) ‘Frailty and famine: Patterns of mortality and physiological stress among victims of famine in medieval London’, *American Journal of Physical Anthropology*, 160(2), pp. 272–283. doi: 10.1002/ajpa.22954.

Zukerman, M., Turner, B. and Armelagos, G. (2012) ‘Evolutionary though in Paleopathology and the rise of the Biocultural Approach’, in Grauer, A. (ed.) *A Companion to Paleopathology*. Oxford: Blackwell Publishing, Inc, pp. 34–57.

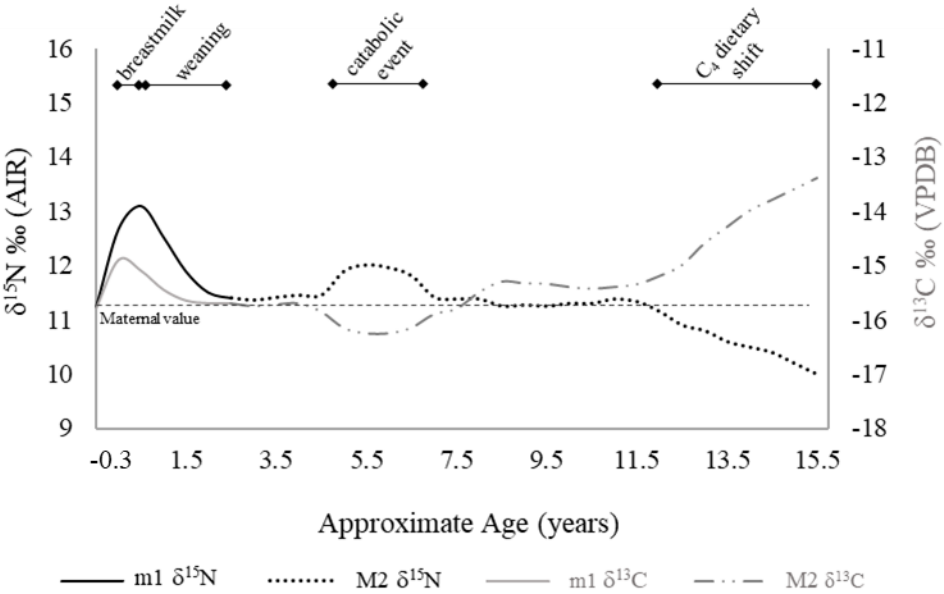

Figure 1. Theoretical illustration showing the possible variations in an isotopic life-history profile. Adapted from Beaumont et al., 2015 and King et al., 2018.

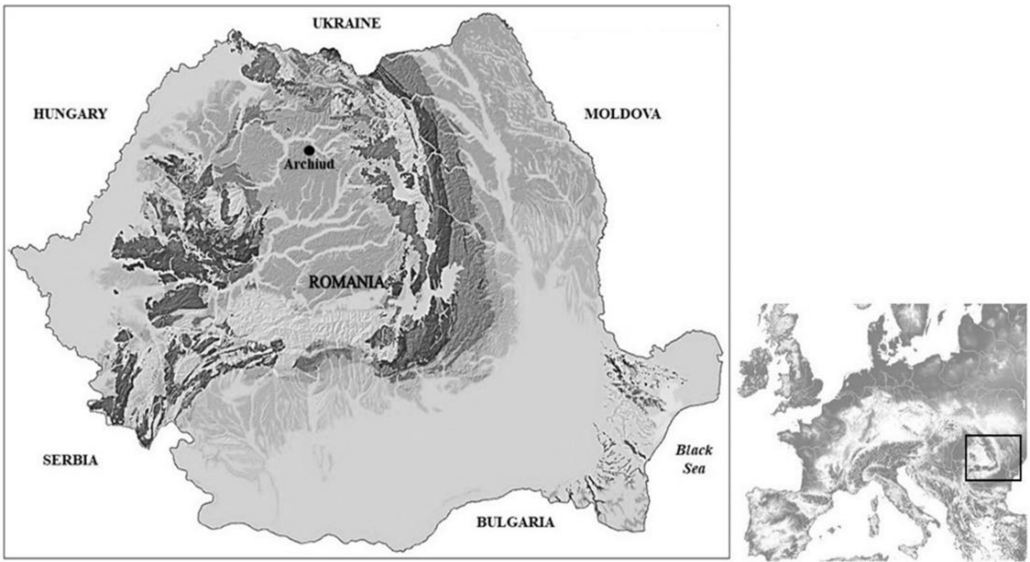

Figure 2. The location of the Archiud Cemetery in the Transylvania Basin, Romania (Tudor, 2012).

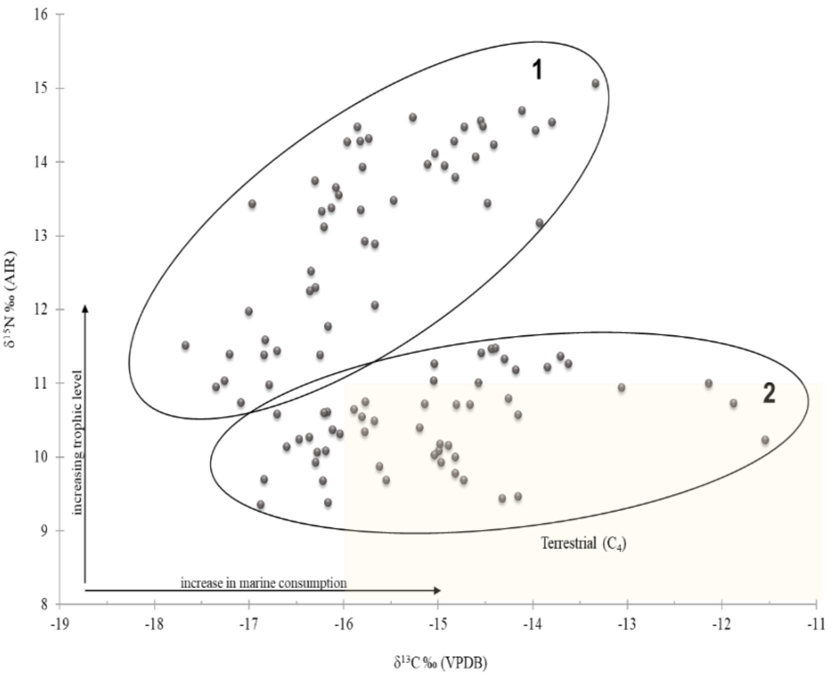

Figure 3. Graph indicating the two trajectories of the incremental dentine results. Isotopic ranges for resources were modelled after Schoeninger and DeNiro (1984) and Schwarcz and Schoeninger (1991).

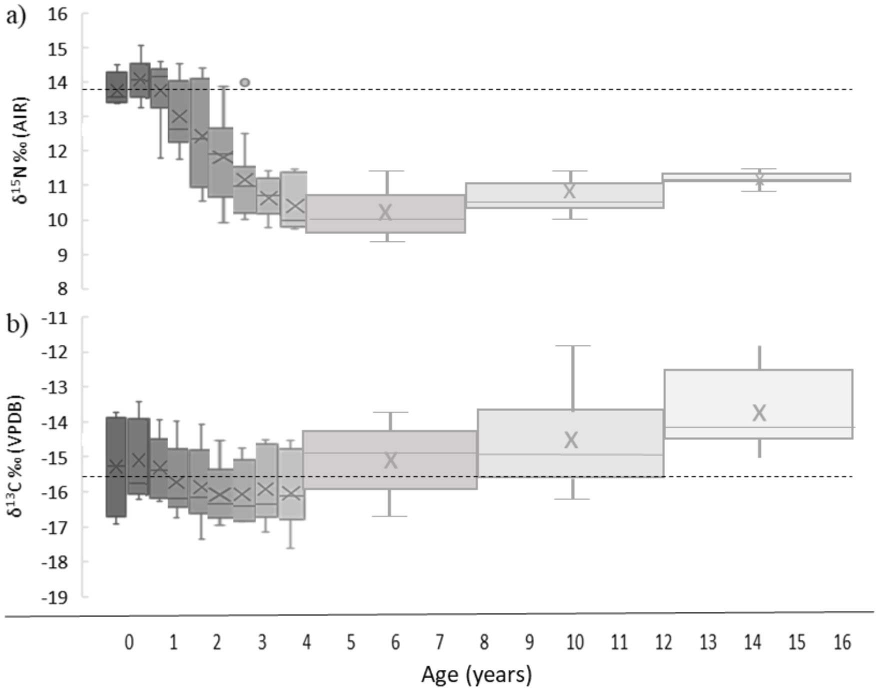

Figure 4. Box and whisker plot demonstrating the change in δ^{15}*N (a) and* δ^{13}*C (b) by age category. The dotted line represents the PFM. The boxes represent the inner quartile range (Q1-Q3) and the whiskers show the smallest and largest values in each category. Within each box, the* x *indicates the mean values and the line (–) indicates the median values. The single outlier was 1.5 times greater than the upper quartile.*

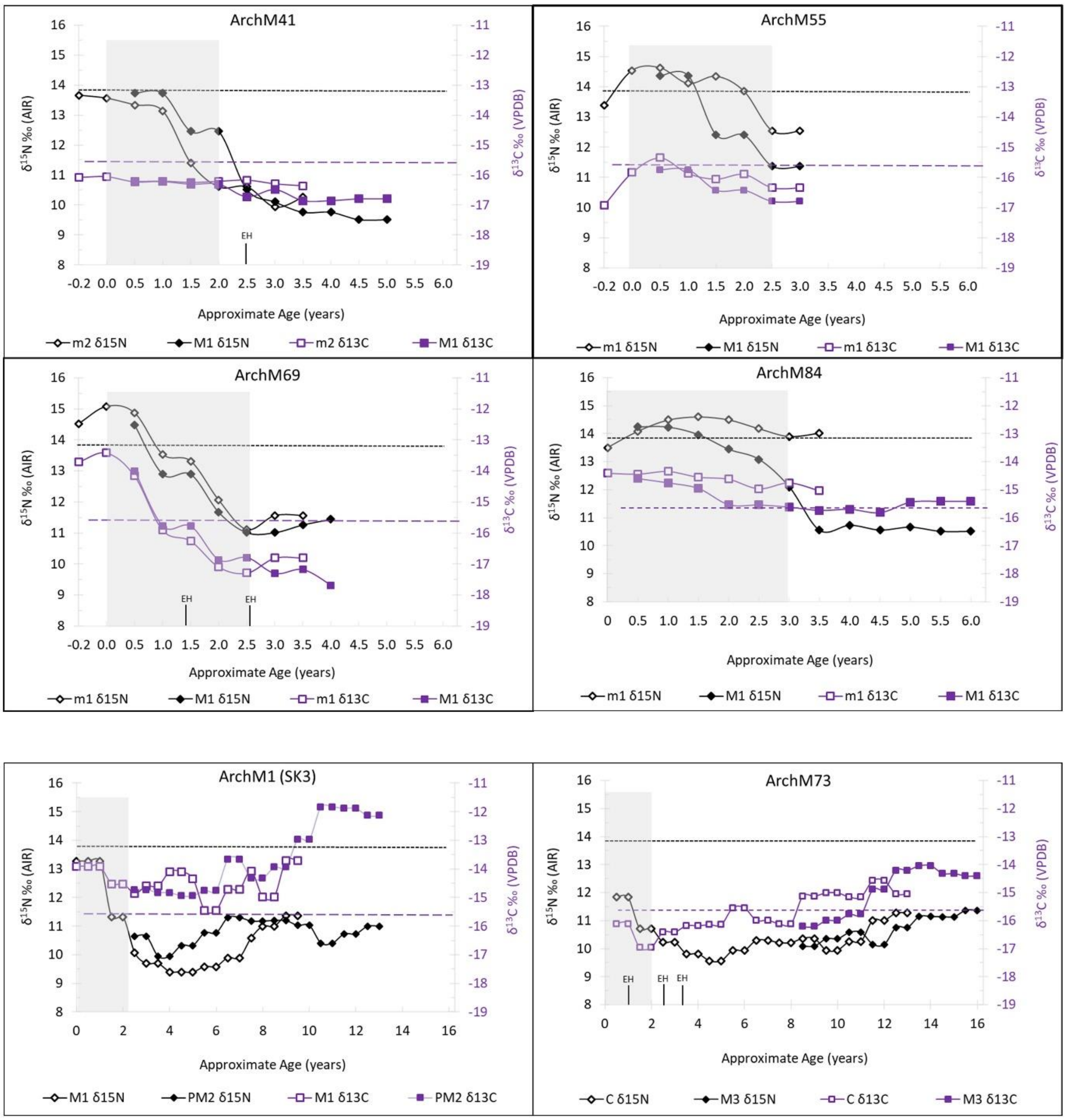

Figure 5. Isotopic life-history profiles for the six non-adult individuals with stress lesions. The shaded area indicates the probable breastfeeding and weaning period and the horizontal lines represent the PFM (dotted line - δ^{15}N, dashed line – δ^{13}C). EH –approximate timing of enamel hypoplasia.

Table 1. Summary of the six non-adult individuals sampled for stable carbon and nitrogen stable isotope analysis of incrementally sampled dentine.

	Cemetery (AD)	**Age at death (years)**	**Indicators of "stress"**	**Sample**
ArchM1	4th – 5th cent.	12 - 14	lower limb bowing; endocranial new bone formation	1. permanent mandibular M1 2. permanent mandibular PM2
ArchM41	6th – 7th cent.	4 – 6	dental enamel hypoplasia; possible bowing of arms and legs	1. deciduous mandibular M2 2. permanent mandibular M1
ArchM55	6th – 7th cent.	3 - 5	possible scurvy; cribra orbitalia; endocranial new bone formation; bowing in lower limbs	1. deciduous mandibular M1 2. permanent mandibular M1
ArchM69	6th – 7th cent.	4 - 6	possible scurvy; dental enamel hypoplasia; cribra orbitalia; endocranial new bone formation	1. deciduous mandibular M1 2. permanent maxillary M1
ArchM73	6th – 7th cent.	15 - 17	possible scurvy; cribra orbitalia; dental enamel hypoplasia	1. permanent mandibular C 2. mandibular M3
ArchM84	6th – 7th cent.	5 - 7	dental enamel hypoplasia	1. deciduous mandibular M1 2. permanent mandibular M1