Stable isotope analyses of human skeletal remains:

Enlightening different origins, diets and weaning patterns among the population of South Bavaria during the "Dark ages"

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Maren Velte

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Summary

The transition from Roman times to the Middle Ages in Europe is characterized by profound changes in all spheres of life, related to the decline of the Western Roman Empire and the emergence of medieval kingdoms. It is often assumed that the migration of various Germanic *gentes* played an important role in this transition. However, little is known about the local history of the Roman frontier province of *Raetia secunda* (*Raetia II*) in present-day southern Bavaria due to the lack of historical sources, so it can be referred to as a "Dark Age." Therefore, the anthropological study of human skeletal remains from this period is of particular importance to gain more insight into historical events and developments.

The stable isotope analyses of human bones and teeth carried out in this thesis investigate the different origins, diets, and weaning practices of the population living in *Raetia II* between Late Antiquity and the Early Middle Ages.

The results suggest that a large number of migrants came to *Raetia II* in the second half of the 5th century. The emerging differences in mobility between men and women, as well as the available evidence for multiple distinct regions of origin, suggest that immigration and mobility in South Bavaria after the fall of the Roman Empire were extremely complex. In particular, it has been noted that the tracked consumption of food sources atypical of the Bavarian region, such as marine resources and millet, may indicate a non-local origin of individuals.

It is implied that the Bavarian population subsisted on a mixture of different types of C3 plants and terrestrial animals. There is separate evidence that economic practices changed during the Early Middle Ages, but further analysis is needed to confirm this.

The observed breastfeeding and weaning practices vary considerably, even within local communities. Most individuals are weaned by the time they reach the age of three. However, in some migrants, probably from a population with a different cultural background and lifestyle, different weaning practices with longer breastfeeding periods are observed. Furthermore, analysis of physiological stress during and after breastfeeding shows that childhood stress increases when there is little or no breastfeeding.

Overall, the results of this study have contributed to enlightening the "Dark Ages" in South Bavaria.

Zusammenfassung

Der Übergang von der Römerzeit zum Mittelalter in Europa ist durch tiefgreifende Veränderungen in allen Lebensbereichen gekennzeichnet, die mit dem Niedergang des Weströmischen Reiches und der Entstehung mittelalterlicher Königreiche zusammenhängen. Es wird oft angenommen, dass die Migration verschiedener germanischer *Gentes* eine wichtige Rolle bei diesem Übergang spielte. Über die lokale Geschichte der römischen Grenzprovinz *Raetia secunda* (*Raetia II*) im heutigen Südbayern ist jedoch aufgrund des Mangels an historischen Quellen wenig bekannt, so dass sie als "dunkles Zeitalter" bezeichnet werden kann. Die anthropologische Untersuchung menschlicher Skelettfunde aus dieser Zeit ist daher von besonderer Bedeutung, um mehr Einsicht in die historischen Ereignisse und Entwicklungen zu erhalten.

Die in dieser Arbeit durchgeführten Stabilisotopenanalysen an menschlichen Knochen und Zähnen untersuchen die unterschiedliche Herkunft, Ernährung und Entwöhnungspraxis der in *Raetia II* lebenden Bevölkerung zwischen Spätantike und Frühmittelalter.

Die Ergebnisse deuten darauf hin, dass in der zweiten Hälfte des 5. Jahrhunderts eine größere Zahl von Migranten nach *Raetia II* kam. Die sich abzeichnenden Unterschiede in der Mobilität zwischen Männern und Frauen sowie die vorliegenden Hinweise auf mehrere, unterschiedliche Herkunftsregionen deuten darauf hin, dass Einwanderung und Mobilität in Südbayern nach dem Untergang des Römischen Reiches äußerst komplex waren. Insbesondere wurde festgestellt, dass der nachweisliche Konsum von für die Region Bayerns untypischen Nahrungsquellen, wie marine Ressourcen und Hirse, auf eine nicht-lokale Herkunft der Menschen hinweisen kann.

Es wird angenommen, dass sich die bayerische Bevölkerung durch eine Mischung aus verschiedenen Arten von C3-Pflanzen und Landtieren ernährte. Es gibt vereinzelte Hinweise, dass sich die Wirtschaftspraktiken im Laufe des Frühmittelalters veränderten, für deren Bestätigung es allerdings weiterer Analysen bedarf.

Die beobachteten Still- und Entwöhnungspraktiken variieren erheblich, auch innerhalb lokaler Gemeinschaften. Die meisten Individuen sind beim Erreichen des dritten Lebensjahres abgestillt. Bei manchen Migranten, die wahrscheinlich aus einer Bevölkerungsgruppe mit einem anderen kulturellen Hintergrund und Lebensstil stammen, werden jedoch andere Stillpraktiken mit längeren Stillzeiten beobachtet. Darüber hinaus zeigt die Untersuchung von physiologischem Stress während und nach der Stillzeit, dass Stress vor allem dann auftritt, wenn wenig oder gar nicht mehr gestillt wird.

Insgesamt konnte durch die Ergebnisse dieser Arbeit ein Beitrag zur Erhellung des "Dunklen Zeitalters" in Südbayern geleistet werden.

1. **Introduction**

Prehistoric anthropology studies human populations in time and space through the analysis of skeletal remains (e.g., Grupe et al. 2015). The subject is part of the biological anthropology which also addresses the interactions between humans and the environment in which they lived. More generally, the information obtained from the examination of human bones and teeth by natural science methods can be linked to cultural studies and thus substantially complement historical and archaeological research.

The present thesis aims to contribute to the broad goal of anthropological research by analyzing human remains at the macroscopic and molecular level to investigate aspects of human diet and movement during the transition from Roman to Medieval times in southern Germany.

The following sections outline the basics and different approaches of applied analyses. Thereby, the analyses of stable isotopes, which represent the methodological focus of this thesis, are discussed in more detail. Subsequently, the historical background and previous research results most relevant to the region and time of the thesis are summarized.

1.1 The informative value of human skeletal remains

Human skeletal remains represent the only source providing direct evidence of past human life histories, which can become amazingly detailed. Their osteological examination on macroscopic to microscopic level provides basic individual data, such as sex and age at death, but can also provide information on the physiological stress level and health status of individuals. Moreover, human bones and teeth are tissue banks for molecular analyses, which have recently gained importance in parallel to the technical and methodological progress. For example, ancient DNA (aDNA) analyses can be used to reconstruct family relationships, but also to investigate the individual's genetic ancestry. Furthermore, stable isotope analysis of heavy and light elements in the organic and inorganic fractions of bones and teeth can provide information about individual's provenance and diet (Figure 1).
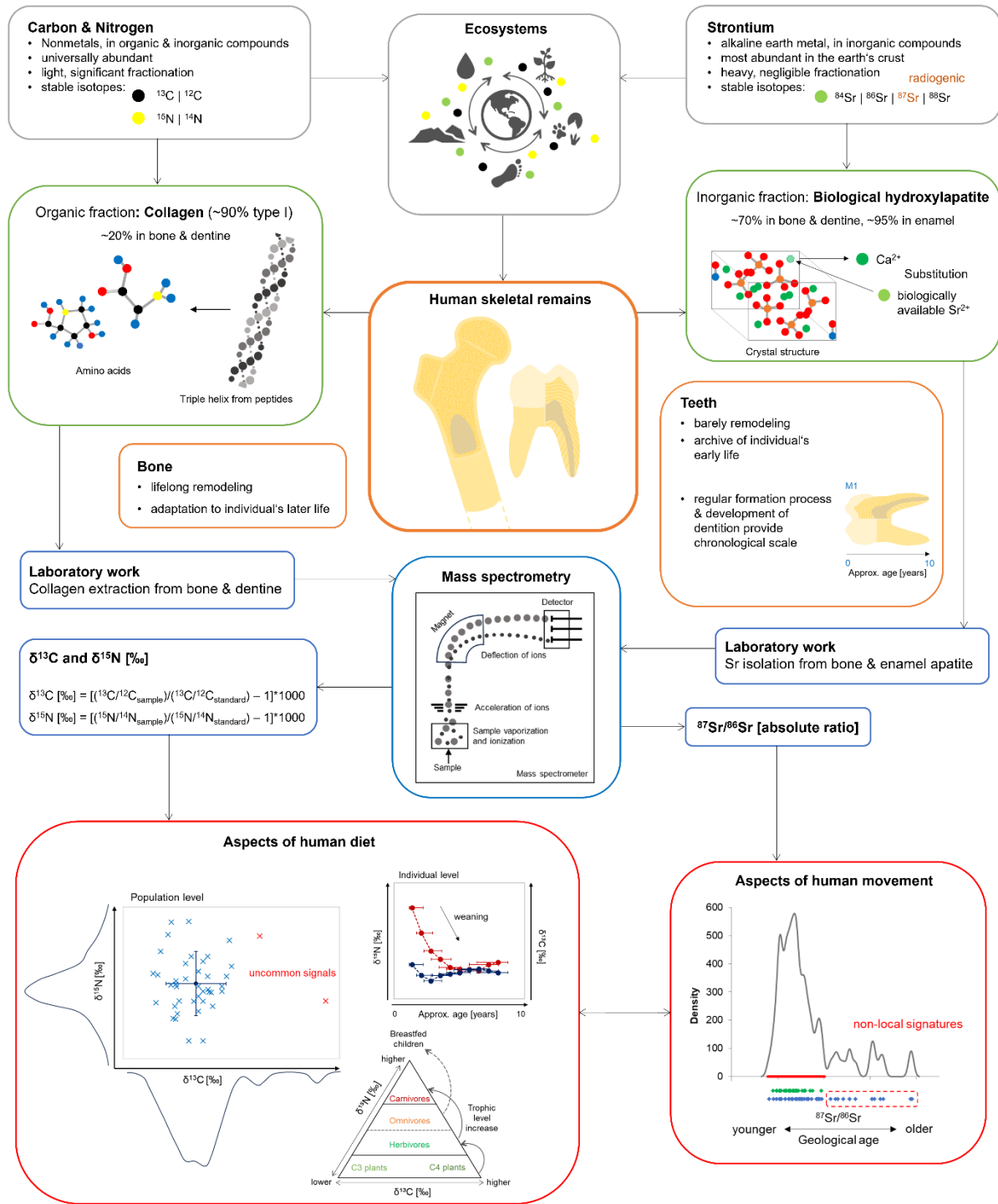
Figure 1: Graphical outline of stable light carbon and nitrogen as well as stable heavy strontium isotope analyses on human skeletal remains.

1.1.1 Stable isotopes

In Earth's ecosystems, isotopes of various elements circulate within and among the geo-, hydro-, and biospheres (e.g., Bentley 2006). These isotopes exhibit distinct behavior in chemical reactions due to differences in their masses (e.g., Fry 2006, Hoefs 2010), resulting in varying distributions among reaction partners, and thus between the different compartments of the ecosystem. This phenomenon is known as isotope fractionation. Fractionation processes are particularly crucial for light elements, where the relative mass difference between their isotopes is substantial.

The light elements carbon (C) and nitrogen (N) are nonmetals, each having two stable isotopes: ^{13}C and ^{12}C, as well as ^{15}N and ^{14}N, respectively. They are among the most common elements, and they are universally abundant and available to organisms in both organic and inorganic forms. The heavy element strontium (Sr), on the other hand, is an alkaline earth metal, which possesses four stable isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr. Notably, ^{87}Sr is a decay product of radioactive rubidium (^{87}Rb, $t_{1/2}=48.8^{x}10^9$ years) and thus radiogenic (Faure 1986). Consequently, Earth's ^{87}Sr content increases over time. Strontium (Sr) is most abundant in the bedrock of the Earth's crust, primarily in inorganic compounds such as minerals (e.g., Turekian & Wedepohl 1961). It (Sr) becomes mobile and thus biologically available to organisms through processes like mineral weathering (e.g., Sillen & Kavanagh 1982, Price 1989, Price et al. 2002).

Isotopes that enter the human organism, primarily through dietary intake, become partially incorporated into mineralized tissues of the body (e.g., Ericson 1985). Bones and teeth largely consist of the inorganic phosphate mineral hydroxylapatite (e.g., Hillson 1996, Fratzl et al. 2004). It is usually described as 'biological apatite' because it can contain several substitutions, e.g., calcium ions (Ca^{2+}) can be substituted by strontium ions (Sr^{2+}) (e.g., Comar et al. 1957, Bentley 2006). In addition, bones and teeth also contain an organic structure protein named collagen, mainly of type I (e.g., Hillson 1996, Nanci 2003). Collagen molecules consist of triple helices formed by three polypeptide chains, each composed of various amino acids. These amino acids and all other existing amino acids (and thus all proteins) contain carbon (C) and nitrogen (N) (e.g., Barrett 1998).

Bone tissue is subject to continuous and lifelong remodeling (e.g., Jowsey 1971, Hedges et al. 2007, Raggatt et al. 2010). Consequently, the isotope composition of bone primarily reflects the status of an individual in the period leading up to death. This implies that the isotopic signal in bone can adapt to changes, e.g., in diet and residence. In contrast, teeth are barely remodeled after their formation (e.g., Goodman & Rose 1990, Buikstra & Ubelaker 1994, Nanci 2003), making them an archive of an individual's early life history. As the formation of the human dentition follows a regular process and schedule (e.g., Moorrees et al. 1963a &

b, AlQahtani et al. 2010), it is possible to assign a rather precise chronological age range to single teeth or several parts of a tooth (e.g., Reid & Dean 2000, Reid & Dean 2006). This, in turn, allows one to investigate isotopic changes between and within specific life periods (e.g., Balasse 2002, Czermak et al. 2018, Czermak et al. 2020). Analyzing both, bone and tooth samples from an adult individual offers the opportunity to study various aspects of his or her early and later life and thus identify potential changes.

Archaeological skeletal remains may be altered by decomposition processes that can also isotopically alter both organic and inorganic fractions. However, well-preserved material can be prepared in the laboratory to determine the isotope ratio of elements by mass spectrometry.

For Sr, the ratio of ^{87}Sr to ^{86}Sr (^{87}Sr/^{86}Sr) in a sample is given as absolute value to the fifth decimal place, because isotope fractionations are considered negligible (e.g., Grupe et al. 2015). For C and N, which show comparatively clear isotope fractionation, the ratios of their heavy to light isotopes of a sample are referred to a standard and given in ‰ according to the international delta (δ) notation. Each measured value is a number reflecting the average of all Sr, C, or N that have been contributed to the sample. Each atom has "journeyed" through many different stages into a person's skeleton where it joins atoms that have followed other routes (Bentley 2006). Therefore, the isotope ratios themselves have a proxy character.

Sr data are commonly used to study aspects of human movement (e.g., Price et al. 1994, Price et al. 2002, Bentley et al. 2004, Wright 2005, Frei et al. 2015). Strontium isotope analysis allows one to empirically identify non-local individuals, i.e., individuals with a strontium signal in bone or tooth mineral different from the range of the local bioavailable ^{87}Sr/^{86}Sr signature, which can vary significantly between different regions. This range is largely defined by the prevailing geological system but also depends on nongeological sources (e.g., Bentley 2006). There is ongoing discussion about how to best determine the local ^{87}Sr/^{86}Sr range, either from different proxies (e.g., geological data, modern environmental samples, archaeological bones and teeth from animal) or directly from human data (e.g., Toncala et al. 2020, Frei et al. 2022, Lugli et al. 2022), to which individual values can then be compared. Usually, ^{87}Sr/^{86}Sr ratios of enamel (and not bone) are used to examine the provenance of the individuals for two main reasons. First, most highly mineralized enamel is more resistant to taphonomic changes than bone. Bone is more likely to be affected by mineral and groundwater contamination of the burial soil, which can alter the ^{87}Sr/^{86}Sr signature of bone toward the local range of the burial site (e.g., Bentley 2006). Thus, also originally non-local bone ratios may be changed and remain undetected. Second, it is generally assumed that children are less mobile than adults. Therefore, it is more useful to investigate the ^{87}Sr/^{86}Sr signal in enamel referring to the region where individuals spend their childhood than to use the ^{87}Sr/^{86}Sr signatures from

bone which, in turn, rather correspond to the region of their later residence. Thus, non-local $^{87}Sr/^{86}Sr$ signals can adapt to the local $^{87}Sr/^{86}Sr$ range due to lifelong remodeling and therefore mask the change of location if it happened longer before death.

In a refined approach, presented in this thesis, the bone and enamel values of animals and humans of different sites and time periods are combined to determine the local $^{87}Sr/^{86}Sr$ range for the inhabitants of a certain region (Figure 2). This range is probably represented by the mode of the data's Gaussian kernel density estimates (KDE) of the data. The limits of this range are defined by the highest density interval that spans 99% of the distribution (99% HDI) below the mode. Individuals who show $^{87}Sr/^{86}Sr$ ratios in enamel that lay outside the limits are defined as non-locals.

Study site and neighboring places of archaeological findings

$^{87}Sr/^{86}Sr$ of human and animal bones and teeth

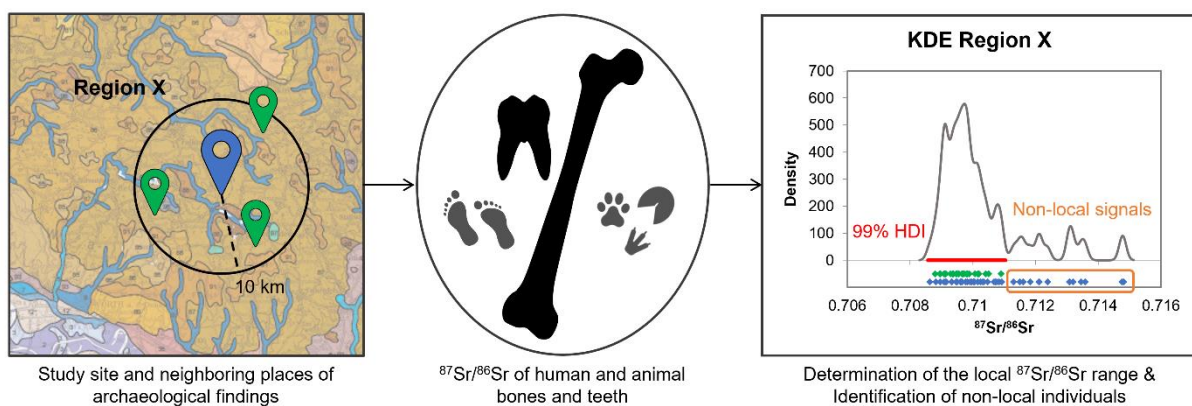Determination of the local $^{87}Sr/^{86}Sr$ range & Identification of non-local individuals

Figure 2: Graphical outline of performed strontium isotope analyses.

C and N isotope data is commonly used to investigate the human diet. Analysis of $\delta^{13}C$ and $\delta^{15}N$ ratios can provide information on the type of resources used primarily by humans, e.g., it is possible to differentiate terrestrial and aquatic (freshwater and marine) resources, as well as C3 and C4 plants (e.g., van der Merwe 1992, Lee-Thorp 2008, Price 2014). More detailed statements, for example, about the proportion of animal protein to plant content, are only possible if a sufficient number of comparative samples of contemporaneous animals and, ideally, also plants are available (e.g., Fraser et al. 2013, Fernandes et al. 2014). $\delta^{13}C$ and $\delta^{15}N$ ratios measured in human bone or dentine collagen are largely dependent on the prevailing ecogeographical conditions of the habitat i.e., external factors such as climate, soil material, water bodies etc. influencing local dietary resources. Despite this, human ratios can also be affected by internal factors, e.g., different practices in agriculture and husbandry, fishing and hunting, food trading, as well as social factors which may determine the access to resources (e.g., Fraser et al. 2011, Le Huray & Schutkowski 2005, Privat et al. 2002). Thus, among a population $\delta^{13}C$ and $\delta^{15}N$ values are assumed to scatter within a certain range resulting from both external and internal factors.

To determine this range, a completely new approach similar to the described procedure of strontium isotope analysis can be used, which is also presented in this thesis. In this way, the KDEs of adult human $\delta^{13}C$ and $\delta^{15}N$ bone data from single sites or combined data from closely neighboring sites are applied, whose modes most likely represent the main populations and therefore the "common intra-populational diet variability" (Figure 3). Individual ratios clearly differing from the main population i.e., which are found outside the 99% HDIs of KDEs, are defined as unusual signals, which can potentially be explained by the assumption that these individuals moved there from a region with a different food base (e.g., Cox et al. 2001, Schroeder et al. 2009).
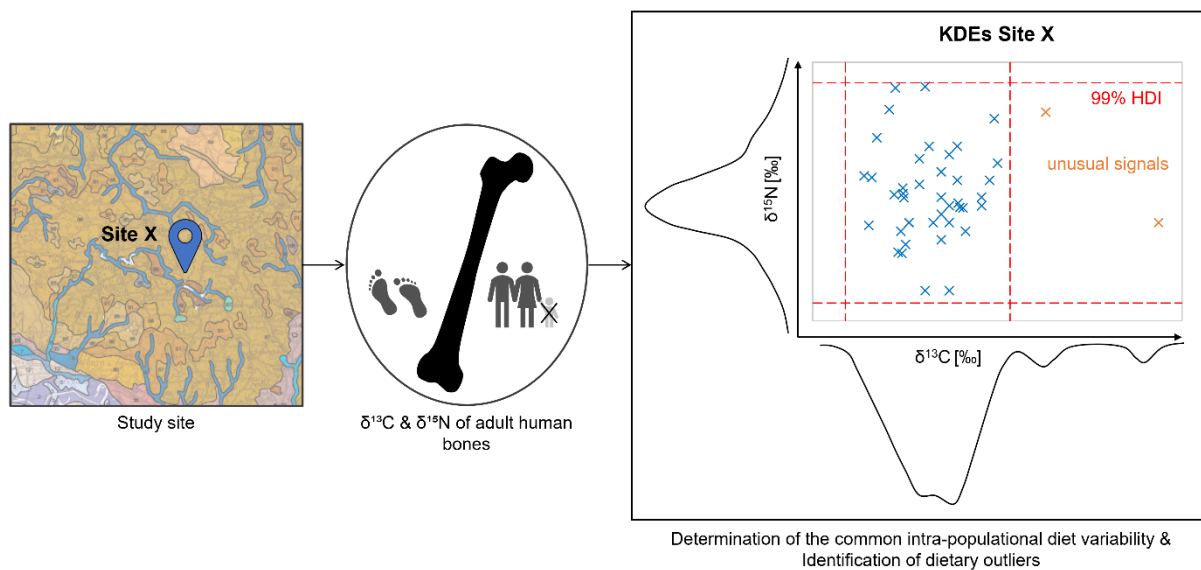

Determination of the common intra-populational diet variability &
Identification of dietary outliers

Figure 3: Graphical outline of performed carbon and nitrogen analyses from bone.

However, caution must be exercised in the interpretation of deviating ratios because $\delta^{13}C$ and $\delta^{15}N$ values can also be influenced by physiological or metabolic aspects, for example, related to stress, chronic diseases, or growth (e.g., Fuller et al. 2004, Fuller at al. 2005, Mekota et al. 2006, Waters-Rist 2009, Neuberger et al. 2013, D'Ortenzio et al. 2015). This is especially true for the ratios of young children because it is unclear how the circumstances of their early death influence $\delta^{13}C$ and $\delta^{15}N$ ratios in developing bones and teeth. Despite this, breastfeeding can also significantly increase an infant's $\delta^{13}C$ and $\delta^{15}N$ ratios. A newborn that is exclusively breastfed after birth shows an increase in $\delta^{15}N$ by one trophic level of ~3.0‰ compared to the breastfeeding female (e.g., Fogel et al. 1989, Millard 2000, Fuller et al. 2006, Herrscher et al. 2017) while the observed rise of in $\delta^{13}C$ varies in studies between around one trophic level of ~1.0‰ (e.g., Richards et al. 2002, Fuller et al. 2003, Fuller et al. 2006) over less increased values of ~0.5‰ (Herrscher et al. 2017) to no increase at all (Fogel et al. 1989). With the increasing consumption of complementary foods $\delta^{15}N$ and $\delta^{13}C$ values decrease over time to the level of the breastfeeding female, that is, until weaning is completed (e.g., Fogel et al. 1989, Fuller et al. 2006, Fernández-Crespo 2018). The

signal of breast milk is also apparent in samples of adult's tooth dentine that formed while individuals were breastfed e.g., first permanent molars. Therefore, the serial sampling of first permanent molar dentine offers the possibility of illustrating the changes of $\delta^{13}C$ and $\delta^{15}N$ ratios within the first ten years of life and retrospectively investigating breastfeeding and weaning practices (e.g., Eerkens et al. 2011, Czermak et al. 2018). Replacement of breastmilk with non-breastmilk liquids and solid foods is visible in isotope profiles through the so-called "weaning dip" (e.g., Tsutaya 2017, Scharlotta et al. 2018). In general, analysis of serial dentine allows one to examine short-term changes of $\delta^{13}C$ and $\delta^{15}N$ ratios over a certain time, potentially linked to dietary shifts and physiological aspects (e.g., Crowder et al. 2019, Beaumont et al. 2016, Craig-Atkins et al. 2020). The time frame for these analyses can be extended by serially sampling several teeth with different formation periods.

The set up of stable light isotope profiles from serial dentine data (Figure 4) requires an age determination of dentine sections from molars, for which this thesis presents a new scheme based on *The London Atlas of Human Tooth Development and Eruption* (AlQahtani et al. 2010). It is also relevant to the estimation of weaning ages from isotope profiles, which is commonly based on changes in $\delta^{15}N$. Weaning ages are often descriptively estimated, for example, as the lowest $\delta^{15}N$ during the initial weaning dip (Scharlotta et al. 2018). This thesis presents a mathematical model to estimate individual weaning ages from the initial drop of $\delta^{15}N$ values i.e., the "weaning dip".
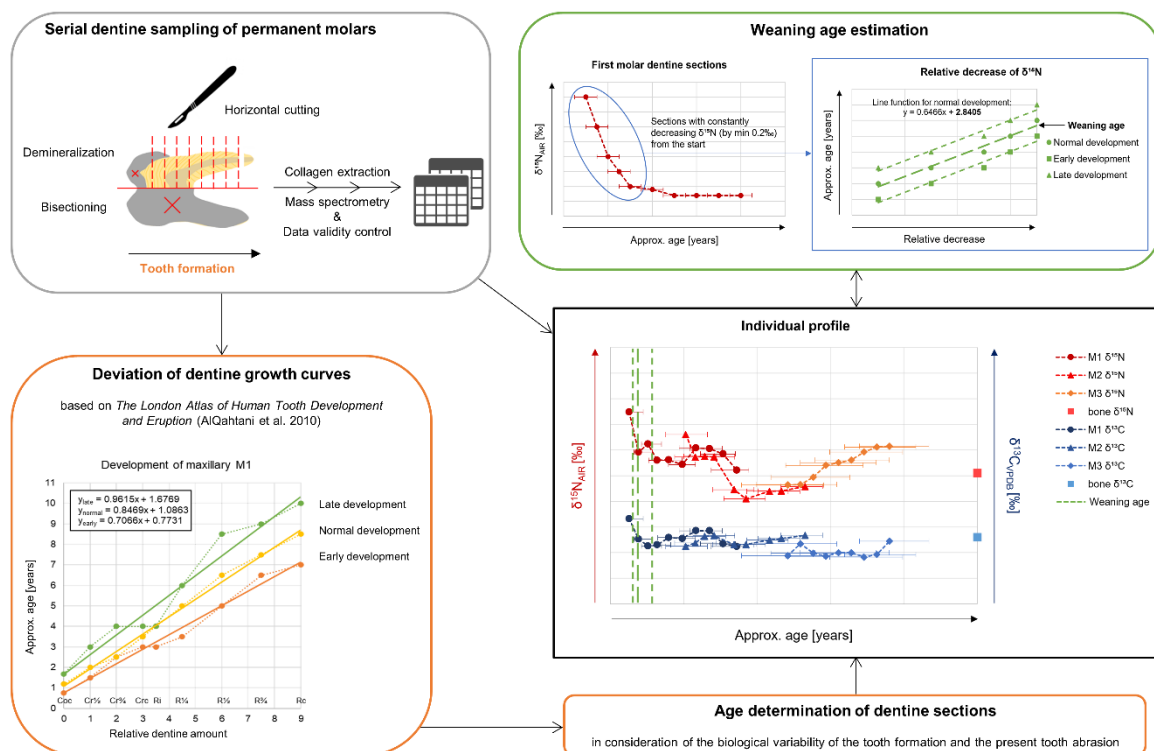

Figure 4: Graphical outline of performed carbon and nitrogen analysis from tooth dentine.

1.1.2 Osteological stress markers

One of the most studied markers for estimating physiological stress is linear enamel hypoplasia (LEH) (e.g., King et al. 2005, Floyd & Littleton 2006, Temple 2016, Bereczki et al. 2019). Enamel hypoplasia is generally defined as any macroscopic defect in the enamel surface (e.g., Pindborg 1970), but defects predominantly appear as band like depressions i.e., LEH. LEH results from a disruption of the enamel secretion of ameloblasts during tooth development (e.g., King et al. 2002) that can have various causes, including nutritional deficiencies, infectious diseases (e.g., Hillson 2008). Once formed, LEH remains unchanged. Thus, LEH found in the permanent dentition of individuals indicates unspecific systemic physiological stress during childhood and adolescence (Goodman & Rose 1990, Guatelli-Steinberg & Lukacs 1999). They can occur to varying degrees, from only subtle lines to defects with strong edging, which is likely related to the duration rather than the severity of the causal crisis (e.g., Hillson 2008). Of greater importance, however, is that the position of defects in the tooth crown roughly indicates the timepoint of a stress event (e.g., Buikstra & Ubelaker 1994, Reid & Dean 2000).

The analysis of LEH within early forming teeth of the anterior dentition is also suitable to investigate stress potentially resulting from the replacement of breastmilk by less nourishing supplementary foods or the exposure to pathogens from contaminated foods and drinks (e.g., Humphrey et al. 2008, Black et al. 2008). More recently, the occurrence and timing of this supposed "weaning stress" has been examined more critically (e.g., Kendall et al. 2021, Fernández-Crespo et al. 2022).

Analysis of LEH in the anterior dentition in parallel to analysis of stable carbon and nitrogen isotopes from serial first molar dentine (Figure 5) allows further investigation of the link between weaning and possible associated stress. To estimate the timing of stress, i.e., the formation age of LEH and thus of the corresponding crown part, is necessary, for which this thesis also presents a new scheme based on *The London Atlas of Human Tooth Development and Eruption* (AlQahtani et al. 2010).
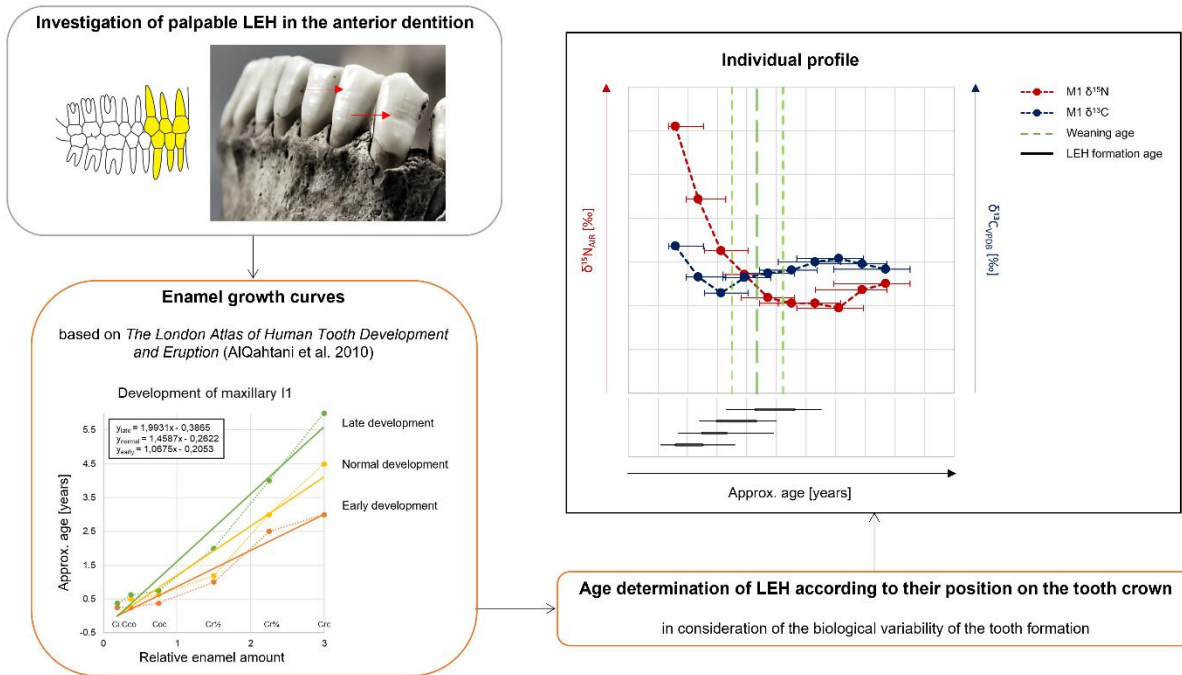
Figure 5: Graphical outline of the performed analysis on the occurrence of LEH in comparison to temporal changes of stable light isotopes and estimated weaning ages.

1.2 The "Dark Ages" in South Bavaria

"Dark Ages" has long been a common term for the time of the Early Middle Ages (or occasionally the entire Middle Age) in Western Europe marked by a supposed cultural and intellectual decline after the end of the Western Roman Empire (e.g., Mommsen 1959). However, the simplistic view and pejorative label of this historical epoch has become fundamentally questioned in recent decades (e.g., Snyder 1998, Rahman 2003). Through a growing body of historical, archaeological, and anthropological research the Middle Ages undergo a reevaluation revealing a much more complex picture of this often misunderstood era.

The new findings emphasize the profound upheavals in Europe during the transition from Roman times to the Middle Ages, which were not limited to politics and economics but also concerned religion, social structures and everyday culture (e.g., Postel 2004, Fehr & von Rummel 2011).

There is also much discussion about the role of migration in the transition because the period between the 3rd to the 6th century is marked by the movement of various ethnic groups within or into the territories of the Roman Empire and Europe as a whole (e.g., Halsall 2009, Fehr & von Rummel 2011). Therefore, it is often referred to as the "Migration period", but also here, the term but especially its German translation "Völkerwanderungszeit" evokes certain associations that are not plausible from today's scientific point of view. First, the idea that large and closed units of whole people left their former home and completely repopulated large parts of Europe is outdated. Furthermore, there has been a paradigm change from "people" to *gens* in recent ethnogenesis research. *Gentes* are not determined by biological origin but seen as initially polyethnic communities resulting from complex historical development processes, i.e., they rather represent mutable groups of interest rather than long-lived, unchanging entities (Postel 2004, Brather 2004). Nevertheless, the extent and nature of these migrations are not fully understood and still subject of substantial debate (e.g., Geary 2002, Noble 2006, Halsall 2009).

Overall, several questions about the events and developments of the transition remain unanswered due to a lack of historical records. Therefore, the Early Middle Ages are sometimes still called the "Dark Ages" (e.g., Sallares 2007). But also this usage, especially as a synonym for the entire period is also criticized due to the growing number of discovered sources in many places and insights provided (e.g., Halsall 2005). However, the history of today's South Bavaria has been only poorly explored between the 4th and 6th centuries due to the nearly complete absence of written sources and the incomplete tradition of archaeological findings (e.g., Kellner 1981, Steinacher 2012, Haberstroh 2019). This makes the transition period in this particular region as "Dark Ages" in this sense.

Southern Bavaria was integrated into the Roman Empire in the 1ˢᵗ century when the Romans expanded their territory north of the Alps. It became part of the province of *Raetia* (Figure 6) which, as a whole, comprised large areas of the northern Alpine foreland (e.g., Meyer 1972). The new territories experienced intense "Romanization" throughout the following decades which included a strong urbanization and economic development (e.g., Fehr 2010, Haas-Gebhard 2013, Boschung et al. 2015).

The area beyond the Roman border was inhabited by several Germanic *gentes* to whom the growing success and wealth of the Roman Empire exerted great attraction. As a result, some of them attempted to gain access through violent attacks (e.g., Fehr & von Rummel 2011, Halsall 2009), which became more frequent in the 3ʳᵈ century and brought devastating destruction and territorial losses (e.g., Mommsen 1993, Wolff 1916). Related to these conflicts, but also due to other factors, internal crises (e.g., supply shortages) also occurred in the course of the 3ʳᵈ century (e.g., Brown 1971).

To get past these "Imperial crises" wide-ranging reforms were initiated (e.g., Demandt 2004), in the wake of which *Raetia* was divided into two parts. South Bavaria henceforth belonged to the province of *Raetia secunda* (*Raetia II*), which comprised the northern foothills of the Alps between the rivers Iller, Danube, and Inn (e.g., Heuberger 1931).

In the first half of the 4ᵗʰ century more stable and peaceful conditions prevailed during which the Roman Empire recovered through reformation and reconstruction. But around the middle of the century Germanic invasions started again (e.g., Haas-Gebhard 2013).

At the same time the Empire of the Huns arose in Eastern Europe, which later was extended over the entire steppe belt north of the Black Sea towards the lower Danube (e.g., Maenchen-Helfen 1973). Germanic groups living in this area were subjected by them or fled towards the Roman territories, which further increased the pressure on the northern border of the Roman Empire (e.g., Thompson 1996, Heather 2005).

At the end of the 4ᵗʰ century the power over the Roman Empire was divided between the two sons of the deceased emperor, who henceforth ruled over a western and an eastern part (e.g., Degen 1987, Meier 2012). While the Eastern Roman Empire further regained in power throughout the 5ᵗʰ century, the Western Roman Empire suffered again from increasing conflicts and crisis, heading towards its decline (e.g., Haas-Gebhard 2013).

The final end of the Roman administration and military defense is often dated onto the deposition of the last Roman emperor by a Germanic leader in 476 AD (e.g., Degen 1987) whose emerging kingdom also covered the region of South Bavaria. A few years later, in 493 AD, the region was conquered by the Ostgoths, who lost power again to their rival tibe, the Franks, only a short time later. Since 537 AD South Bavaria belonged to a dukedom under Frankish rule, which persisted until 751 AD (e.g., Haas-Gebhard 2013).
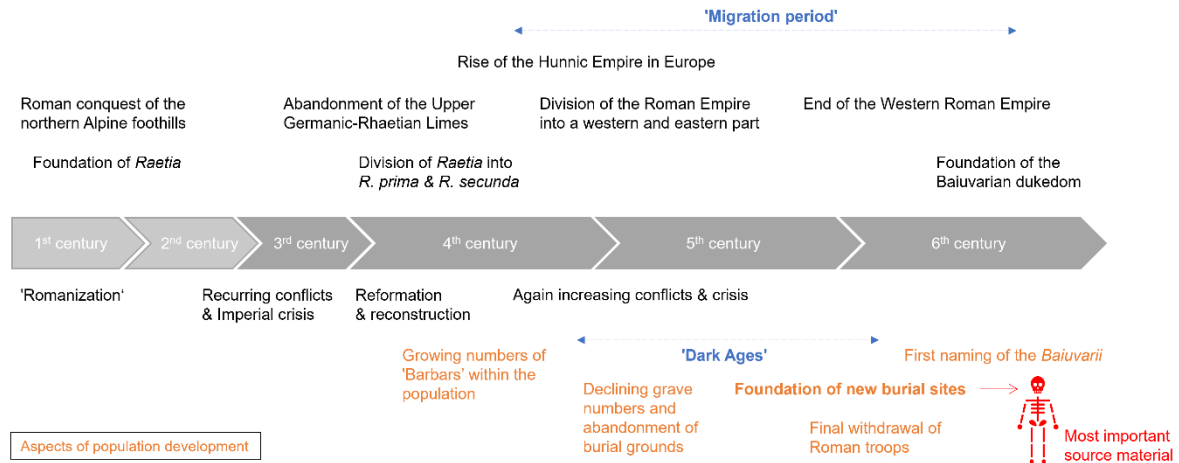
Figure 6: Overview of the historical background.

Perhaps the most important question asked by historians, archaeologists, and anthropologists alike is how these events affected the lives of humans. Previous research from all three fields provides some indications on the development and composition of the population in *Raetia II* during the "Dark ages" (also see Figure 6).

Already in the 4th century, the number of written sources decreased significantly (e.g., Kellner 1981, Degen 1987), resulting in an already fragmentary description of the developments. The crisis of the 3rd century probably left a weakened army and already reduced civilian population in *Raetia II* (e.g., Degen 1987). Due to the reoccurring conflicts with hostile Germanic *gentes* in the second half of the 4th century the remaining population more and more withdraw from rural areas to the better protected urban centers (e.g., Degen 1987, Haas-Gebhard 2013).

Furthermore, it is assumed that a growing number of Germanic people settled in the region, whose excess could not be averted or was tolerated in order to avoid further conflicts (e.g., Haas-Gebhard 2013). In addition, a profound number of Germanic soldiers supposedly were integrated into the Roman army in the 4th century to strengthen border defense (e.g., Schmidts 2004). Thus, especially bigger Roman fortifications along the Danube, like Regensburg (*Castra Regina*) and Straubing (*Sorviodurum*), are thought to became increasingly inhabited by Germanic people (Fischer & Rieckhoff-Pauli 1982).

For the 5th century, historical sources are almost completely missing (e.g., Steinebacher 2012). The most important writing that provides some indirect insights into the history of *Raetia II* during this time is the *Vita Sancti Severini* written around 511 AD by Eugippius (translation Noll 1981), which tells about the life and work of the monk Severinus in the neighboring province *Noricum*. It reports that most Roman border forts along the Danube in *Noricum* were abandoned in the middle of the 5th century, less due to military strikes but rather due to the lack of supplies and soldier's payments (e.g., Degen 1987, Haas-Gebhard 2013). A similar situation could also have arisen in *Raetia II*, but this has so far been unclear.

However, archaeological findings indicate some structural changes in *Raetia II* that could be associated with the gradual withdrawal of the Romans. In the rural hinterland the occupation of most Late Roman burial sites ends in the middle of the 5th century after a clear decline in the number of burials was recognizable. On the contrary, in cemeteries in the vicinity of urban sites, such as the bases along the Danube, grave numbers remain constantly high (Haberstroh & Harbeck 2013). A remarkable number of new burial sites were founded at several places in *Raetia II* towards the end of the 5th century. Some of them arise with a temporal gap of less than one generation and in close geographical distance to previous Late Roman cemeteries (Haberstroh & Harbeck 2013). Initially, occupation density in newly founded necropolises was loose, and the number of individual graves per generation was low. However, at some sites, the number of graves increased and the occupation density became higher in the first half of the 6th century (Haberstroh 2019). Therefore, the so-called "Reihengräberfelder" ("linear cemeteries") developed (e.g., Haas-Gebhard 2013), although a strict row of graves can rarely be observed in some cases (Haberstroh & Harbeck 2013). The use of these cemeteries often continued until the 7th century, with the result that a profound number of inhumations were found in a large area. On the other hand, the occupancy of some new burial sites ends earlier, commonly in the first half of the 6th century, resulting in a comparatively small number of graves. Although traces of the settlements associated with the explored cemeteries are largely missing (e.g., Haberstroh 2019), these observations overall indicate different structural changes for the hinterland and the border region of *Raetia II*. In the hinterland, a "new beginning" in the middle of the 5th century is indicated, while at single rural sites a continuous settlement is also observed (e.g., Haberstroh 2019). In contrast, a certain continuity of settlement from Late Roman to Early Medieval times at the same sites or in close vicinity is assumed for the border region (Haberstroh & Harbeck 2013, Harbeck et al. 2016). The latter is often assumed to be related to the supposed large numbers of people of Germanic origin who already lived there (e.g., Schutz 2001).

More large-scale migrations to *Raetia II* possibly set in shortly after the middle of the 5th century (e.g., Fischer 1993, Bierbrauer 1987) while the Roman rule still persisted but the border already became increasingly leaky. From the late 5th century onwards probably several migration waves occur (e.g., Rettner 2012, Fischer & Geisler 1988, Strömer 2007) after the final decline of the Western Roman Empire. Notably, these results are mainly based on the analysis of grave goods whose interpretation as evidence of individuals' origin is generally viewed more critically today (e.g., Brather 2004, Theune 2008).

For some time, it was also suspected that incoming migrants found empty, abandoned settlement land upon their arrival and completely repopulated the former province *Raetia II* (e.g., Schutz 2001). Nowadays, it is commonly believed that parts of the Roman population stayed in the region even after the Roman administration and military officially withdrew from

that area (e.g., Losert 2003, Strömer 2007). Considered together with the indicated structural changes (see above), it can be assumed that migrants who entered the region in the second part of the 5th century predominantly settled in the rural areas of the hinterland.

In the 6th century, written records started to reappear. Among the most important writings of the 6th century are also the *Getica* written by Jordanes in 551 AD, which describe the history of the Goths (translation Möller 2012). The text first named the legendary *Baiuvarii* as inhabitants within the region that was once *Raetia II*. They were long suspected to be a cohesive migrated group (e.g., Hartung 1983, Lotter 1985, Fischer & Geisler 1988). However, since some time now, the more common opinion is that the Baiuvariian tribe was an ethnically and culturally diverse group (e.g., Haas-Gebhard 2013, Rettner 2004) that already formed years before their first naming (e.g., Störmer 2007), most probably from a mixture of locals who stayed in the region and incoming migrants (e.g., Christlein 1980, Fischer 1993). Nevertheless, it is still questionable where potential migrants came from and how high their number has been related to the remaining residents.

With the increasing number of historical sources, but also archaeological finds in the following period, knowledge about the developments of the region improves. Even if there are indications that in some areas Roman traditions were initially adhered to (e.g., Degen 1987, Mackensen 2013), there are probably more significant changes from the second half of the 6th century onwards. For instance, the strongly growing population apparently also opens up new settlement chambers (Haberstroh 2019), and the so-called "agricultural revolution" begins, which was of great importance for the era as a whole (e.g., Fehr 2019, Henning 2014).

The only way to gain more information on the events and developments during the "Dark ages" in South Bavaria and test historical and archaeological hypotheses is the anthropological examination of human skeletal remains. In contrast to historical and archaeological sources, they are available in large numbers. Of particular interest for the "Dark Ages" are the remains of burial sites which were founded in the second half of the 5th century.

For instance, in several of the cemeteries the remains of women with modified skulls dating into the late 5th or early 6th century were recovered (e.g., Hakenbeck et al. 2010, Trautmann et al. 2017, Veeramah et al. 2018). The practice of artificially modifying the skull shape is commonly associated with the nomadic peoples of the Huns (e.g., Werner 1956), which probably also spread among the ethnic groups that became politically associated with the Huns (e.g., Päffgen et al. 2018). The isolated occurrence of only females with artificial cranium deformation (ACD) in South Bavaria is seen as a sign of exogamous marriage migration (e.g., Hakenbeck 2009). But only their reexamination with modern scientific methods revealed that these women have a different genetic ancestry (Veeramah et al. 2018) and consumed different foods (Hakenbeck et al. 2010) supporting this assumption.

2. Objectives

The aim of this thesis is to further enlighten the history of *Raetia II* during the "Dark ages" through the examination of human remains, especially by archaeometric analyses of stable isotopes that provide valuable information about human movement and diet (cf. 1.1.1).

Publication I focuses on human movement, through the analysis of strontium (Sr), carbon (C), and nitrogen (N) isotopes from teeth and bones of 171 individuals, including the data from previous research to expand the context of this study. The human remains were excavated at seven burial sites of different sizes and periods of use in South Bavaria, but all burials date from the same time, around 500 AD. Notably, this sample also comprises the remains of women with modified skulls (ACD) whose foreign origin has already been indicated by earlier archaeological and anthropological studies (cf. 1.2).

$^{87}Sr/^{86}Sr$ data is used to define local ranges and identify migrants based on their signatures in tooth enamel (cf. Figure 2).

$\delta^{13}C$ and $\delta^{15}N$ bone ratios are analyzed to characterize the human diet in Early Medieval Bavaria. They are also used to define their ranges, which show the overall variability of the diet within a population at a site and thus identify deviating signals as additional indicators of migration (cf. Figure 3). Moreover, $\delta^{13}C$ and $\delta^{15}N$ from tooth dentine were analyzed for a group of individuals to investigate suspected deviating signals throughout childhood and the time in life of associated dietary shifts (cf. Figure 4).

Although the study also addresses some methodological issues, the performed analyses primarily aim to determine the extent and nature of immigration to *Raetia II* towards the end of the 5th century AD by addressing the following questions:

- How many migrants came to *Raetia II* during this time? Is there more migration than at other times?
- Where did the migrants settle within the region of *Raetia II*? Are there local or regional differences in migration rates?
- Who are those migrants? Is there a higher number of female or male migrants? What can be derived about the origin of migrants? Are there indications about the life phase during which migrants moved to *Raetia II*?

Publication II focuses on breastfeeding and weaning practices and the supposedly associated "weaning stress" (cf. 1.1.2) through the analysis of carbon (C) and nitrogen (N) from serial first molar dentine from 38 individuals. Most individuals date around 500 AD and were also included in the first publication providing information on the individuals' provenance. Besides, the sample also contains smaller groups of Late Roman people and individuals dating into the 7th century. With the exception of the graves of a few migrants, all remains were

excavated within the same region that was once *Raetia II* (Erding). In sum, the sample contains different subgroups of individuals who grew up in South Bavaria during different times or in other places.

$\delta^{13}C$ and $\delta^{15}N$ ratios obtained from serial dentine samples are used to set up individual profiles that display temporal changes in isotope ratios throughout the first ten years of life (cf. Figure 5) and thus also cover the time in which weaning happens. Weaning ages are determined by a new approach based on the initial decrease of $\delta^{15}N$ ratios i.e., the "weaning dip" (cf. Figure 5) while post-weaning levels $\delta^{13}C$ and $\delta^{15}N$ are also used to characterize the non-breastmilk nutrition of infants.

Additionally, the study includes the analysis of linear enamel hypoplasia (LEH) to investigate the occurrence and timing of stress throughout the period of weaning and in the post-weaning phase (cf. Figure 5).

On the one hand, the study concerns some methodological aspects and aims to improve our general knowledge about infant feeding strategies practiced in historical times and the occurrence of "weaning stress". On the other hand, it addresses chronological changes of practices during the transition from Roman to Early Medieval times in South Bavaria, as well as differences between spatially or culturally separated Early Medieval communities. It asks the following questions:

- How long were children breastfed in Late Roman to Early Medieval times?
- Do children experience stress while weaning? More precisely, during which phase of the weaning process?
- Are there differences between migrants and Bavarian inhabitants from around 500 AD in infant feeding practices and stress levels?
- Are there chronological changes in infant feeding practices and stress levels among the inhabitants of *Raetia II*?

The insights provided by both studies are jointly summarized in the chapter Discussion focusing on:

(1) different diets among the population of South Bavaria during the "Dark ages"

(2) different origins among the population of South Bavaria during the "Dark ages"

(3) different weaning practices among the population of South Bavaria during the "Dark ages"

3. Results

The results of this dissertation are presented in two peer-reviewed articles for which I served as the first author.

3.1 Publication I

The first article was published on the 5th April 2023 in the Open Access Journal PLOS ONE:

Velte M, Czermak A, Grigat A, Haas-Gebhard B, Gairhos A, Toncala A, Trautmann B, Haberstroh J, Päffgen B, von Heyking K, Lösch S, Burger J, Harbeck M. Between *Raetia Secunda* and the dutchy of Bavaria: Exploring Patterns of human movement and diet. PLoS ONE. 2023; 18(4):e0283243. https://doi.org/10.1371/journal.pone.0283243.

My contribution: I was involved in data collection and was jointly responsible for the curation of data. I developed novel methodological approaches applied in this study and conducted substantial parts of the formal data analysis as well as data visualization. I wrote the original draft together with Andrea Czermak and Michaela Harbeck and subsequently edited the manuscript following input from other co-authors. I also contributed conceptualization of the publication.
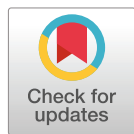
PLOS ONE

RESEARCH ARTICLE

Between *Raetia Secunda* and the dutchy of Bavaria: Exploring patterns of human movement and diet

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Abstract

During the transition from Late Antiquity to the Middle Ages, the Roman Empire dissolved in the West and medieval empires were founded. There has been much discussion about the role that migration played in this transition. This is especially true for the formation of the Baiuvariian tribe and the founding of this tribal dukedom, which took place from the 5th to the 6th century in what is now Southern Bavaria (Germany). In this study, we aimed to determine the extent of immigration during the beginning of this transformation and to shed further light on its character. To achieve this goal, we analyzed stable isotope values of strontium, carbon, and nitrogen from the teeth and bones of over 150 human remains from Southern Germany, dating from around 500 AD. This group of individuals included women with cranial modifications (ACD) which can be found sporadically in the burial grounds of this period. Our results showed an above-average migration rate for both men and women in the second half of the 5th century. They also indicate that a foreign background may also be assumed for the women with ACD. The demonstrably different origins of the immigrants from isotopically diverse regions, and the identification of local differences in detectable migration rate, as well as indication for different timing of residential changes, highlight the complexity of immigration processes and the need for more studies at the regional level.

Introduction

The transition period between Late Antiquity to the Middle Ages had a substantial influence on the formation of today's European settlements, and genetic structures in Europe (e.g., [1, 2]). During this time the western part of the Roman Empire disintegrated and was replaced by medieval kingdoms. Historical sources describe the migration of entire communities, such as

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the tribes of the Alemanni, the Ostrogoth, the Lombards, or the Huns. Although the ethnic attributions of these associations are now considered outdated [3], the period between the 3rd and the 6th century is still commonly referred to as the "Migration Period," and the extent and nature of these migrations are still discussed today [3–5].

During this time, the Roman province of *Raetia secunda (Raetia II)* dissolved in what is now Southern Bavaria, and the first tribal dukedom of the Baiuvarii emerged. In *Raetia II*, a functioning Roman administrative structure and border defenses are thought to have existed in the province until 476 AD [6]. However, throughout the 5th century, and especially toward its end, there was a decline in Roman administrative and military structures and associated way of life. Historical sources for this region are lacking during this time, but archaeological findings indicate that the Roman lifestyle persisted well towards the end the 5th century. Especially in larger settlements, such as the provincial capital Augusta Vindelicum (Augsburg), and also in border towns like Castra Regina (Regensburg) or Sorviodurum (Straubing), continuity from the late Roman period to the time of the first dukedom of the Baiuvarii can be observed (Fig 1).

Early studies attempting to explain the origins of the Baiuvarii argued that migration processes played a major role [11, 12], more recent studies suggest that local political factors, rather than migration, were key factors in their ethnogenesis [13, 14]. The extent of migration remains unclear and under debate. Whereas some authors argue for an increased immigration
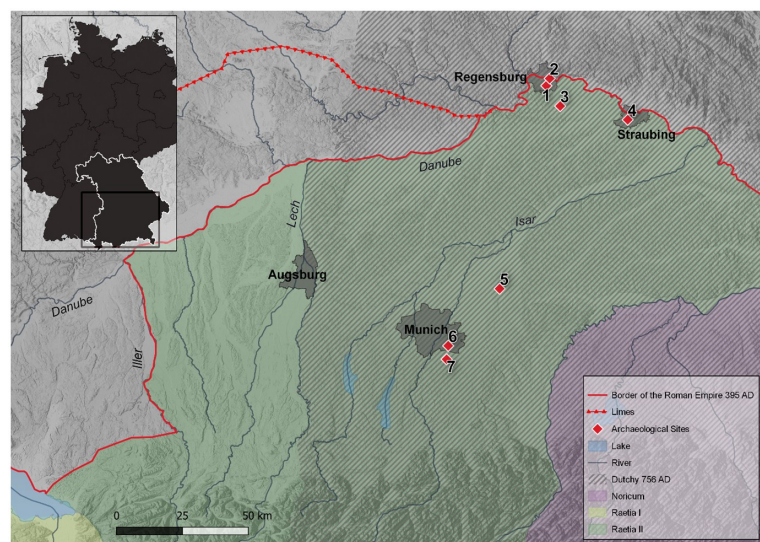
Fig 1. Map of the study area and location of archaeological sites. The study area is located south of the river Danube in the present German state of Bavaria. The region includes the part of Roman *Raetia II* that later became part of the first tribal duchy of the Baiuvarii. The Limes, the Roman-Germanic border established in the 1st century AD, was abandoned in the middle of the 3rd century, and the river boundaries of the Rhine, Iller, and Danube became the new imperial borders. Archaeological sites in the border zone: 1 Burgweinting A and B (BWA, BWB), 2 Irlmauth (IRM), 3 Alteglofsheim (AEH), 4 Straubing-Bajuwarenstraße (STB). Archaeological sites in the hinterland: 5 Altenerding (AED), 6 Munich-Perlach (PEL), 7 Unterhaching (UTH). (Map data: WMS Digital Terrain Model https://gdz.bkg.bund.de/index.php/default/wms-digitales-gelandemodell-gitterweite-200-m-wms-dgm200.html), Data licence Germany–attribution–Version 2.0 and GeoBasis-DE / BKG (2022) [7, 8]. Boundaries of provinces and course of the Limes shown as displayed in Harbeck et al. [9], dukedom drawn as displayed in Grollmann et al. [10], (created with QGIS 3.18.3 Zürich).

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into the Roman province from the late 5th century onwards [15], others contend that only marginal migration occurred during the second half of the 5th century [16].

Most work on this topic has relied on the interpretation of grave goods as evidence of the non-local origins of some individuals, most of whom were women. This is primarily because brooches, belonging to the female costume, have been typologically associated with certain regions of Europe ([17, 18], but see [19, 20]). By contrast, the grave goods of men are rather uniform and thus allow for fewer inferences about mobility and migration [20]. Consequently, female migration is more likely to be detected, and male immigrants are likely underrepresented in the archaeological record.

This topic is further complicated by the fact that terms such as "migration" and "mobility" are not standardized and may cover a variety of different processes connected with residential change [21]. Variation includes the spatial extent of movement, the social context of movement or the number of people involved. In this study, we refer to migration as a long-term relocation of one or more persons who cross cultural and/or political boundaries, while mobility means individual or group movement across shorter distances that typically takes place within the own cultural and/or political region (see [21]).

Hakenbeck et al. [22] and Veeramah et al. [23] analyzed human remains instead of grave goods to address the question of mobility and migration for this region. Both studies reported evidence of migration predominantly for women and suggest an exogamous marriage system that involves women traveling over considerable distances. In particular, the hypothesis applies to a group of women with artificial cranial deformation (ACD). The custom of shaping the skull into an elongated shape in early childhood using bandages or other material is commonly associated with the Huns and their allies [24]. In Bavaria, ACD is mainly observed in women, and there is only disputed evidence for ACD in men or children. Veeramah et al. [23] demonstrated that in most cases, women with ACD buried in Bavaria showed a strong genetic resemblance to present-day South-Eastern European populations that was absent in individuals without ACD.

Hakenbeck et al. [22] determined carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values from the bone bulk collagen of various individuals from Early Medieval Bavaria. δ^{15}N values are indicative of the trophic level of the consumer, and provide a proxy for the amount and type of dietary protein intake (e.g., [25]). δ^{13}C values are generally used to indicate the relative contributions of C3 and C4 plant foods to the diet (e.g., [26]). Hakenbeck et al. [22] suggest that the average diet is mainly based on terrestrial C3 plants, with a large contribution of animal protein, but found little evidence of the usage of freshwater or marine resources. They also found evidence of an alternative diet in 5% of the individuals tested, most of whom were women with ACD and interpret this as a sign of migration.

Here, we build on the results of these prior studies by expanding the dataset to more than 100 additional individuals. We carried out strontium isotope (^{87}Sr/^{86}Sr) analyses on enamel to identify non-local individuals. ^{87}Sr/^{86}Sr varies substantially in regions with different bedrock geology and can thus serve as a geochemical signature. The natural distribution of strontium isotopes in soil, groundwater, and organisms in an ecosystem is primarily a function of the underlying geological system [27], and the contribution of exogenous sources, especially the input of atmospheric aerosols, may play a role [28, 29]. Bioavailable strontium isotopes that enter humans through the food chain are incorporated in the mineral structure of the skeletal system and display a blend of the strontium isotopes abundant in the habitat. Individuals showing a geochemical signature outside the local range of their burial site can be considered to be of non-local origin (e.g., [30]).

In addition, besides δ^{15}N and δ^{13}C from bone collagen, we additionally analyzed δ^{13}C and δ^{15}N in incremental dentine on a cohort of individuals, mainly composed of women with

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Results: Publication I

ACD and individuals for which a deviant diet in childhood was previously suggested. Whereas bone is remodeled throughout life, teeth formed during childhood or adolescence are barely remodeled and thus provide an isotopic archive of nutrition in early life. Accordingly, the analysis of dentine in comparison with bone samples allows for the detection of changes in dietary intake [31, 32], which can also provide indications of mobile behavior in some cases (e.g., [33, 34], see also [21]).

Material

A total of 171 human skeletal samples were analyzed from seven archaeological sites in Southern Germany (Bavaria). The majority of samples dated to about 500 AD, according to archaeological or ^{14}C dating (see [23, 35] for further details). About half of the selected burial sites are located near the border of the former Roman province; the other half corresponds to the hinterland.

Cemeteries vary in size and duration of use (Table 1), with the occurrence of two general types. Smaller to medium-sized burial groups came into use in the middle, or towards the end, of the 5th century and were abandoned in the middle of the 6th century. Large cemeteries, containing a substantially higher number of individuals ("Reihengräberfelder"), appeared at the same time and were in use until the middle of the 7th century. The burial sites selected for this study captured this variety. If possible, all skeletons from smaller cemeteries were sampled. From large cemeteries, burials dating to around 500 AD were selected.

Isotopic data from bone collagen and/or the enamel of some individuals were available from previous studies [9, 22, 42, 44–46] (see S1.1 Table in S1 Text for detailed list). To complement isotopic data from these studies the following samples were selected:

(1) Enamel samples from various teeth of 94 individuals for strontium isotope analysis: Preferably the second permanent molar (M2) was sampled to avoid the influence of breastfeeding. If M2 was not available, a tooth with a similar mineralization period was chosen (e.g., second

Table 1. Archeological sites investigated in this study.

Nr.	Name [Literature]	Total N of burials	Included n of burials	Period of use	Settlement zone	Specifics
1	Burgweinting A (BWA) [36]	15	15	Late 5th to early 6th centuries	Border	Small necropolis
1	Burgweinting B (BWB) [36]	19	19	Late 5th to early 6th centuries	Border	Small necropolis
2	Irlmauth (IRM) [37]	29* (+?)	16	First half of the 6th century?	Border	Small to medium necropolis
3	Alteglofsheim (AEH) [38]	1* (+?)	1	Late 5th to early 6th centuries	Ambiguous	Poorly documented single finding of an individual with ACD
4	Straubing-Bajuwarenstraße (STB) [39]	Over 800	33	Mid-5th to mid-7th centuries	Border	Large cemetery
5	Altenerding (AED) [40, 41]	Over 1400	46	Mid-5th to mid-7th centuries	Hinterland	Large cemetery
6	Munich-Perlach (PEL) [42]	32* (+max 45)	29	Late 5th to early 6th centuries	Hinterland	Small to medium necropolis
7	Unterhaching (UTH) [43]	10* (+?)	10	Late 5th to early 6th centuries	Hinterland	Small necropolis including some individuals with remarkable grave goods indicating a higher social status

Including the total numbers of burials (N) and individuals studied (n).

*not completely excavated, maximum number of buried individuals can (+max N) or cannot (+?) be estimated,? = uncertain, see Fig 1 for locations of the cemeteries.

Cent. = century, ACD = artificial cranial deformation.

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premolar (P2), with approximate formation times at 2–8 and 2–7 years of age respectively. Only if the preferred teeth were not available other types of teeth were used. The mineralization of tooth enamel is completed at youth latest (third molar (M3): 16 years) ([47], see also [48]).

(2) Bone samples from 102 individuals for carbon and nitrogen isotope analysis: Preferably ribs without pathological bone formation were sampled. If ribs were not available cross sections of long bones were collected. Collagen in bone has a rather slow tissue turnover rate e.g., collagen from long bones reflects the average adult diet of the last 20–30 years before death [49]. The turnover of collagen in ribs is faster and thus reflects the average diet of a shorter time period before death [50]. However, since bone turnover rates generally decrease with age [49] it is difficult to estimate an exact timespan the measured isotope values actually reflect.
Our own data combined with data from literature resulted in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from a total of 166 individuals, and $^{87}Sr/^{86}Sr$ ratios from the enamel of 164 individuals.

(3) In addition, we analyzed $\delta^{13}C$ and $\delta^{15}N$ from bulk dentine of the roots of first molars (M1) from a subsample of 24 individuals to obtain information about nutrition during early childhood. Crown sections were excluded to minimize the effect of breastfeeding and weaning. Root dentine reflects the diet between the age of 3.5 and 9.5 (see S2.2.2 section in S2 Text). The selection of individuals was then narrowed down to individuals with at least one M1 with little caries. Subsequently 15 Individuals were selected by means of presence of ACD or (potentially) deviating isotopic ratios in bone or enamel. Also, nine other individuals without ACD or deviating isotopic ratios were randomly selected (S3.2.1 Table in S3 Text). Six of the 24 individuals had all three molars preserved which were additionally analyzed.

The complete data set is provided in detail in S1.1 and S1.3 Table in S1 Text.

Methods

Osteological examination

Human remains for this and previous studies [23, 45, 51, 52] were provided by the State Collection of Anthropology Munich. Osteological data were recorded following the guidelines of the State Collection [53]. The presence of ACD was evaluated by Trautmann et al. [51]. For detailed osteological data see S1.1 Table in S1 Text.

Strontium isotope analysis

Sample preparation and analysis. Procedures used for strontium extraction in this and previous studies followed the protocol described by Toncala et al. [54]. Enamel was removed from dentine using a drill, sonicated in concentrated CH_2O_2, washed twice with double distilled water, and incinerated at 500˚C. Subsequently, samples were dissolved in concentrated HNO_3 (69%) at 100˚C for at least 24 h. After the acid evaporated, the samples were dissolved in 6N HNO_3 at 100˚C for 20 min. Sr-resin SR-B25-S (Eichrom) was used to separate strontium from other elements. Strontium was then eluted from the matrix with 0.05N HNO_3.

Mass spectrometry were carried out at the RiesKraterMuseum Nördlingen (ZERIN). For mass spectrometry, extracts were loaded onto wolfram single filaments and analyzed with a thermal ionization mass spectrometer (MC-TIMS, MAT 261, Finnigan). Each sample was measured once, counting the isotopes 19 times in three blocks. Possible isotope mass fractionations during the analysis were corrected by normalizing the $^{88}Sr/^{86}Sr$ ratio to 8.37521 [55].
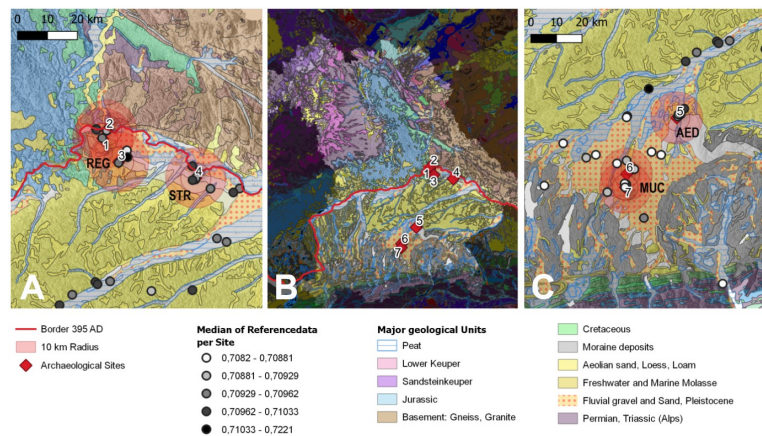
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Fig 2. Geological maps of the study region. Only the main geological units are named (Illustration: QGIS, map base GK1000 © BGR Hannover (2014) [60]. Left (A) and Right (C) Corresponding details. Grey: Location and median of strontium values of the reference data. Red circles: 10-km radii (2x5 km) around the archaeological sites (overlapping for two sites). This resulted in four regional groups: Regensburg (REG, with Burgweinting, Irlmauth and Alteglofsheim), Straubing (STB), Erding (AED), and Munich (MUC, with Munich-Perlach and Unterhaching). Center (B) Geological map of Bavaria and locations of archaeological sites: 1 Burgweinting A and B (BWA, BWB), 2 Irlmauth (IRM), 3 Alteglofsheim (AEH), 4 Straubing-Bajuwarenstraße (STB), 5 Altenerding (AED), 6 Munich-Perlach (PEL), 7 Unterhaching (UTH). (created with QGIS 3.18.3 Zürich).

Strontium standard SRM 987 (^{87}Sr/^{86}Sr 0.71034±0.00026; National Institute of Standards and Technology) was used for quality control. The certified reference value was corrected to a generally accepted value of 0.71025±0.00001 [56, 57]. The mean ^{87}Sr/^{86}Sr ratio of SRM 987 during the measurements was 0.71022±0.00005 (n = 25, 1σ).

Determination of biologically available ^{87}Sr/^{86}Sr ranges. The study area in the North Alpine foreland of Bavaria has a fairly uniform, relatively young geological surface and is surrounded by regions of, at least partly, different geology e.g., with exposed older rocks ([58], Fig 2, S2.2 section in S2 Text). Based on an extensive data set of over 900 skeletal samples from various time periods, bioavailable strontium values between 0.70800 and 0.71050 are to be expected ([59] and S2.3.2 section in S2 Text).

The range of bioavailable strontium is usually defined by inferring a local isotopic range ('baseline') using proxies e.g., geological data, strontium values of archaeological bones and teeth from animals, modern plants and shells, or a combination of it [54, 61, 62]. Currently, there is little agreement on how to best establish a baseline and which proxies to be used (e.g., [63–66]). All approaches show some weaknesses e.g., environmental samples may be altered due to anthropogenic contaminations, limited sample size may underestimate the local bioavailable ^{87}Sr/^{86}Sr ratios [54, 62, 67, 68]. A number of studies draw inferences directly from human data using the distribution of human enamel data to determine the local range of ^{87}Sr/^{86}Sr (e.g. [69–73]). The main peak of the distribution of the data can be interpreted as the local ^{87}Sr/^{86}Sr ratio for humans [74], provided by some temporal depth of the data and the absence of evidence that most of the population was non-local (e.g., [75]).

Other studies use data from human [76, 77] bones. Bone, however, is more permeable compared to tooth enamel and thus strontium from groundwater can alter the Sr-values towards a "local" signal [27]. Thus, bones are less likely to show a non-local signal than teeth

(see S2.3.2 section in Text for more details). However, if diagenetically incorporated strontium increasingly masks the autochthonous signal, the acutal variability of bioavailable strontium in the population investigated is shifted toward groundwater value. Hence bone values alone are not suitable for determining the range of bioavailable $^{87}Sr/^{86}Sr$.

In this study, we combine the bone values of animals and humans with the enamel ratios of of animals and humans of different sites and time periods to determine the local baseline of a region. Data of human enamel from one of the sites (Altenerding) already proofed suitable for the determination of local isotopic variability of the population [54]. Additionally, the very largest part of strontium isotope data is within the typical range for the Northern Alpine foreland (see S2.3.2 section and S2.3.3.1 Fig in S2 Text), providing no evidence that the largest proportion of individuals are immigrants.

To identify potential small-scale differences, we established baselines for the regions surrounding the burial sites. A region was defined as area within a 10 km radius around the burial place. Neighboring sites were combined into one regional group if their 10 km radii overlapped (Fig 2). Faunal and human remains (enamel and bone) were used and strontium isotope data from other time periods was included to ensure greater temporal depth (see S2.3.3.1 Table in S2 Text).

The central tendency of the data was determined using Gaussian kernel density estimates (KDEs). The mode in KDEs most likely represents the values of the main population. Highest density intervals (HDIs) were calculated to indicate which interval covers most of the distribution and which point are most credible ([78], see also Statistical methods below). Intervals that span 99% of the distribution (99% HDIs) define the biologically available $^{87}Sr/^{86}Sr$ range of the individual region.

Non-locals and migrants. Individuals are defined as "non-local" if their strontium isotope ratio in tooth enamel is outside the 99% regional HDI. However, an $^{87}Sr/^{86}Sr$ ratio within the local biologically available range does not necessarily mean that the individual can be considered local. The ratio could also derive from a region with similar geology and biologically available $^{87}Sr/^{86}Sr$ signature. Due to the geographical redundancy of $^{87}Sr/^{86}Sr$ it can be assumed that a certain proportion of non-local individuals remain undetected and thus only the minimum proportion of non-locals can be indicated. Similarly, it is not possible to pinpoint a specific region of origin for detected non-locals. Yet, regions of origin can be excluded e.g., if the $^{87}Sr/^{86}Sr$ ratio of an individual exceeds the range of the alleged region of origin. If the $^{87}Sr/^{86}Sr$ ratio is within the range of an alleged region of origin, however, it might as likely derive from a region with similar conditions.

Non-locals whose $^{87}Sr/^{86}Sr$ ratios fit into the range of another region within the pre-Alpine area and thus likely moved across shorter distances within South Bavaria are referred to as "mobiles". We only refer to individuals as "migrants" if other regions in the Northern pre-Alpine area can be excluded as their region of origin, following the concept that migration occurs over longer distances and cultural and/or political boundaries.

Comparing migration over time. The number of non-locals that can be reliably detected via strontium can vary depending on the geological conditions of both, the region of origin and burial site, as well as the type of data available and methods used. Thus, comparing migration rates is problematic. To minimize this issue and to estimate the extent of migration in South Bavaria over time we calculated a minimum frequency of migrants (MFM = percentage of specific immigrants) in the North Alpine area for different time periods (Late Neolithic, Early Neolithic, Early Bronze Age, Iron Age, Late Antique, around 500 AD and Early Middle Ages) based on the enamel data of adults from the literature (N = 519), using the range of the bioavailable $^{87}Sr/^{86}Sr$ calculated for the whole area (based the adjusted reference data set (N = 865) (for more detail see S2.3 section in S2 Text).

As strontium ratios from the region of Regensburg significantly exceeded the upper limit for the Northern pre-Alpine region due to their high variability (see Results) we excluded this region from calculations of MFM.

Carbon and nitrogen isotope analysis

Sample preparation and analysis. Bone collagen was extracted following the protocol described in Siebke et al. [79]. Bones were cleaned in an ultrasonic bath with distilled water, air-dried, and ground into powder. 250–300 mg of powder were demineralized in 1M HCl at room temperature for 20 min, washed with distilled water, and transferred into 0.125M NaOH for 20 h at room temperature. After washing with distilled water until neutralization, they were gelatinized in 0.001M HCl at 90˚C for 10–15 h. Solubilized collagen was filtered and lyophilized.

For serial incremental dentine sampling molars were bisected longitudinally. Samples were demineralized in 0.5M HCl at 4˚C and then washed with distilled water until pH-neutral. The tooth halves were cut into 1-mm thick slices with a scalpel (see S2.2.1 section S2 Text). Dentine sections were incubated in 0.125M NaOH for 24 h at room temperature, and rinsed with distilled water until neutral pH and then gelatinized in 0.01M HCl for 17 h at 80˚C [80]. Liquid fractions were frozen and lyophilized. 0.3–0.8 mg of bone or dentine collagen was transferred into tin capsules for mass spectrometry.

Samples were analyzed in singles at the GeoCenter (Friedrich-Alexander University, Erlangen-Nürnberg) with a Flash EA 2000 elemental analyzer connected to a ThermoFinnigan Delta V Plus mass spectrometer. Some measurements were carried out at the Isolab GmbH in Hanau using an Elementar Vario Cube EL connected to an Isoprime mass spectrometer (see S1.1 Table in S1 Text for detail). Results are reported in the conventional δ-notation in permil (‰) relative to internationally accepted standards, VPDB for carbon and AIR for nitrogen. Accuracy and precision were checked by replicate analyses of laboratory standards (e.g., Isolab: Collagen STD R (USGS 89), Collagen STD S, Collagen STD BRA; GeoCenter: Casein, Cyclo) calibrated to international standards USGS40 and 41. Analytical precision ensured by the Geo-Center is 0.1‰ for δ^{13}C and δ^{15}N as well as 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N by the Isolab GmbH. Comparing data from different laboratories results in an expanded uncertainty which is generally specified as twofold analytical precision. The collagen quality of samples was evaluated through the C/N atomic ratio (between 2.9 and 3.6 [81]) as well as carbon (in excess of 13%) and nitrogen content (in excess of 4.8%) to indicate sufficient collagen quality [81–84]. Samples were excluded if they had elevated carbon (>50%) and/or nitrogen (>19%) contents, indicative of contamination.

Approximate ages were assigned to dentine sections using a customized scheme based on *The London Atlas of Tooth Development and Eruption* [47]. For detailed description of dentine sampling and age assignment of samples see S2.2.2 section in S2 Text.

Determining basic human diet. To characterize the main components of the diet in Bavaria we used human δ^{13}C and δ^{15}N bone collagen data of adults from all investigated sites in comparison to faunal samples (see S3.1 section in S3 Text for details). We take inter- and intra-populational variability of δ^{13}C and δ^{15}N values into account, which can be influenced by various factors: Isotope signals in bone represent a mixture of dietary intake over a longer time due to tissue turnover (see above) and nutritional stress can influence δ^{13}C and δ^{15}N values [85–87]. Also, divergent natural conditions in the different areas that directly influence the isotopic ratios in plants (e.g., [88–90]). Inside natural limits, different subsistence strategies such as different practices in agriculture and husbandry, fishing and hunting as well as trading food, access to resources (possibly linked to sex or age), social position etc. (e.g., [91–93]) can

result in intra-populational differences. However, variability in diet can also be linked to the mixed composition of communities including individuals of different origins and ethnicities [94].

Determining the "common intra-populational diet variability". The study region is spatially rather narrow and has fairly uniform ecogeographic conditions and thus is likely inhabited by the same type of plants and animals. Human data therefore generally cluster in a range that is limited by the characteristics of the ecosystem and food resources. However, small inter-populational differences are possible.

Therefore, we subdivided population groups by their location. Data from neighboring sites (<5 km) like Munich-Perlach and Unterhaching, as well as Burgweinting, Irlmauth, and Alteglofsheim were combined. These sites have similar external conditions e.g., ground conditions (Munich gravel plain or river valleys covered with loess soils) and/or overlapping catchment areas for crops and animals [95, 96]. The scattering of $\delta^{13}C$ and $\delta^{15}N$ values of adults of combined sites supports this approach. Additionally, the sample size is increased, reducing the risk that the variability is underestimated.

To describe intra-populational variability we use KDEs and HDIs. The 99% intervals cover the variability determined by both external and internal factors and thus are suitable to define the "common variability" of a population's diet.

Detecting dietary outliers. Individuals who show $\delta^{13}C$ and/or $\delta^{15}N$ values outside the 99% HDIs of the respective population are defined as outliers. The diet of these individuals was unusual for the region of their burial site, either because of food imports or because they moved there from a region with different diet [33, 34]. Similar to strontium isotope analysis, it is impossible to pinpoint the region where non-local diet or individuals originally came from. A specific kind of ecosystem can be suspected at the very most. It is also likely that people move from one land-locked region to another within the same ecosystem in temperate Europe without significantly changing their diet [22].

Different diet during early life is likely no longer evident in bones of an older individual because of the turnover of bone collagen. However, it can be detected through the analysis of tooth dentine.

Comparing childhood and adult diet. We investigated inter- and intra-individual variability aiming to detect shifts in isotope ratios possibly be linked to a change in residency. Bone samples from adults show dietary pattern in a later life stage. To analyze diet in childhood we used collagen data from dentine of the first molar. Serial dentine samples provide high-resolution biographical data pointing out dietary changes during an individual's life (e.g., [97]). Fluctuations in micro-sample profiles can be linked physiological stress and migration [98]. Bulk dentine represents the mean values of root dentine sections from the serial analysis.

First, we compared $\delta^{13}C$ and $\delta^{15}N$ values of bulk dentine and bone samples from every individual and identified outliers from the predefined "common variability" of a population's diet in the bulk dentine dataset. Then we tested the hypothesis whether these outliers also show deviating $^{87}Sr/^{86}Sr$ in enamel, deviating $\delta^{13}C$ or $\delta^{15}N$ in bone or the presence of ACD as all these features can indicate foreign origin. Individuals with evidence of foreign origin are also likely to show deviating stable light isotopic ratios in tooth dentine due to their potential stay in a different ecosystem. However, individuals that changed residency during early childhood can only be detected using sequential dentine analysis.

Statistical methods

KDEs and HDIs. Gaussian KDEs and corresponding HDIs of $^{87}Sr/^{86}Sr$, $\delta^{15}N$, and $\delta^{13}C$ data groups were calculated with RStudio 1.4.1717 for Windows using hdr.den function of

package hdrcde [99]. The optimal bandwidth was selected using the "solve-the-equation" method of Sheather & Jones [100] based on the adjusted reference sample set for ^{87}Sr/^{86}Sr (S2.3.2 Text) and combined sample sets of all sites for δ^{13}C and δ^{15}N (S2.4 Text). We calculated 99%, 95% and 90% HDIs below the most prominent mode in KDEs using Hyndman's density quantile algorithm [99] but used the 99% HDIs to determine cutoffs most conservatively and minimize the risk of overestimating the number of individuals with deviating ratios due to methodological uncertainties (see S2.3.3 and S2.4 section in S2 Text for more detail).

Statistical tests. Non-parametric statistical tests like Mann-Whitney U test [101], Kruskal-Wallis test [102], and Dunn's post hoc test [103] were primarily conducted on metric data due to small sample sizes and the properties of the data (e.g., no normal distribution). Parametric tests like t-test [104], ANOVA [105], Hochberg post hoc test (for equal variances, [106, 107]), and Dunnett T3 (for unequal variances, [108]) were used when appropriate (e.g., normal distribution). Nominal data were analyzed by non-parametric Chi-square tests [109]. Significance values for multiple comparisons were adjusted by the Bonferroni correction [110] to counteract alpha error accumulation. Calculations were made with SPSS 26.00 for Windows. Interpretations are based on explanations given by Wilcox [111].

Results

Strontium isotope analysis

Local ranges and outliers. The strontium ratios of the human enamel samples in our study had a mean of 0.71023±0.00149 (range: 0.70658 to 0.71481).

For the entire pre-Alpine region south of the Danube (N = 865) a local range of 0.7081–0.7110 could be determined (S.2.3.2 Text). The following ranges were calculated for smaller-scale regions (S.2.3.3 Text): Straubing (STB): 0.70805–0.71096, Regensburg (REG): 0.70813–0.71195, Erding (AED): 0.70857–0.71104, and Munich (MUC): 0.70802–0.71021. In Fig 3, calculated ranges are shown in red and relevant values are presented in groups.

Regional strontium ranges showed some shifted limits that partially exceeded the range of bioavailable strontium determined for the entire pre-Alpine region south of the Danube. This is especially true for the span of Regensburg. Compared to the Straubing, Erding, and Munich sites, which showed distinct main peaks at similar values, Regensburg had a broader and multi peaked distribution shifted toward higher ^{87}Sr/^{86}Sr values (also see S2.3.2 section in S2 Text).

As expected, in all regions human and animal bone values, as well as enamel values of children (which most likely not relocated) show a narrower range than values of dental enamel of adults (see S2.3.2 section in S2 Text and Methods). For some individuals, ^{87}Sr/^{86}Sr was measured in both bone and enamel (indicated by numbers in Fig 3A–3D). In all these cases bone ^{87}Sr/^{86}Sr values show a signal within the local range, even if the corresponding enamel values are far outside this range.

The range determined from the total set of regional biomineral samples (red area) exceeds in each case the variation of environmental samples. Except some soil samples (region MUC, Fig 3D) which show implausible high values and let suspect methodological problems (as discussed in Toncala et al. [54]). The range of bioavailable strontium based on the total biomineral of the region also exceeds those ranges covered by materials usually taken as reference for local values (tooth enamel of children and animals, bone values). Thus, we consider the chosen method for estimating the local range not only as reliable but also suitable to not overestimate potential newcomers.

For a total of 36 individuals, an origin outside a 10 km radius of their burial site (regional range) can be assumed on the basis of their strontium values in tooth enamel (Table 2). Of these, the ^{87}Sr/^{86}Sr values of nine individuals from three sites exceeded the local ranges but fall
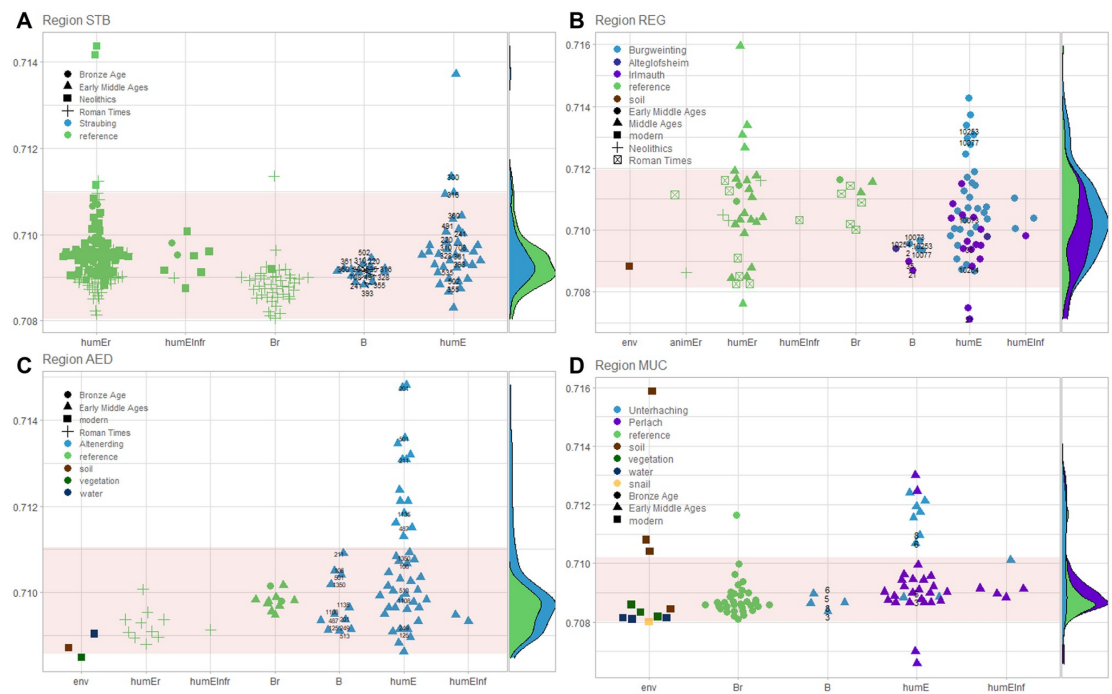
Fig 3. Local ranges and distribution of ^{87}Sr/^{86}Sr values per region and site. (A) Region Straubing (STB), (B) Region Regensburg (REG), (C) Region Erding (AED) and (D) Region Munich (MUC). ^{87}Sr/^{86}Sr (y-axis) of samples from predefined study region divided into groups (x-axis): env = environmental samples, animE = animal enamel, humE = human enamel, humEInf = human enamel of infans (0–12 years), B = bone (animal or human) (followed by an r = part of the reference data set, no r = archaeological site investigated). Red area: calculated range of bioavailable strontium based on all biomineral samples of the region. Symbols provide a chronological classification. Numbers = grave numbers of individuals from which both bone and enamel were measured. If values from environmental samples were known from the literature, they were added for comparative reasons: Lengfelder et al. [112]: soil, vegetation, water for AED and MUC, Hoogewerff et al. [113]: soil for REG, Neumann et al. [114]: soil and snail MUC. Right side: Distribution of samples separated by reference and respective site: Straubing (STB): N = 217, Regensburg (REG): N = 80, Erding (AED): N = 78, and Munich (MUC): N = 76 (illustration: R with packages ggplot2 [115, 116], ggbeeswarm [117], ggside [118], ggrepel [119]).

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into the local ranges of another region in the Northern Alpine foreland of Bavaria (see Table 2). For the majority of these individuals (N = 7) the region around Regensburg with its higher ^{87}Sr/^{86}Sr range can be considered.

Overall, the majority of outliers (N = 32) referred to regions with higher strontium signatures than in the Northern Alpine foothills, such as geologically older regions with granite bedrock in the Bavarian Forest or Bohemia. Only four isotope values were below the local range of our study region. This indicates an origin from regions partially shaped by younger rocks, such as volcanic stone, or those dominated by chalk/dolomite/limestone or impure carbonate sedimentary rocks (e.g., [64]), such as found in South Tirol.

Non-locals and migrants. In total 23% (95% CI: 17–30%) of all individuals are of non-local origin with an equal number of non-local females (23%, 95% CI: 14–32%) and males (24%, 95% CI 14–34%) (Fig 4A, S3.4.1.2 Table in S3 Text). Of the nine mobile individuals whose strontium values are not in the local range of their burial region, but who show values within the Northern Alpine region (especially Regensburg), no differences between the sexes can be detected (5 men and 4 women, see Table 2). If these nine individuals are excluded, in the group of individuals who show strontium ratios from geologically older or younger regions

Results: Publication I

Table 2. Characteristics of assumed newcomers according to deviating isotopic ratios or presence of ACD.

Individual (N = 50)	Stable isotopes					
	$^{87}Sr/^{86}Sr$ enamel	$^{87}Sr/^{86}Sr$ bone	$\delta^{13}C$ [‰] bulk bone	$\delta^{15}N$ [‰] bulk bone	$\delta^{13}C$ [‰] bulk dentine	$\delta^{15}N$ [‰] bulk dentine
STB_228* ♀,m	-	0.70883	-18.7	9.0	-	-
STB_300 ♀,m	0.71135	0.70921	-19.9	8.8	-18.9	9.5
STB_328* ♀,m	0.70960	-	-19.6	8.8	-	-
STB_361* ♀,m	0.70953	0.70921	-19.6	9.2	-15.3	10.0
STB_395 ♂,s	0.71372	-	-20.0	10.2	-	-
STB_535* ♀,m	0.70923	0.70919	-20.1	9.1	-16.0	9.2
AED_94 ♀,m	0.71211	-	-20.0	8.7	-	-
AED_125* ♀,m	0.70908	0.70913	-19.3	9.3	-16.8	10.3
AED_160 ♀,s	0.71481	-	-20.4	9.6	-	-
AED_201 ♀,m	0.71475	0.70937	-20.0	9.0		
AED_211 ♀,m	0.71308	0.71090	-19.3	9.3	-19.4	11.7
AED_321 ♂,m	0.71238	-	-19.9	8.8		
AED_343 ♀,m	0.71345	-	-19.1	7.9	-17.3	9.6
AED_344 ♂,m	0.71130	-	-19.3	9.6	-19.7	9.7
AED_421 ♀,s	0.71092	-	-20.0	11.2		
AED_487 ♂,m	0.71150	0.70935	-19.5	9.7		
AED_492 ♂,m	0.71161	-	-19.5	8.9	-19.8	9.6
AED_501 ♂,m	0.71358	0.71041	-19.4	9.9	-19.8	9.8
AED_513* ♀,m	0.71006	0.70910	-16.8	9.1	-15.9	11.2
AED_521 ♀,m	0.71320	-	-18.8	9.8	-	-
AED_1108* ♀,s	0.70982	0.70950	-18.2	10.4	-	-
AED_1123 ♂,s	0.71212	-	-19.8	9.6	-	-
AED_1135 ♀,a	0.71183	0.70965	-20.3	10.1	-	-
AED_1143 ♂,a	0.71309	-	-19.7	10.0	-19.6	9.0
AED_1350* ♀,m-s	0.71076	0.71019	-18.9	9.6	-	-
IRM_20 ♀,j	0.70750	-	-20.0	8.9	-	-
IRM_21 ♀,m	0.70714	0.70870	-19.9	9.0	-	-
IRM_33* ♀,m	-	-	-19.8	9.5	-	-
BWB_3734 ♂,a	0.71247	-	-21.0	9.2	-	-
BWB_3735 ♀,m	0.71426	-	-20.2	9.5	-	-
BWB_3739 ♀,m	0.71340	-	-19.8	8.7	-	-
BWB_3740 ♀,a	0.71373	-	-20.7	9.2	-	-
BWB_3741 ♀,m	0.71278	-	-20.9	9.0	-	-
BWA_10071 ♀,a	0.71076	-	-15.0	7.7	-14.2	8.4
BWA_10077 ♀,a	0.71297	0.70936	-20.2	9.7	-	-
BWA_10253 ♂,m	0.71309	0.70941	-19.7	9.3	-	-
BWA_10254* ♀,s	0.70873	0.70956	-19.5	10.0	-16.8	11.9
AEH_145* ♀,m	0.70979	0.70941	-19.4	10.0	-	-
PEL_12 ♀,a	0.70658	-	-19.1	8.2	-18.2	9.9
PEL_18 ♀,s	0.70866	-	-18.1	8.9	-	-
PEL_19 ♀?,i II	0.70699	-	-19.1	8.2	-	-
PEL_22 ♂,a-m	0.71247	-	-19.7	9.0	-	-
PEL_27 ♂,m	0.71300	-	-19.2	9.0	-	-
UTH_1 ♀,m	0.71156	-	-19.2	9.3	-	-
UTH_2 ♀,m	0.71214	-	-	-	-	-
UTH_6 ♂,m-s	0.71068	0.70897	-19.5	9.0	-	-

(*Continued*)

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Table 2. (Continued)

Individual (N = 50)	Stable isotopes					
	^{87}Sr/^{86}Sr enamel	^{87}Sr/^{86}Sr bone	δ^{13}C [‰] bulk bone	δ^{15}N [‰] bulk bone	δ^{13}C [‰] bulk dentine	δ^{15}N [‰] bulk dentine
UTH_7 ♂,m	0.71175	-	-19.7	9.3	-	-
UTH_8 ♀,m	0.71096	0.70863	-	-	-	-
UTH_9 ♀,a-m	0.71195	-	-19.2	8.7	-	-
UTH_10 ♀,a	0.71241	-	-	-	-	-

Skull shape: * = modified (ACD) highlighted in bold, no information = not modified. Sex: ♀ = female, ♀? = rather female, ♂ = male. Age at death: i-II = infans II, j = juvenile, a = adult, a-m = adult-mature, m = mature, m-s = mature-senile, s = senile. Isotope data that identify individuals as noticeable are highlighted by grey background. Italic ^{87}Sr/^{86}Sr enamel: Although these Individuals were identified as not being local to their burial site, an origin from another site of the North Alpine Bavarian foreland cannot be excluded.
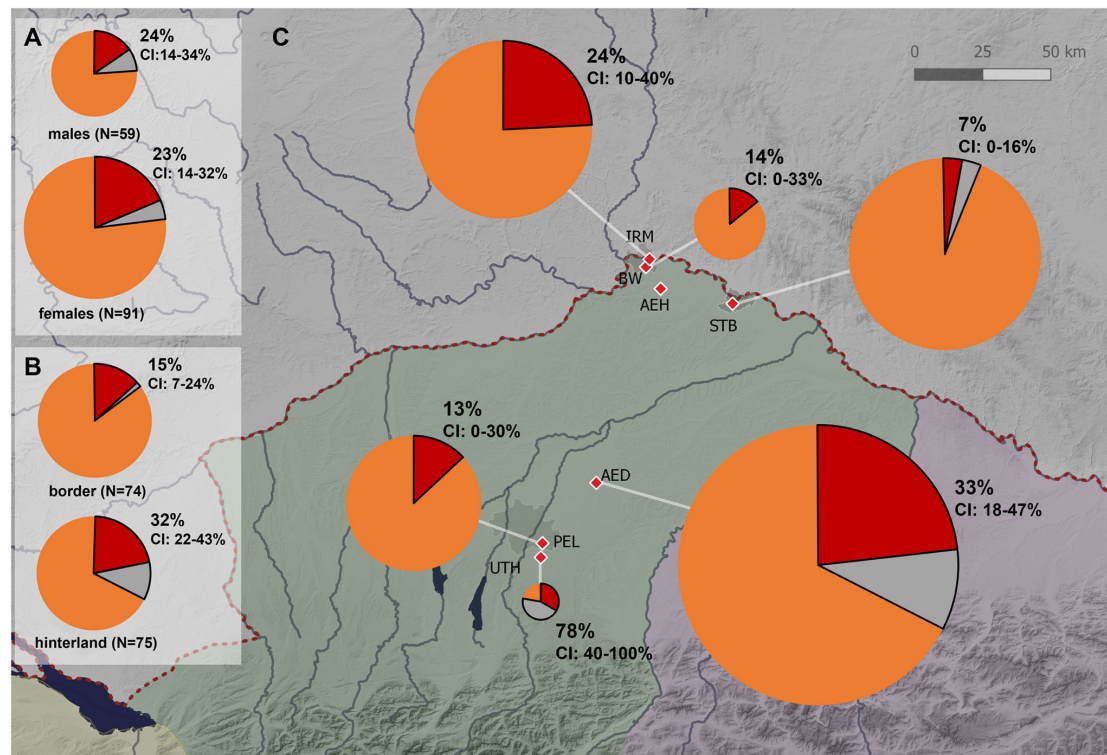
Fig 4. Proportion of non-locals. Circular charts illustrating the proportion of non-locals (A) in gender groups (females vs. males), (B) in settlement zones (border vs. hinterland), (C) for archaeological sites (VW Burgweinting, IRM Irlmauth, STB Straubing-Bajuwarenstraße, AED Altenerding, PEL Munich-Perlach, UTH Unterhaching). Given numbers are frequencies of all non-locals, whose strontium values are found outside the range of their burial region, which summarize the frequencies of individuals who show strontium ratios outside the local range of the Northern Alpine area in South Bavaria (filled in dark red) and individuals whose strontium values are found in other study regions in South Bavaria (filled in grey). (Map data: see Fig 1). (created with QGIS 3.18.3 Zürich and Excel).

than South Bavaria females (19%, 95% CI: 11–28%) and males (15%, 95% CI: 7–25%) are still equally represented (S3.4.1.4 Table in S3 Text). Further characteristics (e.g., age at death etc.) for identified non-locals are given in Table 2. Notably, no woman with ACD has a strontium isotope ratio that falls outside the range of local bioavailable strontium of the respective regions or the Northern Alpine region.

Spatial pattern. A significant difference (Chi-Square Test: p = 0.014) between the prede-fined settlement zones was apparent; specifically, the frequency of non-locals in the border area (15%, 95% CI: 7–24%) was lower than in the hinterland (32%, 95% CI: 22–43%) (Fig 4B, S3.4.2.2 Table in S3 Text). In addition, some local differences (Chi-Square Test: p<0.001) in the proportion of non-locals between archaeological sites were observed (Fig 4C, S3.4.3.2 Table in S3 Text). Pairwise comparisons (S3.4.3.1 Table in S3 Text) of sites revealed a signifi-cantly higher number of non-locals in Unterhaching (UTH) than expected (Post Hoc Chi-Square Test: p<0.001). These differences are also visible if the frequency was calculated only for migrants, however not significant (S3.4.2.4, S3.4.3.4 Table in S3 Text).

MFM rates over time. The average frequency of MFM across all periods was around 15% (95% CI:14–22%), but we observed chronological variations (Fig 5, Chi-Square Test: p = 0.011). The MFM calculated for the cemeteries around 500 AD amounted to 23% (95% CI:15–31%), which was higher than the average, and also higher than the proportions in most other periods (Post Hoc Chi-Square Test: p = 0.0039, S3.5.2 Table in S3 Text).

Carbon and nitrogen analysis

Bone collagen: Variability of diet and outliers. The human bone $\delta^{15}N$ values clustered around a mean of 9.2±0.7‰, ranging from 7.4‰ to 11.2‰. The $\delta^{13}C$ values had a mean of -19.7±0.7‰, with an overall range of -21.0‰ to -15.0‰. The scattering of stable light isotopes suggests that individuals consumed different proportions of plant and animal food sources. We found no significant differences between the $\delta^{13}C$ or $\delta^{15}N$ values of men and women (S3.1.1.1.1 Table in S3 Text), but females showed a higher variance in $\delta^{13}C$ values (S3.1.1.1.2 Table in S3 Text).

While the human isotope ratio values from human bone collagen generally overlapped (Fig 6), we also observed some site-specific variation (Kruskal-Wallis Test: p<0.001 for both $\delta^{13}C$ and $\delta^{15}N$ values). Samples from Munich-Perlach were shifted towards decreased $\delta^{15}N$ values and increased $\delta^{13}C$ values compared to most other sites (S3.1.1.4.2 Table in S3 Text). More-over, Burgweinting, Irlmauth, and Altenerding showed more variable $\delta^{13}C$ and $\delta^{15}N$ values compared to those from Straubing-Bajuwarenstraße, Munich-Perlach, and Unterhaching. Six individuals, solely females, had isotopic values outside the common variability of the popula-tion (Table 2). Five outliers had increased $\delta^{13}C$ bone values, but two $\delta^{15}N$ bone values below or above the "common variability" were also found.

Bulk dentine collagen: Childhood diet. The mean differences between the bone and den-tine collagen of individuals without notable attributes (Fig 7A) were 0.7‰ for $\delta^{13}C$ and 0.5‰ for $\delta^{15}N$, reflecting normal dietary variation over a person's lifetime. The mean differences for individuals who show notable attributes (isotopic outliers or presence of ACD) (Fig 7B), by contrast, were more than twice as large (1.5‰ for $\delta^{13}C$ and 1.1‰ for $\delta^{15}N$), which is signifi-cant for $\delta^{15}N$ (Mann-Whitney-U-Test: p = 0.001, S3.2.1 Table in S3 Text). Dentine $\delta^{13}C$ values of nine individuals, again solely females, exceeded the "common intra-populational variability" (Table 2). Most of these outliers had increased carbon ratios, but some also showed increased $\delta^{15}N$ values.

Only some migrants (determined by Strontium isotope analysis) showed a deviating dietary pattern in childhood. However, uncommon dietary pattern in childhood and the presence of
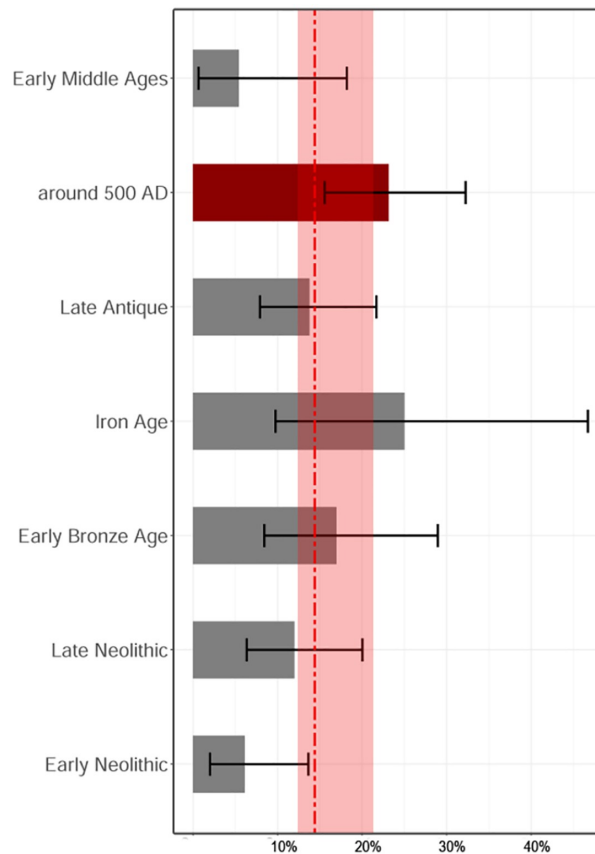
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Fig 5. Minimum Frequency of Migrants (MFM) in different periods. Late Neolithic N = 108, Early Neolithic N = 82, Early Bronze Age N = 59, Iron Age N = 24, Late Antique N = 109, around 500 AD N = 108 and Early Middle Ages N = 37. Error bar = confidence interval, red line = mean frequency of immigrants if all periods are considered together, red box = confidence interval for this average.

notable attributes in individuals was significantly correlated (Chi-Square Test: p = 0.011, S3.2.3 Table in S3 Text). Interestingly, deviating dietary patterns from childhood were not observable in the bone collagen of these individuals, except for in two females (AED_513*, BWA_10071).

Serial dentine collagen: Isotope profiles. Selected stable C and N isotope profiles from incremental dentine serial samples of six individuals are shown in Fig 8. All profiles displayed normal variation in diet such as the decline in both isotopes at the beginning due to weaning or phases with rather low fluctuations (see S3.3 section in S3 Text for more detail). AED_105, previously determined to be "local," showed the most stable profile, lacking any profound dietary changes in stable light isotope values within the local range. The isotopic dentine values of all predefined migrants were outside local ranges over longer periods, and their profiles included remarkable shifts in stable light isotopes outside of normal variability, especially in $\delta^{13}C$.
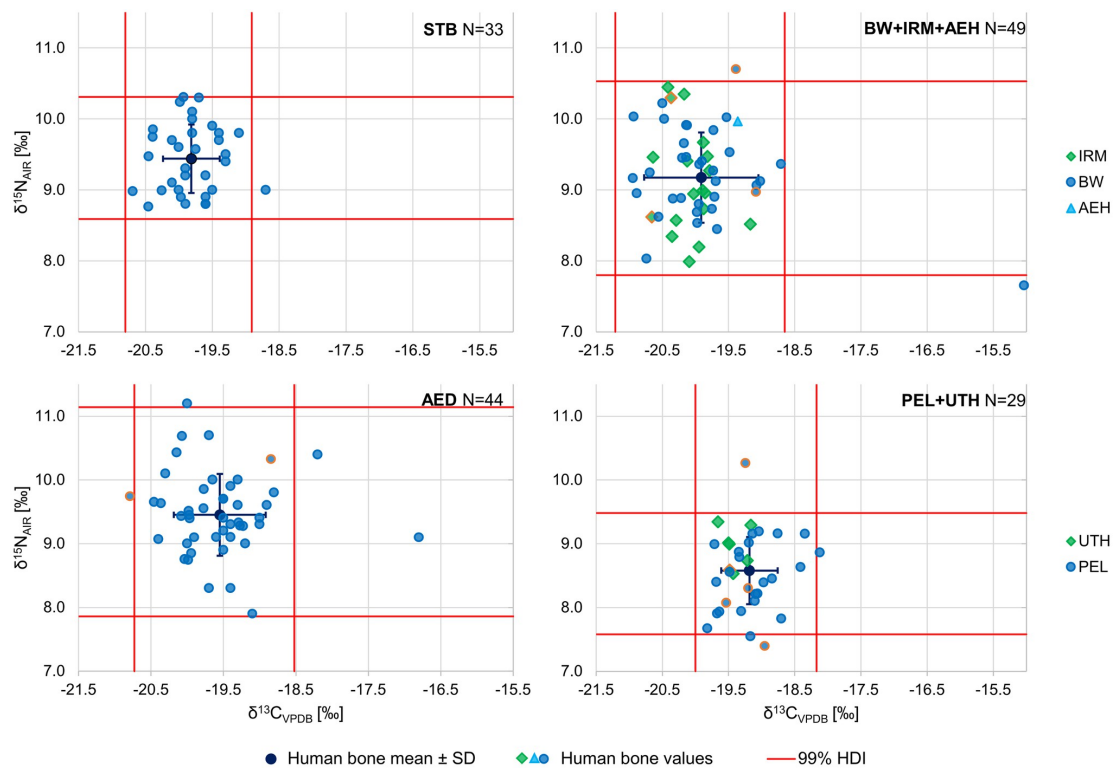
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Fig 6. Variability of carbon and nitrogen isotopic ratios. Scatter plots of $\delta^{13}C$ and $\delta^{15}N$ values of human bone collagen: Straubing-Bajuwarenstraße (STB, N = 33), Burgweinting, Irlmauth and Alteglofsheim (BW+IRM+AEH, N = 49) in the border zone, and Altenerding (AED, N = 44), Munich-Perlach and Unterhaching (PEL+UTH, N = 29) in the hinterland. Mean values and standard deviations (SD) are based on postweaning individuals (excluding infans I children = values outlined in orange) and were calculated using SPSS. Red lines represent 99% HDIs (calculations: hdr.den function of R package hdrcde [99]) (illustration: Excel).

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In the profiles of BWA_10071 and BWA_10254*, there was a shift in $\delta^{13}C$ indicating a change in diet around the age of 15 years. Whereas BWA_10071 reached isotope values that corresponded to those in her burial environment, this was not the case for BWA_10254*. Here, the value only approached the burial environment in the bone, indicating that a change of diet must have occurred between the age of 20 and her death at an advanced age. The same observation was made for two other women (PEL_12 and AED_343). BWA_10075 showed a temporary shift in $\delta^{13}C$ that slightly exceeded the common intra-populational variability.

Discussion

Diet

Average diet (bone collagen). Overall, the human isotope ratio data from Early Medieval Bavaria reflected a mixed diet in a temperate C3 plant-based ecosystem. Archaeological evidence for Southern Germany from Late Antique and Early Medieval times shows that a wide range of C3 plants (cereal crops, oil and fiber plants, pulses) were cultivated [120–123]. The amount of animal protein in the diet was generally moderate and may also have been derived
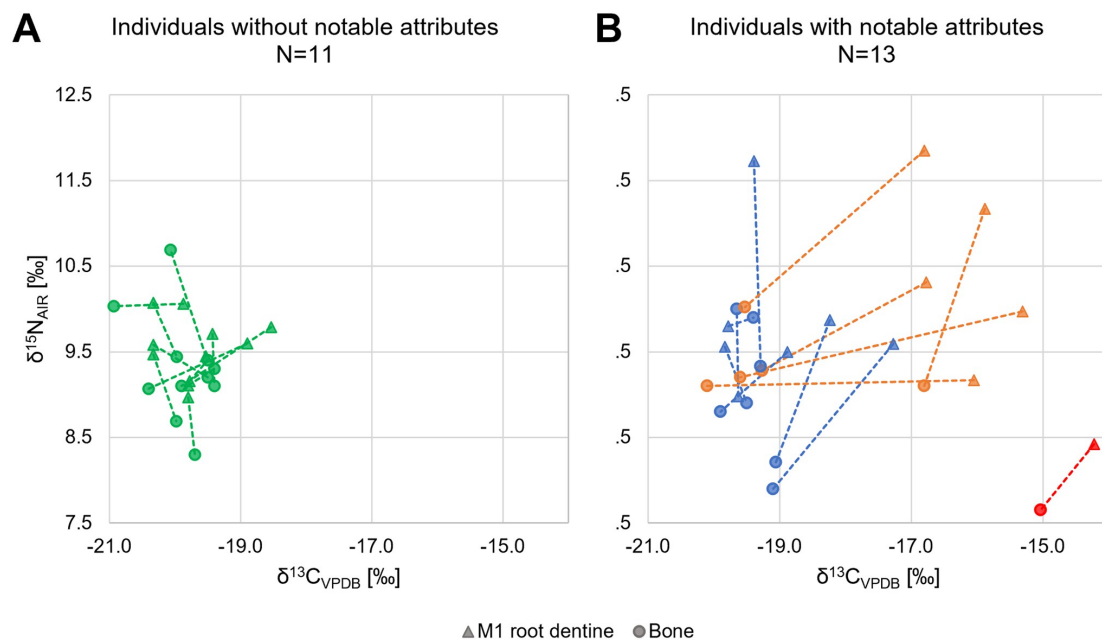
Fig 7. Differences between bulk dentine and bone collagen. (A) Differences between bulk dentine and bone collagen of individuals without notable attributes (N = 11, illustrated in green), and (B) individuals with notable attributes (outliers in enamel ^{87}Sr/^{86}Sr illustrated in blue, outliers in bone collagen δ^{13}C and/or δ^{15}N illustrated in red, or the presence of ACD illustrated in orange) (illustration: Excel).

from low amounts of freshwater fish in addition to terrestrial animals (for more detail see S3.1 section in S3 Text).

In general, isotopic human bone collagen data from different sites show a large overlap as expected for nearby sites with rather similar ecogeographic conditions (S3.1 section in S3 Text). A notable exception is Munich-Perlach with lower δ^{15}N values and higher δ^{13}C values in human bone. Dietary features e.g., lower amounts of animal-derived foodstuffs and higher contents of plants, or plant parts with increased carbon ratios and/or lower amounts of freshwater fish in the diet, or different practices in agriculture e.g., land use could have been less intensive without or even with less manuring [91] (S3.1 Text).

The common assumption that men consume more animal protein or higher trophic level protein than women (e.g., [124]) is not supported by our data in general, but δ^{15}N and δ^{13}C mean values of males are slightly increased compared female ratios at most burial sites, except for the small necropolis in Burgweinting (BW) and Unterhaching (UTH) (S3.1.1.1.4 Table in S3 Text). The higher variability of δ^{13}C values in females suggests a more variable diet, especially regarding plant resources. These findings correspond with the results of Hakenbeck et al. [22].

Diet in subadult age. More than half (67%) of the individuals show only slight deviations (up to 1.5‰ in δ^{13}C and/or δ^{15}N) between later childhood (3.5 to 9.5 years) and adulthood. This can be explained by a varying protein intake and/or varying intake of photosynthetically inactive parts of C3 plants such as roots, seeds and fruits [125] or cereal grains [126] between childhood and adulthood.

Individuals that show greater differences (more than 1.5‰ in δ^{13}C and/or δ^{15}N) in nutrition between childhood and adulthood also show non-local strontium isotope signals or ACD.
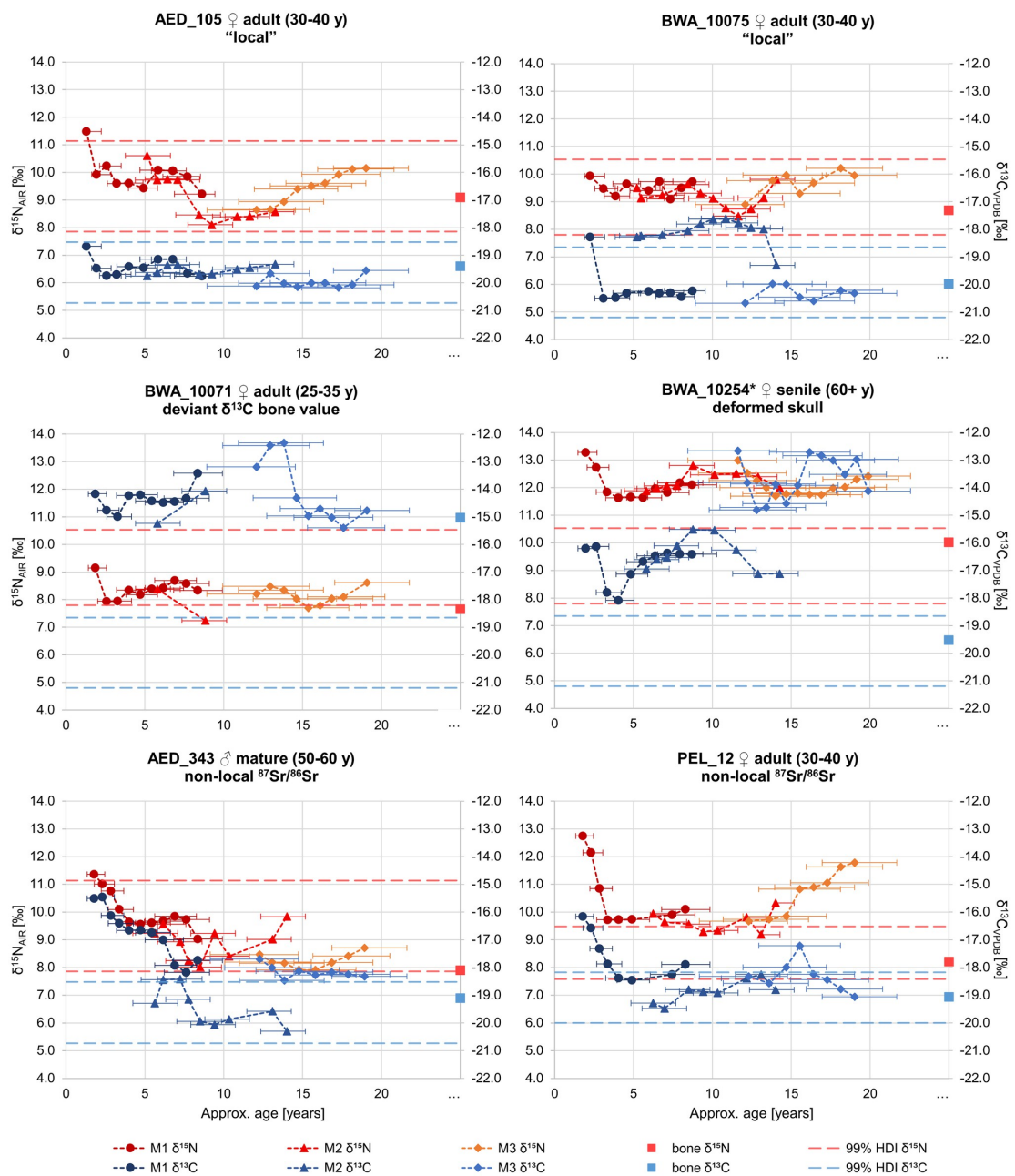
Fig 8. Stable carbon and nitrogen isotope profiles of six selected females (AED_105, AED_343, BWA_10071, BWA_10075, BWA_10254*, PEL_12). δ¹³C (blue shades) and δ¹⁵N (red shades) values of collagen from incremental dentine serial sections of molars (M1-M3) and bone collagen (square). Error bars represent the putative timespan each datapoint covers. Dotted lines represent common dietary signals at burial sites (99% HDIs) (illustration: Excel).

https://doi.org/10.1371/journal.pone.0283243.g008

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Moreover, childhood dietary signals outside the common intra-populational variability significantly correlate with the presence of a notable attribute (ACD, deviant diet in adulthood, non-local $^{87}Sr/^{86}Sr$) which indicate foreign origin (Chi-Square Test: p = 0.011).

A finer resolution of childhood and adolescence diets through serial dentine sampling of six women show high intra-individual variability within the life phases, in particularly in $\delta^{13}C$ values. Previous studies only found higher intra-individual variability in $\delta^{15}N$ [94, 127], making general growth and developmental processes unlikely to be the cause for the high variability of $\delta^{13}C$ values observed in our study. Hence, we believe that strongly divergent diets in childhood and adulthood are rather caused by a change in the ecosystem than by physiological processes or culturally determined differences in the dietary habits of children and adults.

Major changes in $\delta^{13}C$ appear largely around the age of 13 to 15, an age at which a social transition from childhood to adulthood can be assumed for women [128, 129]. However, the changes are not uniform, they move in different directions in different individuals. This makes traditional, local or culture-related behavioral patterns of eating as causes of these fluctuations less likely and again rather points towards individual movements between areas with different ecosystems and food bases (for detailed discussion of each individual, see S3.3 section in S3 Text).

Interpreting deviant diet. Significantly increased (>2‰) $\delta^{13}C$ values, as detected in some individuals are usually interpreted as an indicator for the consumption of C4 plants, such as millet [130, 131]. In Late Antiquity and the Early Middle Ages millet was not unknown in Central Europe [132–134], but its cultivation was rather uncommon [121, 130, 135, 136]. Also according to isotope studies, C4 plants are known to comprise only an insignificant part of the human diet in Early Medieval Central Europa [22, 137], but was commonly consumed in Southern and Eastern Europe [e.g., 138–143], as well as in Northern Central Asia at the time [e.g., 144].

However, millet is known to be used as "back-up-crop" in case of poor harvest or crop failure of more commonly cultivated C3 crops (e.g., [124]) and archaeobotanical evidence from Early Medieval sites in Bavaria showed an isolated and unregular occurrence of millet [145]. Thus, the cultivation of millet in Southern Bavaria, at least temporarily, cannot be excluded (for details see S3.1 section in S3 Text). Such short-term use of millet can lead to an increase of $\delta^{13}C$ for a short period, e.g., as seen in the nutritional profile from serial dentin samples of individual BWA_10075. In bone collagen, however, sporadic consumption of millet causes a slight increase of $\delta^{13}C$ at most, since this represents a mixed signal spanning several years. The distinctly elevated $\delta^{13}C$ values observed in bone values or in dentine values over several years of some individuals can only be explained by long-term consumption of a substantial amount of millet. This may have happened locally due to special circumstances (e.g., several years of continuous crop failure or exclusive consumption of traded food), or due to a previous residency in an area where the consumption of C4 plants was more common practice. Given the uneven distribution of millet consumption in Europe during this time, the latter is the most common interpretation of increased $\delta^{13}C$ values in bones of isolated individuals in Early Medieval central European cemeteries (e.g., [124, 130, 146, 147]). Although extremely deviant dietary practice of individuals, which may have a cultural, religious, or pragmatic background, cannot entirely be excluded, there is little evidence for it in this time and region. Due to this, the archaeobotanical evidence and fact that increased $\delta^{13}C$ values are most frequently found in women with ACD (see below), a change of residence seems to be the most likely explanation for elevated $\delta^{13}C$ values in our sample as well.

The occasionally found elevated $\delta^{15}N$ values in bone or dentin collagen on the other hand might be also a result of an individually different local diet, which is on average richer in animal proteins or includes more seafood. An origin from a region with a higher nitrogen

baseline (such as marine coastal areas or terrestrial ecosystems with greater aridity or higher growth season temperatures [148–150]) can also be an explanation. This is likely to be the case for the elevated bone values of a woman AED_421. Her deviant isotopic signal compatible with a further marine diet together with her grave goods commonly found in Scandinavian regions showed a clear signal for a marine diet which strongly indicate a migration event (see Hakenbeck et al. [22]).

Overall, we believe that these observed association of highly deviant diets with foreign adjuncts or customs, suggest that isotopic values ranging clearly outside 99% of the population can be explained by change of residency of these individuals in most of the cases. This is especially likely if the C and N isotopes of dentin and bone in an individual differ substantially. As C and N isotopes from bone display a composite of the dietary intake over long periods (e.g., [49]), previous uncommon diets are masked. By including tooth dentin analysis, we were able to identify potential migrants, who would not have been detected by bone bulk analysis only.

Women with ACD. While ACD is a worldwide phenomenon, during the Migration period in Europe its spread is often associated with the expansion of the Hunnic Empire [24, 151]. For the 5th and the beginning of the 6th century, the findings of skulls with ACD are mainly centered in the Pannonian Basin, where more than half of the skulls found in graveyards show ACD. It was equally common among males and females and across all age classes [151, 152]. In contrast, only isolated individuals with ACD can be found in Bavarian burials of the late 5th and early 6th century AD and these are mainly adult females and never children or juveniles [51]. Already early on, this pattern was interpreted by some scholars as an indication that the isolated finds of ACD in Bavarian cemeteries around 500 were the remains of immigrant women from the East [24, 152, 153]. Veeramah et al. [23] have recently been able to show that these women have a strong genetic resemblance to present-day South-Eastern European populations, which was absent in individuals without ACD buried in the same graveyards. However, a deviant genetic ancestry alone may not be sufficient to infer a change of location during a person's life, but this is also supported by our observation that all examined women with ACD in this study show signs of increased millet consumption in their childhood, and just some of them show such signals in their later life strongly supports this assumption. Early Medieval populations from the Pannonian basin show similar δ^{13}C values (Hungary: [70, 142, 154, 155]; Romania: [98]) as well as some individuals with ACD from Croatia and Hungary [70, 156]. Additionally, the mean δ^{15}N value in the bulk dentine of women with ACD was often slightly elevated, which also corresponds well with δ^{15}N data of burials found in South-Eastern Europe (e.g., [70, 142, 154–156]).

Our serial dentine analysis provides interesting insights into the life history of women with ACD. One woman (BWA_10254*) who was buried at old age (over 60 years) shows elevated δ^{13}C and δ^{15}N values in childhood, but her bone value does not differ from the "common variability" (Fig 8 center right). A first remarkable dietary change of hers can be detected around the age of 15 years, which could be explained by a change of location, yet, neither the place of origin nor the place where she supposedly first moved to could have been Southern Bavaria. Her tooth isotope values before and after this event are far outside the "common variability", indicating that she reached the region where she was buried, after the age of 20 years, when the formation of her wisdom tooth was completed. Whereas her bone collagen values inside the "common variability" indicated that her movement to Bavaria must have happened long before her death.

Detecting migration and mobility by strontium

Local ranges. The geological conditions are relatively uniform in Southern Bavaria (Fig 2). This agrees with the fact that little variability exists in the local strontium isotope ratios of

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the different archaeological sites investigated. However, this is not the case for Regensburg (S2.3.3.2d Fig in S2 Text), what might be explained by its proximity to regions with elevated strontium isotope signatures, which are located just north of the Danube. The same is true for the site of Straubing, however, without showing a similar pattern. There are also indications that the strontium isotope values of the old town of Regensburg could be higher than the surroundings [9]. This area was built on Roman rubble, which might have influenced local strontium isotope ratios. To resolve this question, further analyses of environmental samples from the region would be necessary. Until then, the assessment of a local range for Regensburg should be considered preliminary.

It is noticeable that the determined local ranges derived from biomineral samples (reference as well as actual sample set), always exceed the range covered by the environmental samples. This may indicate that the local range is overestimated (Fig 3A–3D). However, only very few environmental samples per site are available, too few to capture the entire variability of $^{87}Sr/^{86}Sr$ on site (discussion of this aspect see Toncala et al. [54]). The local range in humans can also be increased by imported food sources. Using environmental samples only would not include this, but the distribution of the isotope values in the population (on which our determination of the local area is based on) does. Overall, the approach used here minimizes the risk of overestimating the number of non-local individuals but might increase in the risk of not recognizing non-local individuals.

Underestimation of human movement.   The quite uniform geology and the use of rather broad local ranges results in small-scale mobility within South Bavaria staying mostly undetected. Furthermore, due to the geographical redundancy of the isotope values it is likely, that some individuals with "local" signature come from an area outside of Southern Bavaria with a similar geological substratum. Thus, the number of non-locals is most certainly underestimated regardless of the approach used to determine the local range. This becomes particularly clear in the case of women with modified skulls. Despite they most likely did not grow up in the region where they were buried, they show "local" $^{87}Sr/^{86}Sr$ isotope values. But their $^{87}Sr/^{86}Sr$ values can also be found in other parts of Europe, such as the Eastern European lowlands or the Eurasian steppe (e.g., Sjögreen et al. [58]).

Nature of human movement.   Except a few individuals (6%, 95% CI: 3–10%), mainly non-locals originating from outside of the region between the Danube and the Alps were detected. The nearest areas with significantly deviating strontium isotope values outside the study area are quite close and north of the Danube (e.g., in the Bavarian Forest, ca. 140 km). Although geographically close, even immigration from these nearby regions into *Raetia II* meant crossing a cultural border, namely from Barbaricum to the former Roman province. Therefore, at least 17% (95% CI:12–23%) can be considered migrants (since migration is defined as long-term relocation of persons who cross cultural and/or political boundaries).

Extent of migration.   An interpretation of the detected extent of migration is difficult. Comparisons with today's migration rates or migration rates from other sites are not feasible. The number of migrants that can be reliably detected in a region not only depends on the number of individuals that actually migrated, but also on the geological and environmental conditions of both, region of origin and the burial site, as well as the type of data available and methods used to determine local isotope ranges. By taking archaeological sites from the Bavarian alpine foothill for comparison and calculating a local range that encompasses the entire region, we avoided some of the interpretation problems.

The depicted fluctuations of MFM over time (Fig 5) can be explained by both (1) variation in the actual number of non-locals, or (2) differences in the migrants' region of origin. Since in different periods different places of origin might have dominated, which could have been detectable to varying degrees by means of strontium, the second explanation can never be

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Human movement and diet in early medieval Central Europe

excluded. But the average frequency, which equals the percentage of detectable migrants considering the individuals of all time periods together (red line in Fig 5) should balances out fluctuations based on differently detectable regions of origin. Because the rate of detectable immigrants for the second half of the 5th century was not only well above this average but also exceeded any other time period, we suggest that an above-average migration rate is indeed evident for the North-Alpine foreland around 500 AD.

Regional differences in migration rates. The lower frequency of migrants in the border region (14%, 95% CI: 7–22%) indicates that, compared with the hinterland, immigration played a minor role there.

Only a few migrants were detected in the border region cemeteries of Straubing-Bajuwarenstraße (3%, 95% CI: 0–10%) and Irlmauth (14%, 95% CI: 0–36%) although Germanic grave goods were found there [37, 39]. This may support the hypothesis that the presence of Germanic grave goods is better explained by increased trade in goods, and only to a limited extent by migration. The site of Burgweinting show the highest proportion of identifiable migrants in the border region (24%, 95% CI:10–41%). Whether this rather high number, which is more comparable to those in the hinterland, can be related to a higher social status (as indicated by Codreanu-Windauer [36]) and thus to migration of elites (analogous to Unterhaching, see below) must remain unclear, since finds and grave goods have not yet been published in a complete and interpretable form. However, this site is also the furthest away from the former border and thus may not actually be subject to the dynamics of the border region.

A higher proportion of migrants in the hinterland (21%, 95% CI: 12–31%) supports the idea that the area was preferentially settled by newcomers, as also shown by 23% migrants (95% CI: 12–37%) in Altenerding. The highest proportion of migrants (33%, 95% CI: 0–67%) from outside Bavaria was found in the group of burials from Unterhaching. Including all non-locals, as much as 78% (95% CI: 44–100%) do not come from the region. The high proportion of non-locals among the group and the rich equipment of the graves suggests that these individuals belonged to an elite class that was sent to represent the new rulers of the region at the end of the 5th century [43]. In Munich-Perlach, just 5 km away, we found a substantially lower frequency of migrants (13%, 95% CI: 0–26%). Consideration of the stable light isotope values suggests that individuals from Unterhaching are only found in the upper half of the data range for Munich-Perlach. The higher average of $\delta^{15}N$ in Unterhaching could be explained by a higher intake of animal protein associated with a higher social status (e.g., [157, 158]) and might therefore be interpreted as a sign of the elite status of the individuals. An alternative explanation for the observed differences in stable light isotopes between Perlach and Unterhaching could be that subsistence strategies or environmental conditions were somewhat different in the region of origin of the non-locals detected in Unterhaching.

Again, it cannot be ruled out that the regional differences observed were caused, not only by actual differences in the number of migrants but also by the different detectability of the potential places of origin. Interestingly, at sites with a relatively low frequency of detectable migrants (PEL, STB, IRM) the $\delta^{13}C$ values from bone bulk collagen were less variable than those at sites with a higher frequency of non-locals (BW, AED). Hence, the variability of carbon isotope ratios at excavation sites seems to reflect the frequency of non-locals.

Again, only the site of Unterhaching is an exception in this pattern. This anomaly can be explained as being due to the exceptionally low number of individuals analyzed from this site, or by the fact that the cemetery was the only one mainly occupied by non-local individuals. If all of those individuals came from an identical or similar region of origin, variation in values is not to be expected. This pattern suggests that different proportions of non-locals reflect real differences in the frequency of immigrants and are not caused by different origins.

Identity of non-locals

Sex differences. Using strontium isotope analysis, we were able to identify not only women but also several men, as migrants. Indeed, in our study, the proportions of male and female migrants did not differ significantly (Fig 4A). However, it should be repeated here, that mobility between settlements within the study area is mostly not detectable with this approach and here gender related mobility rates might have been different. Overall, our results showed that in the Early Middle Ages, both sexes traveled to Southern Bavaria from outside of the former province *Raetia II*. Interesting in this context is, that we found strong deviating $\delta^{13}C$ and $\delta^{15}N$ values only in women. This indicates that men and women have partly migrated from different regions. Since we strongly assume that such a deviating diet is a sign of migration (migration, not mobility, because they must have come from a region outside of *Raetia II*), this would increase the proportion of women with a migration background.

Origin. Genetic associations and elevated $\delta^{13}C$ values (indicating regular millet consumption) of women with ACD suggest an origin from Southeastern Europe as already discussed. This is also true for two other females (BW_10071, STB_310) without ACD [23].

The genetic ancestry of another female buried in Straubing (STB_300) with $^{87}Sr/^{86}Sr$-values different from the study region and the region of origin of the women with ACD indicates an origin somewhere in Southern Europe [23].

For a woman who was buried in Altenerding (AED_421), a region of origin in Northern Europe has been suspected (see Hakenbeck et al. [22]).

Despite limitations on being able to define places of origin more precisely, the further observed variability of non-local $^{87}Sr/^{86}Sr$ but also of $\delta^{13}C$ and $\delta^{15}N$ values indicates that migrants originated from several different, geologically distinct areas and/or ecosystems with different food bases.

Life-stage when moving. We assume that the observed distinct changes in the diet of some women, especially if it happens quite abruptly, represent a change in residence in most cases, it is possible to reconstruct at what life stage the movement took place. For instance, some females (BWA_10254*, AED_343, PEL_12) show an "uncommon" diet in childhood but do not differ from native locals in their bone values. This indicates they grew up elsewhere and immigrated to the region a long time before they were buried there. As their bone value became indistinguishable from the rest of the local population, they apparently adopted common dietary habits.

In contrast, four females (AED_421, AED_513*, AED_1108*, STB_228) were identified as "newcomers", with diverging stable isotope ratios in their bones. Since they were of late adult or senior age at the time of their death. These women moved to Bavaria at rather advanced ages, they must have moved to Bavaria at rather advanced ages. These women were most likely past the reproductive phase, which contests marriage migration as the only reason for a change of location of women.

Serial sampling of the dentin from M1 to M3 sometimes allows even further delineation of the time of change of residence. The isotope profile of one female (AED_10071) revealed a dietary change around the age of 15 years, which can be associated with migration into Southern Bavaria.

This clearly demonstrates the complexity of women's life histories during the Early Middle Ages in Bavaria.

Conclusion

Regardless of how high the actual immigration rate may have been, this study shows that migrants increasingly entered the former province of *Raetia II* towards the end of the 5th century. Therefore, the assumption that only marginal migration took place during this time can

be rejected. Our results correspond well with archaeological evidence regarding the continuity of settlement at the provincial borders, and the profound changes that affected settlement structures in more distant rural areas. We could also show that both sexes undertook migration. Furthermore, the reconstructed life histories of some women hint at the diversity of female life paths during the Early Middle Ages.

Emerging differences in the mobility among sexes, as well as the finding that individuals migrated from a diversity of regions of origin, suggests that immigration and mobility in *Raetia II* after the fall of the Roman Empire was highly complex, and will likely only gradually be uncovered.

Supporting information

S1 Text. Data set.
(XLSX)

S2 Text. Detailed methods.
(DOCX)

S3 Text. Statistics and detailed results.
(DOCX)

Acknowledgments

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Results: Publication I

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3.2 Publication II

The second article was published in open access on the 22nd November of 2023 in the Journal Archaeological and Anthropological Sciences.

Velte M, Czermak A, Grigat A, Neidich D, Trautmann B, Lösch S, Päffgen B, Harbeck M. Tracing early life histories from the Roman to the Medieval era: Weaning practices and physiological stress. Archaeol Anthropol Sci. 2023; 15:190. https://doi.org/10.1007/s12520-023-01882-6.

My contribution: I was involved in data collection and was jointly responsible for the curation of data. I developed novel methodological approaches applied in this study and conducted most of the formal data analysis as well as data visualization. I wrote the original draft with the help of Andrea Czermak and Michaela Harbeck and subsequently edited the manuscript following input from other co-authors. The study design was done by me and Michaela Harbeck.

RESEARCH

Tracing early life histories from Roman times to the Medieval era: weaning practices and physiological stress

Maren Velte[1] · Andrea Czermak[1,2] · Andrea Grigat[1] · Deborah Neidich[3] · Bernd Trautmann[1] · Sandra Lösch[4] · Bernd Päffgen[5] · Michaela Harbeck[1]

Abstract

In humans, breastfeeding and weaning depend on the infant's needs and physiology but are also influenced by environmental and cultural factors. While infant feeding strategies vary across different regions and historical eras, the associated transition from breastmilk to solid foods is universally thought to be stressful. However, still little is known about infant feeding practices and possibly associated stress in former times. This also applies to the period of transition from classical antiquity to medieval times, which shaped modern Western civilization. To enhance the understanding of childhood nutrition and stress during this period, we first analyzed stable carbon and nitrogen isotopes in serial dentine samples from the first molars of 38 individuals buried in the region once known as the Roman frontier province of *Raetia secunda*, now encompassing Southern Bavaria. In addition, we investigated the presence of linear enamel hypoplasia (LEH), known to be a marker of unspecific physiological stress, within their dentition. We used this data to create isotope profiles that display dietary changes in comparison with the occurrence of LEH. We found highly variable $\delta^{15}N$ and $\delta^{13}C$ values and different shapes of isotope profiles which indicate different nutrition of breastfeeding individuals, complementary foods and post-weaning diets, and individual weaning patterns. For most individuals, the weaning process was completed between the ages of two and three. Interestingly, some females of non-local origin show longer weaning periods, likely displaying the influence of different cultural practices in other communities. We also found that LEH most frequently occurred in the post-weaning phase, which supports the assumption that children were at increased risk once breastfeeding had ceased completely. Furthermore, a change in the post-weaning diet in the seventh century coincided with an increased prevalence of LEH, indicating that the foods chosen or available during this time affected the susceptibility of children to stress. In conclusion, our study unveiled diverse infant feeding strategies practiced across various communities, both in different historical eras and geographical locations.

Keywords Early childhood · Serial dentine isotope analysis · Weaning process · Post-weaning diet · Post-weaning stress · Linear enamel hypoplasia (LEH)

Introduction

Early childhood is a critical stage in human life. During these initial years, the development and growth of children are significantly influenced by their living conditions, particularly their diet and exposure to stress (e.g., diseases), two factors that are closely intertwined (Caulfield et al. 2006).

Typically, breastmilk serves as the primary source of nutrition for infants. It offers a secure and hygienic supply of essential macro- and micronutrients such as proteins, fats, carbohydrates, vitamins, and minerals (e.g., Ballard and Morrow 2013; Walker 2010). Moreover, it contains substances that help to protect an infant against infection and inflammation (e.g., Walker 2010), while also contributing to

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the development of the infant's immune system (e.g., Lönnerdal 2000; McDade 2003). However, breastmilk alone cannot meet the requirements for optimal development of an infant after approximately six months (e.g., Kramer and Kakuma 2004; Jay 2009; Pérez-Escamilla et al. 2019), necessitating the inclusion of complementary foods.

Weaning is the process that starts with the gradual or immediate introduction of non-breastmilk liquids and solid foods, replacing breastmilk, which can result in nutritional stress and raise the risk of exposure to pathogens from contaminated food (e.g., Humphrey et al. 2008, Black et al. 2008). The tradeoff between these risks and the potential impediment to development associated with prolonged exclusive breastfeeding is often termed the "weanlings dilemma" (e.g., Rowland et al. 1978; Katzenberg et al. 1996). Many early bioarcheological studies have inferred that "weaning stress" represents a significant health risk or even threat to survival to infants (e.g., Katzenberg et al. 1996; Dittmann and Grupe 2000).

However, Kendall et al. (2021) criticize that a lot of these studies underrate the complexity of breastfeeding and the processes involved. They argue that the period of greatest risk to children does not occur during weaning but after complete discontinuation of breastfeeding. Fernández-Crespo et al. (2022) have referred to this as the "post-weanling's conundrum": a phase of heightened risk characterized by increasing dietary needs and exposure to pathogens with a simultaneous loss of nutritional and antimicrobial support provided by breastmilk while the child's immune system is not yet fully developed. Additionally, they point out that food choices are critical to health in the post-weaning phase, but that even continued breastfeeding may not fully protect against health risks stemming from contaminated, unsuitable, or quantitatively insufficient foods during the weaning phase.

In any case, the practices of infant feeding and supposedly associated stress are influenced by various cultural, socioeconomic, and environmental factors (e.g., Fildes 1986; Quandt 1995; Sellen 2009; Britton et al. 2015; Eerkens et al. 2017), including dietary traditions, resource availability, or infant sex and health (Bereczkei 2001, Fernández-Crespo et al. 2018, Tsutaya 2017, Chinique de Armas and Roksandic 2018) which can result in different patterns of weaning and stress across different temporal and geographical contexts.

In this study, we investigate dietary changes and physiological stress experienced during the first ten years of life in a group of 38 individuals buried in Late Roman and Early Medieval times within the former Roman province of *Raetia secunda* (*Raetia II*). Although these individuals were buried in the same region, some of them spent their childhood in different places, supposedly adhering to various cultural backgrounds and distinct dietary habits (Velte et al. 2023). We characterize infant feeding strategies

through serial stable isotope analysis and assess their correlation with the formation of linear enamel hypoplasia (LEH) as an indicator of stress. This not only provides information on different early life histories of people living in Bavaria between the fourth and seventh centuries but also contributes to the ongoing discussion of "weaning stress" and its timing.

Reconstruction of early childhood diet with serial isotope analysis

Analysis of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes from preserved human tissues offers a unique opportunity to study breastfeeding and weaning practices in past populations (e.g., Fogel et al. 1989, Fuller et al. 2006, Lösch 2009, Eerkens et al. 2011, Bourbou et al. 2019, Siebke et al. 2019, Ganiatsou et al. 2023). A newborn who is exclusively breastfed after birth exhibits an increase in $\delta^{15}N$ by approximately one trophic level of around 3.0‰, compared to the breastfeeding female (e.g., Fogel et al. 1989, Millard 2000, Fuller et al. 2006, Herrscher et al. 2017) (Fig. 1). Some studies also noted a rise of approximately one trophic level of around 1.0‰ in $\delta^{13}C$ (e.g., Richards et al. 2002; Fuller et al. 2003, 2006) for exclusively breastfed infants, while others found slightly smaller increases of around 0.5‰ (Herrscher et al. 2017) or no increase at all (Fogel et al. 1989). Nonetheless, increasing consumption of complementary foods leads to a gradual decrease in $\delta^{15}N$ and $\delta^{13}C$ down to the level of the breastfeeding female, i.e., until weaning is completed (e.g., Fogel et al. 1989, Fuller et al. 2006, Fernández-Crespo et al. 2018) (Fig. 1). However, this pattern can deviate when the infant's diet significantly differs from that of the breastfeeding female. Still, individual variations in physiological and metabolic processes can also affect $\delta^{13}C$ and $\delta^{15}N$ in the body tissues (e.g., Neuberger et al. 2013, Fuller et al. 2004, 2005, Crowder et al. 2019) of both the breastfeeding female and the infant.

Employing serial isotope analysis of tooth dentine allows us to monitor the course of isotopic values throughout the period of dentine formation within an individual (e.g., Eerkens et al. 2011; Beaumont et al. 2013; Czermak et al. 2020). The development of first molars starts around birth and continues throughout the initial 10 years of life, providing information about the phase of exclusive breastfeeding, the complementary feeding (weaning) phase, and the post-weaning phase. Isotope data derived from specific dentine sections can be assigned to their approximate formation ages to create isotope profiles (e.g., Czermak et al. 2020). Consequently, this approach enables us to estimate the age at which supplementary foods were introduced and when weaning was completed.
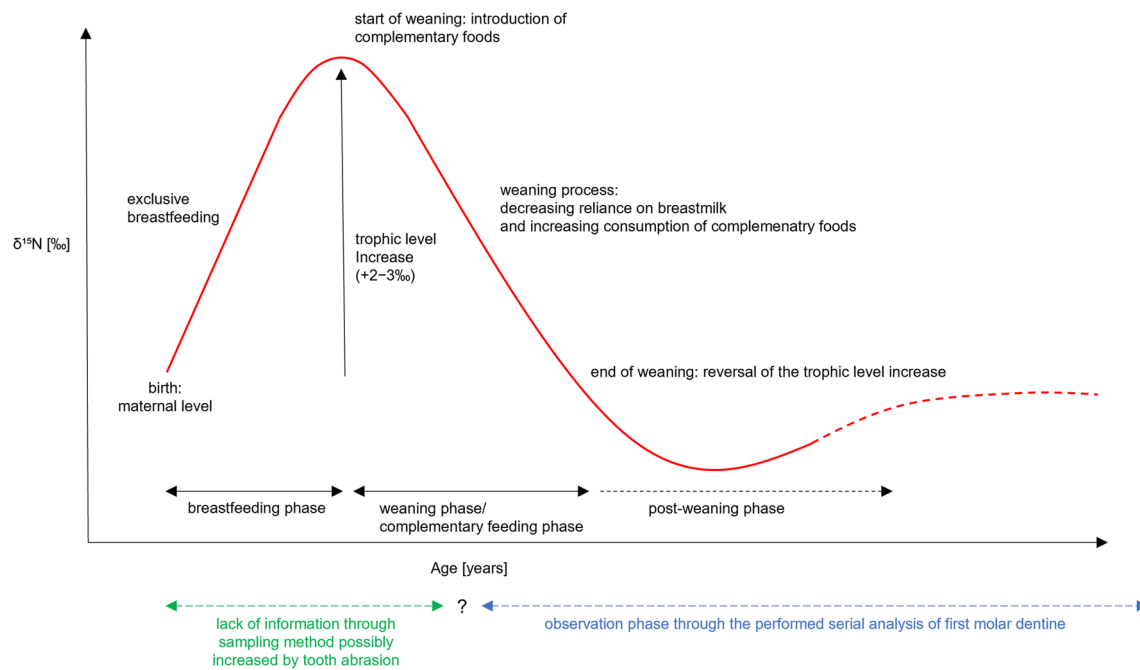
Fig. 1 Schematic course of an infant's δ^{15}N values during the phases of exclusive breastfeeding, weaning, and post-weaning diet.

Linear enamel hypoplasia as an indicator of physical stress

Linear enamel hypoplasia (LEH) results from a disruption in the enamel secretion by ameloblasts (e.g., King et al. 2002) during tooth development. Thus, LEH presumably indicate unspecific physiological stress during childhood (e.g., Goodman and Rose 1990, Guatelli-Steinberg and Lukacs 1999, King et al. 2005, Hillson 2008). Since tooth enamel is not remodeled, it is possible to estimate the approximate age at which any observed defect was formed.

Over the last decades, several studies have implied a connection between weaning and the occurrence of LEH. While many of them have remained rather vague about during which phase "weaning stress" occurred (e.g., Goodman et al. 1984, Corruccini et al. 1985, Berbesque and Hoover 2018), some have observed an increase in the number of LEH towards or shortly after the end of complementary feeding (e.g., Moggi-Cecchi et al. 1994, Crowder et al. 2019, Orellana-González et al. 2020).

South Bavaria between Roman times and the Middle Ages

Southern Bavaria was initially integrated into the Roman Empire in the first century, forming a part of the province *Raetia.* After the province was subdivided in the late fourth century, it became part of *Raetia II*. During the Roman Imperial period, the region experienced progressing urbanization and intensive trade led to the development of a healthy economy that presumably formed a thriving society in Southern Bavaria (e.g., Fehr 2010). However, this situation potentially changed as several crises began to accumulate, such as recurring attacks of German tribes from the mid-third century onwards and increasing supply shortages during the fourth century. The Roman administration and frontier defense for the entire pre-Alpine region eventually ended in 476 AD when the Germanic leader Odoacer replaced the last Western Roman Emperor. Little is known about the subsequent events in the region that was once *Raetia II*. But in the middle of the sixth century, Bavaria became a dukedom under Frankish rule, which lasted until 751 AD (e.g., summarized in Haas-Gebhard 2013).

In a previous study, we showed that part of the individuals buried in the former province of *Raetia II* around 500 AD spent their childhood in a region with differing strontium isotope signatures (Velte et al. 2023). Our findings also indicated that individuals exhibiting unusual dietary habits, e.g., frequent consumption of millet, a C4 plant that was rather uncommon in Bavaria during that time (e.g., Hakenbeck et al. 2010), likely have foreign origins. This was especially notable among some women with artificial cranium deformation (ACD) (Velte et al. 2023). Interestingly, Veeramah et al. (2018) demonstrated that most of these women show

a genetic similarity to people living in the South-East European region.

In this study, our aim is to explore whether the profound political, social, and economic changes that occurred during the transition from Antiquity to the Middle Ages, or potential cultural differences related to people's origins, had any influence on child feeding strategies.

Material

The human remains of the individuals studied ($n = 38$) were excavated at four burial sites in Bavaria (Fig. 2). Most of these individuals ($n = 32$) were uncovered at two cemeteries in Erding: Late Roman Erding-Kletthamer Feld (EKF, $n = 4$) and Early Medieval Altenerding-Klettham (AED, $n = 28$). The small cemetery of EKF (13 inhumations) dates from the second half of the fourth century AD until the first half of the fifth century AD (Sofeso et al. 2012). The burial site of AED came into use in the second half of the fifth century, and burials there continued well into the seventh century (Sage 1984, Losert and Pleterski 2003). It stands as one of

the biggest Early Medieval cemeteries excavated in Bavaria, comprising about 1450 graves. We selected 17 individuals dating around 500 AD, including two women with ACD and five other individuals of documented non-local origin (Veeramah et al. 2018; Velte et al. 2023), and eleven burials dating from the seventh century AD. In addition, we included individuals from two sites located less than 100 km away, namely, Straubing-Bajuwarenstraße (STB) and Burgweinting (BW), due to the presence of ACD in three cases and unusual dietary patterns likely linked to non-local origin (Codreanu-Windauer and Harbeck 2016, Trautmann et al. 2017, Veeramah et al. 2018, Velte et al. 2023). The cemetery STB was occupied from the mid-fifth until the mid-seventh century AD, containing over 800 graves (Geisler 1998). From this site, we included three individuals dating around 500 AD (Veeramah et al. 2018; Velte et al. 2023). Furthermore, we analyzed three individuals of a small grave group (15 inhumations named grave group A) from BW (BWA), dating between the late fifth to the early sixth century AD (Zintl 2012, Codreanu-Windauer and Schleuder 2013).

Many individuals included in this study had been previously investigated, providing valuable data on age at death
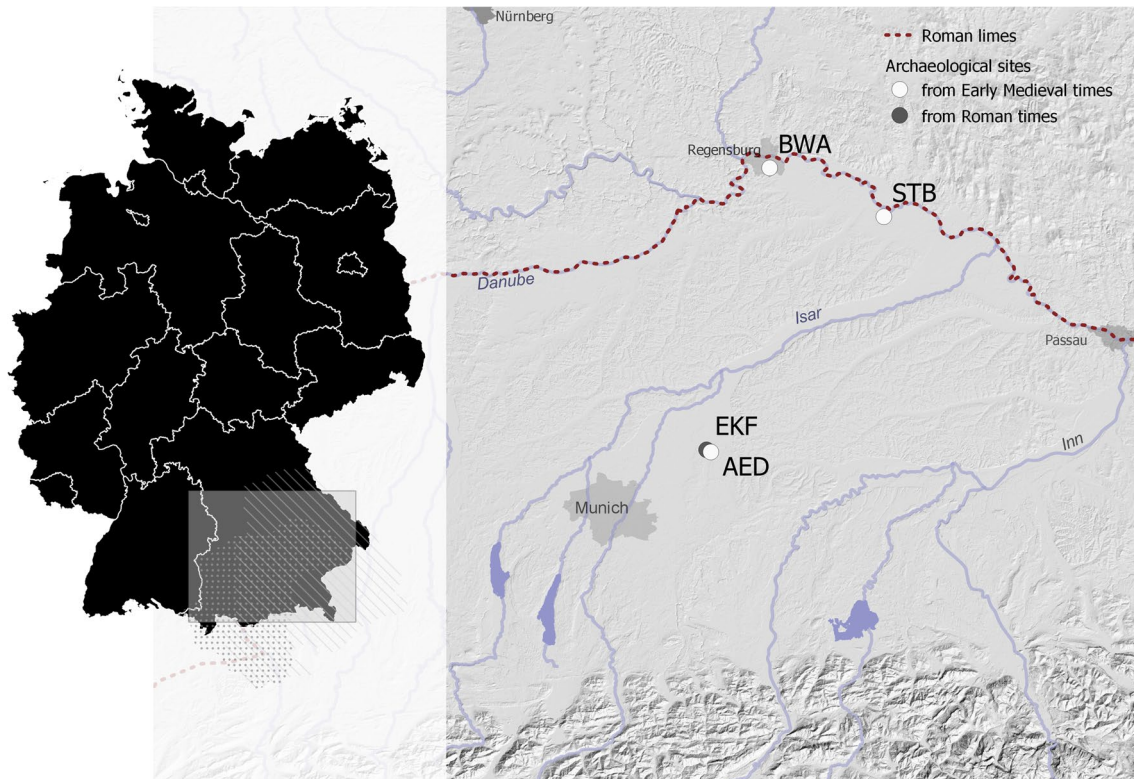
Fig. 2 (Left) Map of Germany with its federal states; the study area (shown in detail on the right) is backed by a gray box; dashed lines indicate the extent of the first duchy of Bavaria (after Weiß 2021) and dotted areas the presumed territory of Raetia II (after Haberstroh and Harbeck (2013)). (Right) Part of southern Bavaria with the geograph-ical positions of the archaeological sites included in this study (AED, Altenerding-Klettham; EKF, Erding-Kletthamer Feld; STB, Straubing-Bajuwarenstraße; BWA, Burgweinting Group A). Map data: EU-DEM © European Union, Copernicus Land Monitoring Service 2017, European Environment Agency (EEA).

and sex, the presence of LEH, the presence of ACD, strontium isotopes, genetic ancestry, dating and $\delta^{13}C$ and $\delta^{15}N$ ratios in bone, and bulk values of first molar dentine (Hakenbeck et al. 2010, Sofeso et al. 2012, Codreanu-Windauer and Schleuder 2013, Codreanu-Windauer and Harbeck 2016, Harbeck et al. 2016, Trautmann et al. 2017, Veeramah et al. 2018, Toncala et al. 2020, Neidich 2023; Velte et al. 2023). Detailed information on all data used and the corresponding literature are listed in the Supplementary (Table S2).

All individuals in the study were adults (> 20 years), except one juvenile individual (AED_1053: 13–20 years). The sample set comprises a slightly higher number of females ($n = 21$) compared to males ($n = 14$), with sex being indeterminable for three individuals (AED_100, AED_217, AED_724) due to poor preservation.

Based on the analysis of strontium, carbon, and nitrogen isotopes as well as genetic data, some individuals from around 500 AD in this study ($n = 23$) can be reasonably assumed to be migrants ($n = 13$, AED + STB + BWA), which includes five females with ACD (as discussed in Velte et al. 2023). Those individuals who showed no indication of foreign origin are considered part of the "local" population ($n = 10$, AED), although it cannot be ruled out that unrecognized migrants are among them (also see Velte et al. 2023).

For burials dating from the first half of the seventh century ($n = 11$, AED), or between the second half of the fourth century and the first quarter of the fifth century ($n = 4$, EKF), such a classification cannot be made, because the corresponding data are not available for all samples.

Methods

$\delta^{13}C$ and $\delta^{15}N$ ratios in collagen were analyzed from both bone and serial dentine samples. Bone samples from adults provide information about dietary patterns during a later life stage, due to tissue turnover, but a more precise timespan reflected by measured isotope values cannot be determined due to varying turnover rates (Hedges et al. 2007). In contrast, dentine is not remodeled once formed during early life, which allows us to associate measured values with the specific examination of chronological age (e.g., Czermak et al. 2020).

Bone collagen was extracted following the protocol described in Siebke et al. (2019) (also see Velte et al. 2023 and Supplementary S1.1). Measurements were carried out at the Isolab GmbH in Hanau using an Elementar Vario Cube EL connected to an Isoprime mass spectrometer. Accuracy and precision were verified through replicate analyses of laboratory standards (e.g., Collagen STD R (USGS 89), Collagen STD S, Collagen STD BRA), calibrated to international standards USGS40 and 41. The Isolab GmbH ensures an analytical precision of 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Serial dentine sampling from the first molars and collagen extraction followed the procedure described in Velte et al. (2023) (for detailed method description see Supplementary S1.1). The samples were analyzed at the GeoCenter (Friedrich-Alexander University, Erlangen-Nürnberg), utilizing a Flash EA 2000 elemental analyzer connected to a ThermoFinnigan Delta V Plus mass spectrometer. Analytical precision ensured by the GeoCenter is 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$ checked by replicate analyses of laboratory standards (e.g., Casein, Cyclo) calibrated to international standards USGS 40 and 41.

The results are reported in the conventional δ-notation in per mil (‰) relative to internationally accepted standards, VPDB for carbon, and AIR for nitrogen. The collagen quality of each sample was evaluated through the C/N atomic ratio, as well as carbon and nitrogen content.

Chronological age assignment of sampled dentine sections to illustrate changes in stable isotopes over time was made using a scheme developed in a prior study (Velte et al. 2023). This scheme is based on *The London Atlas of Tooth Development and Eruption* (AlQahtani et al. 2010) and takes potential wear into account for each tooth.

We defined weaning age as the approximate age at which either breastfeeding is finally terminated, reduced to a low amount, and/or when the isotope signal is masked by the consumption of higher protein sources. To estimate individual weaning age, a customized scheme based on the relative decrease in $\delta^{15}N$ values at the beginning of the isotope profiles is employed: Starting with the first dentine section, any dentine section showing a depletion in $\delta^{15}N$ by at least 0.2‰ (analytical error) compared to the previous section (n_1, n_2, ..., $n_{\downarrow end}$) (Fig. 3A) is given a new value representing the relative decrease in $\delta^{15}N$ ($\downarrow\delta^{15}N_{rel}$). This value is calculated as the difference between the ratio of the last section in the series with continuously decreasing values ($n_{\downarrow end}$) and the ratio of the respective section ($\downarrow\delta^{15}N_{rel}(n_1, n_2, ..., n_{\downarrow end}) = \delta^{15}N(n_{\downarrow end}) - \delta^{15}N(n_1, n_2, ..., n_{\downarrow end})$). $\downarrow\delta^{15}N_{rel}$ values are then plotted against the approximate formation age of dentine sections (Fig. 3B). The intersection of the linear trend line for normal tooth development and the y-axis is determined as the weaning age (line function: $f(x) = m \times x + t$; with t = weaning age, x = decline, m = slope). The error caused by the biological variation in tooth development is determined by using dentine formation ages for both early and late tooth development (Fig. 3B).

This approach can be used to estimate individual weaning ages (as exemplarily shown in Fig. 3B) but it can also be applied to evaluate weaning age on a population level by combining data from all individuals or groups, enabling the assessment of inter- or intra-populational differences (also see Figure S1.2.2).

Data on linear enamel hypoplasia (LEH) were collected on permanent maxillary and mandibular teeth. Rather than

analyzing general stress levels, we aimed to investigate a link between LEH formation and weaning. Thus, we only analyzed teeth that develop during the supposed weaning period: incisors and canines. We recorded the number and position of observable palpable LEH (a rippled pattern was detected by scratching the surface with the fingernail) on teeth from the right side of the mouth, using the corresponding teeth from the left side only if the right tooth was unavailable. Teeth that had lost over 50% of their crown height, e.g., due to wear, or were missing, were marked "not assessable."

The SNSB, Bavarian State Collection of Anthropology, which houses the human remains, specifies in its osteological record form (Harbeck et al. 2020) that the position of LEH should be assessed by dividing the tooth into three equal parts: top, middle, and bottom. For some of the individuals in our study, this data was already available in this format, and we recorded LEH for the remaining individuals following the same manner. To determine the age span during which these crown parts are formed, we provide enamel growth curves for early, normal, and late tooth development in incisors and canines of the upper and lower jaw, based on AlQahtani et al. (2010) (see S1.3 for more details). Linear trend line equations from these curves were used to calculate the age span during which a crown part is most commonly formed (normal development) and the error resulting from the biological variation in tooth development (early and late development).

Statistical data analysis was performed using IBM SPSS 29.00 and RStudio 1.4.1717 for Windows. Statistical tests on metric data were exclusively non-parametric (independent-sample Mann–Whitney U and Kruskal–Wallis tests) due to small sample sizes. Nominal data was analyzed by a non-parametric chi-square test. For multiple comparisons, significance values have been adjusted by the Bonferroni correction

Fig. 4 Carbon and nitrogen isotope profiles ($n = 38$). Colored frames: ▶ dating of burials (orange = second half fourth century to first half fifth century AD; blue = around 500 AD; green = seventh century). Data points: $\delta^{13}C$ or $\delta^{15}N$ values of the first molar dentine sections assuming normal dentine development, small dashed lines highlight changes in $\delta^{13}C$ or $\delta^{15}N$ between successive sections. Error bars: variations in early or late tooth development. Horizontal red and blue dotted lines represent individual ^{13}C or $\delta^{15}N$ bone values. Shaded red and blue areas display intra-populational variability ranges of $\delta^{13}C$ or $\delta^{15}N$ in Erding ($\delta^{13}C$: -20.7 to $-18.5‰$, $\delta^{15}N$: 7.9 to 11.1‰), Straubing ($\delta^{13}C$: -20.8 to $-18.9‰$, $\delta^{15}N$: 8.6 to 10.3‰), and Regensburg ($\delta^{13}C$: -21.2 to $-18.7‰$, $\delta^{15}N$: 7.8 to 10.5‰) (Velte et al. 2023). Vertical green lines: big dashed line = calculated individual weaning age, smaller dashed lines = variation assuming early or late tooth development. Dental charts show the present teeth (filled teeth), sampled molars (yellow), and evaluated anterior teeth for the presence of LEH (dark grey). The formation of LEH is shown below the isotope profiles. Filled bars represent the age span in which the corresponding crown third was formed (assuming normal enamel development), error bars display variations assuming early or late tooth formation. Red signs indicate individuals with incomplete anterior dentition, so the absence of LEH must be evaluated with caution.

to counteract alpha error accumulation. Graphics were created using QGIS 3.22 and Microsoft Excel for Windows.

All statistical analysis and more detailed results are given in the Supplementary (S1.2–3).

Results

Collagen quality

Collagen quality of dentine ($n = 350$) and bone specimens ($n = 11$) was monitored, generally accepting C/N atomic ratios between 2.9 and 3.6 (DeNiro 1985), as well as a
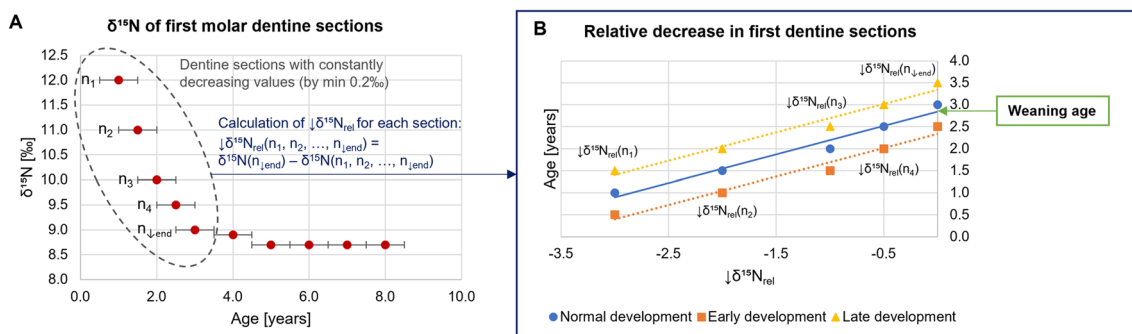

Fig. 3 Schematic illustration of the model used to estimate weaning ages based on the relative decrease in $\delta^{15}N$ ratios of successive dentine sections. **A** Example of dentine sections from a stable light isotope profile. Only dentine sections with a constant decline in $\delta^{15}N$ ratios (by min 0.2‰) at the beginning of the isotope profiles are selected. In this example down to a total drop of 3.2‰. Samples are given a new value, representing the relative decrease in $\delta^{15}N$. This value is calculated as the difference between the ratio of the last section in the series ($n_{\downarrow end}$) with continuously decreasing values and the ratio of the respective section ($\downarrow\delta^{15}N_{rel}(n_1, n_2, ..., n_{\downarrow end}) = \delta^{15}N(n_{\downarrow end}) - \delta^{15}N(n_1, n_2, ..., n_{\downarrow end})$). **B** Plotting of the $\downarrow\delta^{15}N_{rel}$ ratios of dentine sections against the chronological formation age of dentine samples (early, normal, and late development) including linear trend lines. Weaning age is defined as the intersection of the linear trend line for normal development and the y-axis: $f(x) = m \times x + t$, with t = weaning age, x = decline, m = slope/linear trend. The error is shown by the functions for early and late development.
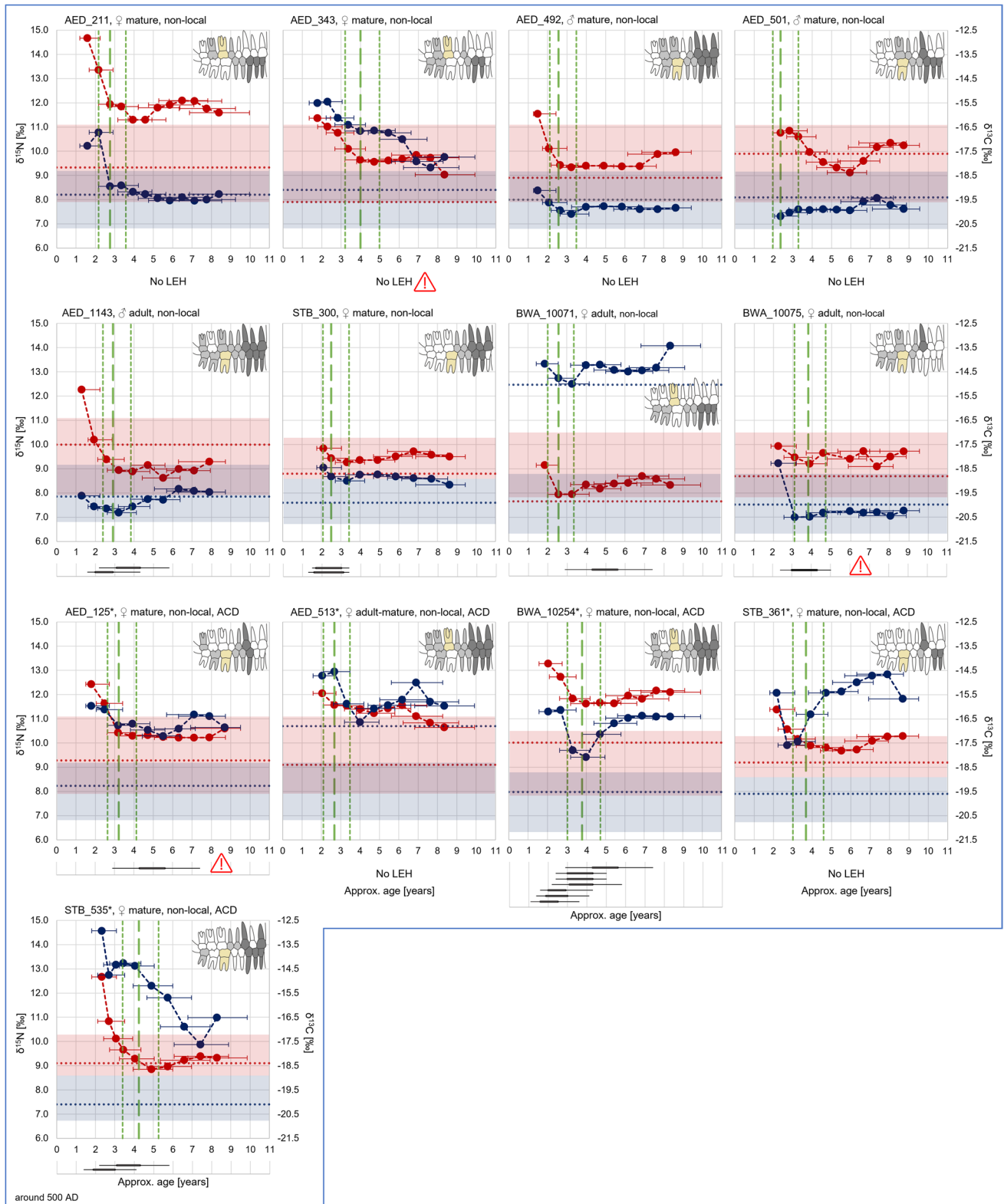
Fig. 4 (continued)

Fig. 4 (continued)

Isotope profiles

Intra-tooth isotopic patterns (isotope profiles) of all 38 individuals, illustrating changes in stable light isotope ratios throughout the first 10 years of life are shown in Fig. 4. We
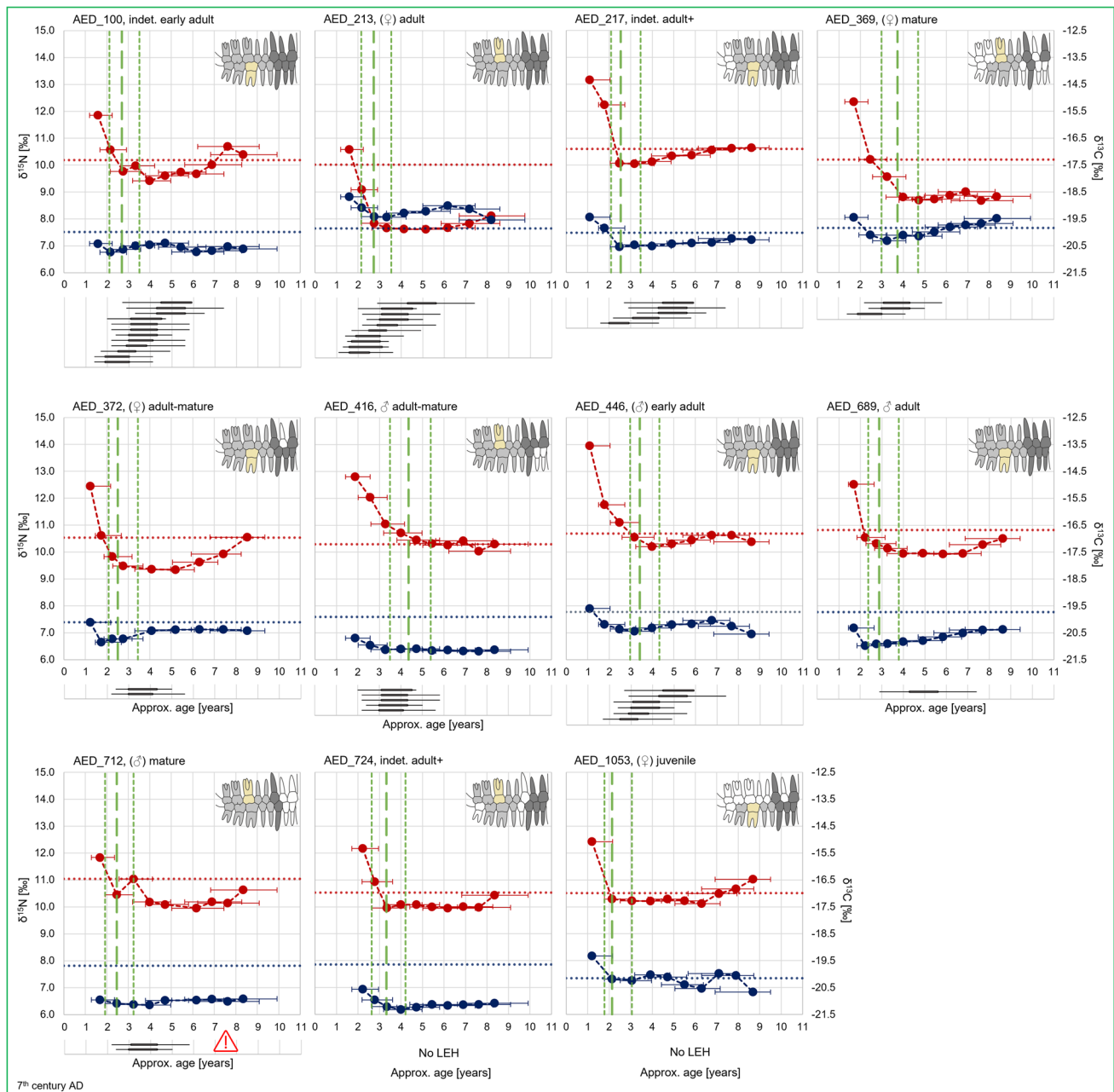
content of carbon from 13 to 50% and of nitrogen from 4.8 to 19%) (largely following DeNiro 1985, Ambrose 1990, Rand et al. 2015). Dentine samples ($n = 15$) with higher contents were excluded.

proceed with the assumption that tooth development was normal and identify changes of isotopy only accepting a minimum difference of at least 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ values.

Weaning phase

We observe no period of exclusive breastfeeding, indicated by the first rising $\delta^{15}N$ values (Fig. 4). In most isotope profiles ($n = 36$), $\delta^{15}N$ ratios continuously decrease from the beginning of observation (Table 1, Fig. 4), marking the

Table 1. Observation start (Ob start) AQ = approximate age of the first dentine section, with the condition of normal tooth development, whereby potential wear was taken into account for each tooth. Individual δ15 N and δ13 C ratios of the first dentine sections (δ15N/ δ13C first section) compared to individual bone ratios (Diff bone) and post-weaning ratios (Diff post-w). Total ↓δ15N/↓δ13C = total decrease of δ15N/δ13C in first dentine sections with continuous (con) decline (by min 0.2‰ per section) and inconstant (incon) decline including second drops. W age = estimated individual weaning ages. Individual post-weaning levels (post-w δ15N/δ13C) are defined as the mean value of dentine sections within three years after the approximate individual weaning age compared to individual bone values (Diff bone). δ13C and δ15N ratios were measured in individual bone samples, primarily ribs (δ15N/ δ13C bone). Values highlighted in gray correspond to minimum and maximum values within groups. Values in bold correspond to the mean value and the standard deviation (sd) within groups and total. Groups are initially defined by their chronological dating (Late Roman, 500 AD, seventh century). Within the group of burials dating around 500 AD, the division into migrants and "locals" is made according to Velte et al. (2023)

Group	Individual *ACD	Ob start [years]	δ15N first section [‰] value	Diff bone	Diff post-w	δ13C first section [‰] value	Diff bone	Diff post-w	Total ↓δ15N [‰] con (incon)	Total ↓δ13C [‰] con (incon)	W age [years]	post-w δ15N [‰] value	Diff bone	post-w δ13C [‰] value	Diff bone	δ15N bone [‰]	δ13C bone [‰]
Late Roman	EKF_1663 ♀	1.5	11.6	2.5	2.1	-19.7	-0.2	-0.9	-2.2 (-2.7)	no	3.0	9.5	0.4	-18.8	0.7	9.1	-19.5
	EKF_1700 ♀	1.4	11.2	1.8	1.2	-15.2	4.4	1.5	-1.1	-2.2	2.3	10	0.6	-16.7	2.9	9.4	-19.6
	EKF_1703 ♂	1.6	12.1	3.2	4.0	-19.1	0.4	0.4	-3.9	-1.1	4.3	8.1	-0.8	-19.5	0.0	8.9	-19.5
	EKF_1719 ♂	2.0	9.7	0.0	0.2	-19.8	-0.4	-0.4	no (-0.4)	no	<2.0	9.5	-0.2	-19.4	0.0	9.7	-19.4
	mean (N=4)	**1.6**	**11.2**	**1.9**	**1.9**	**-18.5**	**1.1**	**0.2**	**-2.4**	**-1.7**	**2.8**	**9.3**	**0.0**	**-18.6**	**0.9**	**9.3**	**-19.5**
	sd	**0.2**	**1.0**	**1.4**	**1.6**	**2.2**	**2.6**	**1.1**	**1.4**	**0.8**	**1.1**	**0.8**	**0.6**	**1.3**	**1.4**	**0.4**	**0.1**
"Locals" ~500 AD	AED_92 ♂	1.6	12.5	3.1	3.1	-20.0	-0.5	-0.3	-2.9 (-3.6)	-0.6	2.2	9.4	0.0	-19.7	-0.2	9.4	-19.5
	AED_105 ♀	1.3	11.5	2.4	1.8	-18.7	0.7	0.9	-1.6 (-1.9)	-1.0	1.9	9.7	0.6	-19.6	-0.2	9.1	-19.4
	AED_154 ♂	2.0	13.7	4.6	4.0	-16.8	3.1	1.6	-4.2	-2.3	4.1	9.7	0.6	-18.4	1.5	9.1	-19.9
	AED_204 ♀	1.3	10.0	0.7	0.9	-19.9	-0.5	-0.4	-1.2	no	2.4	9.1	-0.2	-19.5	-0.1	9.3	-19.4
	AED_249 ♂	2.0	11.9	2.5	1.9	-20.4	-0.4	0.0	-1.3 (-2.3)	-0.3	2.4	10.0	0.6	-20.4	-0.4	9.4	-20.0
	AED_280 ♂	1.3	11.7	3.4	3.2	-19.3	0.4	0.6	-3.3	-1.0	3.0	8.5	0.2	-19.9	-0.2	8.3	-19.7
	AED_825 ♀	1.6	13.5	4.4	3.5	-18.6	1.8	0.4	-3.1 (-3.8)	-0.6	3.4	10.0	0.9	-19.0	1.4	9.1	-20.4
	AED_1119 ♀	1.3	12.2	3.0	2.8	-19.6	-0.1	0.9	-3.4	-0.7	3.4	9.4	0.2	-20.5	-1.0	9.2	-19.5
	AED_1129 ♀	1.4	13.3	2.6	3.0	-19.0	1.1	0.5	-1.2 (-4.6)	-0.4	2.3	10.3	-0.4	-19.5	0.6	10.7	-20.1
	AED_1138 ♂	1.6	11.9	2.2	1.9	-19.6	0.9	0.4	-2.0	-0.3	2.6	10.0	0.3	-20.0	0.5	9.7	-20.5
	mean (N=10)	**1.5**	**12.2**	**2.9**	**2.6**	**-19.2**	**0.7**	**0.5**	**-2.4**	**-0.8**	**2.8**	**9.6**	**0.3**	**-19.7**	**0.2**	**9.3**	**-19.8**
	sd	**0.3**	**1.1**	**1.1**	**1.0**	**1.0**	**1.2**	**0.6**	**1.1**	**0.6**	**0.7**	**0.5**	**0.4**	**0.6**	**0.8**	**0.6**	**0.4**
	mean ♂ (N=5)	1.7	12.3	3.2	2.8	-19.2	0.7	0.5	-2.7	-0.9	2.9	9.5	0.3	-19.7	0.2	9.2	-19.9
	sd	0.3	0.8	0.9	0.9	1.4	1.5	0.7	1.1	0.8	0.8	0.6	0.3	0.8	0.8	0.5	0.4
	mean ♀ (N=5)	1.4	12.1	2.6	2.4	-19.2	0.6	0.5	-2.1	-0.7	2.7	9.7	0.2	-19.6	0.1	9.5	-19.8
	sd	0.1	1.4	1.3	1.0	0.6	0.9	0.5	1.1	0.3	0.5	0.5	0.5	0.6	0.9	0.7	0.5
Migrants ~500 AD	AED_125* ♀	1.8	12.4	3.1	2.1	-16.0	3.3	1.0	-2.0	no (-0.7)	3.2	10.3	1.0	-17.0	2.3	9.3	-19.3
	AED_211 ♀	1.6	14.7	5.4	3.1	-17.3	2.0	2.0	-2.8 (-3.4)	no (-2.3)	2.8	11.6	2.3	-19.3	0.0	9.3	-19.3
	AED_343 ♀	1.8	11.4	3.5	1.7	-15.5	3.6	1.6	-1.7	no (-1.2)	4.0	9.7	1.8	-17.1	2.0	7.9	-19.1
	AED_492 ♂	1.5	11.6	2.7	2.2	-19.1	0.4	0.8	-2.2	-1.0	2.6	9.4	0.5	-19.9	-0.4	8.9	-19.5
	AED_501 ♂	2.4	10.8	0.9	0.7	-20.2	-0.8	-0.3	no (-1.8)	no	<2.4	10.1	0.2	-19.9	-0.5	9.9	-19.4
	AED_513* ♀	3.0	12.1	3.0	0.7	-14.7	2.1	1.4	-0.4	no (-2.0)	2.7	11.4	2.3	-16.1	0.7	9.1	-16.8
	AED_1143 ♂	1.3	12.3	2.3	3.4	-19.6	0.1	0.3	-3.4	-0.5	2.9	8.9	-1.1	-19.9	-0.2	10.0	-19.7
	STB_300 ♀	2.1	9.8	1.0	0.4	-18.5	1.4	0.3	-0.4	-0.5	2.5	9.4	0.6	-18.8	1.1	8.8	-19.9
	STB_361* ♀	2.2	11.4	2.2	1.7	-15.4	4.2	-0.1	-1.5	-2.2	3.7	9.7	0.5	-15.3	4.3	9.2	-19.6
	STB_535* ♀	2.3	12.7	3.6	3.5	-12.9	7.2	3.8	-3.8	-1.9	4.3	9.2	0.1	-16.7	3.4	9.1	-20.1
	BWA_10071 ♀	1.9	9.2	1.5	0.9	-14.2	0.8	0.3	-1.3	-0.8	2.6	8.3	0.6	-14.5	0.5	7.7	-15.0
	BWA_10075 ♀	2.3	9.9	1.2	0.3	-18.3	1.7	2.0	-0.7	-2.2	3.8	9.6	0.9	-20.3	-0.3	8.7	-20.0
	BWA_10254* ♀	2.0	13.3	3.3	1.5	-16.2	3.3	0.5	-1.7	no (-2.0)	3.8	11.8	1.8	-16.7	2.8	10.0	-19.5
	mean (N=13)	**1.9**	**11.7**	**2.6**	**1.7**	**-16.8**	**2.3**	**1.1**	**-1.8**	**-1.3**	**3.2**	**10.0**	**0.9**	**-17.8**	**1.2**	**9.1**	**-19.0**
	sd	**0.3**	**1.5**	**1.3**	**1.1**	**2.3**	**2.1**	**1.1**	**1.1**	**0.8**	**0.7**	**1.1**	**1.0**	**2.0**	**1.6**	**0.7**	**1.5**
	mean ♂ (N=3)	1.7	11.6	2.0	2.1	-19.6	-0.1	0.3	-2.8	-0.6	2.6	9.5	-0.1	-19.9	-0.4	9.6	-19.5
	sd	0.6	0.8	1.0	1.4	0.6	0.6	0.6	0.9	0.4	0.3	0.6	0.9	0.0	0.2	0.6	0.2
	mean ♀ (N=10)	2.0	11.7	2.8	1.6	-15.9	3.0	1.3	-1.6	-1.5	3.3	10.1	1.2	-17.2	1.7	8.9	-18.9
	sd	0.2	1.7	1.4	1.1	1.8	1.8	1.2	1.1	0.8	0.7	1.2	0.8	1.8	1.5	0.7	1.7
7th century	AED_100 indet	1.6	11.9	1.7	2.2	-20.4	-0.4	0.1	-2.1 (-2.5)	-0.3	2.7	9.7	-0.5	-20.5	-0.5	10.2	-20.0
	AED_213 ♀	1.6	10.6	0.6	3.0	-18.7	1.2	0.6	-2.8	-0.7	2.7	7.6	-2.4	-19.3	0.6	10.0	-19.9
	AED_217 indet	1.1	13.2	2.6	3.0	-19.4	0.6	1.1	-3.1	-1.2	2.5	10.2	-0.4	-20.5	-0.5	10.6	-20.0
	AED_369 ♀	1.7	12.4	2.2	3.6	-19.5	0.3	0.5	-3.6	-0.8	3.7	8.8	-1.4	-20.0	-0.2	10.2	-19.8
	AED_372 ♀	1.2	12.5	2.0	3.1	-20.1	0.0	0.3	-3.0	-0.8	2.5	9.4	-1.1	-20.4	-0.3	10.5	-20.1
	AED_416 ♂	1.9	12.8	2.5	2.5	-20.7	-0.8	0.5	-2.5	-0.3	4.4	10.3	0.0	-21.2	-1.3	10.3	-19.9
	AED_446 ♂	1.1	14.0	3.3	3.6	-19.6	0.1	0.6	-3.8	-0.8	3.4	10.4	-0.3	-20.2	-0.5	10.7	-19.7
	AED_689 ♂	1.7	12.5	1.7	2.5	-20.3	-0.6	0.5	-2.4	-0.7	2.9	10.0	-0.8	-20.8	-1.1	10.8	-19.7
	AED_712 ♂	1.7	11.8	0.8	1.4	-21.0	-1.3	0.1	-1.3 (-1.6)	no	2.5	10.4	-0.6	-21.1	-1.4	11.0	-19.7
	AED_724 indet	2.2	12.2	1.7	2.1	-20.6	-1.0	0.6	-2.2	-0.6	3.3	10.1	-0.4	-21.2	-1.6	10.5	-19.6
	AED_1053 ♀	1.2	12.4	1.9	2.2	-19.3	0.9	0.8	-2.1	-0.9	2.1	10.2	-0.3	-20.1	0.1	10.5	-20.2
	mean (N=11)	**1.5**	**12.4**	**1.9**	**2.7**	**-20.0**	**-0.1**	**0.5**	**-2.6**	**-0.7**	**3.0**	**9.7**	**-0.8**	**-20.5**	**-0.6**	**10.5**	**-19.9**
	sd	**0.4**	**0.9**	**0.8**	**0.7**	**0.7**	**0.8**	**0.3**	**0.7**	**0.3**	**0.7**	**0.9**	**0.7**	**0.6**	**0.7**	**0.3**	**0.2**
Total	min	1.1	9.2	0.0	0.2	-21.0	-1.3	-0.9	-4.2	-2.3	1.9	7.6	-2.4	-21.2	-1.6	7.7	-20.5
	max	2.4	14.7	5.4	4.0	-12.9	7.2	3.8	-0.4	-0.3	4.4	11.8	2.3	-14.5	4.3	11.0	-15.0
	mean (N=38)	**1.7**	**12.0**	**2.4**	**2.2**	**-18.5**	**1.0**	**0.7**	**-2.3**	**-1.0**	**3.0**	**9.7**	**0.2**	**-19.2**	**0.4**	**9.6**	**-19.5**
	sd	**0.4**	**1.2**	**1.2**	**1.1**	**2.1**	**1.8**	**0.8**	**1.0**	**0.6**	**0.7**	**0.9**	**1.0**	**1.7**	**1.4**	**0.8**	**1.0**

weaning phase with complementary feeding. A concomitant initial decrease in δ13C values is also observed in many profiles (n = 28), but which often ends earlier than the drop in δ15N (n = 13). In contrast, in some individuals, δ13C remains stable (n = 6) or increases (n = 2) in the earliest part of the isotopic profile, while δ15N simultaneously decreases (Table 1).

Overall, values and intra-tooth patterns of δ13C and δ15N are highly variable in the initial part of the profiles. The levels of the first dentine sections, as well as the extent and rate of the continuous decrease over two or more sections, vary markedly (Table 1).

The δ15N ratios of the first dentine sections are almost universally increased compared to both bone ratios (n = 37) and post-weaning levels (n = 38). This is in line with the expectation that the trophic level increase due to breastfeeding can still be observed at the beginning of the intra-tooth isotopic pattern. The δ13C ratios, however, are similar or

lower compared to bone ($n = 15$) and/or post-weaning levels ($n = 9$) in some cases (Table 1), as anticipated, given the lesser impact of weaning on carbon.

In most isotope profiles, the extent of the observable decrease in $\delta^{15}N$ and $\delta^{13}C$ ($n = 25$) falls within the range of the expected trophic level effect of breastfeeding ($\sim 3.0‰$ in $\delta^{15}N$, $\sim 1.0‰$ in $\delta^{13}C$, e.g., Fuller et al. 2006). In some cases, values decrease to a greater extent in $\delta^{15}N$ ($\geq 3.2‰$, $n = 8$) and $\delta^{13}C$ ($\geq 1.2‰$, $n = 6$) (Table 1). Non or minimally decreasing $\delta^{15}N$ values ($< 1.0‰$) are only found in individuals ($n = 5$) with missing first dentine samples, consequently showing the weaning phase only towards its end or not at all.

The decrease of $\delta^{15}N$ and $\delta^{13}C$ is generally faster at the beginning and slows down towards the end (Table S2.2). Higher rates are predominately found in individuals whose profiles show a greater total decrease in isotope ratios, while lower rates are mostly found in profiles with a smaller total decrease (Kruskal–Wallis test $p(\delta^{15}N) < 0.001$, $p(\delta^{13}C) = 0.003$, Table S1.2.9). In some cases (AED_343, AED_416, STB_361*, BWA_10254*), $\delta^{15}N$ decreases rather gradually at a smaller rate ($\leq 1.5‰$ per year) but still exhibits a notable extent of decline (1.5 to 2.5‰) (Table S2.2). Nevertheless, in certain cases $\delta^{15}N$ ($n = 11$) or $\delta^{13}C$ ratios ($n = 5$) decrease discontinuously, i.e., ratios decrease after a short stabilization or even increase. However, this second decrease mainly ($n = 9$) follows a prior constant decline in $\delta^{15}N$ (e.g., AED_105, AED_1129), whereas a discontinuous drop in $\delta^{13}C$ only occurs if values were either stable or increasing at the very beginning (e.g., AED_211, AED_343) (Table 1, Fig. 4).

Age of complete weaning

At the population level, weaning was completed by the age of 3.0 years (Figure S1.2.1). This corresponds to the average of individual weaning ages (3.0 ± 0.7 years (sd)). Notably, individual weaning ages vary significantly, ranging from as early as 1.9 years to as late as 4.4 years (Table 1).

Fig. 5 Estimated individual weaning ages considering normal tooth development. Error bars display deviations for early and late tooth development. The Kernel density estimation (KDE) in the lower part is based on a Gaussian kernel. The optimal bandwidth for the Gaussian kernel was calculated using the "solve the equation" method by Sheather and Jones (1991).
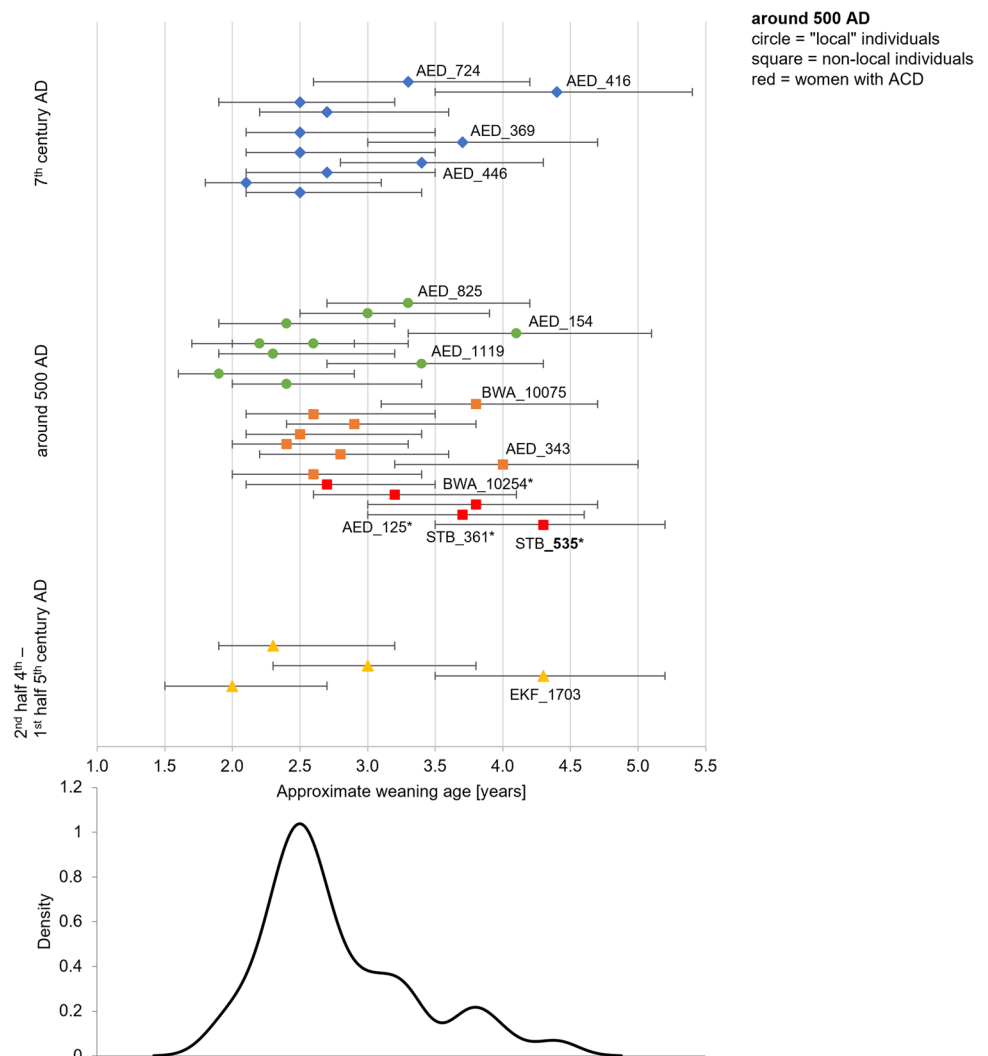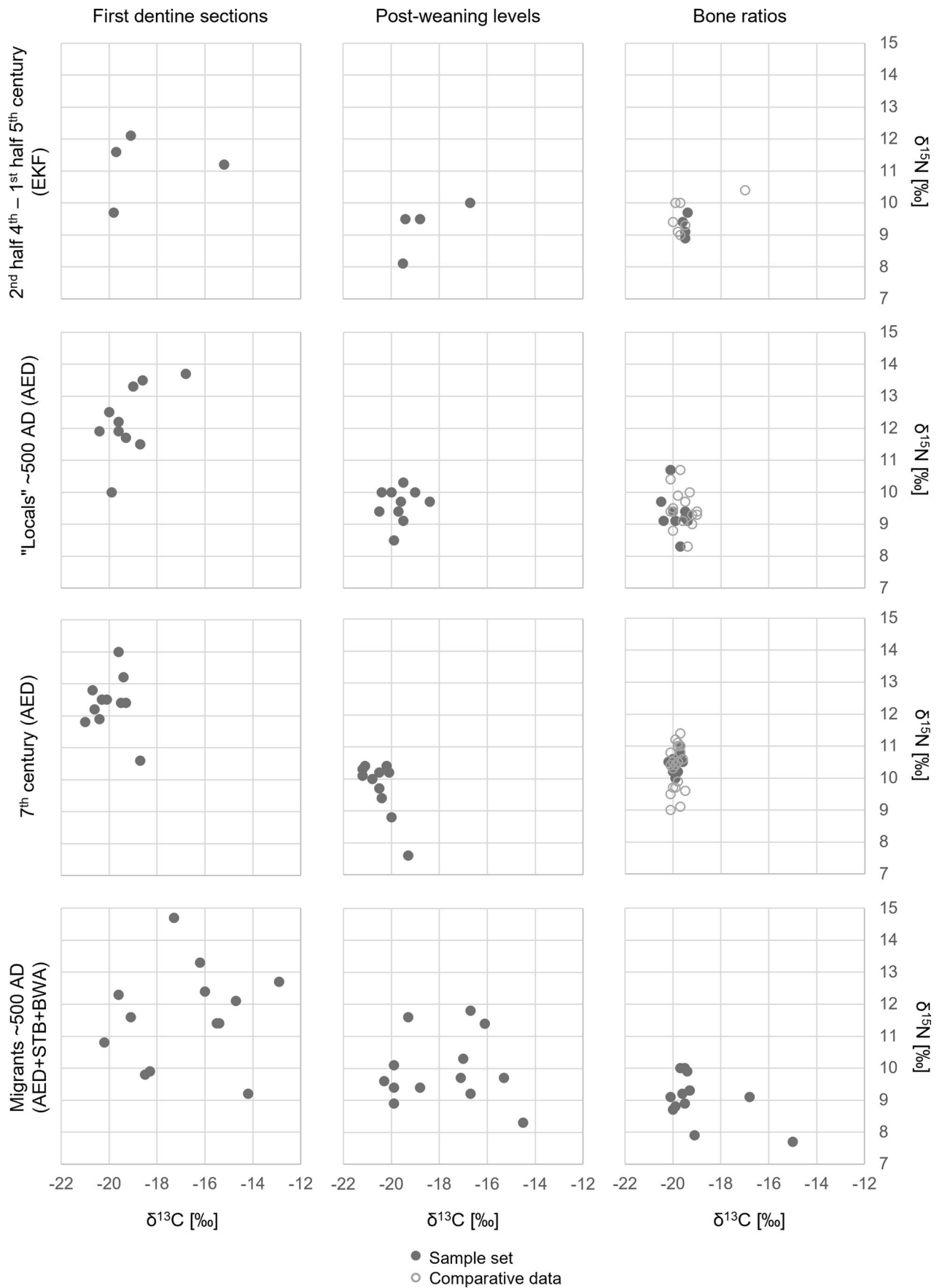
◄**Fig. 6** Group differences of stable light isotope ratios in populations from Erding (EKF, AED). Filled data points show individual values for each stage. Additional comparative bone data are given as unfilled points. Comparative data are taken from Sofeso et al. (2012) for Late Roman individuals (EKF), Hakenbeck et al. (2010), and Velte et al. (2023) for individuals from around 500 AD, and Neidich (2023) for individuals dating from the seventh century (AED). Information about individual's provenance is taken from Velte et al. (2023).

Additionally, the Gaussian kernel density distribution of weaning ages shows two side peaks at over 3.0 years, however, a clear main peak is found around 2.5 years (Fig. 5).

Post-weaning phase

Values and intra-tooth patterns of $\delta^{13}C$ and $\delta^{15}N$ are also significantly variable within the post-weaning phase. Post-weaning levels, assigned as the mean value of dentine ratios covering 3 years following individual weaning ages, show a great range and different extent and direction of fluctuations (Table 1, Fig. 4).

$\delta^{15}N$ ($n = 3$) or $\delta^{13}C$ ($n = 5$) post-weaning levels only rarely match individual bone ratios ($\pm 0.1‰$). Instead, they tend to be more or less equally frequent found above ($\delta^{15}N$: by 0.2 to 2.3‰, $n = 20$; $\delta^{13}C$: by 0.5 to 4.3‰, $n = 15$) or below bone ($\delta^{15}N$): by -0.2 to $-2.4‰$, $n = 15$; $\delta^{13}C$: by -0.2 to $-1.6‰$, $n = 18$) (Table 1). The $\delta^{15}N$ values frequently ($n = 30$) exhibit an increase from the lowest point of the initial decrease, including the observed second drops, towards the end of the profiles, creating the impression of a post-weaning dip (e.g., AED_249, AED_1119) (Fig. 4). However, the visibility of this dip varies; in some cases, it is not clearly visible due to generally more fluctuating ratios (e.g., EKF_1663, BWA_10075), or because the later increase is of lower extent (e.g., EKF_1719, AED_204) or shorter time (e.g., AED_125*, AED_724) (Fig. 4). Four individuals (AED_154, AED_249, AED_501, AED_1053) show covariant decreasing $\delta^{13}C$ values during the later increase in $\delta^{15}N$.

Group differences

Intra tooth patterns are highly variable within all defined groups. However, we identified some general characteristics and group differences.

Late Roman individuals (EKF, $n = 4$) and the "locals" from around 500 AD (AED, $n = 10$) show similar ranges of ratios in the first dentine sections, as well as the extent or rate of decreasing values, post-weaning levels, and bone ratios (Table 1, Fig. 6). Their post-weaning levels often align or are slightly above their individual bone values (Table 1). In both of these groups, single individuals display increased $\delta^{13}C$ values in dentine, at least temporarily

(EKF_1663, EKF_1700, AED_154), corresponding with the levels observed in some migrants from around 500 AD.

In contrast, generally lower $\delta^{13}C$ dentine ratios are found in individuals dating from the seventh century (AED, $n = 11$) (Table 1, Fig. 6). The $\delta^{13}C$ ratios in their first dentine sections show a trend towards lowered values, often ($n = 5$) falling below their individual bone values (Table 1), which are found within a range similar to that of earlier times (Table 1, Fig. 6). Furthermore, their post-weaning $\delta^{13}C$ levels following the weaning dip are significantly decreased in comparison to the levels of the "locals" from around 500 AD (pairwise comparison $p = 0.004$, Table S1.1.23). Although the mean difference is even greater when compared to Late Roman individuals (Table 1), it remains non-significant, probably due to the small sample size and increased variability within the group. Additionally, $\delta^{15}N$ ratios show similar ranges in the first dentine sections, extent of decrease, and post-weaning levels in comparison to individuals dated into Late Roman times (Table 1, Fig. 6). However, the post-weaning $\delta^{15}N$ levels are significantly lower than their individual bone values (paired samples test $p = 0.004$, Table S1.2.25). Moreover, the bone ratios of individuals from the seventh century are significantly increased compared to Late Roman individuals (pairwise comparison $p = 0.002$, Table S1.2.38) and the "locals" from around 500 AD (pairwise comparison $p = 0.015$, Table S1.2.38).

In summary, the estimated weaning ages are remarkably variable across all periods, including prolonged weaning (weaning age > 3.0 years) (Fig. 5). Thus, no significant chronological change in weaning age is found. Instead, chronological differences in $\delta^{13}C$ and $\delta^{15}N$ ratios result from changes in the seventh century.

However, we found some significant differences linked to individuals' origin around 500 AD. Migrants (AED + STB + BWA, $n = 13$) generally exhibit more variable results at the beginning of the observation and in post-weaning levels compared to the "locals" (AED, $n = 10$). While $\delta^{15}N$ ratios of first dentine sections and post-weaning levels tend to differ only slightly from "locals," $\delta^{13}C$ ratios are, on average, significantly increased in the first dentine sections (Mann–Whitney U test $p = 0.006$, Table S1.2.1) and in post-weaning levels (independent-samples Mann–Whitney U test $p = 0.003$, Table S1.2.1) (Fig. 6).

Moreover, discontinuously and more strongly decreasing $\delta^{13}C$ ratios in the earliest parts of profiles are exclusively found in migrants (Table 1), but also stronger fluctuations in $\delta^{13}C$ after weaning were primarily observed in non-locals (Fig. 4). Both $\delta^{15}N$ or $\delta^{13}C$ values in post-weaning dentine of migrants differ significantly from their bone ratios (paired samples test $p(\delta^{15}N) = 0.006$, $p(\delta^{13}C) = 0.019$, Table S1.2.25), which in turn do not differ from bone ratios of the "locals" (Fig. 6). Differences between post-weaning and bone ratios are more pronounced in female

migrants (AED + STB + BWA, $n = 10$) compared to male migrants (AED, $n = 3$) (independent-samples Mann–Whitney U test $p = 0.028$, Table S1.2.19). This difference can be linked to a sex-dependent difference among migrants: $\delta^{13}C$ ratios of the first dentine sections of female migrants are, on average, significantly increased compared to male migrants (Mann–Whitney U test $p = 0.007$, Table S1.2.2), as well as post-weaning $\delta^{13}C$ levels (independent-samples Mann–Whitney U test $p = 0.049$, Table S1.2.19). Additionally, more increased post-weaning $\delta^{15}N$ levels are primarily found in female migrants (Table 1). Although we did not find a significant difference between migrants and "locals" in estimated weaning ages, a clear trend towards later weaning in migrants is noticeable. Among the group of non-locals, females, particularly those with ACD, more often show prolonged weaning (weaning age > 3.0 years) (Fig. 5). Thus, the observed differences between migrants and "locals" are primarily based on non-local females, while male migrants and single female migrants do not exhibit major differences to the "local" population from around 500 AD, in which no differences between men ($n = 5$) and women ($n = 5$) were found.

Linear enamel hypoplasia (LEH)

The preservation of the anterior dentition was highly variable (Fig. 4). LEH data from individuals with an incomplete dentition (less than three teeth assessable) is labeled with an exclamation mark ($n = 6$) in Fig. 4. One individual with a completely missing dentition was excluded from the analysis (Table 2). The incidence of LEH varies, with the highest count being 12. Most individuals have fewer than four ($n = 14$), averaging to 1.9 ± 3.0 (sd) LEH. Slightly over half of the individuals display at least one LEH ($n = 21$). The development of LEH happened between 0.8–1.9 years and 4.5–5.9 years, on average at age 3.0–4.2.

The number of LEH significantly correlates with the mean formation age (midpoint of determined formation span) of the first LEH (Pearson correlation $p = 0.014$, Table S1.3.7), thus, individuals with LEH appearing at an early age tended to develop more LEH.

The average age at the formation of the first LEH (2.7–3.8 years, Table 2) corresponds well with the mean age at the cessation of weaning. However, on an individual level, the initial formation of LEH only rarely matches the estimated weaning age ($n = 5$, Fig. 4). In most individuals, the first LEH developed with a temporal offset (≥ 0.5 years) relative to the estimated weaning ages, either before ($n = 8$) or after ($n = 8$) weaning (Fig. 4).

Regarding the total number of LEH, only a few defects appear during the weaning phase ($n = 13$, 17.6%), primarily observed in individuals ($n = 6$, 75.0%) with noticeable late weaning (≥ 3.4 years). The largest number of LEH formed around the age of complete weaning ($n = 25$, 33.8%) or in the post-weaning phase ($n = 36$, 48.7%). Apparently, they most frequently occur during the supposed post-weaning dip in $\delta^{15}N$ ratios (Fig. 4).

Group differences

We found no indications of differences in the presence, number, or timing of LEH associated with individuals' origin or between sexes among the "locals" from around 500 AD (Table 2). Regarding the timing of defects, there is also no clear difference between the diachronic time phases (Table 2).

However, we observe a difference in individuals from the seventh century ($n = 11$, AED) compared to earlier diachronic phases (Late Roman: $n = 3$, EKF; "Locals" ~ 500 AD: $n = 10$, AED), at least in the presence and number of LEH. They display LEH more frequently (Table 2) and with significantly higher prevalence (independent samples Kruskal Wallis test $p = 0.026$, Table S1.3.10), compared to the "local" individuals from around 500 AD (pairwise comparison $p = 0.027$, Table S1.3.11), but not in comparison to Late Roman individuals, most likely due to the small sample size (Table 2).

Table 2 LEH data. Each observed LEH has a formation span not a time point. Therefore, the mean formation ages are calculated as: (mean of startpoint of formation span) − (mean of endpoint of formation span)

LEH data	Presence of LEH			Mean number of LEH ± sd	Mean formation age of all LEH (years)	Mean formation age of first LEH (years)
Group	N evaluated	N present	% observed (95% CI)			
All individuals	37	21	56.8 (40.5–70.3)	1.9 ± 3.0	3.0–4.2	2.7–3.8
(1) Late Roman (EKF)	3	1	33.3 (0–100)	1.3 ± 2.3	3.0–4.2	1.6–2.5
(2) "Locals" ~ 500 AD	10	4	40 (10–70)	0.6 ± 0.8	3.6–4.9	3.4–4.6
Men	5	2	40 (0–80)	0.6 ± 0.9	3.7–4.9	3.1–4.3
Women	5	2	40 (0–80)	0.6 ± 0.9	3.9–5.2	3.4–4.6
(3) Migrants ~ 500 AD	13	7	53.8 (30.8–76.9)	1.2 ± 1.9	2.8–4.0	2.7–3.8
(4) Seventh century	11	9	81.8 (54.5–100)	4.3 ± 4.1	3.0–4.2	2.6–3.6

Moreover, it is noteworthy that the two individuals from the seventh century with the highest numbers of LEH (AED_213: 11, AED_100: 12) died in early adult to adult age (Fig. 4).

Discussion

General observations and limitations

Unsurprisingly, we could not detect the phase of exclusive breastfeeding or the introduction of complementary foods in any individual in our sample (see Fig. 1), as our observation begins only after the age of 6 months. From that age onward, complementary food is necessary to satisfy the growing need of the infant for energy and nutrients (e.g. Michaelsen et al. 2000, Dewey and Brown 2003). Nevertheless, the weaning process, indicated by the "weaning dip" becomes visible in almost all profiles: it is clearly noticeable in δ^{15}N but less obvious in δ^{13}C. As the trophic level effect is smaller and the impact of supplementary foods is stronger on δ^{13}C, the values align faster to the breastfeeder's value (e.g., Fuller et al. 2006).

The observed variability of both isotope ratios within the weaning phase can be attributed to various factors, including the potential onset of the weaning before observation starts, the progression of the weaning process after the start of observation, the nutrition and physiology of the breastfeeding female, different breastfeeding practices, different weaning foods, as well as variations in the physiology and health of the infant (e.g., Fuller et al. 2006; Crowder et al. 2019; Henderson et al. 2022). However, this variability increased significantly with the inclusion of individuals who grew up in different geographic locations or during different historical periods.

A distinct pattern is apparent in most profiles in all groups: increasing δ^{15}N ratios after cessation of weaning creates the impression of a "post-weaning dip." Depleted δ^{15}N values in the post-weaning phase before a subsequent rise were observed in many other studies across diverse populations from various periods and socio-economic backgrounds (Beaumont et al. 2012; Eerkens et al. 2011; Fernández-Crespo et al. 2018; Henderson et al. 2014). This pattern indicates that the proportion of animal protein only later reaches a level comparable to that of adults. However, post-weaning values below bone level must not exclusively be linked to diet but may also be influenced by a general cause, such as physiological features (e.g., Henderson et al. 2022) e.g., related to growth (e.g., Waters-Rist and Katzenberg 2010). In our study, δ^{15}N values of the "post-weaning dip" drop less frequently below individual adult bone values in contrast to previous studies, but we assume that although less clear, this is still comparable.

In our method for calculating the weaning age, we include the lowest point of the δ^{15}N curve in the calculation. Since this point often lies within the post-weaning dip, which is also influenced by factors that are independent of breastfeeding, it can be assumed that the weaning age may be overestimated. Furthermore, some patterns within profiles can be misinterpreted using our method, e.g., discontinuously decreasing δ^{15}N ratios during the weaning process. These values may result from variable breastfeeding frequencies, changing trophic levels of weaning foods, or physiological stress (Fuller et al. 2005; Mekota et al. 2006; Reitsema 2013). Thus, subsequent decreasing δ^{15}N ratios may also belong to the actual weaning dip but are not considered in the calculations of our weaning ages.

However, these problems are not unique to our method. Especially the inclusion of the lowest δ^{15}N ratios of the "post-weaning dip" is a common practice (e.g., Scharlotta et al. 2018). While a heavily pronounced post-weaning dip could also pose an issue for the method recently published by Ganiatsou et al. (2023), it can be assumed that this method is less sensitive to discontinuously decreasing δ^{15}N ratios. The tool they developed, the WEaning Age FiNder tool (WEAN), employs fitted curves that compensate for such fluctuations. Nevertheless, compared to Ganiatsou et al. (2023), our method offers the advantage of presenting results as age ranges, allowing for a better illustration of the uncertainty of weaning ages due to the biological variability of tooth development.

Regardless of the methodological approach, the calculation of weaning ages should only be seen as an approximation for various reasons. Changes in the protein source potentially appear with a temporal offset of several weeks to month in isotope profiles (e.g., Waters-Rist et al. 2006, Chinique de Armas et al. 2022). This individually delayed onset can hardly be reconstructed, making precise weaning age calculations nearly impossible. Additionally, the temporal resolution of the isotope profiles is limited by the sampling method and by the age assignment of the samples, resulting in rather broad age ranges for certain data points or events derived (Figs. 4 and 5).

The latter also applies to the calculation of LEH formation ages. The less precise determination of the position of the LEH used here (see Methods) further increases the error beyond normal biological variability.

Weaning time and process

Most individuals exhibit a rather abrupt weaning process, with rapidly decreasing δ^{15}N ratios. A rapid decline can result from low-trophic complementary foods with limited protein supply leading to less expressed fractionation of ^{15}N/^{14}N and thus to a decrease in δ^{15}N of the body's nitrogen pool (e.g., Henderson et al. 2022). Another possibility

is that higher amounts of protein in complementary foods result in a faster decline of $\delta^{15}N$ as it more efficiently dilutes the infant's nitrogen pool (e.g., Fuller et al. 2006). However, we consider the first scenario to be more likely, as many typical weaning foods (e.g., bread and cereal porridge) are high in carbohydrates and low in protein (e.g., Sellen and Smay 2001), which is also consistent with Roman and Medieval written sources (e.g., Prowse et al. 2008, Bourbou and Garvie-Lok 2009, Dasen 2015).

We observe a high variability in weaning ages within predefined groups from Late Roman times, around 500 AD and the seventh century. Although some of this variation may be related to the methodological limitations mentioned above, it underscores that breastfeeding and weaning practices can differ significantly within a population, e.g., through adaptation to infant's health and needs, the availability of resources probably dependent on the season, but also social ideals and status, etc. (e.g., Bourbou and Garvie-Lok 2009, Bourbou et al. 2013, Bourbou et al. 2019). Our findings indicate that most individuals are fully weaned around the middle of the third year, although we also observe more extended weaning periods of up to 4 years.

This pattern is consistent with the historical context of the complete cessation of breastfeeding at around 3 to 4 years of age in the Roman provinces, as recommended in ancient texts by the Greek medic Galen, while at the Center of the Roman Empire, breastfeeding typically ceased earlier around 2 years, following the recommendations of another Greek physician, Soranus, which is corroborated by bioarcheological data from the Classical, Hellenistic, and Roman periods (summary in Fulminante 2015). Although mainly shorter weaning ages between 1 and 2 years were observed in Continental Europe during the Early Middle Ages (Fulminante 2015), some studies suggest that breastfeeding also ended rather late around the age of three in some communities (Haydock et al. 2013, Herrscher 2003). Dittmann and Grupe (2000) calculated the weaning age based on bone collage values from the remains of deceased children buried in a cemetery from the same time and region of our study. Interestingly, they also found a rather late weaning age of 3 years for these "non-survivors." In contrast, it is often assumed that individuals who were not breastfed or only for a short period tend to have a lower survival rate (e.g., Stuart-Macadam & Dettwyler 1995; Bourbou et al. 2019; Fernández-Crespo et al. 2022). Consequently, one might expect to observe a higher average weaning age in our study, which in turn exclusively examined survivors.

This underscores the need for additional data on subadults and adults from the same graveyards to get a more detailed picture of infant diet and associated stress in Late Roman and Early Medieval Europe.

Weaning stress

LEH observed in our sample indicates that stress, which manifests as enamel defects, only rarely occurs during the progressing replacement of breastmilk with non-breastmilk liquids and foods. This demonstrates that the weaning process does not represent an intrinsic stress factor for infants (Kendall et al. 2021), but also that continued breastfeeding may not fully protect them from all stressors. The increase in LEH formed at the end of the weaning process or in the post-weaning phase in our sample supports the assumption that the final cessation of breast milk as a source of nutrients and immune protection initiates a high-risk phase, a "weaning conundrum," as suggested by Fernández-Crespo et al. (2022).

An increase in $\delta^{15}N$ (Fuller et al. 2005; Mekota et al. 2006; Reitsema 2013), especially with a simultaneous decrease in $\delta^{13}C$ (Beaumont et al. 2018; Beaumont and Montgomery 2016, Craig-Atkins et al. 2020) is often considered a sign of physiological stress. In our study, this pattern is observed in only a few individuals and is never clearly associated with LEH formation. This correlation is perhaps not necessarily to be expected, since different types, severities, and durations of physiological stress do not necessarily have the same effect on enamel formation and isotope composition. However, LEH formation is often correlated with the observed post-weaning dip in $\delta^{15}N$ ratios, suggesting that infants may be more susceptible to stressors during this phase, possibly related to growth or a low-trophic diet (see above). Nevertheless, far more data is needed to confirm this connection.

Furthermore, we found a positive correlation between the formation age of the first LEH and the total frequency of LEH, a trend also reported previously (e.g., Berbesque and Hoover 2018). This supports the hypothesis that individuals exposed to stressors earlier in life may be less resilient to subsequent crises due to damaged immunological competence (Goodman and Armelagos 1989; Armelagos et al. 2009, Larsen 2015). Previous studies have also linked higher LEH frequencies to an earlier age-at-death (e.g., King et al. 2005; Boldsen 2007; Armelagos et al. 2009), which could explain why the two individuals with the highest numbers of LEH died at early (adult) ages (AED_100, AED_213).

Nutrition and early life histories: links to diachronic changes

We did not find significant differences in weaning patterns or weaning ages related to the considered diachronic phases.

However, we observe variations in $\delta^{13}C$ and $\delta^{15}N$ values in dentine as well as bone collagen values among individuals who potentially grew up in the same region around Erding but during different chronological times. We assume that this

is probably related to diachronic changes in infant feeding practices and/or economics.

While the isotope ratios of diachronic groups generally show great overlap (Fig. 6), we only observe increased $\delta^{13}C$ ratios in the dentine of single individuals dating into Late Roman times or around 500 AD (Figs. 4 and 6). This could be related to millet consumption, either indirectly through breastmilk or directly as weaning foods. Hence, this may also be a potential hint at a previously undetected non-local origin of these individuals. However, sporadic millet cultivation cannot be ruled out, although millet was not commonly used in Bavaria during this time (e.g., Hakenbeck et al. 2010; Velte et al. 2023).

People living in Bavaria between Roman and Medieval times primarily consumed a C3 plant-based terrestrial diet (e.g., Hakenbeck et al. 2010, Sofeso et al. 2012, Velte et al. 2023). The ratio of animal protein to plant content can only be estimated by comparing contemporaneous material, ideally from the archaeological context. However, only animal remains are available for the Late Roman site EKF, where concentration-dependent mixture models suggest an average of 25% animal vs. 75% plant content in the human diet (Harbeck and von Heyking 2014). Similar human bone isotope values of individuals from AED dating around 500 AD suggest comparable ratios if we assume the same "baseline" with unchanged $\delta^{15}N$ values of the consumed plants and thus animals.

Assuming an unchanged baseline, the significant increase in $\delta^{15}N$ bone ratios of individuals from AED dating into the seventh century indicates a higher proportion of animal protein. Increased $\delta^{15}N$ bone ratios were also found in other burial sites in Bavaria dating to the seventh century (Strott 2007, Czermak 2011) and were interpreted as an increase in protein content associated with intensification of livestock and dairy farming (e.g., Fehr 2019). However, this increase can just as well display a baseline shift related to changes in mixed farming practices through the beginning agricultural revolution (e.g., Fehr 2019), e.g., more intensive land use and increased fertilization (e.g., Bogaard et al. 2007; Fraser et al. 2011). Confirmation of a baseline shift would require sufficient data of different animal genera from the site, which is currently lacking.

The two interpretations of the bone collagen isotopes ("higher protein" vs. "different baseline hypothesis") also affect the understanding of the observed differences from the post-weaning diet. In individuals from Late Roman times and around 500 AD, we see post-weaning $\delta^{15}N$ levels within the range of adult collagen ratios, suggesting that infants had an adult-like diet during this time (e.g., Fournier et al. 2022). However, for the seventh century, we observe post-weaning $\delta^{15}N$ levels that are lower than the comparatively increased values we observe in adults of this time but consistent with the post-weaning $\delta^{15}N$ levels in children from the earlier times. If we follow the "higher protein hypothesis," i.e., the increased bone levels in adults result from a higher amount of animal protein, it raises the question of why the protein intake increased in adulthood but not in childhood in the seventh century compared to earlier times. Additionally, the post-weaning diet in the seventh century appears not different from the previous century in $\delta^{15}N$ values but in $\delta^{13}C$ ratios, which are lower. If we consider the "higher protein hypothesis," additional explanations are needed. Lower $\delta^{13}C$ values can indicate a changing plant spectrum, e.g., towards a greater diversification (e.g., Lewit 2009, Zach 2019) or smaller amounts of specific plants (e.g., millet) or plant parts with increased $\delta^{13}C$ (e.g., cereal grains see Cernusak et al. 2009), but this should then also be reflected in bone collagen ratios, at least to some extent, which is not observed.

If we follow the "different baseline hypothesis," in which higher environmental $\delta^{15}N$ levels are assumed for the seventh century, the observed pattern becomes more straightforward to explain. In this scenario, there was no increase in the protein content of the human diet, and the $\delta^{15}N$ values of children's post-weaning diet can be interpreted as a low protein diet because they fall below adult bone collagen ratios (e.g., Eerkens et al. 2011). This also explains the decreased post-weaning $\delta^{13}C$ levels compared to earlier times, potentially influenced by a change in plant foods but masked by the higher proportion of animal protein and thus not visible in bone isotope values. Furthermore, the observed increase in LEH in the seventh century can also be explained by a low-trophic diet, which is not supported by the "higher protein hypothesis." A low-trophic post-weaning diet may not be sufficient to meet the needs of children, especially during growth phases (e.g., Henderson et al. 2022), and may also make them more susceptible to external stressors.

In summary, the hypothesis that an increase in environmental $\delta^{15}N$ occurred in the seventh century and thus children of this period had a lower protein diet compared to adults, which can in turn be related to the increased formation of the LEH, seems more reliable. However, further research in the region on human remains and especially on animal bones is needed to validate this conclusion.

Early life histories: links to a foreign origin

Profiles of migrants who spent their childhood in other geographical locations, with divergent ecogeographic conditions and thus distinct isotopic baselines, as well as probably different dietary traditions, show the most variable isotope profiles. While some of the increased $\delta^{15}N$ ratios are possibly related to regions with higher nitrogen baselines, e.g., marine areas (Fig. 6), the fluctuating $\delta^{13}C$ ratios at significantly higher levels suggest a more frequent consumption of C4 plants such as millet (Fig. 4). However, these unusual signals are primarily limited to the diet during childhood,

whereas most of the bone collagen ratios of migrants adapted to the common signal of a C3 plant-based terrestrial diet (Fig. 6) (for detailed discussion see Velte et al. 2023).

Despite differences in the nutritional spectrum of migrants during childhood, it is notable that only about half of the non-local individuals were completely weaned within the third year of life, all males included. For the remaining women, especially those with ACD, the cessation of weaning was later. Additionally, three female migrants, including two with ACD, show a rather gradual weaning transition curve, which was only observed in four individuals in our sample. The difference between non-local men and women, along with the remarkable variability within the group of migrants, can be attributed to the fact that those migrants originate from different geographical regions in which people likely had a different lifestyle and culture (Velte et al. 2023). Although some migrants potentially grew up in the same cultural environment as people from southern Bavaria, women with ACD likely spent their childhood in a community with different traditions.

Cases of intentional cranial modification in pre- and early history Europe are known since the fifth to fourth centuries BC but become more widespread with the arrival of nomadic populations from the Eurasian Steppe. Thus, ACD is interpreted as an indication of nomadic incomers, e.g., the historically documented Huns and other nomadic groups associated with a pastoralist way of life (for review see Hakenbeck 2009). Some studies suggest that prolonged weaning times are found in such non-sedentary populations (e.g., Clayton et al. 2006, Waters-Rist et al. 2011). Rationales are still debated, ranging from a lack of appropriate weaning foods and the distribution of subsistence activities and workload to fertility control (e.g., Sellen and Smay 2001; Fouts et al. 2005; Sellen 2007; Bocquet-Appel 2011). Studies of pastoral societies indicate that infant diets are supplemented with animal products, including milk, meat, and blood rather than cereals or nuts (Ventresca Miller et al. 2014, 2017, Sellen 2001, Sellen and Smay 2001), which can result in a more gradual weaning curve. Furthermore, a study of pastoralists from Bronze Age Eurasia found that weaning was completed by the age of four (Ventresca Miller et al. 2017). Isotope data from an Early Medieval nomadic culture of Tuva (Southern Siberia) also suggests weaning after two to three years of age (Milella et al. 2022). Consequently, growing up in a community with a pastoralist and/or nomadic lifestyle is a possible explanation for the observed more gradual weaning pattern and prolonged weaning times observed in several migrants. However, to our knowledge, no data on weaning patterns of Early Medieval nomadic-pastoralists or individuals with ACD other than those presented here are available to substantiate this assumption.

To date, no men with ACD or who have a genetic origin or dietary pattern similar to these women have been identified in Bavarian cemeteries. This may indicate that men and women came to the region from different areas, at least partly as a result of distinct, gender-specific migration processes.

Conclusion

The analysis of weaning patterns and childhood stress through stable light isotope analysis of serial dentine samples is an intricate task, with many aspects that are still far from being sufficiently understood.

However, our study has revealed that more abrupt weaning occurred within the third year of life of most of the individuals buried between Roman and Medieval Southern Bavaria. Although we found no differences in weaning ages between males and females or diachronic time phases, we observed a trend towards prolonged weaning in some migrants.

Moreover, we found some evidence for a diachronic change in isotope baselines linked to changes in economic practices, which likely also affect infant feeding strategies, reflected in the post-weaning diet and stress level in the seventh century. If individuals experienced stress associated with the weaning process, it manifests itself primarily in the post-weaning phase, after the complete cessation of breastfeeding. This demonstrates that the choice of foods during this time is important as low-trophic foods can potentially facilitate the susceptibility of infants to stressors.

In summary, we were able to show different weaning practices in various communities, both by chronological distance and cultural differences, although weaning patterns within a community can also vary significantly. However, more data are needed including the remains of subadults and animals to obtain a more detailed and complete picture of infant feeding strategies and related stress.

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Data availability All data analyzed in this study are included in the submitted supplementary information files.

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

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4. Discussion

4.1 Different diets among the population of South Bavaria during the "Dark ages"

The diet of historical populations is closely related to the ecogeographical conditions of their habitat, which determine the presence of native plants and animals as food. However, humans began to shape their habitat early on e.g., by the development of usable areas, cultivation of specific plants, domestication and breeding of different animals, etc. strongly affecting their food base and spectrum. Both, prevailing external conditions as well as subsistence strategies of communities can vary significantly between different regions, especially between more distant regions and cultural groups (e.g., Barer-Stein 1979). Besides the overall availability of resources for a population, also political, social, and cultural aspects can determine the individual access to specific resources (e.g., Alt 2018, Le Huray & Schutkowski 2005, Privat et al. 2002).

References to cultivated crops and domesticated livestock, technical practices in agriculture, animal husbandry, fishing, and hunting in past communities of Roman and Medieval times are also found in historical illustrations and texts (e.g., *De re conquinaria* written by Apicius in the 3rd or 4th century AD translation by Maier 1991, Salzburger Calendar around 818 AD as shown in von den Driesch & Boessneck 1988) as well as the archeozoological (e.g., Benecke 1994, Peters 1994) and archaeological record (e.g., Rösch et al. 1992, Zach 2019). However, the most accurate information is provided by isotope analyses of human skeletal remains, complemented by isotope data of plants and animals (e.g., Hakenbeck et al. 2010, Knipper et al. 2013).

The analyses of carbon (C) and nitrogen (N) isotopes from human bones and teeth carried out in this thesis provide some general information about the nutrition of children and adults in South Bavaria from Late Roman and Early Medieval times (Publication I, Publication II). Although this was not the primary focus of this thesis, this information is crucial for the interpretations of alternative diets as indicators of human movement.

Common diet in Late Roman and Early Medieval Bavaria

People living in South Bavaria during Late Roman and Early Medieval times likely consumed mainly C3 plants, corresponding to the fact that most of the native plants in temperate Central Europe are C3 plants but also aligning with archaeological evidence for Late Roman and Early Medieval Southern Germany that shows a wide range of cultivated C3 plants e.g., cereal crops, oil and fiber plants and pulses (Rösch et al. 1992, Rösch 1998, Stika 1996, Rösch 2008). More generally, the Romans are said to have also brought a more distinctive garden culture for the cultivation of different fruits, vegetables, and herbs to their territories

north of the Alps (e.g., Körber-Grohne 1979, Knörzer & Meurers-Balke 1990). But in terms of isotopies, it is barely possible to distinguish between different species of C3 plants, in contrast to differentiation between C3 and C4 plants (e.g., O'Leary 1981, Farquhar et al. 1989).

Archaeobotanical research at Early Medieval sites in Bavaria also showed the isolated occurrence of a C4 plant – millet (e.g., Zach 2019). C4 plants are adapted to warm and dry climates, so millet cultivation is probably limited to spring or summer in the region of Bavaria. Due to its short growing period, millet is particularly suitable as "back-up-crop" in the case of low yields from early crops or crop failures (e.g., Kirleis et al. 2022). Consequently, regular consumption of millet in higher amounts is not indicated for people living in South Bavaria, neither from the archaeological record nor from the human isotopic data, and thus is considered unlikely.

A statement about the consumption of animal foods is particularly limited because samples of contemporaneous animals from archaeological sites are nearly completely lacking, which hampers the ability to infer an isotopic baseline of human resources. The most frequently mentioned domestic animals in sedentary communities of Roman and Early Medieval times include horses, cattle, pigs, sheep, goats and chicken, whereas not all of them served primarily as meat suppliers (e.g., Kokabi 2001, von den Driesch & Boessneck 1988, Schubert 2006, Benecke 1994). When comparing human ratios with isotope data of these animal species from other cemeteries of the Late Roman and Early Medieval Bavaria it suggests that the consumption of animal protein in Early Medieval South Bavaria was moderate and may also have been derived from low amounts of freshwater fish in addition to terrestrial animals. Although the animal data from other sites is less suitable for comparison, moderate consumption of animal protein also corresponds to the assumption about plant food typically dominating in non-industrialized populations (e.g., Ebersbach 2002, Ebersbach 2007). A sufficient number of contemporaneous domestic animals was only available for a Late Roman excavation site also included in this thesis and concentration-dependent mixture models also indicate a rather low amount of animal protein in the human diet (25% animal vs. 75% plant content, Harbeck & von Heyking 2014). Notably, even if a sufficient number of animal samples is available, it is only rarely possible to distinguish between different animal species and almost impossible to differentiate between meat and other high-protein animal-derived foods like milk or eggs.

The comparison of the isotope data from different phases of the transition from the Roman period to the Early Middle Ages suggests that in the 7th century AD there may have been a change in diet of adults and children. This change could have affected both the range of plants used and their content to animal protein, overall, potentially related to supposed changes in practices and strategies in arable farming as well as livestock and dairy farming (e.g., Fehr 2019). However, different trends in isotope ratios of considered groups and small

sample sizes, but most of all, the lack of animal reference prevents clarification of the actual causes. Nevertheless, one hypothesis best explains all observed isotopic shifts and is thus considered more likely. According to this hypothesis, the isotopic ratios of cultivated plants changed, e.g., due to more intensive land use and increased fertilization (e.g., Bogaard et al. 2007, Fraser et al. 2011). Since plants represent the primary resource of the local food net, changes in their isotopic ratios are passed on to animals; thus, the assumed shift represents a change in the baseline of human resources.

Comparing the diet of adults and infants suggests that children in the late 4th and 5th centuries had an adult-like mixed diet (e.g., Fournier et al. 2022). Whereas infants had only limited access to animal foods compared to adults but were given a more plant-based diet in the 7th century (e.g., Eerkens et al. 2011).

Alternative diets as indicators of human movement to South Bavaria

The alternative diets of individuals displayed by deviating $\delta^{13}C$ and $\delta^{15}N$ ratios that clearly differ from the "common intra-populational diet variability" of the site they were buried can be explained by the consumption of foods from other regions, most likely due to a previous stay in these regions (e.g., Cox et al. 2001, Schroeder et al. 2009).

The carried-out approach to define ranges of $\delta^{13}C$ and $\delta^{15}N$ within the population of a specific region is completely new, and therefore, ratios from outside these ranges are not directly considered as proof of the non-local origin of individuals.

Alternative diets identified in this thesis point toward the consumption of foods from more arid or marine areas (e.g., Shishlina et al. 2012, Liu 2014, Fenner 2014) but in most cases refer to the more frequent consumption of C4 plants or millet, respectively (e.g., Lightfoot et al. 2013, Guede et al. 2018). The local consumption of marine resources in South Bavaria is generally assumed to be unlikely due to the great distance from the sea. But even if some marine foods were imported, their contribution probably is too low to have a significant effect on human isotope ratios (e.g., Knipper et al. 2013). As already mentioned, frequent consumption of millet in higher amounts on site is also indicated to be unlikely (see above). Consequently, it is assumed that individuals with alternative diets likely are of non-local origin.

In principle, it is not possible to derive a specific geographical location of the region of origin of migrants based only on isotopic ratios. However, the particular constellation of different clues allows at least one assumption for the women with modified skulls (ACD). These women are assumed to originate from South-Eastern Europe. First, most of them exhibit a strong millet signal in their diet, in childhood or in adulthood. In Early Medieval times, the cultivation of millet was more common in Southern and Eastern Europe (e.g., Gyulai 2006, Gyulai 2014, Iacumin et al. 2014, Miller 2015, Hakenbeck et al. 2017, Paladin 2020). Second, many of these females also show a strong genetic resemblance to present-day South-Eastern

European populations (Veeramah et al. 2018). Third, this region for a long time belonged to the territory of the Huns and associated groups (e.g., Maenchen-Helfen 1973) whose cultural influence on these females is likely visible through the intentional modification of the skull shape (e.g., Werner 1956).

4.2 Different origins among the population of South Bavaria during the "Dark ages"

People are and have always been mobile. However, human movements not only involve overcoming spatial distances, but are complex processes guided by different agential motivations influenced by culturally specific social structures, economic and political circumstances, or environmental forces which may also change over time (Gregoricka 2021). Residential changes can be made voluntary or forced by single persons or groups over short or longer distances in single or multiple steps. While migration is defined as a long-term relocation of one or more persons who cross cultural and/or political boundaries, mobility means individual or group movement across shorter distances that typically takes place within the own cultural and/or political region (also see Gregoricka 2021).

However, proving human movement in history is generally difficult. Historical written sources that report invasion or migration must be examined for their credibility. Gaps in the archaeological record of a region must not be related to an actual decline in settlement activity, but are present because the missing finds have simply not been discovered yet. Grave goods can rarely be assigned to a specific ethnic group, and even if they are, this does not necessarily mean that the buried person has the same origin. These are just a few examples of the overall problem, which also played a role in the history of *Raetia II* during the transition from Roman to Medieval times (c.f. 1.2).

Evidence of human movement through strontium isotope analysis of human remains, as well as, indications of carbon and nitrogen analysis as part of this thesis, has made a decisive contribution to this story.

The results clearly indicate that there was increased migration to *Raetia II* in the second half of the 5th century AD, which is probably related to the retreat of the Romans and the abandonment of border security in *Raetia II*, similar to the developments in the neighboring province *Noricum* reported by Eugippius (e.g., Noll 1963, Degen 1987, Haas-Gebhard 2013).

However, there are different indications that the extent of human movement is still underestimated. For instance, the refined approach used in this work has a certain weakness, e.g., there are some indications that ranges are slightly overestimated. However, it seems more reasonable to underestimate the number of non-local individuals than taking the risk of falsely turning local inhabitants into migrants. Regardless of the approach used to determine local ranges, the relatively uniform geology of South Bavaria (e.g., Glaser 2004) and the associated low variation of local $^{87}Sr/^{86}Sr$ ranges at different sites condition that human movement within *Raetia II* remains largely invisible. Regarding the presence of alternative diets as an indicator of movement, it remains questionable whether the ranges should be defined more narrowly. But it also applies that some movement remains invisible if people move from

one region to another without significantly changing their diet due to similar ecogeographic conditions, e.g., land-locked regions within the C3 plant-based ecosystem of temperate Europe (e.g., Hakenbeck et al. 2010).

Nevertheless, the rate of detectable migration for the hinterland of *Raetia II* is higher than the migration rate of the border region, supporting the idea that rural areas were preferentially settled by newcomers derived from archaeological evidence on settlement structures (Haberstroh & Harbeck 2013, Haberstroh 2019).

Although no exact geographical region of origin can be assigned to migrants, due to the redundancy of isotope ratios, it is shown that migrants originate from different regions. There is no clear difference in numbers between female and male migrants; however, there are indications that some women originate from certain regions from which none of the male migrants originate. This especially applies to the women with modified skulls who originate from a community with a visibly different culture. However, this cannot be easily explained by previously suspected marriage migration (e.g., Hakenbeck 2009, Veeramah et al. 2018), since these women often migrated to South Bavaria only in advanced age, at least significantly above the common age of marriage but partly even past the reproductive phase.

What remains largely unclear, is how much mobility there was within the region due to the largely overlapping ranges of ^{87}Sr/^{86}Sr as well as δ^{13}C and δ^{15}N ratios of different sites within the region of *Raetia II* resulting from overall similar geological and ecogeographic conditions. However, human communities in neighboring regions within the same political territory or cultural society likely maintain lively exchange, of information, goods, as well as people.

However, independent of several movements that remained invisible, the "local" individuals probably contain a certain number of Romans who are suspected to have stayed in the region even after the administration and military had left (e.g., Losert 2003, Strömer 2007). Together with the proven migration, this is consistent with the theory that the *Baiuvarii* formed from a mixture of people who stayed in the region and incoming migrants (e.g., Christlein 1980, Fischer 1993).

4.3 Different weaning practices among the population of South Bavaria during the "Dark ages"

From a biological point of view, breastfeeding is of great importance for the health of infants. This applies in particular to historical periods, when no such good substitute for mothers milk was available as it is today. Children who were not breastfed at all or only briefly probably had a lower chance of survival (e.g., Katzenberg et al. 1996, Dittmann & Grupe 2000). However, breastmilk alone can also not fully meet the needs of infants from a certain point of time (e.g., Kramer & Kakuma 2004, Jay 2009, Pérez-Escamilla et al. 2019). Thus, complementary feeding becomes necessary, which at the same time initiates the weaning process. The abrupt or gradual replacement of hygiene, nutritious and immunizing breastmilk by solid foods and non-breastmilk liquids can be stressful for infants due to a possible resulting nutrient deficiency and increased contact with pathogens in parallel with the loss of immune protection (e.g., Humphrey et al. 2008, Black et al. 2008).

Both breastfeeding and weaning are flexible behavioral patterns. Therefore, practices can be adapted to the infant's health and acute needs, the availability of resources (probably dependent on the season), but can also be affected by cultural and social aspects possibly related to individuals sex or status, etc. (e.g., Bourbou & Garvie-Lok 2009, Bourbou et al. 2013, Bourbou et al. 2019). In general, infant feeding practices can then and now differ significantly within and across communities.

The carried-out analysis of carbon (C) and nitrogen (N) isotopes from serial samples of first molar dentine provides insights into different weaning patterns of individuals who were buried in *Raetia II* but lived there during different time phases of the transition from Late Roman to Early Medieval times or originate from other communities. The parallel analysis of LEH significantly contributes to the recent discussion of "weaning stress" (Publication II).

The collection of individual profiles displaying changes of $\delta^{13}C$ and $\delta^{15}N$ rations within the first ten years of life, approximate weaning ages derived from $\delta^{15}N$ weaning dip, and the occurrence of LEH as a marker of physiological stress show great variability. In particular, the highly variable courses and levels of isotope ratios remain inconclusive in some respect. Despite some resulting uncertainties, it was nonetheless possible to draw conclusions about the period of weaning and the possible associated stress.

Weaning ages

The determination of weaning ages is difficult, regardless of the approach used. The approach of this study also has perceived weaknesses, e.g., for strong fluctuations. However, it offers the advantage of presenting results as age ranges, allowing for a better illustration of the uncertainty of weaning ages due to the biological variability of tooth development.

Overall, the potential local population of *Raetia II* shows quite similar weaning ages despite an overall high variability. The end of weaning around the middle of the third year corresponds to the textual recommendations of the historical physician Soranus and the results of previous isotope research from the Roman period and the Early Middle Ages in Bavaria and other regions in Continental Europe (e.g., Dittmann & Grupe 2000, Haydock et al. 2013, Herrscher 2003, Fulminante 2015). Also many migrants who exhibit similar weaning ages may come from a community of the same cultural environment.

However, most of the women with modified skulls (ACD) included were obviously weaned longer than until the third year. This could be explained by their growing up in a society that had a nomadic and/or pastoralist lifestyle, which is suspected of the Huns and associated communities (review in Hakenbeck 2009) of the Eurasian Steppe from which they potentially originate and for whom longer weaning times are suspected (e.g., Clayton et al. 2006, Waters-Rist et al. 2011). Nevertheless, rationales are still debated, ranging from a lack of appropriate weaning foods and the distribution of subsistence activities and workload to fertility control (e.g., Sellen & Smay 2001, Fouts et al. 2005, Sellen 2007, Bocquet-Appel 2011).

"Weaning stress"

The results on the occurrence of stress throughout the period of weaning support the assumption that children were at increased risk once breastfeeding had ceased completely (e.g., Fernández-Crespo et al. 2022), therefore, "weaning stress" can better be called "post-weaning stress". It also shows that the weaning process does not represent an intrinsic stress factor for infants (e.g., Kendall et al. 2021), but also that continued breastfeeding may not fully protect from all stressors.

Although the assumption that children had a more plant-based low-trophic post-weaning diet in the 7th century is not proven (c.f. 4.1), this is also supported by the fact that they experienced more stress. A low-trophic post-weaning diet may not be sufficient to meet the needs of these children, especially during growth phases (e.g., Henderson et al. 2022), and may also make them more susceptible to external stressors. However, it may also be applicable that the external stress level was generally increased while these individuals grew up.

However, the analysis of osteological stress markers such as the considered linear enamel defects (LEH) is particularly affected by some interpretative difficulties generally stemming from the fact that a collection of individuals buried together do not reflect the experience of a living population, referred to as the "osteological paradox" (e.g., Wood et al. 1992, Milner & Boldsen 2018). The formation of LEH requires that the infants survived the causal stress event at least for some time. In this thesis, only individuals who survived their childhood and grew into adults are examined. Thus, it can also apply that the youngest part of the living population has been exposed to much higher stress, causing some of them to die

earlier (whereby the stress level can also fluctuate over time). However, these early deceased children are not included in this thesis and, more generally, can hardly be included in studies of LEH. Overall, this could rotate the entire image. Similar interpretative restrictions also apply to determined weaning ages. It remains unclear how long infants who died earlier were breastfed. This underscores the need for additional data on subadults and adults from the same graveyards to get a more detailed picture of infant diet and associated stress in Late Roman and Early Medieval Europe.

Conclusion

The findings of this thesis emphasize that stable isotope analyses provide extensive conclusions about past human life histories of single individuals and entire communities. In particular, the parallel analysis of heavy and light elements significantly extends the informative value of the respective analyses.

It could be shown that migrants increasingly entered the region that was once *Raetia II* after the fall of the Roman Empire. Incoming men and women originate from multiple divergent regions of various geographical locations and different cultural influence. In particular, some non-local women probably originate from South-Eastern Europe, where communities more frequently used millet and potentially had a nomadic lifestyle more oriented towards pastoralism. Whereas supposedly sedentary communities living in early medieval South Bavaria subsisted on a mixture of different types of C3 plants and terrestrial animals.

The different weaning practices observed are likely also related to different lifestyles of communities. While most individuals were weaned before the age of three, women originating from nomadic and/or pastoral communities were breastfed even longer. Furthermore, the investigation of "weaning stress" indicates that infants are only exposed to an increased risk when they are no longer breastfed or hardly breastfed at all.

Some assumptions that could not be clarified based on the current data situation, such as a chronological change of economic practices, offer good starting points for further research.

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Acknowledgments

Appendix

Supplementary Material of Publication I

The Supplementary Material of Publication I consisting of **S1 Data set**, **S2 Detailed methods**, and **S3 Statistics and detailed results** is freely available at the following link: https://doi.org/10.1371/journal.pone.0283243.

Supplementary Material of Publication II

The Supplementary Material of Publication II consisting of **S1 Detailed methods and results**, and **S2 Data set** is freely available at the following link: https://doi.org/10.1007/s12520-023-01882-6

List of additional contributions

Peer-reviewed publications

Toncala A, Trautmann B, **Velte M**, Kropf E, McGlynn G, Peters J, Harbeck M. On the premises of mixing models to define local bioavailable $^{87}Sr/^{86}Sr$ ranges in archaeological contexts. Sci Total Environ. 2020; 745:140902.

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Poster & presentation

Velte M, Grigat A, Neidich D, Czermak A, Harbeck M. Patterns of Linear enamel hypoplasia and Weaning. 23rd Paleopathology Association (PPA) European Meeting in Vilnius, Lithuania 2022.

Presentations

Velte M, Grigat A, Harbeck M. Auf den Zahn gefühlt: Stillpraxis im frühmittelalterlichen Erding – neue Ergebnisse aus dem Reihengräberfeld Altenerding-Klettham. 6th Archaeological Summer Symposium of the Archaeoloical Society Erding (AVE) at the Museum Erding, Germany 2019.

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