Journal of Archaeological Science 188 (2026) 106521

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: www.elsevier.com/locate/jas

Documenting the extent of intra-bone isotopic variation

Olivia Hall [a,*], Alexandra A.Y. Derian [b], Alexis Rausch [a], Julia McCuaig [a], Paul Szpak [a]

[a] Department of Anthropology, Trent University, 1600 West Bank Drive, Peterborough, K9L 0G2, Ontario, Canada
[b] Environmental and Life Sciences Graduate Program, Trent University, 1600 West Bank Drive, Peterborough, K9L 0G2, Ontario, Canada

ABSTRACT

Bone collagen is a common material used for stable isotope analysis in archaeology, palaeontology, and ecology to generate insights about an individual's life history. The extent of intra-bone isotopic variation has generally been assumed to be small, and bone sample size is minimized to avoid unnecessary sample destruction. In this study, we extracted hundreds of subsamples ($n = 1226$) from various regions of the midshaft of two kangaroo tibiae and two cattle radii to systematically assess the extent of isotopic variation in bone collagen δ^{13}C and δ^{15}N within a single skeletal element. We found that within a single element, δ^{13}C varied by up to 10.6 ‰ and δ^{15}N by up to 3.6 ‰. Isotopic variation within an element likely reflects the range of isotopic compositions of foods that were regularly consumed throughout life, with variation in remodeling rates throughout the bone driving intra-element isotopic variation. As bone sample size decreases, the probability of obtaining an isotopic composition that differs significantly from the lifetime average increases. Rather than conceptualizing stable isotope measurements from bone collagen as being akin to those derived from tissues such as muscle, liver, or skin, they may be better understood as being similar to sequentially sampled hair or tooth samples that have lost their specific temporal information.

1. Introduction

Stable isotope analysis is often used to reconstruct the past diet and mobility patterns at the level of populations and individuals. Soft tissues typically degrade and disappear over time, and so bone collagen – which can be preserved in the archaeological record for over 100,000 years – is the most common tissue analyzed in archaeological and palaeontological contexts (Jones et al., 2001). The period of an individual's life that is assessed through stable isotope analysis is mediated by the turnover rate of the tissue sampled (Vander Zanden et al., 2015). Bone is a dynamic tissue, meaning that it is constantly remodeling and replacing itself (Hill, 1998), making collagen homogeneity across an entire skeletal element unlikely. Bone remodeling occurs at the cellular level; osteoclasts break down old collagen and osteoblasts build new collagen by incorporating new molecules (and their constituent atoms/isotopes, for carbon, nitrogen, and sulfur) introduced through diet and through some old tissue recycling. The rate at which this process occurs across the entire bone is referred to as the 'bone turnover rate'. Since different regions of a bone experience varying demands, such as differences in mechanical loading, it is unlikely that osteoclastic and osteoblastic activity—and consequently isotopic composition—will be uniform across an entire skeletal element. Within a single element, there are variations in thickness, type of bone tissue, and muscle attachments, all of which can influence the rate at which any area may turn over (Hill, 1998; Huiskes et al., 2000; Montoya-Sanhueza and Chinsamy, 2017). Within the context of selecting a sample of bone for isotopic analysis, significant variation in turnover rates across the element can bias the results. Samples could be biased to reflect a more recent dietary history (if turnover is faster in the sampled area), the long-term average diet of the individual (if turnover is slower in the sampled area), or a diet characteristic of a period earlier in life (if turnover was negligible in the sampled area). Without knowing the specific turnover rate of the sampled region, it is unclear which period of the individual's life the sample represents, making comparisons to other individuals difficult and potentially leading to misinterpretations of dietary, mobility, or other life history patterns.

To understand the potential scale of stable isotope variation within an individual, it is important to evaluate existing knowledge about inter-element variation. Multiple studies have used various archaeological human skeletal elements to assess intra-skeletal differences. Fahy et al., (2017) examined multiple long bones as well as ribs, clavicles, vertebrae, and other smaller bones and found the maximum variation within a skeleton to be 0.9 ‰ for δ^{13}C and 1.7 ‰, for δ^{15}N. Dauven et al. (2017) looked at a range of archaeological elements, including most long bones, the ilium, rib, vertebra, and some cranial bones, finding variation of up to 1.10 ‰ for δ^{13}C and 1.17 ‰ for δ^{15}N. Valenzuela et al. (2025) found maximum inter-element differences of 3.7 ‰ for δ^{13}C (vertebra and femur) and 1.9 ‰ for δ^{15}N (vertebra and parietal/occipital). Initially, we

---

* Corresponding author.
E-mail addresses: oliviahall@trentu.ca (O. Hall), alexderian@trentu.ca (A.A.Y. Derian), alexisrausch@trentu.ca (A. Rausch), juliamccuaig@trentu.ca (J. McCuaig), paulszpak@trentu.ca (P. Szpak).

https://doi.org/10.1016/j.jas.2026.106521
Received 9 October 2024; Received in revised form 18 February 2026; Accepted 20 February 2026

expected that the isotope variation between skeletal elements should exceed the possible intra-element variation, as the structural and functional differences among separate skeletal elements are likely greater than the differences observed within any one bone.

Previous research has shown inconsistent evidence for intra-bone isotopic variation. Past studies have not sampled at a fine enough spatial resolution to accurately assess the degree of isotopic variation, nor have they sampled individuals or species with sufficient dietary variation that would reflect these differences in the isotopic composition of the collagen. Kontopoulos et al. (2022) sampled multiple sites from multiple human tibiae, humeri, radii, and femora and found only one significant difference in δ^{13}C and δ^{15}N between sample sites in all bones. Sykut et al. (2020) assessed the isotopic variability within red deer (*Cervus elaphus*) mandibles and found that the highest difference between sampled sites was 0.83 ‰ for δ^{13}C and 0.72 ‰ for δ^{15}N. A similar pattern was found by Hyland et al. (2022), where three samples each were taken from a harp seal (*Pagophilus groenlandicus*) humerus, and the average Euclidean distance (the length of the line between two points in bivariate space) within an element was 0.63 ‰; the greatest Euclidean distance between two samples within a single element was 1.25 ‰.These small intra-bone differences in both δ^{13}C and δ^{15}N were also observed in a study conducted on human subadult femora (maximum difference 0.2 ‰; Hedges et al., 2007). In many of these studies, the minimal variability in stable isotope composition was attributed to a consistent diet throughout the individual's life.

Other studies have found a larger degree of isotopic variation within a bone. Matsubayashi and Tayasu (2019), found a maximum difference of 1.7 ‰ in δ^{13}C (incrementally-sampled sika deer [*Cervus nippon*] femur) and 2.2 ‰ in δ^{15}N (incrementally-sampled brown bear [*Ursus arctos*] femur). They suggested that midcortical and pericortical sections of bone remodel minimally, or do not remodel, and perimedullary bone significantly remodels. They also note that trabecular bone in mammals displayed the most isotopic variability. Generally, it is accepted that trabecular bone turns over faster than cortical bone and therefore it is expected that isotopic analyses incorporating trabecular and cortical bone samples have more potential to capture isotopic variability within the life of an individual than pairs of cortical bone samples (Hedges et al., 2007; Manolagas, 2000). De Gruchy et al. (2024) sampled the anterior midshaft of a human femur and sampled from the periosteal, midcortical, and endosteal sections, finding an absolute difference of 1.7 ‰ for δ^{13}C and 2.9 ‰ for δ^{15}N. They also noted that the greatest variability for δ^{13}C was found in the periosteal region and the greatest range for δ^{15}N was found in the endosteal, with the periosteal most likely reflecting the oldest region of bone. Matsubayashi et al. (2025) found maximum variations of 2.4 ‰ and 1.7 ‰ in δ^{13}C and δ^{15}N, respectively, across multiple segments of one bear humerus. Overall, few studies have observed intra-element differences of more that ∼2.5 ‰ in δ^{13}C and ∼3 ‰ for δ^{15}N in bone collagen.

There is, therefore, conflicting evidence with respect to the amount of isotopic variation that can exist within a single skeletal element. Researchers have, however, generally held the assumption that isotopic variation within a skeleton is not a major concern. This assumption stems from the work of DeNiro and Schoeninger (1983), who found that individuals raised on monotonous diets had a maximum range of 1.4 ‰ for δ^{15}N and even less for δ^{13}C throughout their whole skeleton, implying that isotopic variation within a single element should be comparable to or less than these values. While there has been some consideration of the potential magnitude of intraskeletal isotopic variability, this issue has not yet been thoroughly investigated.

We hypothesized that the amount of isotopic variation that is typical within a single bone has been underestimated because.

1. Past investigations attempting to quantify intra-element isotopic variability have not sampled elements at a high enough resolution and/or,
2. Past studies have used bones that have little capacity to display isotopic variability.

The goal of this project was to use stable isotope analysis of modern kangaroo (*Macropus/Osphranter* sp.) and cattle (*Bos taurus*) skeletal remains to investigate the degree of isotopic variability that exists within a skeletal element. Our objectives were to.

1. Characterize the stable carbon and nitrogen isotope composition of kangaroo and cattle long bones through exhaustive intra-element sampling; and
2. Quantitatively assess the magnitude and distribution of isotopic variability within a mammalian long bone at multiple analytical scales.

This research contributes to the understanding of intra-bone isotopic variability and has important implications for sample selection procedures for future stable isotope research involving bones.

2. Methods

2.1. Sample selection

Two cattle radii (one left and one right) and two left kangaroo tibiae (representing four individuals) were analyzed. The two cattle are denoted as Cattle 1 and Cattle 2 (lab ID numbers TEAL 11516 and TEAL 12092, respectively) and the kangaroo are denoted as Kangaroo 1 and Kangaroo 2 (lab numbers TEAL 12989 and TEAL 12996, respectively). All individuals sampled were adults. The distal radius of cattle fuses by age 3.5–4 years, and both distal radii in this study were completely fused (Gilbert and Steinfeld, 1977). The timing of kangaroo epiphyseal fusion is not well understood, but the tibiae sampled in this study were fully fused (Mein et al., 2022). While we can be confident that all individuals were adults, we do not know their precise age at death, as is the case in nearly all studies of post-cranial animal remains. To quantify isotopic variation within a skeletal element, we chose samples from a wild biped (kangaroo) and domestic quadruped (cattle) species. These represented a broad spectrum of behavioral and dietary ecologies, which we expected to be characterized by different levels of intra-bone isotopic variation. The bones are modern (i.e., the animals died in the past ∼ five years) and were obtained from a pet supply store in Canada. The manufacturer of the kangaroo bones indicated that they are free from preservatives and added chemicals, and are dried using low heat. Because the samples used in this study are derived from modern bones, this rules out the possibility that intra-bone isotopic variability is due to degradation or contamination of collagen in the burial environment. The isotopic and elemental compositions of a total of 1226 bone collagen samples from these four individuals were analyzed in this study.

We considered the possibility that the assimilation of food isotopic composition into consumer tissues may occur in a non-uniform manner, and therefore we propose a conceptual model for the factors that could result in different levels of intra-bone isotopic variation based on different collagen turnover properties (Fig. 1).

Kangaroos have variable diets that change in response to season and plant growing conditions. They mainly consume a mixture of C_3 and C_4 grasses, and the relative abundance of different grasses is variable depending on seasonal and geographic availability of different plants (Murphy et al., 2007). Because the relative abundance of C_3 to C_4 plants consumed varies at different times of the year, it is possible that these changes would be recorded isotopically within bones. We are not able to reconstruct a life history for the kangaroos in this study as their specific geographic origins, date of birth, and date of death are all unknown.

Some cattle also experience dietary changes that could be detected in their stable isotope composition. For example, cattle raised for meat are sometimes fed a diet of C_3 grasses throughout their lives, and then switched to a C_4-based diet (i.e., maize) for a brief period prior to death

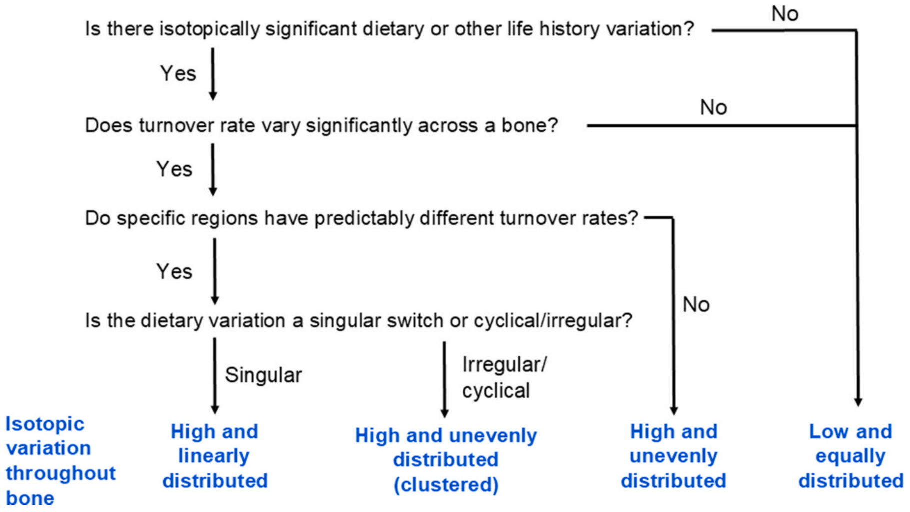

Fig. 1. Conceptual model of the factors driving intra-bone isotopic variation. Note that the model does not account for isotopic variation that is the product of post-burial alteration or contamination.

(Bahar et al., 2005; Williams et al., 1983). Grass-finishing is sometimes used after midlife bulking periods, during which cattle are fed corn-based silage, before they are ultimately slaughtered for beef (Van Elswyk and McNeill, 2014). Dairy cows are sometimes fed a diet that includes grass, grain, soybeans, and oilseeds, and experience short (~60 days) periods of restricted nutrient intake called "drying off" (Erickson and Kalscheur, 2020; Franchi et al., 2019). Because some of the foods often fed to cattle are isotopically distinct from one another, it is possible that changes from one type of feed to another may be reflected in the isotopic compositions of their bone collagen. In the case of a singular change from C_3 grasses to maize, it may be possible to suggest areas of bone that reflect the diet closer to the animal's death. Regions of bone with isotopic compositions that indicate a higher concentration of C_4 plant consumption could be associated with later life diets and indicate more recent replacement of bone. In the case that the cattle experienced more varied foods, with maize or soybeans being introduced at some points but other grains still being used, isotopic variation within a bone could still be detected, but would be more random and less indicative of specific feeding practices closer to death (Fig. 1). There are no life history data available for the cattle included in our study, so feeding

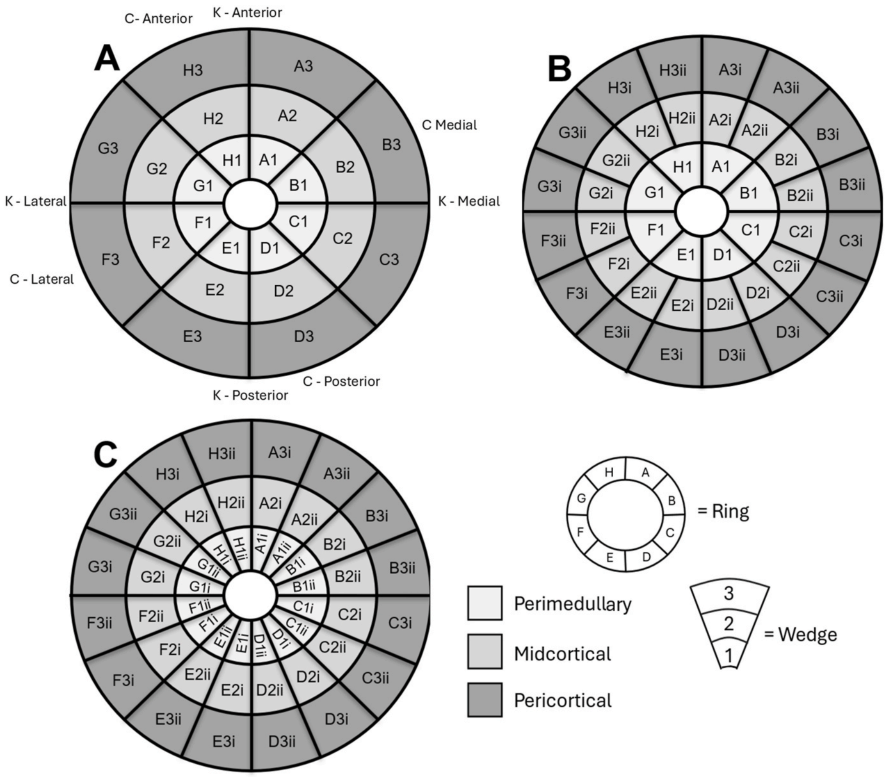

Fig. 2. Visualization of the sampling strategy used to divide the donuts into separate samples. A) Sampling strategy for cross sections with 24 subsamples, with indication of bone direction as they relate to the wedge placements across all donuts of the same species. K is for the kangaroo bones and C is for the cattle bones. B) Sampling strategy for the kangaroo cross sections with 40 subsections C) Sampling strategy for cattle cross sections with 48 subsections. Descriptive terms used throughout the text for the various subsamples are also included in the bottom right of the figure.

regimes cannot be used to link specific bone regions to early versus late dietary inputs, from which relative turnover rates might otherwise be inferred. However, potential shifts between isotopically distinct diets offer a plausible, though non-directional, explanation for the intra-skeletal isotopic variation observed.

2.2. *Sample preparation*

The exterior surface of each bone sampling location was cleaned through manual abrasion with an NSK Ultimate XL-D rotary tool equipped with a 918 PB.11.220 HP medium flexible perforated double-sided diamond disc (Brasseler Canada). Epiphysis samples of 200–300 mg were removed from the most anterior, posterior, medial, and lateral edges of the distal surface for cattle and proximal surface for kangaroos using the dental drill and cutting wheel described above. Diaphyses were cut perpendicular to the cortical surface into 1 cm cross-sections (these cross-sections are termed "donuts" here, Fig. 2) using the dental drill. Cattle 1 had 9 donuts sampled from the mid-section of the diaphysis, Cattle 2 had 7, Kangaroo 1 had 11, and Kangaroo 2 had 18. Each of these donuts were cut into eight wedges of roughly equal size (A–H) (Fig. 2). Standardizing these wedges to an equal size was not possible due to irregularities in bone shape (Fig. 3). The wedges were each subdivided into 3 samples from the medullary cavity to the periosteum (1, 2, 3; Fig. 2). Of the 45 donuts included in this study, 38 were divided into 24 total samples, four into 40 samples, and four into 48 samples (Fig. 2). We refer to the eight donuts cut into 40 and 48 sections as "double cut" (Fig. 2c). The double cut donuts were used to assess whether variability in intracortical and pericortical samples was being obscured by greater sample mass (i.e., because the perimedullary pieces of the wedges in Fig. 2a are smaller than the pericortical pieces). This sampling strategy allowed us to investigate whether there was variability in the stable isotope composition among different regions of the bone (around the circumference of the diaphysis, or among the perimedullary, intracortical, and pericortical sections).

To accurately quantify isotopic variation in bone collagen, lipids must be completely removed as their presence, especially when variable, will artificially inflate the amount of isotopic variation within a skeletal element (Guiry and Szpak, 2020; Post et al., 2007). To extract lipids, the full donuts were submerged in 50 mL beakers that contained ~40 mL of 2:1 chloroform:methanol. The donuts were sonicated for 3 h with the chloroform:methanol solution being refreshed each hour. The chloroform:methanol solution was discarded, and the donuts were left in a fume hood for 24 h while residual solution evaporated. Samples were then demineralized in approximately 40 mL of 1 M HCl for 5–8 days at room temperature until they could be cut with a single edge steel razor blade (009"/0.23 mm). The kangaroo samples were demineralized as whole donuts and then cut into subsections. Due to the greater bone volume relative to surface area compared to the kangaroo donuts, the cattle samples were cut into eight (A–H) wedges prior to demineralization, and further divided after demineralization. Following demineralization, the samples were rinsed with 9 mL of Type I water, and then refluxed in 3.5 mL of 0.01 M HCl at 60 °C for 36 h. The refluxed collagen was centrifuged to separate the solution containing the collagen from any residual solids, transferred to 4 mL glass vials, frozen, and then lyophilized for 48 h.

The quality control criteria recommended by Guiry and Szpak (2020) were used to assess lipid contamination in the modern collagen samples. The criteria in this paper are highly conservative, as the authors suggest excluding any collagen extracts with $\mathrm{C{:}N_{atomic}} > 3.30$ due to the variable presence of lipids. Preliminary data showed that many $\mathrm{C{:}N_{atomic}}$ ratios were >3.30, suggesting the presence of variable amounts of lipids, which would have the effect of artificially increasing the amount of variation in δ^{13}C observed. An additional lipid extraction procedure adapted from Bligh and Dyer (1959) was performed on each of the samples in order to ensure that there were no lipids present.

For this additional lipid extraction step, lyophilized collagen was rehydrated with 1.6 mL of 70 °C Type I water, and combined with 6 mL of 2:1 chloroform:methanol. The solution was sonicated for 1 h, and then centrifuged at 2500 rpm for 30 min. The solution separated itself into three distinct layers: a chloroform-methanol layer on the bottom, a thin layer of solid material (lipids), and a layer of collagen, water, and methanol on top. The top layer was pipetted away and deposited into a separate tube. The solution containing the collagen was again combined with 2:1 chloroform:methanol, sonicated, centrifuged, and the top layer was added to the tubes already containing the water, collagen, and methanol solution. The tubes containing the solution were heated at 60 °C overnight until the methanol had evaporated and the amount of liquid left in each tube was ~1.6 mL (containing only the water and collagen). The samples were frozen for ~24 h and then lyophilized for 48 h. Finally, 550 ± 50 μm of lyophilized collagen was weighed into tin caps for analysis. δ^{13}C, δ^{15}N, and elemental compositions were determined with a Nu Horizon Isotope Ratio Mass Spectrometer (IRMS) coupled to a EuroVector 3300 elemental analyzer (EA) at the Water Quality Center at Trent University.

The δ^{13}C and δ^{15}N values were calibrated relative to Vienna Pee Dee Belemnite (VPDB) and Atmospheric Inhalable Reservoir (AIR), respectively, with a three point calibration curve using USGS40 ($\delta^{13}\mathrm{C} = -26.39 \pm 0.04$ ‰, $\delta^{15}\mathrm{N} = -4.52 \pm 0.04$ ‰), USGS63 ($\delta^{13}\mathrm{C} = -1.17 \pm 0.04$ ‰, $\delta^{15}\mathrm{N} = +37.83 \pm 0.06$ ‰), and USGS66 ($\delta^{13}\mathrm{C} = -0.67 \pm 0.04$ ‰, $\delta^{15}\mathrm{N} = +40.83 \pm 0.06$ ‰) (Qi et al., 2003; Schimmelmann et al., 2016). In-house quality control reference materials were interspersed regularly among the samples to determine accuracy and precision of the isotopic measurements: caribou bone collagen (SRM-1, $\delta^{13}\mathrm{C} = -19.41 \pm 0.11$ ‰, $\delta^{15}\mathrm{N} = 1.89 \pm 0.16$ ‰), polar bear bone collagen (SRM-14, $\delta^{13}\mathrm{C} = -13.62 \pm 0.09$ ‰, $\delta^{15}\mathrm{N} = 21.50 \pm 0.22$ ‰), commercial fish collagen (SRM-26, $\delta^{13}\mathrm{C} = -16.17 \pm 0.10$ ‰, $\delta^{15}\mathrm{N} = 14.70 \pm 0.17$ ‰) and alanine (SRM-28, $\delta^{13}\mathrm{C}\ -16.27 \pm 0.10$ ‰, $\delta^{15}\mathrm{N} = -1.91 \pm 0.18$ ‰). Precision, or *u(Rw)* was calculated to be ±0.13 ‰ for δ^{13}C and ±0.23 ‰ for δ^{15}N. This determination was derived from replicates of calibration standards, check standards, and sample duplicates. The systematic error, or *u(bias)* was calculated at ±0.08 ‰ for δ^{13}C and ±0.21 ‰ for δ^{15}N by referencing the observed compared to the known values of the standards. The overall analytical uncertainty was determined to be ±0.15 ‰ for δ^{13}C and ±0.31 ‰ for δ^{15}N (Szpak, 2026).

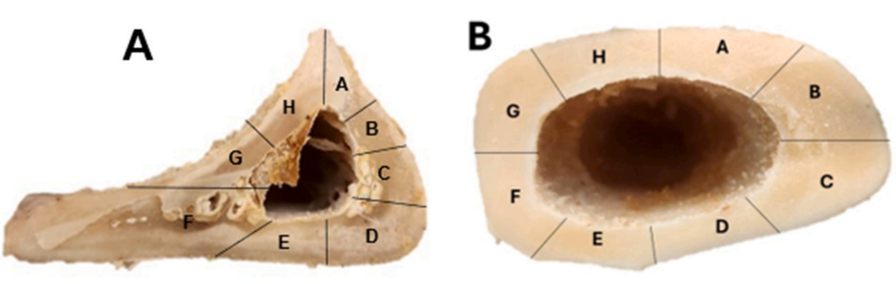

Fig. 3. Images of a kangaroo (A) and cattle donut (B) with the associated region that each wedge would cover indicated in the cross-section. These photos highlight the imperfect nature of the circular donut as skeletal features make each wedge slightly different sizes.

A traditional sample start mass could not be calculated for these samples because the donuts were split after demineralization. When samples were divided, care was taken to make each sample visually equal in size within a wedge. To determine the weighted average δ^{13}C and δ^{15}N values for the entire skeletal element, we calculated the weighted average for all individual subsamples across all donuts. To ensure that any isotopic variation did not reflect variable lipid contamination, we prioritized purifying the collagen through a rigorous lipid extraction that caused variable levels of sample loss. Rather than base the weighted average on subsample collagen masses that were variably impacted by sampling handling rather than starting mass, we applied the same weighting for the perimedullary, micortical, and pericortical segments. The weight for regular-cut segments was 1.00 for all perimedullary samples, 1.59 (cow) and 1.17 (kangaroo) for midcortical samples, and 1.81 (cow) and 1.71 (kangaroo), reflecting the relative difference in size between samples from the various layers. Double-cut samples were further weighted by 0.5. The weighted average for each bone (x) was calculated according to Equation (1) where x_i refers to the isotopic composition of a single sample and w_i refers to the weight.

$$x = \frac{\sum_{i=1}^{n} w_i x_i}{\sum_{i=1}^{n} w_i} \qquad \text{Equation 1}$$

Samples were grouped by ring and wedge to assess spatial patterns in isotopic compositions or to quantify the extent of isotopic variation within bone. When making comparisons among groups, they were tested first for normality using a Shapiro Wilk test, and if distributions were not normal, a Mann-Whitney *U* test was conducted. If distributions were normal, an ANOVA test was performed, with homogeneity of variances assessed with a Levene's test. If the variance was unequal, a Dunn's *post hoc* test was preformed and if the variance was equal, a *post hoc* Tukey's HSD test was applied.

3. Results

The carbon and nitrogen elemental and isotopic compositions from each subsection of bone collagen are presented in supplement A and summarized in Table 1. The isotopic compositions for each individual are summarized according to each ring (as described in Fig. 2) in Table 2. Each skeletal element was characterized by a wide range in both the δ^{13}C and δ^{15}N values (Fig. 4). The bone with the highest range in δ^{13}C was from Cattle 2, with a range of 10.6 ‰, spanning from −23.0 ‰ to −12.3 ‰. The highest range in δ^{15}N was seen in Kangaroo 1, with a range of 3.6 ‰. Cattle 1 had the lowest range for both δ^{13}C and δ^{15}N, with ranges of 2.9 ‰ and 2.2 ‰, respectively.

Some of the skeletal elements exhibited high isotopic variability, even in samples that were directly adjacent to one another (Figs. 5 and 6). In Cattle 2, for example, adjacent bone collagen samples differed from one another by as much as 9.2 ‰ (20080 B2 and 20081 B2) for δ^{13}C and 2.2 ‰ for δ^{15}N (20082 A2 and B2). The pericortical ring of Cattle 2 had δ^{13}C values that were consistently higher than the mean for the overall bone, and δ^{15}N values that were either much higher or much lower than the whole bone.

The probability of any one sample's isotopic composition differing from the mean for the entire skeletal element varied widely among the four individuals that we sampled (Tables 3 and 4). For the skeletal element with the lowest amount of isotopic variation (Cattle 1), the probability of sampling an increment that had a δ^{13}C and δ^{15}N value that was >1 ‰ different from the overall mean was only 7% and 2%. Conversely, for the skeletal element with the most isotopic variation (Cattle 2) the probability of any one sample being >1 ‰ different from the overall mean was 79% for δ^{13}C and 23% for δ^{15}N; the value of 1 ‰ is noteworthy as this is often taken to be a meaningful isotopic difference in isotopic analyses of vertebrate tissues (Hyland et al., 2022; Berg et al., 2022). The level of intra-bone isotopic variation observed in this study far exceeds what has traditionally been assumed to exist.

There were no consistent patterns in the amount of isotopic variation across the rings (pericortical-midcortical-perimedullary) for these four individual skeletal elements (Supplement C). Depending on the individual skeletal element, the isotopic variation could have been highest within any of the three rings. For those individuals that displayed the highest amount of overall isotopic variation (δ^{13}C for Kangaroo 1, Kangaroo 2, and Cattle 2), the pericortical ring was generally characterized by less isotopic variation than either of the other two rings. The pericortical area of mammalian long bones may therefore be the best region to sample for consistent results across individuals. Differences in isotopic variation among wedges (A–H) could be due to morphological features of bone causing irregularities in the sizes of wedges (Fig. 3). This was most prevalent in wedge F within the kangaroo donuts, as it encapsulated the protuberance of the bone, leading to a larger overall wedge. This difference in size did not lead to any consistent differences in the isotopic variability of the F wedge (i.e., having consistently the lowest or highest isotopic variability) for the kangaroo bones (Supplement C, box E-H).

There were no consistent longitudinal (i.e., samples of the same letter) patterns of isotopic variation within the skeletal elements sampled. In some cases, there were relatively large differences in δ^{13}C or δ^{15}N moving from the proximal to distal ends, but in others there was negligible variation along this axis. For example, in Cattle 1, the two most proximal donuts had δ^{15}N values that were consistently below the weighted average for the bone, while the most distal donuts were either similar to the skeletal element mean or slightly above it. Other elements had more distal-proximal variability, with Kangaroo 2 having δ^{13}C values that were lower than the weighted mean for the 5th and 4th most proximal donuts, but the 2nd and most proximal donuts having higher δ^{13}C than the weighted mean. In Cattle 2 sample site G2 always had relatively low δ^{15}N and high δ^{13}C values whereas B3, E3 and G3 always had relatively low δ^{13}C and high δ^{15}N values. Overall, however, no proximal-distal pattern was consistent enough to confidently explain these trends or link them to specific bone features. Regions that shared similar values along the length of the skeletal elements are likely due to muscle insertions at different areas along the bone. Since no specific muscle attachment sites were identified prior to sampling, it is not possible to link the observed isotopic variation to these features. However, future research should investigate how muscle attachments may influence bone turnover rates at specific regions and contribute to isotopic variation within skeletal elements overall.

Similar degrees of isotopic variation were observed within the entire bone using both a "double cut" and standard technique. These smaller sized samples (for the “double cut”) still showed high amounts of isotopic variation in adjacent chunks only a few mm away from one

Table 1
Summarized δ^{13}C and δ^{15}N data across the four individuals analyzed. N indicates the number of subsections analyzed for a given skeletal element.

Individual	Taxon	Skeletal Element	# of donuts	N	δ^{13}C (‰, Mean ± 1σ)	δ^{13}C (‰, Range)	δ^{15}N (‰, Mean ± 1σ)	δ^{15}N (‰, Range)
Cattle 1	Cattle	Radius	7[a]	210	−22.5 ± 0.5	2.9	+5.5 ± 0.3	2.2
Cattle 2	Cattle	Radius	9	264	−18.5 ± 2.9	10.6	+5.7 ± 0.8	3.2
Kangaroo 1	Kangaroo	Tibia	11	274	−17.0 ± 1.1	7.3	+11.7 ± 0.3	2.4
Kangaroo 2	Kangaroo	Tibia	18[a]	481	−16.5 ± 1.1	6.9	+9.3 ± 0.6	3.6

[a] Including 4 double cut donuts.

Table 2
Summarized $\delta^{13}C$ and $\delta^{15}N$ for ring data across the four individuals analyzed.

Individual	$\delta^{13}C$ (‰, Mean ± 1σ)			$\delta^{15}N$ (‰, Mean ±1σ)		
	Perimedullary	Midcortical	Pericortical	Perimedullary	Midcortical	Pericortical
Cattle 1	−22.5 ± 0.1	−22.2 ± 0.3	−22.8 ± 0.2	+5.6 ± 0.1	+5.5 ± 0.1	+5.4 ± 0.1
Cattle 2	−17.5 ± 0.7	−16.9 ± 1.8	−21.3 ± 0.5	+5.4 ± 0.1	+5.2 ± 0.5	+6.5 ± 0.2
Kangaroo 1	−17.1 ± 0.9	−16.8 ± 0.5	−17.0 ± 0.3	+11.8 ± 0.3	+11.6 ± 0.2	+11.7 ± 0.2
Kangaroo 2	−16.5 ± 0.3	−16.7 ± 0.7	−16.5 ± 0.4	+9.5 ± 0.4	+9.2 ± 0.2	+9.2 ± 0.1

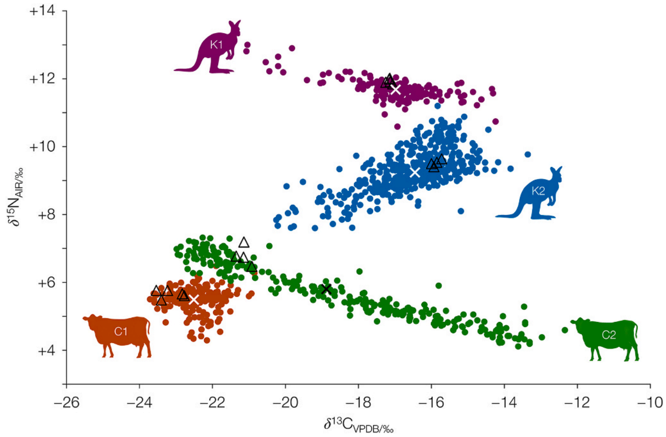

Fig. 4. Bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ of all bone collagen samples for the four individuals included in this study ($N = 1226$). Bone mean and epiphysis isotopic compositions are indicated with × and Δ, respectively.

another. Kangaroo 2 and Cattle 2 had multiple subsections that were divided in two, and both were analyzed. The maximum difference in $\delta^{13}C$ between the two samples of the divided chunk from Cattle 2 was 7.2 ‰ (samples 20079s C1i and C1ii), while in $\delta^{15}N$, it was 2.0 ‰ (samples 20081s C1i and C1ii). This represents about two-thirds of the maximum difference observed in this individual, an especially noteworthy pattern considering these samples are adjacent (separated by a few mm). For Kangaroo 2, the maximum difference between the two samples was 3.4 ‰ in $\delta^{13}C$ and 2.2 ‰ in $\delta^{15}N$ (both from samples 19423s D2i and D2ii). This roughly accounts for half of the total isotopic variation seen within this kangaroo's bone. This finding of large isotopic variation at a very small spatial scale suggests that even with a standardized approach to sampling location on a consistently selected skeletal element, researchers may capture very different periods in the life history of different individuals, especially when the sample being removed is small in size.

Multiple subsamples of the epiphysis tended to produce very similar isotopic compositions to one another (Fig. 4). The isotopic compositions of the epiphysis samples were sometimes very similar to the weighted average for the entire element and other times differed by nearly 3 ‰ (Fig. 4). There was much more isotopic variability observed within the diaphyses of the bones than there was within the epiphyses. Furthermore, there was more isotopic variation observed within the diaphysis samples than there was observed between the epiphysis and diaphysis samples.

Increasing the size of a sample improved the likelihood of obtaining a sample indicative of the skeletal element isotope mean in some cases. For the most isotopically variable bone (Cattle 2), combining an increasing number of subsamples (thus increasing the starting mass) improved the likelihood of the combined collagen sample producing $\delta^{13}C$ and $\delta^{15}N$ values near the mean of the entire skeletal element. However, these larger aggregated samples (e.g., 12 double cut or 6 regular segments) still frequently produced isotopic compositions that differed substantially from the overall bone's average δ values (Fig. 7). For Cattle 2, at 12 combined samples, or a sample mass of roughly 1.1 g,

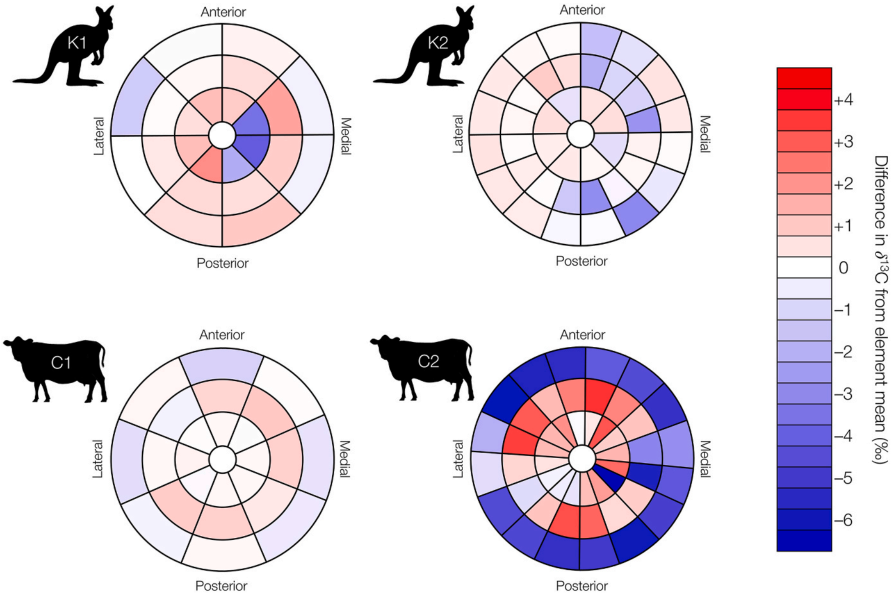

Fig. 5. Differences between the whole bone weighted mean and each subsample for $\delta^{13}C$. One donut from each of the four individual animals is represented. Kangaroo 1 is from donut number TEAL 19237, Kangaroo 2 was from 19424, Cattle 1 was TEAL 19283, and Cattle 2 was TEAL 20079. These donuts were chosen to demonstrate the high $\delta^{13}C$ variability within a single donut for each individual. Colour maps for all samples are available in supplement B. We have plotted the difference in $\delta^{13}C$ from the skeletal element mean so that the isotopic variability within each donut can be easily compared. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

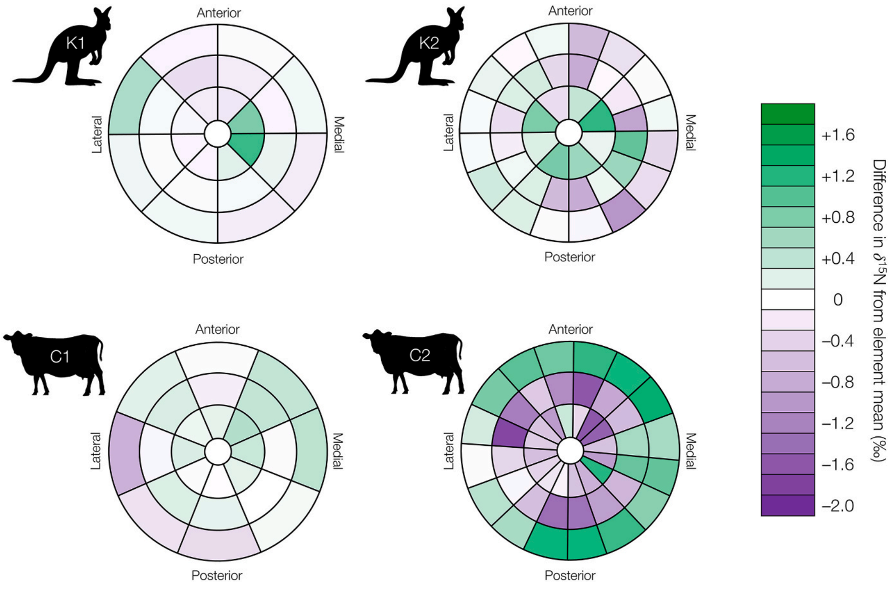

Fig. 6. Differences between the whole bone weighted mean and each subsample for δ^{15}N. One donut from each of the four individual animals is represented. Kangaroo 1 is from donut number TEAL 19699, Kangaroo 2 was from 19424, Cattle1 was TEAL 19283, and Cattle 2 was TEAL 20079.T hese donuts were chosen to demonstrate the high δ^{15}N variability within a single donut for each individual. Colour maps for all samples are available in supplement B. We have plotted the difference in δ^{15}N from the skeletal element mean so that the isotopic variability within each donut can be easily compared. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3

The probability of any individual incremental sample having a δ^{13}C different than the weighted mean by increasing increments.

Individual	> |0.5 ‰| from mean	> |1.0 ‰| from mean	> |2.0 ‰| from mean	> |3.0 ‰| from mean	> |4.0 ‰| from mean
Kangaroo 2	0.64	0.31	0.06	0.02	0
Kangaroo 1	0.42	0.22	0.08	0.03	0.01
Cattle 2	0.92	0.81	0.63	0.35	0.12
Cattle 1	0.36	0.06	0	0	0

Table 4

The probability of any individual incremental sample having a δ^{15}N different than the weighted average by increasing increments.

Individual	> |0.5 ‰| from mean	> |1.0 ‰| from mean	> |2.0 ‰| from mean	> |3.0 ‰| from mean
Kangaroo 2	0.34	0.14	0.02	0
Kangaroo 1	0.06	0.02	0	0
Cattle 2	0.71	0.25	0	0
Cattle 1	0.14	0.02	0	0

there was still a 72% chance that the sample δ^{13}C value would not fall within ±0.5 ‰ of the skeletal element's weighted average, and a 28% chance of the same for δ^{15}N. For Kangaroo 2, which had less isotopic variation than Cattle 2, the probability of producing an isotopic composition similar to the overall weighted mean was more readily achieved with a smaller number of segments, but there was still a 52% chance that the sample δ^{13}C value would not fall within ±0.5 ‰ of the skeletal element average at 12 segments, or a sample of 1.4 g (Fig. 7).

4. Discussion

We observed a significant level of stable isotopic variation within the bone collagen of a single skeletal element, far surpassing observations in prior studies (e.g., Hyland et al., 2022; Holdaway et al., 2011; Olsen et al., 2013). Notably, the maximum variation in δ^{13}C was observed in Cattle 2, with a range of 10.6 ‰, while the highest range in δ^{15}N was found in Kangaroo 2, with a range of 3.6 ‰. In both instances, the observed isotopic variation was most likely caused by an isotopically variable diet throughout the lives of these individuals. The variation in δ^{13}C from Cattle 2 approaches the difference that has been observed between C_4 grazers and C_3 browsers (Ambrose and DeNiro, 1986) and the variation in δ^{15}N from Kangaroo 2 likely captures shifts between environments with very different water availability in Australia (Murphy and Bowman, 2009). While not every skeletal element we analyzed showed extreme amounts of isotopic variation, the collagen from the bone with the least amount of isotopic variation still showed a maximum range of 2.9 ‰ in δ^{13}C and 2.2 ‰ in δ^{15}N, which is enough to affect interpretations about the individual's life history. While we do not know the dietary history of the animals involved, we suspect that the ranges of δ^{13}C and δ^{15}N we observed likely reflect nearly the maximum extent of dietary isotopic variation consumed by these individuals. The implication of this finding is that the upper limit on intra-bone isotopic variation should closely approximate the isotopic variability in foods that were regularly consumed.

Minimal isotopic variation within a single skeletal element has previously been observed (Hyland et al., 2022; Kontopoulos et al., 2022; Sykut et al., 2020), leading to the assumption that any sample taken from an element, regardless of its size or location, would yield relatively similar stable isotope ratios. It has even led to the belief that fragmented samples with more than 1.5 ‰ difference in bivariate (δ^{13}C, δ^{15}N) space can be assumed to be from a different individual all together (Hyland et al., 2022); a similar approach for differentiating individuals from forensic contexts was adopted by Berg et al. (2022). The research presented here, however, challenges these assumptions. These data indicate that different sections of bone undergo remodeling at different times, potentially preserving isotopic signals from various periods of the individual's life, with some areas remaining unchanged for prolonged periods. The substantial isotopic variation observed within a skeletal element in this study suggests that dietary changes are incorporated into bone at different rates across regions, reflecting variations in assimilation over time.

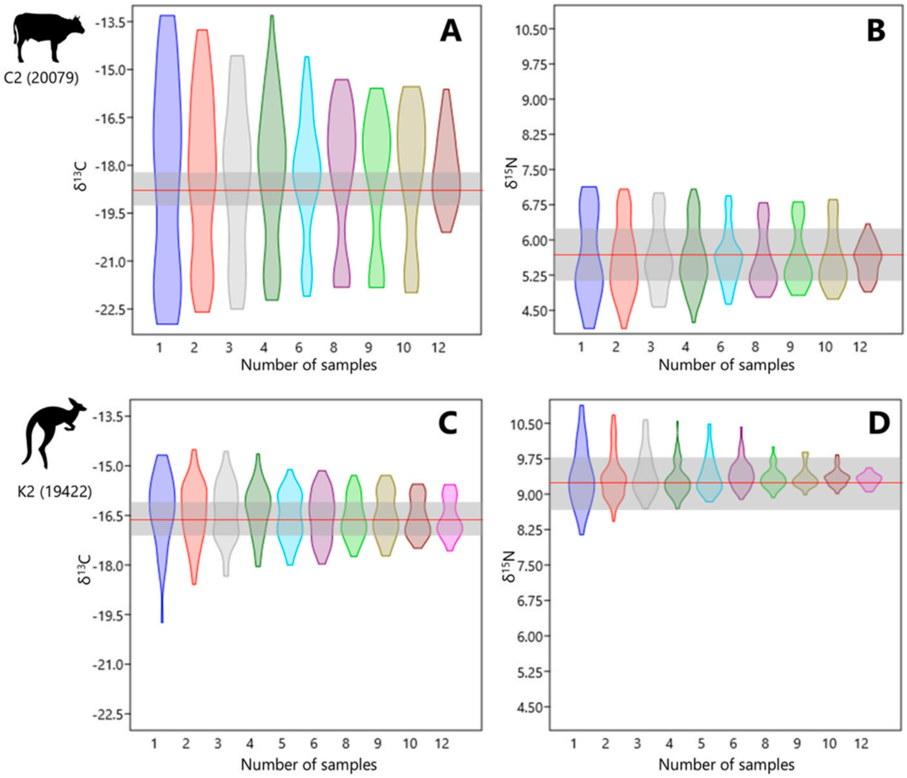

Fig. 7. Averaged $\delta^{13}C$ and $\delta^{15}N$ values of samples from donut 20079 of Cattle 2 (A, B) and donut 19422 of Kangaroo 2 (C, D). The averaging process combined adjacent samples within the donut, as well as those adjacent either proximally or distally. Both donuts were 'double cut' and have segments half the breadth of a regular segment. The red line represents the overall skeletal element weighted average and the shaded areas represent values within 0.5‰ of the weighted average. Combinations of samples that could not reasonably be produced during sampling (e.g., H3 and D2) were not considered. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Wolff's law states that differences in function and form of bones will lead to differences in cellular mechanics (Safadi et al., 2009; Wolff, 1892). Consequently, the chronological pattern of bone formation is likely closely linked to the functional demands placed on it. For instance, as bones age without undergoing remodeling, they experience water loss and increased mineralization. This process enhances the bone's rigidity, which is essential in regions where higher rigidity is required. In these areas, bone tends to be denser, and the rate of collagen turnover is likely slower (Burr, 2019). Non-uniform stress patterns arise within a skeletal element due to variations in muscle attachment sites, gait, and differences in loading demands across the element's structure (e.g., proximal versus distal strength requirements). These factors influence the need for site-specific remodeling to balance the dual needs of strength adaptation and the repair of microcracks (Skedros et al., 2003), which will influence the density of bone (Lai Qin et al., 2005; Skedros et al., 2003).

The relationship between bone loading and remodeling also has implications for comparing isotopic variation between bipedal and quadrupedal taxa. Our study includes both bipeds and quadrupeds, and it is possible that the greater stress placed on hindlimb elements of bipeds (in this case, kangaroos) differently affects the turnover of bone than in the hindlimbs of quadrupeds. Our study, however, did not include both fore- and hindlimb elements of cattle and kangaroo, and therefore we can only speculate on the potential differences between bipeds and quadrupeds. Better understanding the stresses for different regions of bone will assist in connecting specific areas of bone with distinct remodeling rates, and therefore periods of life, but much more research is needed in this area.

Using ^{14}C data, Matsubayashi and Tayasu (2019) proposed that the midcortical section of adult mammalian diaphyses reflects diet during earlier life, and therefore the slowest turnover, while the pericortical and perimedullary sections exhibit a faster turnover rate (see also de Gruchy et al., 2024; Manolagas and Parfitt, 2010). It has been hypothesized that this pattern of variable intrabone turnover is a product of the functional strength needs of midcortical bone and the proximity of internal/external bone surfaces to the vasculature associated with remodeling cells; more accessible surfaces in the pericortical/perimedullary areas relative to the midcortical region may also be a factor (Bolamperti et al., 2022). Differences in remodeling rates between rings could therefore explain variations in the distribution of the stable isotope ratios we measure. While we do not directly assess turnover rates, the above research supports the likelihood that the denser, midcortical layers represent slower turnover, while the perimedullary layers are associated with faster turnover.

Although inconsistent among individuals, the pericortical sections often had the lowest variance in $\delta^{13}C$ and $\delta^{15}N$ (Table 2; Supplement C), potentially indicating that this region was characterized by slower remodeling and may therefore partially reflect an earlier life period. No one ring stood out as being most representative of the weighted average of the skeletal element across all four individuals (Table 2). One individual (Cattle 2) had a dietary history that likely involved a change from two isotopically distinct foods (e.g., C_3 and C_4 plants). Matsubayashi and Tayasu (2019) suggested that the midcortical ring represented the earliest period in life. If this is true, it may be possible that cattle 2 had a C_3 heavier diet earlier in its life. We cannot confirm any trends in turnover rate with our stable isotope data, and we only present possible ways in which our data may hint at different periods in the life history being reflected across the rings. It should also be noted that ensuring that each of the samples across rings were equal in mass was not possible because the samples were demineralized before they were cut. Differences in the mass of samples across the rings could have led to some of the differences in isotope compositions across rings through an averaging effect, particularly for larger samples.

Trabecular bone is known to turn over more quickly than cortical bone (Cox and Sealy, 1997; Johnstone-Belford et al., 2022; Katsimbri, 2017; Quinn, 2024). Therefore, we expected that the epiphysis samples—which are predominantly composed of trabecular bone— to pinpoint which part of Cattle 2's diet occurred later in life. The trabecular bone-rich epiphysis of Cattle 2 produced lower δ^{13}C values than the skeletal element average (Fig. 4). This observation suggests that Cattle 2 may have eaten a more C_3-rich diet closer to death. The epiphysis most closely resembled the pericortical ring in its isotopic composition, consistent with the findings of Matsubayashi and Tayasu, who demonstrated that the pericortical region exhibits a faster turnover rate than the midcortical section. It is important to reiterate that we do not know what Cattle 2's feeding practices were during life, and it is, therefore, difficult to confidently suggest the possible regions of bone to attribute to different rates of turnover. We cannot do more than provide a range of possibilities that are informed by the existing literature on bone turnover. Additionally, variation in bone turnover patterns between species, age, health, bone types (i.e., cortical vs. trabecular), and skeletal elements could further confound the identification of any universal pattern of bone turnover (Hall et al., 2026). Additional research with animals that have undergone a known diet switch or ^{14}C analyses of bone collagen from cortical and trabecular regions of the bones would help in clarifying the variation in turnover patterns throughout the skeleton.

The extent of intra-bone isotopic variation observed in this study is far higher than has previously been documented (Fig. 8), and is much higher than most studies have reported across the skeleton (e.g., Berg et al., 2022; Cheung Jing et al., 2017). The degree of isotopic variation that we observed in our data is likely due to the high resolution and extensive sampling within each individual, as well as including some individuals that would have experienced a diet with a relatively high degree of isotopic variability. The high level of isotopic variation for both kangaroos that were sampled suggests that this high isotopic variability would not be limited to unusual circumstances such as a one-time dietary shift from C_3 to C_4 plants (as observed in Cattle 2 and in the Balasse et al., 1999 study). It is therefore probable that some previous studies have unintentionally underrepresented the amount of isotopic variation within a skeletal element as even our least isotopically variable individual (Cattle 1) demonstrated a greater range of isotopic compositions than has been previously observed in most studies. There may be a need to review previous work and reflect on the potential impact of intraskeletal isotopic variation on interpretations of human and animal life histories.

Bone samples that are selected for stable isotope analysis must be suited to the research questions or must explicitly recognize the uncertainty with respect to the time period represented by the sample's isotopic composition. If the goal is to understand an individual's average lifetime diet, the sample of bone analyzed should be sufficiently large to average intra-element stable isotopic variation. Taking a sample that is only large enough to yield the minimum amount of collagen needed for stable isotope analysis is far more likely to produce misleading isotopic compositions that are not reflective of an individual's lifetime average, or perhaps even long term, diet. The amount of organic material required to generate accurate and precise stable isotope measurements continues to decrease over time with advances in instrumentation. This fact, combined with the destructive nature of the analyses and the desire to preserve archaeological materials for future analyses creates an impetus to take the smallest samples possible. Such an approach must be tempered by the findings of this study. Notably, chasing ever smaller bone sample sizes will increase the probability of producing misleading results, devaluing the very insights provided by the isotopic analysis.

The possibility that different regions of a skeletal element represent diet during different periods of an individual's life implies that bone can be strategically sampled to reconstruct life history. Teeth have been serially sampled in stable isotope studies for longitudinal analyses of diet and mobility over the period of tooth formation (Balasse and Ambrose, 2005; Drucker et al., 2012; Wooller et al., 2021). While not derived from controlled feeding studies, the data presented in Fig. 4 likely approximate the variation in the isotopic composition of dietary items consumed over years of the lives of these individuals. Thus, rather than conceptualizing stable isotope measurements from bone collagen as being akin to those derived from tissues such as muscle, liver, or skin, they may be better understood as being similar to sequentially sampled hair or tooth samples that have lost their specific temporal information. In other words, sampling multiple sites from the same skeletal element is likely to reflect some measure of temporal variation in the life of an individual, as suggested by previous authors (e.g. Balasse et al., 1999; Matsubayashi and Tayasu, 2019; Matsubayashi et al., 2024). Given that the extent of intra-bone isotopic variation that we observed in this study was comparable to or greater than most studies examining inter-bone isotopic variation, we suggest that multiple samples from the same skeletal element (e.g., from the pericortical and perimedullary areas of long bones) may represent a better source of reliable diachronic variation in life history traits than samples derived from different bones,

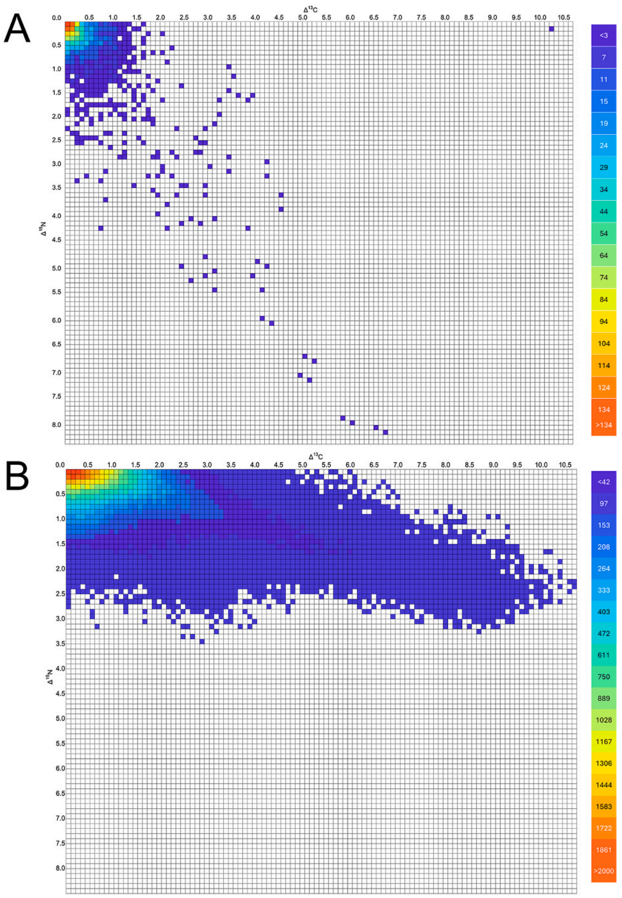

Fig. 8. Heat maps documenting the distribution of recorded intrabone isotopic differences, where $\Delta^{13}C = \delta^{13}C_{sample\ i}$-$\delta^{13}C_{sample\ j}$ and *sample i* and *sample j* are any two paired samples from the same individual. The colors on the heatmaps indicate the absolute abundance of each intrabone isotopic comparison as indicated by the legends on the right. (A) Data extracted from published literature (Balasse et al., 1999; Berg et al., 2022; Cheung Jing et al., 2017; Clark et al., 2017; Dauven et al., 2017; de Gruchy et al., 2024; Hyland et al., 2022; Matsubayashi and Tayasu, 2019; Matsubayashi et al., 2024; Olsen et al., 2013; Waters-Rist et al., 2011). Note that we excluded modern samples with $C:N_{atomic}$ ratios greater than 3.30 to reduce the possibility that lipids were the cause of isotopic variation (Guiry and Szpak, 2020), but this was not always possible as some studies did not present any elemental data. It is therefore likely that some of the variation along the x-axis in the top panel are exaggerated due to lipid contamination. (B) Data for this study. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

particularly in the absence of well-characterized turnover rates among different skeletal elements. Because such analyses would not require different skeletal elements that are clearly associated with the same individual, such an approach could easily be applied to finds of isolated skeletal elements, as is common with faunal materials recovered from middens. The primary limiting factor on realizing the full diachronic potential of bone is, of course, that skeletal elements cannot yet be "mapped" to determine which regions of bone correspond with earlier and later periods of life. Future research should focus on understanding how specific bone regions turn over and whether it is possible to consistently associate regions of bone with periods of an individual's life.

The extent of isotopic variation observed here suggests that care should be taken during the sampling process to ensure that intrabone variation is properly considered. While it is true that taking a larger sample does achieve an isotopic composition that is closer to the skeletal element mean and therefore better represents an averaged picture of an individual's life history, Cattle 2 has shown that even large samples do not preclude the possibility of unintentionally sampling regions that are isotopically quite distinct from the overall average. We recommend taking multiple samples from a bone to capture a range of possible values from the individual, as this can help assess whether the bone exhibits significant variation and whether the diet was relatively stable or highly variable. If locations of muscle attachments are not known, we recommend taking at least 3 samples of bone, one from the perimedulliary, one from the mid-cortical, and one from the pericortical. This sampling style should allow for different "ages" of bone to be captured (sampling both relatively newly laid down and relatively old bone) and create a picture of how the life history of the individual sampled may vary over time. Since our epiphyseal samples were typically consistent with element mean isotope values, it may also be beneficial to sample from this location for constant lifetime average data, however whether or not the epiphysis will always reflect the mean is uncertain. Targeting the same locations across each of the bones sampled would be crucial with such a sampling strategy. The degree to which one sample can differ from an adjacent sample indicates that while carefully selecting sample locations can help mitigate some variation, even minor shifts in sampling position can substantially alter the measured isotope value. At a minimum, more care should be taken to communicate the sampling locations when publishing stable isotope data derived from bone collagen.

5. Conclusions

The lack of a comprehensive assessment of intra-bone isotopic variation has led to an assumption that variability in stable isotope values of bone collagen would be inconsequential, with the exception of unusual circumstances (Balasse et al., 1999). This study demonstrates that intrabone isotopic variability can be much higher than previously thought. There was significant variability in stable isotopic compositions (δ^{13}C and δ^{15}N) of bone collagen within a single skeletal element (up to 10.6‰), which indicates that collagen is not remodeled at a similar rate across a skeletal element, clearly capturing different phases in the life history of an individual. Our results suggest that the depth of a sample (pericorical, midcortical, and perimedullary) and location relative to muscle attachments may be reflective of differing periods of the individual's life. Bones may provide an opportunity for high resolution longitudinal analyses of individual life histories if the relative turnover rates within skeletal elements can be adequately characterized. Future research should look to comprehensively sample skeletal elements from other species with well-documented life histories to better understand how the potential for large variation differs across animals and specific feeding patterns.

CRediT authorship contribution statement

Olivia Hall: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Alexandra A.Y. Derian:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Alexis Rausch:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Julia McCuaig:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Paul Szpak:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Data curation.

Reproducible results

The Associate Editor for Reproducibility could download all materials and was able to reproduce the results presented by the authors.

Declaration of competing interest

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

Acknowledgements

The quality of this manuscript was improved thanks to the comments of three anonymous reviewers. Funding was provided by an NSERC Discovery Grant (2020-04740) and the Canada Research Chairs program. This is Trent Environmental Archaeology Lab Graduate Research on Isotopic Methodologies project #2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2026.106521.

References

Ambrose, S.H., DeNiro, M.J., 1986. The isotopic ecology of east African mammals. Oecologia 69, 395–406. https://doi.org/10.1007/BF00377062.

Bahar, B., Monahan, F.J., Moloney, A.P., O'Kiely, P., Scrimgeour, C.M., Schmidt, O., 2005. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. Rapid Commun. Mass Spectrom. 19 (14), 1937–1942.

Balasse, M., Bocherens, H., Mariotti, A., 1999. Intra-bone variability of collagen and apatite isotopic composition used as evidence of a change of diet. J. Archaeol. Sci. 26 (6), 593–598.

Balasse, M., Ambrose, S.H., 2005. Distinguishing sheep and goats using dental morphology and stable carbon isotopes in C_4 grassland environments. J. Archaeol. Sci. 32 (5), 691–702.

Berg, G.E., Chesson, L.A., Yuryang, J., Youngsoon, S., Bartelink, E.J., 2022. A large-scale evaluation of intraperson isotopic variation within human bone collagen and bioapatite. Forensic Sci. Int. 336, 111319–111319.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37 (8), 911–917.

Bolamperti, S., Villa, I., Rubinacci, A., 2022. Bone remodeling: an operational process ensuring survival and bone mechanical competence. Bone Res. 10 (1), 48.

Burr, D.B., 2019. Bone morphology and organization. In: Burr, D.B., Allen, M.R. (Eds.), Basic and Applied Bone Biology, second ed. Elsevier/Academic Press.

Cheung Jing, Z., Tang, J., Weston, D.A., Richards, M.P., 2017. Diets, social roles, and geographical origins of sacrificial victims at the royal cemetery at Yinxu, Shang China: new evidence from stable carbon, nitrogen, and sulfur isotope analysis. J. Anthropol. Archaeol. 48, 28–45.

Clark, C.T., Horstmann, L., Misarti, N., 2017. Quantifying variability in stable carbon and nitrogen isotope ratios within the skeletons of marine mammals of the suborder Caniformia. J. Archaeol. Sci.: Report 15 (Complete), 393–400.

Cox, G., Sealy, J., 1997. Investigating identity and life histories: isotopic analysis and historical documentation of slave skeletons found on the cape town foreshore, South Africa. Int. J. Hist. Archaeol. 1 (3), 207–224.

Dauven, E., Montrieux, C., O'Boyle, L., Ditchfield, P., Pollard, A.M., 2017. Carbon and nitrogen isotopic variation in bone collagen within the human skeleton. In: Kluiving, S., Kootker, L., Hermans, R. (Eds.), Interdisciplinarity Between Humanities and Science, vol. 2. Sidestone Press, pp. 113–139.

de Gruchy, Y.A., Faillace, K.E., Van de Vijver, K., Schotsmans, E.M., Seifert, J., Bricking, A., et al., 2024. Bone of contention: intra-element variability in remodelling of human femora based on histomorphometric and isotope analyses. PLoS One 19 (6), e0305089.

Deniro, M.J., Schoeninger, M.J., 1983. Stable carbon and nitrogen isotope ratios of bone collagen: variations within individuals, between sexes, and within populations raised on monotonous diets. J. Archaeol. Sci. 10, 199–203.

Drucker, D.G., Hobson, K.A., Münzel, S.C., Pike-Tay, A., 2012. Intra-individual variation in stable carbon (δ 13 C) and nitrogen (δ 15 N) isotopes in mandibles of modern caribou of Qamanirjuaq (Rangifer tarandus groenlandicus) and Banks Island ((Rangifertarandus pearyi): implications for tracing seasonal and temporal changes in diet. Int. J. Osteoarchaeol. 22 (4), 494–504.

Erickson, P.S., Kalscheur, K.F., 2020. Nutrition and feeding of dairy cattle. In: Bazer, W. F., Lamb, G.C., Wu, G. (Eds.), Animal Agriculture: Sustainability, Challenges, and Innovations. Academic Press, pp. 157–180.

Fahy, G.E., Deter, C., Pitfield, R., Miszkiewicz, J.J., Mahoney, P., 2017. Bone deep: Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. J. Archaeol. Sci. 87, 10–16.

Franchi, G.A., Herskin, M.S., Jensen, M.B., 2019. Dairy cows fed a low energy diet before dry-off show signs of hunger despite *ad libitum* access. Sci. Rep. 9 (16159), 1–9.

Gilbert, A.S., Steinfeld, P., 1977. Faunal remains from Dinkha Tepe, northwestern Iran. J. Field Archaeol. 4 (3), 329–351.

Guiry, E.J., Szpak, P., 2020. Quality control for modern bone collagen stable carbon and nitrogen isotope measurements. Methods Ecol. Evol. 11 (9), 1049–1060.

Hedges, R.E.M., Clement, J.G., Thomas, C.D.L., O'Connell, T.C., 2007. Collagen turnover in the adult femoral mid-shaft: modelled from anthropogenic radiocarbon tracer measurements. Am. J. Phys. Anthropol. 133 (2), 808–816.

Hall, O., Forbes, S.L., Szpak, P., 2026. Variation in human bone collagen turnover among skeletal elements. Am. J. Biol. Anthropol. 189, e70199. https://doi.org/10.1002/ajpa.70199.

Hill, P.A., 1998. Bone remodelling. Brit. J Orthodon. 25 (2), 101–107.

Holdaway, Hawke, D.J., Bunce, M., Allentoft, M.E., 2011. Identification of an optimal sampling position for stable isotopic analysis of bone collagen of extinct moa (Aves: emeidae). Notornis 58 (1), 1–7.

Huiskes, R., Ruimerman, R., van Lenthe, G.H., Janssen, J.D., 2000. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. Nature 405 (6787), 6787.

Hyland, C., Scott, M.B., Routledge, J., Szpak, P., 2022. Stable carbon and nitrogen isotope variability of bone collagen to determine the number of isotopically distinct specimens. J. Archaeol. Method Theor 29 (2), 666–686.

Johnstone-Belford, E., Fallon, S.J., Dipnall, J.F., Blau, S., 2022. The importance of bone sample selection when using radiocarbon analysis in cases of unidentified human remains. Forensic Sci. Int. 341, 111480.

Jones, A.M., O'Connell, T.C., Young, E.D., Scott, K., Buckingham, C.M., Iacumin, P., Brasier, M.D., 2001. Biogeochemical data from well preserved 200 ka collagen and skeletal remains. Earth Planet Sci. Lett. 193 (1–2), 143–149.

Katsimbri, P., 2017. The biology of normal bone remodelling. Eur. J. Cancer Care 26 (6), e12740. https://doi.org/10.1111/ecc.12740.

Kontopoulos, I., Van de Vijver, K., Robberechts, B., von Tersch, M., Turner-Walker, G., Penkman, K., Collins, M.J., 2022. Histological and stable isotope analysis of archeological bones from st. Rombout's cemetery (Mechelen, Belgium): intrasite, intraindividual, and intrabone variability. Int. J. Osteoarchaeol. 32 (5), 1142–1156.

Lai Qin, L., Hung, V.W.Y., Chan, K.M., 2005. Regional differences in cortical bone mineral density in the weight-bearing long bone shaft—A pQCT study. Bone 36 (3), 465–471.

Manolagas, S.C., 2000. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. 21 (2), 115–137.

Manolagas, S.C., Parfitt, A.M., 2010. What old means to bone. Trends Endocrinol. Metabol.: TEM (Trends Endocrinol. Metab.) 21 (6), 369–374. https://doi.org/10.1016/j.tem.2010.01.010.

Matsubayashi, J., Miura, K., Yamamori, E., Arimoto, Y., Yoshimizu, C., Tayasu, I., Tsuruga, H., 2025. Multiscale dietary analysis of brown bears in Hokkaido, Japan. J. Wildl. Manag., e70047

Matsubayashi, Tayasu, I., 2019. Collagen turnover and isotopic records in cortical bone. J. Archaeol. Sci. 106, 37–44.

Matsubayashi, J., Tsutaya, T., Sato, T., 2024. Retrospective isotope analysis of ancient remains to distinguish between tamed and wild animals. Archaeol. Anthropol. Sci. 16. https://doi.org/10.1007/s12520-024-02042-0.

Mein, E., Manne, T., Veth, P., Weisbecker, V., 2022. Morphometric classification of kangaroo bones reveals paleoecological change in northwest Australia during the terminal Pleistocene. Sci. Rep. 12, 18245.

Montoya-Sanhueza, G., Chinsamy, A., 2017. Long bone histology of the subterranean rodent *Bathyergus suillus* (Bathyergidae): ontogenetic pattern of cortical bone thickening. J. Anat. 230 (2), 203–233.

Murphy, B.P., Bowman, D.M., 2009. The carbon and nitrogen isotope composition of Australian grasses in relation to climate. Funct. Ecol. 23 (6), 1040–1049.

Murphy, B.P., Bowman, D.M., Gagan, M.K., 2007. Sources of carbon isotope variation in kangaroo bone collagen and tooth enamel. Geochem. Cosmochim. Acta 71 (15), 3847–3858.

Olsen, K.C., White, C.D., Longstaffe, F.J., von Heyking, K., McGlynn, G., Grupe, G., Rühli, F.J., 2013. Intraskeletal isotopic compositions (Δ13C, Δ15N) of bone collagen: nonpathological and pathological variation. Am. J. Phys. Anthropol. 153 (4), 598–604.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189.

Qi, H., Coplen, T.B., Geilmann, H., Brand, W.A., Böhlke, J.K., 2003. Two new organic reference materials for δ13C and δ15N measurements and a new value for the δ13C of NBS 22 oil. Rapid Commun. Mass Spectrom. 17, 2483–2487.

Quinn, Rhonda L., 2024. Bomb pulse 14C evidence for consistent remodeling rates of cortical femur collagen in middle-late adulthood. Am. J. Biol. Anthropol. 183 (4), e24887.

Safadi, F.F., Barbe, M.F., Abdelmagid, S.M., Rico, M.C., Aswad, R.A., Litvin, J., Popoff, S. N., 2009. Bone structure, development and bone biology. In: *Bone pathology*. Humana Press, Totowa, NJ, pp. 1–50.

Schimmelmann, A., Qi, H., Coplen, T.B., Brand, W.A., Fong, J., Meier-Augenstein, W., Kemp, H.F., Toman, B., Ackermann, A., Assonov, S., Aerts-Bijma, A.T., Brejcha, R., Chikaraishi, Y., Darwish, T., Elsner, M., Gehre, M., Geilmann, H., Gröning, M., Hélie, J.-F., Herrero-Martín, S., Meijer, H.A.J., Sauer, P.E., Sessions, A.L., Werner, R. A., 2016. Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: caffeines, n-Alkanes, fatty acid methyl esters, glycines, l-Valines, polyethylenes, and oils. Anal. Chem. 88, 4294–4302.

Skedros, J.G., Sybrowsky, C.L., Parry, T.R., Bloebaum, R.D., 2003. Regional differences in cortical bone organisation and microdamage prevalence in rocky Mountain mule deer. Anat. Rec. 274A (1), 837–850.

Sykut, M., Pawełczyk, S., Borowik, T., Pokorny, B., Flajšman, K., Niedziałkowska, M., 2020. Intraindividual and interpopulation variability in carbon and nitrogen stable isotope ratios of bone collagen in the modern red deer (Cervus elaphus). J. Archaeol. Sci.: Report 34, 102669.

Szpak, P., 2026. An automated spreadsheet for determining analytical uncertainty of stable isotope measurements. J. Archaeol. Sci. 186, 106457. https://doi.org/10.1016/j.jas.2025.106457.

Valenzuela, L.O., Plens, C., Otero, F., Loupias, L.L., Soriano, E.P., de Carvalho, M.V.D., Ubelaker, D.H., 2025. Intra-individual stable isotope variation tracks Brazilian contemporary dietary and nutritional transition. Am. J. Biol. Anthropol. 186 (1), e25057.

Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T., Weidel, B.C., 2015. Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS One 10, e0116182. https://doi.org/10.1371/journal.pone.0116182.

Van Elswyk, M.E., McNeill, S.H., 2014. Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: the US experience. Meat Sci. 96 (1), 535–540.

Waters-Rist, A.L., Bazaliiskii, V.I., Weber, A.W., Katzenberg, M.A., 2011. Infant and child diet in Neolithic hunter-fisher-gatherers from cis-baikal, siberia: intra-long bone stable nitrogen and carbon isotope ratios. Am. J. Phys. Anthropol. 146 (2), 225–241.

Williams, J.E., Wagner, D.G., Walters, L.E., Horn, G.W., Waller, G.R., Sims, P.L., Guenther, J.J., 1983. Effect of production systems on performance, body composition and lipid and mineral profiles of soft tissue in cattle. J. Anim. Sci. 57 (4), 1020–1028.

Wolff, J.,1892. Das gestetz der transformation der knochem. *Verlag von August Hirschwald.*

Wooller, M.J., Bataille, C., Druckenmiller, P., Erickson, G.M., Groves, P., Haubenstock, N., Howe, T., Irrgeher, J., Mann, D., Moon, K., Potter, B.A., Prohaska, T., Rasic, J., Reuther, J., Shapiro, B., Spaleta, K.J., Willis, A.D., 2021. Lifetime mobility of an arctic woolly mammoth. Science 373, 806. https://doi.org/10.1126/science.abg1134.