Isotopic analysis of the human skeletal remains

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This chapter summarises the isotope analysis undertaken on the human remains from RUX6, in order to estimate their diet and physiological changes during life. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratio analysis was carried out using incremental dentine sectioning for collagen for individuals SF 26319 and SF17642 (giving values for childhood and early adolescence) and bulk sampling of bone collagen for these two individuals plus an ulna from a third, disturbed burial from Square G, SF 17437 (providing an average for each of the diet during last 10 years of life). Contemporary faunal bone collage samples were also measured in order to provide an isotopic baseline. In addition, enamel from the teeth of SF 26319 and SF 17642 was sampled for strontium isotope ratios to assess individual migration.

Stable isotope measurements for diet, physiology and migration

The interpretation of variation in the ratios of stable light isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in skeletal and dental tissues has been well–established as a robust method of reconstructing the dietary habits of individuals, and has parallels in modern clinical studies. Fractionation is the key to understanding the dietary pathway of carbon and nitrogen within the biosphere through the food chain, and allows the reconstruction of the diets of fauna and humans.

When an element is incorporated in the tissues of a plant or animal, fractionation occurs and the newly–formed tissue will contain more of the lighter isotope. This means that ratio of the heavier isotope of nitrogen to the lighter $\delta^{15}N$ increases as a result of metabolic fractionation by 2–5‰ and the ratio of the heavier isotope of carbon $\delta^{13}C$ by about 1–2‰ at each trophic level of a food chain and can therefore provide information about the relative consumption of plant, animal and marine protein (Schoeninger and DeNiro 1984). For that reason, faunal bone and tooth from the site have also been analysed.

Isotopic values can also be affected by physiological changes. Raised $\delta^{15}N$ values of body tissues may be the result of extreme nutritional stress (Hobson *et al*. 1993; Guthrie and Picciano 1995). Mekota *et al*. (2006) showed how body mass index (BMI) was related to changes in the $\delta^{15}N$ values of individuals deliberately depriving themselves of food, while Duška *et al*. (2007) demonstrated a negative nitrogen balance (catabolism) in acute starvation in a clinical setting. Hatch *et al*. (2006) advised the use of isotopic values from hair as a diagnostic tool for anorexia and bulimia. Powanda and Beisel (2003) noted that the metabolic effects of infection on nitrogen balance were masked or reduced when protein and calorie intake was increased, suggesting that the effects of any illness on nitrogen balance would be greater when nutrition was also insufficient. It has also been suggested that chronic illness may cause changes in the $\delta^{15}N$ values of pathological bone (Katzenberg and Lovell 1999). Finally, dietary changes revealed by temporal changes in the isotopic values may also be evidence for migration of an individual (e.g. Müldner *et al*. 2011; Beaumont *et al*. 2013).

Materials and methods

The human samples consisted of rib bone from two articulated individuals (SF 26319 and SF 17642), the disarticulated ulna of a third (SF 17437) the upper left second premolar (ULPM2) from SF 17642, the lower right first permanent molar (LRM1) from SF 26319. The faunal samples were bone from one cattle (SF 23102) and a sheep (SF 26514), and dentine from a juvenile cattle (SF 26321) and dentine from a red deer tooth (SF 26185).

All samples were cleaned with a rose–head bur in a slow–speed rotary hand drill to remove adherent surface contamination. The human teeth were sectioned to select a single root from the M1 and divide the root of the PM2 in half. Any secondary dentine and cementum were removed, and a sample of the enamel removed for strontium analysis. The animal teeth were cleaned and as much enamel removed as possible without damaging the dentine.

The bone and dentine samples were demineralised in 0.5M HCl at 4°C. The softened human tooth samples were then divided into 1mm sections using a scalpel (Beaumont *et al*. 2013: method 2). All samples were denatured at 70°C in a pH3 solution, frozen at −80°C and then freeze–dried to produce collagen (Brown *et al*. 1988).

The collagen was weighed into tin capsules (in duplicate where possible) and measured combustion

Sample Nr	Species	Tissue	Age	Sex	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
26319	human	bone (rib)	*c.* 45+ years	M	11.1	−17.7
26319	human	dentine ULPM2 (average)			10.3	−19.4
17642	human	bone (rib)	*c.* 19–25 years	M	9.6	−20.1
17642	human	Dentine LRM1 (average)			9.7	−19.4
17437	human	bone (ulna)			9.9	−20.5
23102	cattle	bone			5.0	−21.8
26514	sheep	bone			6.2	−21.4
26321	juvenile cattle	bone			7.4	−21.4
26321	juvenile cattle	dentine			7.4	−21.1
26185	deer	bone			5.4	−22.2

TABLE 3.5:
RUX6 human and faunal samples, age and sex (Bohling and Buckberry 2016) and bulk $\delta^{15}N$ and $\delta^{13}C$.

in a Thermo Flash EA 1112 and introduction of separated N_2 and CO_2 to a Finnigan Delta plus XL via a Conflo III interface. The collagen samples were interspersed throughout the run with both internal standards and international standards. Calibrated against these standards, the analytical error at 1 standard deviation was ±0.2‰ or better.

Results

The results of the isotopic analysis of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in the skeletal and dental tissue samples can be found in Table 3.5.

Animal baseline

The isotopic animal baseline can indicate or confirm a trophic level shift within diet.

Specimen SF 26321, a calf (juvenile cattle), was located in close proximity to individual SF 26319. SF 26321 has two data points representing the bulk sampling for dentine collagen and bone collagen, with very similar $\delta^{15}N$ and $\delta^{13}C$ values reflecting that these tissues are forming at the same time. As seen in Figure 3.21, SF 26321 fits within the trophic level shift offset explained above.

In Figure 3.21 the faunal data is situated in the lower left portion of the plot, with relatively low $\delta^{15}N$ values, in comparison to the human $\delta^{15}N$. The offset is described by Szpak *et al*. (2012) as 3.6 ± 1.3‰ for $\delta^{15}N$ and 3.7 ± 1.6‰ for $\delta^{13}C$.

This supports the hypothesis that this small sample of individuals may have had a diet which included the consumption of sheep, and possibly deer. However, the bone collagen $\delta^{15}N$ and $\delta^{13}C$ values for SF 26319 are much higher and would suggest that this individual has had input from marine sources in his diet as an adult, reflected in the bone collagen in the rib, which reflects the last 3–5 years of life (Beaumont and Montgomery 2016).

Bone collagen data from contemporaneous British sites

Two of the individuals from Udal (SF 17437 and SF 17642) fall into the terrestrial $\delta^{13}C$ range of the Neolithic/Bronze Age/Iron Age individuals from broadly contemporaneous British sites; Sumburgh (Montgomery *et al*. 2013), Quarterness (Schulting *et al*. 2010), Cladh Hallan (Pearson *et al*. 2005) and Yarnton (Lightfoot *et al*. 2009). The third, SF 26319, appears to be similar in his $\delta^{13}C$ range to the Iron Age male adults from Newark Bay (Richards *et al*. 2006), suggesting this individual had a more marine–based diet. This could be interpreted as evidence that he lived at a different time period, or that he resorted to a marine diet during a time of nutritional distress: the marine value is from rib (turnover time last 3–5 years of life) in contrast to early life values from the dentine, and a further short term period of marine input age 6.5–8 years in the dentine profile. All three have lower $\delta^{15}N$ values than the sites that they match for $\delta^{13}C$ (Figure 3.22).

Dentine profile SF 26319

For individual SF 26319, the incremental dentine $\delta^{15}N$ and $\delta^{13}C$ data is presented in Figure 3.23 using a time line to show the data against approximate age in years. The $\delta^{15}N$ and $\delta^{13}C$ values for each incremental sample generally appear to co–vary suggesting that these are dietary values.

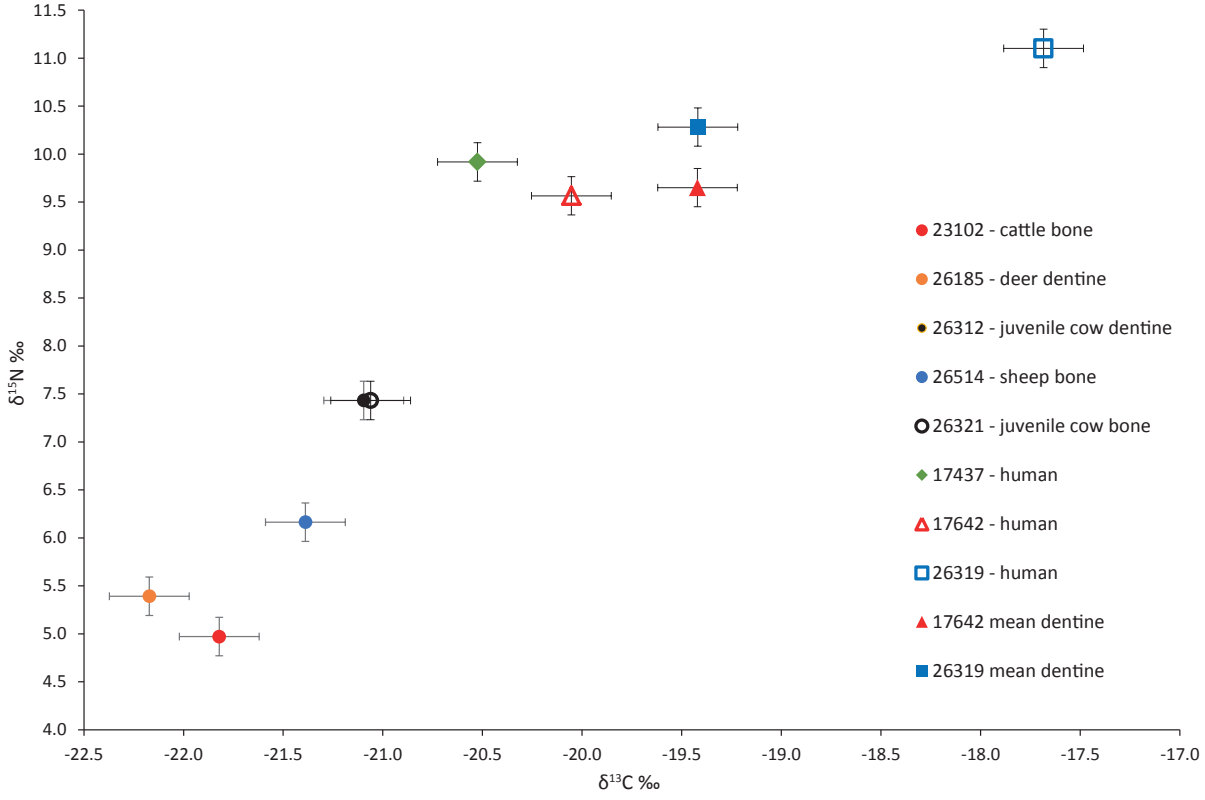

FIGURE 3.21:
A plot of animal dentine and bone collagen $\delta^{15}N$ and $\delta^{13}C$ alongside the human dentine collagen (averaged) and bone collagen. Error bars set ±0.2‰

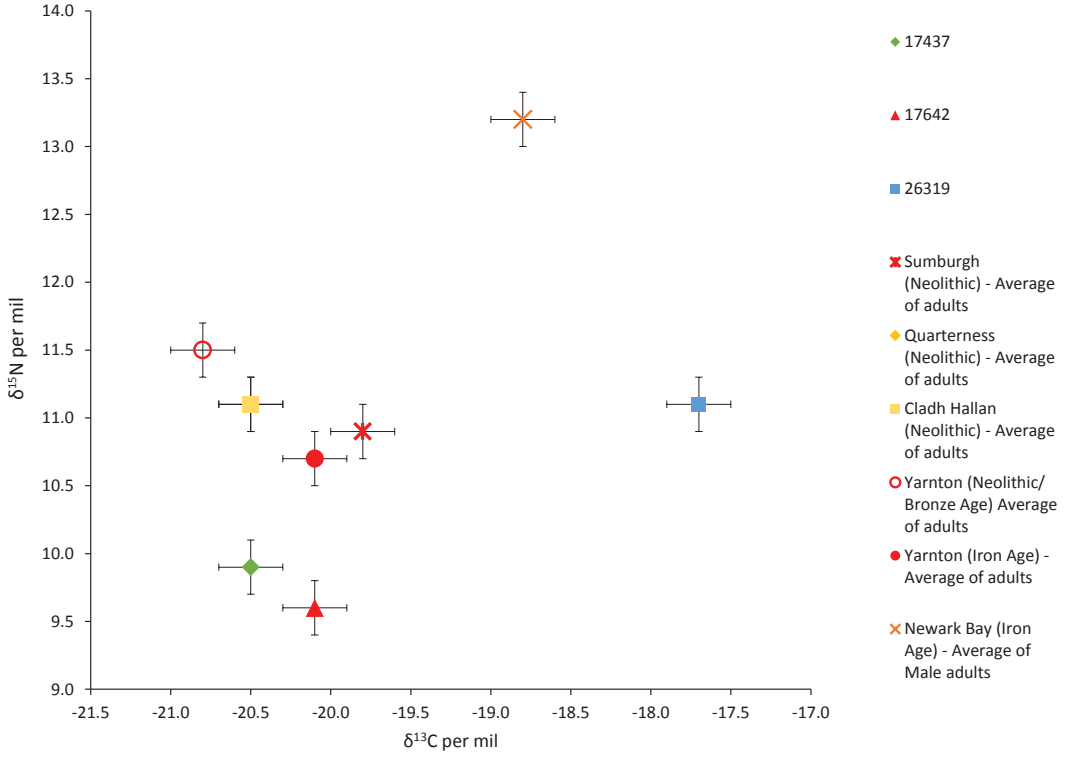

FIGURE 3.22:
Plot showing bone collagen $\delta^{15}N$ and $\delta^{13}C$ for SF 17437, SF 17642, and SF 26319 with bone collagen data from contemporaneous British sites. Sumburgh (Montgomery *et al.* 2013), Quarterness (Schulting *et al.* 2010), Cladh Hallan (Pearson *et al.* 2005), Yarnton (Lightfoot *et al.* 2009) and Newark Bay (Richards *et al.* 2006). Error bars set at ±0.2‰.

In more detail, the early increments suggest a short period of breastfeeding between birth and 6 months of age (a falling value for both $\delta^{15}N$ and $\delta^{13}C$), followed by a period from 0.5 to 1.5 years of opposing covariance which suggests a short period of nutritional distress (Beaumont *et al*. 2015; Beaumont and Montgomery 2016). Both isotope values then remain fairly stable until the age of 6 when both rise reaching values over −19‰ for $\delta^{13}C$ and over 11‰ for $\delta^{15}N$, suggesting a marine input to the diet (Montgomery *et al*. 2013). This is consistent with the bone collagen values for SF 26319, which also suggest that the marine input continued into adult life (and see Figure 3.21). There is a divergence of the values at the age of 9 years with rising $\delta^{15}N$ and falling $\delta^{13}C$, suggesting further nutritional distress.

Dentine profile SF 17642

Compared to SF 26319, the $\delta^{13}C$ values for both the bone (−20.1‰) and dentine (bulk −19.4‰) from SF 17642 remain within the expected terrestrial values from the faunal remains (Figure 3.24). There is evidence for a high marine input to the diet from 8.5–10 years, although the bulk isotope ratio values suggest that the individual was eating meat throughout life. The bone values are close to the bulk dentine values.

In detail, it appears that there is a period of opposing covariance from the age of 3.5 to 6.5 years, which could be a period of nutritional distress with marine input followed by recycling of body tissues shown by continued rise in $\delta^{15}N$ and fall in $\delta^{13}C$. A further period of opposing covariance at the end of the profile suggests some nutritional distress (13.5–14.5 years).

Strontium isotope ratios

Lab No	Sample No	Type	Species	$^{87}Sr/^{86}Sr$	std Error
H667	GU42089	Enamel	Human	0.7095 ± 0.0016	0.002
H668	GU42090	Enamel	Human	0.7095 ± 0.0015	0.002

TABLE 3.6:
Strontium isotope ratios for the two individuals, SF 26319 and SF 17642

Enamel from the teeth of SF 26319 and SF 17642 was sampled for strontium isotope ratios. Both individuals had a Sr value of 0.7095 (see Table 3.6). This is similar to the value of 0.7092 for individuals from Cnip (Montgomery *et al*. 2003) and for the plants living on the machair of South Uist (0.7100). Sr isotopic values for plants growing on the gneiss were reported as 0.7095, 0.7102 and 0.7103 (Montgomery *et al*. 2007). Overall the Sr results are consistent with individuals from the Uists.

Discussion

The individuals from RUX6 have a range of $\delta^{15}N$ and $\delta^{13}C$ values. Comparing these values with those from the contemporaneous faunal remains at the site, it appears that all three were consumers of these animals, with SF 23619 changing from a terrestrial diet from about the age of 6 years to a diet with a higher marine input and SF 17642 with a short−term episode of marine input between about 8.5–11.5 years. The matching variance in $\delta^{15}N$ and $\delta^{13}C$ confirms this to be a dietary change.

Both SF 23619 and SF 17642 show opposing covariance (when the $\delta^{15}N$ and $\delta^{13}C$ change but in opposing directions) within their dentine profiles, suggesting that each had a period of nutritional insufficiency during the period of tooth formation (Beaumont and Montgomery 2016). It is not possible to diagnose what caused this, but SF 23619 is recorded as having enamel hypoplasia present on his dentition at approximately the same age as the opposing covariance in the dentine profile, about the age of 1 year, another sign of non−specific stress during childhood (Bohling and Buckberry 2016). SF 17642 has the start of a similar pattern at the end of tooth formation, at about 13.5 years. This is similar to individuals reported at Sumburgh cist (Neolithic) where the short−term change was interpreted as a response to a failure of the terrestrial food sources (Montgomery *et al*. 2013).

Two of these individuals, SF 17642 and SF 17437, have bone collagen $\delta^{13}C$ values (reflecting their adult diet) which are consistent with the values from adults from contemporaneous British sites where the diet is mainly terrestrial. The third, SF 23619, has $\delta^{13}C$ values which suggest a higher marine input to the diet as an adult, similar to the isotopic values from the Iron Age adult males from Newark Bay.

It is possible that the two terrestrial consumers were near contemporaries, sharing the same diet, and that SF 23619 lived and died at a different time when the local diet was consistently more marine−based.

In all three cases, the $\delta^{15}N$ is lower than the contemporary sites (Figure 3.22). This could reflect the consumption of lower trophic level foods at

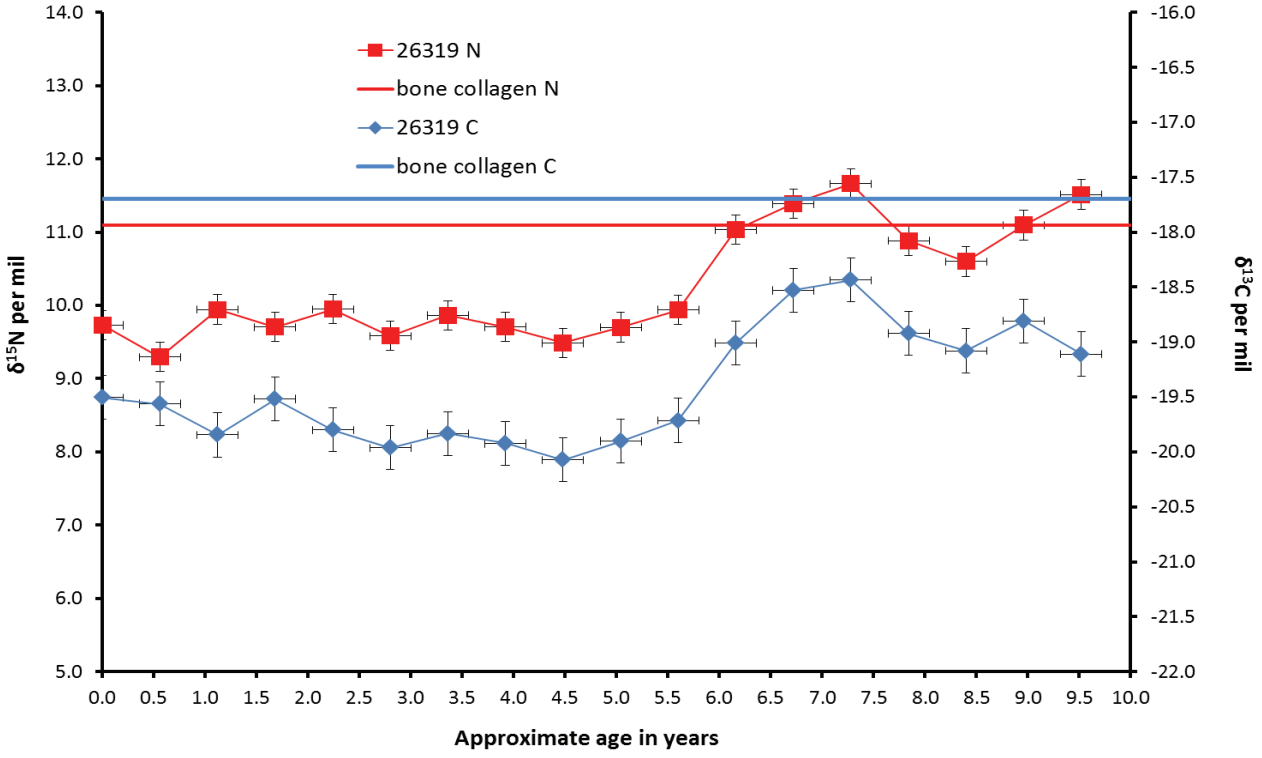

FIGURE 3.23:
Incremental dentine and bone collagen $\delta^{15}N$ and $\delta^{13}C$ profile for SF 26319 plotted against approximate age. Error bars set to 0.2‰.

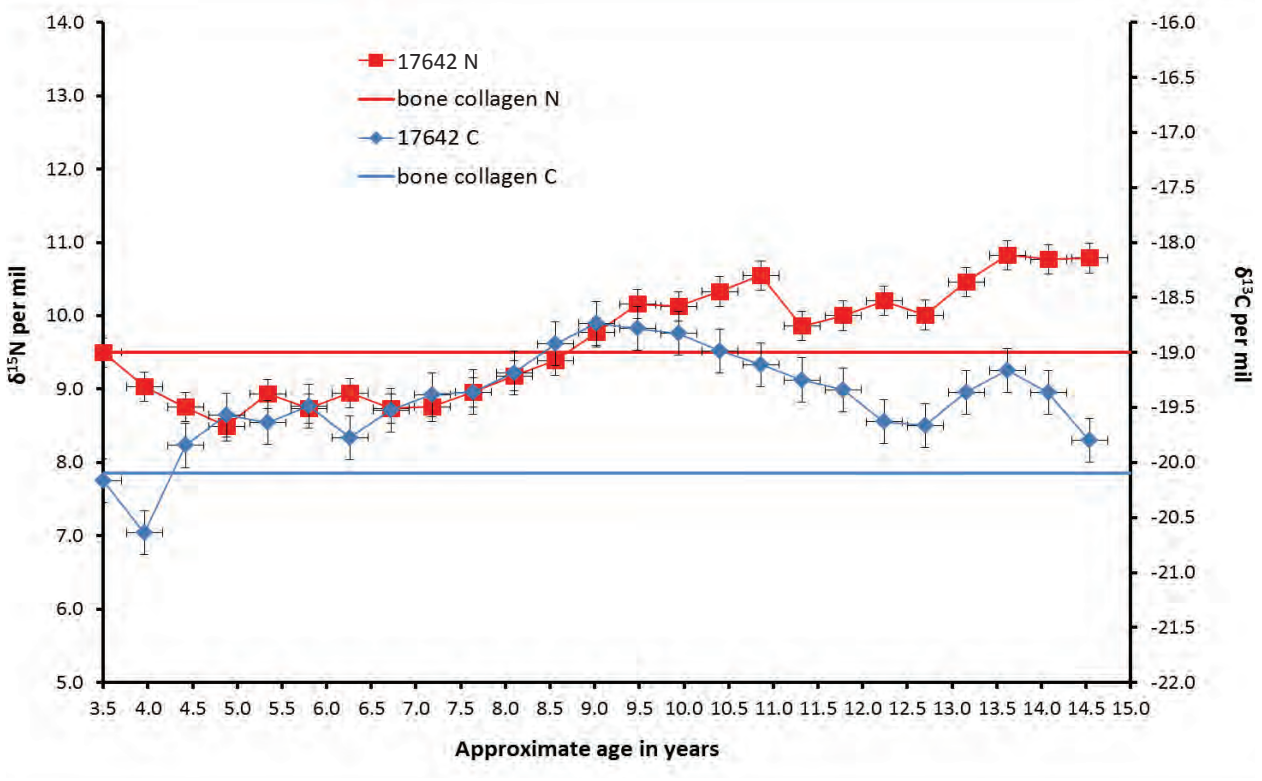

FIGURE 3.24:
Incremental dentine and bone collagen $\delta^{15}N$ and $\delta^{13}C$ profile for SF 17642 plotted against approximate age. Error bars set to 0.2‰.

RUX6, or a difference in the management of crops and animals: for example, manuring of crops or vegetation fed to the animals would result in higher $\delta^{15}N$ values in the faunal and human tissues, as would growing crops or feeding animals in areas of salt marsh (Bogaard *et al*. 2007; Britton *et al*. 2008).

While there is no evidence of migration between areas with different geology in the Sr results, he may have migrated from a location with the same Sr isotope ratio.