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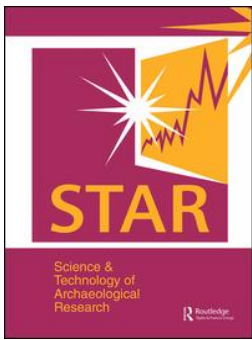
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Strontium concentration, radiogenic ($^{87}\text{Sr}/^{86}\text{Sr}$) and stable ($\delta^{88}\text{Sr}$) strontium isotope systematics in a controlled feeding study

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ABSTRACT

Transhumance and palaeodiet are two central themes in archaeology and using chemical analysis of bones and teeth to reconstruct trends and patterns in diet and mobility has become a cornerstone of bioarchaeology. This study has investigated strontium concentration ([Sr]), radiogenic ($^{87}\text{Sr}/^{86}\text{Sr}$) and stable strontium ($\delta^{88}\text{Sr}$) isotope systematics in a controlled feeding experiment on domestic pigs designed to simulate terrestrial versus marine protein consumption. The results of the radiogenic ($^{87}\text{Sr}/^{86}\text{Sr}$) analysis offer a validation of the strontium isotope methodology. The study confirms that the radiogenic strontium isotope composition of dental enamel does represent the radiogenic strontium isotope composition of the diet. The results of the $\delta^{88}\text{Sr}$ analysis have revealed a distinct shift of 0.322 ± 0.060 ‰ towards isotopically light Sr with trophic level. The magnitude of this shift is consistent with the predictions from the analogous shift observed in calcium isotopes. This is the first time that trophic level fractionation in $\delta^{88}\text{Sr}$ has been identified in a controlled setting. Although still in its infancy, $\delta^{88}\text{Sr}$ analysis has great potential to inform on trophic level systematics, to investigate dietary trends in early life and is potentially useful in examining diagenetic alteration.

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Introduction

Provenance and palaeodietary reconstruction are two central themes in (bio)archaeology and the use of isotopic techniques to elucidate trends and patterns in these areas has become a cornerstone of bioarchaeological research. Use of the mass fractionation corrected ratio of ^{87}Sr to ^{86}Sr abundances, hereafter radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ or simply $^{87}\text{Sr}/^{86}\text{Sr}$, in dental enamel to provenance the geographical source of dietary Sr in order to assess mobility in archaeological populations is now an established technique (Bentley, 2006; Ericson, 1985; Price et al., 2002; Sealy et al., 1991). Studies using this technique have a broad scope, ranging from those assessing a few individuals from a single site to answer specific questions (e.g. Taylor et al., 2013; Montgomery, Budd, and Evans 2000) to larger scale regional studies aimed at investigating broader social trends at the population level (e.g. Haverkort et al., 2008; Bentley et al., 2012). There has also been an increasing use of this technique to study the provenance and management strategies of archaeological fauna (Viner et al., 2010; Knipper 2011; Stephan et al., 2012).

Strontium isotopes do not as yet have a substantial role in the field of palaeodietary reconstruction. This is principally addressed through analysis of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in bone collagen (DeNiro and

Epstein, 1978; Van der Merwe and Vogel, 1978; Chisholm et al., 1982; Schoeninger et al., 1983; Schoeninger and DeNiro, 1984; Nehlich et al., 2011), as well as $\delta^{13}\text{C}$ in bone and tooth apatite structural carbonate ($\delta^{13}\text{C}_{\text{SC}}$) (Lee Thorpe and Van der Merwe, 1991) and, to a lesser extent, by analysis of other stable isotopes in calcified tissues e.g. $\delta^{44}\text{Ca}$ (Skulan and DePaolo, 1999; Reynard et al., 2010). The range of isotopic techniques available for archaeologists studying palaeodiet has recently been expanded by the measurement of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on the individual amino acids which collagen comprises (Howland et al., 2003; Jim et al., 2003). Amino acid analysis has been shown to be particularly useful in resolving marine protein consumption in archaeological populations where aridity and the presence of C_4 plants render bulk collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements ineffective (Corr et al., 2005; Styring et al., 2010; Styring et al., 2015).

Analysis of strontium concentrations in calcified tissues, expressed relative to the major element calcium, has been used as a palaeodietary proxy (Sealy, 2001). The technique relies on biogeochemical cycling of strontium from the biosphere into the food chain but exploits the successive decrease in the elemental Sr/Ca ratio of calcified tissues with trophic level as a result of Ca biopurification in the digestive track

(Comar et al., 1957; Sillen, 1981; Elias et al., 1982). This has been used to assess the relative proportion of plant and meat components in an individual's diet (Toots and Voorhies, 1965; Burton and Wright, 1995; Burton et al., 1999; Balter et al., 2012).

Initial work on biogeochemical cycling of strontium utilised natural experiments in ecosystems (Price et al., 1985) and small scale laboratory experiments (Price et al., 1986). Much of this work focused on assessing the magnitude and controls on Sr/Ca discrimination with trophic level and this work was subsequently incorporated into $^{87}\text{Sr}/^{86}\text{Sr}$ methodologies for migration studies. Whilst the validity of $^{87}\text{Sr}/^{86}\text{Sr}$ in assigning geographical provenance to individuals has been proven in archaeological and modern studies (e.g. Britton et al., 2009; Tütken et al., 2011) we are aware of no published study to date that has attempted to demonstrate this under controlled conditions.

Strontium isotopes undergo mass-dependent fractionation in nature. Although the magnitude of the fractionation is small compared to light stable isotope systems (e.g. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), Sr isotopes do fractionate in a mass-dependent manner as a result of small differences in the thermodynamic properties of individual isotopes which are an inherent function of their mass (Urey, 1947). In radiogenic strontium isotope ($^{87}\text{Sr}/^{86}\text{Sr}$) studies, any mass-dependent fractionation that occurs both in nature and during analysis is corrected for by normalisation to a fixed $^{86}\text{Sr}/^{88}\text{Sr}$ ratio (0.1194, Nier 1938) using an assumed mass-fractionation law. Thus, mass-dependent strontium isotope fractionation does not compromise mobility studies using $^{87}\text{Sr}/^{86}\text{Sr}$.

Measurements of the mass-dependent isotopic fractionation of strontium, $\delta^{88}\text{Sr}$, have been used to study the composition of the early solar system as preserved in meteorites (Patchett, 1980a; Patchett, 1980b; Moynier et al., 2010; Charlier et al., 2012) and used alongside $^{87}\text{Sr}/^{86}\text{Sr}$ as a geological tracer (Pearce et al., 2015; Pearce et al., 2015). $\delta^{88}\text{Sr}$ has been identified as a potential palaeo-thermometer with studies showing variation in the magnitude of $\delta^{88}\text{Sr}$ fractionation between seawater and cold water corals as a function of ocean temperature (Fietzke and Eisenhauer, 2006; Rüggeberg et al., 2008; Krabbenhöft et al., 2010; Vollstaedt et al., 2014; Stevenson et al., 2014). $\delta^{88}\text{Sr}$ has also been measured in fossil apatites (Conodonts) with such samples having the potential to be highly enriched in ^{86}Sr with $\delta^{88}\text{Sr}$ values in excess of -1 ‰ (Neymark et al., 2014).

Strontium isotopes have also been shown to undergo mass-dependent fractionation between soils and plants growing in those soils with plants being enriched in the lighter ^{86}Sr isotope by approximately 0.3 ‰ (de Souza et al., 2010).

Recently, $\delta^{88}\text{Sr}$ has been introduced as a potential palaeodietary tracer for archaeology by Knudson

et al., (2010). Building on geological studies of strontium isotope fractionation, and by analogy with calcium isotope fractionation, Knudson et al., posited that Sr should also undergo mass-dependent fractionation with trophic level. Knudson et al. tested this hypothesis on series of Chiribaya-affiliated sites from southern Peru. Their results showed an approximately 0.3 ‰ difference in $\delta^{88}\text{Sr}$ between molluscs and both small and large herbivores confirming the potential for $\delta^{88}\text{Sr}$ as a palaeodietary tracer.

Regarding tracking trophic level with $\delta^{15}\text{N}$, it has been apparent for some time that for a full understanding of the contribution of plant versus meat protein in the diet it is essential to have an accurate estimate of $\Delta^{15}\text{N}_{\text{diet-tissue}}$ (the magnitude of the trophic level shift) (Hedges and Reynard, 2007; O'Connell et al., 2012). Furthermore, it is also necessary to know how $\Delta^{15}\text{N}_{\text{diet-tissue}}$ can vary with dietary protein source (e.g. marine vs. terrestrial) and total amount of protein in the diet. Although $\delta^{88}\text{Sr}$ should track trophic level across the whole diet rather than just the protein component, similarly to $\delta^{13}\text{C}_{\text{SC}}$, the potential for misinterpretation remains unless $\Delta^{88}\text{Sr}_{\text{diet-tissue}}$ can be adequately constrained.

In this study we present strontium concentration ([Sr]), Sr/Ca, $^{87}\text{Sr}/^{86}\text{Sr}$, and $\delta^{88}\text{Sr}$ determinations made on a series of pigs raised in a controlled feeding study in order to (i) demonstrate under controlled conditions a fixed relationship between $^{87}\text{Sr}/^{86}\text{Sr}_{\text{diet}}$ and $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ and (ii) investigate and characterize the fractionation of $\delta^{88}\text{Sr}$ with trophic level.

The feeding experiment

The controlled feeding experiment from which the samples for this study were obtained was designed to assess the effect of marine protein consumption on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition of various pig tissues at the bulk and single amino-acid level. A detailed description of the feeding experiment design and results including growth rates, for the pigs are presented elsewhere (Webb et al., 2016; Webb et al., 2017); only brief summary is given here.

All pigs were raised at Harper Adams University (Shropshire, UK). Pigs in the controlled feeding experiment were each fed exclusively on one of five diets of known protein source composition ranging from a 100% marine source (fish meal) to a 100% terrestrial source (soy). The five diets consist of 100%, 50%, 25%, 12.5% and 0% marine protein with the remainder of the dietary protein being made up from the terrestrial source. All diets are nutritionally equivalent, have the same amount of total protein and differ only in the source of the dietary protein (Webb et al., 2016; Webb et al., 2017, Fig. 1).

To ensure the pigs reached equilibrium with the diets the experiment was run over two successive generations.

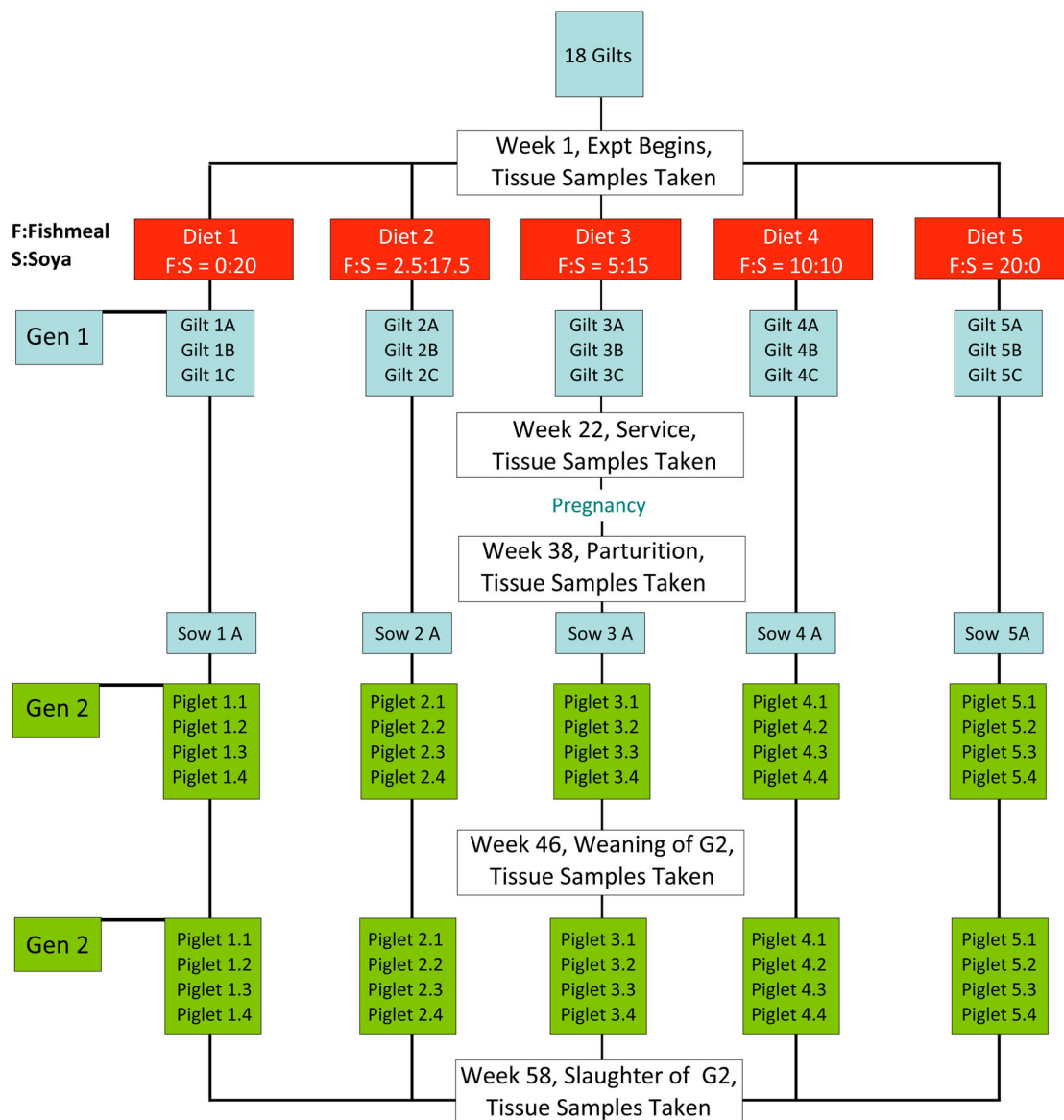


Figure 1. Schematic of the controlled feeding experiment after Webb et al. 2016.

The first generation (G1) gilts (young female pigs that have not yet reproduced), 3 per feeding group, were weaned on to the diets and fed on them until sacrifice as adults (>18 months). The gilts were artificially inseminated and the second generation of pigs (G2) fed on the same test diets as their mothers (including sow milk from the same feeding group) from weaning until they were sacrificed as adolescents in two groups at 5 and a half and 6 and half months old respectively.

Although the feeding experiment represents a complete range of marine to terrestrial protein diets with individuals of a range of ages and with replicated individuals on the same diet it was not initially conceived to examine the uptake of dietary strontium. Therefore feed ingredients were not specifically selected for their strontium isotope compositions prior to the experiment starting. Teeth from the feeding experiment were initially sampled as a potential $^{87}\text{Sr}/^{86}\text{Sr}$ standard for laser ablation (Lewis et al., 2014). It was hoped that the strontium isotope signature of the

terrestrial protein source would differ sufficiently from the marine protein source ($^{87}\text{Sr}/^{86}\text{Sr}$ 0.70918–0.70920, McArthur et al., 2001, $\delta^{88}\text{Sr}$ 0.381 ‰ Fietzke and Eisenhauer, 2006) and that the strontium isotope composition of the pigs on mixed diets would plot between the two end members.

2. Material and methods

2.1 Samples

A range of materials from the feeding experiment have been analysed for [Sr], [Ca], $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88}\text{Sr}$ to assess the dietary uptake of strontium. Each of the 5 feeds were analysed as well as drinking water from a borehole at the Harper Adams site, which is common to all feed groups. Tooth enamel was sampled in 5 G1 adults and 14 G2 adolescents with paired dentine measurements made on 5 of the G2 adolescents. In each case the latest erupting lower left molar was sampled, M₃ for G1 and M₁ for G2. One sample of

faeces was analysed from each feeding group, four of the faeces samples can be paired to individual G2 pigs.

2.2 Sample preparation

Tooth enamel and dentine samples (10–20 mg) were sawn from whole teeth and weighed into clean perfluoroalkoxy (PFA) beakers and dissolved in 7N HNO₃ (Romil SpA). Approximately 500 ml of drinking water from the site was evaporated to dryness in a PFA beaker and the residue dissolved in 1:1 7N HNO₃/6N HCl (Romil SpA).

Faeces were freeze-dried to remove moisture and picked under a microscope to remove straw, which is used for bedding at the Harper Adams site. Approximately 20 mg of faeces were weighed into PFA beakers. Feed pellets (100–200 mg) were crushed in a mortar and pestle and then weighed into PFA beakers. Due to their high organic contents feed and faeces were digested in a high-pressure asher (HPA-S, Anton Paar). Feed and faeces were pre-digested in 3:1 7N HNO₃/6N HCl then transferred to clean quartz glass vials. The vials were sealed and ashed at 300°C and ~100 bar for three hours. Solutions are then transferred back to PFA beakers. Following digestion all samples were evaporated to dryness and re-dissolved in 3N HNO₃.

2.3 Double-spike isotopic tracer

$\delta^{88}\text{Sr}$ determinations are made by using a double-spike isotope tracer (Dodson, 1963; Dodson, 1970). The double-spike tracer method involves the mixing of samples with a tracer artificially enriched in two isotopes. The advantage of this method is that any isotopic fractionation resulting from the sample preparation, chemical separation and mass spectrometry, which also fractionates the tracer, is corrected for. Consequently, incomplete recovery of the strontium, which may fractionate the isotopes, does not affect the $\delta^{88}\text{Sr}$ determination (Rudge et al., 2009).

The tracer, enriched in ^{84}Sr and ^{87}Sr , was prepared from two 'single-spike' solutions, enriched in ^{84}Sr and ^{87}Sr respectively, prepared from isotopically enriched strontium carbonate purchased from Oak Ridge National Laboratory. Solutions of the single-spikes were mixed in such proportions to minimise the uncertainty in the calculated isotope composition of samples in sample-tracer mixtures (Rudge et al., 2009). To back-out the sample composition from a measurement of the mixture, the precise tracer composition must be determined, a procedure called the calibration of the tracer (Coath et al., 2017; Rudge et al., 2009).

The calibration procedure consists of the preparation and analysis of a series of J mixtures of standard and tracer ranging from pure standard to pure tracer. Following Rudge et al., 2009, the instrumental

fractionation corrected measurements must lie on a line in ($^{84}\text{Sr}/^{86}\text{Sr}$, $^{87}\text{Sr}/^{86}\text{Sr}$, $^{88}\text{Sr}/^{86}\text{Sr}$)-space passing through the standard composition. The algorithm solves for the J instrumental fractionation parameters and the unit vector in the direction of the line. The solution for the unit vector is critical to accurate measurements whereas the position on the line where the tracer composition lies is much less so, the latter only having a second-order effect for compositions whose fractionation relative to the standard is small. Nevertheless, the actual tracer composition can be determined as that point on the line lying closest, in a maximum-likelihood sense, from the instrumental fractionation corrected measurement of the pure tracer. Using this method to characterise the tracer, the composition was found to be 48.7815% ^{84}Sr , 2.5144% ^{86}Sr , 38.0902% ^{87}Sr and 10.6139% ^{88}Sr .

Any isotopic fractionation resulting from dissolution may be corrected for by adding the tracer prior to dissolution. However, Lewis (2015) has shown that no measurable fractionation occurs, even when the high-pressure asher is used to dissolve organic-rich samples. Therefore, the tracer is added to an aliquot of the dissolved solution for $\delta^{88}\text{Sr}$ determinations and further aliquots of the same solution are used for the [Sr] and $^{87}\text{Sr}/^{86}\text{Sr}$ determinations. Double-spike is added to the sample to give a final spike/sample proportion of approximately 0.3:0.7, which minimises the uncertainty in the calculated sample isotopic composition (Rudge et al., 2009).

2.4 Sample chemistry and mass spectrometry

Strontium separation was accomplished using Sr Spec resin (Horwitz et al., 1992). Approximately 100 μl of pre-cleaned resin was loaded into PFA micro-columns. Between 100 and 500 ng of strontium was loaded on to columns in 0.5 ml of 3N HNO₃, matrix was eluted in 2 ml of 3N HNO₃ and Sr collected in 1.5 ml of ultra-pure water. Separate micro-columns were used for $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88}\text{Sr}$ samples.

For [Sr] and [Ca] determinations 5 μL of the final dissolved solution was taken, 25 μL of 200 ppb indium solution added as an internal standard and the solution was then diluted to a final volume of 2 ml with 0.3N HNO₃. Concentrations were measured on a Thermo-Electron Element 2 ICPMS, internally normalised to indium and quantified by external calibration relative to a gravimetrically prepared in-house multi-element standard.

The determinations of $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88}\text{Sr}$ were made following the procedure outlined in Lewis et al. (2014), purified Sr was loaded onto rhenium filaments with a TaCl₅ activator (Birck, 1986). Strontium isotope ratios were measured on a Thermo-Finnigan Triton thermal ionisation mass spectrometer. Amplifier gains were calibrated before each analytical session and all isotope ratios were determined in static collection mode with a

4.194 s integration time per cycle and 12 cycles per block, ^{87}Sr beams were corrected for isobaric ^{87}Rb by monitoring ^{85}Rb and using a $^{85}\text{Rb}/^{87}\text{Rb}$ ratio of 2.59265 modified to approximate the effect of instrumental mass bias (Steiger and Jäger, 1977).

For $^{87}\text{Sr}/^{86}\text{Sr}$, data were collected as 30 blocks and corrected for mass fractionation using an exponential mass bias law and a $^{86}\text{Sr}/^{88}\text{Sr}$ of 0.1194 (Nier, 1938; Russell et al., 1978). All data were externally normalised by measuring NIST SRM 987 and assuming a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.710248 (Avanzinelli et al., 2005). Long-term reproducibility of $^{87}\text{Sr}/^{86}\text{Sr}$ for NIST SRM 987 analyses (not externally normalised) was 0.710232 ± 0.000036 (2 SD, $n = 146$) and a modern seal tooth 0.709180 ± 0.000033 (2 SD, $n = 25$).

For the $\delta^{88}\text{Sr}$ analyses, data were collected as 75 blocks and the sample fractionation solved using the double-spike inversion function provided in the 'Double Spike Toolbox' (Rudge et al., 2009) running under MATLAB. All data were externally normalised to measured NIST SRM 987 and fractionation of the $^{86}\text{Sr}/^{88}\text{Sr}$ reported in permil deviation from NIST SRM 987 with $(^{86}\text{Sr}/^{88}\text{Sr})_{\text{SRM987}} = 0.1194$. Long-term reproducibility of $\delta^{88}\text{Sr}$ for NIST SRM 987 was $-0.0016 \text{‰} \pm 0.031 \text{‰}$ (2 SD, $n = 87$) and for a modern seal tooth $-0.2383 \text{‰} \pm 0.033 \text{‰}$ (2 SD, $n = 12$).

3. Results

3.1 Sr concentration and Sr/Ca ratio

For all substrates except drinking water, there is a strong positive correlation (PCC, $R^2 > 0.96$, $p < 0.001$) between the marine protein content of the diet and the [Sr] of the substrate (Fig. 2, table 1a). This is consistent with the marine protein source (fish meal) being enriched in Sr relative to the terrestrial protein source (soya) and the remaining non-protein components of the diets which is identical in each feeding group.

The pig feed has low [Sr] ranging from 13 ppm ($\mu\text{g/g}$) in the 100% terrestrial protein diet to 36 ppm in the 100% marine protein diet. Dental tissues, with [Sr] ranging from 75 ppm to 300 ppm, are concentrated in Sr relative to feed. This is consistent with strontium in mammals being concentrated in calcified tissues. For each feeding group the [Sr] of the faeces is enriched over the feed and has [Sr] similar to teeth. This is initially surprising given the low [Sr] of the feed however absorption via the gut discriminates against strontium relative to nutrients from the bulk feed hence digestion concentrates strontium in the faeces.

The correlation between [Sr] and the marine protein content of the diet is mirrored in the Sr/Ca ratio (expressed as $\text{Sr}/\text{Ca} \times 1000$) of the pig feed and pig dental tissues (PCC, $R^2 > 0.98$, $p < 0.001$, table 1b). However, irrespective of the initial Sr/Ca of the individual diets a consistent decrease was observed in Sr/Ca between the feed and the dental tissues of 0.242 ± 0.044 (2 SD, $n = 5$) as a result of calcium biopurification. This value is within the uncertainty of the canonical 5 fold decrease in Sr/Ca with trophic level and in good agreement with the range of values in the literature for this decrease (Elias et al., 1982; Sillen and Kavanagh, 1982; Burton et al., 2003).

There has been some debate regarding the investigation of resource consumption with Sr/Ca ratios (Schoeninger and Peebles, 1981; Burton and Price, 1999). Although these new data would suggest that marine consumption could be detected with Sr/Ca ratio we do not make this assertion. We consider that the correlation between marine protein content and Sr/Ca ratio is a result of ingredient selection for the experimental feeds. We further note that in our feeds only the protein component of the diet is substituted for a marine source, in cases of marine resource exploitation the whole diet would be from a marine source. Therefore, these results are not relevant in determining if Sr/Ca can be used to detect marine resource consumption.

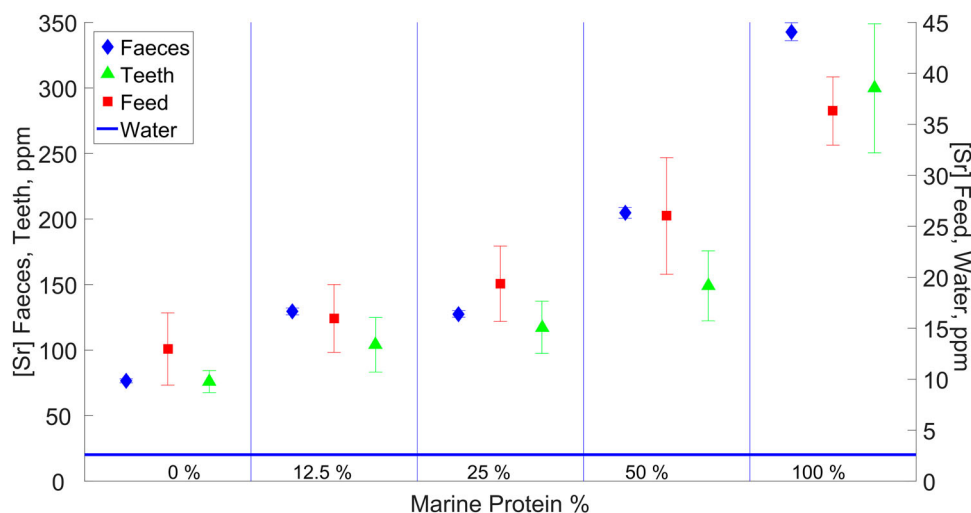


Figure 2. Strontium concentrations for feed, teeth, water and faeces from the five feeding groups in the feeding experiment.

Table 1. Tabulated strontium concentration, Sr/Ca × 1000, ⁸⁷Sr/⁸⁶Sr and δ⁸⁸Sr results from the feeding experiment.

| a. Strontium concentration and 2 σ uncertainty (ppm) | | | | | | | | | | | |
|---|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|
| Marine Protein % | | | | | | | | | | | |
| | 12.5 | 25 | 50 | 100 | | | | | | Mean | 2 SD |
| 0 | | | | | | | | | | | |
| Feed | | | | | | | | | | | |
| 12.96 | 3.55 | 15.96 | 3.32 | 19.37 | 3.69 | 26.01 | 5.72 | 36.31 | 3.34 | | |
| G2 teeth | | | | | | | | | | | |
| 75.95 | 8.40 | 104.03 | 20.79 | 117.36 | 19.90 | 149.01 | 26.69 | 299.73 | 49.18 | | |
| Faeces | | | | | | | | | | | |
| 76.57 | 1.53 | 129.53 | 2.59 | 127.58 | 2.55 | 204.74 | 4.09 | 342.90 | 6.86 | | |
| b. Sr/Ca × 1000 and 2σ uncertainty | | | | | | | | | | | |
| Marine Protein % | | | | | | | | | | | |
| | 12.5 | 25 | 50 | 100 | | | | | | Mean | 2 SD |
| 0 | | | | | | | | | | | |
| Feed | | | | | | | | | | | |
| 0.948 | 0.112 | 1.352 | 0.136 | 1.629 | 0.155 | 2.230 | 0.231 | 3.233 | 0.205 | | |
| G2 teeth | | | | | | | | | | | |
| 0.251 | 0.064 | 0.356 | 0.061 | 0.384 | 0.068 | 0.473 | 0.060 | 0.755 | 0.041 | | |
| Sr/Ca _{teeth/diet} | | | | | | | | | | | |
| 0.265 | | 0.263 | | 0.236 | | 0.212 | | 0.234 | | 0.242 | 0.044 |
| c. ⁸⁷ Sr/ ⁸⁶ Sr and 2σ uncertainty in ppm | | | | | | | | | | | |
| Marine Protein % | | | | | | | | | | | |
| | 12.5 | 25 | 50 | 100 | | | | | | Mean | 2 SD |
| 0 | | | | | | | | | | | |
| Feed | | | | | | | | | | | |
| 0.709092 | 12.2 | 0.709063 | 10.9 | 0.709052 | 19.1 | 0.709061 | 13.8 | 0.709011 | 19.6 | 0.709056 | 58.4 |
| G1 teeth | | | | | | | | | | | |
| 0.709124 | 12.4 | 0.709097 | 9.2 | 0.709074 | 6.2 | 0.709121 | 7.9 | 0.709058 | 16.4 | | |
| G2 teeth | | | | | | | | | | | |
| 0.709098 | 72.9 | 0.709048 | 17.4 | 0.709061 | 12.3 | 0.709078 | 5.4 | 0.709076 | 59.0 | 0.709089 | 75.3 |
| 0.709144 | 8.5 | 0.709108 | 13.4 | 0.709041 | 6.6 | 0.709143 | 6.2 | 0.709072 | 5.7 | | |
| 0.709178 | 12.4 | 0.709061 | 6.5 | 0.709064 | 5.7 | 0.709139 | 5.6 | 0.709047 | 6.3 | | |
| | | 0.709106 | 10.7 | 0.709032 | 5.8 | | | 0.709075 | 4.9 | | |
| | | | | 0.709094 | 5.6 | | | | | | |
| Faeces | | | | | | | | | | | |
| 0.709077 | 6.1 | 0.709103 | 9.9 | 0.709074 | 17.9 | 0.709010 | 12.1 | 0.709013 | 8.1 | 0.709055 | 83.3 |
| Water | | | | | | | | | | | |
| | | | | 0.711572 | 6.0 | | | | | | |
| d. δ ⁸⁸ Sr and 2σ uncertainty in ‰ | | | | | | | | | | | |
| Marine Protein % | | | | | | | | | | | |
| | 12.5 | 25 | 50 | 100 | | | | | | Mean | 2 SD |
| 0 | | | | | | | | | | | |
| Feed | | | | | | | | | | | |
| 0.115 | 0.010 | 0.088 | 0.026 | 0.095 | 0.024 | 0.132 | 0.014 | 0.061 | 0.009 | 0.098 | 0.054 |
| G1 teeth | | | | | | | | | | | |
| -0.255 | 0.007 | -0.225 | 0.006 | -0.254 | 0.007 | -0.232 | 0.009 | -0.190 | 0.008 | | |
| G2 teeth | | | | | | | | | | | |
| -0.228 | 0.019 | -0.211 | 0.019 | -0.256 | 0.008 | -0.227 | 0.011 | -0.260 | 0.005 | -0.224 | 0.044 |
| -0.207 | 0.003 | -0.206 | 0.004 | -0.231 | 0.010 | -0.200 | 0.018 | -0.231 | 0.002 | | |
| -0.201 | 0.022 | -0.184 | 0.006 | -0.212 | 0.003 | -0.246 | 0.005 | -0.229 | 0.003 | | |
| | | -0.199 | 0.003 | -0.241 | 0.009 | | | | | | |
| | | | | -0.218 | 0.004 | | | | | | |
| Δ ⁸⁸ Sr _{diet-teeth} | | | | | | | | | | | |
| 0.337 | | 0.293 | | 0.331 | | 0.359 | | 0.289 | | 0.322 | 0.060 |
| Faeces | | | | | | | | | | | |
| 0.130 | 0.015 | 0.150 | 0.007 | 0.112 | 0.021 | 0.113 | 0.005 | 0.131 | 0.009 | 0.127 | 0.031 |
| Water | | | | | | | | | | | |
| | | | | 0.274 | 0.005 | | | | | | |

3.2 Radiogenic strontium (⁸⁷Sr/⁸⁶Sr)

Dental tissues from the feeding experiment have a mean ⁸⁷Sr/⁸⁶Sr of 0.709089 ± 0.000075 (2 SD, n = 25) whilst the pig feed and faeces have a mean ⁸⁷Sr/⁸⁶Sr of 0.709056 ± 0.000058 and 0.709055 ± 0.000083 (both 2 SD, n = 5) respectively (Fig 3, table 1c). Thus, there is no significant difference in ⁸⁷Sr/⁸⁶Sr between the pig teeth and either the diets or the faeces (Kruskal-Wallis, p = 0.132). In addition, there is no difference between the G1 or G2 pigs indicating that both generations of the feeding experiment are at equilibrium with the dietary strontium (Mann-Whitney, p = 0.644). The agreement between dietary ⁸⁷Sr/⁸⁶Sr and ⁸⁷Sr/⁸⁶Sr in dental tissues supports the current use of strontium isotopes

in dental enamel as a proxy for dietary strontium at the time of mineralisation.

Across all substrates there is no correlation between a modern marine ⁸⁷Sr/⁸⁶Sr ratio (0.70918–0.70920, McArthur et al., 2001) and the marine protein content of the diets. Two reasons are postulated for this.

I) The bioavailable ⁸⁷Sr/⁸⁶Sr of the area where the soya was grown may be similar to that of modern seawater and thus the isotopic difference between the marine and terrestrial protein sources is minimal which is quite possible if the soya was grown in a soil derived from a young carbonate geology. Unfortunately, we do not know the provenance of the soya. Furthermore soya is added as ground soya flour thus we cannot rule out that the soya used in the feeds is

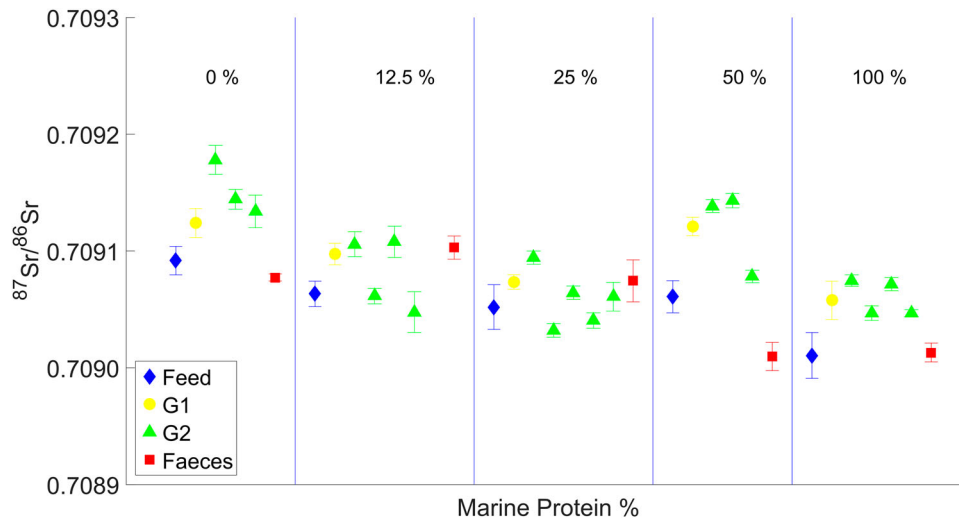


Figure 3. $^{87}\text{Sr}/^{86}\text{Sr}$ in feed, G1 teeth, G2 teeth and faeces from the pig feeding experiment.

from multiple locations, with different bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$, which are then homogenised during processing. This unsatisfactory from a Sr isotope perspective but was acceptable for the C and N isotope systems the feeding experiment was originally designed to investigate. In this case the terrestrial end member was required to have a C_3 carbon isotope signature (soya is a C_3 plant) in order to be distinct from the marine end member.

II) The mean $^{87}\text{Sr}/^{86}\text{Sr}$ of the non-protein component (principally starch, calcium phosphate, wheat fibre and vitamin/mineral supplements) of the diet is similar to that of seawater and that the contribution of this Sr to the total diet means that the signal from the terrestrial protein source is not well resolved in the dietary mean. Indeed, the above scenarios are not mutually exclusive and that the soya may or may not have a modern seawater $^{87}\text{Sr}/^{86}\text{Sr}$ value but also may be depleted in Sr such that it contributes very little Sr to the dietary average. In either case, both scenarios account for the observation that Sr from the fish meal is detected in the [Sr] but not in $^{87}\text{Sr}/^{86}\text{Sr}$. It is unfortunate that the marine and terrestrial diets are so similar in $^{87}\text{Sr}/^{86}\text{Sr}$ that this study offers little insight into how the $^{87}\text{Sr}/^{86}\text{Sr}$ of dental tissues in terrestrial animals is affected by marine resource consumption.

Across all feed groups, G1 and G2 pig teeth have marginally more radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ than their respective feeds. Although this difference is not statistically significant (see above), this small offset is attributed to the contribution of strontium from the pigs' drinking water, which is more radiogenic than the feeds ($^{87}\text{Sr}/^{86}\text{Sr}$, 0.711572). Taking the mean $^{87}\text{Sr}/^{86}\text{Sr}$ of the feed and the dental tissues, water contributes only 1.4% of the strontium by mass and, even in the most radiogenic tooth observed, water contributes <5% of the total strontium. It is also noted that this result is for a 2.6 ppm Sr water source, which is reasonably concentrated, the mean values for ground waters

in England and Wales is 0.64 ppm with a 5 to 95% range of 0.02 ppm to 5 ppm (Shand et al., 2007).

3.3 Stable strontium ($\delta^{88}\text{Sr}$)

The pig feeds and faeces have mean $\delta^{88}\text{Sr}$ of 0.098 ± 0.054 ‰ and 0.127 ± 0.031 ‰ (both 2 SD, $n = 5$) respectively. The pig dental tissues have mean $\delta^{88}\text{Sr}$ of -0.224 ± 0.044 ‰ (2 SD, $n = 18$) (Fig. 4, table 1d), which is 0.322 lower than the feed. As with $^{87}\text{Sr}/^{86}\text{Sr}$, there is no significant difference between the G1 and G2 teeth (Mann-Whitney, $p = 0.412$).

The mean $\Delta^{88}\text{Sr}_{\text{diet-teeth}}$ of 0.322 ± 0.060 ‰ (2 SD) is attributed to a trophic level fractionation in strontium isotopes. The magnitude of the observed fractionation is in excellent agreement with predictions from the analogous fractionation which has been observed in $\delta^{44/40}\text{Ca}$. Skulan and DePaolo (1999) have demonstrated that $\Delta^{44/40}\text{Ca}_{\text{diet-bone}}$ is 1.3 ‰. Based on the mass difference between Ca and Sr isotopes for a 1.3 ‰ shift in $\delta^{44/40}\text{Ca}$ a kinetic fractionation law predicts a 0.314 ‰ fractionation in $\delta^{88/86}\text{Sr}$, which is within the uncertainty of the observed fractionation. This measurement of the magnitude of the fractionation is also consistent with the estimations of de Souza et al. (2010) and Knudson et al. (2010) who have both shown that $\Delta^{88}\text{Sr}$ is approximately 0.3 ‰.

As with $^{87}\text{Sr}/^{86}\text{Sr}$, no trend is observed towards a modern seawater $\delta^{88}\text{Sr}$ (0.381 ‰, Fietzke and Eisenhauer, 2006) with increasing marine protein content of the diet. However, this may not necessarily be expected as the fish meal will itself be fractionated from that of modern seawater and the remainder of the diet and/or the terrestrial protein source have a $\delta^{88}\text{Sr}$ value sufficiently similar to that of the fish meal that no trend may be observed. Measurements of the individual ingredients that comprise the feeds may elucidate this.

The five samples of faeces have $\delta^{88}\text{Sr}$ within the uncertainty of the feeds. One might expect excreta to

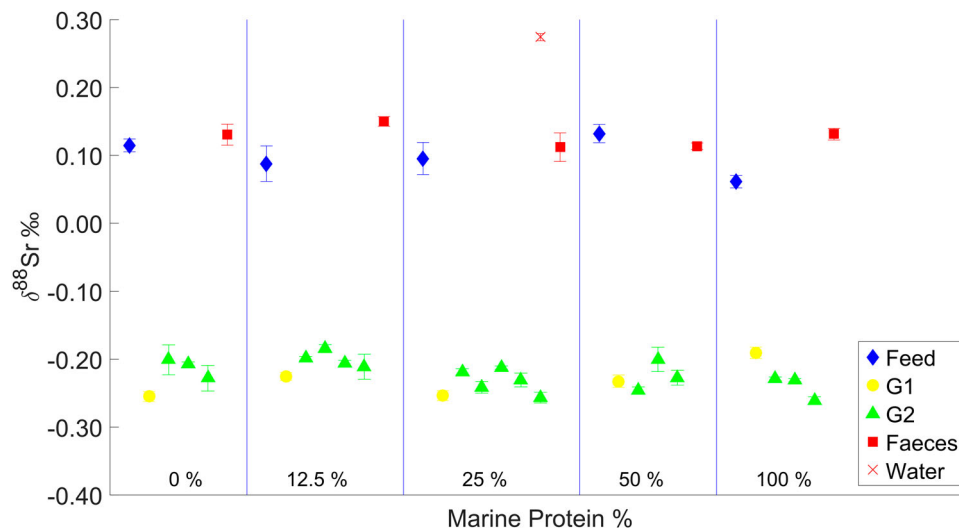


Figure 4. $\delta^{88}\text{Sr}$ in feed, water, G1 adult teeth, G2 adolescent teeth and faeces from the pigs in the feeding experiment.

be elevated in $\delta^{88}\text{Sr}$ to balance fractionation observed in the calcified tissue. However, there are two points to consider. First, from the $[\text{Sr}]$ and Sr/Ca results it is clear that a large proportion of the dietary strontium is not absorbed into the body. Second, by analogy with calcium isotopes, it is suspected that $\delta^{88}\text{Sr}$ fractionation occurs during mineralisation when hydroxyapatite crystals are precipitated. Thus, strontium that is not absorbed from the gut will remain un-fractionated and obscure the signal from any isotopically heavy strontium being excreted. Furthermore, if Sr fractionation does occur at the point of mineralisation then the pig urine is a more likely candidate for the isotopic mass balance as the isotopically heavy Sr resulting from the mineralisation will be removed as urine rather than faeces.

4. Discussion

4.1 Defining the local bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ and the minimum $^{87}\text{Sr}/^{86}\text{Sr}$ variation in a population

At the broadest scale this study offers a validation of the archaeological $^{87}\text{Sr}/^{86}\text{Sr}$ method. The excellent agreement between $^{87}\text{Sr}/^{86}\text{Sr}_{\text{diet}}$ and $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ confirm that with respect to $^{87}\text{Sr}/^{86}\text{Sr}$ “you are what you assimilate”. Whilst the validity of this assertion has been proven in both archaeological contexts and modern natural experiments this is the first study where this has been demonstrated under controlled conditions.

Beyond the validation of the $^{87}\text{Sr}/^{86}\text{Sr}$ method this study can also be used to inform on the minimum variation that can be expected from the local $^{87}\text{Sr}/^{86}\text{Sr}$. Numerous methods have been used to estimate the local bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ range; from measurements of the geology (Budd et al., 2004; Copeland et al., 2010), measurements of plants, soils, and water sources (Bentley et al., 2004; Hodell et al., 2004; Evans et al., 2009; Evans et al., 2010) and the use of paired enamel and dentine

samples (Montgomery et al., 2000; Taylor et al., 2013). Of these, no single technique has risen to dominance.

The results from the feeding experiment can be used to answer a more subtle question, namely what is the minimum variation in $^{87}\text{Sr}/^{86}\text{Sr}$ that can be expected from two individuals raised on identical diets in the same location? Given that the five feeds are isotopically identical and all feeding groups have the same water source, each of the 19 individuals can be said to have consumed a single identical diet. Across all feeding groups the G1 and G2 pig teeth have a 2 standard deviation variability of 75 ppm and thus a conservative estimate of 100 ppm for the minimum range could be made, and it could be argued that two individuals that differ by less than this cannot be distinguished on the basis of their $^{87}\text{Sr}/^{86}\text{Sr}$ alone.

However, factors outside the controlled setting of this experiment may contribute additional variability to $^{87}\text{Sr}/^{86}\text{Sr}$ such as unknown metabolic differences between individuals, and different taxa or the differences in the quantity of food and water consumed, given that there may be isotopic difference between the two. However, this is likely also to be true in most archaeological contexts, especially where there may be multiple water sources (e.g. spring vs. rain-water). We therefore feel it is unlikely that for archaeological studies two locals will exhibit less variability than the c.100 ppm we see in this study.

4.2. The potential for $\delta^{88}\text{Sr}$ in archaeology

The potential for more precisely tracking the dietary source of strontium using $\delta^{88}\text{Sr}$ and determining the trophic level of marine and terrestrial resources has already been highlighted by Knudson et al. (2010). Here we expand on the range of possibilities for $\delta^{88}\text{Sr}$.

Strontium has no biological function in mammals and is only present as an impurity associated with Ca (Pors Nielsen, 2004). Therefore $\delta^{88}\text{Sr}$ may be robust

against the effects of malnutrition and aridity, which have been shown to be problematic for $\delta^{15}\text{N}$, as N is biologically essential (Sealy et al., 1987; Fuller et al., 2005; Hedges and Reynard, 2007). The experimental results, shown in table 1, reveal no trend in magnitude of $\Delta^{88}\text{Sr}_{\text{diet-teeth}}$ with the [Sr] of the diet over the range of [Sr] in the experimental feeds. Thus, $\delta^{88}\text{Sr}$ may be less susceptible to changes in the dietary [Sr] than $\delta^{15}\text{N}$ is to changes in the protein content of the diet, especially in cases of malnutrition. Further, if $\delta^{88}\text{Sr}$ values in faeces can be shown to be similar to that of diet in other animals, especially ruminants, then $\delta^{88}\text{Sr}$ may be less sensitive to manuring which has been shown to increase the $\delta^{15}\text{N}$ baseline (Bogaard et al., 2007).

$\delta^{88}\text{Sr}$ determinations are made on the mineral fraction of tooth enamel and this may have the following advantages.

- (i) Tooth enamel survival in the archaeological record is generally robust and therefore $\delta^{88}\text{Sr}$ may be useful when bone collagen survival is poor, such as in very wet or arid environments (Nielsen-Marsh et al., 2007; Smith et al., 2007).
- (ii) $\delta^{88}\text{Sr}$ should be representative of the whole diet, similar to $\delta^{13}\text{C}_{\text{SC}}$, thus $\delta^{88}\text{Sr}$ should represent the trophic level of the whole diet (with individual components weighted by their Sr concentrations) and not just the protein portion. It is hoped that $\delta^{88}\text{Sr}$ may be used for a more complete assessment of dietary trophic level, but that $\delta^{88}\text{Sr}$ may prove to be robust against the problems caused by different digestive physiologies in different animals which give rise to variable trophic level effects in between $\delta^{13}\text{C}_{\text{SC}}$ and $\delta^{13}\text{C}_{\text{diet}}$ (Lee-Thorpe, 2008; Passey et al., 2005).
- (iii) Measurements of $\delta^{88}\text{Sr}$ on tooth enamel will give information on the dietary trophic level at the time in life when the enamel was being mineralised as enamel does not remodel once mineralised (Budd et al., 2000). This means that $\delta^{88}\text{Sr}$ will give dietary information on early life and through adolescence. Such information may not necessarily be available to archaeologists using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen although recent work on dentine collagen may address this (Beaumont et al., 2013; Sandberg et al., 2014).
- (iv) Sequential mineralisation of individual teeth in a dental arcade and the sequential mineralisation of an individual tooth from the tooth crown to root represent a time resolved signal. Inter-tooth measurements of $\delta^{88}\text{Sr}$ in a dental eruption sequence or intra-tooth measurements of $\delta^{88}\text{Sr}$ along the growth axis of an individual tooth, where the crown height permits, could be used to address changes in trophic level within an individual's lifetime. Specifically this might be used to investigate the effects of weaning.

- (v) $\delta^{88}\text{Sr}$ could be of potential use in the detection of diagenesis in bone and enamel. Bone and dentine have been shown to be susceptible to Sr diagenesis whilst enamel is more resistant (Budd et al., 2000; Trickett et al., 2003; Hoppe et al., 2003). One of the critical considerations in accurately detecting diagenesis has been identifying samples where accurate *a priori* assumptions about the biogenic and diagenetic $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can be made. This has mainly limited studies of diagenetic uptake to marine mammals which have been buried in soils with an $^{87}\text{Sr}/^{86}\text{Sr}$ different from that of modern seawater (e.g. Nelson et al., 1986). However, if one considers that $\delta^{88}\text{Sr}$ fractionates with trophic level then there should be a large difference in $\delta^{88}\text{Sr}$ between the buried bone/tooth and the burial soil, which should be at least two, possibly three, trophic levels. Furthermore, these differences will be systematic and should exist in bones and teeth even when the biogenic and the diagenetic $^{87}\text{Sr}/^{86}\text{Sr}$ are equivalent.

As a counterpoint to the potential benefits of $\delta^{88}\text{Sr}$ there may be some potential concerns for working with $\delta^{88}\text{Sr}$.

- (i) The potential of $\delta^{88}\text{Sr}$ relies on understanding and characterising the variation in geological and bioavailable $\delta^{88}\text{Sr}$. To some extent variation in the geological $\delta^{88}\text{Sr}$ within a site catchment may be muted by the same processes of differential weathering of rocks and subsequent preferential leaching of Sr from soils to soil pore waters that reduces the variation in $^{87}\text{Sr}/^{86}\text{Sr}$ (Capo et al., 1998; Sillen et al., 1998; Price et al., 2002). If this is the case, then current methods for determining the local bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$, such as measuring local plant, soils, water and archaeological small fauna, should be sufficient to characterise the local $\delta^{88}\text{Sr}$. However this assumption needs to be tested and more detailed environmental and trophic level studies, particularly using natural experiments would be invaluable.
- (ii) Wider scale variation in geological $\delta^{88}\text{Sr}$ may still be problematic when comparing between sites. The presently available studies on geological $\delta^{88}\text{Sr}$ have already demonstrated that $\delta^{88}\text{Sr}$ varies between rock types (Ohno et al., 2008; Charlier et al., 2012; Pearce et al., 2015) and that in rocks derived from oceanic carbonates (e.g. Limestone) variation will be systematic with the temperature of the water that the carbonate was precipitated from (Fietzke and Eisenhauer, 2006; Rüggeberg et al., 2008; Vollstaedt et al., 2014). It may be the case that $\delta^{88}\text{Sr}$ suffers from the same limitation as Sr/Ca and whilst $\delta^{88}\text{Sr}$ may be effective at a single site, comparisons between

sites on different geologies may be problematic. Hopefully, careful characterisation of the bioavailable $\delta^{88}\text{Sr}$ can mitigate this.

- (iii) Linked to the variation in geological/bioavailable $\delta^{88}\text{Sr}$ signal is the effect that non-local individuals might have on a population. Knudson et al. (2010) correctly point out that $\delta^{88}\text{Sr}$ can be of value in constraining the dietary source of Sr for $^{87}\text{Sr}/^{86}\text{Sr}$ studies. We would add to this that $^{87}\text{Sr}/^{86}\text{Sr}$ is also important in $\delta^{88}\text{Sr}$ studies. Consider, for example, an allochthonous individual buried at an archaeological site. If this individual has been consuming Sr from an area with a different bioavailable $\delta^{88}\text{Sr}$ then this may lead to an incorrect dietary interpretation in the context of the local bioavailable $\delta^{88}\text{Sr}$. Therefore, having an understanding of the provenance of individuals is important where palaeodietary reconstruction is being assessed and where $\delta^{88}\text{Sr}$ is used for this $^{87}\text{Sr}/^{86}\text{Sr}$ and possibly $\delta^{18}\text{O}$ should also be determined. We note that, whilst the use of the double-spike technique to determine $\delta^{88}\text{Sr}$ necessitates the measurement of $^{87}\text{Sr}/^{86}\text{Sr}$, other techniques such as sample standard bracketing, do not necessarily require the measurement of the ^{87}Sr isotope.

5. Conclusion

In this study we have investigated the [Sr], Sr/Ca, $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88}\text{Sr}$ systematics in a controlled feeding experiment, which was designed to test the effect of marine resource consumption on dietary isotopes.

The results of [Sr] analysis have detected a strong trend in both [Sr] and Sr/Ca with the marine protein content of the diet. However, rather than being a useful indicator of marine resource consumption, we believe that this is a fortuitous result of the feed preparations and is not significant outside of this specific study.

The results of the radiogenic Sr isotope analysis confirm under controlled conditions that the isotopic composition of dental tissues, modified marginally by drinking water, is equivalent to that of dietary strontium. Data from the feeding experiment also constrain the minimum variation between two individual pigs raised on identical diets. We conclude that in archaeological contexts for two individuals who differ by less than 100 ppm in $^{87}\text{Sr}/^{86}\text{Sr}$ the most parsimonious explanation is that they are from the same geographical provenance and that they cannot be differentiated by this technique alone.

Results from stable strontium isotopes determined by the double-spike isotopic tracer method have identified a 0.322 ± 0.060 ‰ shift in lighter values of $\delta^{88}\text{Sr}$ from strontium in diet to strontium in dental tissues, across all the control diets, as a result of trophic level fractionation. The magnitude of this fractionation is

consistent with that which would be predicted from the analogous shift observed elsewhere in calcium isotopes. This is the first time this has been determined in a controlled setting.

Although still in its infancy $\delta^{88}\text{Sr}$ has great potential for use in archaeological isotope investigations. $\delta^{88}\text{Sr}$ may be useful for examining trends in palaeodiet in concert with provenance studies using $^{87}\text{Sr}/^{86}\text{Sr}$ or where collagen preservation is poor and for investigating dietary trends in early life. Furthermore with the demonstration of $\delta^{88}\text{Sr}$ fractionation with trophic level $\delta^{88}\text{Sr}$ may be useful distinguishing biogenic and diagenetic strontium. $\delta^{88}\text{Sr}$ may be key in accurately identifying Sr diagenesis in archaeological enamel.

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