CHAPTER 3

MATERIALS AND METHODS

Conventional environmental samples, consisting of stream waters, soils and plant materials are collected routinely by geochemical surveys. They are effectively ubiquitous, offer a high degree of spatial resolution where needed and thus provide the basis of the biosphere $^{87}Sr/^{86}Sr$ characterisation exercises carried out within this thesis (Chapters 4, 5 and 6). The methods used to prepare these materials for analysis are outlined in Section 3.2, and documented as step-wise procedures in Appendix A. Quality control measures are also reported below.

Archaeological faunal (Chapter 7) and human data (Chapter 8) obtained from dental enamel are also used within this thesis to assess the broad geographic biosphere predictions derived from modern biosphere data. Details of the selected archaeological sites, and sample records are documented in Appendix B. The methods of preparation for archaeological dental tissues are described in Section 3.3 and are fully documented in Appendix A. Section 3.1 describes the analytical method (TIMS) used to analyse all reported samples.

3.1 Analytical method

The details outlined below (Sections 3.11–3.13) are common to each sample medium analysed as part of this work. All measurements were obtained using thermal ionisation mass spectrometry (TIMS). Where necessary, strontium concentrations were determined by isotope dilution (ID-TIMS), using an appropriately calibrated ^{84}Sr-enriched spike solution (Oak Ridge Dilute Strontium). The analyses were carried out using a Thermo Finnigan Triton, multi-collector, magnetic-sector instrument, housed within clean facilities at the NERC Isotope Geosciences Laboratory (NIGL) at the British Geological Survey (Keyworth, Nottingham).

Thermal ionisation mass spectrometry (TIMS) provides the benchmark for high-precision $^{87}Sr/^{86}Sr$ analysis. The history of the developments in instrumentation, principals and techniques are covered in considerable detail within a range of standard texts, such as Dickin (1995) and Faure and Mensing (2005). Whilst ID-TIMS is capable of simultaneously providing accurate measurements of strontium concentration and $^{87}Sr/^{86}Sr$ composition, the strontium must first be separated from the sample matrix, and isolated from other elements that would otherwise retard emission or interfere with the measured strontium masses (^{84}Sr, ^{86}Sr, ^{87}Sr, ^{88}Sr). Accordingly, the chromatographic separation of analytes (Section 3.1.1) is a routine part of sample preparation. This provides a refined strontium salt, which is then evaporated onto a solid-state ion source (Section 3.1.2).

3.1.1 Cation exchange chemistry

The aim the sample preparation methods outlined in Sections 3.2 and 3.3 is to produce a solution from which strontium can be extracted using standard cation exchange methods. This represents the final stage in achieving a sample of strontium that is appropriate for analysis by TIMS. The prepared samples are dried down and converted to chloride using 1 ml of HCl (6 M). This solution is dried down and diluted with 2.5 M HCl to an appropriate volume; one millilitre of the solution is then applied to a calibrated cation exchange column (Dowex AG 50W-X12) and the strontium is eluted in the specified volume of acid (Appendix A). As with all preparation methods involving sample dissolution, this process was carried out within clean facilities at the NIGL, using ultrapure reagents and pre-cleaned acid-leached Savilex^{TM} vessels.

In most geological and archaeological applications strontium is recovered from an essentially inorganic matrix, with a minimal number of interfering constituents. For example, the separation of strontium from waters (Section 3.2.2) and other dilute aqueous solutions, such as soil leaches (Section 3.2.3), simply requires acidification of the sample with HCl. Although the strontium in dental enamel is carried within a high-calcium matrix,

excellent separation and recovery of strontium can usually be achieved without exceeding the capacity of the column. However, more complex organic mixtures derived from plant materials suffer from a range of chromatographic interferences that can make separation and analysis of strontium problematic (Rosner 2010).

Even when high-temperature, concentrated acid is used to digest plant-material, significant organic components will still remain in solution (Araújo *et al.* 2002; Walas *et al.* 2004). Some of the problems that may be encountered in running vegetation samples on TIMS (early emission, weak or unstable beam, catastrophic beam collapse) can potentially be attributed to these effects as well as the presence of organic residues within the eluate (Dickin 1995: 19–32). However, the microwave-assisted method of vegetation digestion developed as a part of this project (Section 3.2.2) results in good strontium separation.

3.1.2 TIMS analysis of $^{87}Sr/^{86}Sr$

During TIMS analysis a solid source (Re filament) is heated to a temperature at which the sample is simultaneously volatilised and ionised. This is achieved by passing a current through the metal filament under high vacuum, heating it to over 1480 °C. This high-temperature process results in mass-dependent fractionation. However, as the naturally occurring isotopes ^{86}Sr and ^{88}Sr are non-radiogenic, fractionation can be monitored using the measured $^{88}Sr/^{86}Sr$ ratio; by international convention, this has a constant value of 0.1194 (Steiger and Jäger 1977). Accordingly, the deviation of the measured $^{88}Sr/^{86}Sr$ ratio from 0.1194 provides a fractionation-factor that allows on-line correction of the $^{87}Sr/^{86}Sr$ ratio throughout each analytical run. Thus, although small levels of biological fractionation (equivalent to around 0.10–0.25 ‰ per mass-unit) have been reported within terrestrial ecosystems (Knudson *et al.* 2010; de Souza *et al.* 2010), this does not influence conventionally measured $^{87}Sr/^{86}Sr$ values reported within this thesis.

In addition to internal correction for mass-dependent fractionation, the $^{87}Sr/^{86}Sr$ data documented in Appendix C – collected in static mode – have been normalised to the results achieved for replicate analysis of the international standard NBS 987. Usually, around three such standards were included within each analytical batch (consisting of up to 21 samples). This allows the data collected during different analytical periods and from different laboratories to be compared directly. The standard results (NBS 987) used to normalise the data reported in this thesis (Appendix C) are summarised in Table 3.01 (accepted $^{87}Sr/^{86}Sr$ value = 0.710250). The standard deviations (2 SD) of the means used to normalise the data obtained within each analytical period provide an indication of the long-term reproducibility of $^{87}Sr/^{86}Sr$ measurements. Across the range of biosphere $^{87}Sr/^{86}Sr$ values reported within this thesis the relative standard deviation (2 RSD) associated with each mean standard result (NBS 987) is equivalent to an absolute 2σ interval of approximately ± 0.00001.

Table 3.01: Mean values for replicate measurements of NBS 987 used to normalise $^{87}Sr/^{86}Sr$ data collected in static mode.

Standard ID*	Start	End	n	Mean	2 SD	2 RSD
149	24/04/2008	06/06/2008	30	0.710284	0.000015	0.0021 %
161	06/01/2009	02/03/2009	21	0.710273	0.000010	0.0015 %
162	20/04/2009	14/08/2009	42	0.710223	0.000013	0.0019 %
168	16/11/2009	17/12/2009	43	0.710226	0.000013	0.0018 %
172	25/02/2010	28/05/2010	18	0.710225	0.000008	0.0012 %
174	30/06/2010	02/09/2010	34	0.710230	0.000011	0.0015 %

*Standard ID referred to in data tables in Appendix C, used to normalise the measured $^{87}Sr/^{86}Sr$ values.

Both the samples and the standards (NBS 987) were loaded onto out-gassed single rhenium (Re) filaments and dried down in a HEPA-filtered fume cupboard. Each sample was taken up and loaded with 2 µl of a TaF activator solution following the method of Birck (1986). Under typical conditions each filament was heated to at least 1480 °C in order to achieve a beam size of 3 V or greater. Each analysis consisted of 150 static measurements broken into 10 blocks, each of 15 cycles. Rejection criteria were in place which allowed up to 10 % (n = 15) of the measurements to be

excluded from each block as outliers. The final output represents the mean of the remaining measurements, corrected internally for fractionation. Within Appendix C the ± 2σ error quoted for each measurement is the standard error (SE) of the individual analytical result (internal error). These conditions provided analytical results precise to ± 0.00002 (2 SE) or better, within run (i.e. internal precision). This is not the highest level of precision that can be achieved, but it can be considered to be adequate for most environmental applications.

It is well within the capabilities of the available instrumentation to provide precise and highly reproducible measurements the $^{87}Sr/^{86}Sr$ composition of strontium, with an internal error affecting only the filth significant figure. However, although it can be reasonably by assumed that sample solutions, including soil leachates and natural waters (stream water and rainwater) are homogenous, plant materials or dental tissues, contain incremental growth structures, which may confer some level of isotopic heterogeneity. In Section 3.2, an attempt is made to place realistic constraints on external precision, based primarily on replicate analysis of the NIST Standard Reference Material 1515 (Apple Leaves).

3.1.3 Isotope dilution

The concentration of strontium in vegetation (Section 3.2.1), in some stream waters and rainwaters (Section 3.2.2), in all soil leachates (Section 3.2.3), and in all dental tissues (Section 3.3.) was determined by ID-TIMS. This involves introducing a known quantity of a ^{84}Sr-enriched spike solution (Oak Ridge Dilute Sr) at the stage at which a representative aliquot of a given sample was weighed/measured; the aim is to achieve a homogenous mixture of the spike and the sample strontium. As the natural abundance of ^{84}Sr is very low (0.56 %), the naturally occurring ^{84}Sr in the sample is swamped by the spike. The isotopic composition of the spike is known, as is the amount of spike strontium added relative to the sample mass/volume. Thus, the composition of the spike-sample mixture (in respect of ^{84}Sr, ^{86}Sr, ^{87}Sr, ^{88}Sr)

can be used to calculate the concentration of strontium in the sample and return its $^{87}Sr/^{86}Sr$ composition.

The calibration of the ^{84}Sr enriched spike is such that the isotope dilution method is capable of achieving accurate results at a level of precision routinely of ± 1 % or better (Dickin 1995: 28–31). However a variety of sources of error mean that, as applied within the current study, the level of variation associated with replicate measurements is likely to be more substantial. These factors include variations in the moisture content of the samples, combined weighing errors during sample preparation, sample heterogeneity, and incomplete homogenisation. This means, for example, that the expanded 2σ error applied to dental enamel may approach ± 10 % or more (Montgomery *et al.* 2000). Comparable levels have been observed within the current study with respect to replicate analysis of the NIST Standard Reference Material 1515 (Apple Leaves).

3.2 Modern environmental sample media

This thesis examines the level at which it is possible to characterise geographic biosphere $^{87}Sr/^{86}Sr$ variation, for application primarily within archaeological research. Plant materials have long been used to map the geochemical properties of the surface environment (e.g. Lax and Selinus 2005) and represent the principal biosphere proxy used within this thesis (Section 3.2.1). Stream water and rainwater were also analysed in bulk (Section 3.2.2). However, an aqueous soil-leach (Milli-Q™ deionised water) was used to characterise labile strontium within surface soils (Section 3.2.3).

3.2.1 Vegetation (Wood, Leaf, and Grass samples)

The preparation methods for the plant materials referred to throughout this thesis – including microwave digestion-vessel cleaning – are documented as step-wise procedures in Appendix A. These were developed using material provided by the G-BASE sample archive, which includes the terminal branches of mature woody shrubs, and in some cases their

accompanying foliage (see Chapter 2). These represent composite samples collected at approximately shoulder height from locations proximal to G-BASE drainage sample sites. The samples were air-dried while sealed within the original Kraft paper sample bags prior to further treatment.

Geochemical mapping exercises indicate that it is appropriate to treat bark, foliage and woody material as separate sample media (Reimann *et al.* 2007b). Accordingly the 'Wood' samples referred to in this thesis represent only the internal composition of the respective plants, as all outer surfaces were removed. Any foliage (referred to as 'Leaf' samples) was left in an untreated state. Additional samples of un-washed grass (referred to as Grass samples) were also collected in the course of two additional field projects (Chapters 3 and 6). Three iteratively developed microwave-assisted wet digestion methods were used to extract the strontium from these sample media, documented in Appendix A as Versions 1, 2 and 3.

3.2.1a Disaggregation and homogenisation

For each vegetation sample (Wood, Leaf and Grass) 2–3 g of dry matter was homogenised, providing at least ten times the amount of material needed for analysis by TIMS (ca. 200 mg). The disaggregation and mixing of a relatively large sub-sample allows a representative aliquot of plant material to be prepared for analysis. It also has the advantage of increasing the surface area of the sample, making the material more vulnerable to chemical attack. Moreover, for monitoring purposes it is desirable to matrix-match the NIST plant powder standard (SRM 1515) as closely as possible. Although smaller aliquots could improve oxidation efficiency during wet digestion (Wasilewska *et al.* 2002) the sample size is consistent with the general recommendations for the use of SRM 1515 (NIST 1993).

To ensure that the Wood and Leaf samples from the G-BASE archive were directly comparable to one another these were both homogenised using a cryogenic mill (Appendix A). Each sample was pulverised separately within a self-contained grinding vial. The closed milling vials eliminate cross

contamination and are easy to clean. Given the large sample size it is assumed that the steel impactor and end-plugs make insignificant contribution to the strontium content of the samples. However, it is possible to disaggregate dry foliage without recourse to such extreme methods. Thus, the Grass samples were prepared, following the methods used by Evans *et al*. (Evans *et al.* 2010a). Each subsample was homogenised by manipulating the dry material within a re-sealable zip-lock polythene sample bag, until a flaky texture resembling that of broken-leaf tea was achieved. This minimised any need to transfer material between different containers and working areas outside of a clean environment.

3.2.1b Microwave-assisted extraction of strontium

The extraction of strontium from plant materials was accomplished using an acid digestion technique with closed system microwave digestion vessels. Digestion procedures (Appendix A) were based on the recommendations of the U.S. Environmental Protection Agency Method 3052 (EPA 1996), and made use of 5 ml of 8 M nitric acid (HNO_3) raised to at least 175 °C for 20 minutes. Dilute acid was used as the addition of water is thought to result in a more predictable pressure/temperature profile during heating (Wu *et al.* 1997). Each sample was weighed and spiked under clean conditions, and subjected to a period of pre-digestion at room temperature to improve analyte recovery (Walas *et al.* 2004). Samples were sealed before being transferred to the microwave facility. A range of temperatures were reached by the samples, which peaked at around 180 °C within any one microwave batch. Comparable single-step, single-acid procedures have been shown to result in good recovery of strontium from a range of NIST plant reference materials without the use of hydrofluoric acid to dissolve siliceous phytoliths (Wu *et al.* 1997).

In the first iteration of the digestion method (Version 1), which was applied to batch N_620 (see Appendix C), the microwaved plant material was dried down with 0.5 ml of hydrogen peroxide (H_2O_2 at 30 % w/w) and then converted to chloride using 1 ml of 6 M hydrochloric acid (HCl) before being

subjected to column chemistry (Section 3.1.1). To counter poor analytical characteristics experienced within this batch, Version 2 included a step in which the dried-down, microwaved samples were re-dissolved and oxidised in a small volume of HNO_3 (8 M) and H_2O_2 (30 % w/w) in a 1:10 (v/v) mixture (Roane *et al.* 2003: 233). In the final iteration of the method (Version 3), a small quantity (0.1 ml) of H_2O_2 (30 % w/w) was added to the digestion vessels prior to microwaving. This quantity of reagent was not intended to oxidise the sample directly, but it is thought to help reduce the vapour pressure of the digest and maintain acid concentrations (Wu *et al.* 1997; Araújo *et al.* 2002).

3.2.1c Blank characteristics

The influence of a procedural blank contribution on a $^{87}Sr/^{86}Sr$ measurement is controlled by the relative size of the blank with respect to sample strontium, and the degree of contrast between the two components. Accordingly, blank contributions should be assessed in terms of the purpose for which a given procedure is fit; that is, a study that requires low levels of precision can tolerate a greater blank contribution than one that tests the limits of detectable variation. The closed-vessel microwave system used within the current study allows procedural blank contributions to be fully and routinely monitored, whereas contamination during furnace ashing may be more difficult to detect and quantify. However, microwave procedures may confer a significant addition to the blank because of the large surface area of the pressure vessels. The first batches of samples processed using the microwave system had blank values of around 2 ng (Table 3.02, overleaf). Although high by normal standards, the effect on the measured $^{87}Sr/^{87}Sr$ composition of a 0.2 g sample containing 10 µg/g of strontium would still fall within the range of the analytical error (cf. Section 3.2.1).

The procedural blank improved as the digestion method was developed (Table 3.02), and the microwave vessels were iteratively cleaned following Version 1 and subsequently, Version 2 of the methods detailed in Appendix A. Three measurements indicated that the final microwave-assisted

leach used to clean the digestion vessels (Version 2) removed 83.0–141 pg of strontium (Batch 513). In contrast, 5 ml of nitric acid subjected to the digestion program in three clean vessels removed only 51.7–65.4 pg of strontium (Batch P_520). These data allowed a significant proportion of the blank contribution to be traced to two bottles of hydrogen peroxide with strontium concentrations of 971 pg/ml and 809 pg/ml, and $^{87}Sr/^{86}Sr$ compositions of 0.70859 ± 0.00001 and 0.70862 ± 0.00001 respectively (Batch P522). Once these were eliminated, the blank contributions fell routinely below 0.1 ng. Blank correction had no significant effect on any of the measured $^{87}Sr/^{86}Sr$ values.

Table 3.02: Procedural blanks for the microwave-assisted digestion of plant material (Wood, Leaf and Grass).

Vessel cleaning method	Sample digestion method	Batch*	Sr (pg)
Version 1	Version 1	N620	2400
	Version 2	P488	2090
		P488	2430
		P488	2430
Version 2	Version 2	P492	671
		P492	700
		P492	714
	Version 3	P513	581
		P513	630
		P513	793
		P501	881
		P501	888
	Version 3	P584	26.1
		P529	26.3
		P584	34.6
		P543	35.8
		P529	49.7
		P543	69.3
		P543	85.4
		P529	97.9

*Chemistry batch referred to in data tables in Appendix C.

3.2.1d Procedural reproducibility

Whilst it is clear that the differences between the $^{87}Sr/^{86}Sr$ compositions of biosphere samples collected within similar geological

domains routinely exceed the analytical errors associated with TIMS analyses (Evans *et al.* 2010b), few data are available that pertain to the external reproducibility of individual biosphere measurements. Although there are currently no international $^{87}Sr/^{86}Sr$ biosphere standards available, NIST Standard Reference Material 1515 (Apple Leaves), represents a well homogenised bulk vegetation sample, and provides a reasonable matrix-match for the plant materials used within this study. To validate the procedures used within this study, a total of 20 aliquots of SRM 1515 were prepared and analysed alongside other vegetation samples (Table 3.03, overleaf).

Although no attempt was made to standardise the moisture content of samples, the mean concentration of strontium in SRM 1515 determined by ID-TIMS (Table 3.03) falls within error of the certified concentration of 25 ± 2 µg/g (NIST 1993). The results are also similar to reported values, which were obtained using comparable wet digestion methods (Wu *et al.* 1997), and show that the sample strontium is well homogenised with the ^{84}Sr-enriched spike during the procedure. However, the relative standard deviation associated with this data-set (± 11 %) indicates that, even when applied to well homogenised plant powders, combined procedural errors may result in an expanded total uncertainty (Section 3.1). This figure is applied to the error bars used in the graphs shown throughout this thesis.

The mean $^{87}Sr/^{86}Sr$ composition of SRM 1515 is 0.71394 ± 0.00005 (2 SD, n = 20). The standard deviation associated with this data-set – equivalent to a relative 2σ interval of ± 0.0065 % – shows that there is a significant degree of isotopic heterogeneity within the standard. The vegetation standards produced by the NIST are generated from large well mixed bulk samples and are considered to be suitably homogenous for trace-element studies. Accordingly, these data provide a conservative estimate of the standard deviation associated with replicate analysis of plant materials, which incorporates both procedural error and long-term analytical

reproducibility. Across the range of biosphere values reported in this thesis, this is equivalent to an absolute interval of approximately ± 0.00005.

Table 3.03: Results of the replicate $^{87}Sr/^{86}Sr$ analysis of NIST Standard Reference Material 1515 (Apple Leaves) analysed by TIMS.

Batch	Sr (µg/g)	$^{87}Sr/^{86}Sr$	Standard ID‡
P513	23.4	0.713920 ± 0.000009	168
P488	23.4	0.713922 ± 0.000012	161
P513	23.3	0.713922 ± 0.000007	168
P488	23.0	0.713926 ± 0.000009	161
P529	23.4	0.713928 ± 0.000006	172
P543a	23.1	0.713931 ± 0.000008	174
N620	24.1	0.713933 ± 0.000013	161
P501	23.3	0.713933 ± 0.000010	162
P488	28.8	0.713934 ± 0.000012	161
P529	23.2	0.713937 ± 0.000007	172
P501*	—	0.713941 ± 0.000006	162
P492	23.1	0.713944 ± 0.000007	162
P513	23.3	0.713945 ± 0.000007	168
P492	23.0	0.713947 ± 0.000011	162
P488	23.5	0.713949 ± 0.000014	161
P488	23.3	0.713950 ± 0.000009	161
P492*	—	0.713958 ± 0.000014	162
N620	22.9	0.713985 ± 0.000013	161
P543a	23.2	0.713986 ± 0.000009	174
P488	23.8	0.714006 ± 0.000012	161
Mean	**23.6 (n = 18)**	**0.71394 (n = 20)**	
2 SD	**2.6 (n = 18)**	**0.00005 (n = 20)**	
2 RSD	**11 %**	**0.0065 %**	

*Un-spiked samples.
‡cf. Table 3.01

The difference between the replicate analyses of Wood samples, with relatively low strontium concentrations and prepared for analysis initially within batches associated with high procedural blanks (Table 3.02: N620; P488; and P492), failed to exceed the relative standard deviations (2σ) for concentration (± 11 %) and $^{87}Sr/^{86}Sr$ (± 0.0065 %) estimated using SRM 1515 (Table 3.04, overleaf). The differences between these samples are no greater than would be expected to result from sample heterogeneity. Likewise, although the difference in strontium concentration between field

duplicates (cf. Chapters 4 and 5) may be more substantial, there is no evidence that differences in $^{87}Sr/^{86}Sr$ composition are any more substantial than would be expected on the same basis (Table 3.05).

Table 3.04: Results of the initial $^{87}Sr/^{86}Sr$ analysis of vegetation samples (Wood), and replicate analyses undertaken in chemistry batch P492.

G-BASE number	Initial analysis (subsample A)			Replicate analysis (subsample B)		Difference	
	Batch	Sr (µg/g)	$^{87}Sr/^{86}Sr$	Sr (µg/g)	$^{87}Sr/^{86}Sr$	Sr (µg/g)	$^{87}Sr/^{86}Sr$
461093	P492	1.83	0.709261	1.85	0.709272	0.02	0.000011
461109	P488	4.76	0.709433	4.70	0.709444	0.06	0.000011
461150	P488	3.18	0.708745	3.05	0.708756	0.13	0.000011
461084	N620	3.46	0.709247	3.28	0.709241	0.18	0.000006
461492	N620	4.53	0.708711	3.68	0.708731	0.85	0.000020
461258	N620	18.9	0.709441	19.5	0.709470	0.60	0.000029

Table 3.05: Results of the $^{87}Sr/^{86}Sr$ analysis of field duplicates of vegetation samples (Wood).

Primary field sample			Field duplicate			Difference	
G-BASE number	Sr (µg/g)	$^{87}Sr/^{86}Sr$	G-BASE number	Sr (µg/g)	$^{87}Sr/^{86}Sr$	Sr (µg/g)	$^{87}Sr/^{86}Sr$
461131*	10.4	0.709586	—	—	—	—	—
461476	11.8	0.707846	461481	22.6	0.707880	10.8	0.000034
461204	6.71	0.709175	461296	5.78	0.709217	0.93	0.000042
Primary field sample			**Field duplicate**			**Difference**	
Sample ID	**Sr (µg/g)**	**$^{87}Sr/^{86}Sr$**	**Sample ID**	**Sr (µg/g)**	**$^{87}Sr/^{86}Sr$**	**Sr (µg/g)**	**$^{87}Sr/^{86}Sr$**
WhtLf_F	3.63	0.707630	WhtLf_G	4.23	0.707616	0.60	0.000014

*Field duplicate for 461131 (i.e. 46117) not present within sample archive (cf. Table 3.08, page 51).

3.2.2 Waters (Water and Rain samples)

All stream water (Water) and rainwater (Rain) samples were stored under refrigerated conditions in acid-leached HDPE bottles and required minimal preparation (Appendix A). Stream waters – provided solely by the G-BASE project (see Section 2.3) – had previously been filtered (< 0.45 µm), and acidified with nitric acid (1 % w/w) in the field to inhibit biological activity during extended storage periods (Giblin 1994: 300). Rainwaters collected by the Centre for Ecology and Hydrology (CEH) as part of the Rural Heavy Metals Monitoring Network (Chapter 6), were also acidified with ultra-pure nitric acid (1 % w/w) (Haley Guyatt pers. comm.). The G-BASE stream water,

and CEH rainwater collection and treatment protocols are designed to detect trace and ultra-trace analytes by ICP-MS. Thus, routine determination of strontium concentrations by isotope dilution (ID-TIMS) was not necessary. Additional rainwater samples, for which strontium concentrations were not available (Chapter 6), were analysed by ID-TIMS.

3.2.2a Water sample treatment

Prior to further sample treatment, sealed water sample containers were allowed to equilibrate to room temperature within the clean facilities at the NIGL. All preparation was undertaken using ultra-pure reagents in acid-leached Savilex^{TM} vessels. The waters were transferred from the storage vessels to drying vessels using an appropriately sized and rinsed pipette, prepared following the step-wise methods detailed in Appendix A. Where it was necessary to determine strontium concentration the sample was transferred to a drying-vessel previously spiked with a known quantity of the ^{84}Sr-enriched spike (Oak Ridge Dilute Strontium). One millilitre of HCl (6 M) was added to each sample prior to dry-down to provide strontium in the chloride form suitable for application to the cation exchange column (Section 3.1).

Selected samples within the first batch of G-BASE stream water samples analysed within the current study were spiked to provide a comparison with the strontium concentrations supplied by the G-BASE project and determined by ICP-MS. These data show that results agreed within error (Table 3.06, overleaf); that is, there is no systematic difference between the G-BASE concentration data and that determined by ID-TIMS. Accordingly, throughout the remainder of this thesis (Chapters 4 and 5) the reported strontium concentrations for G-BASE stream water samples (Water), and CEH rainwater samples (Rain) are those provided by the respective projects by IC-MS. As trace element data were not available for an additional six rainwater samples collected during 2010 (Chapter 6), five of these were spiked to provide ID-TIMS data, having first achieved a reliable ^{87}Sr/^{86}Sr measurement.

Table 3.06: Results indicating agreement between ID-TIMS and G-BASE concentration of strontium in stream water samples (Water) from chemistry batch P455.

G-BASE number	G-BASE Sr (mg/l)	ID-TIMS Sr (mg/l)
461545	0.355	0.365
461513	0.451	0.454
461599	0.674	0.682
461568	1.31	1.33
461571	1.34	1.34

G-BASE Sr concentration data are provided by the BGS Geochemistry Database (Johnson *et al.* 2005); The concentrations of strontium determined by G-BASE ('G-BASE Conc') are subject to a combined error of approximately ± 5 %, or greater. A combined error has not been calculated for the concentrations determined by isotope dilution, which may be as low as ± 1 %.

3.2.2b Stream water (Water samples)

Two 5 ml aliquots of water, taken as blanks by the G-BASE project, were measured along with procedural blanks to assess the contribution from the field and the laboratory (Table 3.07): the procedural blank contributed 59 pg of strontium; the unfiltered field blank provided 68 pg and the filtered blank 172 pg. Procedural blanks varied from 20–73 pg, across a range of stream water strontium concentrations equivalent to 0.66–13 µg of strontium in 5 ml. Although, in some cases this has the potential to influence the $^{87}Sr/^{86}Sr$ of the samples in the 5th decimal place the magnitude of any bias is unlikely to exceed the long-term reproducibility of the measurement.

Table 3.07: Results for G-BASE field blanks and procedural blank for preparation of stream water samples (Water) in chemistry batch P455.

G-BASE number	Blank type	Sr (pg)
—	Procedural blank	59.2
461514	Unfiltered field blank	68.1
461562	Filtered field blank	172

Since it was assumed that stream waters are likely to be homogeneous, replicate analysis was not undertaken. However, a limited number of G-BASE filed duplicates were available (Table 3.08). These were prepared for $^{87}Sr/^{86}Sr$ analysis within the same chemistry batch (P538), associated with procedural blanks of 23–34 pg, and analysed within the same analytical batch (Appendix C). The difference between the $^{87}Sr/^{86}Sr$

values of the samples fails to exceed the reproducibility even of the standard (NBS 987) during the analytical period, which was 0.710225 ± 0.000008 (2 SD, n = 18) (Table 3.01). It is also assumed that the estimated standard deviation associated with replicate analysis of all waters is unlikely to exceed the long-term reproducibility of the analytical method.

Table 3.08: Analysis of G-BASE stream water field duplicates.

Primary field sample		Field duplicate		Difference
G-BASE number	$^{87}Sr/^{86}Sr$	G-BASE number	$^{87}Sr/^{86}Sr$	$^{87}Sr/^{86}Sr$
461131	0.709101	461137	0.709111	0.000010
461204	0.708918	461296	0.708922	0.000004
461476	0.707839	461481	0.707833	0.000006

3.2.2c Rainwater (Rain samples)

All acidified rainwater samples (Rain) were prepared using a 20 ml aliquot, but due to the low concentrations of strontium in rainwater (ppb range) all lab-ware was subjected to additional leaching in high purity 8 M nitric acid before use (Appendix A). Having first achieved a reliable $^{87}Sr/^{86}Sr$ measurement for the rainwater (Chapter 6) the concentrations of strontium in the remaining five rainwater samples were determined by isotope dilution. Procedural blanks associated with these six samples (batches P536 and P538) varied from 20–34 pg (n = 5). Rainwater samples supplied by CEH were analysed in Batches P542 and P547, returning procedural blanks of 38–40 pg (n = 2) and 19–26 pg (n = 3) respectively. The samples contained strontium in concentrations equivalent to 0.01–0.18 µg in 20 ml and thus, may be subject to a blank influence in the fifth decimal place.

3.2.3 Surface soil samples (Soil samples)

Soils sit at the interface between the geosphere and the biosphere and represent the primary growth medium for terrestrial plants. However, bulk soil analyses rarely provide $^{87}Sr/^{86}Sr$ data that are directly related to biosphere values, because of differences in the composition and the stability of soil-forming minerals (see Chapter 2). Within this study a deionised water

leach (Milli-Q^{TM}) was used to extract labile strontium from pre-prepared surface soil samples provided by the G-BASE project (Appendix A), each representing a composite A-horizon sample of around 1 kg (Johnson 2005). Following initial air drying, the soil is freeze dried, sieved to pass a 2 mm mesh and a representative aliquot is pulverised in an agate planetary ball-mill. The reported soil-leach data (Appendix C) are produced from a subsample of milled material that was prepared for the analysis of loss-on-ignition (archived as the 'LOI split').

3.2.3a Soil-leach method

Within this study 10 ml of deionised water (Milli Q^{TM}) was used to leach 1 g of soil for 24-hours, under constant agitation at room temperature (Appendix A). The soil samples were weighed into centrifuge tubes (10 ml PP) under clean-room conditions and sealed during the leaching period. Particulate material was removed from suspension by centrifugation, which was repeated using clean centrifuge tubes until no additional material was precipitated. In order to avoid disturbing any precipitate a measured volume of each leachate (< 10 ml) was transferred to a pre-cleaned Savilex^{TM} beaker and mixed with a known quantity of the ^{84}Sr-enriched spike solution (Oak Ridge Dilute Strontium) to determine strontium concentrations by ID_TIMS. The leachate/spike mixture was acidified with HCl (6 M) and dried down prior to cation exchange chemistry (see Section 3.3.1).

The method provided leachates with strontium concentrations of 0.03–0.26 mg/l (median = 0.07 mg/l, n = 35); these are reported as leachable concentrations (mg/kg), normalised to the initial mass of each soil sample (Appendix C). Comparisons with G-BASE data (bulk strontium concentrations) from the British Geological Survey (BGS) Geochemical Database (Johnson *et al.* 2005) show that the soil-leach liberated typically around 1 % or less of the strontium contained by each sample. Nonetheless median topsoil pore-waters from various sites in central southern England, summarised by Campbell *et al.* (1989), range from 0.05 mg/l (woodland soils) to 0.17 mg/l (agricultural soils), whilst Chalk grassland pore-waters have

been reported with intermediate strontium concentrations of around 0.08 mg/l (Kinniburgh and Miles 1983). Accordingly, the soil-leach strontium concentrations can be considered to resemble soil pore-waters collected at field capacity.

3.2.3b Blank characteristics

Procedural blanks for the soil-leach method varied between 324–703 pg in batch P496 (n = 3) and 107–327 pg in batch P496 (n = 2). Although this compares favourably with procedures reported in the literature – for example Castorina and Masi (2008) report procedural blanks of 2 ng – the relatively small quantity of strontium extracted from each sample (0.3–4 µg) means that this represents a relatively large fractional contribution. The most likely source of contamination is transfer between leaching vessels at the weighing stage of the procedure. However, although it was not possible to make an accurate measurement of the $^{87}Sr/^{86}Sr$ compositions of the blanks, it is possible to estimate the value needed to affect a given shift in the sample $^{87}Sr/^{86}Sr$, as a proportion of the strontium measured in each sample. An informed decision can then be made about the likely significance of the blank contribution.

Natural soils represent complex mixtures of mineral sediments and organic matter and thus, by nature, are likely to be isotopically heterogeneous. Although steps are taken during sample collection and preparation to improve sample homogeneity, it is unlikely that any soil-leach method would ever provide uniform $^{87}Sr/^{86}Sr$ values. Although replicate soil leaches were not undertaken in the current study, the NIST Apple Leaf standard (SRM 1515) indicates a realistic level of reproducibility that may be associated with biosphere sample media (see Section 3.2.1d). Bulk $^{87}Sr/^{87}Sr$ analysis of SRM 1515 returned a standard deviation equivalent to a 2σ confidence interval of ± 0.0065 %. In the case of the aims of the present study, a shift in isotopic composition equivalent to the standard deviation associated with replicate analysis of SRM 1515 does not represent a confounding factor in the interpretation of soil leach $^{87}Sr/^{86}Sr$ compositions.

Assuming that the blank contribution represents approximately 0.7 ng of the strontium measured in each sample, the blank $^{87}Sr/^{86}Sr$ compositions needed to effect a downwards shift equivalent to 0.0065 % (0.4560–0.6903) are too low to represent a plausible fall-in blank, as the range of modern $^{87}Sr/^{86}Sr$ ratios in natural materials are constrained by the primordial composition of strontium and radiogenic in-growth. The blank $^{87}Sr/^{86}Sr$ composition required to have shifted the sample ratios up by the same proportion varies between 0.7312–0.9608; that is, blank $^{87}Sr/^{86}Sr$ compositions of 0.7312 and lower are unlikely to have had a significant influence on measured values. Although it is possible that individual mineral fractions could provide radiogenic strontium, all of the soil leachate $^{87}Sr/^{96}Sr$ compositions (0.7077–0.7162) fall well below this value (Appendix C). It is therefore difficult to attribute any significance to cross-contamination. Accordingly, the blank contribution can be considered to be insignificant.

3.3 Archaeological sample media

Due to its resistance to diagenesis, dental enamel has become the tissue choice for most archaeological applications of $^{87}Sr/^{86}Sr$ analysis (Bentley 2006). Mature dental enamel is a highly mineralised tissue consisting of relatively large crystallites of biological apatite, with a well-ordered structure (Hillson 2005: 155–184). Biological apatite (bioapatite) is a non-stoichiometric calcium phosphate mineral that resembles hydroxyapatite, but with a carbonate ion substitutions (ca. 3–6 %) at several locations within the crystal Lattice (Lee-Thorp 2002). Although this mineral is common to all osseous tissues, bone and dentine are poorly crystalline in comparison to enamel, and more porous (Koch *et al.* 1997). Moreover, both bone and dentine contain a substantial organic component, unlike mature enamel, which may contain less than 1 % organic matter (Hillson 2005: 148–150). Whilst bone and dentine can provide an indication of the diagenetic trajectory associated with a given burial environment, they are unsuitable for the assessment of endogenous $^{87}Sr/^{86}Sr$ values (Budd *et al.* 2000). Thus, all dentine was carefully separated from each enamel sample (Appendix A).

3.3.1 Faunal material

All of the faunal material analysed in this work thesis was obtained from assemblages made up of the commingled, disarticulated skeletal remains of a number of individuals. Only complete mandibles or mandibular fragments bearing a significant proportion of each tooth row were selected for analysis. This aided the accurate identification of the teeth, the accurate attribution of the selected teeth to one individual and ensured that the risk of duplication was eliminated. In the absence of articulated post-cranial remains, the use of mandibular teeth also allows an estimate of the age at death of each individual to be made using standard zooarchaeological methods (Grant 1982). Records of the archaeological sites from which material was obtained for analysis, and records of the selected samples are documented in Appendix B.

Due to the inherent difficulty in distinguishing sheep and goat remains all such material, belonging to the *Caprinae* sub-family is recorded as sheep/goat. In addition the metric characteristics and the tooth-wear scores of the selected specimens are fully documented in Appendix B, following the recommendations of von den Dreisch (1976); due to the fragmentary nature of much of the material, it was not possible to accurately record the full suite of measurements. The teeth held by each of the specimens provided enamel in good apparent macroscopic condition, judged by visual inspection following Montgomery (2002); i.e. the crowns provided suitable regions of hard, white enamel that showed minimal evidence of cracking. On extraction from the mandible each tooth showed extensive root development beyond the cervical margin of the crown, providing confidence that each tooth crown was fully mineralised.

The faunal sampling exercise undertaken within this study (Chapter 7) was designed to maximise the length of the continuous record of $^{87}Sr/^{86}Sr$ variation available from each individual using the minimum number of high-crowned (hypsodont) check teeth. This introduced an age related bias,

leading necessarily to the selection of relatively long-lived individuals and restricting the number of mandibles deemed suitable for analysis:

- As the first molar of cattle begins to form *in utero* and in sheep during the first few months of life, and as there is considerable overlap between the crown formation times of the first permanent molar and the second later in the first year (Hillson 2005: 207–256) this tooth was not considered for analysis.
- As it appears that the eruption of the teeth through the alveolar, and even the clinical emergence of the crown into the oral cavity can occur prior to the completion of enamel maturation within the cervical region of each tooth (Hillson 2005: 207–256) only mandibles within which the third molar displayed some degree of wear were selected.

3.3.1a Sampling regime used to allow $^{87}Sr/^{86}Sr$ variation in sheep and cattle teeth to be plotted on common time-related axis

The formation of the enamel of the high crowned (hypsodont) teeth of sheep and cattle follows a gross directional axis, from the cusp of the crown to the cervix (Hillson 2005). However, following the secretion of the enamel matrix, an extended period of enamel maturation mitigates against the calculation of a precise and accurate mineralisation time for a given macroscopic sample (Montgomery *et al.* 2010). Nonetheless, In order to compare time-related data from different teeth, different animals, or the teeth of more than one species it is useful to be able to plot intra-tooth data on a common time-related axis. Accordingly, the apparent time of formation that can be calculated for any given vertical position on a given crown represents a gross approximation, and is undertaken in this thesis primarily as a scaling exercise (Chapter 7).

In ungulates (i.e. sheep, goats, cattle etc.) bearing full adult dentition, the earliest forming enamel will be lost due to dental attrition (Hambleton 1999). This means that the only chronological baseline available for each tooth is provided by the cervical margin (Balasse 2003). In a fully mineralised crown showing occlusal wear and significant root development, this location represents a fixed point in time at which the crown of a worn tooth can be

assumed to have been complete (Hoppe *et al.* 2004). In order to convert the measured distance of a sample from the cervical margin into an estimate of the position on a common developmental axis, a number of assumptions must be made:

- Literature sources provide an accurate estimate of the time at which mineralisation is completed.
- Literature sources provide an accurate estimate of the length of time taken for each crown to form.
- The enamel of each tooth forms at an approximately constant rate.
- An accurate estimate can be made of the original height of each crown.

Table 3.09: Mandibular molar formation times of cattle (Brown *et al.* 1960) and sheep (Milhaud and Nézit 1991).

	Stage	First molar (M_1)	Second molar (M_2)	Third molar (M_3)
Cattle	Onset of mineralisation	In utero ⅓ complete at birth	1 month	9–10 months
	Crown complete	2–3 months	12–13 months	23–24 months
Sheep	Onset of mineralisation	In utero ½ complete at birth*	1 month soon after birth*	9–10 months
	Crown complete	6–7 months	11–12 months	20–22 months

*Additional detail described by Hillson (2005: 229–232)

Table 3.09 shows the gross developmental pattern of crown formation of the permanent mandibular molars of cattle and sheep used within this study. These timings are based principally upon radiographic data and clinical observations. By making use of the lower second (M_2) and the third molar (M_3) of each taxon (cattle and sheep/goat) it is possible to obtain enamel from regions of the tooth that began to mineralise within the first few months after birth through to a period approaching the end of the second year of the life of each animal. It should be noted that the range of variation in the timing of mineralisation recorded in Table 3.09 cover the expected variation within a population. One should not necessarily infer from this data that a specific tooth will necessarily form across maximum time interval

implied; that is, a tooth that starts to mineralise late may, in all likelihood also finish late and *vice versa*.

Brown *et al.* (1960) indicates a considerable degree of consistency in the development of the cheek teeth of cattle, both between sexes and modem domestic breeds. In contrast, tooth formation in sheep/goat may be more variable. Hillson (2005: 229–232) summarises the developmental sequence of the dentition of sheep and goat, suggesting that the most reliably established events are the eruption of the permanent molars. Radiographic and morphological studies on the duration of tooth formation in sheep provided by Weinreb and Sharav (1964) indicate that mineralisation of the crown of the third molar is visible from 12 months after birth and completed by 24–26 months. However, Milhaud and Nézit (Milhaud and Nézit) suggest that formation can begin at nine months and be completed as early as 22 months after birth.

Zazzo *et al.* (2010) report that at an age of between 11–12 months the crowns of the second molars of sheep within their study were complete and that the crowns of the third molars had achieved approximately one third of their final height; mineralisation of the third molar crowns was complete at between 18 and 25 months. The authors (*ibid.*) state that their observations are in line with those of Milhaud & Nézit (1991), who found that second molar crowns began to form 1 month after birth were complete by 11–12 months and that third molar crowns, which began to mineralise at between 9–10 months were complete between 20–22 months. This suggests that the second molars of sheep may take typically 10.5 months to form and the third molars 11.5 months. On this basis the time-related relative position of samples from a second molar can be calculated as:

$$\textbf{Sheep } M_2 \textbf{ sample formation time} = 11.5 + (-10.5 \times (H_{smp} / H_{crn})$$

Where H_{crn} represents the fully formed crown height and H_{smp} represents the distance at which the sample was collected from the cervical margin. Third molar sample formation times can be calculated as:

$$\textit{Sheep } M_3 \textit{ sample formation time} = 21 - (11.5 \times (H_{smp} / H_{crn})$$

Following (Brown *et al.* 1960) the crowns of the second molars of cattle form typically over a period of 11.5 months: between 1 month and 12.5 months after birth. The third molar crown forms between 9.5 months and 23.5 months, representing a period of 14 months (Brown *et al.* 1960). The time-related, relative position of intra-tooth second molar samples of cattle can be calculated as:

$$\textit{Cattle } M_2 \textit{ sample formation time} = 12.5 + (-11.5 \times (H_{smp} / H_{crn}))$$

And third molar samples as:

$$\textit{Cattle } M_3 \textit{ sample formation time} = 23.5 + (14 \times (H_{smp} / H_{crn}))$$

This method of scaling requires an estimate of the original crown height of each tooth (H_{crn}). Unfortunately, there is very little published data available to allow this estimate to be made. The average crown heights of the second and third maxillary molars of the Bronze Age cattle referred to by Towers *et al.* (2010) were estimated at 43.0 mm and 43.3 mm respectively and lower third molar crown height estimated as 51.0 mm (Towers pers. comm.). The average lower third molar crown height of prehistoric cattle from the sites of Mine Howe, Pool Earl's Bu and Old Scatness Broch in the Northern Isles have also been measured by Towers (unpublished data) and have an mean height of 44.3 mm. Towers (*ibid*.) indicates that these datasets contain at least a ± 5 mm range, equivalent to an error of ≥ 10 %. Although Bison have larger teeth than domestic cattle, for comparative purposes Widga *et al.* (2010) indicate that the heights of the lower second and third molar crowns of Bison lie well within ± 5 mm of each other.

In this thesis (Chapter 7), a limited number of loose fully mineralised lower molars were identified that showed little or no wear and from which initial crown heights could be estimated. These are documented in Appendix B. As the available data suggest that second and third molar crown heights may be very similar, the unworn crown heights have been combined to calculate a mean crown height for each taxon:

- Mean cattle molars: 48.9 mm (n = 3)
- Mean sheep/goat molars: 36.4 mm (n = 10)

On the basis of the crown height data cited above, the crowns of sheep teeth are around 9 % shorter than those of cattle. However, as the formation times of the cattle crowns are longer than those of sheep, the growth rates for each species appear to be similar (Table 3.10, overleaf). As similar sized samples have been analysed from each tooth, the apparent time interval associated with each sample is presumably similar for sheep and for cattle. For the purpose of plotting the data, these values suggest that the ≈ 2 mm height of each enamel sample is approximately equivalent to ± 0.3 months. Although this interval provides an indication of the relationship between samples within a single tooth crown, it does not represent accurately the combined error associated with the constants used to estimate of apparent formation time. To reflect this, each estimate is reported to the nearest 0.5 months.

Table 3.10: Estimated rate of formation of the enamel crowns of the second (M_2) and third (M_3) mandibular molars of cattle and sheep/goat. Formation time is quoted as a central value within the ranges cited in Table 3.09.

	Cattle		Sheep/goat	
	M_2	M_3	M_2	M_3
Estimated height (mm)	48.9	48.9	36.4	36.4
Formation time (months)	11.5	14.0	10.5	11.5
mm/month	4.3	3.5	3.5	3.2

3.3.1b Hypsodont tooth sampling methods

As the crowns of the teeth selected for this study form after birth and are retained as part of the permanent dentition the chronology of tooth development allows a series of samples to be obtained from each individual that can be compared on the same time-related axis. The sampling methods are based on the low-density approach adopted by Viner *et al.* (2010), who excised pieces of enamel of approximately 2 mm in height from the top (cusp), middle (mid lobe) and bottom (cervix) of each tooth crown within their study. Data provided by Montgomery *et al*. (2010) suggest that the averaging processes involved in enamel mineralisation result in a highly smoothed record of variation within the pool of strontium available for crown formation.

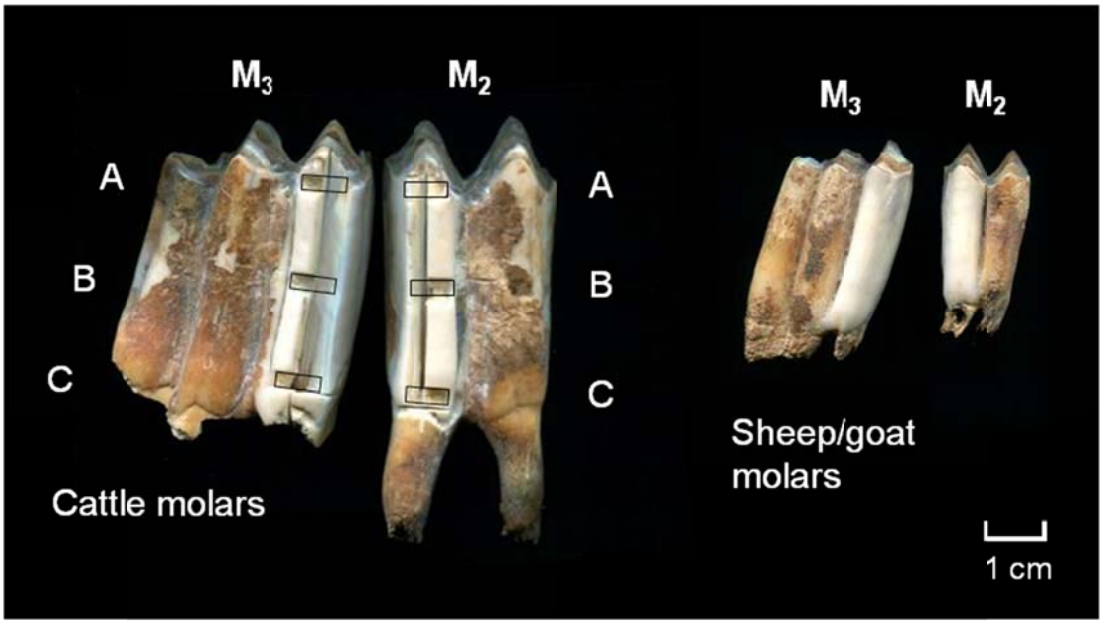

Figure shows the permanent lower second molars (M_2 and M_3) extracted from cattle mandible BIFF_01, from which both serial (black boxes) and bulk enamel samples were excised from the same lobes; A = cusp, B = mid-lobe; C = cervix. Sheep teeth are shown for comparative purposes.

Figure 3.01: Illustration showing the macroscopic sampling method uses to obtain serial enamel samples removed from cattle and sheep teeth.

In contrast to the somewhat flat lingual surfaces, the deeply folded morphology of the buccal lobes of sheep and cattle molars provide easy access to the enamel using a flexible, rotary dental saw. All samples, from both sheep and cattle, were collected from the distal buccal lobe of the second molar and mesial lobe of the third molar (Figure 3.01). This policy

generally provided the greatest height of enamel available in each worn tooth and maximised the spatial separation between intra-tooth samples. However, the teeth contained within this study are subject to different levels of wear, both between teeth from the same mandible (the M_2 is in wear for longer than the M_3) and between animals of different age at death. In order to maintain an approximately equivalent time-related spacing between intra-tooth samples, only two samples (cuspal and cervical enamel) were excised from teeth that presented in an advanced stage of wear.

Serial, intra-tooth enamel samples were collected following the procedure documented in Appendix A. The sampling locations were identified and measured as a vertical distance from the cervical margin, perpendicular to the growth axis of the crown; a sample height of ≈ 2 mm was chosen for serial cattle and sheep/goat samples in order to fulfil the requirements for the removal of dentine and mechanical cleaning. These were prepared from a longitudinal strip of enamel, excised from the selected lobe and incorporating each sample location. To ensure the removal of all dentine, exposed enamel surfaces and any enamel that appeared to be pitted or discoloured, each sample was heavily abraded under a low power binocular microscope. The surfaces of beaks that occurred during cleaning were also abraded.

Samples of 5–10 mg were digested in HNO_3 (8 M), and subjected to conventional chromatographic methods in order to isolate the strontium for analysis by TIMS. Details of the suite of samples taken from each tooth are documented in Appendix B. In addition to the samples of enamel, primary dentine retained from three of the cervical samples of sheep enamel were prepared as a diagenetic control for each site; these samples are identified by the 'Dentine' suffix within the 'Sample ID' field and were analysed alongside the enamel samples, following the same preparatory methods.

3.3.1c Comparison of bulk samples and weighted means

Four bulk enamel samples were prepared for comparison with the weighted mean derived from serial samples taken from the same sheep and

cattle teeth. Identified by the 'Bulk' suffix within the 'Sample ID' field (Tables 7.11, and 7.12 overleaf), each parallel sided strip of enamel (< 4 mm wide) represented the portion of the tooth crown between the top of the cuspal sample and bottom of the cervical sample taken from the same tooth. Due to the size of the bulk samples (sheep 60–93 mg; cattle 122–155 mg) a 10 % aliquot of each solution was subject to analysis; after conversion to chloride the sample was re-dissolved in 1000 µl of 2.5 M HCl and A 200 µl aliquot was spiked using a pipette. This mixture was dried down and re-dissolved in the appropriate volume of 2.5 M HCl prior to loading onto a chromatographic column.

Serial and bulk samples were prepared from the teeth of two mandibles detailed in Tables 3.11 and 3.12 using immediately adjacent strips of enamel excised from the same buccal lobe. As can be seen in Figure 3.02 (overleaf) the concentration weighted means of the serial samples from each tooth fall within the estimated 0.0065 % RSD (2 σ) of the bulk $^{87}Sr/^{86}Sr$ composition. There is a consistent off-set in strontium concentration (23–40 µg/g) between the mean and the bulk values for each tooth. Although this falls within estimated ± 11 % RSD (2σ) associated with each measurement the direction of the bias suggests that a systematic loss of strontium took place during preparation of the bulk sample (Figure 3.02, overleaf). This is likely to be an analytical artefact associated with incomplete dissolution, solution heterogeneity or the calibration of the pipettes used to measure the sample and spike solutions.

Table 3.11: Details of bulk and serial samples of cattle enamel obtained from the same crowns.

Mandible ID	Tooth	Crown height (mm)	location	Position (mm)	Sample ID
BIFF_01	M_2	38.2	Cusp	35.4–33.2	BIFF_01_M2_A
			Mid-lobe	20.2–17.4	BIFF_01_M2_B
			Cervix	4.3–2.2	BIFF_01_M2_C
			Bulk	35.4–2.2	BIFF_01_M2_Bulk
	M_3	44.5	Cusp	40.1–38.1	BIFF_01_ M3_A
			Mid-lobe	23.4–21.4	BIFF_01_ M3_B
			Cervix	7.0–4.7	BIFF_01_M3_C
			Bulk	40.1–4.7	BIFF_01_M3_Bulk

Table 3.12: Details of bulk and serial samples of sheep/goat enamel obtained from the same crowns.

Mandible ID	Tooth	Crown height (mm)	location	Position (mm)	Sample ID
BIFF_02	M_2	21.0	Cusp	19.9–17.7	BIFF_02_M2_A
			Mid-lobe	11.9–9.8	BIFF_02_M2_B
			Cervix	4.5–1.8	BIFF_02_M2_C
			Bulk	19.9–1.8	BIFF_02_M2_Bulk
	M_3	27.1	Cusp	22.4–20.3	BIFF_02_M3_A
			Mid-lobe	12.9–10.9	BIFF_02_M3_B
			Cervix	3.5–1.4	BIFF_02_M3_C
			Bulk	22.4–1.4	BIFF_02_M3_Bulk

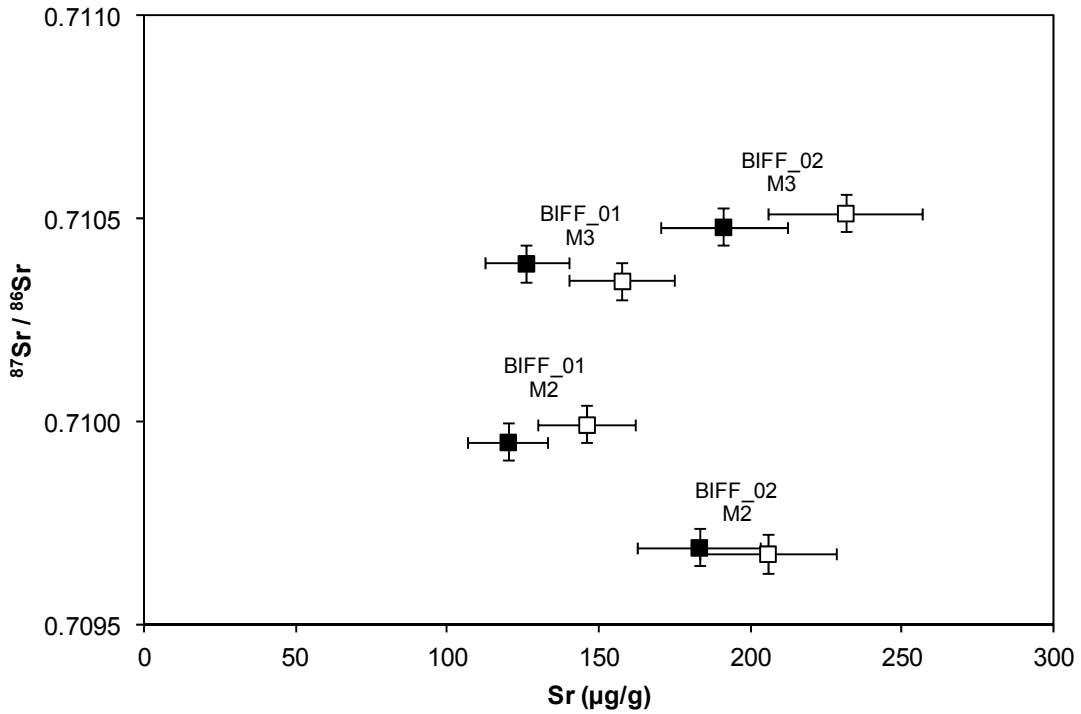

Figure 3.02: Satter plot comparing cattle and sheep/goat teeth from Bicester Fields Farm on the basis of bulk enamel (■), and weighted mean enamel (□) $^{87}Sr/^{86}Sr$ compostion and Sr concentrarion (cf. Tables 3.11 and 3.12).

3.3.1d Duplicate analyses

Replicate samples from the cusp and cervix of the second molar of one cattle mandible (BIFF_04) were also analysed. These were obtained from intact enamel adjacent to the region used to provide the principal intra-tooth series, at locations measured independently from the cervical margin (Table 3.13, overleaf).

Table 3.13: Details of duplicate serial samples of cattle enamel from the same lobe of the second molar (M_2) of cattle mandible BIFF_04.

Tooth	Crown Height (mm)	location	Principal intra-tooth series		Duplicate intra-tooth series	
			Position (mm)	Sample ID	Position (mm)	Sample ID
M_2	42.1	Cusp	40.2–38.0	BIFF_04_M2_A	39.8–37.8	BIFF_04_M2_A2
		Mid-lobe	21.8–19.5	BIFF_04_M2_B	—	—
		Cervix	3.2–1.0	BIFF_04_M2_C	2.8–1.0	BIFF_04_M2_C2

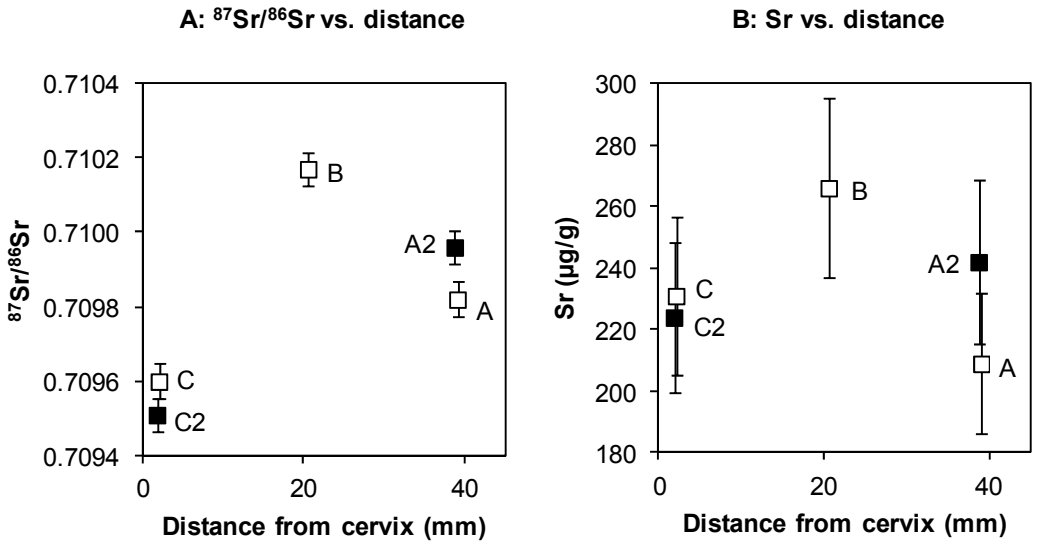

Figure 3.03: Scatter plot comparing principal series of enamel samples A, B, and C (□) with replicate series A2 and C2 (■) from cattle tooth BIFF_04_M2 on the basis of: A) $^{87}Sr/^{86}Sr$ composition; B) strontium concentration (cf. Table 3.13).

Duplicate samples were taken from the cusp (A) and cervix (C) of the tooth identified as BIFF_04_M2. This was intended to provide an indication of the scale of difference in $^{87}Sr/^{86}Sr$ between two samples that can be considered to be significant in terms of a time-related trend within a tooth. The selected tooth displayed a moderate amount of wear and provided enough well preserved enamel to facilitate the collection of additional samples from extreme positions on the same distal lobe. Figure 3.03 shows that the estimated RSD (2 σ) of the $^{87}Sr/^{86}Sr$ composition of the two cuspal samples (BIFF_04_M2_A and BIFF_04_M2_A2) are non-overlapping (i.e. difference in $^{87}Sr/^{86}Sr \geq 0.00010$) and can be considered to be significantly different from one another. There is a marginal degree of overlap between the intervals associated with the cervical samples (BIFF_04_M2_C and BIFF_04_M2_C2).

The mean difference in $^{87}Sr/^{86}Sr$ composition between the samples taken from different vertical zones (0.00038 ≈ 0.054 %) is more than twice as great as the largest difference between the samples taken from comparable positions (0.00014 ≈ 0.020 %), suggesting that the tooth provides evidence of a significant change in the dietary sources of strontium during crown development. As well as differences in strontium isotope composition there are also differences in strontium concentration between each of the samples (Figure 3.03). However, as an approximation of external precision the estimated RSD (2σ) associated with the determination of strontium concentration may approach ± 11 %. This suggests that, although there is a significant difference between BIFF_04_M2_A and BIFF_04_M2_B, the differences between any remaining pair of samples are not necessarily so.

Dental enamel has a complex anisotropic microstructure and is known often to be isotopically heterogeneous (Montgomery *et al.* 2010). Moreover, the geometry of a tooth is such that the relative position of one sample to another in relation to mineralisation history is captured imperfectly by a two dimensional vertical measurement (Balasse 2003). As the preparatory methods used in this study rely on the removal of surface enamel and enamel from the enamel/dentine junction, it is likely that the duplicate samples reported above incorporated volumes of the crown that mineralised at slightly different times to those within the principal series. Under this model, it is likely that the 'noise' associated with a sampling error is captured best by the divergence of the paired samples from the median of those two values, rather than the absolute range recorded at either the cusp or cervix of the tooth. The maximum observed difference is equivalent to an interval of 0.70989 ± 0.014 %. Within the limitations of the sampling method, differences between samples excised from different locations within an enamel sequence that exceed the maximum observed divergence from the median are likely to be highly significant and can be assumed to have a time-related component.

3.3.2 Human material

Selected teeth from the individuals interred at Old Dairy Cottage (ODC89) were provided by Helen Rees, the Curator of Archaeology at Winchester Museums Service (documented in Appendix B). Second molars were prioritised for analysis as they are likely to begin mineralising after the cessation of weaning, but still in early childhood. When second molars were not available, or the teeth could not be easily removed without causing unacceptable damage, first or second premolars were selected. Table 3.14 provides details of the estimated mineralisation time associated with the selected teeth and has been reproduced after Montgomery (2002: 52, Table 3.3), following the work of Gustafson and Koch (1974).

Table 3.14: Permanent tooth formation time following Montgomery (2002: 52, Table 3.3).

Tooth	Onset of mineralisation	Crown complete
Upper first premolar (Pm^1)	1.5–2 years	5–7.5 years
Lower second premolar (Pm_2)	2–2.25 years	6–8 years
Lower second molar (M_2)	2.5–3 years	6.2–8 years
Upper second molar (M^2)	2.5–3 years	7–8 years

Teeth were chosen on the basis of macroscopic condition, avoiding those specimens with extensive cracking and obvious signs of decay and staining; where a small number of caries were present these were restricted to the developmental fissures of the occlusal surfaces and inter-dental locations. A region of each crown was selected for $^{87}Sr/^{86}Sr$ analysis with the aim of maximising the worn crown height. In addition to the enamel samples, one sample of primary dentine (ODC_Sk578_D) was processed, in order to obtain an estimate of the likely diagenetic trajectory associated with the site. In each case between 13–20 mg of fully prepared material was analysed.

The selected teeth were prepared for analysis following the stepwise procedure detailed in Appendix A. The teeth were rinsed in deionised water to remove any loosely adhering material and allowed to dry. A region of each

crown was selected for sampling, on the basis of apparent condition. The outer enamel of the selected cusp was abraded to a depth > 100 μm using a pre-cleaned tungsten carbide dental burr. A slice of enamel was excised, representing the entire available crown height using a flexible, diamond edged rotary dental saw. Where possible the majority of the adhering dentine was cut away as one piece, or collected as powder from a tungsten burr. In each case between 13–20 mg of fully prepared enamel was analysed.

The removal of all remaining dentine from the enamel and the cleaning of all the exposed surfaces, including the edges of any new breaks, was achieved using a diamond impregnated dental burr under a low power binocular microscope. In addition to the enamel samples one core dentine sample (ODC_Sk578_D) was processed similarly, in order to obtain an indication of the likely diagenetic trajectory associated with the site. The resulting samples were transferred to clean vials and removed to a clean working area. All further preparation and analysis was carried out within the clean laboratory facilities at the NERC Isotope Geosciences Laboratory.

3.3.3 Preparation of dental tissues for analysis

The chemical preparation and analysis of human and faunal dental tissues was carried out under clean conditions (Appendix A). All chemical treatments were undertaken within laminar-flow hoods using high purity reagents and acid leached vessels. The same processes were applied to enamel and of dentine, from both faunal and human teeth. To remove organic surface residues each sample was initially rinsed in acetone. This was decanted and the samples rinsed in deionised water. The samples were then cleaned ultrasonically in deionised water and rinsed, before being placed on a hotplate (50 °C) for one hour in deionised water. The samples were rinsed in deionised water again and allowed to dry before being weighed into separate Savillex^{TM} beakers and spiked with a known quantity of the ^{84}Sr-enriched spike solution (Oak Ridge Dilute Strontium). Each sample was dissolved in 2 ml of high purity distilled HNO_3 (8 M) and

converted to chloride to allow Strontium to be extracted using standard cation exchange methods (Section 3.1).

3.3.4 Blank characteristics

During this study up to three procedural blanks were included in each enamel chemistry batch (P534, P540, P545 and P548). The mean strontium blank was 43 pg (n = 14), but varied from 22–78 pg. Enamel strontium concentrations in human enamel were between 45.8–164 µg/g and faunal enamel from 89.8–308 µg/g in samples weighing between 3.1–31 mg. Thus, the very highest blank possible represents less than 0.01 % of the smallest sample of strontium. Blank corrections were therefore considered to be unnecessary.