CHAPTER 4 METHODS AND TECHNIQUES

4.1 Introduction

In this chapter, I present different aspects of the overall methodological approach taken in this study. I begin with a detailed discussion of the sampling design and sample collection strategies, pertaining first to the selection of bioavailable samples (animals and plants) and then to human samples for Sr isotope analysis. This is followed by a presentation of the sample processing protocols employed in this research project for the different sample types and materials. Next, the laboratory methods and procedures for the separation and purification of strontium are provided. Then, I discuss the measurement of strontium isotope compositions using Thermal Ionization Mass Spectrometry (TIMS), including the results of the analyses of standard reference materials and total procedural blanks. Finally, I present the protocols and procedures for the sampling, processing, and measurement of oxygen and carbon isotope ratios in human dental enamel samples via gas source Isotope Ratio Mass Spectrometry (IRMS).

4.2 Sample Collection Strategies

The selection of appropriate ancient human skeletal assemblages from the Caribbean region was an important consideration for addressing the research questions of this study. One of the primary goals was to collect samples from a wide range of geographic, cultural, and chronological settings within the Caribbean region. In order to make comparisons between different groups it was necessary to collect and analyze skeletal assemblages from different islands within the region. However, due to a variety of reasons (from variable conditions of preservation to varying histories of archaeological research) not all regions of the Caribbean possess large, systematically excavated, pre-Columbian skeletal assemblages. For example, some islands have not been subjected to

extensive, research-oriented archaeological investigation while others have been the foci of research but have not yielded large skeletal collections to date. For this project, we focused on the inclusion of large assemblages from controlled contexts, e.g., from which relevant and necessary contextual data were available. Contextual data in this sense refers primarily to demographic (sex and age); mortuary (location, type, and positioning of burials and grave inventories); chronological (radiocarbon and/or relative dating from associated materials); and dietary (stable isotope, paleobotanical, and zooarchaeological results) data.

4.2.1 Collection of Faunal and Botanical Samples

As discussed in Chapter 3, most attempts to interpret strontium isotope results derived from human remains rely on assessments of local ranges of Sr isotope variation. This can be accomplished through various means and for this project we chose to incorporate a number of lines of evidence into our local range estimates. Price and colleagues (2002) have proposed that the remains of certain species of archaeological fauna may provide the most reliable measures of local Sr isotope variation for a given site. Price has also suggested a ranking of various sources of local Sr isotope data with archaeological fauna representing the most robust source (Price et al. 2002).

Archaeological faunal remains are considered representative of local signatures under the assumptions that certain species have restricted feeding ranges, that they are obtaining the vast majority of their subsistence resources (and hence strontium) locally, and that archaeological fauna are much less likely to have consumed imported foodstuffs or environmental pollutants relative to modern faunal samples (Bentley 2006; Price et al. 2002). Modern fauna can be used if archaeological faunal remains are unavailable but are considered somewhat less reliable sources of data owing to the possibility that modern fauna may be consuming food products that have been moved substantial distances through human agency and/or owing to potential sources of modern contamination including a number of widespread environmental pollutants, fertilizers, pesticides, herbicides, and so on.

Macro-botanical remains are rarely preserved in significant quantities in archaeological deposits, especially in tropical contexts, and thus are not considered as a realistic source of Sr isotope data within an extensive sampling strategy. Although, where available, they may constitute another possible source of local Sr data. Modern botanical remains can be utilized for local range assessments but greater care must be taken with sample collection to avoid the previously mentioned problems concerning the ubiquity and widespread use of various modern chemicals and potential anthropogenic-induced environmental changes.

Thus our main sources of information for defining local strontium isotope ranges come from several sources, these include analysis of locally derived samples of: 1) archaeological fauna, 2) modern fauna, 3) and modern flora. Other sources of data are derived from the published literature on Sr isotope variation in the Caribbean derived from geochemical analysis of various geological samples (whole rock, mineral, and soil) samples (Roobol and Smith 1980; Wadge and Wooden 1982). These data are utilized in conjunction with the statistical analysis of patterns in the main data set derived from Sr isotope analysis of human dental enamel.

The vast majority of faunal remains included in this project consist of dental enamel samples of rodents from archaeological deposits and from land snail shells, both archaeological and modern. The rodent remains primarily come from rice rat and hutía remains (*Oryzomys sp*. and *Capromyidae*, respectively) and were chosen based on several parameters. These include the assumptions that: 1) owing to their ubiquitous presence, they were not widely transported from island to island or region to region; 2) they have fairly restricted subsistence ranges; 3) when available they will often consume human food refuse, and thus consume similar foods as humans; and 4) there is evidence that rodents (and possibly also snails) themselves were consumed by humans in the ancient Caribbean (deFrance et al. 1996; Newsom and Wing 2004). In other words, we propose that rodent remains from archaeological refuse deposits represent animals that were probably living and subsisting locally (most likely the same island, and possibly the same catchment or habitat) as the humans living at the site and thus most likely shared in common a similar subsistence base despite potential differences in dietary breadth. Furthermore, it is likely that if these creatures were captured locally that they were also

consuming products from house gardens and midden or refuse deposits and they may have eventually ended up in a site's deposits via the 'dinner plate'.

Modern and archaeological land snail shells of various species were chosen based on the same parameters and assumptions as the rodent remains. They are however, even more ubiquitous and thus even less likely to have been moved from island to island by humans. In addition, land snail shells are still readily available in many areas of the Caribbean, and from a logistical perspective are easy and inexpensive to find, collect, and process. In many cases where archaeological faunal remains were absent or unavailable, modern land snail shells were collected, primarily from extant archaeological sites or uncultivated areas, and are considered to be fairly reliable representatives of local Sr isotope signatures.

Fortunately, we were able to collect a number of faunal remains from all of the sites/regions from which we have collected and analyzed human remains. Therefore, we have robust datasets for local range estimations for most of these skeletal collections. However, an important aspect of this project was to develop a database of biosphere Sr isotope variation for the greater circum-Caribbean region and thus we have attempted to make initial assessments of this variation for most of the Caribbean islands and certain areas of the adjacent coastal mainland, including also islands and regions from where human remains were not available or were not analyzed. Nevertheless, there are several islands and areas within the Caribbean region from which no samples were collected owing to limitations of time, resources, and/or logistical concerns.

In order to broaden our sample collections and to make them more comparable we chose to collect and analyze botanical samples from all of the islands and coastal regions included in this study. This was also done to provide auxiliary or secondary lines of evidence in contexts where faunal and human remains had been incorporated into the study and to provide an initial baseline of Sr variation and ranges in islands/regions from where no other evidence was available. The collected botanical specimens were primarily grasses (family *Poaceae*) but other types of botanical samples (e.g., tree leaves) were also collected in a few instances. In fact, for botanical samples the choice of species is somewhat less important than for faunal sample collection as any plants within a small, geologically homogenous region should share *broadly* similar Sr isotope ratios. However,

some variation does exist between certain species sharing the same ecosystem and even within individual specimens, owing to variable conditions within the local hydrological system, fluid dynamics, soil matrices, and so on (Dijkstra et al. 2003; Pozwa et al. 2000; Pozwa et al. 2002; Pozwa et al. 2004).

Another potential limitation of using botanical samples relative to faunal samples to determine local Sr range estimations is that botanical samples primarily reflect the localized geology of the soils from which they derive their nutrients along with variable contributions from atmospheric sources (Bentley 2006). Thus they lack one of the other main benefits of faunal samples, the tendency of 'bio-averaging' (Price et al. 2002). Any local variations of Sr isotope signatures tend to become 'averaged out' within the tissues of animals as they consume various foodstuffs from the local environment and incorporate trace elements into their bodies, making the Sr values within their tissues more directly comparable to that of human consumers (Price et al. 2002).

Despite these limitations and the previously mentioned potential issues concerning modern environmental contaminants/pollutants, several studies have concluded that the strontium within plant tissues is primarily derived from local soil geology and thus should be broadly reflective of local strontium isotope variation (Bentley 2006). For this reason, we have incorporated them into our analysis with the caveat that these data are considered to be somewhat less reliable than other sources used in this project and that the potential influences of the various environmental parameters need to be taken into consideration in terms of sample selection strategies.

To minimize the potential that the collected botanical samples had been directly subjected to contamination through the application of fertilizers, pesticides, herbicides, or industrial pollutants we generally avoided collecting samples from areas of either urban development or commercial cultivation. These restrictions focused our sample selection and collection primarily to rural areas, for example: trails, forested woodlands, open pastures, inter-property fence-lines, and so on. Our sampling strategy was also influenced by several other important variables. First and foremost, for islands that are less geologically homogenous, we attempted to collect botanical (and where possible faunal) samples from multiple geological regions in order to broaden our coverage and detect intra-island isotopic variations.

Other sampling parameters attempted to account for intra-island variation of strontium isotope signals owing to variable rates of atmospheric contributions of strontium from marine-derived rainwater or sea-spray (Bentley 2006; Price and Gestsdóttir 2006). Therefore, particularly for the more geologically homogenous regions and islands, we also attempted to collect samples based on altitude, distance from coast, leeward/windward sides, and overall coverage. The aim of this sampling strategy was to characterize intra-island isotope variation owing to varying marine effects as opposed to variability in underlying geology per se. In fact, the island of Saba was chosen as a case study for this approach as it is a very small (~13 km^2) and rather geologically homogenous island. Saba is primarily composed of geologically young volcanic bedrock with low-lying areas covered by Quaternary alluvium (van Soest et al. 2002). We systematically and intensively collected over 50 faunal and botanical samples from Saba for the purpose of elucidating the dynamics of marine effects and determine the relative contributions of various strontium sources to the local island ecosystem (Laffoon and Hoogland 2009).

In summary, our sampling strategy for the collection of comparative and representative strontium isotope data for the greater Caribbean was both systematic and opportunistic. We based our sample selection decisions on the suggestions of other prominent researchers in this field, namely that archaeological faunal remains represent the most reliable source of this type of data. Concerted efforts were made to collect archaeological faunal remains, with a specific focus on dental enamel from native rodents and land snail shells, for any site for which we also analyzed human remains. Modern faunal samples (land snail shells) were also collected, even if archaeological samples were available, to broaden our sample set.

Lastly, botanical samples were collected, under strict parameters, from nearly every island of the Caribbean to provide a secondary base-line to constrain the local bioavailable Sr isotope ranges. These baselines can be further refined by future research but provide us with the necessary data for initial attempts at determining potential geographical origins for human individuals identified as nonlocals within our sample set. It is also hoped that other researchers, not only archaeologists but also geologists and ecologists, can make use of this Caribbean Strontium Isotope Database to be constructed

with the extensive data produced by our research. For example, potential applications include research into the provenance of widespread indigenous Caribbean wood-carvings (Ostapkowicz et al. 2009) or investigations of the geographic origins of individuals interred in mixed population colonial era cemeteries (Goodman et al. 2004; Jones et al. 2003; Price et al. 2006).

4.2.2 Collection of Human Dental Samples

For human dental samples, we chose to sample permanent (adult) premolars when present and in good condition, i.e., whole and intact with no large carious lesions. If not present, we sampled any tooth that was available and in good condition with priority placed on first and second molars, then incisors and canines. Third molars were generally avoided unless no other tooth was available because the time of formation and mineralization of M3's occurs later in life relative to most other teeth, with crown formation only beginning around 7-13 years of age (Hillson 1996; White and Folkens 2005) such that they are less obviously reflective of the geochemical environment of early childhood. In fact, any tooth will work from the perspective of our analysis but the identification and interpretation of localness and migration is more problematic for third molars, owing to the somewhat increased likelihood that the individual may have moved during their early childhood years, movement which would not be identified if occurring before the formation of the third molars. In total, more than 60% of the human dental samples are premolars with the remainder represented by a combination of other dental elements.

Permanent teeth were preferentially sampled (see Appendix B), even for children, unless they were not present or not sufficiently mineralized and thus more susceptible to diagenic contamination of the dental enamel by the burial environment. In general, we expect that children will be less mobile than adults, owing to the fact that having died in childhood they had less time (and opportunities) within their relatively brief lives to have migrated. However, this is a hypothesis that needs to be tested. Similar research into past human mobility from a biogeochemical perspective (Cucina et al. 2005; Goodman et al. 2004) suggests that children are generally less mobile than adults. However, as some

children have in fact been identified as nonlocals or migrants, this point remains a rough generalization that probably varies according to the mobility system of the population in question. Nonetheless, we propose that for many contexts the Sr isotope signatures from the remains of children are more likely to be representative of the local isotopic signature than not and thus they can provide an independent assessment of the local range. For this reason, the inclusion of a small number of dental samples from children and infants (including deciduous teeth) was also a component of our sampling strategy. Lastly, relevant information concerning the sample locations for human skeletal materials including detailed descriptions of site histories, contexts and settings are presented in chapter 5.

4.3 Strontium Isotope Analyses: Sample Processing

As the procedures for processing different types of samples (human, faunal, botanical) and different sample materials (enamel, shell, plant tissues) vary, these will be discussed separately. However, after the preliminary sample processing and chemical treatments, all samples are subjected to the same procedures for strontium extraction and isotope analysis. All sample processing was done under controlled conditions at the Faculty of Earth and Life Sciences, The VU, Free University of Amsterdam, The Netherlands. The processing of dental enamel from humans and rodents is essentially the same and so both will be included in this section. If the selected tooth was still covered in soil matrix, it was sonicated for 1 hour in Milli-Q water before processing was begun but this step was only necessary for a small number of samples. For most samples mechanical cleaning and removal of the outer surface (soil, calculus, staining) and outer-most layers of enamel was sufficient to reveal the inner core enamel from which the enamel was extracted for later analysis. The cleaning and extraction of the enamel was accomplished by means of a variable speed dental micro-drill, specifically a Minilor Perceuse M1 hand-held drill with an AFX DC regulated power supply (0-15V, 2A) outfitted with a diamond-tipped rotary burr.

All cleaning procedures were performed wearing protective gloves and a laboratory coat in a designated room away from the clean lab. The work station was cleaned using ethanol and Milli-Q water, and dried with compressed air (the university compressed air supply is filtered and dried to ensure cleanliness and minimal water content). The drill bit was cleaned by successive placement into 1) ethanol, 2) Milli-Q water, 3) 0.15 N HCl, 4) Milli-Q water, and 5) dried with compressed air. This process was repeated twice and was performed for each surface cleaning of the tooth and then again prior to mechanical enamel removal. A specific surface of the tooth was chosen for sample extraction and this area was then thoroughly cleaned using the drill to abrade away any superficial deposits including calculus, soil, stains, and the first several microns of outer enamel. The inner core enamel is readily identifiable owing to its milky-white color and homogenous texture. Usually only the area of the surface around the sample extraction site was cleaned but if necessary the entire surface of the crown and sometimes even the root was cleaned to prevent loose particles from contaminating the extracted enamel sample.

The workstation and drill were then re-cleaned as previously described and the tooth itself was blown clean using filtered, compressed air to remove any remaining dust or dirt. Approximately 1-5 mg of enamel (about the size of a small dental filling) was then extracted by directly abrading the exposed inner core enamel with the drill bit following the protocols of Knudson (2008). The enamel powder was collected on a fresh sheet of weighing paper and then transferred to a pre-cleaned/pre-treated 1 ml centrifuge tube, weighed, and then taken to the clean lab for chemical treatment and processing.

All lab hardware (teflon and quartz beakers, pipette tips, centrifuge tubes, etc...) was pre-cleaned following the methodology outlined in designated sections of the *Laboratory for Geochemical Analysis* (a class 100 clean laboratory). Except where stated, hardware was leached in 6-7 N HCl in a laminar flowhood for several hours to several days depending on container material type, rinsed with demi-water and then Milli-Q water, leached in 3N HNO3, re-rinsed with Milli-Q water and then dried, sealed, labeled, and weighed for later use.

Land snail shells were either drilled or broken by hand within the sample bags and the fragments were sonicated in Milli-Q water for one hour, rinsed with Milli-Q

water, dried, weighed, and then transferred to clean 30 ml Teflon beakers before being taken to the clean lab for further processing (see below).

Plant samples were dried immediately after collection by setting them in the sun, drying over a vent, microwaving, or with a hair-dryer. After transport, bagged samples were frozen for several days and if necessary, dried again on aluminum foil in a drying oven at 50 ^{o}C for 1-2 days to remove all moisture and arrest any decay. Approximately one gram of plant leaf or stem was then removed and placed into a pre-cleaned, sealed titanium crucible. The dried plant samples were ashed within a (Heraeus model) muffle furnace at 500 ^{o}C for 12 hours and then allowed to cool. Ashed samples were then transferred to pre-cleaned 30 ml PFA vials under a laminar flow-hood and weighed again before being taken to the clean lab for further processing (see below).

Dissolution procedures vary between enamel, shell, and ash (plant) samples and each of these will be discussed separately. All chemical procedures were performed under controlled conditions within a laminar flow-hood within a specially designated clean lab (class 100), using specific laboratory procedures for the minimization of sample contamination, including the use of protective gloves, sleeves, eyewear, footwear, and lab coats. All centrifuge tubes, PFA vials, beakers, and pipette tips were pre-cleaned within the clean lab, to remove potential contaminants using previously discussed procedures.

Ash samples were processed as follows: 1) Ash samples were weighed within pre-weighed and pre-cleaned vials; 2) Samples were dissolved in 10ml of concentrated, distilled nitric acid (14N) and then dried down on a hotplate at 120 ^{o}C for around 12 hours. This step was repeated twice; 3) Samples were then dissolved in 2 ml of conc. HNO_3 and 200 µl of H_2O_2 and then dried down again on a hotplate. This step was repeated; 4) samples were re-dissolved in 3N HNO_3, centrifuged at 10,000 rpm for 4 minutes and the supernatant was removed and placed into a new pre-weighed, pre-cleaned vial; 5) Samples were then dried down and re-nitrated with 500 µl of 3N HNO_3 and reweighed; 6) A 10% aliquot was removed for trace element analysis by ICP-MS; 7) The samples were centrifuged and then loaded onto cation exchange columns pre-loaded with strontium-specific resin for isotope separation (see below for details of strontium separation procedures).

Enamel samples are chemically processed somewhat differently than those of ashed botanical materials. As dental enamel consists of approximately 97-99% calcium hydroxylapatite, there are fewer steps required to eliminate the presence of organic components within the sample matrix compared to ashed plant samples. Enamel samples from both human and faunal remains were processed as follows: 1) Powdered enamel was dissolved briefly (20-30 minutes) in 0.5 ml of acetic acid (to remove extant exogenous calcium carbonate), centrifuged at 12,000 rpm for four minutes, and the liquid portion removed and discarded; 2) Samples were washed in 0.5 ml of Milli-Q, centrifuged again, and the supernate removed and discarded; 3) Samples were nitrated with 0.5 ml of 3N HNO_3 and then dried down; 4) A 10% aliquot was removed for future trace element analysis by ICP-MS; 5) Prior to strontium separation, samples are dissolved for 30-60 minutes in 3N HNO_3 and then centrifuged at 12,000 rpm for 4 minutes and then loaded onto pre-prepared strontium separation columns.

Land snail shell samples differ from enamel samples in that they consist primarily of calcium carbonate and thus are processed in a slightly different manner. The procedures for chemical treatment of shell samples are identical to that of enamel samples except that they are not initially subjected to an acetic acid wash and instead are simply rinsed with de-ionized, de-mineralized (Milli-Q) water.

The separation of the strontium component of the samples from other elements in the sample matrix after chemical dissolution is accomplished by running the nitrated samples through specially designed separation (cation exchange) columns. These columns consist of hand-blown quartz glass columns with a 0.8 ml loading reservoir and a tapered column with a small glass frit material at the bottom. These columns are pre-treated/cleaned and then loaded with (~0.12 ml) strontium-specific, crown-ether resin (Eichrom) slurry, which is specifically designed to bind only to strontium. After thorough cleaning of the resin columns, the samples are loaded onto the columns and as the liquid passes over the resin, Sr nitrate molecules bind to the resin while allowing other elements to pass through the column. Milli-Q water is later loaded onto the column which alters the pH as it passes through the column, thereby altering the binding sites and allowing the strontium to be released and pass through the column and be collected in pre-cleaned vials.

The steps for column preparation and strontium separation are as follows: 1) glass columns are normally stored in 3N HNO_3 but prior to column preparation, they are rinsed with Milli-Q water and placed onto a pre-cleaned holding rack; 2) A resin slurry (~0.12 ml), of equal parts Eichrom Sr Spec resin (100-150 um diameter) and Milli-Q water, is then loaded into the column reservoir and allowed to settle into the tapered bottom; 3) The resin is then cleaned by washing alternately with 3N HNO_3 and Milli-Q water, three times each; 4) The resin is then pre-conditioned with 0.5 ml 3N HNO_3 (to ensure that the pH within the column is ideal for the strontium-binding capacity of the resin); 5) The pre-prepared samples (~0.4 ml) are then loaded onto the columns; 6) The samples are washed over the resin by sequentially adding 0.2 ml of 3N HNO_3, five times and then with a final wash of 0.8 ml 3N HNO_3 (this is doubled for ash samples which tend to be 'dirtier' in the sense that they potentially contain more organic components that can interfere with the separation process); 7) After the nitric acid pre-fraction has totally passed through the columns, the pre-fraction collection vials are replaced by pre-cleaned 5 ml collection vials and 0.8 ml of Milli-Q water is added to the column reservoir; 8) The strontium fraction is then collected, dried down on a hotplate, and then as a final step nitrated and dried down twice with concentrated HNO_3. Strontium samples are then ready for loading onto filaments for isotope analysis via TIMS.

4.4 TIMS - Thermal Ionization Mass Spectrometry

Several methods for the analysis of strontium isotopes exist and different types of mass spectrometry can be utilized for the analysis of strontium isotopes from human and animal remains to address various research questions (Balter et al. 2008; Bentley et al. 2007a; Booden et al. 2008; Nowell and Horstwood 2009; Richards et al. 2008; Simonetti et al. 2008). The most common means of measuring strontium isotope compositions of these material types are ICP-MS and MC-ICP-MS (in solution mode or through the direct ablation of solid-state samples via the coupling of a laser to the mass spectrometer) and TIMS. The use of chemical separation procedures and isotopic analysis via TIMS represents the longest and most widely used method for strontium isotope analysis in

archaeological contexts (Ezzo et al. 1997; Grupe et al. 1997; Price et al. 1994a; Price et al. 1994b).

This approach was taken within this research project for a variety of reasons. Several decades of analysis via TIMS, has indicated that this robust method routinely produces precise, reliable, and consistent results (Nowell and Horstwood 2009). TIMS is also relatively inexpensive, especially if one prepares, processes, and analyzes their own samples. Furthermore, the purported advantages of LA-ICP-MS, namely minimization of sample destruction and speed of sample processing relative to other factors, particularly data quality, have not been borne out by recent research. More explicit discussions of the pros and cons of various methods of strontium isotope analyses, can be found in (Copeland et al. 2008; Copeland et al. 2010; Nowell and Horstwood 2009; Simonetti et al. 2008).

4.4.1 TIMS - Filaments

Strontium isotopes ratios within samples were measured on a TIMS by using annealed rhenium ribbons mounted onto metal sample loading brackets. Sample strontium is imbedded within a tiny ceramic bead of tantalum salts, which is loaded onto the filament and eventually into the TIMS machine. The ceramic bead thus generated reduces the rate of strontium evaporation, causing the Sr to evaporate at higher temperatures. This higher temperature causes greater thermal ionization and thus provides higher ionization efficiencies.

The first step in this process is to make rhenium filaments onto which the sample can be loaded and then placed into the machine where it can be heated through the application of an electric current (all previously used Re is removed and the filament holder is cleaned with a file or an abrasive drill). This is accomplished by welding a ~1.2 mm strip of rhenium ribbon onto the filament loading bracket using a high-precision welder with a current of 4 amps. 'Filaments' in this terminology consist of a small metal loading bracket with a thin rhenium ribbon welded onto it.

The metal brackets themselves and their associated plates and shields are all reusable but the ribbons themselves are not. The hardware (brackets, plates, and shields) are pre-cleaned with the following protocol: 1) The hardware is washed by scrubbing the surface with a combination of demi-water and powdered aluminum, and then rinsed thoroughly with demi-water; 2) Then the hardware is boiled in demi-water for 25 minutes and then boiled in Milli-Q water for 25 minutes; 3) The hardware is then dried in an oven at 100 ^{o}C for 1 hour.

The filaments are then further cleaned by removal of contaminants (from the air and water) by subjecting them to high temperatures at vacuum; this is known as 'out-gassing'. The out-gassing procedure consists of loading the filaments into a designated chamber within a ThermoScientific Bakeout Device. This machine de-pressurizes the internal chamber containing the filaments to a pressure of $< 2 \times 10^{-6}$ bar and applies a high current to all of the filaments. This increases the surface temperature to 1.5-2.0k ^{o}C and thus evaporates and/or ionizes any surface contaminants on the Re filament. This step is essential because it is necessary to remove (as much as possible) any potential source of strontium other than the sample which is to be analyzed. These steps and others are thus designed to provide confidence that the measurements produced are derived from the sample itself and not from other sources.

4.4.2 Loading Samples

Strontium samples were loaded onto the prepared filaments according to the following procedures. 1) dried samples were dissolved in 2 µl of 3 N HNO_3; 2) filaments were attached to a specially designed loading device which allows a current to be passed through the filament; 3) a 1.1 A current is applied to the filament and a small amount of parafilm is melted onto the filament in two places to produce 'dams' that prevent the loaded sample from spreading across the entire filament; 4) the current is reduced to 0.9A and 2 µl of $TaCl_5$ and 1 µl of H_3PO_4 is placed onto the filament; 5) 2 µl of nitrated sample (or standard) are then placed onto the filament and the mixture is allowed to dry down; 6) the current is sequentially increased to 1.2, 1.5, and 2 amps to dry down the sample, burn

off the parafilm, and evaporate the phosphoric acid, respectively; 7) the sample is heated to a dull red color to fully dry the sample; 8) the loading bracket with samples is then placed onto a loading turret which is subsequently placed into the source chamber of the TIMS and at least one external standard (NBS-987) is analyzed with every 12 samples.

4.4.3 TIMS – Operating Parameters

All samples were analyzed for strontium isotope composition with a thermal ionization mass spectrometer (TIMS, ThermoFinnigan MAT 262 RPQ plus) at the Faculty of Earth and Life Sciences of the VU, Free University Amsterdam, The Netherlands. The source chamber with loaded samples (and standards and blanks) is pumped down to a vacuum of $< 8 \times 10^{-8}$ torr and a current is slowly applied to the appropriate filament. Samples are generally run at a current of 2.5-3.2 amps, producing temperatures of roughly 1400-1600 ^{o}C. The $TaCl_5/H_3PO_4$ mixture, within which the sample is loaded, creates a tiny ceramic-like bead which causes the strontium atoms to ionize at higher temperatures and thus tends to increase the ionization efficiency compared to previous methods that use only H_3PO_4. The ionization is accomplished thermally, hence the name TIMS, and after a brief interval to permit the burning off of any contaminants (including any residual rubidium), Sr isotope analysis can begin.

The ionization process generates charged particles that can then be manipulated via electromagnetic forces. The ion beam is thus focused and the particles spatially separated by mass such that ions of different masses can be collected and measured via Faraday collectors within the instrument. To avoid biases generated from potential differences between collector cups, for the first year of the project samples were measured with a dynamic triple-jumping method. All measurements were automatically corrected, using an exponential correction factor, to an $^{86}Sr/^{88}Sr$ value of 0.1194. Measurements were only accepted when the $^{85}Rb/^{86}Sr$ ratio was below 0.0002, indicating minimal presence of mass interference from rubidium. A minimum of 60 ratios per sample were collected, more if necessary, with a goal of obtaining a standard error of 0.000010 (2σ) or less for each sample. A small percentage (<3%) of samples produced

standard errors (2σ) > than 0.000020 and were re-analyzed, thus samples included herein yielded signatures with a standard error of < 0.00002 (2σ). After the first year, samples were measured in static mode once it became clear that measurement precision of ±0.00002 was insubstantial in terms of the regional Sr isotope variations.

4.4.4 TIMS – Standards

Each set of samples on the TIMS included one or more standards, in addition to about 10-12 samples and blanks. For external reproducibility and quality control, we used the certified strontium carbonate ($SrCO_3$) reference material (NBS/NIST) SRM-987. Repeat measurements of the international standard over the period of analyses yielded a mean $^{87}Sr/^{86}Sr$ value of 0.710250 ± 0.00003 (1σ, n=81). Over the course of the project, samples and standards were analyzed using two different analytical modes. Analyses of the international standard (SRM-987) in dynamic mode produced more consistent results with a mean $^{87}Sr/^{86}Sr$ value of 0.710231 ± 0.000008 (1σ, n=27) that is within error of the accepted value and a lower standard deviation. Thus all samples analyzed in dynamic mode were not corrected (see Appendix A and B). Over the three year duration of the project, analyses of the international standard (SRM-987) in static mode produced slightly more variable results with a mean $^{87}Sr/^{86}Sr$ value of 0.710259 ± 0.000033 (1σ, n=54). This period included changes in collector performance that led to drift in the Faraday cups used for Sr isotope analysis. These changes caused transient variations in the values of the standard increasing the variance in the standard data. Consequently, all samples analyzed in static mode were normalized (in reference to the in-run value of the external standard) to the generally accepted $^{87}Sr/^{86}Sr$ value for the standard reference material of 0.710240.

4.4.5 TIMS – Blanks

For each series of samples that underwent chemical processing and strontium separation at least one total procedural blank was analyzed to test for the presence of ambient or intrusive strontium which may have potentially contaminated any of the reagents, hardware, or samples. Total procedural blanks were spiked after strontium separation with calibrated ^{84}Sr spike [serial code # 20080117]. Analyses of $^{84}Sr/^{86}Sr$ ratios were conducted with a minimum of one block (10 single scans) in static mode with the TIMS and the overall strontium concentration was calculated using the principles of isotopic fractionation (Faure and Mensing 2005). Over the course of our analyses, total procedural blanks yielded Sr concentrations typically less than 200 pg. Such low concentrations are considered negligible and insignificant relative to the Sr concentrations of the samples themselves, with loaded samples ranging from roughly 0.2 to 1.0 μg. Thus the typical sample contains at least a thousand times more strontium than the typical blank.

4.5 Carbon and Oxygen Isotope Analyses

Samples of human dental enamel from fifty individuals from eight different sites were analyzed for this study. Premolars were sampled when possible as their enamel crowns develop and mineralize roughly between the ages of two and six (Hillson 1996; White and Folkens 1999). Other teeth were sampled when suitable premolars were unavailable. Sampling focused on enamel which has been shown to be less susceptible to diagenic contamination than bone (Budd et al. 2000; Hoppe et al. 2003). Teeth were mechanically cleaned and approximately the first several microns of surficial enamel was removed to expose the inner core enamel which was removed using a hand-held drill dental drill equipped with a diamond-tipped burring tip. No additional pretreatment step was used but diagenic alteration of the enamel was not expected as previous analyses had revealed no correlation between strontium concentrations (ppm) and strontium isotope compositions. Contamination was also monitored via measurements of uranium concentrations via ICP-MS and were consistently low (<1 ppm). Carbon and oxygen isotope compositions were

measured on a Finnigan DeltaPlus Isotope Ratio Mass Spectrometer following reaction of the carbonate sample with H_3PO_4 (100%) and isolation of the produced CO_2 on a Gasbench II universal automated interface (Faculty of Earth and Life Sciences, VU University, Amsterdam). Long term analyses of the NBS-19 international calcite reference standard yielded a reproducibility for $\delta^{13}C$ and $\delta^{18}O$ of <0.1‰ and <0.2‰, respectively. All $\delta^{13}C$ and $\delta^{18}O$ values referenced herein are reported in the δ notation, in parts per thousand (‰) relative to the international PDB (Pee Dee Belemnite) standard.