Building life histories of Cape Town's enslaved, 1700-1850

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Chapter 4

Chemical answers to historical questions

Introduction

A common problem with the historical record in both the study of the Atlantic and Indian Ocean slave trades is that it gives information about slaves' points of departure as opposed to their places of origin (Raben, 1996; Schroeder *et al.*, 2009). It is for this reason that researchers have turned to the archaeological record to complement historical findings and to reconstruct the life histories of enslaved people. At times these records converge tantalisingly, for instance the request by the church to the Cape administration for land for a new graveyard as people were dying at an alarming rate in 1755. This is presumably a crisis that arose from the smallpox epidemic of that year. This event falls within the time period of interest for this study. Could some of the individuals the reader will encounter in later chapters have died from this epidemic?

This project is mainly concerned with isotope analysis as it pertains to the reconstruction of the migration aspect of enslaved people's life histories. In order to do this $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ were determined from different skeletal elements as proxies for migration in the study of populations at the colonial Cape. $\delta^{15}N$ also allowed for a dietary comparison between these populations. The following literature review concentrates on these three elements. The research questions that could potentially be answered by isotopic investigation of these populations were the following:

- Were the individuals local or foreign-born?
- What are their possible geographic origins?
- At what ages were these individuals transport/enslaved
- How many migration events/changes of hands did these individuals experience?
- What did they eat?

Isotope analyses in bioarchaeology

"Isotopic analysis has been an important tool in several fields of anthropology: archaeology, skeletal biology, zooarchaeology, paleoanthropology, primatology, ethnobotany, and forensic anthropology" (Schwarcz *et al.*, 2010:335). Isotopes of an element have the same number of protons and electrons, but different numbers of neutrons. Due to the difference in mass of the isotopes of an element, they react at different rates from one another, a kinetic effect, and form chemical bonds of differing strength, a thermodynamic effect (Price *et al.*, 1985). These effects are most noticeable for the lighter elements as the mass differential is large relative to the atomic mass (Schoeninger and Moore, 1992). The second process that results in isotopic variation is radioactive decay (Faure 1977).

In this study carbon (C) and nitrogen (N) isotope ratios are determined from collagen and used for dietary assessment while carbon and strontium isotope analyses of collagen and enamel

respectively are used as proxies for migration. Carbon and nitrogen isotope ratios are reported in delta (δ) notation where

$$\delta(‰) = \{(R_{sample}/R_{standard})\text{-}1\}*1000$$

and R is the ratio of the heavier to the lighter isotope. Carbon isotope ratios are measured relative to Vienna Pee Dee Belemnite (VPDB), nitrogen isotope ratios are measured relative to Ambient Inhalable Reservoir (AIR), and strontium isotope ratios are expressed as $^{87}Sr/^{86}Sr$.

Principles of strontium isotope analysis

Geosphere

The principals of strontium isotope geochemistry have been extensively discussed in the literature and are briefly reviewed here. The reader is referred to text books by Faure (1977) and Dicken (1995) for a more comprehensive review. Strontium has four naturally occurring isotopes with associated abundances: ^{84}Sr (0.56%), ^{86}Sr (9.87%), ^{87}Sr (7.04) and ^{88}Sr (82.53%) of which ^{87}Sr is radiogenic (Faure, 1977).

Bedrock

^{87}Sr is formed as a result of the radioactive decay of ^{87}Rb in rocks and thus its amount varies with time in a closed system whilst that of the other isotopes remains constant. As a result, older rocks generally have a higher $^{87}Sr/^{86}Sr$ than younger ones. Strontium isotope ratios and the ^{87}Rb-^{87}Sr system therefore lend themselves to the determination of age and origin of geological materials. When these are coupled with variations in the Rb/Sr ratios of different types of rock, Sr isotope ratios at the earth's surface are highly variable, ranging from 0.701 to > 10. Not only are strontium isotopes used to provenance the source of different rocks types in geology but also in archaeology (Dicken, 1995).

Soils

Mineral weathering of rock is the most significant contributor to Sr concentration in soil, followed by contributions from ground and stream water, atmospheric deposition and fertilizers (Bentley, 2006). The strontium isotope ratios of bulk local soils can vary given the different weathering potentials of constituent minerals (Bentley, 2006; Capo *et al*., 1998; Mikova, 2012).

Hydrosphere

Surface Water

Rivers carry weathered material mostly in suspension, but also in dissolved form to oceans. In general, high elevation and weathering rates result in a close correlation between $^{87}Sr/^{86}Sr$ of streams and bedrock. At lower elevation this correlation does not hold as the contribution of precipitation becomes more significant (Bentley, 2006). Aberg's (1994) isotopic study of Swedish lakes demonstrated the effect seasonal inputs can have on surface water. Lake water $^{87}Sr/^{86}Sr$ remained relatively constant throughout the year except in the spring when melting snow reflected a different geological signature contributing to the lake waters. Other seasonal precipitation also caused variations in lake $^{87}Sr/^{86}Sr$ as it constituted a greater proportion of the lake water.

Groundwater

Similarly, strontium enters groundwater as a result of weathering of rocks and soils. The contact between water and rocks results in dissolution and reactions that release Sr into water (Semhi *et al*., 2017). The Sr concentration and signature of groundwater depends on the mixing of constituent waters from different sources (including precipitation) and their residence times (Blum and Erel, 2003; Semhi *et al*., 2017).

Seawater

As mentioned above, rivers are responsible for carrying weathered Sr-containing sediment and some dissolved Sr to the ocean, the largest reservoir of dissolved strontium (Bentley, 2006; Watt and Howe, 2010). Not only do rivers transport continental Sr to oceans, sea water- basalt interaction along with recrystallization of carbonates also contribute to the total amount of oceanic Sr (Wadleigh *et al*., 1985). At any given time, the Sr isotopic signature in oceans is considered to be constant as its residence time is four orders of magnitude greater than the mixing rate of oceanic water (Aberg, 1994).

Atmosphere

Strontium can be transferred from the ocean to the air by sea spray. Sr^{2+} released into the atmosphere is primarily in oxide form which quickly reacts with water and carbon dioxide to form a hydroxide or carbonate respectively (Watts and Howe, 2010). Wind can transport seaspray-derived aerosols inland where they can be deposited on plants and absorbed by soils through precipitation (Whipkey *et al*., 2000). Strontium can also be deposited in marine

carbonate sediment. Finally, hydrated strontium ions can be adsorb onto clay minerals, iron oxides and products of weathering such as amorphous silica (Watts and Howe, 2010).

Biosphere

Plants

Strontium in soil and groundwater enters the food chain as drinking water and in leafy vegetables, grains and dairy products (Watts and Howe, 2010). Plant uptake of labile strontium is mainly through the roots, from where it is transported to different parts of the plant, although aerosol deposition can also be significant (Dijkstra *et al*., 2003). Sandy soils with low clay and organic content are ideal for plant uptake of strontium whilst calcium and potassium retard this process (Watts and Howe, 2010). "The plant-available $^{87}Sr/^{86}Sr$ reflects a more consistent average of the local biologically-available strontium than whole soils" (Bentley, 2006). On the other hand, plants in the same environment but with different root depths may display different Sr isotope ratios due to the differing microenvironment these roots are in contact with (Aberg, 1994). Roots can also alter soil Sr isotope ratios in the long run as they absorb minerals, depleting the soil. Burrowing animals can reveal subsoil Sr and spread it via faecal matter and animal tissue.

The Human Skeleton

Humans ingest strontium-containing plants and water. Once in the skeleton, strontium substitutes for calcium in hydroxyapatite, the main constituent of teeth and bone, as the two elements have similar radii and the same valency (Price *et al*., 1992). Movement of Sr from the geosphere into the biosphere does not alter (fractionate) the strontium ratio greatly by mass dependant processes as the difference in mass between ^{87}Sr and ^{86}Sr is small relative to the mass of the atoms (Ericson, 1985). Strontium isotope ratios will change, however, due to the incongruent break down of minerals during the weathering process, as explained above. Strontium forms complexes with other organic and inorganic ligands that coordinate with calcium. A small amount, 11-30 %, of strontium ingested by humans is absorbed and the rest is subsequently excreted mostly through urine but also through faeces (Watts and Howe, 2010). Strontium is absorbed by a growing human foetus during pregnancy and after birth through mother's milk (ibid).

Determining a local strontium baseline signal

When undertaking strontium isotope studies of a population, it is essential that a local bioavailable strontium range is established for the area in which the individuals are found (Bentley, 2006; Slovak and Paytan, 2011). Possible migrants can be identified in a skeletal population using strontium isotope ratios if a locally bioavailable baseline has been determined for comparison, and if it can be assumed that the population consumed local foods (Schwarcz *et al*., 2010). This can be done by determining the strontium isotope ratios of a variety of archaeological fauna or performing a statistical analysis of the sample set in question (Bentley, 2006; Evans, 2006; Price *et al*., 1985). The development of isotopic landscapes (isoscapes) which map isotopic variation, empirically determined and/or modelled, has improved archaeologists' ability to assign migrants to possible places of origin (Bowen, 2010; West *et al*., 2010,).

Direct substrate measurements are now recognised as inappropriate to determine a local signal as constituent minerals in a heterogeneous rock can exhibit different strontium isotope ratios as stated previously. Differential weathering of these minerals will result in skewed strontium isotope ratio of soils derived from them (Price *et al*., 2002). Due to air/waterborne sediments (inputs) and weathering/leaching (outputs) the bioavailable strontium isotope ratios at any given time will depend on a variety of factors. Skeletons, like plants, have an averaging effect on the local signal (ibid). In this study $^{87}Sr/^{86}Sr$ determined from both archaeological faunal remains and the statistical analysis (trimming to normal distribution after removal of outliers) of the human populations are used to determine the local background strontium isotopic range at The Cape (Balasse *et al*., 2002; Sealy *et al*., 1991). Ideally it would be possible to determine geographic origins of the individuals in this study by comparing their $^{87}Sr/^{86}Sr$ values to reference bioavailable data from the Indian Ocean basin, however, a comprehensive dataset does not yet exist.

Limitations of strontium isotope analysis

Once a local strontium baseline is determined it is easy to ascertain who the migrants are in a population if they display strontium isotope ratios that fall outside the local strontium range. It is much more difficult to ascertain their origins as strontium isotope ratios are not unique and different geographic areas can have identical strontium isotope signatures. This approach will thus underestimate non-locals. Moreover, sea-spray, wind-blown dust, and fertilizer amongst other factors can affect the strontium isotope ratios, confounding attempts to interpret data.

Maurer *et al*. (2012) have demonstrated the difficulties associated with generating bioavailable $^{87}Sr/^{86}Sr$ reference maps (isotopic landscapes or isoscapes) based on samples used in conventional studies. In their area of study, local archaeological fauna were found not to have fed in the area in which they were found, excluding them from the delineation of a local

bioavailable signature. Modern fauna were found to have consumed plants or water that had been in contact with unradiogenic fertilisers, thus their low $^{87}Sr/^{86}Sr$ values (<0.708) relative to the other materials sampled (0.708-0.710). Soil leachates were found to yield consistently higher strontium isotope ratios (by as much as 0.0015) relative to ground vegetation. Snail shell strontium isotope ratios fell in the range of ± 0.0005 of the average ground vegetation value. The authors also warn against the use of ground vegetation which consistently produces lower Sr isotope ratios than tree leaves in theirs and other studies. Even archaeological human teeth as biosphere proxies were found to be questionable in their context. They found that modern water and tree leaves were the most representative of the local bioavailable strontium signal. Ultimately, they came to the conclusion that "It is clear that there is no ideal material to use in this respect, and the choice is probably best made on a case-by-case basis, to avoid misleading inferences in past migration studies. (Maurer *et al*., 2012:227)"

Another limitation associated with strontium isotope analysis is that the extent to which $^{87}Sr/^{86}Sr$ varies naturally in a population is not yet known. Even though individual migration histories have been pieced together by determining isotope ratios of bulk dental elements that develop at different periods in an individual's life, this resolution may not be sufficient to track all migration events. A final confounding factor in the interpretation of $^{87}Sr/^{86}Sr$ is that individuals may have consumed non-locally grown foods. This factor should be of limited importance in this study as the early modern Cape came into existence precisely to do away with the need to transport vast amounts of food and water on the long journey between Europe and the Far East.

Principles of Carbon isotope analysis

The combined analysis of collagen C and N isotopes gives us insight into "the macronutrient components of diets [protein, fats, carbohydrates], as well as the degree of herbivory versus carnivory" (Fizet *et al*., 1995:71; Lee-Thorp *et al*., 1989:593; Schwarcz and Schoeninger, 2011:730). Other lines of evidence are necessary in addition to C and N isotope ratios to determine what foods were consumed by past populations. To this end, isotopic signals of foods that constituted past populations' diets and lipid residues from potsherds can also be determined where available.

Carbon isotopes in terrestrial foodwebs

The main source of carbon in terrestrial foodwebs is atmospheric CO_2. The two stable isotopes of carbon are ^{13}C and ^{12}C. $\delta^{13}C$, the relative amount of ^{13}C to ^{12}C found in human tissue varies as a result of consumption of plants that follow different photosynthetic pathways. These pathways metabolise atmospheric CO_2 differently. C_4 plants follow the Hatch-Slack photosynthetic

pathway and their leaves have two kinds of photosynthetic cells (Hatch and Slack, 1966). The mesophyll cells contain chlorophyll and are where CO_2 is initially fixed (Furbank *et al*., 2000). Malate or aspartate diffuse into the ribulose *bis*-phosphate carboxylase/oxygenase (RuBisCO) enzyme-containing bundle sheath cells and are decarboxylated (Furbank, 2001). The increased concentration of CO_2 in the bundle sheath cells leads to the inhibition of the oxygenase activity of RuBisCO, thus inhibiting photorespiration and increases the efficiency of photosynthesis (Iglesias *et al*., 1986). Examples of such plants are maize, millet and sorghum.

C_3 plants follow the Calvin-Benson cycle and fix carbon into phosphoglyceric acid (PGA) (Chisholm *et al*., 1983; Hall and Rao, 1994). PGA is phosphorylated by adenosine triphosphate (ATP) at the carboxylic acid function, a reaction that is catalysed by phosphoglycerate kinase. The masked acid function is then reduced by $NADPH_2$ to form phosphoglyceraldehyde, a reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase (Hall and Rao, 1994). The ribulose bisphosphate (RuBP) starting material is regenerated via a series of reactions. Although carbohydrates and sugars are the primary products of photosynthesis, other products may form depending on three variables: CO_2 and O_2 concentration and light intensity (ibid). Examples of C_3 plants are wheat, oats, rye, rice and barley.

During photosynthesis, discrimination (Farquhar *et al*., 1989) or fractionation "the non-linear transfer of isotopes from the source to the product" (Leatherdale, 2013: 40) occurs, and typically the heavier isotopes will react more slowly than the lighter ones "leading to different fractionations of carbon isotopes relative to their source of carbon (ibid)." The extent of fractionation diminishes with increasing temperature (Schwarcz and Schoeninger, 1991). The major factors contributing to fractionation in C_3 plants are "the differential diffusivities of CO_2 containing ^{12}C and ^{13}C across the stomatal pathway and the fractionation by Rubisco" (Farquhar *et al*., 1989:509). Fractionation in C_4 plants is a result of CO_2 and/or HCO_3^- leaking from the bundle sheath cells into the mesophyll cells (ibid).

Water stress in plants will lead to stomata partially closing leading to a decrease in the loss of transpired water and a decrease in the circulation of CO_2. The CO_2 reduction reaction to carbohydrates is thus more complete which translates to more of the heavier ^{13}C reacting which in turn leads to elevated $\delta^{13}C$ (Schwarcz and Schoeninger, 2011). This elevation is propagated up the food chain and is more evident in C_3 than C_4 plants resulting, for example, in regional variations in the $\delta^{13}C$ values in wheat across Europe (Suits *et al*., 2005)

Stable carbon isotope analysis has also been used to track maize cultivation in the Americas (Schoeninger and Moore, 1992; Vogel and Van der Merwe, 1977; Wright *et al*., 2010). Consumers of cultivated maize, a C_4 plant, in a predominantly C_3 environment will exhibit a $\delta^{13}C$ value that is greater than consumers who rely more on C_3 plants (Lynott *et al*., 1986). A similar pattern would be observed if sorghum or millet, both C_4 plants, were to be introduced to the diet of

people in a C_3 predominated environment (Vogel and Van der Merwe, 1977). Both C_3 and C_4 plants are ^{13}C depleted relative to atmospheric CO_2, their source of inorganic carbon, but C_3 plants are much more so than C_4 plants. It follows that animals consuming C_3 plants will have $\delta^{13}C$ values that are lower than those for animals consuming C_4 plants (**Figure 2**).

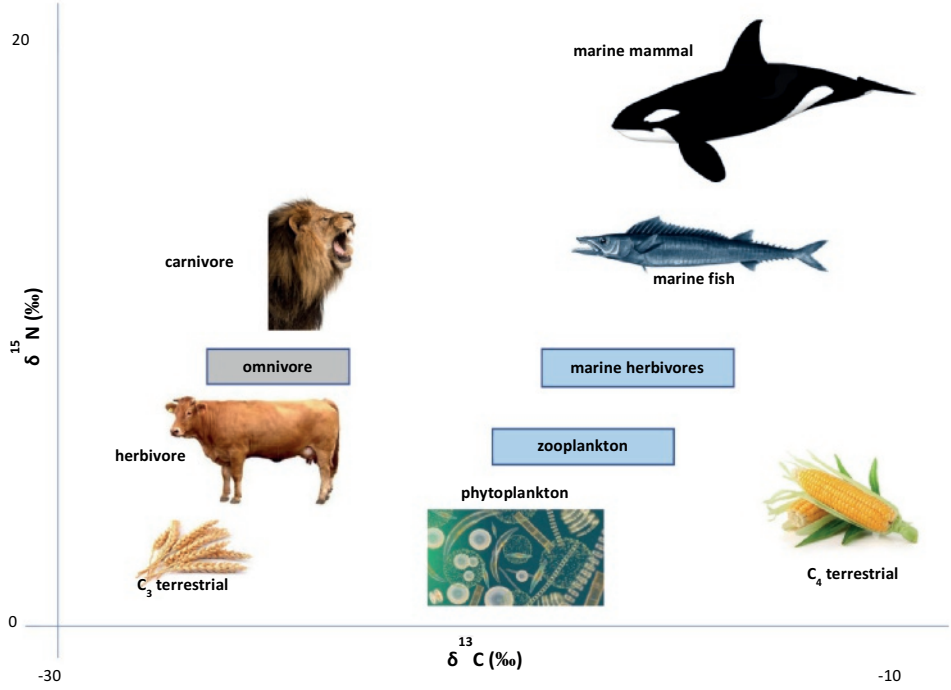

Figure 2: Schematic isotope biplot showing the relative positions of organisms in foodwebs

Not only can $\delta^{13}C$ be used to categorise plants and track cultivation, it can also be used to distinguish forest from non-forest dwellers, and of the forest dwellers to distinguish those living on the forest floor from those living in the canopy (Ambrose, 1986). $\delta^{13}C$ of forest plant leaves is highest in the canopy and decreases in the lower levels of the forest (Medina and Minchin, 1980). One explanation for this "canopy effect" is "photosynthetic recycling of ^{13}C-depleted CO_2 produced by rotting leaf litter on the forest floor" (Van der Merwe and Medina, 1991). Light intensity seems to be a more important factor than temperature or rainfall in low $\delta^{13}C$ in plants. Moreover, an inverse relationship was observed between $\delta^{13}C$ of grasses and light intensity in woodland areas. The $\delta^{13}C$ of plants grown in shaded areas were 4.2-6.2‰ lower than those grown in open areas (Bonafini *et al*., 2013). Other environmental factors affecting plant $\delta^{13}C$ are soil salinity and altitude (Schwarcz and Schoeninger, 2011; Szpak *et al*., 2013).

Finally, there is a carbon trophic level effect resulting in 0-2‰ increase in $\delta^{13}C$ per trophic level within single food webs (Schoeninger, 1985). This effect is more pronounced in herbivores than in carnivores and partly depends on assimilated diet isotope ratio, taxon physiology, macronutrient routing to different tissues, lipid extraction and fractionation (Caut *et al*., 2009; Darr and Hewitt, 2006).

Carbon isotopes in aquatic foodwebs

The main source of carbon in marine foodwebs is dissolved inorganic carbon (including carbonic acid and CO_2). As with terrestrial foodwebs, carbon enters the marine foodweb through photosynthesis. Bacterial biosynthesis and the terrestrial detritus transferred by rivers to the ocean also contribute to the transfer of carbon to the biological realm (Schwarcz and Schoeninger, 1991).

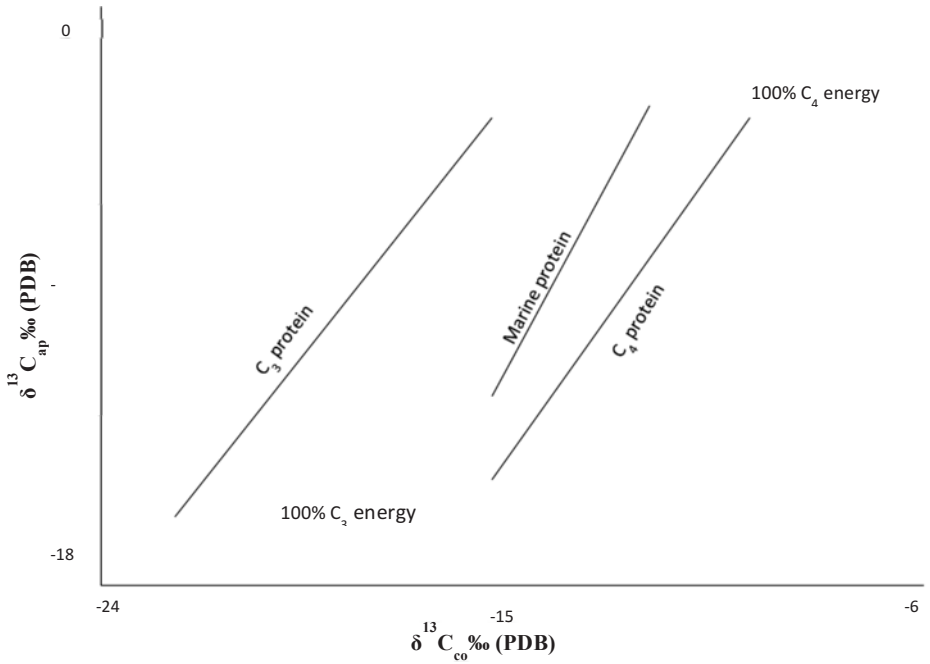

Figure 3: Schematic of three protein source regression lines
Generated by Kellner and Schoeninger (2007)

The relative amounts of marine versus C_3 terrestrial protein intake in an individual's diet can be determined from $\delta^{13}C$ of human bone collagen as marine-based food chains have a higher $\delta^{13}C$ than terrestrial ones. This is a reflection of the higher $\delta^{13}C$ in oceanic bicarbonate versus terrestrial carbon dioxide carbon (Chisholm *et al*., 1982; Chisholm *et al*., 1983). The $\delta^{13}C$ values

of C_4 and marine protein consumers' collagen overlap and are therefore not diagnostic. Diets may consist of a combination of C_3, C_4 and marine protein. To pry apart information on different protein and energy sources from carbon isotope data, Kellner and Schoeninger (2007) generated protein source plots of bone collagen $\delta^{13}C$ vs bone apatite $\delta^{13}C$ (**Figure 3**). Depending on which end of the lines data falls it is possible to determine whether consumers ate C_3 or C_4 energy sources.

Limitations of carbon isotope analysis

Kellner and Schoeninger (2007) developed their model from which it is possible to determine the energy component of palaeodiet from regression lines of C_3, C_4 and marine diet protein as they questioned the predictive power of the spacing between $\delta^{13}C_{collagen}$ and $\delta^{13}C_{apatite}$ alone. Moreover, they note that much research on dietary assessment does not take potential errors into consideration such as differential fractionation and measurement error (Pestle *et al.*, 2015). Hedges (2003) also questions how researchers interpret $\Delta_{apa\text{-}col}$ as it is apparent that that the difference in the spacing between herbivores and carnivores cannot be due to differences in diet and methanogenesis only. It is suggested that perhaps the dynamics of bone formation may contribute to the spacing.

Principles of nitrogen isotope analysis

Nitrogen isotopes in terrestrial foodwebs

The two stable isotopes of nitrogen are ^{15}N and ^{14}N. The main nitrogen reservoir is the atmosphere with a $\delta^{15}N$ defined as 0.0‰. Inorganic nitrogen (N_2) is introduced into the biological realm through bacterial action at the roots of nitrogen fixing plants, particularly legumes. Most plants, however, absorb soil nitrogen (Szpak *et al.*, 2013) in the form of ammonium ions, NH_4^+ or nitrate, NO_3^- which is a product of the bacterial degradation of organic matter and a series of nitrification/denitrification processes (Schwarcz and Schoeninger, 1991).

Plants have the ability to process N in their stems and roots resulting in isotopic fractionation and different $\delta^{15}N$ values between leaves and soil. Moreover, soil N can be fractionated as a result of the aforementioned nitrification (mineralisation) and denitrification processes. Ammonia is an intermediate in the former process and is also volatile, thus, $\delta^{15}N$ can vary as a function of soil depth. The evaporation of NH_3 from soils in arid areas results in elevated $\delta^{15}N$ which is propagated up the food chain (Schwarcz and Schoeninger, 2011).

Nearly all nitrogen in one's diet comes from protein, and thus $\delta^{15}N$ in bone collagen is a reflection of dietary protein (Mays and Beavan, 2012). There is a noticeable difference (3-5‰) in $\delta^{15}N$ between levels of a trophic system (Hedges and Reynard, 2007; Schoeninger and Moore,

1992). Nursing infants are a trophic level above their mothers and therefore exhibit greater $\delta^{15}N$ relative to them (Herring *et al*., 1998; Wright *et al*., 2010). Breastfeeding is reflected in a rise in $\delta^{15}N$ from birth, and the weaning of infants from breast milk to other sources of protein is reflected in the decline in $\delta^{15}N$ to adult levels (Richards *et al*., 2002; Schurr, 1997).

Nitrogen isotopes in aquatic foodwebs

Marine nitrogen is derived from algal and bacterial fixation (Schwarcz and Schoeninger, 1991). Marine organisms tend to have higher $\delta^{15}N$ than terrestrial ones due to a greater influence of bacterial activity, higher $\delta^{15}N$ of nitrate and organic matter in seawater, and the greater lengths of marine food chains relative to terrestrial ones (Lee-Thorp, 2008; Schwarcz and Schoeninger, 2011). Thus, with few exceptions, humans who eat a considerable amount of marine foods will have a higher $\delta^{15}N$ relative to those who do not.

Non-dietary variation in $\delta^{15}N$

A negative correlation was observed between collagen $\delta^{15}N$ and rainfall (Heaton *et al*., 1986). Water stress experienced by animals with an adequate protein intake leads to elevated $\delta^{15}N$. Drought-tolerant water-conserving species have elevated $\delta^{15}N$ relative to water-dependant obligate drinkers (Ambrose, 1991). One explanation for this effect is that animals experiencing water stress preferentially excrete urea rather than water in their urine (ibid). Fractionation during this process results in light ^{14}N depletion and heavy ^{15}N enrichment in the remaining nitrogen pool.

Tissue $\delta^{15}N$ also increases as a result of stresses such as starvation and illness. One of the body's responses to negative nitrogen balance is catabolism, the breaking down of existing amino acids in muscle tissue (Preiser *et al*., 2014). Some of the proteins are recycled for protein synthesis, or they are oxidized, releasing the amino function which is passed as ^{15}N-depleted urea and ammonia (Hobson *et al*., 1993; Katzenberg and Lovell, 1999). The remaining tissue will be enriched in ^{15}N. The resultant carbon skeletons are intermediates that can be converted to glucose in the liver or ketones/fatty acids for energy.

Limitations of nitrogen isotope analysis

Aridity leads to unreliable bulk bone collagen $\delta^{15}N$ values and an overlap between C_4 and high marine protein consumers $\delta^{13}C$ values. In response to the latter limitation, compound-specific measurement of collagen amino acids $\delta^{13}C$ values was carried out (Corr *et al*., 2005). All amino acids', save for glycine, $\delta^{13}C$ values were depleted for a high marine protein consumer relative to the C_4 consumer allowing for isotopic differentiation that was not possible when considering bulk collagen $\delta^{13}C$.

Although $\delta^{15}N$ is used as an indication of human trophic level, there are several confounding factors associated with the interpretation of data. The natural variation in $\delta^{15}N$ between individuals is not known, making it difficult to ascertain whether inter- or intra-population differences are indeed related to diet and other factors. Furthermore, whether diet affects ^{15}N enrichment, and if so how, is not yet understood. (Hedges and Reynard, 2007). Moreover, protein stress and high protein intake both result in elevated $\delta^{15}N$ (Reitsma, 2013). Even the widely accepted $\Delta^{15}N_{diet\text{-}collagen}$ offset of 3-5‰, has been challenged by O'Connell *et al.* (2012) who have reported an upper limit of 6.3‰.

Choice of tissue type for isotope analysis

Development of skeletal elements

Tooth enamel is formed in childhood and does not remodel in later life. Tooth dentin mostly forms in childhood during root formation. The first molars, M1, begin to form in utero. M2, develops until approximately age 8, and M3 forms between ages 8 and 17 (Schwarcz *et al*., 2010). By "taking advantage of the different times of calcification of different teeth and regions within a tooth, it may be possible to estimate the age of individuals at the time of forced migration" (Goodman *et al*., 2009:97). This strategy is employed in this study.

Bone is constantly resorbed and redeposited throughout life (Hadjidakis and Androulakis, 2006; Hedges *et al*., 2005). Carbon and nitrogen isotopic data derived from bone collagen gives a long-term overview of diet, particularly the protein component of an individual's diet (Lee-Thorp, 2008; Schwarcz *et al*., 2010; Sponheimer and Lee-Thorp, 2007). Data from denser cortical bone reflects diet over a period of decades, and that from trabecular bone, which remodels with shorter turnover times, reflecting the diet of the past few years (Pye, 2004). Enamel and bone apatite are more representative of total diet (Loftus and Sealy, 2012, Pate, 1994; Wright *et al*., 2010).

Due to the variation in skeletal element development mentioned above, the body can be seen as an archive. Teeth tell the story of early life as they development in childhood and adolescence. Ever-changing bone tells a more dynamic story, one short-term (trabecular/spongy bone) and the other long-term (cortical/compact bone).

Collagen vs Apatite

$\delta^{13}C$ values of collagen are a reflection of the protein component of diet as collagen mainly consists of amino acids from hydrolysed ingested food protein (Ambrose and Norr, 1993; Chisholm *et al*., 1982). Bone hydroxyapatite carbon is synthesised from respired blood plasma CO_2 and its $\delta^{13}C$ is a weighted average of the isotopic signals of all components of diet (Sullivan and Krueger, 1981).

Diagenesis

Collagen loss through microbial attack or hydrolysis can lead to fractionation of C and N isotope ratios. It has been observed that C:N ratios are also affected by these processes. In general, a C:N ratio of 2.9 to 3.6 is thought to reflect good collagen preservation and these values are required for reliable results along with a collagen yield of at least 0.5-3.5 % depending on the region in which the bone is found (Hedges, 2002; Jans, 2004; van Klinken, 1999; Lee-Thorp *et al*., 1989; Schoeninger and Moore, 1992).

Indicators of bone diagenesis include: histological integrity, protein content, porosity as determined by water retention or mercury inclusion, and crystallinity as determined by splitting factor (Hedges, 2002; Jans *et al*., 2004; Nielson-Marsh and Hedges, 2000; Price *et al*., 1992). The crystallinity index (CI) has been found lacking in terms of its ability to indicate the reliability of C and N isotopic analyses (Schwarcz and Schoening, 2011). Jim *et al.* (2004) proposed using cholesterol $\delta^{13}C$ as an indicator of apatite integrity.

Bone mineral is highly susceptible to diagenesis due to its high organic content, porosity and small crystal size (Lee-Thorp, 2008; Sponheimer and Lee-Thorp, 2007). The ratio Ca:P in the mineral component of unaltered bone is approximately 2, variations from this value can be an indication of bone alteration (Price *et al*., 1992; Slovak and Paytan, 2011). Dissolution and re-precipitation of hydroxyapatite facilitates incorporation of exogenous halides and carbonate (Pye, 2004; Sponheimer and Lee-Thorp, 2007; Stathopoulou *et al*., 2008). The effects of diagenesis can be countered by mechanical cleaning of the sample and chemical cleaning with acid (Lambert *et al*., 1990; Price *et al*., 1992).

Tooth enamel is less susceptible to diagenesis as it is highly crystalline and has little (5%) organic material (Bentley, 2006; Blyth, 2001; Budd *et al*., 2000; Sponheimer and Lee-Thorp, 2007; Pye, 2004; Schwarcz *et al*., 2010; Slovak and Paytan, 2011). This often makes enamel the logical choice of material for isotopic studies (Eckardt *et al*., 2009; Lee-Thorp, 2008; Pye, 2004; Wright *et al*., 2010).

Interpreting human palaeodietary data

Using carbon and nitrogen isotope ratios for the assessment of palaeodiet from collagen, as has been done in this study, requires an understanding of the fractionation associated with collagen formation (Van der Merwe, 1982). This fractionation occurs as a result of differential digestion and absorption of foods or metabolic processes (McCutchan *et al*., 2003). Bocherens and Drucker (2003) advise that researchers consider the use of a range of enrichment values as opposed to using a discrete average value calculated from published data.

A plot of $\delta^{13}C$ of bone apatite vs $\delta^{13}C$ of bone collagen of animals of known diet was found to be linear and it was thus possible to determine the relative contributions of C_3 and C_4 foods to diets from knowledge of the $\delta^{13}C$ of either bone collagen and/or apatite (Sullivan and Krueger, 1981). As the difference in $\delta^{13}C$ collagen-apatite, Δ, is smaller for carnivores (4‰) relative to herbivores (7‰), it can be used as an indication of the importance of meat in ancient diets relative to whole diet (Lee-Thorp *et al*., 1989). Diet composition aside, other factors affecting $\Delta_{co\text{-}ca}$ are: digestive physiology, nutritional status, growth and tissue turnover (Leatherdale, 2014).

Conclusions

The basic principles underpinning the isotope systems of interest to this study have been presented. It is apparent that many physical and chemical factors are at play that determine the ultimate isotope value read from the body archive and that preservation in an archaeological context is a crucial issue. It is also apparent that there are still many confounding factors that limit the value added by these isotope systems. We acknowledge these limitations in future chapters and are cautious in our conclusions. Despite these limitations, however, isotope geochemistry has allowed us to build intricate life histories of enslaved persons at The Cape on the individual and group level.