Rock Hyraxes *(Procavia capensis)* and Their Environments:

$\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ as environmental indicators

Lesa la Grange

Supervised by Dr Deano Stynder and Prof Judith Sealy

Dissertation submitted in fulfilment of the requirements for the degree of
Master of Philosophy (MPhil) in Archaeology

Department of Archaeology

University of Cape Town

20 July 2015

Abstract

Rock hyraxes (*Procavia capensis*) are a common component of faunal assemblages at South African archaeological sites. Previous studies have focused on their remains as proxies for aspects of diet and mobility in prehistoric human populations. They have also been recognized as valuable palaeoenvironmental indicators due to the fact that their stratified communal middens record shifts in stable isotopes, palynology, and molecular biomarkers that appear to reflect environmental change.

Previous studies conducted in east Africa have concluded that rock hyraxes are preferential grazers. There is comparatively little evidence, however, for the dietary preference of South African rock hyraxes, although it has been suggested that they consume more browse. This research aims to quantify the carbon isotopic ratios in their bone collagen to reconstruct diet. Results support preferential browsing in South African rock hyraxes, with a mean $\delta^{13}C$ value for all animals in this study of -20.3 ± 1.9‰ (n = 210) indicating a C_3 dietary contribution of 73.4 ± 13.3%.

Rock hyraxes are drought-tolerant animals. They therefore exhibit high $\delta^{15}N$ values compared to other species, particularly in arid environments. There is uncertainty regarding the extent to which this is the result of high $\delta^{15}N$ in plants in arid areas, and/or of metabolic processes within the animal, possibly including urea recycling as a water conservation mechanism. This study includes rock hyraxes collected across South African environmental gradients, allowing the relative importance of environmental and metabolic determinants of $\delta^{15}N$ to be investigated. Results show that $\delta^{15}N$ decreases by 1‰ for every additional 100 mm of rainfall, although there is a high degree of variability, indicating that $\delta^{15}N$ values are not driven by moisture availability alone. Other climatic variables, and additional fractionation of the isotopes during physiological processes within the rock hyraxes may play a role in determining tissue $\delta^{15}N$.

The fact that $\delta^{13}C_{collagen}$ indicates preferential browsing limits the degree to which one can use rock hyrax remains to interpret shifts in the extent of the winter, summer, and year-round rainfall zones. Furthermore, the relatively low correlation between $\delta^{15}N_{collagen}$ values and rainfall suggests that further investigation into the primary determinants of $\delta^{15}N_{collagen}$ values is required.

Acknowledgements

- I thank my supervisors, Dr Deano Stynder and Prof Judith Sealy, for their guidance and assistance throughout the course of this degree. Their comments on draft versions of this thesis were valuable and constructive.

- I thank Ms Julie Luyt, Ms Nandi Masemula, Mr Ian Newton, and Mr John Lanham for their assistance with sample preparation

- I acknowledge the following persons for their assistance in assembling the hyrax sample analysed in this study:

  Dr Jurie du Plessis, Dr Nico Avenant, Prof Louis Scott, Dr Teresa Kearney, Mr Len Edwards, Mr Morné Brits, Ms Louisa Hutten, Ms Philippa Haarhof, Dr Alexandra Sumner, Mr Mark Duckitt, Mr Pierre Nel, Mr John Adendorff, Mr Nardus du Plessis, Mr Nick de Goede, Mr Adrian Fortuin, Mr Bernard van Lente, Ms Nicole Grunert, Dr Alex Mackay, Mr Ross Lyall, Mr James McGrath, Ms Emily Hallinan, Mr James McPherson, and Mr Philip la Grange

- I acknowledge the following institutions for granting permission to sample skeletal material in their care:

  The West Coast Fossil Park, South African National Parks, The Ditsong National Museum of Natural History, The National Museum of Bloemfontein, and the Western Cape Nature Conservation Board.

- I thank Dr Alastair Potts, Mr Thomas Slingsby, and Ms Ushma Galal for their assistance with statistical analyses and GIS data generation.

- I acknowledge financial support from the South African Research Chairs Initiative (**SARChI**) of the Department of Science and Technology (**DST**) and National Research Foundation (**NRF**) of South Africa, which provided both bursary support and research running costs. Further bursary support was received from the DST-NRF Centre of Excellence for Palaeosciences (**CoE-Pal**) and the Palaeontological Scientific Trust (**PAST**).

- I thank the examiners for their valuable reviews. Their recommendations greatly enhanced the final version of this thesis.

Contents

List of Tables

List of Figures

Chapter One

Introduction

1.1 - The use of rock hyraxes as archaeological and palaeoenvironmental proxies

Extensive research on the remains of rock hyraxes (*Procavia capensis*), or dassies as they are locally referred to, has been carried out, particularly in South Africa. It has been demonstrated that rock hyraxes were extensively exploited for their meat and skins, and their remains are therefore found at many South African archaeological sites (Parkington & Poggenpoel 1971; Smith *et al.* 1991; Jerardino 1995; Texier *et al.* 2010; Wadley 2010). Previous studies have thus focused on their remains as proxies for the behaviour of prehistoric human populations, including aspects of their diet and mobility.

Although it is has been found that rock hyraxes exhibit extensive dietary tolerance (Sale 1960; Skinner & Smithers 1990), disagreement regarding their dietary preferences exists. There are proponents of both preferential grazing (e.g. DeNiro & Epstein 1978a, Walker *et al.* 1978; Hoeck 1989; Zimman 2008) and preferential browsing (Scott & Vogel 2000), but this has yet to be systematically investigated across vegetation and precipitation gradients. Samples analysed here were collected in a transect spanning a variety of South African vegetation biomes and rainfall seasonality zones, and their bone collagen carbon isotope ratios ($\delta^{13}C$) were used as independent indicators of preferential feeding behaviour, particularly in summer rainfall areas where grazers and browsers can be distinguished from one another (Ambrose & DeNiro 1986).

It has been demonstrated that stable nitrogen isotope ratios of plants (dietary sources), and therefore animal tissues (consumers) are a function of moisture availability in environments (Heaton *et al.* 1986; Handley *et al.* 1999; Hedges *et al.* 2004; Hartman & Danin 2010). Analyses of nitrogen isotopic data from communal *P. capensis* middens have relied heavily on this relationship (Chase *et al.* 2010, 2011, 2012, 2013). However, the relative importance of environmental and physiological factors remains uncertain (Schwarcz *et al.* 1999; Makarewicz & Sealy 2015). Rock hyraxes in this study were collected from localities with very different annual precipitation (~55-1100 mm), allowing the relative contribution of metabolic and environmental determinants of their bone collagen $\delta^{15}N$ to be explored.

The abundance and wide distribution of rock hyraxes provides the opportunity to collect modern bone from a variety of ecological contexts to examine the degree to which their stable carbon and nitrogen isotope ratios reflect environmental parameters, and thereby investigate their potential utility as palaeoenvironmental proxies at South African archaeological sites.

1.2 - Thesis outline

The remainder of this thesis is laid out as follows:

Chapter Two provides a systematic overview of the principles and applications of stable isotope ratios of carbon and nitrogen in the atmosphere, plants, and consumers. Some of the primary determinants of these ratios are described, and previous research is summarised.

Chapter Three is a review of publications concerning rock hyrax taxonomy, distribution, ecology, behaviour, and diet. Previous work on stable carbon and nitrogen isotope ratios of their body tissues and excreta are described.

Chapter Four provides details of the collection localities of individuals analysed in this study, including published environmental data, ecological context, and personal observations, and outlines the analytical methods employed.

The results of the stable isotope analyses are presented in Chapter Five. The values are considered in relation to environmental variables, and the main features of the geographical distribution of stable carbon and nitrogen isotope ratios are summarised.

Chapter Six discusses how we may infer grazing/browsing preferences of South African hyraxes from the carbon isotope results, and how these compare with earlier studies reported in the literature. It also discusses factors influencing nitrogen isotope ratios, as well as patterns of variability and overlap within and between different collection localities. Finally, the main conclusions of this study are summarised and suggestions made as to possible future research avenues.

Chapter Two

Stable Carbon and Nitrogen Isotopes

2.1 - Introduction

Carbon and nitrogen occur in a wide range of organic and inorganic compounds worldwide, and wherever these elements are found, they exist as one of several isotopes (Trumbore & Druffel 1995). The most abundant stable isotopes of carbon are carbon 12 (^{12}C) and carbon 13 (^{13}C) (*cf.* Park & Epstein 1960; Farquhar *et al.* 1982; Ehleringer *et al.* 1997), and for nitrogen, they are nitrogen 14 (^{14}N) and nitrogen 15 (^{15}N) (*cf.* DeNiro *et al.* 1985; Schoeninger & Moore 1992; Evans 2001). These pairs are used to calculate stable isotope ratios for different compounds by determining the concentration of the heavier isotope (^{13}C or ^{15}N) relative to the lighter one (^{12}C or ^{14}N). The global relative abundances of these isotope pairs are 98.9% ^{12}C to 1.1% ^{13}C, and 99.6% ^{14}N to 0.4% ^{15}N (Ehleringer & Rundel 1989). Organic and inorganic compounds can have very different stable isotope ratios (Trumbore & Druffel 1995). This is because the ratios of the heavy to light isotopes shift in patterned ways as these elements move through carbon and nitrogen cycles. Isotope ratios can therefore be used to trace flows of carbon and nitrogen through food webs or ecosystems.

Isotope ratios are expressed in the delta (δ) notation as ratios of the heavy compared with the light isotope, relative to a recognised reference material. The delta values are calculated using the equation

$$\left(\frac{Rsample}{Rstandard} - 1\right) \text{ x } 1000\ (‰)$$

where *Rsample* is the ratio of the heavy isotope to the light isotope in the sample material, and *Rstandard* is the equivalent ratio in the reference material (Slater *et al*. 2001). Thus *R* may be $^{13}C/^{12}C$ or $^{15}N/^{14}N$, or another isotope pair. The units are parts per thousand, or per mille (‰). The accepted reference material for isotopes of carbon is Belemnite from the Pee Dee formation (PDB), while nitrogen isotope ratios are expressed relative to atmospheric nitrogen (AIR) (Hayes 1983). These standards have delta values of 0‰. Positive sample delta values therefore indicate that the measured material contains a greater abundance of the heavy isotope than the standard, while negative delta values indicate that the sample is relatively depleted in the heavy isotope (Dawson *et al.* 2002).

2.2 - Stable isotopes of carbon in the atmosphere, plants, and consumers

Atmospheric carbon dioxide (CO_2) is an inorganic compound with a $\delta^{13}C$ value of approximately -8‰ (O'Leary 1993), although this value is becoming more negative due to the burning of fossil fuels (Peterson & Fry 1987). Plants 'fix' CO_2 during photosynthesis, a process which discriminates against the heavy isotope (Farquhar *et al*. 1989). Plants are thus depleted in ^{13}C relative to atmospheric CO_2.

There are three main types of photosynthesis used by plants, namely the Calvin-Benson cycle (C_3), used by most dicotyledons and some monocotyledons, the Hatch-Slack cycle (C_4), used mostly by some monocotyledons, and crassulacean acid metabolism (CAM), used by most xerophytes (Bocherens 1997).

C_3 photosynthesis begins with the diffusion of CO_2 molecules into the leaf, through the stomata. This involves isotopic fractionation, as smaller, lighter $^{12}CO_2$ molecules diffuse slightly more readily than $^{13}CO_2$. The C_3 photosynthetic pathway uses the ribulose bisphosphate (RuBisCO or RuBP) enzyme to fix atmospheric CO_2 (Ehleringer & Cerling 2002). RuBP favours the light ^{12}C isotope (Gannes *et al.* 1998), a kinetic fractionation effect that is reflected in the very negative $\delta^{13}C$ values of C_3 plants, which range from ~-35‰ to -20‰. The most negative values occur at ground level in closed canopy forests, where high concentrations of $^{12}CO_2$ occur due to leaf decomposition and respiration under closed canopies (Ambrose & DeNiro 1986; Hare *et al.* 1991). Diefendorf *et al.* (2010) utilised methods first described in Farquhar *et al.* (1989), where an equation for quantifying the degree to which water-use efficiency (WUE) is a mechanism of carbon isotope fractionation was provided, to conduct an extensive global analysis of carbon isotope fractionation in the leaves of 75 woody plant families. It was found that variability in leaf $\delta^{13}C$ values were primarily due to changes in WUE as a result of the rate of stomatal conductance.

C_4 photosynthesis differs from the C_3 cycle as a result of a number of physiological features that evolved as adaptations to the environment (Cowling 1983; Collatz *et al.* 1998). C_4 plants concentrate CO_2 in the bundle sheath cells, using the phosphoenol pyruvate (PEP) carboxylase enzyme (Gowik & Westhoff 2011), which discriminates against ^{13}C to a lesser degree than RuBP (O'Leary 1993). These bundle-sheath cells also act as CO_2 traps, preventing losses of CO_2 during photorespiration. From the bundle sheath cells, RuBP takes over to produce sugars and other photosynthetic products, as in C_3 photosynthesis. The overall process of C_4 photosynthesis involves a lesser degree of discrimination against ^{13}C than does C_3 photosynthesis. C_4 plants therefore have more positive $\delta^{13}C$ values, averaging -12.5‰ to -13‰ (Ambrose & DeNiro 1986; Cerling *et al.* 1997; Cerling & Harris 1999; Kelly 1999).

CAM plants have adapted to assimilate carbon at night under xeric conditions, thereby reducing the risk of water loss during stomatal opening (Mooney *et al.* 1977). They are able to use both RuBP and PEP carboxylase reactions during carbon fixation (Bloom & Troughton 1979). As a result, they are difficult to distinguish from C_3 and C_4 plants using stable carbon isotope ratios (Bocherens 1997). The $\delta^{13}C$ values more closely resemble those of C_3 plants when CAM plants use more RuBP carboxylase reactions during diurnal photosynthesis. When more PEP carboxylase reactions occur, discrimination against ^{13}C is reduced, and $\delta^{13}C$ values are more positive (i.e. closer to those of C_4 plants) (Ehleringer & Rundel 1989). The $\delta^{13}C$ values of some CAM plants have demonstrated seasonal variability, with more positive (C_4-like) values indicating low moisture levels, resulting in increased nocturnal CO_2 fixation using the PEP carboxylase enzyme (Bloom & Troughton 1979). Similarly, the amount of CO_2 fixed diurnally using RuBP carboxylase reactions when conditions are favourable results in more negative $\delta^{13}C$ values (Winter & Holtum 2002).

Plants following the C_3 and C_4 pathways therefore exhibit a bimodal distribution of $\delta^{13}C$ values that are distinct from one another (Deines 1980). Mean global plant $\delta^{13}C$ values are between -26.5‰ and -27‰ for C_3 plants, and -12.5‰ and -13‰ for C_4 plants (Ambrose & DeNiro 1986; Ambrose & Sikes 1991; Cerling *et al.* 1997; Cerling & Harris 1999; Kelly 1999), suggesting an average C_3 to C_4 spacing of 14‰ to 14.5‰ in most environments (Sponheimer *et al.* 2003a; Passey & Cerling 2006). The $\delta^{13}C$ values of CAM plants overlap those of both C_3 and C_4 plants under different conditions (O'Leary 1981).

The distribution of plants fixing CO_2 via the three photosynthetic pathways is determined by environmental variables (Scholes & Archer 1997). C_3 dicotyledons are distributed across a wide range of habitats (Still *et al.* 2003). C_4 monocotyledons require high temperatures during the growing season to flourish. They are also able to photosynthesise more effectively than C_3 plants when concentrations of CO_2 in the atmosphere are low. C_4 grasses occur mainly in open habitats in biomes with a high degree of summer rainfall (Ehleringer *et al.* 1997). C_3 grasses dominate in winter rainfall areas, and both C_3 and C_4 grasses grow in winter/summer rainfall ecotones (Vogel *et al.* 1978; Scott & Vogel 2000). CAM plants can be found in a range of habitats. They require little water to survive, and therefore tend to thrive better than other plants in hot, dry environments. Their ability to conserve water through opening their stomata for nocturnal carbon fixation also reduces CO_2 loss through photorespiration, affording them an advantage over plants that photosynthesise diurnally (Mooney *et al.* 1977; Bloom & Troughton 1979; O'Leary 1981).

The extent of South African seasonal rainfall zones is shown in Figure 2.1. Most rainfall occurs during winter in the south west (the winter rainfall zone), and in summer in the north and east (the summer rainfall zone). Between the winter and summer rainfall zones is an intermediate zone which experiences year-round rainfall, allowing both C_3 and C_4 grasses to grow. Dicotyledonous plants are predominantly C_3 across all South African rainfall seasonality axes (Vogel 1978).

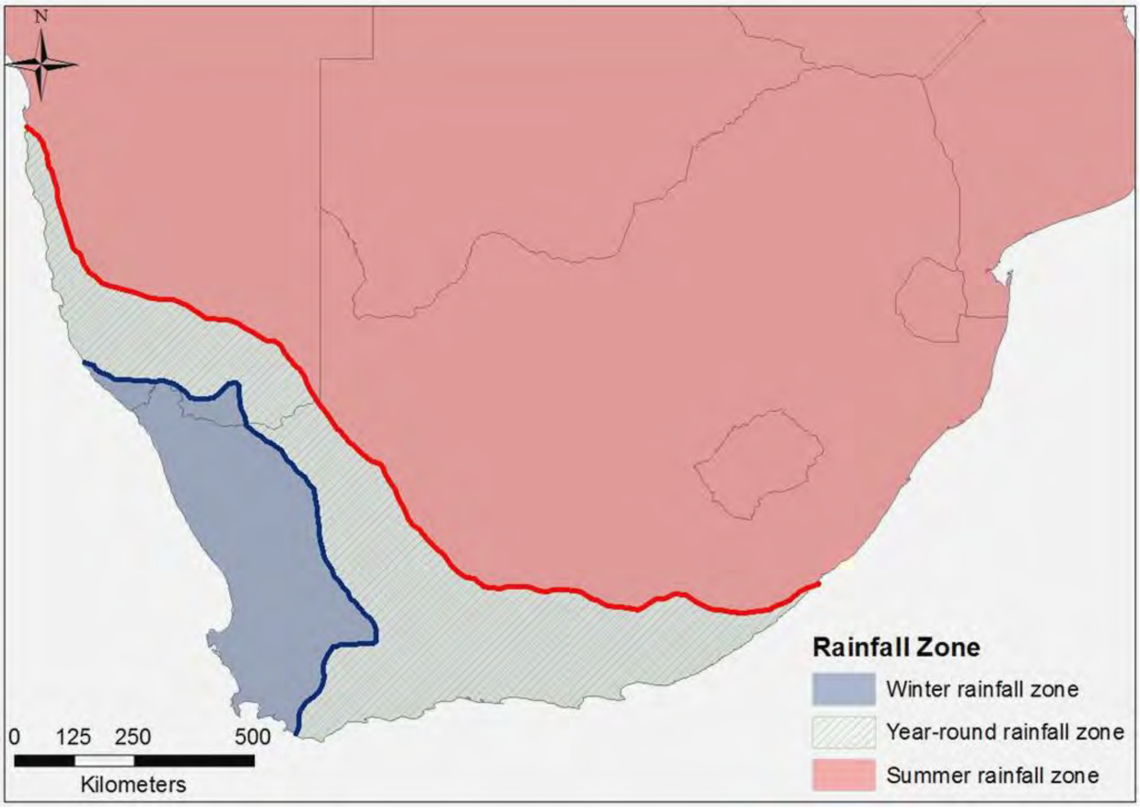


Figure 2.1. Map showing the present rainfall seasonality zones for South Africa (redrawn after Chase & Meadows 2007; Chase *et al.* 2012).

The carbon isotope ratios of plants are passed on to consumers, making it possible to use the $\delta^{13}C$ values of various animal tissues to distinguish between consumers of C_3 and C_4 plants. Additional fractionation between dietary sources and body tissues results in more positive $\delta^{13}C$ values of consumers relative to the plants they eat. Consumer bone collagen $\delta^{13}C$ values ($\delta^{13}C_{collagen}$) can be ~0.5 - 6.1‰ more positive (Vogel 1978; van der Merwe & Vogel 1978; Chisholm *et al.* 1982; van der Merwe 1982; Ambrose & Norr 1993; Kelly 1999; Jim *et al.* 2004) than dietary $\delta^{13}C$ ($\delta^{13}C_{diet}$). In the case of small animals such as rock hyraxes, the expected offset between $\delta^{13}C_{diet}$ and $\delta^{13}C_{collagen}$ is ~3-4‰ (DeNiro & Epstein 1978b; Ambrose & Norr 1993; Caut *et al*. 2009).

Since browsers consume mostly C_3 dicotyledons, the $\delta^{13}C$ values of their tissues reflect the ^{13}C depleted values of their diet. Reported $\delta^{13}C_{collagen}$ values for C_3 browsers range from ~-24.5‰ to -15‰. Monocotyledon consumers (grazers) can have similarly negative $\delta^{13}C$ values when consuming C_3 grasses. In areas with C_4 grass, grazer $\delta^{13}C_{collagen}$ values range from ~-15‰ to -7‰ (Ambrose & DeNiro 1986; van der Merwe 1989; Codron *et al.* 2005; Codron *et al.* 2012). In these areas, browsers and grazers have distinct $\delta^{13}C_{collagen}$ values with little overlap. Grazer $\delta^{13}C$ values can be used to determine the relative abundance of C_3 and C_4 grasses in palaeoenvironmental studies, allowing for the reconstruction of past atmospheric CO_2 concentration and rainfall seasonality (*cf*. Murphy *et al.* 2007).

Since many animals have flexible diets incorporating a mixture of C_3 and C_4 plant taxa, the $\delta^{13}C$ values of their body tissues are positioned along the C_3 to C_4 continuum. Simple carbon mixing models have been used as a means of estimating the relative contributions of isotopically distinct components to a consumer's diet (Cerling *et al.* 1999; Cerling & Viehl 2004; Phillips 2012; Phillips *et al*. 2014). Such models use consumer $\delta^{13}C$ values to calculate the percentages of C_3 and C_4 taxa in diet by calculating their distance from the carbon isotopic endpoints of two distinct dietary inputs (e.g. C_3 and C_4 plants). Vegetation end points can be corrected for diet to tissue fractionation, resulting in end point values that conform to the expectations for the body tissues of pure browsers and grazers in a given environment (Phillips 2012). For example, in an environment where the C_3 endpoint for bone collagen $\delta^{13}C$ is -23‰, the C_4 bone collagen $\delta^{13}C$ endpoint would be -9‰, assuming a 14‰ spacing between endpoints (*cf.* Cerling *et al.* 1999). A consumer in this environment with a $\delta^{13}C_{collagen}$ value of -16‰ lies at the centre of the continuum and is therefore incorporating 50% C_3 and 50% C_4 plants in its diet (Fig 2.3a).

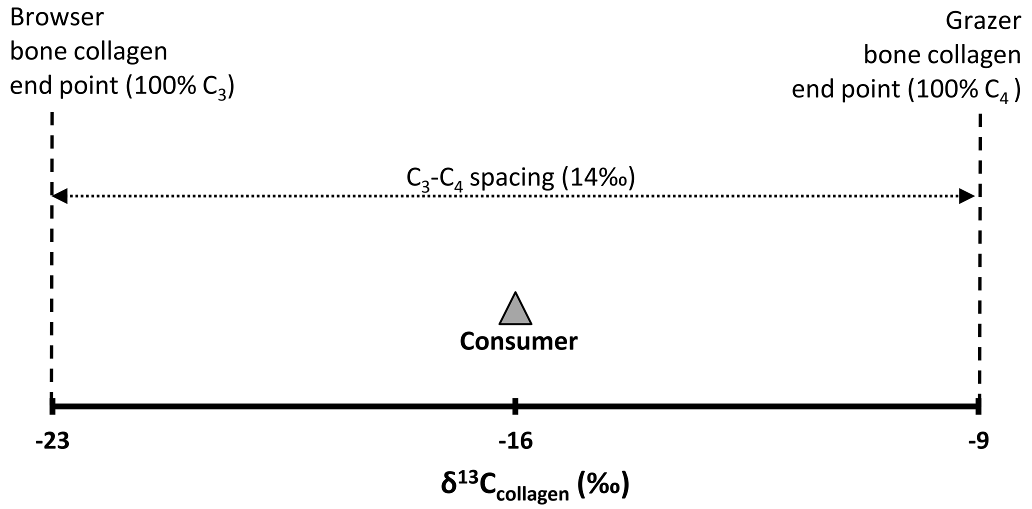
The percentages of C_3 and C_4 taxa can be calculated using the equation

$$a = -100\left(\frac{x - y}{y - z}\right)$$

$$b = 100 - a$$

where a and b are the percentage C_3 and C_4 contributions, and x is the exact (in the case of a single individual) or mean (in the case of multiple individuals) $\delta^{13}C$ value. y and z are the $\delta^{13}C$ values of the C_4 and C_3 source endpoints, respectively (Phillips 2012). Since most $\delta^{13}C$ values are negative numbers, the result of the calculation for the C_3 contribution is multiplied by -100 to obtain a positive percentage. The C_4 contribution is then calculated by subtracting the C_3 percentage from 100 (%). For instance, a consumer living in the same environment as the animal in the first example, but with a $\delta^{13}C$ value of -19.3‰, ate 73.6% C_3 plants, and 26.4% C_4 plants (Figure 2.2b).

(a)



(b)

$$\%C_3 = -100\left(\frac{-19.3+9}{-9+23}\right)$$

$$= 73.6$$

$$\%C_4 = 100 - 73.6$$

$$= 26.4$$

Figure 2.2. Example 1 **(a)** of a simple carbon mixing diagram (redrawn after Phillips 2012) used to determine the relative contribution of C_3 and C_4 taxa in the diet of a consumer with a $\delta^{13}C$ value of -16‰. Endpoints are corrected for diet-tissue fractionation. **(b)** Example (2) of using a carbon mixing equation to calculate the C_3 and C_4 dietary contributions of a consumer with a $\delta^{13}C$ value of -19.3‰ as percentages.

Since vegetation end points are often not known and may vary in different environments, one method of tailoring the approach to a particular case is to use the most negative $\delta^{13}C$ value of a known browsing individual, or the most positive $\delta^{13}C$ value of a known grazing individual, as an endpoint, and then to calculate the opposite endpoint using a 14‰ spacing between source endpoints. This reduces the chances of imprecise approximations of diet to tissue fractionation from producing spurious estimates of dietary composition in consumers for which the fractionation between whole diet and specific tissues is poorly defined (Phillips 2012). However, these assumptions could still yield estimates with a number of fundamental uncertainties. For example, there is some evidence that diet to tissue fractionation does not remain constant along the C_3 to C_4 continuum; the diet to tissue spacing can be up to 2‰ greater in C_4 consumers than it is in C_3 consumers (Hare *et al.* 1991; Ambrose & Norr 1993; Pate *et al.* 1998; Sponheimer *et al*. 2003c).

In order to have confidence in the results of mixing models, the carbon isotopic ratios of both dietary sources and consumers must be known. Precise knowledge of diet to tissue fractionation is also necessary, and it must be assumed that this is constant for all dietary sources. Applications of mixing models that do not meet these criteria produce results with inherent uncertainties and interpretation of such cases should demonstrate an understanding of the limitations associated with each exploration (Phillips *et al.* 2014).

2.3 - Stable isotopes of nitrogen in the atmosphere, soils and plants, and consumers

Atmospheric nitrogen gas (N_2) has a $\delta^{15}N$ value of 0‰ (Mariotti 1981; Robinson 2001). Global nitrogen pools are on average ^{15}N enriched relative to atmospheric N_2 (Handley & Raven 1992). Soils have variable concentrations of ^{15}N derived from fractionation during complex cycling processes, including nitrogen fixation by nitrogen fixing plants and soil microbes (Handley & Raven 1992; Sheng *et al*. 2014).

Diazotrophic plants (primarily leguminous) convert soil and atmospheric N_2 to usable fixed nitrates (NO_3^-) in their root nodules (Shearer *et al*. 1980; Handley & Raven 1992), a process called nitrification (Evans 2007). Most plants are unable to fix their own nitrogen or to use atmospheric N_2; they rely on nitrogen uptake from nitrifying soil microbes that convert N_2 into usable NO_3^- or ammonium (NH_4^+) (Ambrose 1991). Non nitrogen fixing plants generally have $\delta^{15}N$ values that are between 2‰ and 10‰ more positive than those of diazotrophic plants (DeNiro & Schoeninger 1983; Heaton *et al.* 1986; Högberg 1986), although there is some overlap. The ^{15}N depletion in diazotrophic plants is partly due to the fact that the contribution of relatively ^{15}N depleted atmospheric N_2 to the plants results in lower values (Shearer & Kohl 1986; Handley & Raven 1992), although the root nodules tend to be ^{15}N enriched relative to the whole plant (Shearer *et al.* 1980). Denitrification (the loss of nitrogen from systems) is the final step in the nitrogen cycle; the term describes the process of soil microbes converting fixed NO_{3-} or NH_4^+ back to N_2 gas (Zumft 1997). Kinetic fractionation occurs during this process, resulting in the release of ^{15}N depleted N_2 gas to the atmosphere, leaving the substrate enriched in ^{15}N. Other causes of soil ^{15}N enrichment are the deposition of animal excretions and decomposition of organic matter following plant senescence (Shearer & Kohl 1986; Robinson 2001). The nitrogen isotopic composition of soils also varies according to depth (Högberg 1997). Near the surface, soils are depleted in ^{15}N. As decomposing organic matter is mobilised downward in the soil profile, soils become ^{15}N enriched as exchange with atmospheric N_2 decreases (Evans & Ehleringer 1993), resulting in variable plant $\delta^{15}N$ values dependent upon root depth (Högberg 1997).

It has been established that relationships exist between the nitrogen isotope ratios of plants and the availability of water in environments (Heaton *et al.* 1986, 1987; Swap *et al.* 2004; Pate & Anson 2008; Craine *et al.* 2009; Hartman & Danin 2010). Part of the reason for this relationship is that plant $\delta^{15}N$ is a function of the relative 'openness' of the soil nitrogen cycle. Open cycles allow a greater degree of exchange between fixed nitrogen and atmospheric N_2 gas, while closed cycles facilitate limited exchange. The nitrogen cycle in arid localities is open relative to that of wetter localities, resulting in higher plant $\delta^{15}N$ values (Murphy &

Bowman 2009), particularly in C_3 plants (Swap *et al.* 2004). Hence, in mesic environments where the nitrogen cycle is closed, the ratio of ^{15}N to ^{14}N is lower, since less ^{14}N is able to leave the cycle.

While plant $\delta^{15}N$ is partly determined by nitrogen fixation and water availability, the primary determinants of animal tissue $\delta^{15}N$ values can be more difficult to discern. Plant $\delta^{15}N$ is partly responsible for animal $\delta^{15}N$, since the expected ^{15}N enrichment of body tissues ranges from ~0.4‰ to 5‰ relative to the values of the diet (Hare *et al.* 1991; Gannes *et al.* 1998; Roth & Hobson 2000; Murphy & Bowman 2006; Caut *et al.* 2009). This enrichment increases by an average of about 3-4‰ for each step-wise consumer trophic level in terrestrial ecosystems (Schoeninger & DeNiro 1984; Ambrose 1991).

A further contribution to animal $\delta^{15}N$ is fractionation during physiological processes, and it is believed that nitrogen metabolism can strongly influence tissue $\delta^{15}N$ (Heaton *et al.* 1986, 1987; Sealy *et al.* 1987; Ambrose 1991; Gröcke *et al*. 1997). Some animals are able to recycle urea when water stressed, allowing them to survive on very little water for extended periods of time (Hume *et al.* 1980; Chilcott *et al.* 1985). In order to conserve water during droughts, concentrated urea is excreted, and nutrients are recycled to the hindgut. Since the light ^{14}N isotope is preferentially expelled via urea passed on to the kidneys, while the heavier ^{15}N isotope is re-absorbed via the hindgut, this cycle results in ^{15}N-enriched body tissues, and ^{15}N-depleted urea (Ambrose 1991).

The degree to which animals recycle urea remains the subject of debate, although the effects are believed to be particularly pronounced in drought-tolerant species (Gröcke *et al*. 1997). Hence, in dry areas, it is expected that $\delta^{15}N$ values of body tissues (including bone collagen) are higher when animals are driven to recycle urea in order to maintain nitrogen balance and remain hydrated (Ambrose & DeNiro 1987; Foley & Hume 1987). Conversely, urea is not recycled to the same degree in wetter areas, resulting in a lesser degree of re-absorption of ^{15}N, and consequently lower $\delta^{15}N$ values of body tissues. The expectation is therefore that a relationship between aridity and higher $\delta^{15}N$ values of body tissues in drought tolerant animals exists (Heaton *et al.* 1986). Drought tolerant herbivores have $\delta^{15}N$ values that are about 2-4‰ higher than those for water dependent herbivores living in the same environment (Ambrose & DeNiro 1986), and previously published data suggests a decrease of 1.1‰ to 1.3‰ in animal $\delta^{15}N$ for every 100 mm increase in annual rainfall (Heaton *et al*. 1986, 1987).

Other studies, however, suggest that the elevated $\delta^{15}N$ values of animals inhabiting xeric environments are less attributable to metabolic effects, such as urea recycling, than previously thought (Schwarcz *et al.* 1999; Szpak *et al*. 2010; *cf*. Makarewicz & Sealy 2015). An Australian study of the offset between water availability and plant $\delta^{15}N$, and water availability and kangaroo bone collagen $\delta^{15}N$, indicated that drought tolerant herbivores in arid localities mostly reflect the values of their diet (Murphy & Bowman 2006). Since plants tend to be ^{15}N enriched when it is dry (Swap *et al.* 2004; Hartman & Danin 2010), the body tissues of animals eating those plants are correspondingly enriched, and vice versa. Furthermore, Sponheimer *et al.* (2003b) conducted laboratory experiments using four llamas and found that while urine is ^{15}N depleted by between 0.3‰ and 2.1‰ relative to diet, faeces are ^{15}N enriched by about 3‰, indicating that combined excreta are therefore not ^{15}N depleted relative to diet. This suggests that ^{14}N efflux through excretion does not necessarily result in ^{15}N enriched body tissues such as bone collagen.

2.4 - Stable isotope ratios in bone collagen

Since bones and teeth are well-represented in the archaeological record (Lee-Thorp 2008), their utility as sources of carbon and nitrogen for stable isotope analysis has been recognised and applied in many studies (Brown and Brown 2013). Collagen is the primary proteinaceous component of bone (Weiner & Wagner 1998; Taton 2001). Bone collagen consists of peptide chains wound together in a triple-helical structure (Holmgren 1998). The sequence of amino acids in collagen is well known, enabling us to characterise the %C, %N, and C:N ratio of collagen, and to use these as reliable indicators of the structural and isotopic integrity of collagen extracted from bone. As a result, diagenetically altered material can be detected and discarded in the early stages of sample preparation (Tuross *et al.* 1989a; Ambrose 1990; van Klinken 1999). Collagen fibrils are laid down when bone forms, but undergo resorption and re-formation ("turnover") throughout life. The rate of turnover is much slower in bone than in soft tissues (Kelly 1999), resulting in an isotopic composition which reflects a long-term average of the food consumed and the environment inhabited by an animal over much or all of its life (Lee-Thorp 2008). Bone collagen is the chosen material for this study because of the existence of a large body of comparative data for other grazing and browsing mammals, and because of the existence of quality indicators, ensuring that one can have confidence in the isotopic measurements made.

2.5 - Summary

Stable carbon isotope ratios have been recognised as useful for distinguishing grazers and browsers in habitats dominated by C_3 dicotyledons and C_4 monocotyledons. Mixing models can be used to calculate the relative contributions of these two dietary sources, providing estimates of the degree to which consumers prefer one source over another. It has long been established that the ratio of ^{15}N to ^{14}N in consumer body tissues is higher in arid areas, although the precise determinants of this relationship remain uncertain. Considering more recent studies of consumer $\delta^{15}N$, highly resolved analyses of drought tolerant consumer stable nitrogen isotope ratios across South African aridity gradients are a key research avenue that could assist in elucidating the mechanisms that determine ^{15}N content in body tissues.

Chapter Three

Rock hyraxes

3.1 - Taxonomy

Rock hyraxes (*Procavia capensis*) are small drought tolerant mammals in the order Hyracoidea. The Procaviidae are the only family in this order, and there are three genera found in Africa, namely *Procavia, Heterohyrax,* and *Dendrohyrax* (Skinner & Smithers 1990). *Procavia* has been divided into a number of species and subspecies, for example *P. capensis* (Yom-Tov 1993; Barry & Barry 1996; Cunningham 1999; Kotler *et al.* 1999), *P. habessinica* (Rübsamen *et al.* 1979; Hume *et al.* 1980; Kleinschmidt *et al.* 1986)*, P. johnstoni* (Sale 1966; Hoeck 1975; Walker *et al.* 1978; Rübsamen *et al.* 1982; Caro & Alawi 1985; Mahaney 1986; Hoeck 1989; Young & Evans 1993)*, P. capensis welwitschii* (Olds & Shoshani 1982; Grubb *et al.* 1999; Scott *et al.* 2004)*,* and *P. ruficeps* (Spinage 1981; Peters 1989).

The rationale for the naming of subspecies is not explicit. One study used mitochondrial DNA (mtDNA) to distinguish two South African *P. capensis* clades with geographically restricted haplotypes that are thought to have diverged from common ancestral Karoo hyrax populations ~2mya. It was suggested that mtDNA distinctions between populations in the extreme north and north east of South Africa, and the southern and central regions, is due to an absence of gene flow since divergence occurred (Prinsloo and Robinson 1992). Genetic diversity in *P. capensis* awaits further investigation, and the genus *Procavia* is therefore regarded as monospecific here and in other recent publications (Schlitter *et al.* 2014; Butynski *et al.* 2015).

3.2 - Distribution, habitat and colony structure

Procavia capensis occurs throughout most of South Africa (Figure 3.1), with the exception of a small portion of the KwaZulu-Natal east coast, which lies between Durban and the border with Mozambique. They are also absent from a strip at the country's borders with Namibia and Botswana, running from the Kgalagadi in the Northern Cape to the Ramatlabama border post in the North West province (Butynski *et al.* 2015). Their absence from these areas is likely due to the lack of suitable habitats (Skinner & Smithers 1990). Their distribution extends from South Africa to cover a great deal of Namibia, as well as parts of Botswana, Zimbabwe, Angola and Malawi. Rock hyraxes also occur in many other sub-Saharan countries north of the equator, as well as in some countries in North Africa, and on parts of the Arabian Peninsula (Wallach *et al.* 1977; Butynski *et al.* 2015). Since they are remarkably drought tolerant (Rübsamen *et al.* 1979; Hume *et al.* 1980; Eley 1994; Cunningham 1999; Brown & Downs 2006), their occurrence is limited mainly by the availability of food sources and suitable habitat (Skinner & Smithers 1990; Shipley 1999).

Procavia capensis populations occurring in natural environments occupy crevices and hollows in rocky outcrops, rock shelters, cliffs, and caves (Sale 1966; Young & Evans 1993; Brown & Downs 2005, 2007). They have also been known to inhabit the abandoned dens of other animals (Roberts 1951). Those occurring near or within urban human settlements reside in roadside storm water drains, gutters, woodpiles, disused quarries, among concrete blocks, and beneath buildings (Wiid *et al.* 2015; la Grange, pers. obs. 2012).

Well-worn paths connect the living spaces within each colony (Sale 1966). These also lead to a number of communal dung heaps (middens) where rock hyraxes routinely defecate and urinate (Scott & Vogel 2000; Meadows *et al.* 2010; Chase *et al.* 2010, 2015). Middens are thus composed of mixed urino-faecal matter, the relative proportions of which can be variable and challenging to ascertain (Chase *et al*. 2012, 2013). In many cases, these middens are primarily composed of dung pellets with little or no binding material (la Grange, pers. obs. 2013). In arid areas, they are also rich in an adhesive substance called hyraceum (Figure 3.2) (Scott & Bousman 1990; Scott 1996), especially when they are sheltered from rain by sufficiently large rocky overhangs (Chase *et al*. 2012). It has been suggested that humidity in coastal and mesic environments precludes the development of hyraceum-rich midden accretions (Chase *et al.* 2012).

Hyraceum is an excreted metabolic product consisting of concentrated rock hyrax urine and salts (Rübsamen *et al*. 1982; Eley 1994; Prinsloo 2007; Gil-Romera *et al.* 2007; Olsen *et al.* 2008; Carr *et al.* 2010; Gil-Romera *et al.* 2010; Chase *et al*. 2012). When present in sufficient quantities, this consolidates the midden deposit, which can then be preserved for tens of thousands of years (Scott 1996; Carrion *et al.* 1999; Chase *et al*. 2009).

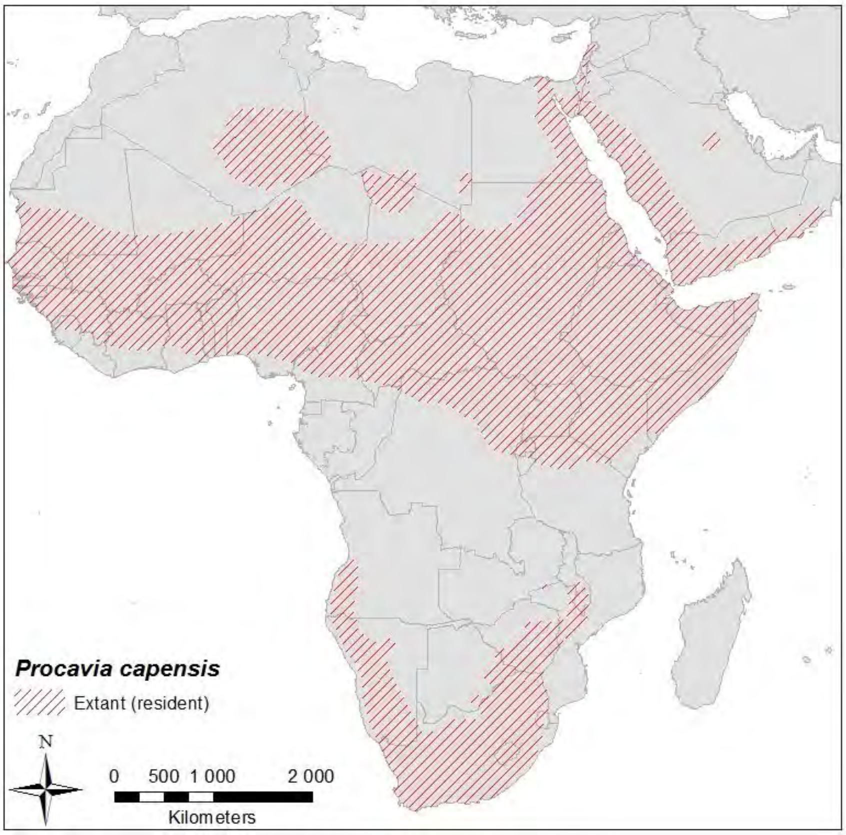


Figure 3.1 - Map showing the distribution of extant *P. capensis* colonies across Africa and the Arabian Peninsula. The map was rendered in ArcGIS using spatial data obtained from the IUCN Red List of Threatened Species (Butynski *et al.* 2015).

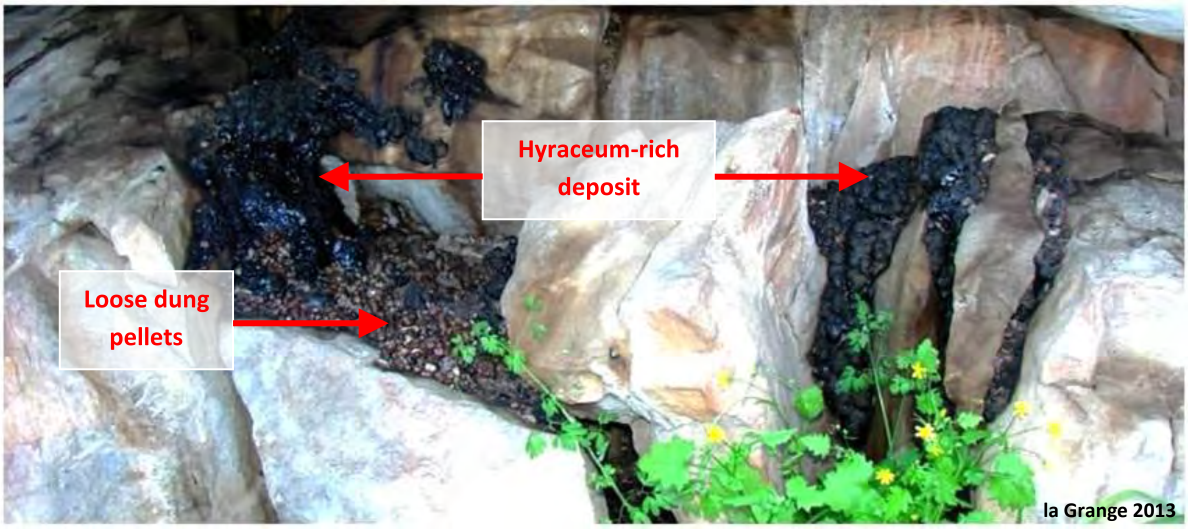


Figure 3.2 - Rock hyrax midden on the farm Mertenhof in the Cederberg. The midden comprises both loose dung pellets, and dung pellets that have been bound by hyraceum.

3.3 - Social structure, behaviour, and reproduction

Rock hyraxes live in groups of up to 80 or more individuals (Hoeck 1989; Kotler *et al.* 1999; Zimman 2008; Wiid *et al*. 2015) with a high ratio of adult females relative to adult males (Fairall & Hanekom 1987; Koren & Geffen 2008). Since they are diurnal, days are spent foraging, basking, huddling, and playing (in the case of juveniles), and nights are mostly spent inside the colony structure (Caro & Alawi 1985; Skinner & Smithers 1990; Brown & Downs 2007).

A complex array of vocalisations allows rock hyraxes to venture beyond the colony structure to forage (Ilany *et al.* 2011; Kershenbaum *et al.* 2012). Warning calls alert foraging hyraxes to the presence of predators, giving the majority of individuals time to return to the colony to seek shelter (Young & Evans 1993; la Grange, pers. obs. 2012). Despite this sentry system, rock hyraxes rarely forage in excess of 15m from the den (Druce *et al.* 2006). In some cases nearby food sources become depleted. This results in nutritional stress and can cause hyraxes to travel up to 150m away from the den to forage (Olds & Shoshani 1982; Kotler *et al.* 1999).

The birthing season of *P. capensis* is restricted by photoperiod, occurring progressively later in the year towards northern latitudes in southern Africa (Millar & Fairall 1976). The gestation period is around 7.5 months (Caro & Alawi 1985), considerably longer than that of other small mammals, following which females give birth to between one and four precocious, fully furred pups (Hoeck 1982). They are dry and able to run, play, and forage within hours of birth (Olds & Shoshani 1982), but are only fully weaned at around six to nine months (Hoeck 1977; Young & Evans 1993).

Males and females are able to reproduce at 16 months (Millar 1972), and dental maturity is reached at around 36 months, once the third molar has erupted (Fairall 1980). There are accounts of hyraxes living for up to 13 years in captivity (Aroch *et al.* 2007), although a maximum age of between 8.5-11 years is more commonplace among wild populations due to predation (Hoeck 1989).

3.4 - Diet

Dietary assessments of rock hyraxes based on field observations indicate that they consume a variety of plant types, including grasses, succulents, forbs, bushes, fruit, and the leaves and bark of trees (Sale 1960; Skinner & Smithers 1990; Klein & Cruz-Uribe 1996; Moran 1996; la Grange, pers. obs. 2013). At some localities, hyraxes forage in residential areas and agricultural fields and are therefore considered pests (Nico Avenant, pers. comm. 2013; Jurie du Plessis, pers. comm. 2013; Wiid *et al*. 2015). Such dietary behaviour, however, is not representative of hyrax feeding patterns in natural environments.

Disagreement regarding the relative contribution of grass and browse vegetation to overall rock hyrax diet exists. Several publications have used data from studies conducted in east Africa to state that hyraxes selectively feed on grass (Janis 1979; Caro & Alawi 1985; Barry & Barry 1996), and that browse is only consumed in significant quantities during periods of grass depletion under conditions of hyper-aridity (Hoeck 1975; Shipley 1999). This idea is founded on the premise that in xeric areas or during periods of water-stress (e.g. low rainfall seasons) when grasses have minimal access to groundwater, rock hyraxes are forced to subsist on plants with deep root-systems that are able to satisfy their moisture requirements during droughts (Chase *et al*. 2009, 2012). It has also been found that rock hyraxes in the Karoo and in southern Namibia consume grasses mostly wet periods when they are most palatable (Fourie 1983; Lensing 1983), although the individuals' diets were dominated by dicotyledon species on average.

A study using bone collagen $\delta^{13}C$ as a direct dietary proxy established that rock hyraxes living in the C_4 grass dominated savannah environment of the Serengeti National Park, Tanzania, consumed more grass relative to bush hyraxes (*Heterohyrax brucei*). Observational data indicated that *P. capensis* grazed 43% of the time during the dry season when grass is less abundant, and 78% of the time during the wet season when grass is widely available. The reported bone collagen $\delta^{13}C$ values for the four rock hyraxes analysed ranged from -16‰ to -12.8‰, reflecting the consumption of substantial amounts of C_4 grass (DeNiro & Epstein 1978a).

Preferential grazing in the Serengeti rock hyraxes was supported by Walker *et al.* (1978) using dental microwear analysis. Observational data indicated that grass comprised 57% and 78% of diet during the dry and wet seasons, respectively. Microscopic enamel wear of 19 individuals was dominated by pitting (indicative of browsing) during the dry season, and striations (indicative of grazing) during the wet season. Hoeck (1989) and Zimman *et al.* (2008) also reported observational data documenting preferential grazing in Serengeti rock hyraxes 78% (n=14) and 70.3% (n=37) of the time, respectively.

Palynological analyses of rock hyrax middens are widely used in palaeoenvironmental studies to reconstruct regional vegetation structure. Wind-borne pollen likely constitutes much of the pollen spectra found in hyrax middens (Scott *et al.* 2004; Gil-Romera *et al.* 2007), resulting in the under-representation of zoophilous plants that transfer pollen directly from the anther to the stigma (Scott & Woodborne 2007a). However, zoophilous pollen taxa are often deposited in the midden from the fur and feet of animals (Meadows *et al.* 2010), potentially rectifying their muted representation. Further midden pollen derives from plants that were consumed and excreted (Carrion *et al.* 1999). These potential biases notwithstanding, some studies have used pollen spectra from hyrax middens to reconstruct hyrax diets. One such study of a hyrax midden at Mount Kenya identified 92%

monocotyledon and 8% dicotyledon species in dung pellets (Mahaney 1986). This result was interpreted as evidence for large-scale grass consumption and thus further corroboration of the widely-held belief that hyraxes are preferential grazers.

The idea that hyraxes primarily consume grass was, however, challenged by other palynological studies. These studies, which were carried out on midden samples collected across various southern African vegetation biomes, probably represent the most comprehensive survey of pollen spectra derived from hyrax middens (e.g. Scott & Bousman 1990; Scott 1996; Scott *et al.* 2004; Scott & Woodborne 2007b). Sampling of pollen grains was in some cases carried out at intervals on hyraceum-rich accretions, and the chronology of accumulation was assessed by means of radiocarbon dating. Plant taxa were then identified, often to genus level, and in some cases to species level, providing valuable palaeoenvironmental information. It was initially concluded that midden pollen spectra are not skewed by dietary preference and are a proxy for available plant taxa only (Scott & Cooremans 1992); an observation that suggests a large degree of site-dependent variability (*cf.* Gil-Romera *et al.* 2010). However, later conclusions drawn from $\delta^{13}C$ values of hyrax dung pellets (considered a direct proxy for dietary preference) contrasted with those of Young & Evans (1993), indicating that although grass pollens were present during certain time periods, the majority of diet consisted of C_3 vegetation in both summer and winter rainfall areas in South Africa (Scott & Vogel 2000).

3.5 - Stable isotope analysis of rock hyrax middens

Numerous large-scale palaeoclimate reconstructions using stable isotope and pollen analyses of hyraceum-rich, stratigraphically coherent southern African rock hyrax middens have been published (Chase *et al.* 2009, 2010, 2011a, b, 2012, 2013; Meadows *et al.* 2010). In many cases, these archives have demonstrated compelling agreement with well-recognised global marine and terrestrial palaeoclimate proxies, such as marine and ice cores, speleothems, global sea-surface temperatures, and insolation and coastal upwelling patterns.

Following previous accounts of rock hyrax graze preference, the $\delta^{13}C$ values obtained from South African middens were interpreted as indicative of changes in the relative contribution of C_3 and C_4 plants to diet (Chase *et al.* 2009). It was suggested that, in the WRZ, where there is little C_4 vegetation, shifts in $\delta^{13}C$ values may be the result of variations in water-use efficiency in plants (*cf.* Farquhar *et al.* 1989) consumed by rock hyraxes (Meadows *et al.* 2010; Chase *et al.* 2015), and local effective precipitation (Chase *et al.* 2011a), with enriched $\delta^{13}C$ values corresponding to relatively drier conditions (Chase 2010). Relationships between $\delta^{13}C$ and aridity were later considered ambiguous, and the emphasis shifted to examining $\delta^{15}N$ as a proxy for the relative palaeo-aridity of environments (Chase *et al.* 2012).

Relationships between midden $\delta^{15}N$ and aridity have thus been emphasised, with higher and lower $\delta^{15}N$ values being associated with dry and wet periods, respectively. Increases of between ~2‰ and 6‰ in $\delta^{15}N$ values are associated with dry periods such as the Younger Dryas (YD) and Heinrich Stadial 1 (HS1), and decreases of between ~1‰ and 6‰ with the relatively mesic conditions of the mid to late Holocene Transition (MLHT), African Humid Period (AHP), and Antarctic Cold Reversal (ACR) (Chase 2010; Chase *et al.* 2011, 2012, 2015). These interpretations of the $\delta^{15}N$ record thus assume that the plants consumed by rock hyraxes are the primary source of $\delta^{15}N$ variability in their middens, a problematic supposition since it does not adequately account for the potential role of physiological mechanisms such as urea recycling in determining $\delta^{15}N$ values.

3.6 - Summary

Procavia capensis is an attractive research subject for isotope studies for a variety of reasons. The fact that they consume a variety of plant types and thrive in a range of habitats, and their consequent widespread distribution provides the opportunity to sample individuals in a transect across a number of environmental gradients. This allows relationships between stable isotope ratios and environmental variables to be investigated. The restricted home range utilised by *P. capensis,* and their relative longevity when compared to other small mammals, makes them particularly useful for characterising local environments and microhabitats at high degrees of geographic and temporal resolution. Conflicting data relating to the diets of east and southern African rock hyraxes, and uncertainties regarding the role of physiology in determining $\delta^{15}N$ values, highlight the need for a systematic investigation into the factors influencing the stable carbon and nitrogen isotopic composition of their body tissues and combined excreta.

As shown in Chapter Two, the use of stable isotopes in addressing questions relating to the relative contribution of C_3 and C_4 plants to herbivore diets is well established. It is therefore hoped that analysis of the carbon isotope ratios of rock hyrax bone collagen will provide insights into the extent to which one can quantify their dietary preference. Comparison between climatic data and bone collagen nitrogen isotope ratios will be used to investigate the degree to which rock hyraxes reflect their environments, so that additional factors potentially influencing the $\delta^{15}N$ values of their bone collagen may be explored.

Chapter Four

Materials and Methods

4.1 - Sample selection criteria, provenance, and accession

Modern *Procavia capensis* individuals were sampled from a variety of localities representing a range of ecological contexts (Figure 4.1). Of these, 210 were suitable for stable isotope analysis (Table 4.1). The primary selection criterion was provenance, so that patterns across environmental gradients could be observed. Samples with imprecise or no provenance data, uncertain species identification, poor bone preservation, or questionable dietary sources (such as those collected near houses, camping sites, or picnic areas where anthropogenic influence may have contributed to diet) were excluded. The precision of provenance data is at worst within 30 km, but in most cases, it is accurate to within 1 m.

A combination of field-collected, donated, and museum samples were included in the dataset. Each sample was assigned a number in the sample register for the Stable Isotope Laboratory at the University of Cape Town (hereafter referred to as UCT number).

The 119 field-collected samples were collected in 2013 and 2014 from rock hyrax colonies located in areas where precipitation and vegetation were deemed representative of undisturbed local patterns (see Table 4.2 for a summary of field-collection site ecology). Field collection involved surveying for suitable colonies and gathering remains from the dens or their immediate surrounds. Remains were collected on the surface, suggesting that they are modern. Environmental stress from factors such as sunlight, temperature, and moisture fluctuations can have a marked effect on the integrity of bone collagen over as short a period as ten years, something which can be detected during the early stages of sample preparation (Tuross *et al.* 1989a). Samples showing evidence of collagen degradation were excluded from the analysis. Geographical positioning systems (GPS) coordinates were recorded in decimal degree (DD) format on the day of collection. In most cases, samples collected within 5m of one another were assigned a single GPS coordinate. Many field-collected samples have been assigned field numbers indicating the year and month of collection, as well as the number of the sample (YYMMNNN). A few field-collected samples were given three digit numbers corresponding to the number of the marked landmark on the GPS device's memory. The remaining field-collected samples were bagged separately on the day of collection but not assigned field numbers. Individuals selected for isotope analysis were limited to a maximum of 20-25 individuals per field-collection locality. In cases where disassociated skeletal remains were collected within close proximity of one another, sampling was restricted to the minimum number of identifiable individuals (MNI).

A total of 35 samples were collected by others and donated to the project. In some cases, these samples do not have exact GPS coordinates. Instead, a GPS coordinate was derived from the vicinity in which each sample was collected. The age and sex of field-collected and donated samples have not been determined, although the majority of individuals were sub-adult or fully adult.

Fifty six samples were taken from sub-adult and fully adult individuals from museum collections. Many museum-accessioned samples were provenanced to the quarter degree square (QDS) (n = 41), while the rest have GPS coordinates (n = 25). Museum samples have identification numbers from the institutions where they are accessioned. Sample identification numbers from the National Museum of Bloemfontein are prefixed NMB, while those from Ditsong Museum in Pretoria start with either TM (Transvaal Museum) or AZ (Archaeozoology Department). These samples were selected from areas that were poorly represented among the field-collected specimens, and especially to increase the number of specimens from summer rainfall areas. Permission to sample at institutions was restricted to specific numbers of individuals that were harvested on site following institutional guidelines to limit the degree of destruction to the remains.

4.2 - General notes relating to environmental data used

ArcGIS was used to generate the maps presented throughout this thesis. A number of data layers were used to elucidate relationships between environmental variables, sample localities, and isotope results. Biome base layers were taken from The Vegetation Map of South Africa, Lesotho and Swaziland (Mucina & Rutherford 2006) and used to assign each sample to its corresponding vegetation biome.

Sample collection localities were also superimposed over a base map of the rainfall seasonality zones of South Africa (redrawn after Chase & Meadows 2007; Chase *et al*. 2012), showing the extent of the winter rainfall zone (WRZ), the year-round rainfall zone (YRZ), and the summer rainfall zone (SRZ). Each sample was thus assigned a rainfall zone designation.

Quantitative rainfall estimates given for localities in this chapter were derived from the South African Atlas of Agrohydrology and Climatology (Schulze 2007) (hereafter referred to as MAP1), and the Water Resources of South Africa Executive Summary, Version One (Middleton & Bailey 2009) (hereafter referred to as MAP2). For samples with GPS coordinates, mean annual precipitation (MAP) values (in mm) taken from the databases were generated for each sample separately. In the case of museum-accessioned samples with QDS data, MAP values from MAP1 were calculated by averaging 25 random points within that QDS, and MAP2 values were taken from the mid-point of each QDS.

Since MAP values derived from the two databases showed no significant difference, MAP1, being the more widely cited, was chosen as the base layer for isotope distribution maps presented in Chapter Five. For comparative purposes, both MAP1 and MAP2 were used for graphs and statistical analyses.

4.3 - Winter rainfall zone samples

The majority of samples from the WRZ were field-collected specifically for this study. The first samples were taken from colonies on or near the south-west coast and in parts of the Cederberg (~100 km from the coast). These localities are within the Fynbos biome, a region dominated by low-growing shrubs, bushes, and small amounts of grass. The dens on the south-west coast had poorly stratified middens with loose dung, while a few of the Cederberg middens had substantial hyraceum accumulations.

Just to the north-west of the Cederberg, in the succulent Karoo biome, lies the Knersvlakte Bioregion. The Moedverloren reserve located there is characterised by a high level of CAM plant biodiversity. Samples were collected from a small colony within the reserve, where the majority of plants were succulents, although small amounts of grass as well as a few shrubs also occur.

The succulent Karoo biome extends further north-west towards the coast. Samples were collected from the inland areas of the Namaqua National Park, which encompasses a portion of the coastline. Although the region is within the succulent Karoo biome, pockets of fynbos vegetation are here interspersed with low-growing succulents and grasses. Individuals collected in Namaqua were from a variety of colonies with predominantly pellet-rich middens.

In the extreme north-west of the country, at the border between South Africa and Namibia, there is a small, arid strip of land that falls within the desert biome. Samples were collected from various localities in the Richtersveld Transfrontier National Park, which is located in this biome on the boundary between the WRZ and the YRZ. A number of the Richtersveld samples had been collected from an eagle nest near the Akkedis Pass and were donated to the project. The rest of the samples were collected from dens on or near the pass. Although the region is generally hyper-arid, higher rainfall in some years results in prolific but short-lived plant growth dominated by small bushes and grass. Hyrax populations tend to decline during particularly dry spells when food becomes limited (Nick de Goede, pers. comm. 2014). At the time of collection, none of the colonies sampled were occupied and the middens were dry and consisted of loose dung deposits, suggesting that hyraxes had been absent from the area for some time. The fact that around seven samples collected here disintegrated during sample preparation further supports the suggestion that the remains had been exposed long enough to undergo diagenesis.

4.4 - Year-round rainfall zone samples

Some of the YRZ samples were collected by others and donated to the project. A handful of these were collected between the town of Mossel Bay and the Robberg Peninsula on the south coast. This region is within the fynbos biome, although these localities are generally grassier than those in the WRZ. Other donated samples were collected from the succulent and Nama-Karoo biomes further inland. The majority of YRZ samples were collected in the Addo Elephant National Park. Addo lies approximately 70 km north of the south coast, and falls within the Albany thicket biome. Samples were taken from a single colony at the south-western boundary of the Colchester section of the park, near the Sundays River. Vegetation surrounding the site is dense. Hyraxes were observed climbing and eating non-indigenous succulents (*Opuntia ficus-indica*), growing at the base of the rocky outcrop (la Grange, pers. obs. 2014), where loose dung pellets deposited within the deeper recesses of the den had been washed out. No hyraceum-rich accretions were observed.

4.5 - Summer rainfall zone samples

The bulk of samples from the SRZ were sourced from museums. The best-represented biome within the SRZ is the grassland biome (Fig. 4.1). A number of samples are from localities within the savannah biome. Both the grassland and savannah biomes are characterised by large amounts of grass interspersed with small numbers of trees and shrubs. Samples were also field collected from a single colony in the Klipriviersberg reserve near Johannesburg. The reserve is dominated by large tufts of dry grass, and hyraxes were observed stripping bark from the trunks and lower branches of trees (la Grange, pers. obs. 2014).

The Indian Ocean coastal belt is a small, mesic section of the east coast where vegetation is comprised of several tropical plant species. A single sample collected from within this biome near the coast was donated to the project.

West of the grassland biome lies the SRZ portion of the Nama-Karoo biome. A few museum samples from this region were donated to the project, but the majority were collected from the Augrabies Falls National Park at the north-west boundary between the SRZ and the YRZ. Vegetation in the park is dominated by grasses with a few tall shrubs and some trees. Large numbers of hyraxes were seen climbing trees to eat leaves and basking on the rocks surrounding the boardwalk that leads to the main falls (la Grange, pers. obs. 2014). The middens at Augrabies were extensive and consisted of unconsolidated dung pellets.

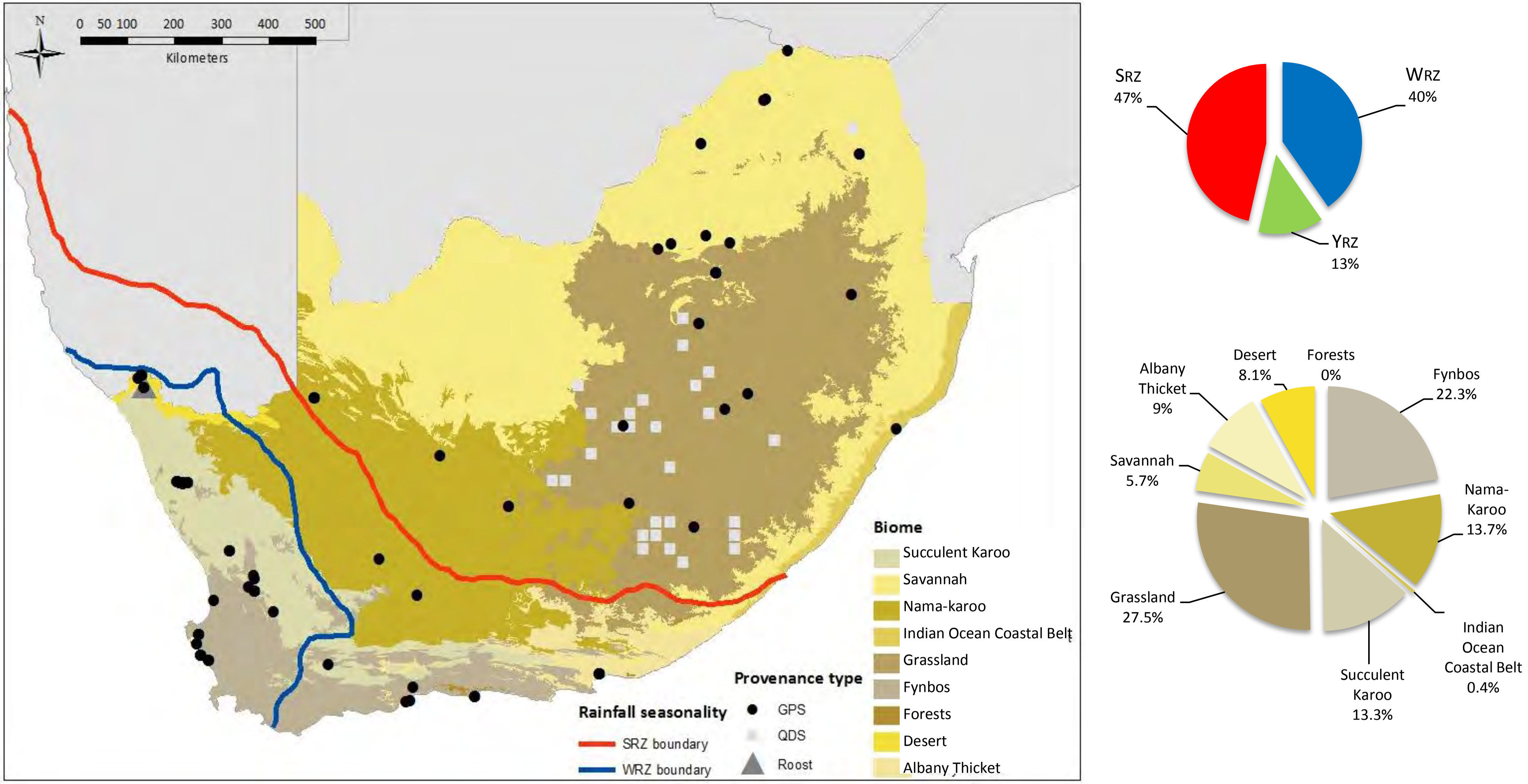


Figure 4.1 –Map showing sampling localities of 210 individuals. The provenance type is indicated using black circles for GPS coordinates, a dark grey triangle for the eagle roost samples, and light grey squares for samples with QDS grid data. Individuals from each rainfall seasonality zone and vegetation biome are indicated as a percentage of the total dataset using pie charts.

Table 4.1 - Table providing accession numbers, rainfall seasonality zone, vegetation biome, provenance, and local MAP values for each sample (n = 210). See guide below for an explanation of the abbreviations used.

Guide to abbreviations

IOCB	Indian Ocean Coastal Belt
NP	National Park
WCFP	West Coast Fossil Park

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
14327			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14328			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14329			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14331			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14332			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14333			WRZ	Fynbos	Yzerfontein	-33.345333	18.154694		388	419
14334			WRZ	Fynbos	WCFP	-32.958320	18.110672		251	322
14335			WRZ	Fynbos	Heuningvlei	-32.161020	19.185062		286	277
14336			WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
14337			SRZ	Nama-Karoo	Prieska	-29.667363	22.727730		272	226
14338			YRZ	Fynbos	Robberg	-34.109857	23.402110		836	642
14339			YRZ	Fynbos	Robberg	-34.109857	23.402110		875	642
14340			YRZ	Fynbos	Robberg	-34.109857	23.402110		870	642
14341			SRZ	Grassland	Oudefontein	-30.54829	26.353778		433	432
14342			SRZ	Grassland	Cathedral cave	-30.974750	27.591760		551	588
14343			YRZ	Fynbos	Cape St. Blaize	-34.186407	22.156849		395	380
14344			YRZ	Fynbos	Pinnacle Point	-34.20634	22.091247		444	473
14345			YRZ	Fynbos	Pinnacle Point	-34.20634	22.091247		444	473

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
14346			WRZ	Fynbos	Biedouw Valley	-32.16102	19.185062		286	277
14347			WRZ	Fynbos	Biedouw Valley	-32.16102	19.185062		286	277
14348			WRZ	Fynbos	Biedouw Valley	-32.16102	19.185062		286	277
14349			WRZ	Succulent Karoo	Aspoort	-32.539982	19.539964		233	166
14350			WRZ	Fynbos	Putslaagte	-31.931815	19.186160		194	146
14351			SRZ	IOCB	Sibudu	-29.172669	31.466118		970	1035
14655		NMB807	SRZ	Grassland	Bloemfontein			2926Aa	476	514
14656		NMB1008	SRZ	Grassland	Bloemfontein			2926Aa	478	514
14657		NMB1452	SRZ	Grassland	Maselspoort			2926Ab	495	521
14658		NMB1453	SRZ	Grassland	Bloemfontein			2926Aa	470	514
14659		NMB1454	SRZ	Grassland	Bloemfontein			2926Aa	471	514
14660		NMB1506	SRZ	Grassland	Skietfontein			3025Aa	332	378
14661		NMB1580	SRZ	Grassland	Wolwespruit			2825Dc	401	405
14663		NMB1616	SRZ	Grassland	Vredefort			2727Ab	558	577
14665		NMB1781	SRZ	Grassland	Ficksburg			2827Dd	699	720
14666		NMB1832	SRZ	Grassland	Vredefort			2727Ab	557	577
14667		NMB2007	SRZ	Grassland	Glen			2826Cd	465	510
14668		NMB2008	SRZ	Grassland	Ficksburg			2827Dd	707	720
14669		NMB2344	SRZ	Grassland	Joubertsgat			3024Bb	334	352
14670		NMB2700	SRZ	Grassland	Avalon			2925Da	363	418
14671		NMB2703	SRZ	Grassland	Arlington			2827Bb	606	586
14672		NMB2704	SRZ	Grassland	Arlington			2827Bb	610	586
14673		NMB2742	SRZ	Grassland	Vanstadensrus			2927Cc	596	616
14674		NMB2811	SRZ	Grassland	Basberg			2825Ad	386	405
14675		NMB2817	SRZ	Grassland	Basberg			2825Ad	387	405
14676		NMB2859	SRZ	Grassland	Eensgevonden			2826Da	471	468
14677		NMB2951	SRZ	Grassland	Thaba Nchu			2926Bb	542	562
14678		NMB3086	SRZ	Grassland	Westleigh			2727Cb	546	557
14679		NMB3127	SRZ	Grassland	No data			2827Bc	545	555

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
14680		NMB4326	SRZ	Grassland	Vineyard			3026Dd	542	518
14681		NMB4333	SRZ	Grassland	Floukraal			3027Cc	593	647
14682		NMB4707	SRZ	Grassland	Dordrecht			3127Ac	564	578
14683		NMB4714	SRZ	Grassland	Brosterlea			3126Bc	518	531
14684		NMB5069	SRZ	Grassland	Jamestown			3126Bb	499	491
14685		NMB5070	SRZ	Grassland	Jamestown			3126Bb	495	491
14686		NMB5261	SRZ	Grassland	Maclear			3128Ab	806	780
14687		NMB5262	SRZ	Grassland	Maclear			3128Ab	793	780
14688		NMB5573	SRZ	Grassland	Umnga Flats			3128Ad	875	637
14689		NMB5632	SRZ	Grassland	Witkop			3126Ba	526	558
14690		NMB5839	SRZ	Grassland	Tsitsana			3028Cd	790	805
14691		NMB6260	SRZ	Grassland	Indwepoort			3127Cb	638	683
14692		NMB6264	SRZ	Grassland	Vineyard			3026Dd	545	518
14693		NMB6991	SRZ	Grassland	Evander			2929Ac	751	585
14694		NMB6991	SRZ	Grassland	Evander			2929Ac	752	585
15401		TM16542	SRZ	Grassland	Voortrekkerhoogte	-25.800000	28.183333		759	814
15403		TM760	SRZ	Savannah	Brits Rooikoppies	-25.605177	27.821470		635	637
15443		TM16637	SRZ	Grassland	Golden Gate	-28.516666	28.616666		798	832
15445		TM13823	SRZ	Savannah	Horseshoe Bend			2327Ac	440	458
15446		TM13827	SRZ	Savannah	Tambotiekloof	-23.916666	27.733333		505	457
15447		TM20374	SRZ	Savannah	Messina	-22.200000	29.383333		314	322
15448		TM4208	SRZ	Savannah	Koster	-25.866666	26.900000		626	648
15449		TM46456	SRZ	Grassland	Koppiesdam	-27.233333	27.683333		600	622
15450		TM20165	SRZ	Grassland	Ngwempisi River	-26.683333	30.600000		894	862
15451		TM46457	SRZ	Grassland	Koppiesdam	-27.233333	27.683333		600	622
15452		AZ693	YRZ	Nama-Karoo	Karoo NP	-32.242385	22.290005		175	179
15454		TM13844	SRZ	Savannah	Magaliesberg	-25.750000	27.166666		614	615
15455		TM42480	SRZ	Savannah	Mica	-24.100000	30.766666		497	495
15456		TM13793	SRZ	Savannah	Buffelshoek	-23.116666	28.916666		482	686

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
15457		TM9330	WRZ	Fynbos	Jonkersberg	-33.934119	22.216687		818	1012
15459		TM41413	SRZ	Savannah	La Cotte			2330Da	557	518
15460		TM13824	SRZ	Savannah	Motlateng	-23.083333	28.966666		471	627
15462		TM42481	SRZ	Savannah	Mica	-24.100000	30.766666		497	495
15463	130911		WRZ	Fynbos	WCFP	-32.958320	18.110672		256	289
15466	13097		WRZ	Fynbos	WCFP	-32.958320	18.110672		256	289
15467	13098		WRZ	Fynbos	WCFP	-32.958320	18.110672		256	289
15468	13099		WRZ	Fynbos	WCFP	-32.958320	18.110672		256	289
15469	130910		WRZ	Fynbos	WCFP	-32.958320	18.110672		256	289
15470			WRZ	Fynbos	Sevilla	-32.072972	19.079585		352	225
15471			YRZ	Fynbos	Robberg	-34.109857	23.402111		846	642
15472			WRZ	Succulent Karoo	Kransgat River	-31.875991	19.176705		242	181
15473	13101		SRZ	Grassland	Naval Hill	-29.104677	26.232269		508	535
15475			SRZ	Savannah	Pretoria Botanical Garden	-25.736378	28.278022		698	726
15476	130912		WRZ	Fynbos	West Coast NP	-33.12232	18.077297		274	402
15477	130913		WRZ	Fynbos	West Coast NP	-33.12232	18.077297		274	402
15478	13091		WRZ	Fynbos	Yzerfontein	-33.346200	18.161700		388	421
15479	13092		WRZ	Fynbos	Yzerfontein	-33.346200	18.161700		388	421
15480	130914		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15481	130915		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15482	130916		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15483	130917		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15484	130918		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15485	130919		WRZ	Fynbos	Rondeberg	-33.426709	18.304592		446	363
15486	130921		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15487	130922		WRZ	Fynbos	Mertenhof	-32.16102	19.185063		286	277
15488	130923		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15489	130924		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15490	130925		WRZ	Fynbos	Mertenhof	-32.158504	19.183318		286	277

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
15491	130926		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15492	130928		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15493	140447		YRZ	Nama-Karoo	Fraserburg	-31.56277	21.564000		147	150
15494	130920		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15495	130927		WRZ	Fynbos	Mertenhof	-32.158504	19.183316		286	277
15496	153		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15497	154		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15498	155		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15499	156		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15500	157		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15501	158		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15502	159		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15503	160		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15504	161		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15505	162		SRZ	Grassland	Klipriviersberg	-26.286136	28.009499		689	776
15506	163		SRZ	Grassland	Klipriviersberg	-26.286136	28.009496		689	776
15507			SRZ	Nama-Karoo	De Aar	-30.599021	24.038112		279	289
15508			SRZ	Nama-Karoo	De Aar	-30.599021	24.038112		279	289
15509			YRZ	Succulent Karoo	Anysberg	-33.510031	20.603944		323	825
15510			WRZ	Fynbos	Diepkloof	-32.336903	18.397490		225	208
15511	14012		WRZ	Succulent Karoo	Knersvlakte	-31.415542	18.707472		184	86
15512	14011		WRZ	Succulent Karoo	Knersvlakte	-31.415542	18.707472		184	86
15513	14013		WRZ	Succulent Karoo	Knersvlakte	-31.415542	18.707472		184	86
15515	14015		WRZ	Succulent Karoo	Knersvlakte	-31.415542	18.707472		184	86
15516	14016		WRZ	Succulent Karoo	Knersvlakte	-31.415542	18.707472		184	86
15517	14018		WRZ	Succulent Karoo	Namaqua NP	-30.165140	17.754990		223	232
15518	140125		WRZ	Succulent Karoo	Namaqua NP	-30.141730	17.707280		199	182
15519	140122		WRZ	Succulent Karoo	Namaqua NP	-30.147180	17.722240		195	181
15520	140121		WRZ	Succulent Karoo	Namaqua NP	-30.165090	17.756400		223	232

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
15521	140120		WRZ	Succulent Karoo	Namaqua NP	-30.165040	17.756450		223	232
15522	140119		WRZ	Succulent Karoo	Namaqua NP	-30.165040	17.756450		223	232
15523	140117		WRZ	Succulent Karoo	Namaqua NP	-30.165140	17.754990		223	232
15524	140116		WRZ	Succulent Karoo	Namaqua NP	-30.163770	17.779660		247	251
15525	140111		WRZ	Succulent Karoo	Namaqua NP	-30.165620	17.757120		223	232
15526	140110		WRZ	Succulent Karoo	Namaqua NP	-30.165620	17.757120		223	232
15527	14/31		WRZ	Succulent Karoo	Namaqua NP	-30.165620	17.757120		223	232
15528	140115		WRZ	Succulent Karoo	Namaqua NP	-30.163800	17.779640		247	251
15529	140124		WRZ	Succulent Karoo	Namaqua NP	-30.141730	17.707280		199	182
15530	140124		WRZ	Succulent Karoo	Namaqua NP	-30.167500	17.809300		252	222
15531	140142		WRZ	Succulent Karoo	Namaqua NP	-30.167500	17.809300		252	222
15532	140139		WRZ	Succulent Karoo	Namaqua NP	-30.164900	17.909860		229	230
15533	140140		WRZ	Succulent Karoo	Namaqua NP	-30.161450	17.807510		246	219
15534	140137		WRZ	Succulent Karoo	Namaqua NP	-30.164900	17.909860		229	230
15535			WRZ	Succulent Karoo	Namaqua NP	-30.164900	17.909860		229	230
15536	140130		WRZ	Succulent Karoo	Namaqua NP	-30.166850	17.758850		234	238
15538	140170		WRZ	Desert	Richtersveld NP	-28.167400	17.020130		79	25
15539	140150		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15540	140159		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15541	140156		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15542	140147		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15543	140155		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15544	140157		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15545	140152		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15546	140151		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15547	140154		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15548	140158		WRZ	Desert	Richtersveld NP	-28.242183	16.949340		64	25
15549	140148		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27
15550	140153		WRZ	Desert	Richtersveld NP	-28.404541	17.066678		64	27

Table 4.1 (continued)

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
15551	140160		WRZ	Desert	Richtersveld NP	-28.167750	17.026130		83	25
15552	140167		WRZ	Desert	Richtersveld NP	-28.167400	17.026130		83	25
15553	140165		WRZ	Desert	Richtersveld NP	-28.167400	17.026130		83	25
15556	140161		WRZ	Desert	Richtersveld NP	-28.167400	17.026130		83	25
15557	140172		SRZ	Nama-Karoo	Augrabies Falls NP	-28.592801	20.330757		120	123
15558	140175		SRZ	Nama-Karoo	Augrabies Falls NP	-28.592801	20.330757		120	123
15559	140174		SRZ	Nama-Karoo	Augrabies Falls NP	-28.592801	20.330757		120	123
15560	1401106		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590770	20.334530		123	127
15561	1401104		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590950	20.334720		123	127
15562	140176		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590550	20.334630		123	127
15563	140181		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590550	20.334630		123	127
15564	140180		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590550	20.334630		123	127
15565	140179		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590550	20.334630		123	127
15566	1401102		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590950	20.334720		123	127
15567	1401110		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590820	20.334610		123	127
15568	140191		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590540	20.334910		123	127
15569	140188		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590540	20.334910		123	127
15570	1401108		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590820	20.334610		123	127
15571	140109		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590820	20.334610		123	127
15572	140184		SRZ	Nama-Karoo	Augrabies Falls NP	-28.591710	20.338120		120	123
15573	140183		SRZ	Nama-Karoo	Augrabies Falls NP	-28.591710	20.338120		120	123
15574	140185		SRZ	Nama-Karoo	Augrabies Falls NP	-28.591710	20.338120		120	123
15575	140182		SRZ	Nama-Karoo	Augrabies Falls NP	-28.591710	20.338120		120	123
15576	140194		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590940	20.334590		123	127
15578	1401101		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590710	20.334500		123	127
15579	1401100		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590710	20.334500		123	127
15580	140199		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590701	20.334500		123	127
15581	1401107		SRZ	Nama-Karoo	Augrabies Falls NP	-28.590800	20.334320		123	127
15977	140313		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348

UCT #	Field #	Museum #	Rainfall zone	Biome	Locality	Latitude (S)	Longitude (E)	QDS	MAP1 (mm)	MAP2 (mm)
15978	140336		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15979	140332		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15980	140310		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15982	140324		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15983	140321		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15984	140320		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15985	140318		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15986	140334		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15987	140331		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15988	140316		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15989	14031		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15990	140314		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15991	140315		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15992	140338		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15993	140336		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15994	14032		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15995	14035		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348
15996	140325		YRZ	Albany Thicket	Addo Elephant NP	-33.680140	25.770522		423	348

Table 4.2 - Summarised site ecology information for field-collection localities with five or more individuals.

Locality name	Rainfall season	Vegetation biome	Latitude, longitude*	Number of individuals	Vegetation density	Vegetation types†	MAP◊ (mm)	Midden composition	Sampling year/month
Addo Elephant National Park	Summer	Albany Thicket	-33.680140, 25.770522	19	Dense	Bushes, grass, succulents	328-423	Dung pellets	2014/March
Augrabies Falls National Park	Summer	Nama-Karoo	-28.591671, 20.334439	24	Intermediate to sparse	Grass, bushes, shrubs, trees	120-127	Dung pellets	2014/January
Klipriviersberg	Summer	Grassland	-26.286136, 28.009496	11	Intermediate	Grass, trees, bushes	689-776	Dung pellets	2013/October
Knersvlakte	Winter	Succulent Karoo	-31.415542, 18.707472	5	Sparse	Succulents, grass, shrubs	86-184	Dung pellets	2014/January
Mertenhof	Winter	Fynbos	-32.159762, 19.184190	10	Dense	Bushes, grass, trees	277-286	Dung pellets and hyraceum	2013/September
Namaqua National Park	Winter	Fynbos	-30.167500, 17.707280	20	Dense to intermediate	Bushes, shrubs, grass	181-252	Dung pellets	2014/January
Richtersveld National Park	Winter	Desert	-28.285971, 17.008009	17	Sparse	Shrubs, grass, bushes, trees	25-83	Dung pellets	2014/January
Rondeberg	Winter	Fynbos	-33.426709, 18.304592	6	Sparse	Bushes, shrubs	363-446	Dung pellets	2013/September
West Coast Fossil Park	Winter	Fynbos	-32.958320, 18.110672	6	Sparse	Shrubs, grass, bushes	251-322	Dung pellets	2013/September
Yzerfontein	Winter	Fynbos	-33.345766, 18.158197	8	Dense to intermediate	Bushes, shrubs	388-421	Dung pellets	2013/September

---

*GPS coordinates averaged for localities with multiple collection sites

†Vegetation types provided in order of prevalence

◊MAP ranges derived from both MAP1 and MAP2 databases.

4.5 - Sample preparation

Once collected, soil and visible organic material were removed from the bone surface. A hand-held Dremel saw fitted with a 24 mm emery cut-off wheel was used to harvest approximately 4-8 mm^3 of bone from each specimen. In some cases (especially for museum-accessioned samples) special effort was made to sample skeletal elements for which the antimere was also present, and samples were taken from minimally invasive or previously damaged parts of the skeleton. Fine sand paper was used to remove remaining particles before the initial bone weight (mg) was recorded using a Sartorius/Mettler Toledo micro balance.

Isotopically depleted lipids were removed from the samples by placing each bone fragment in a separate vial and soaking in ~15ml of defatting solution (two parts chloroform: one part methanol: 0.5 parts distilled water) for 12-20 hours. The solution was replaced if large amounts of lipids were present. Samples were considered lipid-free once an oily film no longer accumulated on the surface of the solution. This step was followed by a thorough rinse with distilled H_2O. From this point onwards, samples were handled with forceps to avoid contamination with finger grease.

In order to remove the isotopically enriched mineral component of bone, samples were treated with 0.1-0.3M hydrochloric acid (HCl) until a collagen pseudomorph remained. The molarity of the acid depended on the size of the harvested bone fragment. Larger samples required a higher molarity solution to accelerate decalcification, whilst smaller samples required a more dilute solution to prevent disintegration. It has been demonstrated that variations in the molarity of HCl has no effect on the isotope ratios of the resultant bone collagen (Pestle 2010). Each sample was tested daily by visual inspection, and by prodding with a needle to check whether or not it had decalcified. If the needle was not able to penetrate the sample effortlessly, fresh HCl solution was added for an additional 24-hour period, after which the test was repeated.

Once samples had fully decalcified they were drained, rinsed with distilled H_2O, and the collagen pseudomorph soaked in a 0.1M solution of sodium hydroxide (NaOH) for 12-20 hours to remove humic acid that may have leached into the sample before it was collected.

The above chemical treatments resulted in a sample pH of ~10. Samples were neutralised to pH 7 over 7-14 days by soaking in distilled H_2O. pH levels were tested every 2-3 days during this period, and the H_2O was replaced every 24-48 hours. Once neutral, samples were drained and each vial sealed using parafilm. The parafilm was pierced and vials placed in a freeze drier at -50°C for 12-20 hours.

Once fully desiccated, samples were left to equilibrate with atmospheric moisture until the weight of each sample remained constant. The freeze dried weight (in mg) was then recorded and the collagen yield calculated as a percentage of the initial bone sample weight. Between 0.45 and 0.65 mg of collagen was weighed into a tin capsule, which was then folded to enclose the sample.

4.6 - Sample analysis and reproducibility of measurements

The samples were combusted in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a continuous flow (ConFlo) IV gas control unit. All three items are made by Thermo Scientific, Bremen, Germany. There were five separate runs with batch sizes ranging from 24-64 individuals. For each run, two empty tin capsules (‘blanks’) and between 16 and 28 standards (materials of known carbon and nitrogen isotope ratios) were included at intervals as controls to measure the precision and stability of the instrument throughout the run. The in-house standards used were chocolate (a commercial chocolate/egg mixture from an associate in the United States), Merck gelatin (a protein gel produced by Merck), seal bone (a seal bone crushed, demineralized and dissolved in acid, and then reconstituted in gel form in the UCT Stable Isotope Laboratory), and Valine (DL Valine purchased from Sigma). A small number of sucrose standards were also used. The sucrose standard was obtained from the stable isotope laboratory at the Australian National University (ANU sucrose). Carbon and nitrogen isotope values of all standards have been established by measurement against internationally accepted reference materials distributed by the National Bureau of Standards and the International Atomic Energy Agency. Nitrogen isotope measurements are expressed relative to atmospheric nitrogen (AIR), while carbon is expressed relative to Pee-Dee Belemnite (PDB).

The standard deviations of repeated determinations of $\delta^{13}C$ and $\delta^{15}N$ values for in-house laboratory standards analysed together with the samples reported here and in Chapter Five were ≤ 0.1‰, with the exception of seal bone, for which the standard deviation was < 0.2‰ for $\delta^{15}N$. $\delta^{13}C$ values of samples in this study are thus reproducible to within 0.1‰, while $\delta^{15}N$ values are reproducible to within 0.2‰ (see Table 4.3 for a summary of standards used and their reproducibility).

Table 4.3 - Details of laboratory standards included in each run, and reproducibility of $\delta^{13}C$ and $\delta^{15}N$ values obtained.

Laboratory standard name	**Expected standard $\delta^{13}C$ (‰)**	**Expected standard $\delta^{15}N$ (‰)**	**Number of standards used (by run number)**					**Standard deviation (across all runs)**	
			1	**2**	**3**	**4**	**5**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Chocolate	-17.6	4.3			16	16	12	0.1	<0.1
Merck gelatin	-20.1	8.0	8	8	4	4	8	<0.1	0.1
Seal bone	-11.0	15.8	4	4	4	4	4	<0.1	<0.2
DL Valine	-26.8	12.1	4	4	4	4	8	<0.1	0.1
ANU Sucrose	-10.6						4	<0.1	<0.1

Chapter Five

Results

5.1 - Collagen quality assessment

Collagen quality indicators include the percentage of collagen by weight relative to the initial bone weight (collagen yield), the elemental ratio of carbon to nitrogen in the bone collagen (C:N), and the percentages of carbon (%C) and nitrogen (%N) in the bone collagen by weight. The collagen pseudomorph can also be examined for signs of collagen degradation; collagen that is degraded appears 'furry' and breaks apart easily, while high quality collagen results in a pliable, translucent pseudomorph of the original bone sample (Tuross *et al.* 1989b; la Grange, pers. obs. 2014; Sealy, pers. comm. 2014). Collagen yields are unfortunately not available for all samples, due to the theft of the computer on which they were recorded. This is not a significantly limiting factor since collagen yields are highly variable depending on the method of collagen extraction used (Sealy *et al.* 2014); they are therefore less reliable measures of collagen quality than C:N ratios, %C, and %N, all of which are available for each sample.

A collagen yield of 22% is considered indicative of well-preserved collagen in modern bone samples, although values as low as 1% and as high as 30% are considered acceptable (van Klinken *et al.* 1999). Low collagen yields are typically indicative of collagen degradation, although these can also result from extended HCl treatment, which causes collagen to dissolve. High collagen yields usually indicate that the sample was not treated with HCl for a sufficient period of time, and that a proportion of the mineral phase of the bone remains. The C:N ratio of well-preserved collagen is ~3.2 (Ambrose 1990), although ratios ranging from 2.9 to 3.6 are considered acceptable (van Klinken *et al*. 1999). Higher C:N ratios indicate contamination by extraneous carbon and/or loss of bone nitrogen. The carbon content of well preserved modern bone collagen is between 15.3% and 47%, and acceptable %N ranges from 5.3% to 17.3%, with lower contents being correlated with decreasing collagen yield (Ambrose 1990).

The collagen yields, C:N ratios, and the %C and %N are presented in Table 5.1. All samples yielded pliable collagen pseudomorphs, indicating that the bone collagen is well preserved. The mean collagen yield is 24.1 ± 3.3%, with a range from 9.5 to 27.8% (n=37). This is indicative of high collagen quality. Sample 14659 had the lowest collagen yield (9.5%). Since this sample had an intact collagen pseudomorph, the low yield is attributed to extended HCl pre-treatment. The mean C:N ratio is 3.3 ± 0.1, with a range of 0.3. All C:N ratios are within the accepted 2.9 to 3.6 range. The %C values range from 37.3% to 53% (mean 43.6 ± 2.2%), and %N ranges from 13.3% to 19.2% (mean 15.6 ± 0.9%) (n=210), indicating that collagen quality is good, and one can have confidence in the carbon and nitrogen isotope ratios measured on these samples.

Table 5.1 - Collagen quality indicators, $\delta^{13}C$ and $\delta^{15}N$ for all samples analysed.

Guide to abbreviations

IOCB	Indian Ocean Coastal Belt
NP	National Park
WCFP	West Coast Fossil Park

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
14327	WRZ	Fynbos	Yzerfontein		42.2	15.3	3.2	-21.9	12.1
14328	WRZ	Fynbos	Yzerfontein		46.6	16.8	3.2	-17.8	11.8
14329	WRZ	Fynbos	Yzerfontein		49.1	17.9	3.2	-19.4	15.0
14331	WRZ	Fynbos	Yzerfontein		43.2	15.9	3.2	-20.4	11.2
14332	WRZ	Fynbos	Yzerfontein		43.6	15.5	3.3	-21.7	13.2
14333	WRZ	Fynbos	Yzerfontein		45.4	16.6	3.2	-20.1	14.4
14334	WRZ	Fynbos	WCFP		51.4	18.2	3.3	-22.0	2.5
14335	WRZ	Fynbos	Heuningvlei		52.5	19.1	3.2	-22.3	7.6
14336	WRZ	Fynbos	Mertenhof		48.0	17.5	3.2	-21.6	13.1
14337	SRZ	Nama-Karoo	Prieska		44.6	16.5	3.2	-19.8	11.5
14338	YRZ	Fynbos	Robberg		42.3	15.5	3.2	-20.1	6.8
14339	YRZ	Fynbos	Robberg		53.1	19.2	3.2	-21.7	6.6
14340	YRZ	Fynbos	Robberg		52.2	18.8	3.2	-20.6	9.0
14341	SRZ	Grassland	Oudefontein		44.6	16.3	3.2	-18.7	8.3
14342	SRZ	Grassland	Cathedral cave		47.1	16.2	3.4	-18.6	5.3
14343	YRZ	Fynbos	Cape St. Blaize		45.9	16.8	3.2	-19.3	9.7
14344	YRZ	Fynbos	Pinnacle Point		46.2	16.9	3.2	-22.1	9.9
14345	YRZ	Fynbos	Pinnacle Point		43.4	15.9	3.2	-21.4	9.5
14346	WRZ	Fynbos	Biedouw Valley		48.1	17.4	3.2	-21.5	13.2

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	δ^{13}C (‰)	δ^{15}N (‰)
14347	WRZ	Fynbos	Biedouw Valley		46.7	16.4	3.3	-23.1	10.9
14348	WRZ	Fynbos	Biedouw Valley		45.0	16.2	3.2	-21.7	14.0
14349	WRZ	Succulent Karoo	Aspoort		46.7	16.8	3.2	-18.5	17.3
14350	WRZ	Fynbos	Putslaagte		44.9	16.2	3.2	-20.9	15.4
14351	SRZ	IOCB	Sibudu		45.1	16.5	3.2	-22.0	8.7
14655	SRZ	Grassland	Bloemfontein	27.8	41.6	14.7	3.3	-16.0	7.7
14656	SRZ	Grassland	Bloemfontein	22.5	44.2	16.0	3.2	-18.4	7.7
14657	SRZ	Grassland	Maselspoort	26.9	44.7	15.9	3.3	-17.2	6.7
14658	SRZ	Grassland	Bloemfontein	24.1	45.1	16.0	3.3	-20.6	7.9
14659	SRZ	Grassland	Bloemfontein	9.5	44.9	15.5	3.4	-20.6	9.0
14660	SRZ	Grassland	Skietfontein	28.1	41.4	14.9	3.2	-19.4	5.9
14661	SRZ	Grassland	Wolwespruit	21.2	43.3	15.2	3.3	-15.9	9.1
14663	SRZ	Grassland	Vredefort	20.9	44.0	15.7	3.3	-18.1	9.3
14665	SRZ	Grassland	Ficksburg	25.5	46.5	16.8	3.2	-21.9	8.1
14666	SRZ	Grassland	Vredefort	23.8	48.8	17.6	3.2	-17.3	10.9
14667	SRZ	Grassland	Glen	25.1	43.1	15.5	3.2	-20.5	8.7
14668	SRZ	Grassland	Ficksburg	27.4	44.0	15.5	3.3	-21.1	6.7
14669	SRZ	Grassland	Joubertsgat	21.9	43.0	15.5	3.2	-23.1	7.8
14670	SRZ	Grassland	Avalon	24.4	47.1	16.6	3.3	-20.5	5.2
14671	SRZ	Grassland	Arlington	26.7	43.4	15.1	3.3	-17.6	10.6
14672	SRZ	Grassland	Arlington	29.1	45.0	15.6	3.4	-19.3	10.3
14673	SRZ	Grassland	Vanstadensrus	25.6	44.5	15.8	3.3	-19.4	9.5
14674	SRZ	Grassland	Basberg	23.5	48.4	17.5	3.2	-16.5	9.3
14675	SRZ	Grassland	Basberg	25.2	45.3	16.2	3.3	-16.1	10.2
14676	SRZ	Grassland	Eensgevonden	27.3	45.6	16.3	3.3	-18.0	8.4
14677	SRZ	Grassland	Thaba Nchu	23.0	45.6	16.2	3.3	-21.4	4.5
14678	SRZ	Grassland	Westleigh	25.7	42.1	14.9	3.3	-22.0	9.5
14679	SRZ	Grassland	No data	27.5	41.7	14.8	3.3	-18.1	7.0
14680	SRZ	Grassland	Vineyard	22.5	47.8	16.9	3.3	-20.9	7.6

Table 5.1 (continued)

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	δ^{13}C (‰)	δ^{15}N (‰)
14681	SRZ	Grassland	Floukraal	19.5	43.4	15.4	3.3	-20.3	7.3
14682	SRZ	Grassland	Dordrecht	24.5	44.1	15.5	3.3	-19.8	6.3
14683	SRZ	Grassland	Brosterlea	23.7	44.2	15.8	3.3	-20.8	8.6
14684	SRZ	Grassland	Jamestown	22.8	42.9	15.3	3.3	-18.0	7.1
14685	SRZ	Grassland	Jamestown	25.3	45.6	16.2	3.3	-18.5	6.9
14686	SRZ	Grassland	Maclear	21.5	45.3	16.2	3.3	-20.1	4.7
14687	SRZ	Grassland	Maclear	26.1	46.0	16.3	3.3	-20.3	5.6
14688	SRZ	Grassland	Umnga Flats	23.8	45.5	16.1	3.3	-20.4	4.7
14689	SRZ	Grassland	Witkop	22.5	44.1	15.8	3.3	-20.2	7.5
14690	SRZ	Grassland	Tsitsana	25.8	44.1	15.9	3.2	-17.1	5.2
14691	SRZ	Grassland	Indwepoort	25.2	45.3	16.4	3.2	-17.8	9.0
14692	SRZ	Grassland	Vineyard	24.1	41.9	14.6	3.3	-19.5	9.3
14693	SRZ	Grassland	Evander		41.9	14.6	3.30	-22.8	6.3
14694	SRZ	Grassland	Evander	26.3	44.7	16.0	3.3	-21.9	5.5
15401	SRZ	Grassland	Voortrekkerhoogte		42.4	15.2	3.3	-18.4	11.9
15403	SRZ	Savannah	Brits Rooikoppies		44.0	15.8	3.2	-14.8	5.6
15443	SRZ	Grassland	Golden Gate		42.5	14.7	3.4	-19.7	7.1
15445	SRZ	Savannah	Horseshoe Bend		41.6	14.9	3.3	-21.4	7.2
15446	SRZ	Savannah	Tambotiekloof		42.1	15.1	3.2	-18.6	8.3
15447	SRZ	Savannah	Messina		42.3	15.1	3.3	-18.2	13.8
15448	SRZ	Savannah	Koster		39.2	13.8	3.3	-20.6	8.6
15449	SRZ	Grassland	Koppiesdam		41.7	14.7	3.3	-21.9	11.5
15450	SRZ	Grassland	Ngwempisi River		38.9	13.8	3.3	-21.0	7.5
15451	SRZ	Grassland	Koppiesdam		42.4	14.7	3.4	-20.4	10.9
15452	YRZ	Nama-Karoo	Karoo NP		42.0	15.2	3.2	-21.0	9.0
15454	SRZ	Savannah	Magaliesberg		44.0	15.6	3.3	-16.7	5.8
15455	SRZ	Savannah	Mica		43.3	15.7	3.2	-17.9	5.5
15456	SRZ	Savannah	Buffelshoek		42.0	14.7	3.3	-18.3	3.7
15457	WRZ	Fynbos	Jonkersberg		41.5	14.8	3.3	-21.8	1.2

Table 5.1 (continued)

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	δ^{13}C (‰)	δ^{15}N (‰)
15459	SRZ	Savannah	La Cotte		42.6	15.5	3.2	-18.8	7.9
15460	SRZ	Savannah	Motlateng		39.1	13.9	3.3	-21.5	1.9
15462	SRZ	Savannah	Mica		43.6	15.6	3.3	-19.0	8.6
15463	WRZ	Fynbos	WCFP		41.8	14.5	3.4	-22.8	2.4
15466	WRZ	Fynbos	WCFP		40.7	14.5	3.3	-22.0	4.1
15467	WRZ	Fynbos	WCFP		41.5	15.0	3.2	-21.7	2.3
15468	WRZ	Fynbos	WCFP		41.6	15.0	3.2	-20.9	18.4
15469	WRZ	Fynbos	WCFP		44.6	15.8	3.3	-22.8	2.5
15470	WRZ	Fynbos	Sevilla		40.1	14.2	3.3	-21.2	12.8
15471	YRZ	Fynbos	Robberg		41.4	14.9	3.3	-21.4	8.9
15472	WRZ	Succulent Karoo	Kransgat River		41.7	15.2	3.2	-22.4	14.5
15473	SRZ	Grassland	Naval Hill		42.5	15.4	3.2	-21.9	5.5
15475	SRZ	Savannah	Pretoria Botanical Garden		42.2	15.2	3.2	-19.9	8.1
15476	WRZ	Fynbos	West Coast NP		41.6	14.8	3.3	-21.0	9.6
15477	WRZ	Fynbos	West Coast NP		42.8	15.0	3.3	-21.7	8.7
15478	WRZ	Fynbos	Yzerfontein		41.6	15.0	3.2	-21.8	14.3
15479	WRZ	Fynbos	Yzerfontein		46.3	14.9	3.6	-20.6	14.7
15480	WRZ	Fynbos	Rondeberg		41.2	14.8	3.2	-22.2	8.1
15481	WRZ	Fynbos	Rondeberg		38.0	13.6	3.3	-21.4	8.1
15482	WRZ	Fynbos	Rondeberg		40.9	14.5	3.3	-23.1	9.4
15483	WRZ	Fynbos	Rondeberg		41.5	15.0	3.2	-24.0	10.1
15484	WRZ	Fynbos	Rondeberg		43.9	15.8	3.2	-23.6	9.5
15485	WRZ	Fynbos	Rondeberg		41.8	15.0	3.2	-24.0	10.4
15486	WRZ	Fynbos	Mertenhof		41.6	15.1	3.2	-21.7	14.5
15487	WRZ	Fynbos	Mertenhof		42.8	15.7	3.2	-22.1	14.3
15488	WRZ	Fynbos	Mertenhof		42.8	15.7	3.2	-20.2	14.9
15489	WRZ	Fynbos	Mertenhof		40.9	14.3	3.3	-21.1	13.2
15490	WRZ	Fynbos	Mertenhof		40.4	13.3	3.5	-21.4	16.2
15491	WRZ	Fynbos	Mertenhof		42.2	15.1	3.3	-20.8	11.9

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
15492	WRZ	Fynbos	Mertenhof		39.1	13.9	3.3	-20.5	14.1
15493	YRZ	Nama-Karoo	Fraserburg		42.9	15.4	3.3	-21.1	11.7
15494	WRZ	Fynbos	Mertenhof		41.7	15.1	3.2	-20.8	11.2
15495	WRZ	Fynbos	Mertenhof		42.7	15.3	3.3	-20.6	11.9
15496	SRZ	Grassland	Klipriviersberg		41.4	14.9	3.2	-21.7	6.0
15497	SRZ	Grassland	Klipriviersberg		41.6	15.1	3.2	-22.1	5.5
15498	SRZ	Grassland	Klipriviersberg		41.5	15.0	3.2	-22.1	6.6
15499	SRZ	Grassland	Klipriviersberg		41.7	15.0	3.2	-21.3	6.8
15500	SRZ	Grassland	Klipriviersberg		39.9	14.3	3.2	-21.5	6.0
15501	SRZ	Grassland	Klipriviersberg		41.3	14.8	3.3	-20.7	8.1
15502	SRZ	Grassland	Klipriviersberg		42.9	15.4	3.3	-21.7	7.0
15503	SRZ	Grassland	Klipriviersberg		41.4	15.0	3.2	-16.8	6.0
15504	SRZ	Grassland	Klipriviersberg		41.5	14.9	3.2	-22.8	6.5
15505	SRZ	Grassland	Klipriviersberg		42.3	15.6	3.2	-22.1	8.3
15506	SRZ	Grassland	Klipriviersberg		42.8	15.5	3.2	-20.4	5.1
15507	SRZ	Nama-Karoo	De Aar		44.3	16.2	3.2	-19.6	6.3
15508	SRZ	Nama-Karoo	De Aar		44.4	16.3	3.2	-18.6	9.7
15509	YRZ	Succulent Karoo	Anysberg		42.8	15.4	3.2	-21.3	4.0
15510	WRZ	Fynbos	Diepkloof		42.0	14.8	3.3	-22.8	16.1
15511	WRZ	Succulent Karoo	Knersvlakte		38.5	14.0	3.2	-20.3	17.5
15512	WRZ	Succulent Karoo	Knersvlakte		41.4	15.0	3.2	-20.4	17.9
15513	WRZ	Succulent Karoo	Knersvlakte		44.3	15.8	3.3	-21.4	19.5
15515	WRZ	Succulent Karoo	Knersvlakte		41.3	14.8	3.3	-19.9	18.8
15516	WRZ	Succulent Karoo	Knersvlakte		44.4	16.2	3.2	-20.2	17.5
15517	WRZ	Succulent Karoo	Namaqua NP		44.5	16.2	3.2	-23.0	11.1
15518	WRZ	Succulent Karoo	Namaqua NP		44.3	16.2	3.2	-20.9	18.2
15519	WRZ	Succulent Karoo	Namaqua NP		44.5	16.2	3.2	-19.5	16.0
15520	WRZ	Succulent Karoo	Namaqua NP		43.8	15.3	3.3	-20.8	12.5
15521	WRZ	Succulent Karoo	Namaqua NP		44.2	15.9	3.2	-22.8	11.6

Table 5.1 (continued)

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	δ^{13}C (‰)	δ^{15}N (‰)
15522	WRZ	Succulent Karoo	Namaqua NP		45.3	15.9	3.3	-22.1	10.4
15523	WRZ	Succulent Karoo	Namaqua NP		44.3	16.1	3.2	-22.2	12.0
15524	WRZ	Succulent Karoo	Namaqua NP		44.8	15.9	3.3	-22.8	10.9
15525	WRZ	Succulent Karoo	Namaqua NP		44.4	16.1	3.2	-21.6	12.1
15526	WRZ	Succulent Karoo	Namaqua NP		44.2	16.0	3.2	-21.1	10.5
15527	WRZ	Succulent Karoo	Namaqua NP		43.5	16.0	3.2	-22.5	11.5
15528	WRZ	Succulent Karoo	Namaqua NP		43.1	15.5	3.2	-21.5	9.9
15529	WRZ	Succulent Karoo	Namaqua NP		44.4	16.1	3.2	-21.4	17.4
15530	WRZ	Succulent Karoo	Namaqua NP		44.7	15.8	3.3	-23.0	11.2
15531	WRZ	Succulent Karoo	Namaqua NP		45.5	15.7	3.4	-23.0	10.9
15532	WRZ	Succulent Karoo	Namaqua NP		44.4	15.8	3.3	-21.3	13.4
15533	WRZ	Succulent Karoo	Namaqua NP		44.7	16.0	3.3	-22.5	11.7
15534	WRZ	Succulent Karoo	Namaqua NP		44.0	16.0	3.2	-20.3	13.9
15535	WRZ	Succulent Karoo	Namaqua NP		44.3	15.9	3.3	-21.3	11.9
15536	WRZ	Succulent Karoo	Namaqua NP		44.4	16.0	3.2	-20.3	13.5
15538	WRZ	Desert	Richtersveld NP		44.1	15.8	3.3	-20.5	10.2
15539	WRZ	Desert	Richtersveld NP		44.2	16.0	3.2	-21.8	9.9
15540	WRZ	Desert	Richtersveld NP		41.9	15.2	3.2	-23.7	9.6
15541	WRZ	Desert	Richtersveld NP		43.3	15.3	3.3	-23.8	10.8
15542	WRZ	Desert	Richtersveld NP		44.9	15.6	3.3	-20.7	10.4
15543	WRZ	Desert	Richtersveld NP		44.5	15.6	3.3	-21.8	10.5
15544	WRZ	Desert	Richtersveld NP		42.6	15.5	3.2	-23.6	10.1
15545	WRZ	Desert	Richtersveld NP		44.0	16.1	3.2	-21.5	11.5
15546	WRZ	Desert	Richtersveld NP		45.0	16.1	3.3	-22.2	11.0
15547	WRZ	Desert	Richtersveld NP		43.7	16.1	3.2	-23.7	9.1
15548	WRZ	Desert	Richtersveld NP		44.4	16.1	3.2	-18.0	9.1
15549	WRZ	Desert	Richtersveld NP		44.4	15.7	3.3	-21.7	8.1
15550	WRZ	Desert	Richtersveld NP		44.7	16.2	3.2	-21.7	9.4
15551	WRZ	Desert	Richtersveld NP		44.7	16.0	3.3	-20.2	9.4

Table 5.1 (continued)

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
15552	WRZ	Desert	Richtersveld NP		43.7	16.0	3.2	-16.1	8.3
15553	WRZ	Desert	Richtersveld NP		43.5	15.6	3.3	-19.3	9.1
15556	WRZ	Desert	Richtersveld NP		43.9	16.0	3.2	-18.3	8.8
15557	SRZ	Nama-Karoo	Augrabies Falls NP		44.1	16.0	3.2	-19.5	10.9
15558	SRZ	Nama-Karoo	Augrabies Falls NP		45.6	15.6	3.4	-19.2	9.6
15559	SRZ	Nama-Karoo	Augrabies Falls NP		44.3	15.9	3.3	-18.4	9.6
15560	SRZ	Nama-Karoo	Augrabies Falls NP		43.8	15.7	3.3	-20.0	8.7
15561	SRZ	Nama-Karoo	Augrabies Falls NP		43.5	16.1	3.2	-19.2	9.4
15562	SRZ	Nama-Karoo	Augrabies Falls NP		44.5	16.3	3.2	-19.5	9.3
15563	SRZ	Nama-Karoo	Augrabies Falls NP		44.1	15.9	3.2	-20.0	9.8
15564	SRZ	Nama-Karoo	Augrabies Falls NP		43.8	15.9	3.2	-20.3	9.5
15565	SRZ	Nama-Karoo	Augrabies Falls NP		41.9	15.3	3.2	-21.5	1.9
15566	SRZ	Nama-Karoo	Augrabies Falls NP		44.8	16.4	3.2	-19.4	7.9
15567	SRZ	Nama-Karoo	Augrabies Falls NP		44.8	16.1	3.3	-20.3	9.4
15568	SRZ	Nama-Karoo	Augrabies Falls NP		44.8	16.2	3.2	-19.5	8.4
15569	SRZ	Nama-Karoo	Augrabies Falls NP		43.7	15.9	3.2	-19.2	8.9
15570	SRZ	Nama-Karoo	Augrabies Falls NP		44.0	16.1	3.2	-20.6	7.9
15571	SRZ	Nama-Karoo	Augrabies Falls NP		44.5	16.2	3.2	-21.1	7.8
15572	SRZ	Nama-Karoo	Augrabies Falls NP		43.1	15.7	3.2	-18.6	9.7
15573	SRZ	Nama-Karoo	Augrabies Falls NP		43.3	15.8	3.2	-18.8	9.0
15574	SRZ	Nama-Karoo	Augrabies Falls NP		44.5	16.0	3.2	-18.1	8.2
15575	SRZ	Nama-Karoo	Augrabies Falls NP		43.4	15.6	3.2	-17.1	8.6
15576	SRZ	Nama-Karoo	Augrabies Falls NP		44.9	16.0	3.3	-19.5	8.9
15578	SRZ	Nama-Karoo	Augrabies Falls NP		41.3	14.7	3.3	-19.4	7.8
15579	SRZ	Nama-Karoo	Augrabies Falls NP		44.8	16.0	3.3	-21.4	9.8
15580	SRZ	Nama-Karoo	Augrabies Falls NP		45.1	16.4	3.2	-18.6	10.3
15581	SRZ	Nama-Karoo	Augrabies Falls NP		41.8	14.9	3.3	-18.2	12.7
15977	YRZ	Albany Thicket	Addo Elephant NP		42.6	15.4	3.2	-16.8	14.4
15978	YRZ	Albany Thicket	Addo Elephant NP		42.1	15.3	3.2	-18.8	14.8

Table 5.1 (continued)

UCT #	Rainfall zone	Biome	Locality	Collagen yield (%)	%C	%N	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
15979	YRZ	Albany Thicket	Addo Elephant NP		42.8	15.4	3.2	-18.8	15.0
15980	YRZ	Albany Thicket	Addo Elephant NP		42.2	15.4	3.2	-17.7	14.8
15982	YRZ	Albany Thicket	Addo Elephant NP		43.2	15.7	3.2	-18.5	12.0
15983	YRZ	Albany Thicket	Addo Elephant NP		42.6	15.5	3.2	-17.6	14.5
15984	YRZ	Albany Thicket	Addo Elephant NP		43.2	15.6	3.2	-18.6	13.7
15985	YRZ	Albany Thicket	Addo Elephant NP		42.9	14.8	3.4	-17.6	14.6
15986	YRZ	Albany Thicket	Addo Elephant NP		43.0	15.4	3.3	-16.8	14.7
15987	YRZ	Albany Thicket	Addo Elephant NP		41.0	14.9	3.2	-18.1	16.1
15988	YRZ	Albany Thicket	Addo Elephant NP		42.8	15.5	3.2	-18.2	14.7
15989	YRZ	Albany Thicket	Addo Elephant NP		42.3	15.2	3.2	-18.1	14.2
15990	YRZ	Albany Thicket	Addo Elephant NP		42.6	15.5	3.2	-19.1	15.0
15991	YRZ	Albany Thicket	Addo Elephant NP		42.9	15.5	3.2	-18.8	14.5
15992	YRZ	Albany Thicket	Addo Elephant NP		37.3	13.3	3.3	-18.0	15.3
15993	YRZ	Albany Thicket	Addo Elephant NP		41.9	14.8	3.3	-18.5	14.4
15994	YRZ	Albany Thicket	Addo Elephant NP		42.6	15.4	3.2	-16.8	13.5
15995	YRZ	Albany Thicket	Addo Elephant NP		42.7	15.5	3.2	-19.8	15.1
15996	YRZ	Albany Thicket	Addo Elephant NP		42.7	15.4	3.2	-18.3	12.1

5.2 - Stable isotope ratios

The $\delta^{13}C$ and $\delta^{15}N$ values for all samples analysed are presented in Table 5.1 and plotted against each other in Figure 5.1 ($n = 210$). The correlation coefficient (R^2 value) for this bivariate plot is 0.01, indicating that no correlation between the regressed variables exists.

The $\delta^{13}C$ values range from -24.0‰ to -14.8‰, with a mean of -20.3 ± 1.9‰. The majority of samples lie within the expected range for browser bone collagen (~-24.5‰ to -15‰), and only one individual falls within the expected grazer range (-15‰ and higher). These are empirically observed ranges for many species of confirmed browsers and grazers collected across a number of habitats in East Africa, including savannah and montane forests (Ambrose & DeNiro 1986). The $\delta^{15}N$ values of samples analysed for this thesis are highly variable, ranging from 1.2‰ to 19.5‰, with a mean of 10 ± 3.6‰. These values span the reported ranges for both obligate and non-obligate drinkers summarised in Ambrose & DeNiro (1986).

5.2.1 - Carbon

Although many individuals fall within the expected $\delta^{13}C$ range for browsers or C_3 feeders, this could be skewed by the large number of individuals collected in the WRZ, where both browse and graze is C_3. To circumvent this bias, samples were grouped according to the rainfall seasonality of their collection locality (Figure 5.2). The distributions of $\delta^{13}C$ values of samples from both the SRZ and YRZ are statistically significantly different from those from the WRZ ($P < 0.001$) but not from each another ($P = 0.111$) (Table 5.2).[1] Samples from the WRZ have $\delta^{13}C$ values that range from -24‰ to -16.1‰ (mean $\delta^{13}C = -21.4 \pm 1.1‰$). They therefore lie closest to the expected range of bone collagen $\delta^{13}C$ values for C_3 consumers. The range of $\delta^{13}C$ values for the YRZ is smaller than that of the WRZ (from -22.1‰ to -16.8‰). This is likely due to the smaller number of samples. The mean $\delta^{13}C$ value of

---

[1] Calculated using the Mann-Whitney test for equal medians.

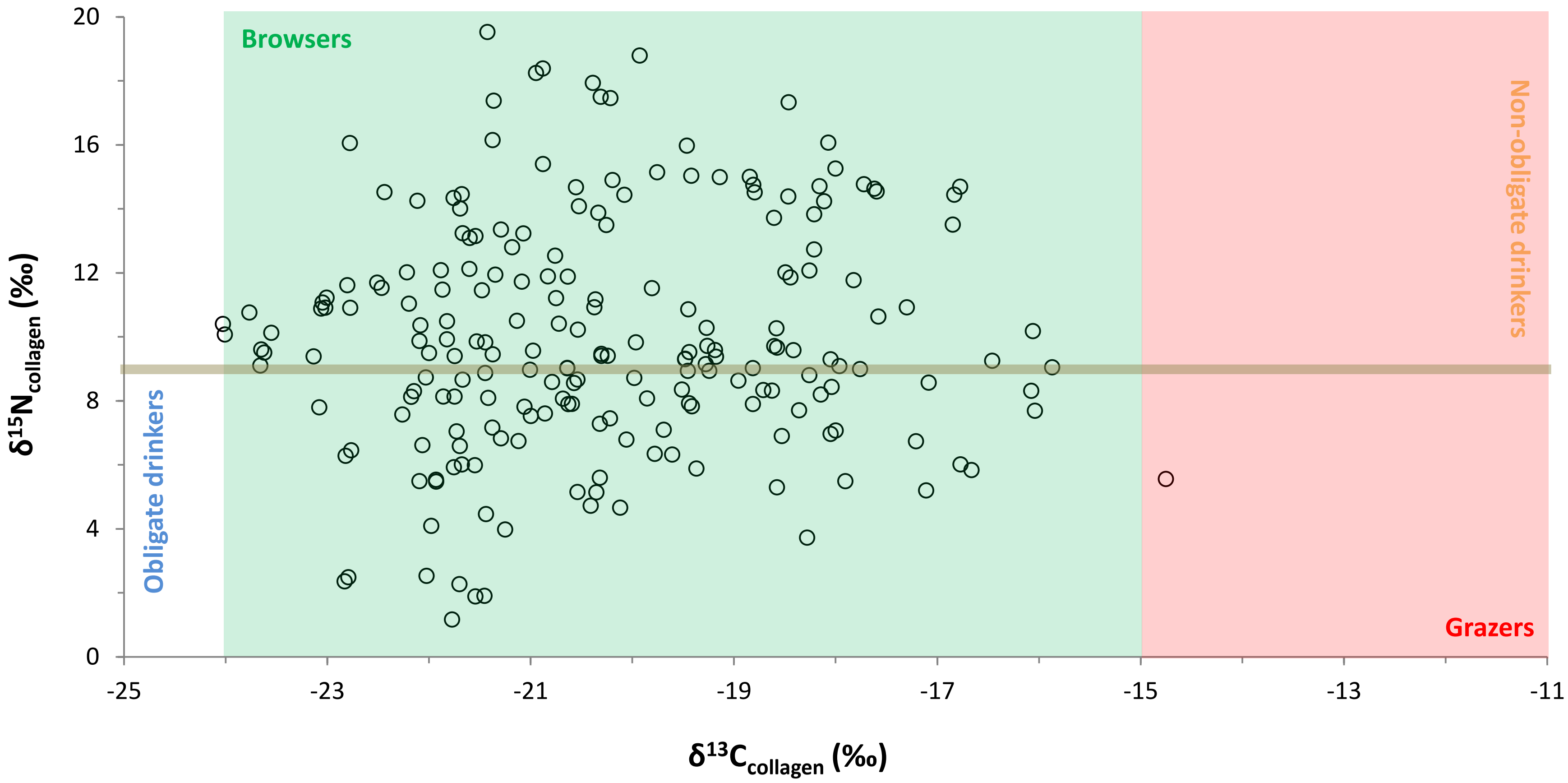


Figure 5.1 – Bivariate plot of bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for all samples. Expected ranges of $\delta^{13}C$ values for browsers and grazers are indicated by the green and red shaded areas, respectively, and the shaded brown line separates the reported $\delta^{15}N$ values of obligate drinkers from non-obligate drinkers (Ambrose & DeNiro 1986).

-19.1 ±1.4‰ is more positive than that of the WRZ ($P < 0.001$), reflecting the availability and consumption of some C_4 grass. SRZ $\delta^{13}C$ values range from -23.1‰ to -14.8‰. This is similar to the range for the WRZ. The mean $\delta^{13}C$ value of -19.8 ±1‰ for SRZ samples is slightly more negative than that of the YRZ, although this is not statistically significant ($P = 0.111$). The majority of samples lie closer to the expected range of bone collagen $\delta^{13}C$ values for browsers than for grazers.

As seen in Figures 5.2 and 5.3, many individuals are within the range of expected bone collagen $\delta^{13}C$ values for browsers across all rainfall seasonality zones and all biomes except Albany Thicket, where the distribution of $\delta^{13}C$ values is significantly more positive relative to all other biomes ($P \leq 0.001$) except the Savannah ($P = 0.173$). There are no individuals within the range of expected bone collagen $\delta^{13}C$ values for grazers. Overall, the majority of rock hyraxes display a strong preference for browse vegetation, with grasses and succulents forming a secondary contribution to their diets.

Relationships between $\delta^{13}C$ values and the two MAP data sets are explored using bivariate plots in Figure 5.4. The strength of these relationships was assessed using trend lines, for which the R^2 values were 0.01 for both MAP1 and MAP2. The lack of correlation between $\delta^{13}C$ and both MAP data sets indicates that rock hyraxes prefer browse vegetation regardless of the amount of annual rainfall; more negative bone collagen $\delta^{13}C$ values are not constrained to arid localities where grass is less abundant during droughts.

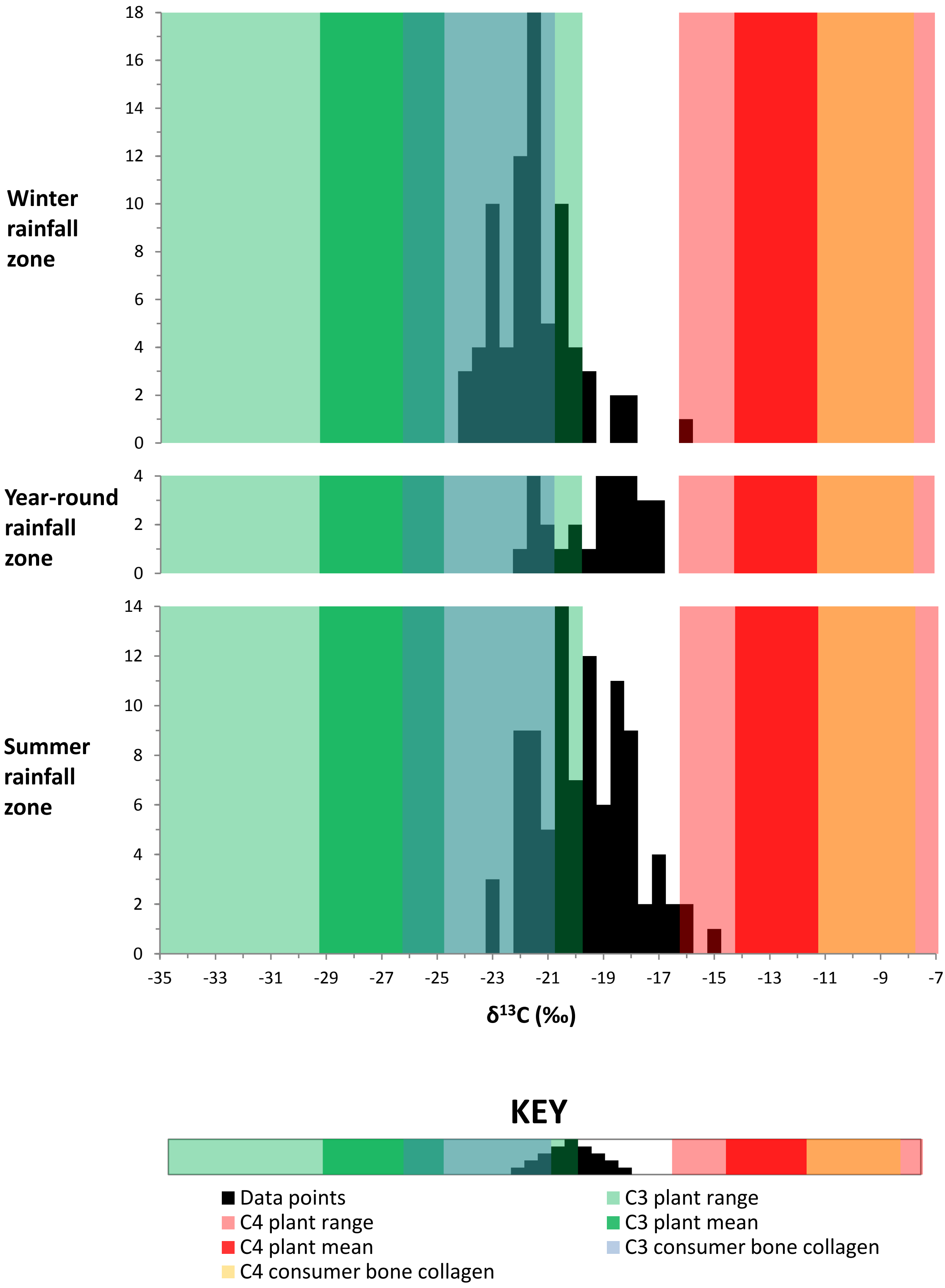


Figure 5.2 - Bone collagen δ^{13}C values (this study) presented as frequency plots by rainfall zone. For plants, ranges and mean δ^{13}C values ± two standard deviations for C_3 plants and ±1 standard deviation for C_4 plants are indicated (cf. Ambrose & DeNiro 1986; Cerling *et al.* 1997; Cerling & Harris 1999; Kelly 1999), and the expected mean bone collagen δ^{13}C values derived from a diet to collagen spacing of 3-4‰ for C_3 and C_4 consumers are shown (cf. DeNiro & Epstein 1978; Ambrose & Norr 1993; Caut *et al.* 2009).

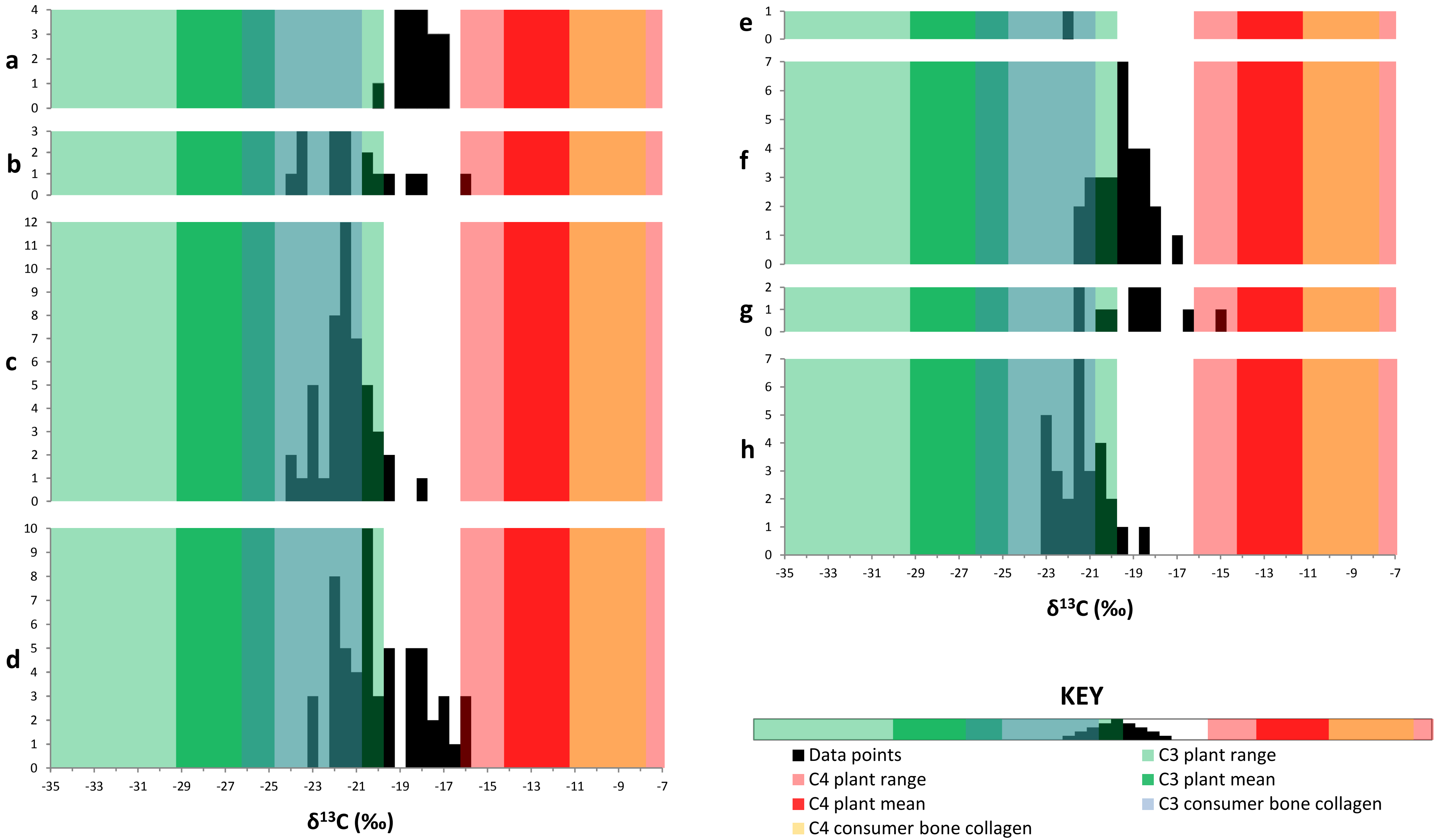


Figure 5.3 - Bone collagen δ^{13}C values presented as frequency plots for vegetation biome sub-samples. **(a)** Albany Thicket, **(b)** Desert, **(c)** Fynbos, **(d)** Grassland, **(e)** Indian Ocean coastal belt, **(f)** Nama-Karoo, **(g)** Savannah h, **(h)** Succulent Karoo. C_3 and C_4 plant ranges, plant means and standard deviations, and derived consumer bone collagen means determined as in Figure 5.2.

Table 5.2 - Summarised $\delta^{13}C$ statistics and Mann-Whitney test P value matrix for rainfall seasonality and vegetation biome sub-samples. The Indian Ocean coastal belt was excluded since there is only a single observation.

*P values of <0.05, indicating that the distribution of $\delta^{13}C$ values is significantly different.

Summary statistics	**Winter rainfall**	**Year-round rainfall**	**Summer rainfall**	**Albany Thicket**	**Desert**	**Fynbos**	**Grassland**	**Nama-Karoo**	**Savannah**	**Succulent Karoo**
N	84	29	97	19	17	47	57	29	12	28
Mean $\delta^{13}C$ (‰)	-21.4	-19.1	-19.6	-18.2	-21.1	-21.5	-19.8	-19.6	-18.8	-21.4
Median $\delta^{13}C$ (‰)	-19.5	-18.8	-21.4	-18.2	-21.7	-21.6	-20.3	-19.5	-18.7	-21.4
Std. deviation (‰)	1.1	1.4	1.0	0.8	2.1	1.2	1.9	1.0	1.8	1.2
$\delta^{13}C$ Range (‰)	7.9	5.3	8.3	3.0	7.7	6.2	7.2	1.6	6.8	4.6

Mann-Whitney test P values	**Winter rainfall**	**Year-round rainfall**	**Summer rainfall**	**Albany Thicket**	**Desert**	**Fynbos**	**Grassland**	**Nama-Karoo**	**Savannah**	**Succulent Karoo**
Year-round rainfall	<0.001*									
Summer rainfall	<0.001*	0.111								
Desert				<0.001*						
Fynbos				<0.001*	0.808					
Grassland				0.001*	0.033*	<0.001*				
Nama-Karoo				<0.001*	0.004*	<0.001*	0.261			
Savannah				0.173	0.006*	<0.001*	0.121	0.173		
Succulent Karoo				<0.001*	0.963	0.657	0.001*	<0.001*	<0.001*	

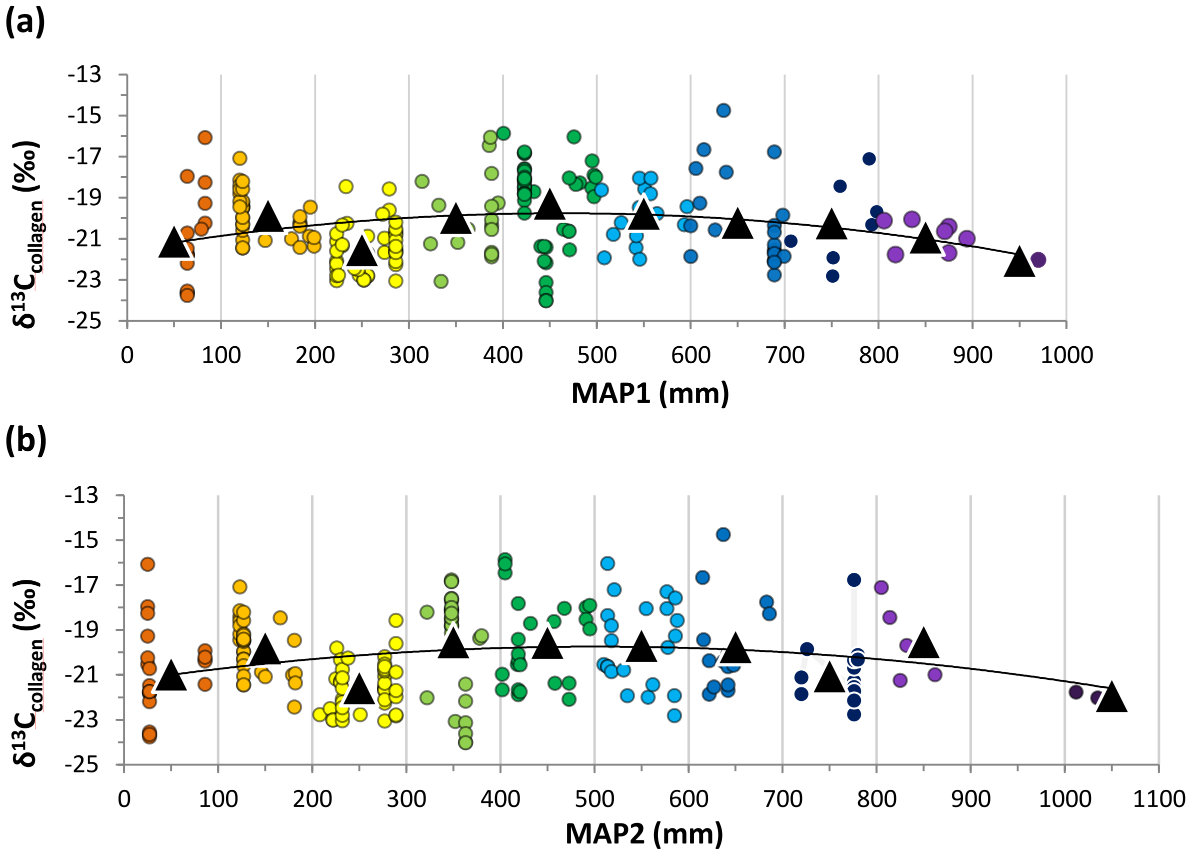


Figure 5.4 – Bivariate plots comparing the δ^{13}C values of individuals with (a) MAP1 and (b) MAP2 values for collection localities. Colours separate samples into 100 mm rainfall bins. Sample means for each rainfall bin are indicated by the black triangles. Non-parametric local regression (LOESS) trend lines were used to assess the relationship between the variables for all data points. The correlation coefficient rounded to 2 decimal places is 0.01 for both plots.

5.2.2 - Nitrogen

δ^{15}N values were first examined by locality to investigate intra-locality variability. A number of localities had five or more observations, and half of these had δ^{15}N ranges above 4‰. Figure 5.5 is a map of the distribution of mean δ^{15}N by locality, divided into the following categories: very depleted (1-5‰), fairly depleted (5.1-7‰), moderate (7.1-11‰), fairly enriched (11.1-15‰), and very enriched (15.1-20‰). The labelled localities have δ^{15}N ranges above 4‰ and five or more observations. Very enriched values occur in the Western (low rainfall) parts of the country, while very depleted values are more widely distributed, a pattern which is described in greater detail below. Bar graphs are used to indicate the number of individuals in each of the $\delta^{15}N$ categories for these localities separately (Figure 5.6). The largest range in $\delta^{15}N$ at a single locality is ~16.1‰ at the West Coast Fossil Park, although this results from a single very enriched individual. This individual's (UCT 15468) C:N ratio is 3.2 and its %C and %N are 41.6 and 15, respectively (Table 5.1). These figures are almost identical to those of other samples collected from the same colony, indicating that there is no evidence to suggest that its unusually high $\delta^{15}N$ value is the result of issues relating to preservation or errors in sample preparation. Augrabies Falls National Park has a $\delta^{15}N$ range of ~10.8‰. The majority of samples collected have intermediate $\delta^{15}N$ values, although there is one sample that is very depleted and one that is very enriched. The range of $\delta^{15}N$ values at the Namaqua National Park is ~8.4‰, where individuals have moderate to very enriched $\delta^{15}N$ values. $\delta^{15}N$ values of samples collected at Mertenhof have a range of ~4.9‰. Most individuals are in the fairly enriched category, and one is in the very enriched category. The Colchester section of the Addo Elephant National Park has a $\delta^{15}N$ range of ~4.1‰. Most individuals are very enriched, and a few are fairly enriched. The ranges at these localities indicate that $\delta^{15}N$ values are highly variable both within and between localities. Intra-locality variability is in one case as high as 16.1‰, indicating that individuals subjected to identical environmental conditions can have very different bone collagen $\delta^{15}N$ values.

Since large ranges were observed at single localities, sample $\delta^{15}N$ values were next compared to environmental data. The strongest relationship found was between $\delta^{15}N$ and MAP ($R^2 = 0.37$ for MAP1, and 0.45 for MAP2), and a weaker relationship was found between $\delta^{15}N$ and mean annual temperature (MAT) ($R^2 = 0.25$). Because of the stronger relationship, the MAP data were extensively analysed in an attempt to quantify the degree to which they influence bone collagen $\delta^{15}N$ values.

Patterns of $\delta^{15}N$ variability across precipitation increments are presented as bivariate plots in Figure 5.7. All results of the regression analysis are statistically significant at the 95%

confidence interval. $\delta^{15}N$ values are on average slightly more positive in individuals living in xeric areas relative to those living in mesic areas. Nevertheless, the range of $\delta^{15}N$ values in the 100 mm precipitation bins in both data sets indicates that MAP is not the sole determinant of $\delta^{15}N$ values in rock hyraxes. Overlap in $\delta^{15}N$ values across MAP bins shows that individuals with very low $\delta^{15}N$ values (1-5‰) occur in areas with MAP of ~120 to 1000 mm. Very high $\delta^{15}N$ values (15.1-20‰), however, are restricted to localities with <500 mm of annual precipitation. This suggests that higher $\delta^{15}N$ can be associated with relatively arid localities, but lower $\delta^{15}N$ is not necessarily an indicator of relatively wet environments.

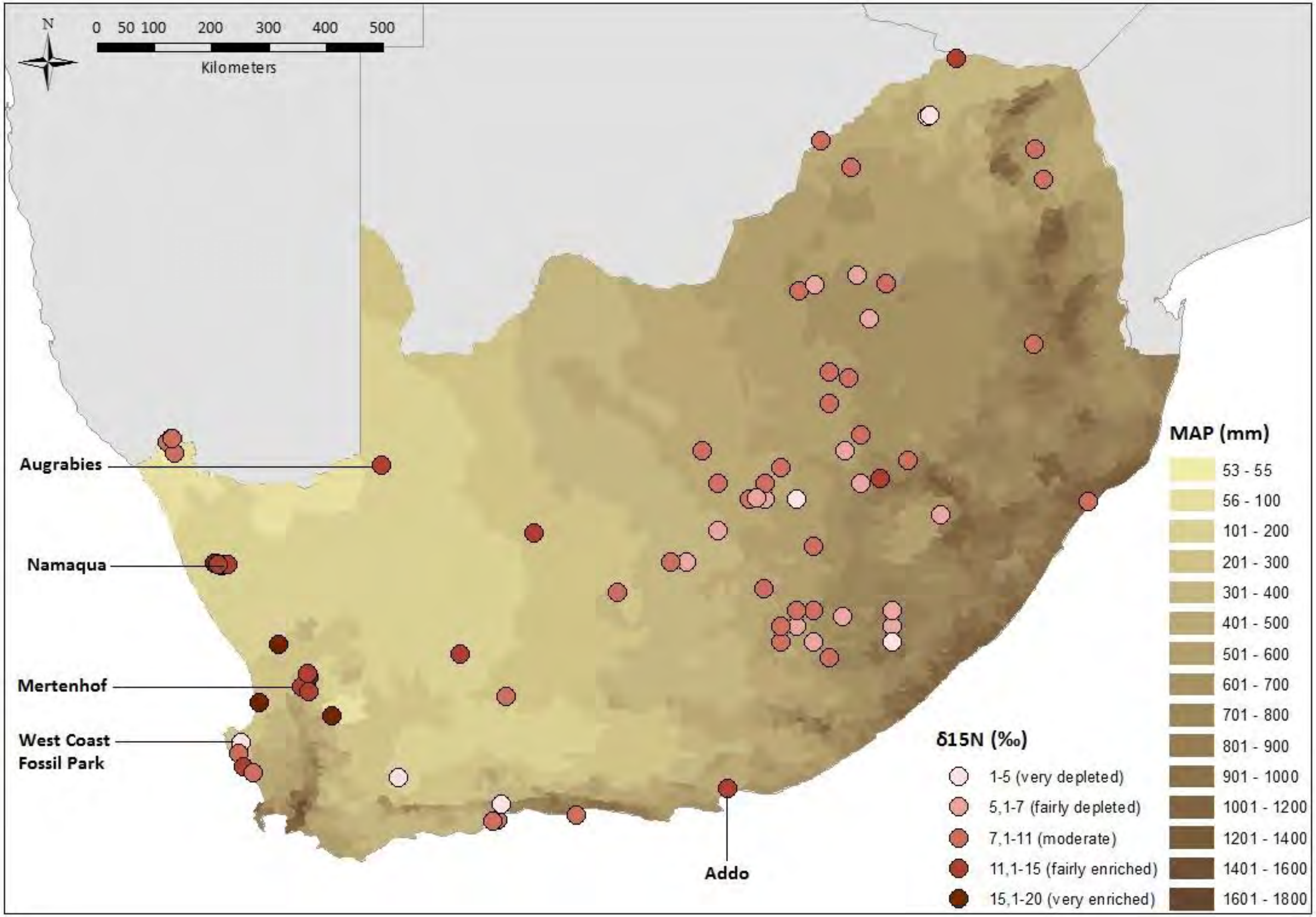


Figure 5.5 - Categorical $\delta^{15}N$ values superimposed on the MAP1 base map. For localities with multiple individuals, the mean $\delta^{15}N$ value is used to assign the whole group to a $\delta^{15}N$ category. Localities where $n \geq 5$, and the range of $\delta^{15}N$ values exceeds 4‰ are indicated using black lines.

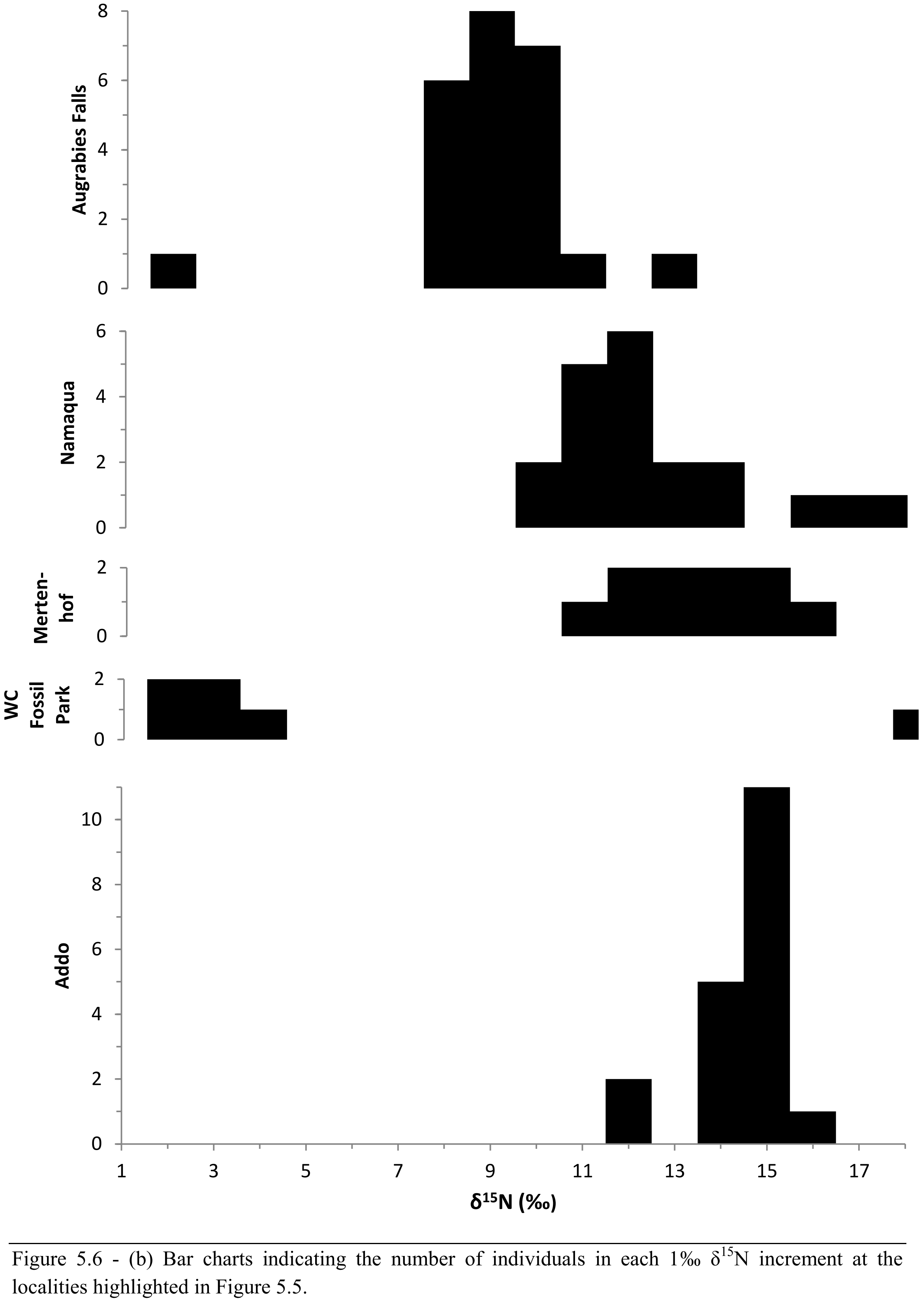


Figure 5.6 - (b) Bar charts indicating the number of individuals in each 1‰ $\delta^{15}N$ increment at the localities highlighted in Figure 5.5.

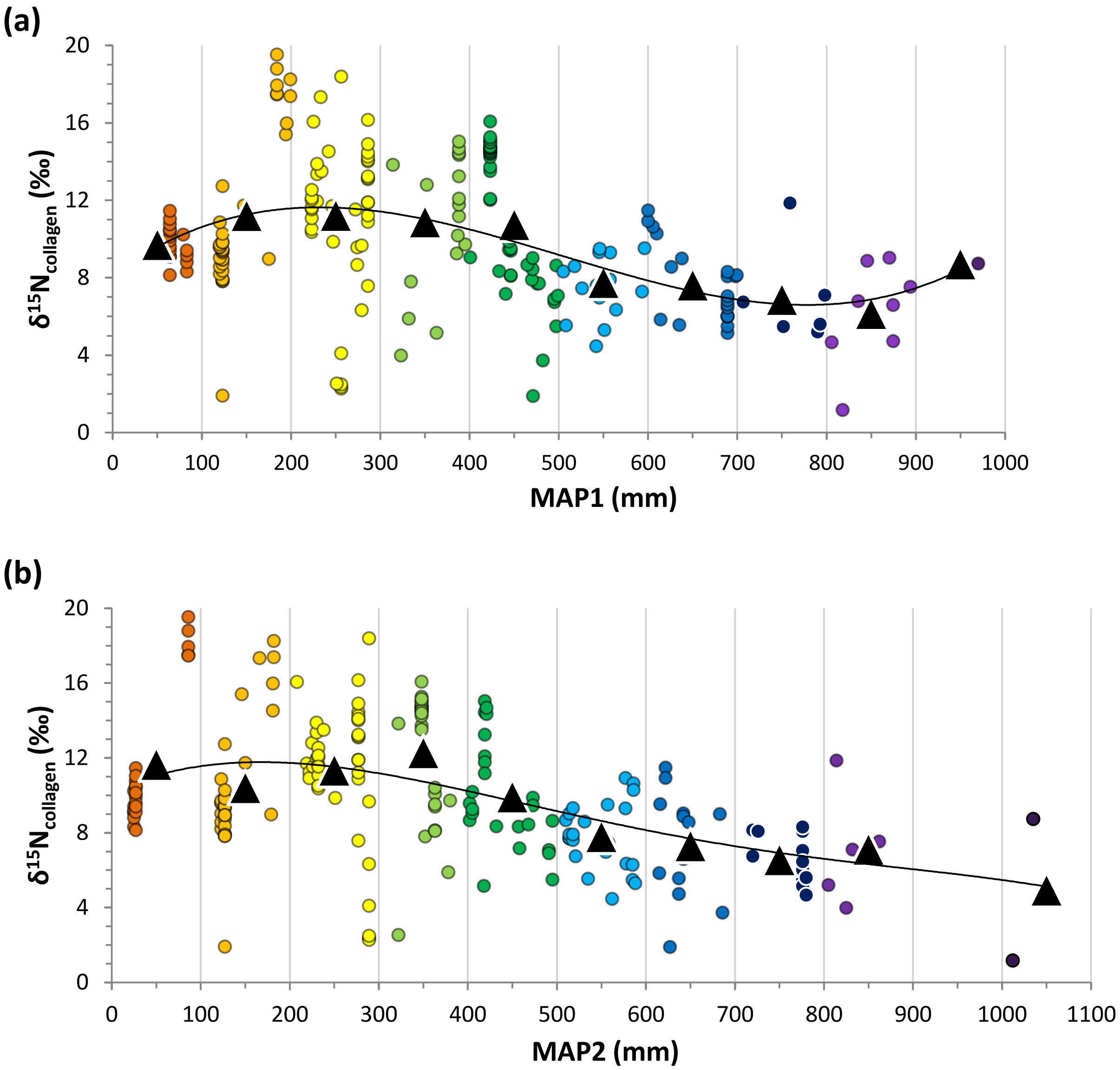


Figure 5.7 –Bivariate plots comparing the δ^{15}N values of all individuals to their corresponding MAP values. Colours separate samples into 100 mm rainfall bins. Mean sample δ^{15}N values for each rainfall bin are indicated by the black triangles. Non-parametric local regression (LOESS) trend lines were used to assess the relationship between the variables for all data points. The R^2 value for (a) MAP1 rounded to 2 decimal places is 0.37. The R^2 value for (b) MAP2 rounded to 2 decimal places is 0.45.

Rainfall values were binned at 100 mm and 200 mm for both MAP1 and MAP2. As shown in Figure 5.8, the majority of samples in the dataset were collected in localities with MAP <500 mm. Arid areas have a low locality to sample ratio relative to mesic localities, where low numbers of observations at localities result in higher locality to observation ratios.

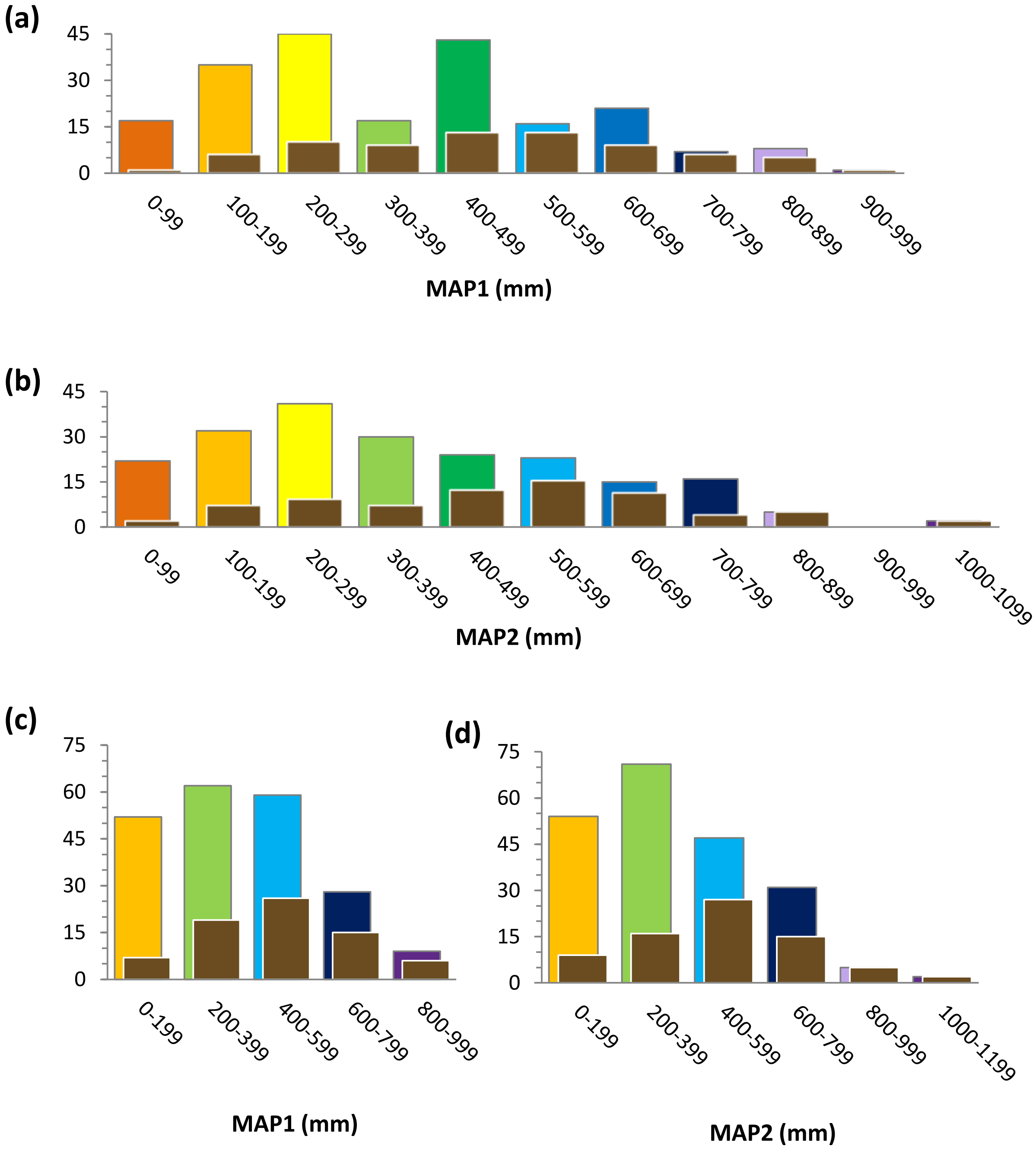


Figure 5.8 - Frequency histograms for observations binned into 100 mm and 200 mm MAP increments. The coloured bars represent the number of individuals collected, and the brown bars show the number of localities sampled. (a) Frequency of observations binned at 100mm MAP1 increments. (b) Frequency of observations binned at 100mm MAP2 increments. (c) Frequency of observations binned at 200mm MAP2 increments. (d) Frequency of observations binned at 200mm MAP2 increments.

The range of δ^{15}N values for each 100 mm MAP increment is presented as a box and whisker diagram in Figure 5.9. Ranges are highest for increments with MAP of 500 mm or less, although this may be due to variations in sample sizes. There is a high degree of intra-bin variability, as well as inter-bin overlap, in $\delta^{15}N$ values. This is particularly salient in arid localities. Enriched values occur most frequently in arid areas, with the majority of arid-zone samples exhibiting moderate to enriched values, and a few exhibiting very depleted values. Depleted values occur in regions where MAP ranges from 100-199 mm to >1000 mm.

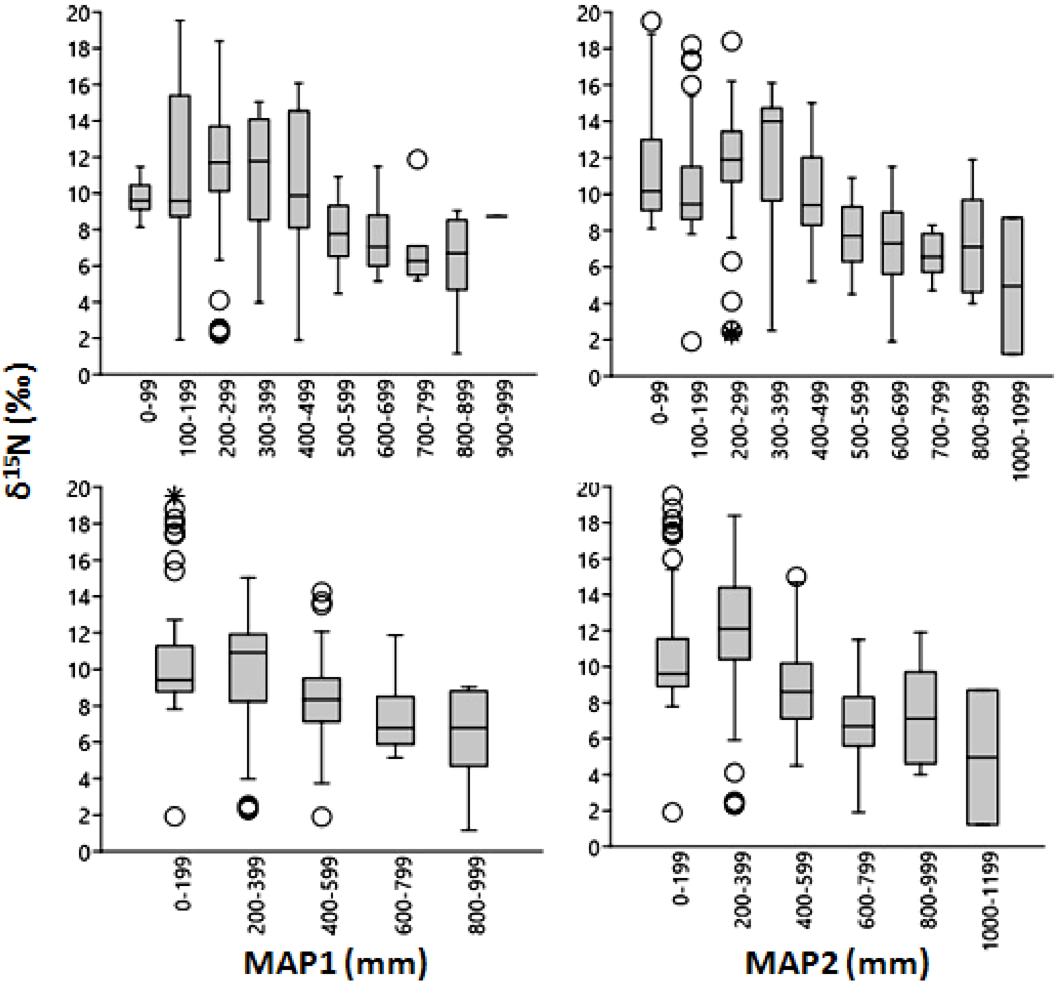


Figure 5.9 - Box and whisker plots of $\delta^{15}N$ values illustrating intra- and inter-gradient variability in $\delta^{15}N$ values binned at 100 mm and 200 mm MAP1 and MAP2 increments. Outliers for each box plot are indicated with open circles, and extreme outliers with asterisks.

A non-parametric regression model was attempted to explore the variability in $\delta^{15}N$ values across rainfall gradients. This model was problematic as it did not adequately characterise the relationships between $\delta^{15}N$ and MAP without the use of complex third order polynomial regressions or splines. Results using the polynomial regressions and splines were not interpretable, and the model was abandoned. A linear regression model was therefore used to estimate the degree to which δ^{15}N values differ between rainfall increments. Such models assume that errors are independent and normally distributed, as well as homoscedastic (having equal variance). They also ignore any clustering of values due to multiple observations at certain localities versus single observations at others.

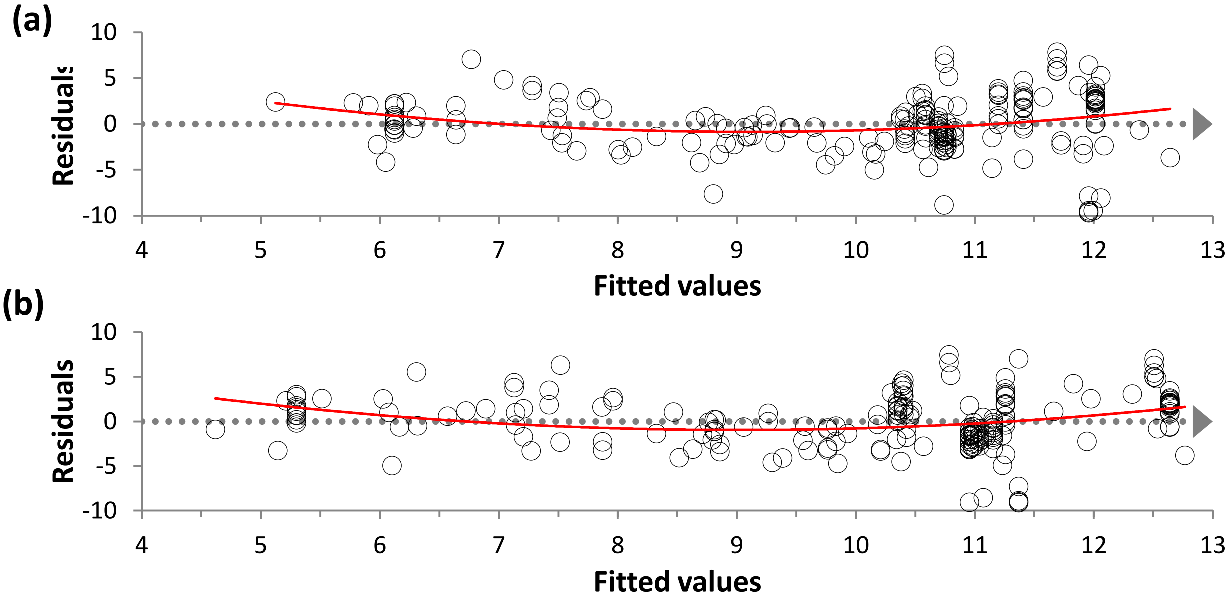


Figure 5.10 - Bivariate plots showing the relationship between the residuals and the fitted values for (a) MAP1 and (b) MAP2 model data.

Residual values (the difference between each actual *y* value and the value predicted by the line of best fit) are used to estimate the errors. To check the fit of the model for the data, it was necessary to check whether the residuals are normally distributed and homoscedastic. To check for homoscedasticity, the residuals were regressed with the fitted values (the predicted *y* values on the regression line). The bivariate plots generated (Figure 5.10) show that the errors are not fully homoscedastic (i.e. they are not randomly clustered around zero). The slight u-shaped pattern seen shows that the higher and lower fitted values are more variable than those in the middle; an indication that not all variables are accounted for by the model.

This further supports earlier suggestions that MAP is not the only determinant of the $\delta^{15}N$ values.

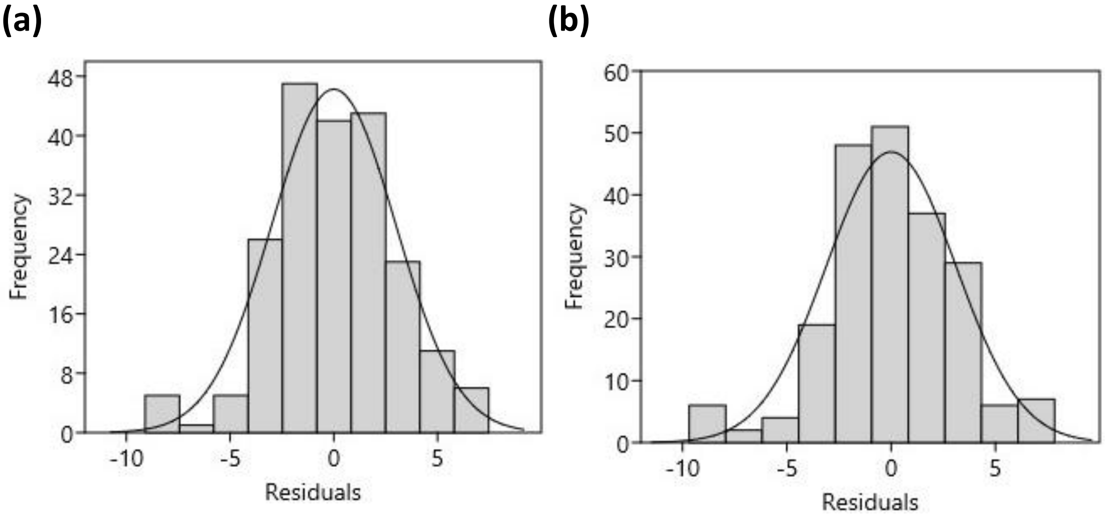


Figure 5.11 - Histograms estimating the normality of the distribution of residuals for (a) MAP1 and (b) MAP2 model data. Although there is a degree of skewness, the distribution of residual values is not so erratic that normality can be ruled out.

Histograms were used to check the normality of the residuals in Figure 5.11. Although the histograms show some variability in the frequency of certain residual categories, the distribution is sufficiently symmetrical that normality may be assumed. The results of the have been interpreted as indicating that for every 100 mm increase in MAP using either MAP1 or MAP2, there is a 1‰ decrease in $\delta^{15}N$. The decrease is statistically significant at the 95% confidence interval, with a standard error of 0.00, and a P value of <0.001 (Table 5.3).

To further quantify the decrease in $\delta^{15}N$ with increasing MAP, the data was binned at 100 mm rainfall increments so that the change could be estimated for each rainfall bin. The $\delta^{15}N$ shift for each bin was measured relative to the most arid rainfall increment (0-99 mm) (hereafter referred to as the reference category). The results for the binned data are presented in Table 5.3. Since there are so few observations in bins with >800 mm MAP, these categories were combined. Using data from MAP1, it was found that the first rainfall increment (100-199 mm) showed a positive shift of 0.76‰ in $\delta^{15}N$ relative to the reference category. This result was, however, not statistically significant at the 95% confidence interval ($P = 0.460$). All other rainfall increments for MAP1 and MAP2 except two showed negative shifts in $\delta^{15}N$ values with increasing MAP relative to both the reference category and their preceding rainfall bin, although not all of the decreases were statistically significant. The first exception is between the 500-599 mm and 600-699 mm bins for MAP1, where there is a positive shift in $\delta^{15}N$ of 0.19‰ in the more mesic bin. This result is statistically significant ($P < 0.001$). The second exception is between the 100-199 mm and 200-299 mm bins for MAP2, where there is a positive $\delta^{15}N$ shift of 0.3‰ in the more mesic bin. This result is, however, not significantly different from the reference category ($P = 0.066$).

As seen in Table 5.3, $\delta^{15}N$ decreases more sharply between the bins with the highest MAP (>500 mm) relative to the more arid bins (<500 mm). This could be due to the smaller numbers of samples from these bins, potentially indicating that variability at mesic localities has not yet been sufficiently resolved.

Table 5.3 - Results of the $\delta^{15}N$ model using data binned at 100 mm MAP increments. Shifts in $\delta^{15}N$ relative to the reference category are shown in the third (MAP1) and fourth (MAP2) columns, with lower and upper estimates indicated in parentheses.

MAP	**Shift in $\delta^{15}N$ (‰)**		**Standard error**		**P value**	
(mm)	**MAP1**	**MAP2**	**MAP1**	**MAP2**	**MAP1**	**MAP2**
100-199	0.76 (-1.27, 2.79)	-1.91 (-3.66, -0.16)	1.03	0.89	0.460	0.033*
200-299	-0.30 (-2.35, 1.75)	-1.61 (-3.33, 0.11)	1.04	0.87	0.773	0.066
300-399	-0.78 (-3.46, 1.90)	-2.53 (-5.12, 0.06)	1.36	1.31	0.567	0.055
400-499	-1.06 (-4.16, 2.05)	-3.59 (-5.81, -1.36)	1.57	1.13	0.502	0.002*
500-599	-2.29 (-5.69, 1.10)	-5.92 (-8.63, -3.20)	1.72	1.38	0.184	<0.001*
600-699	-2.10 (-5.37, 1.18)	-6.34 (-9.12, -3.56)	1.66	1.41	0.208	<0.001*
700-799	-4.04 (-8.19, 0.10)	-6.47 (-9.30, -3.65)	2.10	1.43	0.056	<0.001*
>800	-6.23 (-10.08, -2.37)	-8.02 (-11.45, -4.59)	1.95	1.74	0.002*	<0.001*

*Estimates that have P values of <0.05, indicating that $\delta^{15}N$ values are statistically significantly different from those in the reference category.

5.3 - Summary

The $\delta^{13}C$ values of rock hyraxes analysed in this study are closer to the expected bone collagen values for browsers than grazers. There are small differences in the distribution of $\delta^{13}C$ values between different rainfall seasonality zones and vegetation biomes, some of which are statistically significant, but there is no perceptible distinction in the distribution of $\delta^{13}C$ values across South African MAP increments. Overall, mostly C_3 vegetation was consumed by the majority of individuals collected from all localities, including those with abundant C_4 grass.

The $\delta^{15}N$ values presented were more robustly correlated with MAP than other environmental variables investigated here. Exploring this correlation revealed that the delta values decrease with increasing MAP on average, although xeric localities (less than 400 mm MAP) span the total sample $\delta^{15}N$ range and exhibit high degrees of variability relative to mesic ones. Mean $\delta^{15}N$ values in some cases increase slightly from the aridmost MAP increments up to 400 mm, after which they decrease systematically for each additional 100 mm of MAP.

Chapter Six

Discussion and Conclusion

6.1 - Quantifying rock hyrax dietary preference: carbon mixing models

The $\delta^{13}C$ values presented in Chapter Five reflect preferential browsing in South African rock hyraxes. Their degree of browse preference was explored further with the use of the linear mixing model, as described in Chapter Two. The mixing diagram is presented in Figure 6.1.

Considering the requirements for using mixing models to their best potential (i.e. that vegetation end points and diet to tissue discrimination should be known) it is recognised that the fact that only animals were sampled in this study is a limiting factor. The C_3 consumer end point was set at the most depleted $\delta^{13}C_{collagen}$ value for the dataset (an individual from the WRZ with a $\delta^{13}C$ value of -24‰). The global $\delta^{13}C$ mean for C_3 plants is ~-27‰, and rock hyrax $\delta^{13}C_{diet}$ to $\delta^{13}C_{collagen}$ discrimination is around +3-4‰, consistent with a bone collagen end point of -24‰ for a pure C_3 consumer. In fact, such an end point will, if anything, underestimate the C_3 contribution, since global C_3 plant means include very negative $\delta^{13}C$ values from ground-level in closed canopy forests. Since no data presented here are from such environments, the C_3 plant mean $\delta^{13}C$ for the environments sampled in this study is likely more positive than -27‰. The C_4 consumer end point is predicted to be 10‰, i.e. 14‰ more positive than the C_3 endpoint, following the approximate 14‰ spacing between C_3 and C_4 vegetation (after Cerling & Harris 1999).

Using the end points outlined above, mixing equations were used to quantify the relative contribution of browse and graze to overall diet for the whole sample, and then for the WRZ, YRZ, and SRZ separately. Results show that C_3 contribution to diet is 73.4% ± 13.3% for the sample as a whole, with a total of 200 individuals consuming more than 50% C_3, and 10 individuals consuming more than 50% non-C_3 plants. The amount of C_3 consumed is greatest among WRZ samples, with 81.4% C_3 and 18.6% non-C_3 consumed on average. Individuals from the YRZ consume averages of 64.7% C_3 and 35.3% non-C_3. For individuals collected from localities in the SRZ, where C_4 grass is readily available, the average C_3 contribution to diet is 69.1% and the C_4 or non-C_3 contribution is 31.9%.

The results of the mixing model are superimposed on rainfall seasonality, vegetation biome, and MAP base maps in Figures 6.2 and 6.3. The contribution of C_3 vegetation to rock hyrax diet was divided into categories, with higher percentage C_3 categories reflecting the largest degree of C_3 plant consumption. Figure 6.2 shows that individuals consuming the highest percentages of C_3 vegetation on average (≥76%) occur in all three rainfall seasonality zones and all vegetation biomes except Albany Thicket, where the C_3 dietary component ranges from 48% to 69%.

Although rock hyraxes from the WRZ are characterised by consistently high C_3 consumption (≥76% at most localities), those from three localities in the Succulent Karoo biome (Namaqua National Park, Knersvlakte, and Aspoort) are within the 61% to 75% C_3 dietary contribution category. The ^{13}C enrichment exhibited by these individuals relative to those from other WRZ localities likely reflects CAM consumption, since CAM plants are relatively abundant in the Succulent Karoo. Hyraxes from two localities in the Richtersveld National Park have the lowest mean C_3 dietary contributions for the WRZ (between 51-60%). The Richtersveld is situated in the Desert biome on the boundary between the WRZ and the ecotonal YRZ, suggesting that the more positive $\delta^{13}C$ values are due to increased availability and consumption of C_4 grass during certain periods.

On average, rock hyraxes from the YRZ consume over 76% C_3 vegetation at all but two localities. The first locality is Cape Saint Blaize on the South coast near Mossel Bay, where the dietary contribution of C_3 plants was 66%. This is likely due to opportunistic summer grazing of C_4 grasses. The second locality is in the Colchester section of the Addo Elephant National Park in the Albany thicket biome, where mostly CAM plant consumption was observed (la Grange, pers. obs. 2014). This is likely the cause of their lower mean C_3 dietary contribution, which is in the 51% to 60% category.

Rock hyraxes from the SRZ exhibit the most variable C_3 dietary contributions relative to the other seasonal rainfall zones. However, most localities show preferential C_3 consumption despite the abundance of C_4 grass. There are only four localities where individuals consumed less than 50% C_3 vegetation on average. Three are in the grassland biome (Wolwespruit, Basberg, and Magaliesberg) and one is in the savannah biome (Brits Rooikoppies). Most of the other localities in these biomes are characterised by C_3 consumption of 61% to 75%, many are characterised by C_3 consumption of 76% and above, and a handful consume between 51% and 60% C_3 vegetation.

Comparison between the percentages of C_3 vegetation consumed and MAP increments (Figure 6.3) shows that high mean C_3 contribution to diet is observed across all MAP gradients, and high mean C_4 dietary contribution is not limited to mesic localities. Individuals consuming less than 50% C_3 are found in localities with MAP values ranging from ~100-800 mm, and those consuming more than 50% C_3 are found in all rainfall increments sampled. Individuals incorporating 76% or more C_3 plants occur from the most arid localities in the north-west to the most mesic localities on the south and east coasts. Intakes of over 60% C_3

plants are observed in individuals across a wide range of MAP increments (~54 mm – 1000 mm). Lower %C_3 (<50%) is observed in individuals from localities with moderate to high MAP values. This indicates that neither preferential browsing nor preferential grazing is determined by the amount of annual rainfall.

The high degree of browsing relative to grazing observed in this study is consistent with the observations of Lensing (1983) and Fourie (1983), who found that rock hyraxes in Namibia and the Karoo consume mostly dicotyledon species. These results, however, contrast with the majority of previous studies of rock hyrax diet, conducted in east Africa, which concluded that rock hyraxes are preferential grazers (Hoeck 1975; DeNiro & Epstein 1978a). It was also stated that the degree of grazing was greater during high rainfall periods (Walker *et al.* 1978; Fourie 1983; Lensing 1983; Hoeck 1989; Zimman 2008). Since bone collagen provides an averaged isotopic signal, the data presented here do not refute preferential grazing during the growing season. Seasonal preference may be a factor, but since $\delta^{13}C$ values show no relationship to MAP (Fig. 5.4), this may not be attributable the need to consume C_3 plants with better access to groundwater during droughts, as was suggested elsewhere (Chase *et al.* 2009, 2012). Furthermore, even in the SRZ, hyraxes analysed in this study do not show sufficient ^{13}C enrichment to indicate preferential grazing on average.

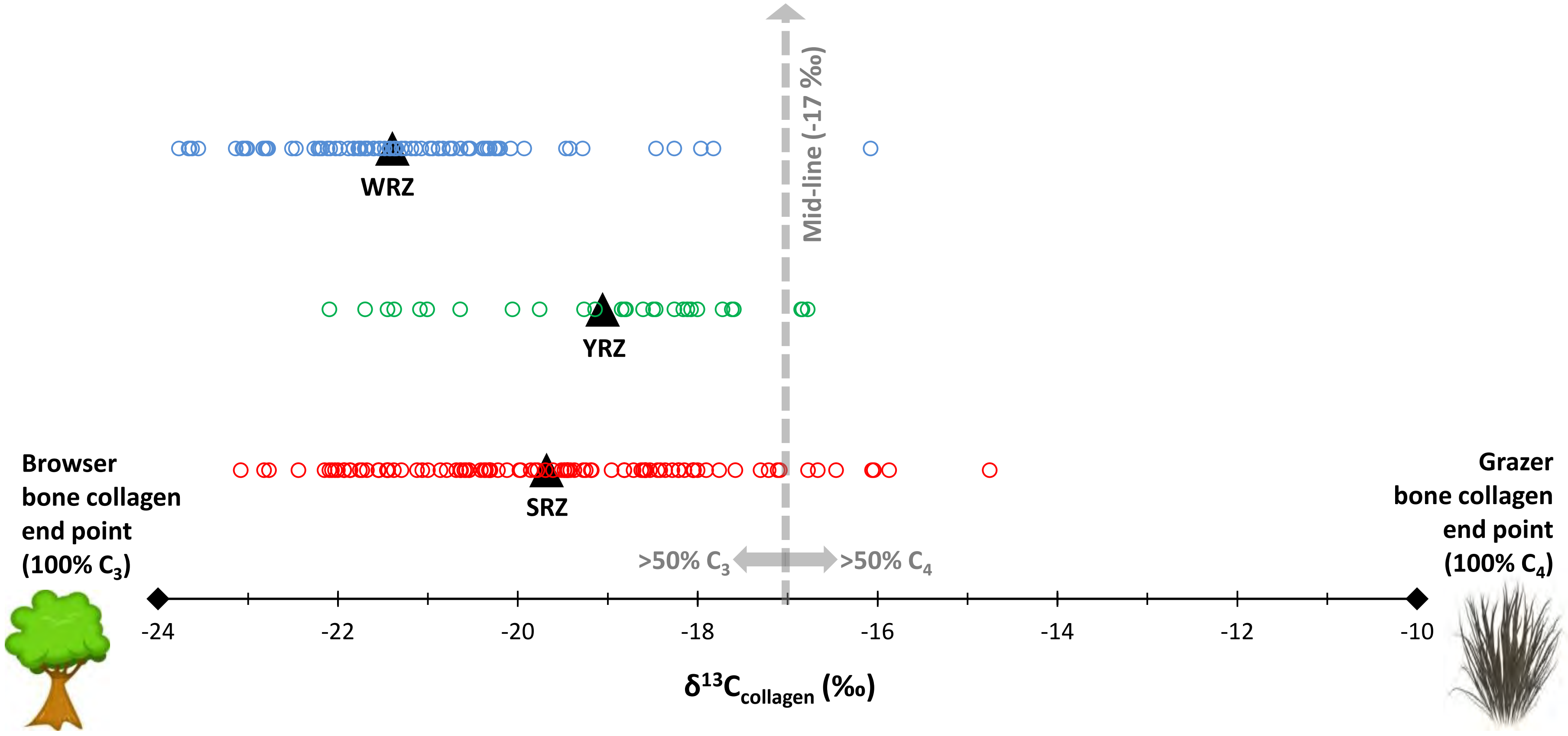


Figure 6.1 – Carbon mixing diagram showing the $\delta^{13}C$ values of all samples relative to browser (100% C_3) and grazer (100% C_4) end points. The black triangles show the mean bone collagen $\delta^{13}C$ values for each rainfall zone. Individuals that lie between the C_3 consumer end point and the dashed grey mid-line indicated at the $\delta^{13}C$ value of -17‰ consumed more than 50% C_3 vegetation, while those to the right hand side of the line consumed more than 50% C_4 vegetation.

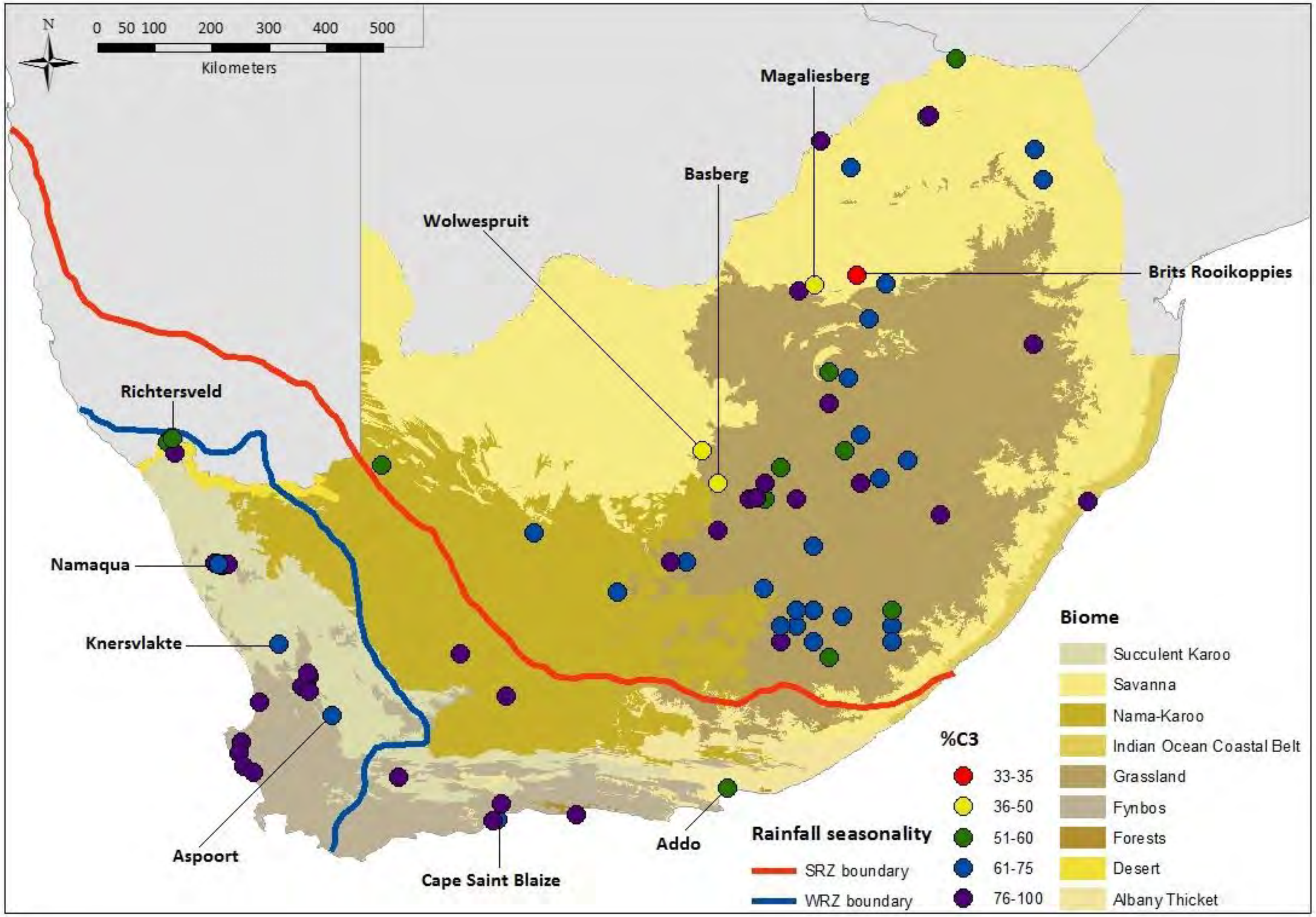


Figure 6.2 –Map showing the distribution of categorical mean %C_3 in hyrax diets across the South African vegetation biomes and rainfall seasonality zones.

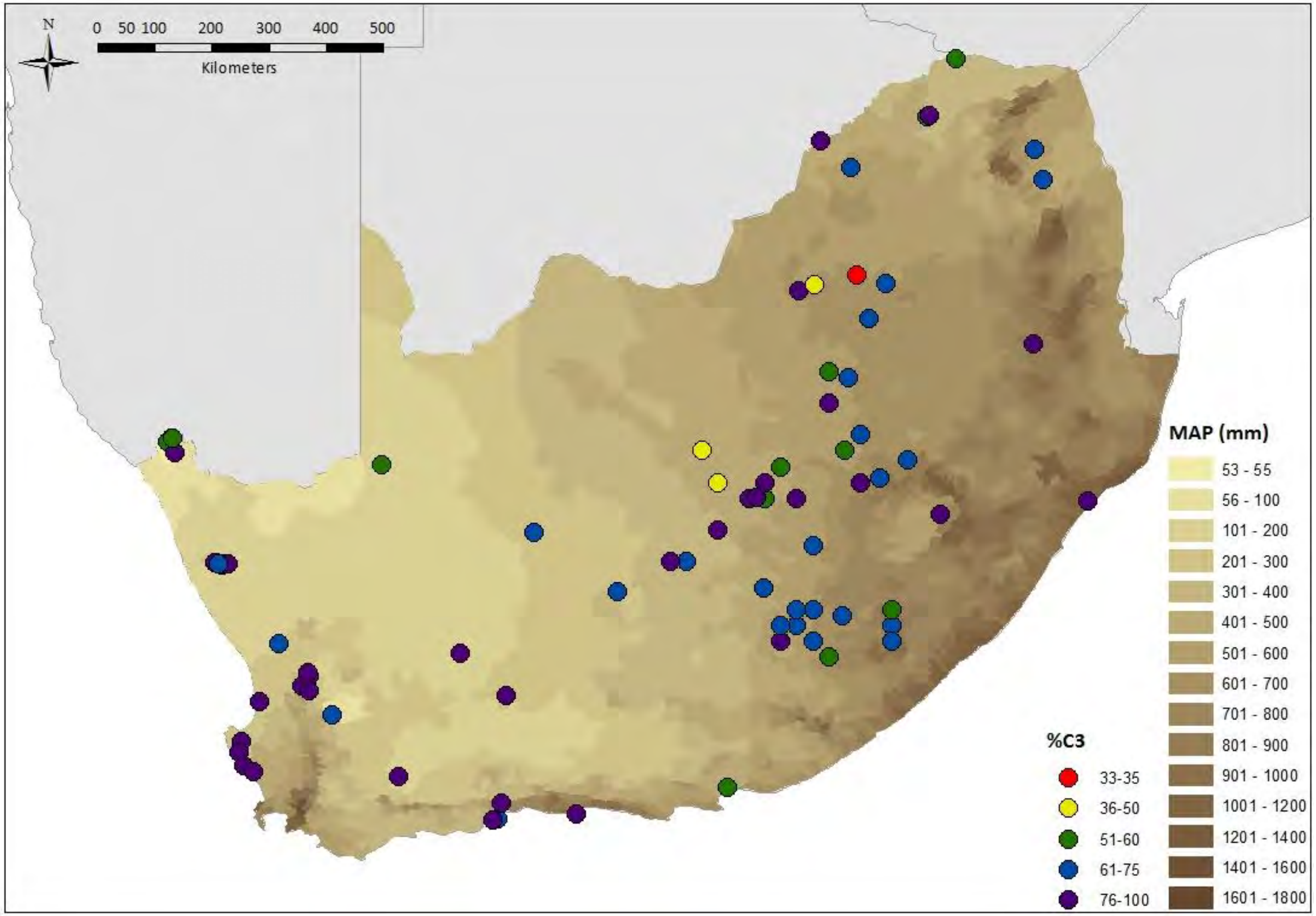


Figure 6.3 – Map showing the distribution of categorical mean %C_3 in hyrax diets across rainfall increments (MAP1).

6.2 - Investigating the determinants of bone collagen $\delta^{15}N$

The first notable feature of the $\delta^{15}N$ results presented in Chapter Five is that the data exhibit a large range of values both within and between localities. Inter-locality variability is unsurprising since samples were collected from diverse environmental contexts. Intra-locality variability spans 87.9% of the range of variation in $\delta^{15}N$ values for the whole sample, indicating that samples collected from the same geographic and temporal context are likely influenced by more than just climate. A proportion of the high degree of nitrogen isotope scatter found in this study could be attributable to the temporally-limited sampling strategy employed. Given that the majority of individuals are from arid or semi-arid environments which experience a large degree of inter-annual variability in the amount of MAP, the scatter is not entirely unexpected, especially when one considers that rock hyraxes may be short-lived relative to inter-decadal fluctuations in climate.

It is clear that MAP has an effect on bone collagen $\delta^{15}N$, although this relationship is somewhat less robust than expected, given that previous studies of mammalian bone collagen $\delta^{15}N$ and MAP have reported R^2 values between 0.53 and 0.98 (Gröcke *et al*. 1997; Murphy & Bowman 2006; Pate & Anson 2008), while R^2 values for this dataset range from 0.37 to 0.45. The lower R^2 values found here could indicate that physiology plays a role in determining hyrax $\delta^{15}N$ values. At this stage, it remains unclear whether varying degrees of fractionation due to urea recycling are contributing to the high degree of variation seen in bone collagen $\delta^{15}N$, and/or whether diet to tissue discrimination against ^{15}N might be highly variable in South African rock hyraxes. The fact that rock hyraxes in this study mostly browse also suggests that additional sources of foliar $\delta^{15}N$ variability, such as tree root depth (Högberg 1997; Evans & Ehleringer 1993), may also influence bone collagen $\delta^{15}N$ values.

Studies of South African plants have demonstrated that C_3 plant $\delta^{15}N$ and MAP are correlated (R^2=0.54) (Swap *et al.* 2004). In C_4 plants, however, the relationship is significantly less robust. Since hyraxes consume mostly C_3 plants, the expectation is that there should be a constant offset between plant and bone collagen $\delta^{15}N$ if plant $\delta^{15}N$ were the primary determinant of tissue $\delta^{15}N$. The relationship between rock hyrax bone collagen $\delta^{15}N$ and MAP is somewhat different to the relationship between South African C_3 plants and MAP, indicating that nitrogen isotopic diet to tissue discrimination may not be constant in rock hyraxes, and that the degree of fractionation and its physiological mechanisms could be variable and poorly understood. The fact that rock hyrax $\delta^{15}N$ is highly variable both at single localities and between localities experiencing the same amount of MAP, and the overlap in $\delta^{15}N$ values between localities with very different MAP, suggests that urea recycling as a response to water stress may not have a predictable or constant influence on tissue $\delta^{15}N$ either.

Previous research on the relationship between bone collagen $\delta^{15}N$ and MAP has found that $\delta^{15}N$ values are higher at arid localities relative to mesic ones . The data presented here show that very high $\delta^{15}N$ values are restricted to localities with less than 500 mm of MAP, but very low $\delta^{15}N$ values were measured in individuals from both mesic and xeric environments. The trend is that $\delta^{15}N$ values decrease very little for each 100 mm increase in MAP, and in some cases become more positive, up to 400 mm, and decrease systematically for each 100 mm increase in MAP thereafter. This contrasts with the findings of Heaton *et al.* (1986), Gröcke *et al.* (1997), Murphy & Bowman (2006), and Pate & Anson (2008), who report robust relationships between lower $\delta^{15}N$ values and increasing MAP at localities experiencing less than 500 mm of MAP, with markedly less steep decreases between more mesic environments. The number of observations for the more mesic localities (500 mm MAP or more) in this study is three times lower than the number of observations for xeric localities. Hence, a possible explanation for the discrepancies between the data presented here and previous research is that variability at mesic localities in this study may not yet be sufficiently resolved.

6.3 - Conclusions and suggestions for future research

Previous studies of *P. capensis* diet have utilised a variety of methods, including field observation, stable isotope analysis of body tissues and excreta, dental microwear analysis, stomach content examination, and midden and dung palynology. These studies were carried out on populations in east and southern Africa. Most results indicated that *P. capensis* is a preferential grazer. The current study presents the first comprehensive quantitative analysis of *P. capensis* feeding preference using a direct dietary proxy for a large number of individuals sampled from a range of environmental contexts.

Several studies conducted in South Africa and Australia have demonstrated robust relationships between plant and animal tissue $\delta^{15}N$ and aridity (e.g. Heaton *et al.* 1986, 1987; Gröcke *et al*. 1997). It was initially suggested that drought tolerant herbivore physiology leads to higher $\delta^{15}N$ values when animals are water stressed, but other research has indicated that higher plant $\delta^{15}N$ values in arid areas are responsible for much, if not all, of the ^{15}N enrichment of animals in these regions (e.g. Murphy & Bowman 2006). Namibian and South African *P. capensis* middens have been analysed, and relationships between midden $\delta^{15}N$ and aridity demonstrated (Chase 2010; Chase *et al.* 2011, 2012, 2015). To further investigate this, this study analysed bone collagen $\delta^{15}N$ values for a large sample of *P. capensis* individuals collected from localities that experience vastly different amounts of annual precipitation.

The $\delta^{13}C$ values for individuals collected from an array of environmental contexts presented here indicate that South African rock hyraxes are preferential browsers. This contrasts with the findings of DeNiro & Epstein (1978a), Walker *et al.* (1978), Hoeck (1989), and Zimman (2008), who report preferential grass consumption by rock hyraxes in east Africa. DeNiro & Epstein (1978a) report $\delta^{13}C$ values for hyrax bone collagen ranging from -16‰ to -12.8‰. Since only four individuals were sampled, it is likely that their dataset did not adequately characterise dietary variability. Observational data from Walker *et al.* (1978), Hoeck (1989), and Zimman (2008) reflect up to 78% C_4 grass consumption. The mixing model employed in this study shows that no individuals consumed over 34% C_4 grass, and that only 10.3% of individuals in areas with C_4 grass preferred grazing to browsing.

It is therefore clear that South African rock hyraxes rely on C_4 grass to a lesser extent than east African ones. One explanation for the discrepancies between this study and the east African studies is that observational data can be subjective - rock hyraxes foraging among grass can appear to be grazing when they are actually selecting dicotyledonous shoots between grasses. Secondly, since bone collagen gives an average life time signal, more negative $\delta^{13}C$ values do not refute seasonal grazing preference. In order to systematically investigate this, less long lived tissues should be sampled during different seasons. Since hyrax incisors grow continuously, resulting in the deposition of new enamel at the cementum-enamel junction, incisors can be serially sampled to determine whether there is variability in $\delta^{13}C$ along the growth axis. If variability exists, the $\delta^{13}C$ value of the most recently formed enamel can be taken as representative of the season of death. Hair samples could be taken from live individuals during different seasons to see if these also reflect this variability. Dental microwear could also be used to discern whether individuals graze or browse during certain seasons, provided that season of death is known.

It would be useful to sample C_3 and C_4 plants at collection localities in the SRZ so that vegetation end points and their isotopic distance from one another can be more precisely determined for each environment sampled. This will help to get a better idea of whether diet to tissue fractionation in rock hyraxes is constant along the C_3 to C_4 continuum, allowing for the more precise interpretation of mixing models.

The correlation between rock hyrax bone collagen $\delta^{15}N$ and MAP indicates an estimated 1‰ decrease in $\delta^{15}N$ for every 100 mm increase in MAP. This is similar to the findings of Heaton *et al.* (1987), who report decreases of 1.1‰ to 1.3‰ for every 100 mm increase in MAP in South Africa, and to Gröcke *et al.* (1997) who report decreases of 0.8‰ to 1.15‰ for Australian macropods. The R^2 values for MAP versus $\delta^{15}N$ in the dataset presented in this study are, however, less robust than those previously reported, hinting at additional physiological factors that may affect $\delta^{15}N$. To determine whether the relationship between bone collagen $\delta^{15}N$ and MAP is influenced by rock hyrax physiology, the $\delta^{15}N$ of hyrax diets needs to be characterized by sampling plants at collection localities and comparing their nitrogen isotope values with those of hyrax bone collagen.

One explanation for the relatively limited correlation between rock hyrax bone collagen $\delta^{15}N$ and MAP may be that MAP is not the best measure of aridity. A myriad of other factors, such as soil composition, number of rain days, temperature, and annual variability in the amount of rainfall could influence soil moisture. For example, hard, clay-like soils are more likely to absorb rain slowly, leaving pools of water on the surface, particularly during long rainfall spells. Looser sediments, on the other hand, would absorb rain very quickly, reducing the amount of surface water available. It is also likely that soils in mesic environments with high temperatures will experience a greater degree of moisture loss through evaporation than those with lower temperatures. This could affect the relative openness of the soil nitrogen cycle, thereby influencing the δ^{15}N values of plants and their consumers. It would be useful to consider these factors in future analyses of rock hyrax δ^{15}N values, since their combined influence on soil moisture may account for a greater proportion of variation in δ^{15}N across environmental gradients.

Studies of South African rock hyrax middens have emphasised relationships between higher δ^{15}N values of hyraceum-rich accretions and increased aridity (Chase *et al*. 2009, 2010, 2011a, b, 2012, 2013; 2015; Meadows *et al.* 2010). In many cases, these records have shown peaks and troughs in midden δ^{15}N that are concurrent with environmental shifts recorded in well-recognised palaeo-aridity proxies. These shifts are usually between ~1 and 6‰. The range of δ^{15}N values observed at single localities in this study shows that rock hyraxes display a more variable response to identical climatic conditions, and that δ^{15}N shifts of between 4‰ and 16‰ could be attributable to natural variation in their tissues and excreta.

Discrepancies between the range of δ^{15}N values presented here and those reported in the studies of rock hyraxes middens could also be due to the nature of midden data, where individual samples potentially average the isotope values of many individuals over several decades, resulting in a smoothed record which could obscure the impact of the degree of inter-individual variation observed in this study.

Sponheimer *et al.* (2003b) demonstrated that while faeces, like body tissues, have higher δ^{15}N values relative to diet, urine typically has lower δ^{15}N values. If rock hyrax physiology were partly responsible for their elevated δ^{15}N values, the effect of urea recycling on nitrogen efflux would result in high body tissue δ^{15}N and low hyraceum δ^{15}N during arid periods. Since the majority of stable isotope analysis of middens has been carried out on hyraceum-rich accretions, it is surprising that δ^{15}N peaks appear to be so well correlated with aridity, although this could be due to the mixing of faeces and urine in middens. The relative proportions of hyraceum and faeces in rock hyrax middens therefore needs to be quantified in order to better understand the relationship between midden $\delta^{15}N$ and MAP. It would also be useful to determine the diet to hyraceum and diet to dung discrimination for hyraxes specifically. Additionally, sampling rock hyrax faeces during different seasons could provide an indication of short-term variations in dietary choice and the carbon and nitrogen isotopic composition of food sources since they reflect the dietary signal of only a few days (Codron *et al.* 2005).

This study highlights the fact that variation in the diets of African mammals in different environments warrants further investigation before firm environmental retrodictions can be made. It has been demonstrated here and elsewhere (Sponheimer *et al.* 2003d) that South African herbivores are less dependent on C_4 grass than their east African counterparts. The browse preference of South African herbivores therefore limits their utility for interpreting past shifts in rainfall seasonality. The relatively low correlation between MAP and bone collagen $\delta^{15}N$ values found here shows that studies quantifying additional determinants of tissue $\delta^{15}N$, such as metabolic effects and climatic variables other than MAP, are fundamental future research avenues.

References

Ambrose, S. H. & Norr, L. 1993. Experimental Evidence for the Relationship of the Carbon Isotope Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. In: Lambert, J.B. & Grupe, G. (eds) *Prehistoric Human Bone:* 1-37. New York: Springer.

Ambrose, S.H. & DeNiro, M.J. 1986. The Isotopic Ecology of East African Mammals. *Oecologia* 69 (3): 395-406.

Ambrose, S.H. & DeNiro, M.J. 1987. Bone Nitrogen Isotope Composition and Climate. *Nature* 325: 201.

Ambrose, S.H. & Sikes, N.E. 1991. Soil Carbon Isotopic Evidence for Holocene Habitat Change in the Kenya Rift Valley. *Science* 253: 1402-1405.

Ambrose, S.H. 1990. Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. *Journal of Archaeological Science* 17: 431-451.

Ambrose, S.H. 1991. Effects of Diet, Climate and Physiology on Nitrogen Isotope Abundances in Terrestrial Foodwebs. *Journal of Archaeological Science* 18: 293-317.

Amundson, R., Austin, A.T., Schuur, E.A.G., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., Brenner, D. & Baisden, W.T. 2003. Global Patterns of the Isotopic Composition of Soil and Plant Nitrogen. *Global Biogeochemical Cycles* 17(1): 1-10.

Aroch, I., King, R. & Baneth, G. 2007. Hemetology and Serum Biochemistry Values of Trapped, Healthy, Free-living Rock Hyraxes *(Procavia capensis)* and Their Association with Age, Sex, and Gestational Status. *Veterinary Clinical Pathology* 36(1): 40-48.

Barry, R.E. & Barry, L.M. 1996. Species Composition and Age Structure of Remains of Hyraxes (Hyracoidea: Procaviidae) at Nests of Black Eagles. *Journal of Mammalogy* 77(3): 702-707.

Bloom, A.J. & Troughton, J.H. 1979. High Productivity and Photosynthetic Flexibility in a CAM Plant. *Oecologia* 38(1): 35-43.

Bocherens, H. 1997. Isotopic Biogeochemistry as a Marker of Neandertal Diet. *Anthropologischer Anzeiger* 55(2): 101-120.

Brown, K.A. & Brown T.A. 2013. Biomolecular Archaeology. *Annual Review of Anthropology* 42: 159-174.

Brown, K. & Downs, C.T. 2005. Seasonal Behavioural Patterns of Free-Living Rock Hyrax *(Procavia capensis)*. *Journal of Zoology* 265: 1-16.

Brown, K. & Downs, C.T. 2006. Seasonal Patterns in Body Temperature of Free-living rock Hyrax (*Procavia capensis)*. *Comparative Journal of Biochemistry and Physiology* 143: 42-49.

Brown, K.T. & Downs, C.T. 2007. Basking Behaviour in the Rock Hyrax *(Procavia capensis)* During Winter. *African Zoology* 42(1): 70-79.

Butynski, T., Hoeck, H., Koren, L. & de Jong, Y.A. 2015. *Procavia capensis*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Downloaded on 13 July 2015.

Caro, T.M. & Alawi, R.M. 1985. Comparative Aspects of Behavioural Development in Two Species of Free-Living Hyrax. *Behaviour* 95(1/2): 87-109.

Carr, A.S., Boom, A. & Chase, B.M. 2010. The Potential of Plant Biomarker Evidence Derived from Hyrax Middens as an Indicator of Palaeoenvironmental Change. *Palaeogeography, Palaeoclimatology, Palaeoecology* 285: 321-330.

Carrion, J.S., Scott, L. & Vogel, J.C. 1999. Twentieth Century Changes in Montane Vegetation in the Eastern Free State, South Africa, Derived from Palynology of Hyrax Dung Middens. *Journal of Quaternary Science* 14(1): 1-16.

Caut, S., Angulo, E. & Courchamp, F. 2009. Variation in Discrimination Factors Δ^{15}N and Δ^{13}C): the effect of isotope values and applications for diet reconstruction. *Journal of Applied Ecology* 46: 443-453.

Cerling, T.E., Harris, J.M. & Leakey, M.G. 1999. Browsing and Grazing Elephants: the isotope record of modern and fossil proboscideans. *Oecologia* 120: 364-374.

Cerling, T.E. & Harris, J.M. 1999. Carbon Isotope Fractionation Between Diet and Bioapatite in Ungulate Mammals and Implications for Ecological and Palaeoecological Studies. *Oecologia* 120: 347-363.

Cerling, T.E. & Viehl, K. 2004. Seasonal Diet Changes of the Forest Hog *(Hylochoerus meinertzhageni)* Based on the Carbon Isotopic Composition of Hair. *African Journal of Ecology* 42: 88-92.

Cerling, T.E., Harris, J.M. & Passey, B.H. 2003. Diet of East African Bovidae Based on Stable Isotope Analysis. *Journal of Mammalogy* 84(2): 456-470.

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V. & Ehleringer, J.R. 1997. Global Vegetation Change Through the Miocene/Pliocene Boundary. *Nature* 389: 153-158.

Chase, B.M. & Meadows, M.E. 2007. Late Quaternary Dynamics of Southern Africa's Winter Rainfall Zone. *Earth Science Reviews* 84: 103-138.

Chase, B.M. 2010. South African Palaeoenvironments During Marine Oxygen Isotope Stage 4: a context for the Howiesons Poort and Still Bay Industries. *Journal of Archaeological Science* 37: 1359-1366.

Chase, B.M., Boom, A., Carr, A.S., Carré, M., Chevalier, M., Meadows, M.E., Pedor, J.B., Stager, J.C. & Reimer, P.J. 2015. Evolving Southwest African Response to Abrupt Deglacial North Atlantic Climate Change Events. *Quaternary Science Reviews* 121: 132-136.

Chase, B.M., Boom, A., Carr, A.S., Meadows, M.E. & Reimer, P.J. 2013. Holocene Climate Change in Southernmost South Africa: Rock hyrax middens records shifts in the southern westerlies. *Quaternary Science Reviews* 82: 199-205.

Chase, B.M., Meadows, M.E., Carr, A.S. & Reimer, P.J. 2010. Evidence for Progressive Holocene Aridification in Southern Africa Recorded in Namibian Hyrax Middens: Implications for African Monsoon dynamics and the "African Humid Period". *Quaternary Research* 74: 36-45.

Chase, B.M., Meadows, M.E., Scott, L., Thomas, D.S.G., Marais, E., Sealy, J. & Reimer, P.J. 2009. A Record of Rapid Holocene Climate Change Preserved in Hyrax Middens from Southwestern Africa. *Geology* 37(8): 703-706.

Chase, B.M., Quick, L.J., Meadows, M.E., Scott, L., Thomas, D.S.G. & Reimer, P.J. 2011. Late Glacial Interhemispheric Climate Dynamics Revealed in South African Hyrax Middens. *Geology* 39(1): 19-22.

Chase, B.M., Scott, L., Meadows, M.E., Gil-Romera, G., Boom, A., Carr, A.S., Reimer, P.J., Truc, L., Valsecchi, V. & Quick, L.J. 2012. Rock Hyrax Middens: A palaeoenvironmental archive for southern African drylands. *Quaternary Science Reviews* 56: 107-125.

Chilcott, M.J., Moore, S.A. & Hume, I.D. 1985. Effects of Water Restriction on Nitrogen Metabolism and Urea Recycling in the Macropodid Marsupials *Macropus eugenii* (Tammar Wallaby) and *Thylogale thetis* (Red-Necked Pademelon). *Journal of Comparative Physiology B* 155(6): 759-767.

Chisholm, B.S., Nelson, D.E. & Schwarcz, H.P. Stable-Carbon Isotope Ratios as a Measure of Marine Versus Terrestrial Protein in Ancient Diets. *Science* 216: 1131-1132.

Codron, D., Sponheimer, M., Codron, J., Newton, I., Lanham, J. & Clauss, M. 2012. The Confounding Effects of Source Heterogeneity on Consumer-Diet and Tissue-Tissue Stable Isotope Relationships. *Oecologia* 169(4): 939-953.

Codron, D., Codron, J., Lee-Thorp, J.A., Sponheimer, M. & de Ruiter, D. 2005. Animal Diets in the Waterberg Based on Stable Isotopic Composition of Faeces. *South African Journal of Wildlife Research* 35(1): 43-52.

Codron, J., Codron, D., Lee-Thorp, J.A., Sponheimer, M., Bond, W.J., de Ruiter, D. & Grant, R. 2005. Taxonomic, Anatomical, and Spatio-Temporal Variations in the Stable Carbon and Nitrogen Isotopic Compositions of Plants from an African Savannah . *Journal of Archaeological Science* 32: 1752-1772.

Collatz, G.J., Berry, J.A. & Clark, J.S. 1998. Effects of Climate and Atmospheric CO_2 Partial Pressure on the Global Distribution of C_4 grasses: Present, Past, and Future. *Oecologia* 114: 441-454.

Cowling, R.M. 1983. The Occurrence of C_3 and C_4 Grasses in Fynbos and Allied Shrublands in the South Eastern Cape, South Africa. *Oecologia* 58: 121-127.

Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamente, M., Dawson, T.E., Hobbie, E.A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo., L.H., Peñuelas, J., Reich, P.B., Schuur, E.A.G., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M. & Wright, I.J. 2009. Global Patterns of Foliar Nitrogen Isotopes and Their Relationships with Climate, Mycorrhizal Fungi, Foliar Nutrient Concentrations, and Nitrogen Availability. *New Phytologist* 183: 980-992.

Cunningham, P.L. 1999. A Population of Rock Hyrax *Procavia capensis* on Jebel Hafit. *Tribulus* 9(2): 29.

Deines, P. 1980. The Isotopic Composition of Reduced Organic Carbon. In: Fritz, P. & Fontes, J-C. (eds) *Handbook of Environmental Isotope Geochemistry: The terrestrial environment* : 329-406. California: Elsevier.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H. & Tu, K.P. 2002. Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics* 33: 507-559.

DeNiro, M.J. & Epstein, S. 1978a. Carbon Isotopic Evidence for Different Feeding Patterns in Two Hyrax Species Occupying the Same Habitat. *Science* 201(4359): 906-908.

DeNiro, M.J. & Epstein, S. 1978b. Influence of Diet on the Distribution of Carbon Isotopes in Animals. *Geochimica et Cosmochimica Acta* 42: 495-506.

DeNiro, M.J. & Schoeninger, M.J. 1983. Stable Carbon and Nitrogen Isotope Ratios of Bone Collagen: Variations Within Individuals, Between Sexes, and Within Populations Raised on Monotonous Diets. *Journal of Archaeological Science* 10: 199-203.

Diefendorf, A.F., Mueller, K.E., Wing, S.L., Koch, P.L. & Freeman, K.H. 2010. Global Patterns in Leaf ^{13}C Discrimination and Implications for Studies of Past and Future Climate. *Proceedings of the National Academy of Sciences* 107(13): 5738-5743.

Druce, D.J., Brown, J.S., Castley, J.G., Kerley, G.I.H., Kotler, B.P., Slotow, R. & Knight, M.H. 2006. Scale-Dependent Foraging Costs: Habitat use by rock hyraxes *(Procavia capensis)* determined using giving-up densities. *Oikos* 115(3): 513-525.

Ehleringer, J.R. & Cerling, T.E. 2002. C_3 and C_4 Photosynthesis. In: Mun, T., Mooney, H.A. & Canadell, J.G. (eds) *Encyclopedia of Environmental Change Volume 2*: 186-190. Chichester: John Wiley & Sons.

Ehleringer, J.R. & Rundel, P.W. 1989. Stable Isotopes: History, units, and instrumentation. In: Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. *Stable Isotopes in Ecological Research*: 1-15. New York: Springer.

Ehleringer, J.R., Cerling, T.E. & Helliker, B.R. 1997. C_4 Photosynthesis, Atmospheric CO_2, and Climate. *Oecologia* 112: 285-299.

Eley, R.M. 1994. The Hyrax: a most mysterious mammal. *Biologist* 41(4): 141-144.

Evans, R.D. & Ehleringer, J.R. 1993. A Break in the Nitrogen Cycle in Aridlands? Evidence from $\delta^{15}N$ of Soils. *Oecologia* 94(3): 314-317.

Evans, R.D. 2001. Physiological Mechanisms Influencing Plant Nitrogen Isotope Composition. *Trends in Plant Science* 6(3): 121-126.

Evans, R.D. 2007. Soil Nitrogen Isotope Composition. In: Michener, R. & Lajtha, K. (eds) *Stable Isotopes in Ecology and Environmental Science*: 83-98. USA: Blackwell Publishing Ltd.

Fairall, N. 1980. Growth and Age Determination in the Hyrax *(Procavia capensis). South African Journal of Zoology* 15(1): 16-21.

Fairall, N. & Hanekom, N. 1987. Population Dynamics and Possible Management Options for the Rock Dassie *Procavia capensis* Population in the Tsitsikamma Coastal National Park. *Koedoe* 30(1): 139-148.

Farquhar, G.D., Ball, M.C., von Cae mmerer, S. & Roksandic, Z. 1982. Effect of Salinity and Humidity on $\delta^{13}C$ Value of Haplophytes - Evidence for Diffusional Isotope Fractionation Determined by the Ratio of Intercellular/Atmospheric Partial Pressure of CO_2 Under Different Conditions. *Oecologia* 52: 121-124.

Farquhar, G.D., Hubick, K.T., Gordon, A.G. & Richards, R.A. 1989. Carbon Isotope Fractionation and Plant Water-Use Efficiency. In: Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. *Stable Isotopes in Ecological Research*: 21-40. New York: Springer.

Foley, W.J. & Hume, I.D. 1987. Nitrogen Requirements and Urea Metabolism in Two Arboreal Marsupials, the Greater Glider *(Peroroides volans)* and the Brushtail Possum *(Trichosurus vulpecula)*, Fed Eucalyptus Foliage. *Physiological Zoology* 60(2): 241-250.

Fourie, L.J. 1983. The Popuation Dynamics of the Rock Hyrax *Procavia capensis* (Pallas, 1766) in the Mountain Zebra National Park. Unpublished PhD thesis, Rhodes University, South Africa.

Gannes, L.Z., del Rio, C.M. & Koch, P. 1998. Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology. *Comparative Biochemistry and Physiology* 119A(3): 725-737.

Gil-Romera, G., Scott, L., Marais, E. & Brook, G.A. 2007. Late Holocene Environmental Change in the Northwestern Namib Desert Margin: New fossil pollen evidence from hyrax middens. *Palaeogeography, Palaeoclimatology, Palaeoecology* 249: 1-17.

Gil-Romera, G., Lamb, H.F., Turton, D., Sevilla-Callejo, M. & Umer, M. 2010. Long-Term Resilience, Bush Encroachment Patterns and Local Knowledge in a Northeast African Savanna. *Global Environmental Change* 20: 612-626.

Gowik, U. & Westhoff, P. 2011. The Path from C_3 to C_4 Photosynthesis. *Plant Physiology* 155: 56-63.

Gröcke, D.R., Bocherens, H. & Mariotti, A. 1997. Annual Rainfall and Nitrogen-Isotope Correlation in Macropod Collagen: Application as a palaeoprecipitation indicator. *Earth and Planetary Science Letters* 153: 279-285.

Grubb, P., Sandrock, O., Kullmer, O., Kaiser, T.M. & Schrenk, F. 2000. Relationships Between Eastern and Southern African Mammal Faunas. In: Bromage, T. & Shrenk, F. (eds) *African Biogeography, Climate Change, and Early Hominid Evolution*: 253-267. New York: Oxford University Press.

Handley, L.L., Austin, A.T., Robinson, D., Scrimgeour, C.M., Raven, J.A., Heaton, T.H.E., Schmidt, S. & Stewart, G.R. 1999. The ^{15}N Natural Abundance (δ^{15}N) of Ecosystem Samples Reflects Measures of Water Availability. *Australian Journal of Plant Physiology* 26: 185-199.

Handley, L.L. & Raven, J.A. 1992. The Use of Natural Abundance of Nitrogen Isotopes in Plant Physiology and Ecology. *Plant, Cell and Environment* 15: 965-985.

Hare, P.E., Fogel, M.L., Stafford, T.W., Mitchell, A.D. & Hoering, T.C. 1991. The Isotopic Composition of Carbon and Nitrogen in Individuals Amino Acids Isolated from Modern and Fossil Proteins. *Journal of Archaeological Science* 18: 277-292.

Hartman, G. & Danin, A. 2010. Isotopic Values of Plants in Relation to Water Availability in the Eastern Mediterranean Region. *Oecologia* 162(4): 837-852.

Hayes, J.M. 1983. Practice and Principles of Isotope Measurements in Organic Geochemistry. *Organic Geochemistry of Contemporaneous and Ancient Sediments* 5: e5.

Heaton, T.H.E. 1987. The ^{15}N/^{14}N Ratios of Plants in South Africa and Namibia: Relationship to climate and coastal/saline environments. *Oecologia* 74: 236-246.

Heaton, T.H.E., Vogel, J.C., von la Chevallerie, G. & Collet, G. 1986. Climatic Influence on the Isotopic Composition of Bone Nitrogen. *Nature* 322(28): 822-823.

Hedges, R.E.M., Stevens, R.E. & Richards, M.P. 2004. Bone as a Stable Isotope Archive for Local Climatic Information. *Quaternary Science Reviews* 23: 959-965.

Hoeck, H.N. 1975. Differential Feeding Behaviour of the Sympatric Hyrax *Procavia johnstoni* and *Heterohyrax brucei*. *Oecologia* 22(1): 15-47.

Hoeck, H.N. 1977. Teat Order in Hyrax *(Procavia johnstoni* and *Heterohyrax brucei). International Journal of Mammalian Biology* 42(2): 112-115.

Hoeck, H.N. 1982. Population Dynamics, Dispersal and Genetic Isolation in Two Species of Hyrax *(Heterohyrax brucei* and *Procavia johnstoni)* on Habitat Islands in the Serengeti. *Zeitschrift für Tierphychologie* 53(3): 177-210.

Hoeck, H.N. 1989. Demography and Competition in Hyrax, a 17 Years Study. *Oecologia* 79(3): 353-360.

Högberg, P. 1986. Nitrogen-Fixation and Nutrient Relations in Savannah Woodland Trees (Tanzania). *The Journal of Applied Ecology* 23(2): 675-688.

Högberg, P. 1997. ^{15}N Natural Abundance in Soil-Plant Systems. *New Phytologist* 137: 179-203.

Holmgren, S.K., Taylor, K.M., Bretscher, L.E. & Raines, R.T. 1998. Code for Collagen's Stability Deciphered. *Nature* 392: 666.

Hume, I.D., Rübsamen, K. & Engelhardt, W.V. 1980. Nitrogen Metabolism and Urea Kinetics in the Rock Hyrax *(Procavia habessinica). Journal of Comparative Physiology* 138: 307-314.

Ilany, A., Barocas, A., Koren, L., Kam, M. & Geffen, E. 2011. Do Singing Rock Hyraxes Exploit Conspecific Calls to Gain Attention? *PLoS One* 6(12): e28612.

Janis, C.M. 1979. Mastication in the Hyrax and its Relevance to Ungulate Dental Evolution. *Palaeobiology* 5(1): 50-59.

Jerardino, A. 1995. The Problem with Density Values in Archaeological Analysis: A case study from Tortoise Cave, Western Cape, South Africa. *The South African Archaeological Bulletin* 50(161): 21-27.

Jim, S., Ambrose, S.H. & Evershed, R.P. 2004. Stable Carbon Isotopic Evidence for Differences in the Dietary Origin of Bone Cholesterol, Collagen and Apatite: Implications for their use in palaeodietary reconstruction. *Geochimica et Cosmochimica Acta* 68(1): 61-72.

Kelly, J.F. 1999. Stable Isotopes of Carbon and Nitrogen in the Study of Avian and Mammalian Trophic Ecology. *Canadian Journal of Zoology* 78: 1-27.

Kershenbaum, A., Ilany, A., Blaustein, L & Geffen, E. Syntactic Structure and Geographical Dialects in the Songs of Male Rock Hyraxes. *Proceedings of the Royal Society of London B: Biological Sciences* 279(1740): 2974-2981.

Klein, R.G. & Cruz-Uribe, K. 1996. Size Variation in the Rock Hyrax *(Procavia capensis)* and Late Quaternary Climate Change in South Africa. *Quaternary Research* 46: 193-207.

Kleinschmidt, T., Czelusniak, J., Goodman, M. & Braunitzer, G. 1986. Paenungulata: a comparison of the Haemoglobin sequences from elephant, hyrax, and manatee. *Molecular Biology and Evolution* 3(5): 427-435.

Kohn, M.J. & Cerling, T.E. 2002. Stable Isotope Compositions of Biological Apatite. In: Kohn, M.J., Rakovan, J. & Hughes, J.M. (eds) *Phosphates, Geochemical, Geobiological, and Materials Importance. Reviews in Mineralogy and Geochemistry (Version 48)*: 455-488. Washington: Mineralogical Society of America.

Kohn, M.J. 2010. Carbon Isotope Compositions of Terrestrial C_3 Plants as Indicators of (Paleo)ecology and (Paleo)climate. *Proceedings of the National Academy of Sciences* 107(46): 19691-19695.

Koren, L. & Geffen, E. 2008. Complex Call in Male Rock Hyrax *(Procavia capensis):* a multi-information distributing channel. *Behavioral Ecology and Sociobiology* 3(4): 581-590.

Kotler, B.P., Brown, J.S. & Knight, M.H. 1999. Habitat and Patch Use by Hyraxes: There's no place like home? *Ecology Letters* 2: 82-88.

Lensing, J.E. 1983. Feeding Strategy of the Rock Hyrax and its Relation to the Rock Hyrax Problem in Southern South West Africa. *Madoqua* 13: 177-196.

Mackay, A., Stewart, B.A. & Chase, B.M. 2014. Coalescence and Fragmentation in the Late Pleistocene Archaeology of Southernmost Africa. *Journal of Human Evolution* 72: 26-53.

Mahaney, W.C. 1986. Environmental Impact in the Afroalpine and Subalpine Belts of Mount Kenya, East Africa. *Mountain Research and Development* 6(3): 247-256.

Makarewicz, C.A. & Sealy, J.C. 2015. Dietary Reconstruction, Mobility, and the Analysis of Ancient Skeletal Tissues: Expanding the prospects of stable isotope research in archaeology. *Journal of Archaeological Science* 56: 146-158.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. & Tardieux, P. 1981. Experimental Determination of Nitrogen Kinetic Isotope Fractionation: Some principles: Illustration for the denitrification and nitrification processes. *Plant and Soil* 62(3): 413-430.

Meadows, M.E., Chase, B.M. & Selaine, M. 2010. Holocene Palaeoenvironments of the Cederberg and Swartruggens Mountains, Western Cape, South Africa: Pollen and stable isotope evidence from hyrax dung middens. *Journal of Arid Environments* 74: 786-793.

Middleton, B.J., Bailey, A.K. 2009. Water Resources of South Africa 2005 Study. *Water Research Commission Report* 380(8).

Millar, R.P. 1972. Degradation of Spermatozoa in the Epididymus of a Seasonally Breeding Mammal, the Rock Hyrax, *Procavia capensis*. *Journal of Reproduction and Fertility* 30: 447-450.

Millar, R.P. & Fairall, N. 1976. Hypothalamic, Pituitary and Gonadal Hormone Production in Relation to Nutrition in the Male Rock Hyrax *(Procavia capensis). Journal of Reproduction and Fertility* 47(2): 339-341.

Mooney, H.A., Troughton, J.H. & Berry, J.A. 1977. Carbon Isotope Measurements of Succulent Plants in Southern Africa. *Oecologia* 30(4): 295-305.

Moran, S. 1996. Efficacy of the repellent RO-PEL in Reducing Damage by the Rock Hyrax *(Procavia capensis)* to Fruit Trees. *International Journal of Pest Management* 42(4): 273-276.

Mucina, L. & Rutherford, M.C. 2006. *The Vegetation of South Africa, Lesotho and Swaziland.* Pretoria: South African National Biodiversity Institute.

Murphy, B.P. & Bowman, D.M.J.S. 2006. Kangaroo Metabolism Does Not Cause the Relationship Between Bone Collagen δ^{15}N and Water Availability. *Functional Ecology* 20: 1062-1069.

Murphy, B.P., Bowman, D.M.J.S. & Gagan, M.K. 2007. Sources of Carbon Isotope Variation in Kangaroo Bone Collagen and Tooth Enamel. *Geochimica et Cosmochimica Acta* 71: 3847-3858.

Murphy, B.P. & Bowman, D.M.J.S. 2009. The Carbon and Nitrogen isotope Composition of Australian Grasses in Relation to Climate. *Functional Ecology* 23: 1040-1049.

O'Connel, T.C., Kneale, C.J., Tasevska, N. & Kuhnle, G.G.C. 2012. The Diet-Body Offset in Human Nitrogen Isotopic Values: a controlled dietary study. *American Journal of Physical Anthropology* 149: 426-434.

Olds, N. & Shoshani, J. 1982. Procavia capensis. *Mammalian Species* 171: 1-7.

O'Leary, M. H. 1981. Carbon Isotope Fractionation in Plants. *Phytochemistry* 20(4): 553-567.

O'Leary, M.H. 1993. Biochemical Basis of Carbon Isotope Fractionation. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (eds) *Stable Isotopes and Plant Carbon-Water Relations*: 82-94. California: Academic Press, Inc.

Olsen, A., Prinsloo, L.C., Scott, L. & Jäger, A.K. 2008. Hyraceum, the Fossilized Metabolic Product of Rock Hyraxes *(Procavia capensis),* Shows GABA-Benzodiazepine Receptor Affinity. *South African Journal of Science* 103: 437-439.

Park, R. & Epstein, S. 1960. Carbon Isotope Fractionation During Photosynthesis. *Geochimica et Cosmochimica Acta* 21(1): 110-126.

Parkington, J. & Poggenpoel, C. 1971. Excavations at De Hangen, 1968. *The South African Archaeological Bulletin*: 3-36.

Passey, B.H. & Cerling, T.E. 2006. In Situ Stable Isotope Analysis (δ13C, δ18O) of Very Small Teeth Using Laser Ablation GC/IRMS. *Chemical Geology* 235(3): 238-249.

Pate, D.F. & Anson, T.J. 2008. Stable Nitrogen Isotope Values in Arid-Land Kangaroos Correlated with Mean Annual Rainfall: Potential as a palaeoclimatic indicator. *International Journal of Osteoarchaeology* 18: 317-326.

Pate, D.F., Anson, T.J., Noble, A.H., Schoeninger, M.J. 1998. Bone Collagen Stable Carbon and Nitrogen Isotope Variability in Modern South Australian Mammals: a baseline for palaeoecological inferences. *Quaternary Australasia* 16(1): 43-51.

Pestle, W.J. 2010. Chemical, Elemental, and Isotopic Effects of Acid Concentration and Treatment Duration on Ancient Bone Collagen: an exploratory study. *Journal of Archaeological Science* 30: 1-5.

Peters, J. 1989. Faunal Remains and Environmental Change in Central and Eastern Sudan from Terminal Pleistocene to Middle Holocene Times. *Academiae Analecta* 4: 121-148.

Peterson, B.J. & Fry, B. 1987. Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* 18: 293-320.

Phillips, D.L. 2012. Converting Isotope Values to Diet Composition: the use of mixing models. *Journal of Mammalogy* 93(2): 342-352.

Phillips, D.L., Inger, R, Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X. & Ward, E.J. 2014. Best Practices for Use of Stable Isotope Mixing Models in Food-Web Studies. *Canadian Journal of Zoology* 92: 823-835.

Prinsloo, P. & Robinson, T.J. 1992. Geographic Mitochondrial DNA Variation in the Rock Hyrax, *Procavia capensis. Molecular Biology and Evolution* 9(3): 447- 456.

Prinsloo, L.C. 2007. Rock Hyraxes: A cause for San rock art deterioration? *Journal of Raman Spectroscopy* 38: 496-503.

Quick, L.J., Chase, B.M., Meadows, M.E., Scott, L. & Reimer, P.J. 2011. A 19.5 kyr Vegetation History from the Central Cederberg Mountains, South Africa" Palynological Evidence from Rock Hyrax Middens. *Palaeogeography, Palaeoclimatology, Palaeoecology* 309: 253-270.

Robinson, D. 2001. δ^{15}N as an Integrator of the Nitrogen Cycle. *Trends in Ecology and Evolution* 16(3): 153-162.

Roth, J.D. & Hobson, K.A. 2000. Stable Carbon and Nitrogen Isotopic Fractionation Between Diet and Tissue of Captive Red Fox: Implications for dietary reconstruction. *Canadian Journal of Zoology* 78: 848-852.

Rübsamen, K., Heller, R., Lawrenz, H. & Engelhardt, W.V. 1979. Water and Energy Metabolism in the Rock Hyrax *(Procavia habessinica). Journal of Comparative Physiology* 131(4): 303-309.

Rübsamen, K., Hume, I.D., Engelhardt, W.V. 1982. Physiology of the Rock Hyrax. *Comparative Biochemistry and Physiology Part A: Physiology* 72(2): 271-277.

Sale, J.B. 1960. The Hyracoidea. *Journal of East African Natural History* 23(5): 185-188.

Sale, J.B. 1966. The Habitat of the Rock Hyrax. *Journal of East African Natural History* 25(3): 205-215.

Schlitter, D.A., Ferguson, A.W. & McDonough, M.M. 2014. Mammals of Africa (Vol. I-VI). *Journal of Mammalogy* 95(6): 1299-1303.

Schoeninger, M.J. & Moore, K. 1992. Bone Stable Isotope Studies in Archaeology. *Journal of World Prehistory* 6(2): 247-296.

Scholes, R.J., & Archer, S.R. 1997. Tree-Grass Interactions in Savannahs. *Annual Review of Ecology and Systematics* 28: 517-544.

Schulze, R.E. 2007. *South African Atlas of Climatology and Agrohydrology*. Pretoria: Water Research Commission.

Schwarcz, H.P., Dupras, T.L. & Fairgrieve, S.I. 1999. ^{15}N Enrichment in the Sahara: In search of a global relationship. *Journal of Archaeological Science* 26: 629-636.

Schweizer, M., Fear, J. & Cadisch, G. 1999. Isotopic (^{13}C) Fractionation During Plant Residue Decomposition and its Implications for Soil Organic Matter Studies. *Rapid Communications in Mass Spectrometry* 13: 1284-1290.

Scott, L. & Bousman, C.B. 1990. Palynological Analysis of Hyrax Middens from Southern Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 76: 367-379.

Scott, L. & Cooremans, B. 1992. Pollen in Recent Procavia (Hyrax), Petromus (Dassie Rat) and Bird Dung in South Africa. *Journal of Biogeography* 19(2): 205-215.

Scott, L. & Vogel, J.C. 2000. Evidence for Environmental Conditions in the Last 20 000 Years in Southern Africa from ^{13}C in Fossil Hyrax Dung. *Global and Planetary Change* 26: 207-215.

Scott, L. & Woodborne, S. 2007a. Pollen Analysis and Dating of Late Quaternary Faecal Deposits (Hyraceum) in the Cederberg, Western Cape, South Africa. *Review of Palaeobotany and Palynology* 144: 123-134.

Scott, L. & Woodborne, S. 2007b. Vegetation History Inferred from Pollen in late Quaternary Faecal Deposits (Hyraceum) in the Cape Winter-Rain Region and its Bearing on Past Climates in South Africa. *Quaternary Science Reviews* 26: 941-953.

Scott, L. 1996. Palynology of Hyrax Middens: 2000 years of palaeoenvironmental History in Namibia. *Quaternary International* 33: 73-79.

Scott, L. 2002. Grassland Development Under Glacial and Interglacial Conditions in Southern Africa: Review of pollen, phytolith and isotope evidence. *Palaeogeography, Palaeoclimatology, Palaeoecology* 177: 47-57.

Scott, L., Marais, E. & Brook, G.A. 2004. Fossil Hyrax Dung and Evidence of Late Pleistocene and Holocene Vegetation Types in the Namib Desert. *Journal of Quaternary Science* 19(8): 829-832.

Sealy, J.C. & van der Merwe, N.J. 1988. Social, Spatial and Chronological Patterning in Marine Food Use as Determined by $\delta^{13}C$ Measurements of Holocene Human Skeletons from the South-Western Cape, South Africa. *World Archaeology* 20(1): 87-102.

Sealy, J.C., Johnson, M., Richards, M. & Nehlich, O. 2014. Comparison of Two Methods of Extracting Bone Collagen for Stable Carbon and Nitrogen Isotope Analysis: Comparing whole bone demineralization with gelatinization and ultrafiltration. *Journal of Archaeological Science* 47: 64-69.

Sealy, J.C., van der Merwe, N.J., Lee-Thorp, J.A. & Lanham, J.L. 1987. Nitrogen Isotopic Ecology in Southern Africa: Implications for environmental and dietary tracing. *Geochimica et Cosmochimica Acta* 51: 2707-2717.

Sharp, Z. 2007. *Principles of Stable Isotope Geochemistry.* New Jersey: Pearson Education Inc.

Shearer, G. & Kohl, D.H. 1989. Estimates of N_2 Fixation in Ecosystems: the need for and basis of the ^{15}N natural abundance method. In: Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. Stable Isotopes in Ecological Research: 342-374. New York: Springer.

Shearer, G., Kohl, D.H. & Harper, J.E, 1980. Distribution of ^{15}N Among Plant Parts of Nodulating and Nonnodulating Isolines of Soybeans. *Plant Physiology* 66: 57-60.

Sheng, W., Yu, G., Fang, H., Liu, Y., Wang, Q., Chen, Z. & Zhang, L. 2014. Regional Patterns of ^{15}N Natural Abundance in Forest Ecosystems Along a Large Terrestrial Transect in Eastern China. *Scientific Reports* 4: 1-6.

Shipley, L.A. 1999. Grazers and Browsers: How digestive morphology affects diet selection. *Grazing Behavior of Livestock and Wildlife*: 20-27.

Skinner, J.D. & Smithers, R.H.N. 1990. *The Mammals of the Southern African Subregion (Second Edition).* Pretoria: University of Pretoria.

Slater, C., Preston, T. & Weaver, L.T. 2001. Stable Isotopes and the International System of Units. *Rapid Communications in Mass Spectrometry* 15: 1270-1273.

Smith, A.B., Sadr, K., Gribble, J. & Yates, R. 1991. Excavations in the South-Western Cape, South Africa, and the Archaeological Identity of Prehistoric Hunter-Gatherers within the Last 2000 Years. *The South African Archaeological Bulletin* 46(154): 71-91.

Spinage, C.A. 1981. Some Faunal Isolates of the Central African Republic. *African Journal of Ecology* 19(1-2): 125-132.

Sponheimer, M., Grant, C.C., de Ruiter, D.J., Lee-Thorp, J.A., Codron, D.M. & Codron, J. 2003a. Diets of Impala from Kruger National Park: Evidence from stable carbon isotopes. *Koedoe* 46(1): 101-106.

Sponheimer, M., Lee-Thorp, J.A., de Ruiter, D.J., Smith, J.M., van der Merwe, N.J., Reed, K., Grant, C.C., Ayliffe, L.K., Robinson, T.F., Heidelberger, C. & Marcus, W. 2003d. Diets of Southern African Bovidae: Stable isotope evidence. *Journal of Mammalogy* 84(2): 471-479.

Sponheimer, M., Robinson, T.F., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D. & Ehleringer, J. 2003c. An Experimental Study of Carbon-Isotope Fractionation Between Diet, Hair, and Feces of Mammalian Herbivores. *Canadian Journal of Zoology* 81: 871-876.

Sponheimer, M., Robinson, T.F., Roeder, B.L., Passey, B.H., Ayliffe, L.K., Cerling, T.E., Dearing, M.D. & Ehleringer, J.D. 2003b. An Experimental Study of Nitrogen Flux in Llamas: is ^{14}N preferentially excreted? *Journal of Archaeological Science* 30: 1649-1655.

Spriggs, A.C., Stock, W.D. & Dakora, F.D. 2003. Influence of Mycorrhizal Associations on Foliar δ^{15}N Values of Legume and Non-Legume Shrubs and Trees in the Fynbos of South Africa: Implications for estimating N_2 fixation using the ^{15}N natural abundance method. *Plant and Soil* 255: 495-502.

Still, C.J., Berry, J.A., Collatz, G.J. & DeFries, R.S. 2003. Global Distribution of C_3 and C_4 Vegetation: Carbon cycle implications. *Global Biogeochemical Cycles* 17(1): 1-14.

Sukumar, R. & Ramesh, R. 1992. Stable Carbon Isotope Ratios in Asian Elephant Collagen: Implications for dietary studies. *Oecologia* 91: 536-539.

Swap, R.J., Aranibar, J.N., Dowty, P.R. & Gilhooly, W.P. 2004. Natural Abundance of ^{13}C and ^{15}N in C_3 and C_4 Vegetation of Southern Africa: Patterns and Implications. *Global Change Biology* 10: 350-358.

Szpak, P., Gröcke, D.R., Debruyne, R., MacPhee, R.D.E., Guthrie, R.D., Froese, D., Zazula, G.D., Patterson, W.P. & Poinar, H.N. 2010. Regional Differences in δ^{13}C and δ^{15}N of Pleistocene Mammoths: Implications for the Paleoecology of the Mammoth Steppe. *Palaeogeography, Palaeoclimatology, Palaeoecology* 286: 88-96.

Taton, T.A. 2001. Boning up on Biology. *Nature* 412: 491-492.

Texier, P.J., Porraz, G., Parkington, J., Rigaud, J-P., Poggenpoel, C., Miller, C., Tribolo, C., Cartwright, C., Coudenneau, A., Klein, R., Steele, T. & Verna, C. 2010. A Howiesons Poort Tradition of Engraving Ostrich Eggshell Containers dated to 60,000 Years Ago at Diepkloof Rock Shelter, South Africa. *Proceedings of the National Academy of Sciences* 107(14): 6180-6185.

Trumbore, S.E. & Druffel, E.R.M. Carbon Isotopes for Characterizing Sources and Turnover of Nonliving Organic Matter. In: Zepp, R.G. & Sonntag, C. *The Role of Nonliving Organic Matter in the Earth's Carbon Cycle* : 7-23. England: John Wiley & Sons, Ltd.

Tuross, N., Behrensmeyer, A.K., Eanes, E.D., Fisher, L.W. & Hare, P.E. 1989a. Molecular Preservation and Crystallographic Alterations in a Weathering Sequence of Wildebeest Bones. *Applied Geochemistry* 4: 261-270.

Tuross, N., Fogel, M.L. & Hare, P.E. 1989b. Variability in the Preservation of the Isotopic Composition of Collagen from Fossil Bone. *Geochimica et Cosmochimica Acta* 52: 929-935.

van der Merwe, N.J. & Vogel, J.C. 1978. ^{13}C Content of Human Collagen as a Measure of Prehistoric Diet in Woodland North America. *Nature* 276: 815-816.

van der Merwe, N.J. 1982. Carbon Isotopes, Photosynthesis, and Archaeology: Different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets. *American Scientist* : 596-606.

van der Merwe, N.J. 1989. Natural Variation in ^{13}C Concentration and its Effect on Environmental Reconstruction Using ^{13}C/^{12}C Ratios in Animal Bones. In: Price, T.D. (ed) *The Chemistry of Prehistoric Human Bone* : 105-125. Cambridge: Cambridge University Press.

van Klinken, G.J. 1999. Bone Collagen Quality Indicators for Palaeodietary and Radiocarbon Measurements. *Journal of Archaeological Science* 26: 687-695.

Vogel, J.C. 1978. Isotopic Assessment of the Dietary Habits of Ungulates. *South African Journal of Science* 74: 298-301.

Vogel, J.C., Fuls, A. & Ellis, R.P. 1978. The Geographical Distribution of Kranz Grasses in South Africa. *South African Journal of Science* 47: 209-215.

Wadley, L. 2010. Were Snares and Traps Used in the Middle Stone Age and Does it Matter? A Review and Case Study from Sibudu, South Africa. *Journal of Human Evolution* 58: 179-192.

Walker, A., Hoeck, H.N. & Perez, L. 1978. Microwear of Mammalian Teeth as an Indicator of Diet. *Science* 201(4359): 908-910.

Walker, A., Ludwig, D., Holling, C.S. & Peterman, R.M. 1981. Stability of Semi-Arid Savannah Grazing Systems. *Journal of Ecology* 69(2): 473-498.

Wallach, J.D., Lagarde, K. & Silberman, M.S. 1977. Haematological and Serum Chemistry Values of the Rock Hyrax *(Procavia capensis). The Journal of Zoo Animal Medicine* 8(3): 30-34.

Weiner, S. & Wagner, H.D. 1998. The Material Bone: Structure-mechanical function relations. *Annual Review of Materials Science* 28: 271-298.

West, J.B., Bowen, G.J., Cerling, T.E. & Ehleringer, J.R. 2006. Stable Isotopes as One of Nature's Ecological Recorders. *Trends in Ecology and Evolution* 21(7): 408-414.

Wiid, R.E. & Butler, H.J.B. 2015. Population Management of Rock Hyraxes *(Procavia capensis)* in Residential Areas. *Pest Management Science* 71: 180-188.

Winter, K. & Holtum, J.A.M. 2002. How Closely do the δ^{13}C Values of Crassulacean Acid Metabolism Plants Reflect the Proportion of CO2 Fixed During Day and Night? *Plant Physiology* 129: 1843-1851.

Yom-Tov, Y. 1993. Does the Rock Hyrax, *Procavia capensis* Conform with Bergmann's Rule? *Zoological Journal of the Linnean Society* 108: 171-177.

Young, T.P. & Evans, M.R. 1993. Alpine Vertebrates of Mount Kenya, with Particular Notes on the Rock Hyrax. *Journal of the East Africa Natural History Society and National Museum* 82(202): 55-79.

Zimman, A.M. 2008. Niche Differentiation of Sympatric Hyraxes *Heterohyrax brucei* and *Procavia johnstoni* in Serengeti National Park, Tanzania. *Bios* 74(1): 1-8.

Zumft, W.G. 1997. Cell Biology and Molecular Basis of Denitrification. *Microbiology and Molecular Biology Reviews* 61(4): 533-616.