Stable isotope ecology of modern herbivores from Mmabolela, Limpopo

by

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Abstract

This thesis explores the stable isotope ecology of 12 species of co-existing wild herbivores (11 mammals and one reptile) (62 individuals) from Mmabolela, a game farm on the northern border of South Africa in the Limpopo Province. The aim is to investigate and interpret patterns in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values of different species of herbivores and to compare these results with previous studies. As expected in this summer-rainfall region, $\delta^{13}C_{\text{bone collagen}}$ values fall into two distinct groups: predominant browsers with mean $\delta^{13}C$ values ranging from -21.37‰ to -18.10‰, and grazing species with mean $\delta^{13}C$ values ranging from -13.25‰ to -10.49‰, the more negative values reflecting some supplementary feeding with lucerne during the dry season. There are no differences in $\delta^{13}C$ between the browsing species eland (*Taurotragus oryx*), bushbuck (*Tragelaphus scriptus*) and kudu (*Tragelaphus strepsiceros*). The $\delta^{15}N$ values for all species (including corrected values for keratin from *Hystrix africaeaustralis*) range from 7.73‰ to 11.20‰. $\delta^{15}N$ values of baboons fall within the range seen in bovids. Amongst bovids, there is no statistically significant difference between the $\delta^{15}N$ values of browsers (drought-tolerant or water-independent) and grazers (obligate drinkers). Zebra have lower $\delta^{15}N$ values. The potential of porcupine quills to provide a record of seasonal dietary changes was investigated by serial sampling of quills from three porcupines (*Hystrix africaeaustralis*), and tooth enamel from nine zebra (*Equus quagga*). The porcupines show short-term, but not necessarily seasonal variations in their diets. $\delta^{13}C$ and $\delta^{18}O$ profiles of the second and third molars of nine *Equus quagga* individuals are highly variable. A better understanding of the isotopic ecology of communities such as this will help to interpret similar analyses of fossil faunal assemblages and assist in reconstructing palaeoenvironments.

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Chapter 1: Introduction

This thesis investigates δ^{13}C, δ^{15}N and δ^{18}O values of various co-existing species of wild herbivores from Mmabolela Estates, a game farm on the northern border of South Africa, on the banks of the Limpopo River (Figure 1.1). This is a summer rainfall region with vegetation classified as part of the Savanna Biome (Mucina and Rutherford, 2006). Most of the grasses in this region are C_4, while the trees and shrubs are mainly C_3 (Sage *et al*. 1999; Sponheimer *et al*., 2003a; Vogel *et al*. 1978).

The fauna analysed for this thesis consist mainly of mammalian herbivores. There are seven species of bovids: bushbuck (*Tragelaphus scriptus*), common eland (*Taurotragus oryx*), gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*), greater kudu (*Tragelaphus strepsiceros*), waterbuck (*Kobus ellipsiprymnus*) and blue wildebeest (*Connochaetes taurinus*) as well as the equid plains zebra (*Equus quagga*). Other mammals are chacma baboons (*Papio ursinus*), Cape porcupines (*Hystrix africaeaustralis*), and warthogs (*Phacochoerus africanus*). In addition, there are four leopard tortoises (*Stigmochelys pardalis*). These specimens were collected in late 2020, and they derive from animals that had lived in the area over the preceding few years.

This thesis reports δ^{13}C and δ^{15}N values of bone collagen from all species except *H. africaeaustralis*, for which the samples available consisted of quills, so δ^{13}C and δ^{15}N values are for keratin. In addition, δ^{13}C and δ^{18}O values are reported for serial samples of tooth enamel from nine *E. quagga*. In savanna environments, stable carbon isotope ratios (δ^{13}C) reflect the proportions of [C_4] graze and [C_3] browse in the diet of an animal (e.g. Cerling *et al*., 2003; Codron *et al*., 2005a; Gagnon and Chew, 2000; Sponheimer *et al*., 2003a). δ^{15}N values reflect protein intake, trophic behaviour, and environmental stress (i.e. nutritional and water). In animals, stable oxygen isotope ratios (δ^{18}O) provide useful information about the source of water (i.e. meteoric or leaf water). δ^{18}O values can also be used to reconstruct palaeoclimates and past environments (e.g., Ayliffe and Chivas, 1990; Crespin *et al*., 2010; Green *et al*., 2018; Longinelli, 1984; Schoeninger *et al*., 2003).

1.1. Aims and Objectives

This thesis aims to learn about the stable isotope values that characterise different co-existing wild herbivore species from a single area. Only a few studies of this type have been reported in the literature from East (e.g. Ambrose and DeNiro, 1986; Cerling *et al*., 2003; Tieszen *et al*., 1979) and South Africa (e.g. Botha and Stock, 2005; Codron *et al*., 2005a, 2007; Sponheimer *et al*., 2003a), some of which include fewer species than this thesis. Isotope ecology is complex, since feeding behaviour may vary in different regions, and shifts as the seasons change. It may also depend on the sex and age of the animal, if there are changes in dietary behaviour over the course of its life. Isotopic 'niches' may vary in different environments offering different resources. The hope is that improving our understanding of the isotopic ecology of contemporary communities such as this will aid in interpreting similar analyses of fossil faunal assemblages and thus assist in reconstructing palaeoenvironments.

Specific goals of this thesis are therefore (i) to investigate and interpret isotopic differences between different species of herbivores (ii) to see how different species within the categories of grazers, mixed feeders and browsers relate to one another (iii) to compare the patterns seen at Mmabolela with those reported in previous studies (Ambrose and DeNiro, 1986; Botha and Stock, 2005; Cerling *et al*., 2003; Codron *et al*., 2005a; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a; Tieszen *et al*., 1979) (iv) to investigate possible seasonal shifts in isotope values in porcupine quills and enamel serial samples of *E*. *quagga*.

Incremental tissues (e.g. teeth and hair) sampled along the growth axis provide information on short-term dietary shifts, over the period of tissue formation (Cerling and Viehl, 2004; Koch, 1989). Cape porcupine (*Hystrix africaeaustralis*) quills and enamel from plains zebra (*Equus quagga*) second and third molars were sequentially sampled. In domestic horses (modern equids), the M2 reflects the diet from approximately 7 (start of mineralisation) to 37 months (end of mineralisation). The M3 provides dietary information from approximately 21 to 55 months (Hoppe *et al*., 2004). However, in this thesis, only the parts of the teeth protruding into the mouth were sampled (i.e. approximately one-third of the entire tooth). Therefore, the M2 is likely to provide information from approximately 7 to 17 months, and the M3 from ~21 to 32 months.

1.2. Thesis Outline

Chapter 1 has introduced the thesis and outlined the aims and objectives of this study. Chapter 2 outlines the history, environment, climate and vegetation of the study area. Chapter 3 provides a background of the chemistry and application of stable isotopes, including diet-to-tissue differences in consumers, and the quality control indicators used to assess the integrity of collagen isolated from bone samples. This chapter also provides an overview of the literature on the habits, habitats and diets of the animals included in this thesis. In addition, it outlines relevant aspects of the formation and development of bone, porcupine quills and teeth. Chapter 4 describes the specimens, sampling methods, preparation of samples and isotopic analyses. Chapter 5 reports the results: $\delta^{13}C_{\text{bone collagen}}$ and $\delta^{15}N_{\text{bone collagen}}$, $\delta^{13}C_{\text{keratin}}$ and $\delta^{15}N_{\text{keratin}}$ for porcupine quills and $\delta^{13}C_{\text{enamel}}$ and $\delta^{18}O_{\text{enamel}}$ for zebra teeth. Chapter 6 provides a discussion of the results, investigates seasonal patterns and draws conclusions.

Map data ©2023 AfriGIS (Pty) Ltd

Figure 1.1: A location map of Mmabolela Estates.

Chapter 2: History, Climate, Environment and Vegetation of the Study Area

Mmabolela Estates is a game farm located in the Limpopo Province, South Africa (22°40'13.5"S 28°14'33.6"E) (Mmabolela, 2021). It lies along the southern bank of the Limpopo River, between the gravel-bedded rivers Suking and Lotsane, and extends over an area of 5375 hectares (Figure 2.1). The first written descriptions date from the early 19th century, documented by hunters and explorers who came across a large quantity of game (Mmabolela, 2021). In his book, *A Hunter's Life Among Lions, Elephants and Other Wild Animals of South Africa*, the famous traveller Roualeyn George Gordon-Cumming wrote: "On the 10th, at dawn of day, I rode down the river, and ordered my wagons to follow. I found sea-cows more and more abundant; every pool had its herd: the margin of the river on each side was trampled down by elephants, rhinoceroses, buffaloes, & c." (Gordon-Cumming, 1857).

Limpopo is a summer rainfall region with dry winters. The vegetation of Mmabolela Estates is Limpopo Sweet Bushveld (SVcb19) which falls into the Central Bushveld Bioregion within the Savanna Biome (Mucina and Rutherford, 2006). Sweetveld occurs in areas characterised by fertile soils, which means that grazing is relatively good even during the dry season (Scholes, 1990). According to Mucina and Rutherford (2006), the Limpopo Sweet Bushveld is a "short open woodland, where thickets of blue-thorn (*Senegalia erubescens*), black-thorn (*Senegalia mellifera*) and sickle-bush (*Dichrostachys cinerea*) are almost impenetrable in disturbed areas". The sweet veld in Limpopo is characterised by plains that are sometimes "irregular and undulating", it has a high grazing capacity regardless of the low rainfall reported (Mucina and Rutherford, 2006). The quality and abundance of the graze create an area that is well-suited for game and cattle farming (Mucina and Rutherford, 2006).

In African savannas, graze consists almost entirely of C_4 grasses while most trees, shrubs and forbs use the C_3 photosynthetic pathway (Cerling and Harris, 1999; Sage *et al*. 1999; Sponheimer *et al*., 2003a; Vogel *et al*. 1978). In southern Africa, C_3 grasses are predominant only at high elevations, e.g. in the summits of the Eastern Cape and the Drakensberg mountains and the winter rainfall region of the Western Cape Province (Ellis *et al*., 1980; Vogel, 1978; Vogel *et al*., 1978).

Rainfall data measured at the Du Plessis Farm (one of the farms in the Mmabolela conservancy) are used. The average annual rainfall from 1980 to 2019 was 359 mm. The animals analysed in this thesis probably lived between 2015 and 2019. The annual rainfall was 444 mm in 2015, 386 mm in 2016, followed by three drier years (2017-2019) with rainfall of 267, 314 and 246 mm respectively (Berry, 2021). Although there is no trend in the amount of rainfall over recent decades (it is not getting wetter or drier), rain is falling in fewer, heavier rainfall events. Since 1980, there are on average 25-30 rain days each year. Over the last 100 years there has been a shift in the local vegetation community, with an increase in bush cover and a decrease in grass. This is partly as a result of a drop in the water table, due to the drilling of boreholes and greater use of underground water. It is also influenced by fencing, more intensive grazing and reduced fire frequency (Berry, 2021).

In the late 1800s, the land was sold to the Verviers family, descendants of colonist settlers who trekked from the Cape (Butler, 2021). In 1920 the farm was sold to businessman and newspaper owner A.V. Lindbergh (Mmabolela, 2021). Lindbergh combined Weerdedooper, Du Plessis farm and the adjacent ‘pondt plase’ (farms that were sold for a pound) to form Mmabolela Estates (Mmabolela, 2021). Lindbergh later bought an adjoining property in Bechuanaland (modern-day Botswana), which was sold in 1980 when the border between Botswana and South Africa was formalised (Mmabolela, 2021). Lindbergh put strict policies in place to conserve wildlife (Mmabolela, 2021). The farm was named after Mabalel, a young woman who was killed by a crocodile. Eugène Marais, a South African naturalist and poet wrote a poem in honour of Mabalel.

Mmabolela Estates is not a pristine ecosystem because it is a game farm, where visitors observe or hunt various animal species. To maintain the animals in good condition through the dry winter months, some supplementary feed is provided, mostly in the form of lucerne, and salt licks are available year-round. In the wetter months, the Limpopo River flows, offering drinking water for animals. In winter, surface flow largely dries up, so that animals can drink only from the remaining pools. Water is also pumped from boreholes to provide drinking water at locations farther from the river; this information is relevant for the study of oxygen isotopes. Despite these environmental alterations, this region still provides an excellent opportunity to study the isotopic ecology of multiple species of large animals from a single community in a summer rainfall region.

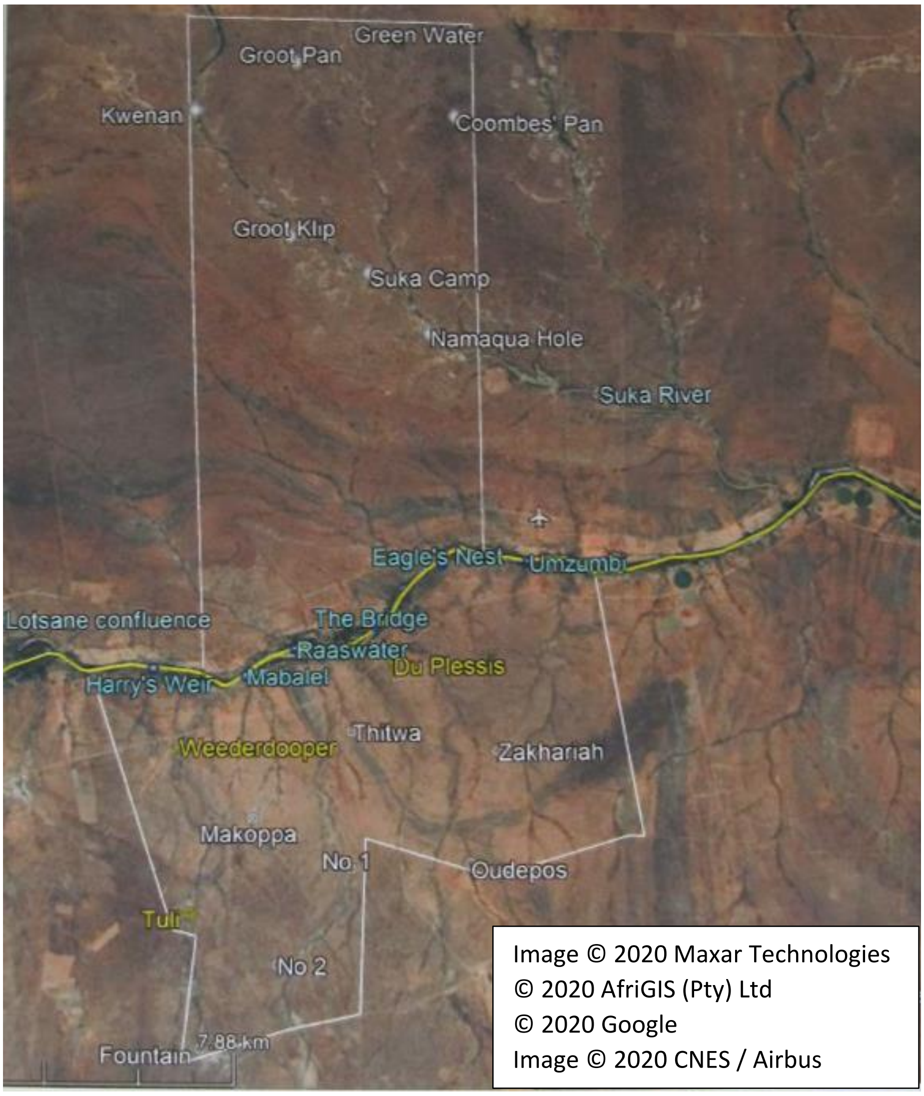


Figure 2.1: A Google image of the Mmabolela conservancy. The conservancy is the area enclosed in white. The yellow line represents the Limpopo River. Image taken from Berry (2021:60).

The following animal species were collected for this research project: chacma baboons (*Papio ursinus*), bushbuck (*Tragelaphus scriptus*), common eland (*Taurotragus oryx*), gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*), greater kudu (*Tragelaphus strepsiceros*), Cape porcupines (*Hystrix africaeaustralis*), leopard tortoise (*Stigmochelys pardalis*), warthogs (*Phacochoerus africanus*), waterbuck (*Kobus ellipsiprymnus*), blue wildebeest (*Connochaetes taurinus*) and plains zebra (*Equus quagga*). The animals analysed in this thesis came from the area south of the Limpopo

River. Other animal species found at the farm include giraffe (*Giraffa giraffa*), steenbok (*Raphicerus campestris*), duiker (*Sylvicapra grimmia*), crocodile (*Crocodylus niloticus*) and hippopotamus (*Hippopotamus amphibius*) (Mmabolela, 2021). Lions, formerly abundant in the area, have been extirpated. Leopards (*Panthera pardus*) are still found in the area, along with smaller carnivores such as the caracal (*Felis caracal*). Mmabolela Estates is also famous for its diverse birdlife; there are approximately 350 bird species (Mmabolela, 2021).

Chapter 3: Background and Literature Review

3.1. Stable Isotopes

Isotopes are different forms of the same chemical element: they have the same atomic number but differ in the number of neutrons found in the nucleus. For example, oxygen has three naturally occurring stable isotopes, oxygen-18 (eight protons and ten neutrons), oxygen-17 (eight protons and nine neutrons) and oxygen-16 (eight protons and eight neutrons). Isotopes can further be classified as radioactive or stable. Radioactive isotopes (e.g. carbon-14) are 'unstable' because they decay over time. Stable isotopes do not decay with time - their abundance remains constant.

During chemical and physical reactions, heavier isotopes require more energy than lighter isotopes to break or create bonds. Consequently, the proportions of different isotopes of an element may differ in the product of a reaction compared to the starting materials (DeNiro, 1987), as long as some starting materials remain. Heavy isotopes tend to remain in the reactant, with light isotopes more readily converted into the product. This process is called isotope fractionation.

Stable isotope ratios are measured on an isotope ratio mass spectrometer (IRMS), and values are reported using the delta (δ) notation, in units of 'parts per thousand' (per mille, ‰). Delta values are calculated as follows:

$$\delta\ (‰) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

Where R_{sample} is the proportion of the heavy isotope to the light isotope (e.g. $^{13}C/^{12}C$) in the sample, and R_{standard} is the proportion of the heavy isotope to the light isotope of an internationally accepted reference standard (materials with known values). The ratios are multiplied by 1000 to make the numbers easier to work with. $\delta^{13}C$ values are reported relative to the VPDB (Vienna PeeDee Belemnite) standard. $\delta^{15}N$ values are reported relative to atmospheric nitrogen (N_2). $\delta^{18}O$ values are reported relative to either VPDB or VSMOW (Vienna Standard Mean Ocean Water). When δ values are positive, this indicates that there is a greater proportion of the heavy isotope in the sample than the standard. When δ values are negative, this indicates that there is a smaller proportion of the heavy isotope in the sample relative to the standard.

Early applications of stable isotopes as dietary tracers focused on a relatively small number of questions to do with humans, such as the establishment and expansion of maize agriculture (Makarewicz and Sealy, 2015). Stable isotopes are now used to explore more complex and diverse topics, including, diet, mobility, livelihood, and social practices of past societies (Lee-Thorp, 2008; Makarewicz and Sealy, 2015). Early studies sometimes had difficulty with archaeological and fossil samples (especially bones) that had undergone diagenesis (i.e. post-depositional alteration), but scholars have found ways to better understand diagenesis, and develop effective pre-treatment methods to mitigate its effects.

Stable isotopes in plant and animal tissues provide useful information on diet and the environment (DeNiro and Epstein, 1978; Hobson and Clark, 1992; Koch *et al*., 1995; Rubenstein and Hobson, 2004). In the past (and still today), animal ecologists study animal diet and feeding behaviour through visual observation and analysis of stomach contents, scat or pellets. The introduction of stable isotope analyses of animal tissues in animal ecology provided an independent means of tracing food and water consumed during the period of tissue formation, reflecting behaviour over longer periods than previously possible. Stable carbon isotope analyses in animal ecology are able to differentiate between browsers, grazers and mixed feeders (Cerling *et al*., 2003; Codron *et al*., 2005a; Gagnon and Chew, 2000; Sponheimer *et al*., 2003a; Vogel, 1978); and nitrogen isotopes allow investigation of the trophic levels at which animals are feeding, as well as aspects of environmental reconstruction (Ambrose, 1991; Bai *et al*., 2022; Codron *et al*., 2006; Rowell *et al*., 2010; Schoeninger *et al*., 1997; Sealy *et al*., 1987; Sponheimer *et al*., 2003a).

3.1.1. Stable Carbon Isotopes

Carbon is a chemical element with two stable isotopes, carbon-12 (^{12}C) and carbon-13 (^{13}C). ^{12}C has six protons, six electrons and six neutrons, ^{13}C has six protons, six electrons and seven neutrons. Carbon-12 makes up approximately 98.93% of all natural carbon found on Earth, while carbon-13 only makes up 1.1%. During photosynthesis, carbon dioxide (CO_2) and isotope fractionation have a strong effect on stable carbon isotope ratios ($^{13}C/^{12}C$) in terrestrial plants (DeNiro, 1987). Plant tissues tend to be enriched with the light (^{12}C) isotope because it requires less energy to react than the heavy (^{13}C) isotope and is thus more favoured by enzymes (Sponheimer and Cerling, 2014; van der Merwe, 1982). There are three types of photosynthetic pathways in terrestrial plants: C_3 photosynthesis (Calvin Cycle), C_4 photosynthesis (Hatch-Slack Cycle) and the CAM (Crassulacean Acid Metabolism) photosynthetic pathway. Isotopic fractionation is different for each photosynthetic pathway (Leatherdale, 2013; O'Leary, 1988; van der Merwe, 1982).

Stable Carbon Isotopes in Plants

Most terrestrial plants use the C_3 photosynthetic pathway, including temperate grasses (e.g. barley, rice and wheat), trees and most shrubs and herbs. C_3 photosynthesis begins with the diffusion of CO_2 from the air into the stomata of the leaf. The enzyme RuBisCo (ribulose-1,5-bisphosphate carboxylase-oxygenase) combines CO_2 and RuBP (ribulose 1,5-bisphosphate, five-carbon compound sugar) into two molecules of 3-phosphoglyceric acid (three-carbon compound). This process occurs in the mesophyll cells. Even though it is the most common photosynthetic pathway, C_3 photosynthesis is not efficient in hot arid environments (Ehleringer and Cerling, 2002). During dry and hot conditions, the stomata close to prevent water loss. The continuation of carbon fixation in the Calvin Cycle results in the reduction of CO_2 concentration. RuBisCo eventually starts fixing O_2 instead of CO_2, in a process called photorespiration. Photorespiration does not produce sugars and reduces the amount of carbon fixed during C_3 photosynthesis, limiting growth. Therefore, plants that use C_3 photosynthesis are abundant in areas with cool growing seasons, i.e. winter rainfall regions.

Plants that use the C_4 photosynthetic pathway are referred to as C_4 plants. These include many grasses that grow in hot climates, such as maize, sorghum, millet and sugar cane. In the first step of the C_4 photosynthetic pathway, the enzyme PEPC (phosphoenolpyruvate carboxylase) fixes CO_2 which reacts with PEP (phosphoenolpyruvate, three-carbon compound) to form OAA (oxaloacetic acid, four-carbon compound) in the mesophyll cell. OAA then enters the bundle sheath cells and releases CO_2, which is then fixed by RuBisCo. C_4 photosynthesis overcomes photorespiration by concentrating CO_2 around RuBisCo in the bundle sheath. This ensures that there is always enough CO_2 for the Calvin Cycle to produce sugars. In hot and arid climates, plants that use the C_4 photosynthetic pathway reduce photorespiration and decrease the loss of carbon (Ghannoum *et al*., 2011; Mallmann *et al*., 2014; Schuler *et al*., 2016; Zhu *et al*., 2008). Consequently, C_4 photosynthetic pathways thrive in regions with high temperatures and low CO_2 concentrations. Therefore, plants that use C_4 photosynthesis are abundant in summer rainfall regions (Vogel *et al*., 1978).

There is a correlation between the distribution of C_3 and C_4 grass species and temperature and rainfall patterns (Bentley and O'Connor, 2018; Ellis *et al*., 1980; Killick, 1963; Morris, 2017; Schulze, 1965; Vogel *et al*., 1978). C_3 grass species thrive in regions with relatively low temperatures during the growing season (i.e. winter rainfall areas) while C_4 grasses prefer warmer temperatures, especially in warmer growing seasons (i.e. summer rainfall areas) (Bentley and O'Connor, 2018; Ehleringer *et al*., 1997; Ellis *et al*., 1980; Killick, 1963; Morris, 2017; Vogel *et al*., 1978). C_3 grass species are more likely to occur at high altitudes and on more shaded slopes (Bentley and O'Connor, 2018; Killick, 1963; Morris, 2017). However, Knapp *et al*. (2020) reported that drought-induced shifts were able to allow for the dominance of C_3 grass species in grasslands (high temperature, low rainfall) where C_4 grasses normally thrive and dominate. C_4 grasses are mostly found at low altitudes or sunnier slopes (Bentley and O'Connor, 2018; Killick, 1963; Morris, 2017).

Plants that use CAM photosynthesis include epiphytes and succulents that grow in hot and extremely arid areas prone to water loss, such as deserts (Ehleringer and Cerling, 2002; Neales and Incoll, 1968). The paragraph above details how C_4 photosynthesis minimises photorespiration by separating the initial CO_2 fixation process from the Calvin Cycle in different cells. Plants that use the CAM photosynthetic pathway also minimise photorespiration by separating these processes. The difference is that, in CAM plants, this separation occurs between night and day (instead of different cells). At night, plants that use CAM photosynthesis collect CO_2 through their stomata into the mesophyll cells. PEPC fixes the CO_2 into a four-carbon compound (OAA) which is then converted into an organic acid that is stored in the vacuole of the plant. During the day, plants that use CAM photosynthesis close their stomata to reduce the loss of water. CAM plants photosynthesise during the day even though their stomata are closed. Plants that use the CAM photosynthetic pathway use the organic acids made at night to release CO_2 for the Calvin Cycle to begin. CAM plants are similar to C_4 plants, in that CO_2 is concentrated around RuBisCo, this reduces the possibility of RuBisCo binding to O_2, thus reducing photorespiration. Plants that use the CAM photosynthetic pathway tend to have $\delta^{13}C$ values that lie in between those observed for C_3 and C_4 plants. The combination of carbon isotopes and hydrogen isotopes enables researchers to distinguish CAM plants from those that use C_4 photosynthesis (Ehleringer, 1991; Kelly, 2000; Lajtha and Marshall, 1994; Sternberg, 1989). It is difficult to distinguish between the two with only carbon isotopic analysis.

There are different modes of the CAM photosynthetic pathway: obligate CAM, facultative CAM, CAM-idling and CAM-cycling (Kerbauy *et al*., 2012). In obligate CAM, plants only open their stomata and assimilate organic acids at night regardless of water availability or other favourable conditions (Kerbauy *et al*., 2012; Kluge and Ting, 1978). Facultative CAM plants are able to switch from the C_3 photosynthetic pathway to CAM, depending on environmental conditions, such as drought (Kerbauy *et al*., 2012). Plants that employ CAM-cycling do not open their stomata at night, the accumulated diurnal CO_2 is recycled by respiration (Herrera, 2009; Kerbauy *et al*., 2012). Plants employing the CAM-idling mode do not open their stomata during the day and at night, similar to CAM-cycling, the recycling of respiratory CO_2 is used to accumulate organic acids (Herrera, 2009; Kerbauy *et al*., 2012).

Isotope fractionation is greater in the C_3 photosynthetic pathway compared with C_4 and CAM photosynthesis, largely because RuBisCo strongly favours $^{12}CO_2$ molecules (O'Leary, 1988; Sponheimer and Cerling, 2014). In C_4 and CAM photosynthesis, RuBisCo is localised in a relatively closed system that prohibits CO_2 enriched with the heavy isotope (^{13}C) from escaping into the atmosphere (Gannes *et al*., 1998). Plants that use the C_3 photosynthetic pathway have a mean $\delta^{13}C$ value of -28.77 ± 2.68‰ (1σ, n=3478) (Cornwell *et al*., 2017). $\delta^{13}C$ values for terrestrial C_3 plants range from -35 to -21‰ (Kelly, 2000). Plants that use the C_4 photosynthetic pathway have a mean $\delta^{13}C$ value of -12.90 ± 1.52‰ (1σ, n=137) (Cornwell *et al*., 2017). $\delta^{13}C$ values for terrestrial C_4 plants range from -14 to -10‰ (Kelly, 2000). Cerling *et al*. (2003) used $\delta^{13}C$ values in C_3 plants to show that species from a single ecosystem can vary depending on the season. They found that C_3 plants in the savanna (East Africa) had an average $\delta^{13}C$ value of -28‰ near the end of the rainy period, and -25‰ in dry conditions (Cerling *et al*., 2003).

Stable Carbon Isotopes in Animals

The carbon isotopic composition of an animal's tissues will reflect the $^{13}C/^{12}C$ ratio of the diet (Ambrose and Norr, 1993; DeNiro and Epstein, 1978). Different animals have different feeding strategies (e.g. grazers or browsers, selective or bulk feeders) and thus select different plants or parts of plants. As a result, in savanna environments, grazers (e.g. *Equus quagga*) will have higher tissue $^{13}C/^{12}C$ ratios than browsers (Sponheimer *et al*., 2003a). Herbivores will reflect the $\delta^{13}C$ values of the plants they eat, and carnivores will reflect the $\delta^{13}C$ values of the herbivores they consume. The study of $\delta^{13}C$ ratios in consumers enables researchers to differentiate between C_3-based and C_4-based diets. $\delta^{13}C$ values may vary depending on the nature of the environment and temperature. Higher $\delta^{13}C$ values are recorded in animals that live in regions with relatively higher temperatures and arid conditions (Kohn, 2010).

Carbon isotope values can vary in different body tissues (e.g. blood, fat, muscle, hair, teeth and bone). Different time periods of diet are represented in different tissues. Muscle, hair and faeces represent shorter time periods (a few years, months or days), with faeces indicating the diet of an organism during its most recent past – a few hours for small insects, and several days for large mammalian herbivores (Tieszen *et al*., 1983). Teeth reflect the diet consumed during the period of tooth formation, with some contribution from recycled carbon from catabolised tissues. In most animals, teeth form during early life. In domestic horses, the first tooth (M1) starts to form about two weeks after birth and erupts at approximately 8 to 12 months of age, the P4 is the last tooth to erupt at 4 years of age (Hoppe *et al*., 2004). Bone (continually remodelling) represents a long-term average of diet over multiple years (Lee-Thorp, 2008).

Application

$\delta^{13}C$ values are used to distinguish between predominantly C_3 diets, predominantly C_4 diets and intermediate diets (DeNiro and Epstein, 1978). In savanna environments, where trees and shrubs are C_3 and grasses are C_4, this amounts to classifying herbivores as browsers, grazers or mixed feeders (Cerling *et al*., 2003; Codron *et al*., 2005a; Gagnon and Chew, 2000; Sponheimer *et al*., 2003a). Sponheimer *et al*. (2003a) used stable carbon isotope ratios to investigate the relationship between the proportion of grass in bovid diets and the degree of molar hypsodonty. Their results supported the positive correlation reported previously (Janis, 1988; Reed, 1996). Stable carbon isotope ratios in archaeological and fossil samples can also be used as palaeodietary and palaeoenvironmental tracers. Cerling *et al*. (1997) analysed fossil tooth enamel in grazers from all over the world to show that although C_4 grasses evolved a long time ago, they only became common worldwide between 8 and 6 million years ago.

This thesis focuses specifically on African (especially southern African) large mammals. Previous isotope studies of southern African large mammals include Codron (2006), who used isotopes (mainly carbon isotopes) to assess the diets of African savanna ungulates in order to test competing models of herbivore evolution (Ambrose and DeNiro, 1986; Botha and Stock, 2005; Cerling *et al*., 2003; Codron *et al*., 2005a; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a; Tieszen *et al*., 1979). Codron *et al*. (2011a) used carbon isotope analysis of faeces to investigate variations in graze and browse proportions in African elephant diets. They found that unlike many generalist herbivores, who feed on various foods to achieve ‘dietary diversity’ (Sorensen *et al*., 2005; Wiggins *et al*., 2006), elephants can switch between graze and browse. Such studies dedicated to the impact on vegetation communities are relevant to the management and conservation of elephant populations.

3.1.2. Stable Nitrogen Isotopes

Nitrogen is a chemical element with two stable isotopes, nitrogen-14 (^{14}N) and nitrogen-15 (^{15}N). ^{14}N has seven protons, seven electrons and seven neutrons, ^{15}N has seven protons, seven electrons and eight neutrons. Stable nitrogen isotope ratios ($^{15}N/^{14}N$) can be expressed as $\delta^{15}N$ relative to the ratio in atmospheric nitrogen (N_2), which makes up approximately 78% of the earth’s atmosphere. Nitrogen-14 makes up

98.93% of all natural nitrogen, while nitrogen-15 only makes up 1.1%. Nitrogen is the building block of DNA (nucleotides) and protein (amino acids).

Stable Nitrogen Isotopes in Plants

N_2 is not available for plants to use, it is an unreactive molecule held by strong triple covalent bonds. In order for nitrogen to be usable by plants, it has to be fixed. The nitrogen cycle involves the movement of nitrogen from the atmosphere to the soil, then the animal and back to the atmosphere. Nitrogen is absorbed into the soil and combined with hydrogen by free-living bacteria to make ammonia (NH_3), in a process called nitrogen fixation (Cocking, 2000; Osadebe *et al*., 2022; Postgate, 1998). The bacteria are found either in the soil or associated with the roots of some plants. Some plants can use ammonium ions (NH_4^+) as a source of nitrogen. Nitrifying bacteria in the soil combine ammonia with oxygen to make nitrites and nitrates, with the latter being a major form of nitrogen for assimilation by plants. Some nitrate is returned to the atmosphere as N_2 gas via a process called denitrification. ^{14}N is preferentially converted to N_2 gas, leaving the soil enriched in the heavy isotope (^{15}N). This nitrogen cycle is similar in both aquatic and terrestrial environments. $\delta^{15}N$ values vary in different parts of the plant, and between different plants (Handley *et al*. 1999). Nitrogen is incorporated into the body tissues of animals as they consume plant foods. When plants and animals die and decompose, ammonia (and other forms of fixed nitrogen) is returned to the soil by decomposers, this form of nitrogen is then recycled. Isotopic fractionation is lower during nitrogen fixation relative to photosynthesis. Evans (2007) reported a factor of 0‰ to 3‰ during the nitrogen fixation process.

There is a negative correlation between leaf $\delta^{15}N$ values and annual precipitation. Many studies observed that plants that grow in warm, dry areas have higher $\delta^{15}N$ values than those that grow in cooler, wetter conditions (Austin and Vitousek, 1998; Handley *et al*., 1999; Martinelli *et al*., 1999; Pardo *et al*., 2006; Szpak, 2014) probably because warm, dry environments tend to lose more nitrogen from the soil through denitrification and are thus left enriched in ^{15}N. $\delta^{15}N$ values can also be affected by the soil depth at which nitrogen is absorbed. Deep-rooted plants tend to have more positive $\delta^{15}N$ values than shallow-rooted plants (Amundson *et al*., 2003; Hobbie and Hogberg, 2012).

Stable Nitrogen Isotopes in Animals

$\delta^{15}N$ values track the protein in the diet of an animal, since nitrogen is not found in carbohydrates or lipids (Koch, 2007; Schoeninger, 1985; Schoeninger and DeNiro 1984). $\delta^{15}N$ values can help to identify the trophic level of organisms (Hedges and Reynard, 2007; Roth and Hobson, 2000; Schoeninger *et al*., 1983). Early studies proposed that nitrogen trophic fractionation (stepwise enrichment), sometimes called the nitrogen discrimination factor ($\Delta^{15}N$) is approximately 3-4‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schulting, 1998). This means that $\delta^{15}N$ values of organisms in a food web are generally 3-4‰ more positive at each successive trophic level, therefore, organisms higher up the food chain are more enriched in ^{15}N (Minagawa and Wada, 1984; Reitsema and Holder, 2018). Subsequently, we have learned that there can be more variation within trophic levels than initially realised: Sponheimer *et al*. (2003c) reported a difference of up to 3.6‰ in the hair $\delta^{15}N$ values of different herbivore species with identical diets, but different types of metabolisms/digestions. The amount of protein in the diet also influences trophic fractionation (Sponheimer *et al*. 2003c), although the effect is not seen in all studies (Ambrose, 2000). Caut *et al*. (2009) analysed and compared the mean nitrogen discrimination factor for different tissues (plasma, liver, muscle, blood, collagen, feather and hair) of different taxa (mammals, birds and fish) and found that there are significant differences. It is important to consider such factors when interpreting $\delta^{15}N$ values.

Stable nitrogen isotopes can be used to investigate the proportion of terrestrial and marine foods in a diet (Schoeninger *et al*., 1983). Marine systems are enriched in ^{15}N because denitrification mostly occurs in the oceans (i.e. the conversion of nitrate to nitrogen gas, in which ^{14}N is favoured) (Brunelle *et al*., 2007; Sweeney *et al*., 1978; Waser *et al*., 1998; Wu *et al*., 1997). $\delta^{15}N$ values of marine organisms therefore tend to be more positive than those of terrestrial organisms (Ambrose, 1991; Amir *et al*., 2019; Gao *et al*., 2012; Gearing, 1988; Lamb *et al*., 2006; Schoeninger *et al*., 1983). In addition, marine food chains tend to have more trophic levels than terrestrial ones (Reitsema and Holder, 2018; Schoeninger and Moore, 1992), so higher trophic level marine organisms reflect multiple stepwise enrichment in ^{15}N.

Since $^{15}N/^{14}N$ ratios in soils and plants are affected by MAP (Mean Annual Precipitation) and MAT (Mean Annual Temperature), as outlined above, these trends are also reflected in animals. In general, fauna show higher $\delta^{15}N$ values due to increases in nutritional stress, protein intake, water stress, age and aridity (Ambrose and DeNiro, 1986; Heaton, 1987; Hobson *et al*., 1993; Kelly, 2000; Richards and Hedges, 2003; Schoeninger et al., 1997; Schwarcz *et al*., 1999; Sealy *et al*., 1987; Sponheimer *et al*., 2003c). It is difficult to distinguish between terrestrial and marine proportions in a diet in arid regions because $\delta^{15}N$ values in animals tend to be more elevated in such conditions (Heaton *et al*., 1986; Sealy *et al*., 1987).

Ambrose and DeNiro (1986) stated that herbivores that are drought-tolerant (not dependent on water) have mean $\delta^{15}N$ values that are 2-4‰ higher than those that are obligate drinkers (mostly grazers). They interpreted this as being due to the loss of more ^{15}N-depleted urea (urea recycling), in order to conserve water in drought-tolerant animals (Ambrose and DeNiro, 1986). Drought-tolerant species are mainly browsers, i.e. consuming relatively deep-rooted plants, so this may also contribute to the pattern documented. In addition, $\delta^{15}N$ values may be affected by animal fertilisers: $\delta^{15}N$ values are increased by up to 10‰ for plants that grow in fertilised soil and animals that consume them (Bogaard *et al*., 2013; Fraser *et al*., 2011).

Application

The combination of stable nitrogen and carbon isotopes produces a better reconstruction of diet than just one of these on its own (Makarewicz and Sealy, 2015; Schoeninger *et al*. 1983). This combination can be used to investigate the effects of nutritional stress and disease on tissues (Hatch, 2012; Hobson *et al*., 1993; Reitsema, 2013). Nitrogen isotope ratios are easily affected by nutritional stress, $\delta^{15}N$ values increase with an increase in the duration of starvation or fasting (Hatch, 2012; Martínez del Rio and Wolf, 2005). Hobson *et al*. (1993) discovered that starvation in birds resulted in body tissues (i.e. liver and muscle) that were more enriched in ^{15}N. Such studies have expanded the use of stable isotopes beyond the reconstruction of diet to understanding health and physiology.

Other uses of nitrogen isotope ratios include the assessment of diet quality. The finding that diet-hair fractionation in $\delta^{15}N$ was 2.3‰ greater in herbivores with a high-protein, as opposed to a low-protein diet (Sponheimer *et al*., 2003c) offers some hope for using $\delta^{15}N$ to assess diet quality, if other possible sources of variation can be controlled. Sponheimer *et al*. (2003c) also used nitrogen isotope ratios to show that not all large fractionations are climatically induced (e.g. by aridity), they found that inter-specific physiological differences may result in enriched $\delta^{15}N_{hair}$ values (i.e. 3.6‰ differences) even when individuals had a similar diet. Physiological factors include the efficiency with which nitrogen can be utilised by the animals (Cantalapiedra-Hijar *et al*., 2018), growth rate (Warinner and Tuross, 2010), and processes such as pregnancy and lactation.

3.1.3. Stable Oxygen Isotopes

The Earth's atmosphere includes approximately 21% oxygen gas (O_2). Oxygen is a chemical element with three stable isotopes, oxygen-16 (^{16}O), oxygen-17 (^{17}O) and oxygen-18 (^{18}O). This thesis focuses on ^{16}O and ^{18}O. ^{16}O has eight protons, eight electrons and eight neutrons, ^{18}O has eight protons, eight electrons and ten neutrons. Stable oxygen isotope ratios ($^{18}O/^{16}O$) can be expressed as $\delta^{18}O$ relative to VPDB (as in this thesis) or SMOW (Standard Mean Ocean Water), which is the standard that oceanographers and hydrologists use. Oxygen-16 makes up approximately 99.76% of all oxygen, while oxygen-18 makes up approximately 0.2%. Like many chemical elements, oxygen is found in different reservoirs (i.e. land surface, cryosphere, hydrosphere, atmosphere and biosphere), with ^{16}O and ^{18}O occurring in different proportions in these different reservoirs. Water bodies across the world may display differences in $\delta^{18}O$ values due to differences in origin and movement in the hydrological cycle (Valdivielso *et al*., 2020). Studying precipitation patterns can enable researchers to investigate $\delta^{18}O$ in water sources.

Hydrologic Cycle

The water cycle begins with the evaporation of water (H_2O) from the surface of the ocean, river or lake, followed by condensation of water vapour into clouds. As the temperature cools, this moisture returns to the earth's surface as precipitation (meteoric water). This may return to a body of water and in time evaporate back into the atmosphere, or the water may become groundwater, then run off into water bodies or go into an aquifer (groundwater reservoir). Isotope fractionation occurs as $H_2{}^{16}O$ and $H_2{}^{18}O$ evaporate from a body of water (e.g. ocean) into the clouds. $H_2{}^{16}O$ evaporates more quickly into the atmosphere because it is lighter, while $H_2{}^{18}O$ is the first to fall as precipitation (Dansgaard, 1964). As a result, the ocean is more enriched in ^{18}O while the atmosphere now contains more ^{16}O. Studies in East Africa showed that seasonal pools and lakes exhibit $\delta^{18}O$ values above 0.0‰ (Barton *et al*., 1987; Janzen *et al*., 2020). In contrast, streams, springs and rivers (less evaporated) have $\delta^{18}O$ values around -3‰ (Cerling *et al*., 2008; Janzen *et al*., 2020; Levin *et al*., 2009).

Some of the factors that affect $\delta^{18}O$ values in precipitation include latitude, temperature, humidity, altitude and seasonal effect (Marshall *et al*., 2007; McGuire and McDonnell, 2007). $\delta^{18}O$ values in precipitation are lower at higher latitudes due to lower temperatures resulting in less evaporation of $H_2{}^{18}O$ (Marshall *et al*., 2007; McGuire and McDonnell, 2007). Relative humidity and temperature are inversely proportional. As a result, low temperatures are accompanied by high relative humidity and thus lower $\delta^{18}O$ in precipitation because these conditions result in less H_218O evaporating (McGuire and McDonnell, 2007). The higher the altitude, the more negative the $\delta^{18}O$ values. Samples from boreholes or springs around Mmabolela in 2006 were collected by the University of Cape Town. The predicted $\delta^{18}O$ values of groundwater (relative to VSMOW) ranged from -4.68‰ to -3.31‰ (see Table 3.1 below) (Waterisotopes, 2023). The following equation from Brand *et al*. (2014) was used to correct these values for comparison with the tooth enamel $\delta^{18}O$ values in this thesis which are relative to VPDB:

$$\delta^{18}O_{x/VPDB} = 0.97001 \times \delta^{18}O_{x/VSMOW} - 29.99‰ \text{ (where } x \text{ represents sample } x\text{)}$$

Table 3.1: δ^{18}O of groundwater in areas around Mmabolela converted from the VSMOW to the VPDB reference scale. Data from Waterisotopes.org.

Site Name	Latitude	Longitude	Sample ID	Collection Date	Sample	δ^{18}O (‰) VSMOW	δ^{18}O (‰) VPDB
Usutu	-22.569167	28.621944	West_ZQMTUG2	2006/10/19 00:00	Borehole	-4.68	-34.53
Usutu	-22.570278	28.622222	West_ZQMTUG4	2006/10/19 00:00	Spring/Eye	-4.13	-34.00
Swartwater	-22.855556	28.202778	West_ZQMSWW1	2006/08/23 00:00	Borehole	-3.62	-33.50
Swartwater	-22.856667	28.21278	West_ZQMSWW2	2006/08/23 00:00	Borehole	-3.31	-33.20

Stable Oxygen Isotopes in Plants

Plants absorb water from the soil. This water is used during the photosynthesis process and to provide nutrients to leaves. Therefore, ^{18}O/^{16}O ratios in plants depend on the ^{18}O/^{16}O ratios of the water source (Barbour, 2007). Oxygen isotope fractionation in plants occurs mainly in leaves. $H_2{}^{16}O$ is favoured over $H_2{}^{18}O$ during transpiration. As a result, leaf water is enriched in $H_2{}^{18}O$ compared with meteoric water (Dongmann *et al*., 1974; Epstein *et al*., 1977; Sponheimer and Lee-Thorp, 1999; Sternberg, 1989; Yakir, 1992).

Helliker and Ehleringer (2002) found that δ^{18}O of bulk leaf water in C_3 dicots was less enriched than C_4 grasses due to the length of the blade in the latter. Leaf water is increasingly enriched from the base towards the tip of the leaf (i.e. the oldest part of the leaf) (Helliker and Ehleringer, 2002). However, woody stems generally do not lose water to transpiration, therefore, δ^{18}O values are similar to those observed in the roots. It is usually only in leaves that transpiration takes place and fractionation occurs (Barbour, 2007; Barbour *et al*., 2000). Some 'base-to-tip' differences in δ^{18}O were over 40‰ (Helliker and Ehleringer, 2002). However, these δ^{18}O values may vary depending on humidity. Sternberg *et al*. (1984) found a difference of 10‰ between the compositions of C_3 and C_4 plants in arid conditions, while Epstein *et al*. (1977) found a difference of less than 1‰ in cool and moist conditions.

Stable Oxygen Isotopes in Animals

$\delta^{18}O$ ratios in animal tissues depend on drinking water (usually meteoric water), food intake (for herbivores, mainly leaf water), and inhalation of atmospheric oxygen (Bryant and Froelich, 1995; Longinelli, 1984; Sponheimer and Lee-Thorp, 1999). Some of the ways in which oxygen is eliminated from the body include water vapour lost during panting and sweating, respiration (CO_2) and excreta (faeces and urine). Some of these processes play an integral role in determining the $^{18}O/^{16}O$ values of animals. For example, Sponheimer and Lee-Thorp (2001) reported that despite equal isotopic inputs, a species that sweats to cool down will have lower $\delta^{18}O$ values than a species that cools by panting.

Some studies reported more positive $\delta^{18}O$ values for animals that eat C_4 grasses compared to C_3 species in regions with low relative humidity (Helliker and Ehleringer, 2000; Kohn *et al*., 1996). Factors such as leaf length anatomy, preferential evapotranspiration and water-use efficiency result in these distinctions. Due to the enrichment of leaf water, higher $\delta^{18}O$ values are usually observed for browsers than grazers (Kohn *et al*., 1996; Sponheimer and Lee-Thorp, 1999). Many browsers are non-obligate drinkers and obtain much or all of their water from plant leaves, while obligate drinkers (including most grazers) obtain their water from an open water source (e.g. lakes, streams etc.). Therefore, $\delta^{18}O$ values of obligate drinkers will track drinking water instead of their food intake (Dansgaard, 1964; Kohn *et al*., 1996). Reid *et al*. (2019) found that non-obligate drinkers (dik-dik) recorded significantly higher tooth enamel $\delta^{18}O$ values than obligate drinkers (warthogs).

Applications

Stable oxygen isotope ratios can be used to reconstruct palaeoclimates and past environments (e.g., Ayliffe and Chivas, 1990; Blumenthal *et al*., 2017; Levin *et al*., 2006; Longinelli, 1984; Schoeninger *et al*., 2003). Oxygen isotope ratios can also be used to reconstruct diet and drinking patterns in animal species. Sponheimer and Lee-Thorp (1999) used $\delta^{18}O_{enamel}$ to investigate drinking behaviours, physiology and diet in fauna from Swartkrans and Equus Cave, South Africa. They found that browsers are more enriched in ^{18}O compared with grazers at both sites. Another study conducted in Morea Estate (South Africa) observed the highest $\delta^{18}O$ values for impala compared to other herbivores (hyena, aardvark, vervet monkey, giraffe, wildebeest, tsessebe, waterbuck and warthog) (Sponheimer and Lee-Thorp, 2001). However, most studies classified *A. melampus* as mixed-feeders (Cerling *et al*., 2003; Codron *et al*.,2007; Gagnon and Chew, 2000; Hofmann, 1973; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a). Sponheimer and Lee-Thorp (2001) suggested that impala from Morea Estate are the most enriched in ^{18}O because they consume more isotopically enriched leaf water and are able to endure high body temperatures (Lee-Thorp *et al*., 2003; Sponheimer and Lee-Thorp, 2001).

The combination of oxygen and carbon isotope ratios enables researchers to provide more detailed answers to complex questions. Tian *et al*. (2013) used $\delta^{13}C$ and $\delta^{18}O$ ratios of tooth enamel of mammals from Jiangsu Province (China) to reconstruct environments, diets and investigate patterns of seasonal variation. Carbon isotope ratios showed that deer and wild pigs primarily ate C_3 plants. In addition, oxygen isotope ratios showed that these two species had different water sources, therefore, they most likely occupied different niches within the same eco-environment (Tian *et al*., 2013). Tian *et al*. (2013) listed two more possible causes for the differences observed in $\delta^{18}O$ values between these two species. The first factor is that these species obtained water from different sources (i.e. leaf water is more enriched in ^{18}O). The second factor is that these species may have eaten different parts of the plant (i.e. different parts of the plant and tissues may have different $\delta^{18}O$ values).

Some studies show that oxygen isotopic compositions may vary in tooth micro samples depending on the season (Zazzo *et al*., 2006; Hoppe *et al*., 2005). Seasonality is recorded in periods where low and high $\delta^{13}C_{enamel}$ and/or $\delta^{18}O_{enamel}$ values alternate at a constant frequency (i.e. cyclicity). In herbivores, $\delta^{18}O$ values of enamel may reflect the meteoric water isotopic composition, and thus reflect the environment and climate during enamel formation (Stuart-Williams and Schwarcz, 1997; Tian *et al*., 2013).

Blumenthal *et al*. (2019) found that in equids, adjacent serial samples (i.e. along tooth row) from the same individual may record different isotopic ratios because they represent different growth periods. Green *et al*. (2018) observed different isotopic signals of body water among sheep (*Ovis aries*) eating an identical diet (food and water). Therefore, individuals living in the same ecosystem and consuming the same food and water may have different isotopic ratios. Such differences may be caused by behavioural, genetic and physiological variability between individuals (Blumenthal *et al*., 2019).

There are a number of studies in the literature that use oxygen isotope ratios of serial samples to investigate seasonality. However, not all of these studies have successfully detected clear seasonal variation in $\delta^{18}O$ values. Tornero *et al*. (2018) used $\delta^{13}C$ and $\delta^{18}O$ analyses to investigate seasonal patterns in modern sheep tooth enamel. In order to interpret the tooth serial samples, this study made use of seasonal patterns of $\delta^{18}O_{\text{meteoric water}}$ (reference data), daily and monthly precipitation records, vegetation changes and $\delta^{13}C$ of surrounding plants. Seasonality was successfully detected, with clear minimum and maximum peaks reflecting cyclical fluctuations. High $\delta^{18}O_{\text{meteoric water}}$ values were observed for warmer months, and low values for cold months. The $\delta^{13}C$ and $\delta^{18}O$ values of the serial enamel bioapatite samples follow a similar pattern (Tornero *et al*., 2018). Such studies where serial sampling of enamel has yielded clear patterns sample the enamel at high resolution (i.e. they take many samples very close together up the height of the tooth crown).

Norwood *et al*. (2023) investigated precipitation seasonality patterns in serial enamel samples of modern zebra (*E. quagga*) from a wildlife conservancy in Kenya. Isotope values were not plotted against distance (crown to root), but rather against the sample age. Additionally, a linear model was applied to the serial enamel samples to determine cyclicity in isotope ratios due to the unknown birthdates of zebra (Norwood *et al*., 2023). Due to noise found in the population, Norwood *et al*. (2023) did not detect any seasonality. This region has two rainy seasons per year, making this kind of study more challenging. The temperature time series pattern was different from the precipitation – the minimum and maximum values did not fluctuate consistently. Norwood *et al*. (2023) note that $\delta^{18}O$ values are more difficult to interpret than $\delta^{13}C$ because as stated above, there are more factors to be considered (e.g. $\delta^{18}O$ values of water source and enamel etc.). Different water sources may have different $\delta^{18}O$ values. The zebra in their study area drank from multiple water sources (ponds, rivers and dams), which further complicates the study.

Other applications in the field include: the use oxygen isotope ratios to investigate weaning and breastfeeding patterns, and to identify human migrants (Britton *et al*., 2015; Lightfoot and O'Connell, 2016); and the analysis of $\delta^{18}O$ in opercula to investigate SST (Sea Surface Temperatures) (Galimberti *et al*., 2017).

3.1.4. Diet-to-Tissue and Tissue-to-Tissue Differences

Most consumer tissues are enriched in ^{13}C relative to diet (DeNiro and Epstein, 1977). In mammalian herbivores, bone collagen is approximately 5‰ more enriched than diet (Ambrose and DeNiro, 1986; Ambrose *et al*., 2003; Codron *et al*., 2018; Krueger and Sullivan, 1984; Lee-Thorp *et al*., 1989; Vogel, 1978; Vogel and van der Merwe, 1977). Bioapatite is 12-14‰ more enriched than diet (Lee-Thorp *et al*., 1989; Passey *et al*., 2005). O'Connell *et al*. (2001) studied modern human isotopic data and found that bone collagen is enriched by +0.86‰ in $\delta^{15}N$ and +1.4‰ in $\delta^{13}C$ compared to hair keratin from the same individual. Bone collagen and hair keratin have different amino acid compositions, which might account for the differences in $\delta^{13}C$ values. They also found that $\delta^{13}C_{\text{nail keratin}}$ and $\delta^{13}C_{\text{hair keratin}}$ from the same individual are similar and do not show a significant difference. However, nail keratin is enriched by +0.65‰ in $\delta^{15}N$ compared to hair keratin (O'Connell *et al*., 2001).

Stable carbon isotope analysis can be applied to various materials, such as faeces, hard tissue (tooth enamel and bone collagen) and hair. Faeces and hair, in particular, are good materials for studying the nutritional ecology of bovids because they are easily obtainable (Schoeninger *et al*., 1999; Sponheimer *et al*., 2003b). Faeces reflect the diet of an animal over a short period of time (Codron *et al*., 2005a; Sponheimer *et al*., 2003b). However, dietary predictions might be compromised because faeces incorporate both waste and undigested plant materials (Codron *et al*., 2005a). Jones *et al*. (1979) reported that it took approximately six days for faeces to reflect the new dietary $\delta^{13}C$ when steers switched from a C_4 to a C_3 diet. Codron and Brink (2007) suggested that it is important to consider the differences between collagen and faecal $\delta^{13}C$ before interpreting the data. Faeces are ~0.9‰ depleted in ^{13}C compared with diet (Ambrose and Norr, 1993). For example, a diet with a $\delta^{13}C$ of -13‰ would yield a $\delta^{13}C$ value close to -8‰ for dentine collagen and a $\delta^{13}C$ value close to -13.9‰ for faeces. Hair sampled in bulk reflects the diet consumed over the period of hair growth (Sponheimer *et al*., 2003b). The combination of faeces, hair and hard tissues enables researchers to investigate dietary information through a wider range of space and time (Codron *et al*., 2005a).

3.1.5. Quality Control Criteria

DeNiro (1985), Ambrose (1990), van Klinken (1999) and Guiry and Szpak (2020) listed several criteria to assess the quality of collagen in samples (i.e. diagenetic alteration and/or contamination). Diagenesis refers to chemical and physical changes that occur due to post-depositional alteration. Diagenetic alteration may affect isotopic signatures, resulting in extremely low $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ values (Ambrose, 1990). The pre-treatment of the samples can mitigate the effect of diagenetic alteration (Ericson, 1985). The quality control criteria include nitrogen content (wt %N), carbon content (wt %C) and elemental C:N (Ambrose, 1990; Guiry and Szpak, 2020; van Klinken, 1999). Guiry and Szpak (2020) observed that modern samples with reliable collagen should have a carbon content of 41.91 ± 0.39%, and a nitrogen content of 15.40 ± 0.20%. Van Klinken (1999) stated that ‘intact collagen’ will have a nitrogen content between 11 and 16 wt %. For modern samples, the elemental C:N of ‘good’ collagen ranges from 3.1 to 3.5 (van Klinken, 1999). Ambrose (1990) and van Klinken (1999) were concerned mainly with developing criteria for deciding when collagen from archaeological samples was sufficiently well-preserved to yield reliable results. Guiry and Szpak (2020) were concerned with modern samples, so their criteria are more stringent. Cape porcupine (*Hystrix africaeaustralis*) quills are more similar to human nail keratin than hair keratin. O’Connell *et al*. (2001) reported atomic C/N ratios that range from 3.0-3.8 for both modern hair and nail keratin samples.

3.2. Fauna and Ecology

In African savannas, plants that use C_3 photosynthesis consist of shrubs, forbs and almost all trees, while nearly all grasses and sedges use the C_4 photosynthetic pathway (Codron *et al*., 2005a; Sponheimer *et al*., 2003a; Vogel *et al*., 1978). Since C_3 and C_4 plants have distinct, non-overlapping ranges of $\delta^{13}C$ values, many studies use stable carbon isotope measurements of consumers to investigate animal ecology, especially the consumption of browse vs graze (Ambrose and DeNiro, 1986; Cerling *et al*., 2003; Lee-Thorp and van der Merwe, 1987; Schoeninger *et al*., 1999; Sponheimer *et al*., 2003a; Vogel, 1978). Sponheimer *et al*. (2003a) stated that although observational studies provide valuable information, stable carbon isotopes can be used to quantify the relative amounts of browse and graze consumed by an animal.

The Bovidae family comprises approximately 137 species throughout the African continent together with species found in North America, Europe and Asia (Gagnon and Chew, 2000; Wilson and Reeder, 1993). Most modern bovid species are endemic to Africa where they are the most observable mammals in savannas (Spinage, 1986; Sponheimer *et al*., 2003a). Studying bovid species helps researchers to better understand neo-ecology and palaeoecology (Sponheimer *et al*., 2003a). Multiple papers have been published on bovid ecology over the years, but there is still an ongoing debate about the diets of some species. This study will compare the following studies on bovid species: Gagnon and Chew (2000); Cerling *et al*. (2003); Sponheimer *et al*. (2003a); Skinner and Chimimba (2005) and Codron *et al*. (2007).

Different groups of bovid species have craniodental and digestive adaptations to facilitate the consumption and digestion of the types of food they consume (Janis, 1995; Mendoza *et al*., 2002; Shipley, 1999). Janis (2008) stated that grass contains silica which results in greater tooth wear. Many studies have shown that the degree to which teeth are high-crowned (hypsodont) corresponds with the proportion of abrasive graze in the diet (Codron *et al*., 2007; Janis, 1988; Janis, 2008). Animals other than grazers may also have high-crowned cheek teeth, including small-bodied browsers such as duikers. Janis (1988) suggested that these were previously mixed feeders that returned to consuming mainly browse in recent evolutionary times. Janis (2008) stated that grazers tend to have larger chewing muscles because grass is more abrasive and fibrous than browse. Therefore, grazers have a longer masseteric fossa and a broader muzzle to aid with massive bites (Janis, 2008). Grazers tend to have thicker and longer enamel than browsers (Archer and Sanson, 2002; Heywood, 2009). Archer and Sanson (2002) did not observe any traits that were unique to intermediate feeders, most species had a craniodental morphology that resembled browsers, with the exception of impala (*Aepyceros melampus*), which displayed an occlusal surface similar to grazers (four shearing ridges).

Ruminants can be divided into categories based on the morphology of their stomachs, which are structured differently to accommodate different diets (Hofmann and Stewart, 1972; Shipley, 1999). Grazers have to digest more fibre than browsers (Pérez-Barbería *et al*., 2004). Some studies (Clauss *et al*., 2006; Hofmann, 1968; Hofmann and Stewart, 1972; Shipley, 1999) showed that grazers tend to have a larger omasum and smaller reticulum than browsers. Gagnon and Chew (2000) suggested that there is a relationship between body mass and diet, with grazers tending to be bigger than browsers and frugivores. In contrast, Sponheimer *et al*. (2003a) found no correlation between body mass and the proportion of monocotyledons consumed by bovid species ($r^2 = 0.076$; $P = 0.164$), because browsers are found at all body sizes.

Researchers group bovid species according to their dietary preferences in several ways. Gagnon and Chew (2000) attempted to determine the dietary preference of 78 species of extant African Bovidae using information compiled from 100 published primary sources. This information was either obtained through observational studies, faeces analysis or gut anatomy. They avoided the traditional ‘browser or grazer’ classification system because it was “too simplistic for most purposes”. Instead, they considered six dietary categories: frugivores, browsers, generalists, browser–grazer intermediates, variable grazers, and obligate grazers based on the estimated proportions of three food types: fruits, dicotyledons (leaves, shrubs, buds etc.), and monocotyledons (grasses, reeds etc.). This enabled Gagnon and Chew (2000) to provide more specific dietary preferences than most earlier studies, e.g. blue wildebeest (*Connochaetes taurinus*) are obligate grazers; gemsbok (*Oryx gazella*) are variable grazers.

Cerling *et al*. (2003) investigated the diets of 37 East African bovid species using stable carbon isotopic analysis of keratin and tooth enamel. These authors classified the bovid species as follows: hypergrazers (diet consists of more than 95% C_4 grass), grazers (diet consists of between 70 and 95% C_4 grass), mixed feeders (diet consists of more than 30% C_4 grass and more than 30% C_3 browse), browsers (consume between 70 and 95% C_3 browse), and hyper browsers or frugivores (consume more than 95% C_3 browse or fruit).

Sponheimer *et al*. (2003a) carried out a similar study in southern Africa (Zimbabwe, South Africa, Namibia, Malawi and Botswana), measuring $\delta^{13}C$ in bone collagen, keratin and tooth enamel from 27 bovid species collected during the 30 years before 2003. They tested the dietary information in Gagnon and Chew (2000) and compared it with the isotopic results from East Africa (i.e. Cerling *et al*., 2003). Sponheimer *et al*. (2003a) reported bovid diets as percentages of C_3 and C_4 foods. Their C_3 category combined fruits and dicotyledons, considered separately by Gagnon and Chew (2000) but with similar $\delta^{13}C$ values. For example, if Gagnon and Chew (2000) reported that a certain bovid species ate 20% fruits, 30% dicotyledons, and 50% monocotyledons, Sponheimer *et al*. (2003a) converted these to 50% C_3 and 50% C_4 foods. Stable carbon isotopes indicated that the difference in diet estimations between South Africa and East Africa is less than 10% for all the bovid species observed except for the sitatunga (*Tragelaphus spekii*) (Cerling *et al*., 2003). Sponheimer *et al*. (2003a) used the results to investigate the relationships between body mass, bovid species diets and morphology. Sponheimer *et al*. (2003a) stated that Gagnon and Chew (2000) made the mistake of assuming that the behaviour of the populations observed was a representation of the species as a whole.

Codron *et al*. (2007) investigated the diets of savanna ungulates from the Kruger National Park (South Africa) using faecal $\delta^{13}C$. The results confirmed the following dietary predictions based on the literature: browsers (bushbuck (*Tragelaphus scriptus*) and kudu (*Tragelaphus strepsiceros*)), mixed-feeders (impala (*Aepyceros melampus*)), and grazers (zebra (*Equus quagga*), warthogs (*Phacochoerus africanus*), blue wildebeest (*Connochaetes taurinus*) and waterbuck (*Kobus ellipsiprymnus*)). Of the 13 bovid species analysed, six had faecal $\delta^{13}C$ values that were different from those predicted in most literature – i.e. the faecal $\delta^{13}C$ indicated that C_4 proportions in the diet differed by more than 10% from those predicted by Gagnon and Chew (2000). These six include *Taurotragus oryx* (3% C_4, n=5) and *Aepyceros melampus* (60% C_4, n=606) (Codron *et al*., 2007), perhaps due to geographical differences in food preferences. For example, eland from southern Africa might prefer different foods than eland from East Africa (Codron *et al*., 2007).

Below is a brief overview of each of the mammal species analysed in this thesis, summarising relevant information about diet, habitat and habits, drinking behaviours and other relevant aspects.

3.2.1. *Bovidae*

Aepyceros melampus

Skinner and Chimimba (2005) describe impala (*Aepyceros melampus*) as a gregarious and diurnal species that tends to live close to water sources (within 1.6 km). When there is a lack of drinking water, they obtain water from succulent plants (Skinner and Chimimba, 2005; Sponheimer and Lee-Thorp, 2001). Sponheimer and Lee-Thorp (2001) stated that out of all the antelopes in their study, *A*. *melampus* obtained less of their water intake from drinking. *A. melampus* uses both sweating and panting for thermoregulation (Maloiy and Hopcraft, 1971).

Gagnon and Chew (2000) classified *A*. *melampus* as mixed feeders and reported a diet consisting of approximately 45% graze. This was supported by the stable carbon isotope data reported by Cerling *et al*. (2003) and Sponheimer *et al*. (2003a). Most literature classified *A*. *melampus* as intermediate mixed-feeders (Cerling *et al*., 2003; Codron *et al*.,2007; Gagnon and Chew, 2000; Hofmann, 1973; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a). Sponheimer *et al*. (2003a) found that *A*. *melampus* displayed the most variable $\delta^{13}C$ values, some individuals ate more than 90% graze, while others ate approximately 80% browse. These $\delta^{13}C$ values might be variable due to sex differences and because C_3 and C_4 plants are not equally available across the southern African region (Sponheimer *et al*., 2003a).

Skinner and Chimimba (2005) stated that *A. melampus* may browse or graze more depending on the season and locality. For example, in the eastern Limpopo Province, *A. melampus* diet consists of 90% grass in the wet season and 65% grass in the dry season (Pietersen *et al*., 1993). Hofmann and Stewart (1972) suggested that *A. melampus* are intermediate feeders that prefer to eat grass. Ables and Ables (1969) stated that *A. melampus* prefer to graze and become browsers during droughts when graze is reduced. Archer and Sanson (2002) classified *A. melampus* as intermediate feeders; they suggested that the impala changed between a graze and browse diet depending on the season (Skinner and Smithers, 1990).

Connochaetes taurinus

Blue wildebeest (*Connochaetes taurinus*) are water-dependent gregarious bovids that follow local rains to find fresh grazing (Skinner and Chimimba, 2005). Taylor and Robertshaw (1969) stated that despite having functioning sweat glands, *C. taurinus* actively pants for thermoregulation.

Gagnon and Chew (2000) classified *C. taurinus* as variable grazers with a diet that consists of 87.5% monocotyledons (grasses, reeds and sedges). Cerling *et al*. (2003) classified *C. taurinus* as hypergrazers, since according to their $\delta^{13}C$ results, *C. taurinus* diet consists of 100% C_4 grasses. Some sources suggested that *C. taurinus* incorporated approximately 10% C_3 foods into their diet (Codron *et al*., 2007; Sponheimer *et al*., 2003a). Other studies have shown that *C. taurinus* are predominant grazers throughout the year except during the wet season when they consume some dicotyledons (Gwynne and Bell, 1968; Stewart and Stewart, 1971).

Codron and Brink (2007) use stable nitrogen and carbon isotopic analyses in faeces and dentine collagen to compare two South African grazers, *C. taurinus* and black wildebeest (*Connochaetes gnou*). Codron and Brink (2007) indicated that *C. taurinus* displayed a more flexible diet, which is expected because of their ability to tolerate a broader habitat range. Faecal and dentine collagen $\delta^{13}C$ in *C. gnou* and *C. taurinus* both indicated a predominantly C_4 diet (Codron and Brink, 2007). Based on the results, the two species of wildebeest were classified as predominant grazers that also incorporated a small proportion of C_3 plants into their diet (Codron and Brink, 2007).

Kobus ellipsiprymnus

Waterbuck (*Kobus ellipsiprymnus*) are gregarious species with high water requirements (Skinner and Chimimba, 2005; Taylor *et al*., 1969). Melton (1978) stated that *K. ellipsiprymnus* select habitats based on the availability of water and the quality of grass. For thermoregulation, *K. ellipsiprymnus* sweat and pant equally (Sponheimer and Lee-Thorp, 2001; Taylor *et al*., 1969). This means that *K. ellipsiprymnus* are relatively depleted in ^{18}O since they retain most of the depleted H_2O that would be lost if cooling was primarily by panting (Wong *et al*., 1988).

Gagnon and Chew (2000) classified *K. ellipsiprymnus* as variable grazers with a diet that consists of 84% monocotyledons. Cerling *et al*. (2003) reported a diet that consists of 94% C_4 grass. Sponheimer *et al*. (2003a) categorised *K. ellipsiprymnus* as pure grazers, with a diet that consists of 100% C_4 foods (grasses and sedges). Skinner and Chimimba (2005) classified *K. ellipsiprymnus* as primary grazers that occasionally add browse and fruits to their diet. Hanks *et al*. (1969) added that these predominant grazers most often browse at the end of a dry season.

Oryx gazella

Gemsbok (*Oryx gazella*) are gregarious species predominantly found in open and arid habitats (Skinner and Chimimba, 2005). They are not water-dependent and respond to a rise in body temperature by panting (Skinner and Chimimba, 2005; Taylor, 1969). During dry seasons, when drinking water is scarce, *O. gazella* dig for succulent roots and bulbs (Diekmann, 1980; Skinner and Chimimba, 2005).

Gagnon and Chew (2000) classified *O. gazella* as variable grazers with a diet consisting of 5% fruits, 20% dicotyledons, and 75% monocotyledons. Cerling *et al*. (2003) classified *O. gazella* in East Africa as grazers with a diet that consists of approximately 88% C_4 grasses. Sponheimer *et al*. (2003a) categorised *O. gazella* as grazers, reporting a diet consisting of 81% C_4 grasses. Skinner and Chimimba (2005) classified *O. gazella* as “selective grass and roughage feeders”. Diekmann (1980) stated that even though *O. gazella* are predominantly grazers, they thrived on a diet consisting of browse and ephemeral plants when they were in areas with low grass cover. Knight (1990) recorded a diet consisting of 89% grass in *O. gazella* in the Kgalagadi Transfrontier Park (Botswana and South Africa) during summer, and 76% during the winter season.

Taurotragus oryx

Eland (*Taurotragus oryx*) are the largest antelopes in Africa, with a mean mass of up to 650 kg recorded from bushveld regions (Skinner and Chimimba, 2005). *T. oryx* are crepuscular, and active during the twilight period. Pappas (2002) stated that *T. oryx* are nomadic, they move from one region to another based on the season and availability of food. Even though *T. oryx* drink a lot of water when it is available, most of the water they consume come from their diet (Pappas, 2002; Skinner and Smithers 1990). For thermoregulation, *T.oryx* use sweating as a means of evaporative cooling (Finch, 1972).

Gagnon and Chew (2000) classified *T. oryx* as mixed-feeders (browser-grazer intermediates) with a diet consisting of 5% fruits, 45% dicotyledons, and 50% monocotyledons, but Skinner and Chimimba (2005) stated that it is a misconception to classify *T. oryx* simply as mixed-feeders. Hofmann and Stewart (1972) classified *T. oryx* as intermediate feeders that prefer shrubs, forbs and tree foliage. Sponheimer *et al*. (2003a) and Codron *et al*. (2007) categorised *T. oryx* in South Africa as browsers, both papers reporting a diet consisting of less than 10% C_4 (monocotyledons) grasses. Cerling *et al*. (2003) classified *T*. *oryx* as browsers with a diet that consists of approximately 18% C_4 grasses. This shows that *T*. *oryx* in East Africa ate more grass than their southern African equivalent (Cerling *et al*., 2003; Codron *et al*., 2007; D'Ammando *et al*., 2015; Sponheimer *et al*., 2003a), perhaps because of different vegetation composition and forage availability (D'Ammando *et al*., 2015; Wallington *et al*., 2007).

Hofmann (1989) suggested that *T. oryx* might switch from browsing to grazing throughout the year depending on the availability of local food. Studies conducted at S.A. Lombard Nature Reserve (North West Province, South Africa) suggested that *T. oryx* graze in summer and browse in winter (Buys, 1987; Skinner and Chimimba, 2005). *T. oryx* take advantage of fresh new grass that grows in summer (Buys, 1990; D'Ammando *et al*., 2015; Watson and Owen-Smith, 2000). Hejcmanová *et al*. (2020) randomly selected six male *T.oryx* which were exposed to two different diets, grass hay (graze) and browse. Based on the results, *T.oryx* is classified as 'strict browsers' that are nevertheless able to survive on a grass diet.

Hejcmanová *et al*. (2020) identified *T. oryx* as 'moose-type ruminants' according to their digestive physiology. The two possible categories for these ruminants were 'moose-type' and 'cattle-type'. In moose-type ruminants, there is not much difference in the flow of small particles and fluids out of the rumen. In cattle-type ruminants, smaller particles flow out of the rumen slower than fluids. Most browsers classify as ‘moose-type’ ruminants, while grazers and intermediate feeders classify as ‘cattle-type’ ruminants (Hejcmanová *et al*., 2020).

Tragelaphus scriptus

Bushbuck (*Tragelaphus scriptus*) are solitary species that inhabit regions that are close to permanent water sources (Skinner and Chimimba, 2005; Wilson and Child, 1964). *T. scriptus* can survive without drinking water, obtaining sufficient moisture from plants (Pallas, 1766). These medium-sized antelopes use sweating as a means of evaporative cooling. Gagnon and Chew (2000) classified *T. scriptus* as predominant browsers with a diet consisting of 10% monocotyledons. This is in good agreement with Codron *et al*. (2007), who estimated from $\delta^{13}C$ values of faeces that *T. scriptus* in the Kruger National Park are browsers that consume approximately 9% C_4 (monocots) foods.

Most studies support Gagnon and Chew (2008) in classifying *T. scriptus* as browsers that occasionally feed on a small proportion of monocotyledons (Allen-Rowlandson, 1985; Seymour, 2002; Skinner and Chimimba, 2005; Wilson and Child, 1964). Cerling *et al*. (2003) and Sponheimer *et al*. (2003a) classified *T. scriptus* as pure C_3 browsers (C_4 proportion in diet = 0%). *T. scriptus* are selective feeders that are flexible enough to survive in unfavourable environments (Simpson, 1974; Skinner and Chimimba, 2005).

Tragelaphus strepsiceros

Skinner and Chimimba (2005) describe greater kudu (*Tragelaphus strepsiceros*) as one of the most ‘resilient’ large-bodied mammals. *T*. *strepsiceros* are persistent even with rising pressure from hunters and settlements (Skinner and Chimimba, 2005; Tilahun, 2019). *T*. *strepsiceros* generally obtain water from waterholes and plants with high water content, such as tsama melons and cucurbits (Skinner and Chimimba, 2005). Large species like *T*. *strepsiceros* tend to cool themselves by sweating rather than panting (Robertshaw, 2006).

According to Gagnon and Chew (2000), the diet of *T*. *strepsiceros* consists of 30% fruits, 55% dicotyledons, and 15% monocotyledons. Cerling *et al*. (2003), Sponheimer *et al*. (2003a), and Codron *et al*. (2007) all report <10% monocotyledons in the diet. Some studies (Anon, 1960; Owen-Smith and Cooper, 1989) suggest that *T. strepsiceros* ate mainly shoots, fruits, shrubs, branches and trees. Wilson (1965) suggested that *T. strepsiceros* consumed mostly browse with a little grass during the rainy season. Several studies conducted in the southern African subregion show that *T*. *strepsiceros* eat a greater variety of browse than any other bovid (Skinner and Chimimba, 2005). Brynard and Pienaar (1960) recorded 148 plant species eaten by *T*. *strepsiceros* in Kruger National Park.

3.2.2. *Equus quagga*

Plains zebra (*Equus quagga*) are gregarious, water-dependent species that move seasonally or daily to find regions with palatable grasses and adequate water supplies (Skinner and Chimimba, 2005; Strani *et al*., 2019). This species is partial to habitats where water is available – they are obligate drinkers (Skinner and Chimimba, 2005; Stevenson-Hamilton, 1947). All equids (including *E. quagga*) sweat to keep cool (Cobb and Cobb, 2019; Jenkinson *et al*., 2006). Codron *et al*. (2007) classified *E. quagga* from the Kruger National Park as grazers, with a diet consisting of 92% C_4 grass. Skinner and Chimimba (2005) classified *E. quagga* as predominant grazers that occasionally browse when forced into unusual habitats.

3.2.3. *Hystrix africaeaustralis*

Cape porcupines (*Hystrix africaeaustralis*) are the largest rodents in southern Africa (Skinner and Chimimba, 2005). *H. africaeaustralis* live up to 10 years in the wild and 20 years in captivity (Ellerman, 1940; van Aarde, 1987). They are monogamous species that are primarily nocturnal, although they have been reported to occasionally sunbathe (Skinner and Chimimba, 2005). *H. africaeaustralis* have bodies that are covered with spines and quills. Pigozzi (1988) marked porcupine quills and observed how long they took to fall off. Most were lost in 5-7 months, with only one left after 11 months. However, it is unknown how long it takes for the quill to form. In summer, when water is in short supply, *H. africaeaustralis* eat plants with high water content, such as Hyacinthaceae bulbs (Barthelmess, 2006). Most rodents cannot sweat or pant effectively (Mancinelli, 2010; Schmidt-Nielsen, 1975). *H. africaeaustralis* can regulate body temperature during winter and summer, they thermoregulate through huddling (Barthelmess, 2006; Skinner and Chimimba, 2005; van Aarde, 1987).

Skinner and Chimimba (2005) stated that *H. africaeaustralis* are predominant vegetarians with a diet that consists of tubers, roots and bulbs, i.e. C_3 plants. Some studies have reported that *H. africaeaustralis* nibble on bones and carrion (Duthie and Skinner, 1986; Pokines *et al*., 2017; Skinner and Chimimba, 2005). *H. africaeaustralis* are regarded as pests by farmers because they are destructive eaters that damage crops and debark trees (Barthelmess, 2006; de Villiers and van Aarde, 1994; Skinner and Chimimba, 2005).

3.2.4. *Papio ursinus*

Chacma baboons (*Papio ursinus*) are gregarious species that occupy a certain space based on the availability of water (Skinner and Chimimba, 2005; Stoltz and Saayman, 1970). Baboons are obligate drinkers (Funkhouser *et al*., 1967). In arid conditions, baboons drink water almost daily (Barton *et al*. 1992; Stoltz and Saayman 1970). Barton *et al*. (1992) stated that all baboons drink meteoric water (i.e. evaporation insensitive). Baboons primarily sweat to maintain core temperatures (Mitchell *et al*., 2009; Newman *et al*., 1970). Other studies found that baboons may dissipate heat by panting (Barton *et al*., 1992; Funkhouser *et al*., 1967).

P. ursinus are omnivores that feed on leaves, bulbs, rhizomes, tubers, grass, invertebrates and fruits (Moolman and Breytenbach, 1976; Skinner and Chimimba, 2005). De Vore and Hall (1965) mentioned that it is difficult to list the foods that *P. ursinus* eat because the list is so long. Codron *et al*. (2008a) used stable isotope data to study baboon feeding ecology in South Africa in order to learn more about early hominins occupying similar habitats. The results confirmed that baboons and early hominins had a diet that consists of a significant amount of C_4 food items (Codron *et al*., 2008a).

3.2.5. *Phacochoerus africanus*

Although common warthogs (*Phacochoerus africanus*) are found in areas with water nearby, water availability is not an important requirement for their habitat (Skinner and Chimimba, 2005). Drinking activities were observed throughout the day, especially after noon (Cumming, 1975). *P. africanus* are diurnal species that remain in holes at night for protection from predators and unfavourable climates (Skinner and Chimimba, 2005; Stevenson-Hamilton, 1947). Cumming (1975) found that there was a decrease in the amount of time warthogs spent basking in the sun when temperatures were high. Cumming (1975) stated that wallowing is a thermoregulatory function in *P. africanus*. There is a positive correlation between the frequency of wallowing (lying or rolling in the mud to keep cool) and the average temperature of the environment (observed at 14:00).

Codron *et al*. (2007) classified *P. africanus* from the Kruger National Park as grazers, with a diet consisting of 91% C_4 food. Skinner and Chimimba (2005) stated that *P. africanus* are generally vegetarians who favour freshly sprouted grass after the ground has been burnt. *P. africanus* tend to consume a wide range of grass species in summer (wet season) and feed on bulbs, rhizomes and roots in winter (Cumming, 1975).

3.2.6. *Stigmochelys pardalis*

The leopard tortoise (*Stigmochelys pardalis*) is the largest tortoise species in southern Africa. This is a diurnal species found in a range of habitats across Africa, ranging from semiarid to savannah (Boycott and Bourquin, 2000), although it tends to avoid very hot and arid conditions (McMaster and Downs, 2013). *S. pardalis* is ectothermic, depending on the environment to regulate body temperature (McMaster and Downs, 2013; Rose and Judd, 1975). McMaster and Downs (2013) investigated seasonal and daily activities of leopard tortoises in the semi-arid Nama-Karoo (South Africa). They observed that this species drinks primarily in summer during the afternoon. No drinking was recorded in winter (McMaster and Downs, 2013).

S. pardalis is a generalist herbivore that feeds on a variety of grasses, occasionally eating forbs, legumes and fruit depending on the availability of species (Hailey, 1997; McMaster and Downs, 2008; Milton, 1992). Many studies have reported that tortoises play an important role in dispersing seeds over long distances because many types of seeds are not digested in their gut (Blake *et al*., 2012; Falcón *et al*., 2020; Strong and Fragoso, 2006).

3.2.7. Niche Dynamics

The type and amount of food an animal consumes will determine its interaction with and the effect it will have on other species around it (Potter *et al*., 2022). A niche refers to the role a species plays in a community. This includes how organisms interact with the environment and one another (i.e. competition or predation). Niche partitioning is the process in which competing organisms use the environment (i.e. resources) in different ways in order to co-exist. Competition and the difference in resource use in a habitat lead to a rise in isotope niche dynamics across populations (Codron *et al*., 2011b). Codron *et al*. (2023) investigated how multiple species of large mammal herbivores coexist in a single community. Species with similar niches may drive each other to extinction (Codron *et al*., 2023). However, species with similar competitive abilities are capable of coexisting (Codron *et al*., 2023).

Ecologists are interested in sympatric large mammalian herbivores from Africa because they are diverse and have different diets (Potter *et al*., 2022). Ecological diversity extends beyond the classification of large herbivores as browsers, grazers or intermediate feeders. Other factors that may result in different biomass and qualities of forage include the differences in the species' gut and craniofacial morphology (Bell, 1971; Cromsigt and Olff, 2006; Janis and Ehrhardt, 1988; Potter *et al*., 2022). Lastly, the differences in herbivore height may result in individuals feeding on different plant species at different heights (du Toit, 1990; du Toit and Olff, 2014; Potter *et al*., 2022; Skinner and Smith, 1990). Species such as waterbuck, with similar dietary requirements throughout their life (e.g. concentrating near water bodies), are likely to escape competition (Codron, 2006).

Codron *et al*. (2016) used carbon and nitrogen isotope ratios to investigate niche partitioning and niche variation in herbivores and carnivores from South African savannas. They found that herbivores have narrower isotopic niche widths and lower isotopic differentiation in individual species than carnivores (Codron *et al*., 2016). Codron *et al*. (2023) found that species frequently cluster around regions on the niche axis with high resource availability. This leads to an imbalance and results in competition (Codron *et al*., 2023). Resource partitioning may reduce the effects of competition (niche overlap) because it allows species to use resources differently (Codron *et al*., 2023; Holt, 2009; MacArthur and Levins, 1967). In the absence of niche partitioning, species may survive if they have similar tolerance to competition (Hubbell, 2001).

3.3. Bone Formation

Bone is made up of inorganic and organic components (Figure 3.1). The organic component of the bone is responsible for strength and flexibility; it consists mostly of collagen (fibrous protein) and a small quantity of non-collagenous proteins (Aiello and Dean, 1990; Fratzl *et al*., 2004; Koch, 2007; Lee-Thorp, 2008). The inorganic component makes up most of the bone and consists mainly of bioapatite (calcium phosphate) (Sponheimer and Cerling, 2014). Bone composition and structure may vary depending on the age and type of the bone (Aiello and Dean, 1990; Pate, 1994). The bone tissue formation process is called ossification. This process involves the use of osteoblast cells to produce new bone material (Boskey, 1981). Flat bones such as the skull, mandible and clavicle are formed by intramembranous ossification (without a cartilage model), while long bones form by endochondral ossification (Clarke, 2008; Koch, 2007; Rolian, 2020).

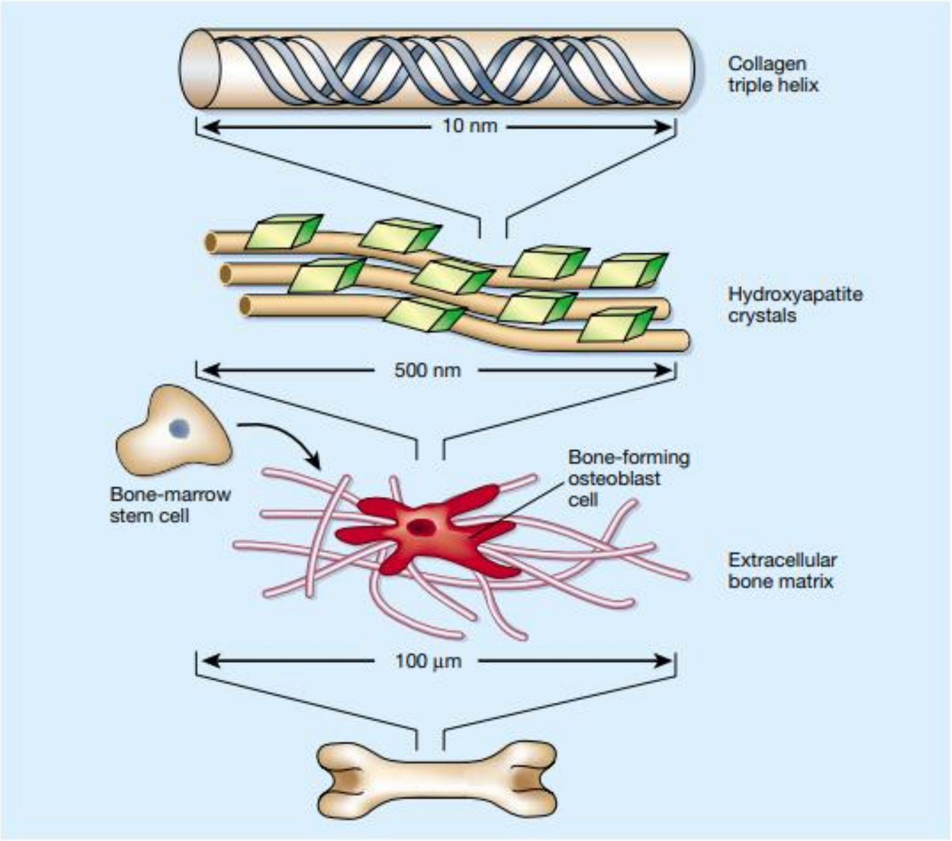


Figure 3.1: The structure of bone, from Taton (2001).

During intramembranous ossification, osteoblasts produce a matrix of organic materials consisting of collagen and other proteins (Percival and Richtsmeier, 2013; Ross *et al*., 1993). This matrix eventually calcifies and traps the osteoblasts. A collection of osteoid around the capillaries form trabeculae, which connect with one another to form trabecular (cancellous or spongy) bone (Evian *et al*., 1982; Kanczler and Oreffo, 2008). Cancellous bone congregates around bone marrow, which is made of stem cells. The outside of the bone is made up of compact (cortical) bone which is covered by a layer of fibrous membrane called the periosteum, where muscles and tendons attach to bone (Clarke, 2008). Intramembranous ossification occurs from the early stages of embryogenesis into adolescence.

During endochondral ossification, an initial structure made of hyaline cartilage is eventually entirely replaced by bone (Frost, 1990; Jukes *et al*., 2008; Provot and Schipani, 2005; Usmani *et al*., 2012). As the cartilage is replaced, chondrocytes and cartilage continue to form at the ends of the long bones, forming what will later become the epiphyses (Provot and Schipani, 2005). This process enables the bone to increase in length as the animal grows.

Bone constantly remodels (turns over) throughout an individual’s lifetime as osteoclasts resorb old bone, while osteoblasts form new bone material (Gocha *et al*., 2018; Matsuoka *et al*., 2014; Stout, 1989). Bone turnover rates vary according to species (Hedges *et al*., 2007), skeletal element (Gocha *et al*., 2018; Hedges *et al*., 2007) and the age, diet and health of the individual (Pasteris *et al*., 2008). For example, the mid-shaft cortical bone in the femur turns over slowly, but trabecular bone in the epiphyses turns over much faster. Newly formed bone incorporates material derived from food consumed at that time, together with some material from resorbed body tissues. The period of diet reflected in bone isotope values therefore depends on the time of formation and rate of turnover of the tissue analysed. Therefore, the isotopic composition of bones that remodel slower reflects a long-term diet (i.e. more years prior to death) compared to bones with faster turnover rates (i.e. later stages of life) (Cox and Sealy, 1997; Hedges *et al*., 2007). Consequently, bone will reflect the diet of an individual over a longer time period compared to teeth (i.e. enamel), which do not remodel (Lee-Thorp, 2008).

3.3.1. Quill Formation in *Hystrix africaeaustralis*

The body of the Cape porcupine (*Hystrix africaeaustralis*) is covered with spines, quills and bristly brown-black fur (Skinner and Smithers, 1990). There are five quill types observed in this species: spines, true quills, tactile bristles, transitional quills, and rattle quills (Barthelmess, 2006; Pigozzi, 1988). Spines and quills are modified hairs made up of keratin, and act as the main defence mechanism against predators. Spines can be up to 500 mm long, while quills reach up to 300 mm (Skinner and Chimimba, 2005). Quills and spines cover the tail, sides and back region of *H*. *africaeaustralis* (Barthelmess, 2006; Smithers, 1983). The quills are banded white and black, the black bands are slightly broader, and the tips are white (de Graaff, 1981). Quills may be shed naturally or lost during fights. New quills grow to replace those that have been shed or removed.

3.3.2. Shell and Bone Formation in *Stigmochelys pardalis*

The skeleton of the leopard tortoise (*Stigmochelys pardalis*) is made up of the endoskeleton (internal bones) and the exoskeleton (shell). The shell consists of the ventral plastron, the intermediate bridge and the dorsal carapace (see Figure 3.2) (Heinrich and Heinrich, 2016; Holt *et al*., 2019). The carapace is an extension of the ribcage and vertebrae, with the exception of the first seven cervical vertebrae which are mobile and not attached to the carapace (Holt *et al*., 2019; Sobolik and Steele, 1996). The bony carapace is covered by an outer layer made up of keratinous plates called scutes (Holt *et al*., 2019).

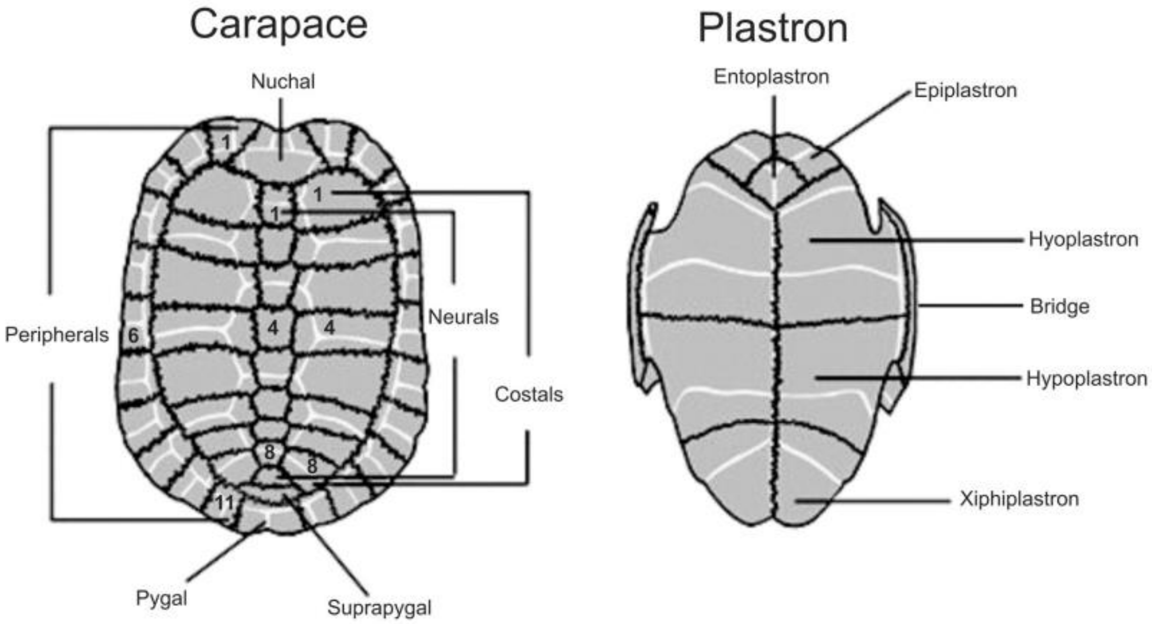


Figure 3.2: The different components of a tortoise shell and the positions of the keratinous scutes (white lines), from Holt *et al*. (2019).

3.4. Tooth Development

3.4.1. Tooth Enamel and Dentine Collagen

A tooth is composed of a crown and root(s). Teeth start to develop from the occlusal surface to the roots. Consequently, the enamel is older than the base of the root. Teeth consist of hard (calcified) tissues: enamel, dentine, and cementum, and soft (non-calcified) tissue: pulp. The pulp houses the blood vessels and nerves (Hillson, 1986;

2005). The enamel covers the dentine in the crown of the tooth. Cementum covers the dentine in the root, an unexposed part of the tooth below the crown that secures the tooth into the jaw. Alveolar bone, gums and periodontal ligaments are responsible for supporting the tooth into the jaws. In mammals, tooth enamel is the hardest part of the body (Ungar, 2010). Tooth enamel is mainly made up of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) within an organic matrix (Boyde, 1967). Dentine is also made up of the mineral hydroxyapatite (approximately 70% of dentine) and organics, primarily collagen, which make up approximately 20% of dentine (Beaumont and Montgomery, 2015; Ungar, 2010).

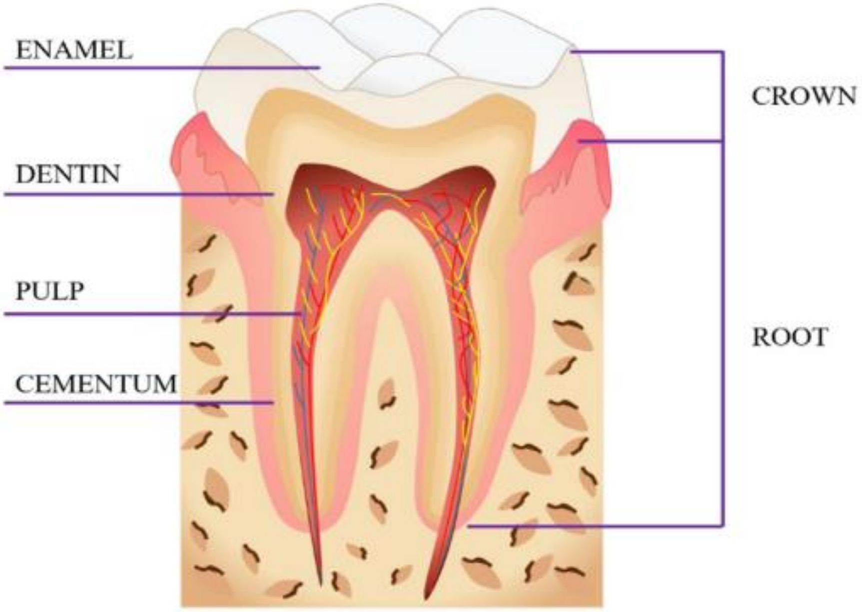


Figure 3.3: The structure of a tooth, from Fu *et al*. (2022).

Alveolar bone has a high turnover rate due to the constant stress imposed on teeth during mastication. Unlike bone, teeth do not turn over. In most mammals, specifically in species with teeth that are not continuously developing, the isotopic composition of dental tissues provides dietary information about the earlier stages of the individual's life. Teeth represent a single distinct period of an individual's life, while bone merges old and new isotopic signals (Sponheimer and Cerling, 2014). The combination of these different tissues can provide dietary information about various phases of an animal's life (Ericson, 1985; Price *et al*., 1994; Sealy *et al*.,1995).

Tooth enamel grows in increments and does not remodel after development. In mammals, the mineralisation of enamel can be summarised into two parts: “matrix production” (also known as appositional stage) followed by “enamel maturation” (Blumenthal *et al*., 2019; Hillson, 1986; Robinson *et al*., 1978; Simmer *et al*., 2012). Initially, an organic matrix is laid down in layers from the occlusal surface to the enamel-dentine junction (EDJ). As the enamel matures and mineralisation increases, matrix proteins and fluid content are steadily removed. Isotope analysis of serial samples of enamel collected perpendicular to the growth axis of the tooth can be used to investigate seasonal shifts in diet or environment during tooth development (Balasse *et al*., 2002; Blumenthal *et al*., 2019; Hoppe *et al*., 2004; Wiedemann *et al*., 1999).

Most mammals have molars, premolars, canines and incisors. The dental formula, sequencing and patterning of teeth varies across mammals. However, in most mammals, deciduous teeth erupt fully prior to the eruption of permanent teeth (Smith, 2000). The first permanent molars (M1) are the first to erupt, while the third permanent molars (M3) erupt last across most mammals (Asher *et al*., 2017; Swindler, 2002; Veitschegger and Sánchez-Villagra, 2016). Smith (2000) reported that although mandibular teeth emerge marginally earlier than maxillary, there is little difference between the two.

3.4.2. Tooth Development and Eruption in *Equus quagga*

Tooth development and eruption in *Equus quagga* are important for this thesis. The patterns are likely to be very similar to those reported by Hoppe *et al*. (2004) for modern domestic horse (*Equus caballus*) mandibles, in an investigation of the timing and pattern of mineralisation in equid premolars and molars. They found that enamel continues to mineralise after each tooth begins to erupt, with mineralisation extending over ~1.5 to 2.8 years. According to Hoppe *et al*. (2004, Fig. 3), the M1 (first molar) is the first permanent tooth to erupt (at age 8-12 months). The M2 (second molar) is the second permanent tooth to erupt at about 20 to 26 months of age. The M3 (third molar) is the second to last tooth to erupt (39-50 months of age). This thesis focuses on M2 and M3 teeth in *Equus quagga*. In modern equids, the M2 enamel mineralisation begins at 7 (± 1.5) months and ends at 37 (± 3) months. The M3 starts to mineralise at 21 (± 3) months and ends at 55 (± 2) months. However, in this thesis, only the parts of the teeth protruding into the mouth were sampled (i.e. approximately one-third of the entire tooth). Therefore, values for the M2 reported below are likely to provide information from approximately 7 to 17 months, and the M3 from ~21 to 32 months. The teeth are lightly worn and are likely to preserve a record close to the mineralisation period.

The M2 grows vertically at a rate of approximately 3.5 to 4 cm per year, and the M3 has a growth rate of ~3 cm/year (Hoppe *et al*., 2004). Higgins and MacFadden (2004) suggested that modern horse molars grow at a constant rate of ~22 mm/year. In contrast, Bendrey *et al*. (2015) suggested that the growth rate of horse teeth decreases exponentially with time. Serial samples of hypsodont equid teeth allow for the reconstruction of seasonal climatic changes because they represent multiple annual cycles (Blumenthal *et al*., 2019; Fricke and O'Neil, 1996; Hoppe *et al*., 2004).

Many studies of fossil equids have used $\delta^{13}C_{enamel}$ for the reconstruction of palaeo-vegetation and diets (Cerling *et al*., 1993; MacFadden *et al*., 1999; Wang *et al*., 1994). $\delta^{18}O_{enamel}$ can be used to estimate annual environmental conditions (Fricke and Rogers, 2000; Stuart-Williams and Schwarcz, 1997). Blumenthal *et al*. (2019) discovered that modern and Early Pleistocene fossil equid teeth from East Africa show variable patterns of isotopic seasonality, as seen in the climate of contemporary East Africa. Equids are water-dependent, so stable oxygen isotope ratios of their enamel represent the source of the drinking water (Crowell-Davis *et al*., 1985; Fricke and Rogers, 2000; Sharp and Cerling, 1998). Studies of modern equids help to understand the relationship between climate/environment and enamel isotope profiles, thus providing useful information that can be applied to fossil species.

Chapter 4: Materials and Methods

Isotope analyses were performed on bone collagen, keratin and tooth enamel of 11 species of large mammals and one reptile from a single ecosystem (game farm). This chapter outlines the laboratory techniques used, including pretreatment of the specimens and isotopic analysis.

4.1. Specimens

A total of 62 individual animals were collected from Mmabolela Estates in the Limpopo Province. The collection consists of seven bovid species: impala (*Aepyceros melampus*, n=4), blue wildebeest (*Connochaetes taurinus*, n=5), waterbuck (*Kobus ellipsiprymnus*, n=5), gemsbok (*Oryx gazella*, n=3), eland (*Taurotragus oryx*, n=3), bushbuck (*Tragelaphus scriptus*, n=4), greater kudu (*Tragelaphus strepsiceros*, n=12), one equid (*Equus quagga*, n=11), plus Cape porcupines (*Hystrix africaeaustralis*, n=3), chacma baboons (*Papio ursinus*, n=3), common warthogs (*Phacochoerus africanus*, n=5) and leopard tortoises (*Stigmochelys pardalis*, n=4). The bovids, equids and suid had been shot over several years by hunters and after butchery, the remains of the carcasses were dumped at a particular location in the veld. At the time of sampling, most soft tissue had decomposed but some crania still had adhering skin. The bones had therefore been exposed to the elements and undergone a degree of weathering, but they had not been buried. For the bovids, equids and suids, samples available consisted of mandibles and/or maxillae, most with teeth still present. The tortoises died natural deaths. Tortoise carapace fragments and vertebrae and porcupine quills were surface-collected in the veld. The three porcupine true quills were found at different localities on the farm, so very likely come from three different animals. No live animals were killed for the purposes of this study. There have been no translocations of these species onto the farm in the period during which these animals were alive.

4.2. Bone Collagen and Keratin

4.2.1. Preparation and Pre-treatment

Bone Collagen

The collagen samples were prepared using standard techniques (as practiced at the University of Cape Town) described by Sealy *et al*. (2014). A Dremel hand drill fitted with an emery disc (replaced between each sample) was used to clean the surfaces of the bones. Approximately 1 cm of bone was sampled from each individual (except for *Stigmochelys pardalis* and *Hystrix africaeaustralis*, see relevant sections below). Cancellous bone was avoided, since this is more prone to contamination from soil, roots, etc. The samples were placed in vials and labelled accordingly. For example, the sample from the first zebra individual was named 'zebra 1'. The initial weight (g) of the samples was recorded to enable calculations of the collagen yield (the weight percentage of collagen relative to the weight of the starting material) later. Treatment with de-fatting solution seemed unnecessary, because the bone appeared clean and dry after having been exposed to the elements. The chunks of bone were reacted with 0.2M HCl (hydrochloric acid), replaced every second day, to decalcify the bone and isolate the collagen (Sealy *et al*., 2014). This took approximately six days. Decalcified bone is flexible and translucent. The samples were then rinsed twice with distilled water and soaked overnight in 0.1M NaOH (sodium hydroxide) to eliminate lipids and humic acids (Ambrose, 1990). The samples were removed from the NaOH and rinsed twice with distilled water. The samples were then soaked in distilled water (replaced every second day) for approximately seven days, or until the pH of the water remained constant. The distilled water was poured out and the vials were covered with parafilm. Holes were poked into the parafilm, and the samples were freeze-dried overnight, then removed from the freeze-dryer and left on the bench for 24 hours with the parafilm on, to equilibrate with the atmosphere before re-weighing.

For the leopard tortoise (*Stigmochelys pardalis*), approximately 2.5 cm of vertebral bone was sampled, weighed, and reacted with 0.2M HCl (replaced every second or third day) until the bone was decalcified and the collagen was isolated (which took approximately three weeks). The samples were then rinsed twice with distilled water and soaked overnight in 0.1M NaOH. Most of the tortoise samples (except Tortoise 4) had black particles due to contamination (possibly rootlets), which were scraped off with a scalpel. Lower collagen yields are expected because small amounts of bone were also removed in the process. The samples were then rinsed three times with distilled water and soaked in distilled water (changed every third day) for approximately twelve days (until the solution remained at the pH of distilled water). Tortoise carapaces grow incrementally, and previous studies of tortoises have therefore homogenized the collagen by “gelatinising” it. This procedure was also followed here. Distilled water was replaced with a pH 3 solution (HCl diluted with distilled water) and the vials were covered loosely with foil to reduce evaporation. The vials were placed on a hotplate set to 72°C and stirred with magnetic stirrers. The temperature in the vials, as measured with a thermometer, was lower than the temperature to which the hotplate was set, so this required adjustment. By the following day, gelatinisation was complete. The four vials were removed from the hotplate and allowed to cool, then covered with perforated parafilm and placed in the freezer overnight before being freeze-dried overnight. Three of the samples had the consistency of sugar brittle, while one looked like cotton candy because it had parafilm with bigger holes (more air coming in and out).

Keratin

Three porcupine (*Hystrix africaeaustralis*) quills were wiped with ethanol and then acetone to remove surface contaminants. The quills were then cut in half lengthways, and one half was further divided for analysis while the other was kept for reference. The quills were cut into different sizes because it was not clear which sampling interval would be best. Porcupine quill 1 was ~36 cm long and cut into ~0.85 cm sections, Porcupine quill 2 was ~34 cm long and cut into ~0.25 cm sections, and Porcupine quill 3 (~37 cm) into ~0.35 cm sections. The samples were placed into labelled vials, where serial sample P1.1 is the end of the quill closest to Porcupine One’s body (the proximal end), and P1.42 is the end furthest away from Porcupine One’s body (the distal end). The samples were defatted, and then washed with distilled water as done for other types of keratin samples (O’Connell *et al*., 2001). Each section of the quills was treated with a defatting solution (2 methanol: 1 chloroform: 0.8 water) for 48 hours to remove lipids. The vials were constantly swirled on the rotator to mix the solution. The defatting solution was poured out into the waste containers and the samples were washed in distilled water, before freeze-drying overnight as indicated above.

4.2.2. Isotopic Analysis

The collagen and keratin samples were weighed into tin cups in preparation for the mass spectrometer. A set of internal laboratory standards (Chocolate, Valine, Sucrose and New Merck Gel) was weighed out at the same time. The range of weights was between 0.35 and 0.45 mg for both samples and standards. These were combusted in a Flash 2000 organic elemental analyser set to 1020°C. The CO_2 and N_2 gases produced were purified and passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit, using helium as the carrier gas.

Internal laboratory standards had previously been calibrated against international standard materials from the IAEA and USGS. The standards were analysed in triplicate and showed reproducibility $\leq 0.2‰$ for both $\delta^{13}C$ and $\delta^{15}N$.

4.3. Tooth Enamel

4.3.1. Preparation and Pre-treatment

The enamel samples were prepared using methods described by Lee-Thorp *et al*. (1997), with modifications. The Dremel 8000 diamond-tipped drill was used to clean the surface of the *Equus quagga* (zebra) teeth and to collect the series of enamel powders. The drill bit was cleaned between each sample using a small paintbrush. The samples were obtained up the height of the tooth from the cervix to the occlusal surface (Figure 4.1). For seven animals, both the M2 (second molar) and M3 (third molar) could be sampled, but for two animals (zebras 2 and 8) only the M2 was present. Serial sample Z1.1M2 indicates the sample of the M2 closest to the occlusal surface for zebra 1, and Z1.6M2 indicates the sample closest to the alveolar bone for the same individual. Only the part of the tooth that extended into the oral cavity was sampled. Approximately 10 mg of enamel powder was collected for each serial sample and placed in a microcentrifuge tube. The samples were soaked in 1.75% NaOCl (sodium hypochlorite) for 45 minutes to remove any organic materials (adjusted from

Lee-Thorp *et al*. 1997), then centrifuged for one minute at 100 000 rpm. The NaOCl was removed using a Pasteur pipette and the samples rinsed with distilled water three times, centrifuging between each rinse. Next, the samples were reacted with 0.1M acetic acid for 10 minutes to eliminate post-depositional diagenetic carbonates, centrifuged and rinsed three times with distilled water. The microcentrifuge tubes were covered with perforated parafilm and freeze-dried overnight. After being allowed to equilibrate with the atmosphere, 2 mg of each sample was weighed into a cleaned borosilicate glass tube (see cleaning procedure below), in readiness for loading into the gas bench.

Figure 4.1: Sampling procedure of the M2 (second molar) tooth enamel of *E. quagga* second molar. Serial samples were drilled perpendicularly to the axis of tooth growth.

Cleaning procedure of the vials

Round-bottomed borosilicate glass tubes (12 ml) fitted with screw caps with permeable septa were thoroughly cleaned in preparation for use on the gas bench. The screw caps were removed, and the vials were filled with 1 M phosphoric acid. Water was squirted over the bottom of the vials to wash off spilt acid. The vials were loosely covered with foil and placed in the oven overnight at 72°C. On the following day, the dilute acid was poured out and the vials rinsed with hot tap water, and then twice with distilled water. The vials were filled with distilled water, covered with foil and placed in the oven for an hour at 72°C. The distilled water was then replaced with MILLIQ water and the vials returned to the oven for an additional hour at 72°C. The MILLIQ water was poured out and the vials were tipped upside down on the tray and placed in the oven overnight at 72°C to dry. The vials were then wrapped in foil and baked out in a furnace for approximately 3 hours at 500°C.

4.3.2. Isotopic Analysis

Between 1.9 and 2.1 mg of each pre-treated enamel sample was weighed into a cleaned borosilicate glass tube using a tin disc. A set of standards (IAEA-CO8, Carrara Z New and Cavendish Marble) was weighed out at the same time and run together with the samples. The range was between 0.18 and 0.20 mg for the standards. The vials were capped and placed in the sampler tray in the Thermo Finnigan GasBench II at 72°C, then flushed with helium to remove air. A syringe was used to inject warm 100% phosphoric acid (H_3PO_4) manually through the septa: five drops to the standards and seven to the samples. The samples and standards were left for a minimum of two and a half hours to react with the H_3PO_4 and to release CO_2 (carbon dioxide). A Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS), controlled by IsoDat software was used to measure carbon and oxygen isotope ratios in the purified CO_2. The reproducibility of repeated measurements of the standard material was ≤ 0.1‰ for $\delta^{13}C$ and $\delta^{18}O$ across all runs.

Data analyses were conducted using IBM SPSS Statistic Data Editor software (version 28.0.1.1). Since stable isotope values are usually not normally distributed, non-parametric tests (Mann-Whitney U and Kruskal-Wallis) were used. A p-value of <0.05 was taken to indicate a statistically significant difference.

Adjusted $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values were calculated in order to compare the porcupine quills directly with bone collagen of the other species. Consequently, +1.4‰ was added to porcupine $\delta^{13}C_{keratin}$ values, and +0.21‰ to $\delta^{15}N_{keratin}$ values (O'Connell *et al*., 2001).

Mean $\delta^{13}C$ values were converted to percentage C_4 grass-intake (%C_4) using the following formula from Codron (2006):

$$\%C_4 = (\delta^{13}C_{C3}\text{ plants} + \Delta\delta^{13}C - \delta^{13}C_{animal}) / (\delta^{13}C_{C3}\text{ plants} - \delta^{13}C_{C4}\text{ plants}) \times 100$$

Where ‘$\delta^{13}C_{C3}$ plants’ and ‘$\delta^{13}C_{C4}$ plants’ are the mean $\delta^{13}C$ values of plants that use C_3 and C_4 photosynthetic pathways respectively. $\Delta\delta^{13}C$ is the isotopic enrichment factor for bone collagen compared with diet.

Chapter 5: Results

This chapter reports the carbon and nitrogen isotope ratios of bone collagen and keratin (*Hystrix africaeaustralis*) in all species (62 individual animals) listed in the Fauna and Ecology chapter. The carbon and oxygen isotope ratios of tooth enamel serial samples in *Equus quagga* are also reported.

5.1. δ^{13}C and δ^{15}N of Bone Collagen and Keratin

Table 5.1 lists the bone collagen quality control indicators and the $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ values for all the individuals included in this study, with the exception of *H. africaeaustralis*, for which the samples consisted of quills made up of keratin. The carbon content (wt %C) of bone collagen in Table 5.1 ranged from 41.5% to 44.6%, and the nitrogen content (wt %N) ranged from 14.3% to 16.0%. The elemental C:N for the bone collagen samples ranged from 3.2 to 3.4. None yielded high %C and C/N values indicative of lipid contamination. The elemental C:N for the keratin samples ranged from 3.5 to 3.7 (Appendix 2). All samples meet the collagen quality control criteria proposed by van Klinken (1999) (see section 3.1.5), although not all fall within the narrower ranges proposed by Guiry and Szpak (2020) for modern collagen. These samples were not, however, collected as fresh or frozen tissues, which is how Guiry and Szpak define “modern” (2020: 2). They are more similar to Guiry and Szpak’s category of “archival” samples. As such, the criteria of van Klinken (1999) are considered more appropriate for this study.

Table 5.1: $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$ and bone collagen quality indicators for all species (except *Hystrix africaeaustralis*) included in this thesis.

UCT No.	**Scientific Name**	**Common Name**	**δ^{13}C (‰)**	**δ^{15}N (‰)**	**Wt %C**	**Wt %N**	**% Collagen Yield**	**C/N elemental**
24293	*Tragelaphus strepsiceros*	Kudu 1	-21.1	10.7	43.9	16.0	29.1	3.2
24294		Kudu 2	-21.1	9.7	44.0	15.9	27.1	3.2
24295		Kudu 3	-21.5	11.4	43.0	15.7	25.5	3.2
24296		Kudu 4	-21.3	10.2	43.1	15.7	31.7	3.2
24297		Kudu 5	-21.2	9.8	43.2	15.7	22.2	3.2
24298		Kudu 6	-21.6	9.0	43.3	15.9	25.9	3.2
24299		Kudu 7	-21.4	10.3	43.1	15.7	26.3	3.2
24300		Kudu 8	-20.8	11.1	43.8	15.2	31.2	3.4
24301		Kudu 9	-21.4	9.4	43.4	15.6	29.7	3.3
24302		Kudu 10	-21.8	10.7	43.6	15.6	26.6	3.3
24303		Kudu 11	-20.9	10.1	43.3	15.8	28.0	3.2
24304		Kudu 12	-20.7	8.9	43.9	15.8	26.8	3.2
24305	*Taurotragus oryx*	Eland 1	-21.0	8.7	43.5	15.3	20.7	3.3
24306		Eland 2	-21.4	9.8	43.2	15.2	17.9	3.3
24307		Eland 3	-21.7	10.0	43.7	15.4	20.0	3.3
24308	*Oryx gazella*	Gemsbok 1	-11.1	10.6	43.8	15.2	24.4	3.4
24309		Gemsbok 2	-12.2	9.8	44.4	15.4	21.4	3.4
24310		Gemsbok 3	-14.7	10.0	43.8	15.4	21.0	3.3
24311	*Tragelaphus scriptus*	Bushbuck 1	-21.2	11.3	43.2	15.6	32.6	3.2
24312		Bushbuck 2	-21.5	10.1	43.2	15.6	26.0	3.2
24313		Bushbuck 3	-21.0	11.2	43.2	15.5	25.4	3.2
24314		Bushbuck 4	-21.2	10.3	43.3	15.5	23.5	3.3
24315	*Aepyceros melampus*	Impala 1	-17.8	8.9	43.1	15.5	25.8	3.2
24316		Impala 2	-17.8	9.2	43.6	15.7	23.6	3.2
24317		Impala 3	-17.1	9.8	44.6	15.3	28.1	3.4
24318		Impala 4	-19.7	9.8	43.6	15.5	29.0	3.3
24319	*Connochaetes taurinus*	Wildebeest 1	-10.0	9.6	43.3	15.5	26.4	3.3
24320		Wildebeest 2	-11.3	8.3	44.3	15.5	27.7	3.3
24321		Wildebeest 3	-9.5	10.3	43.4	15.5	19.9	3.3

24322		Wildebeest 4	-12.1	9.7	43.4	15.6	20.7	3.2
24323		Wildebeest 5	-9.5	9.3	44.1	15.9	25.4	3.2
24324	*Equus quagga*	Zebra 1	-13.1	7.8	42.7	15.5	25.1	3.2
24325		Zebra 2	-12.7	8.1	44.2	15.4	25.7	3.3
24326		Zebra 3	-13.0	7.4	44.0	15.6	23.8	3.3
24327		Zebra 4	-14.5	7.3	43.5	15.5	28.5	3.3
24328		Zebra 5	-12.8	7.7	44.2	15.3	21.9	3.4
24329		Zebra 6	-14.3	6.8	43.4	15.7	25.9	3.2
24330		Zebra 7	-14.4	6.9	44.1	15.3	25.5	3.4
24331		Zebra 8	-12.1	7.4	43.4	15.6	21.8	3.2
24332		Zebra 9	-12.5	8.4	44.3	15.5	22.6	3.3
24333		Zebra 10	-15.4	6.6	43.9	15.4	18.5	3.3
24334		Zebra 11	-10.9	10.6	43.4	15.7	22.8	3.2
24335	*Phacochoerus africanus*	Warthog 1	-11.1	8.7	44.4	15.3	18.2	3.4
24336		Warthog 2	-11.2	8.3	43.8	15.4	24.5	3.3
24337		Warthog 3	-12.0	9.4	44.0	15.9	24.2	3.2
24338		Warthog 4	-10.9	9.5	44.3	15.2	26.9	3.4
24339		Warthog 5	-11.3	9.3	43.9	15.4	19.7	3.3
24340	*Papio ursinus*	Baboon 1	-20.4	10.2	43.6	15.4	24.7	3.3
24341		Baboon 2	-19.6	9.2	43.0	15.5	22.7	3.2
24342		Baboon 3	-20.0	8.6	43.2	15.6	24.4	3.2
24343	*Kobus ellipsiprymnus*	Waterbuck 1	-9.6	8.6	43.1	15.5	16.1	3.2
24344		Waterbuck 2	-11.2	10.3	42.8	15.6	25.1	3.2
24345		Waterbuck 3	-12.0	9.6	42.7	15.5	27.0	3.2
24346		Waterbuck 4	-9.6	9.8	42.7	15.5	27.4	3.2
24347		Waterbuck 5	-11.8	9.9	42.7	15.5	28.5	3.2
24351	*Stigmochelys pardalis*	Tortoise 1	-20.5	9.8	42.1	14.7	24.0	3.3
24352		Tortoise 2	-17.5	12.9	42.6	15.0	15.6	3.3
24353		Tortoise 3	-22.0	8.2	42.1	14.3	22.5	3.4
24354		Tortoise 4	-18.4	13.8	41.5	14.7	23.1	3.3

Table 5.2 provides the summary statistics for $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ per species, and for keratin from porcupine (*H. africaeaustralis*) quills. Both raw (uncorrected) and corrected values are given for *H. africaeaustralis*. These corrections make $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values comparable with $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ values. Modern human isotopic data showed that bone collagen is enriched by +0.86‰ in $\delta^{15}N$ and +1.4‰ in $\delta^{13}C$ compared with hair keratin from the same individual (O'Connell *et al.*, 2001). There is no significant difference between $\delta^{13}C$ of nail and hair keratin from the same individual, but nail keratin is enriched by +0.65‰ in $\delta^{15}N$ compared to hair keratin (O'Connell *et al.*, 2001). Porcupine quills are likely more similar to human nails than hair keratin. As a result, +1.4‰ was added to porcupine $\delta^{13}C_{keratin}$ values, and +0.21‰ to $\delta^{15}N_{keratin}$ values.

The $\delta^{13}C_{collagen}$ and corrected $\delta^{13}C_{keratin}$ values across all species ranged from -22.2‰ (in *H. africaeaustralis*) to -9.5‰ (in *C. taurinus*). $\delta^{15}N$ values ranged from 6.3‰ (in *H. africaeaustralis*) to 13.8‰ (in *S. pardalis*) (see Table 5.2). *H. africaeaustralis* exhibited the largest range of $\delta^{13}C$ (5.1‰) and $\delta^{15}N$ values (6.6‰). This is not surprising, considering that the serial sampling of *H. africaeaustralis* quills captured short-term variation, whereas analysis of bulk collagen from other species averaged this out.

Table 5.2: Summary statistics for $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ for all species. The uncorrected (keratin) and corrected values for Cape porcupines (*H. africaeaustralis*) are included. Values for porcupines were calculated by combining all the samples from three individuals (see **Table 5.3**).

Fauna	No. of individuals	Mean $\delta^{13}C$ (‰)	Median $\delta^{13}C$ (‰)	Std Dev $\delta^{13}C$ (‰)	Min $\delta^{13}C$ (‰)	Max $\delta^{13}C$ (‰)	Range $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	Median $\delta^{15}N$ (‰)	Std Dev $\delta^{15}N$ (‰)	Min $\delta^{15}N$ (‰)	Max $\delta^{15}N$ (‰)	Range $\delta^{15}N$ (‰)
Kudu	12	-21.23	-21.2	0.32	-21.8	-20.7	1.1	10.11	10.1	0.78	8.9	11.4	2.5
Eland	3	-21.37	-21.4	0.35	-21.7	-21.0	0.7	9.50	9.8	0.70	8.7	10.0	1.3
Gemsbok	3	-12.65	-12.2	1.86	-14.7	-11.1	3.6	10.15	10.0	0.43	9.8	10.6	0.8
Bushbuck	4	-21.21	-21.2	0.18	-21.5	-21.0	0.5	10.71	10.7	0.63	10.1	11.3	1.2
Impala	4	-18.10	-17.8	1.12	-19.7	-17.1	2.6	9.42	9.5	0.44	8.9	9.8	0.9
Wildebeest	5	-10.49	-10.0	1.20	-12.1	-9.5	2.6	9.44	9.6	0.75	8.3	10.3	2.0
Zebra	11	-13.25	-13.0	1.30	-15.4	-10.9	4.5	7.73	7.4	1.10	6.6	10.6	4.0
Warthog	5	-11.32	-11.2	0.44	-12.0	-10.9	1.1	9.03	9.3	0.50	8.3	9.5	1.2
Baboon	3	-20.01	-20.0	0.40	-20.4	-19.6	0.8	9.33	9.2	0.83	8.6	10.2	1.6
Waterbuck	5	-10.86	-11.2	1.16	-12.0	-9.6	2.4	9.64	9.8	0.65	8.6	10.3	1.7
Tortoise	4	-19.57	-19.4	2.03	-22.0	-17.5	4.5	11.20	11.4	2.64	8.2	13.8	5.6
Porcupine (uncorrected)	3	-21.38	-21.5	1.10	-23.6	-18.5	5.1	8.16	7.7	1.71	6.1	12.7	6.6
Porcupine (corrected)	3	-19.98	-20.1	1.10	-22.2	-17.1	5.1	8.37	7.9	1.71	6.3	12.9	6.6

According to Figure 5.1 below, there are two distinct groups based on the δ^{13}C values. The δ^{13}C values of these groups do not overlap. The species that fall in the blue circle are primarily grazers, and the ones that lie within the red circle are primarily browsers. As expected, the browsing species are clustered at the negative end of the x-axis, while the grazing species are more positive. Mean δ^{13}C values for browsing species ranged from -21.37‰ to -18.10‰. Those for grazing species ranged from -13.25‰ to -10.49‰.

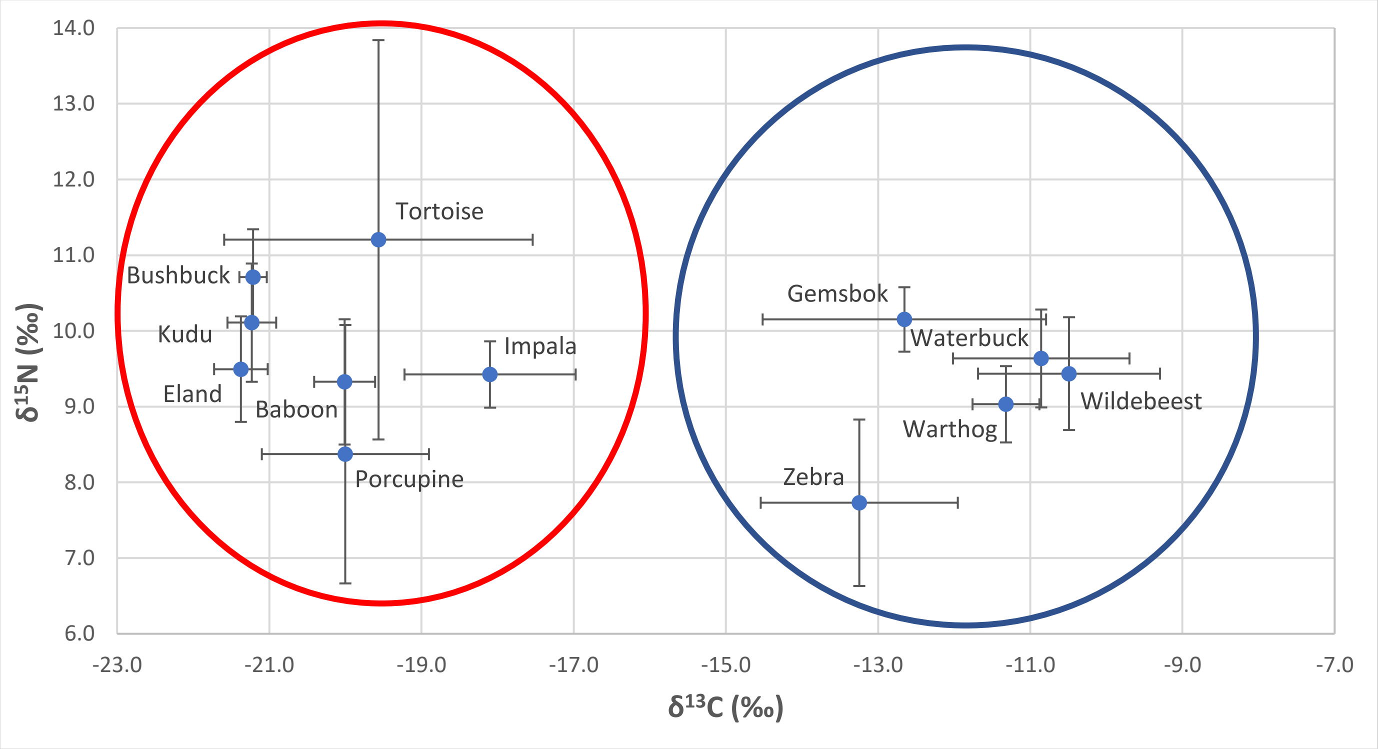


Figure 5.1: Scatter plot of δ^{15}N$_{collagen}$ against δ^{13}C$_{collagen}$ for all species. The dots show the means, and the error bars show one standard deviation. Values for porcupines have been adjusted to make them comparable to bone collagen (O'Connell *et al*., 2001). The species that fall in the blue circle are primarily grazers, and the ones that lie within the red circle are primarily browsers.

For both 'browsers' and 'grazers', Kruskal-Wallis and Mann-Whitney U (where applicable) tests with pairwise comparisons were run for each of the δ^{13}C and δ^{15}N datasets. Pairwise comparison post hoc tests were used to determine where the differences came from when the null hypothesis was rejected (i.e. statistically significant differences). The analyses were performed using the IBM SPSS Statistic

Data Editor software. A p-value of <0.05 indicates a statistically significant difference. The chi-square distribution was used to determine the p-value.

Within the grazing group, plains zebra (*Equus quagga*) have the most negative mean $\delta^{13}C$ and the lowest mean $\delta^{15}N$ values, and plot some distance away from the other species. Waterbuck (*K*. *ellipsiprymnus*) and blue wildebeest (*C*. *taurinus*) have similar $\delta^{13}C$ and $\delta^{15}N$ values. A Mann-Whitney U test showed no significant differences among the two groups in $\delta^{13}C$ (Mann-Whitney Z = 15.000, p = 0.690) or $\delta^{15}N$ (Mann-Whitney Z = 9.000, p = 0.548) (see Figures 5.2 and 5.4). Values for waterbuck and blue wildebeest were therefore combined and compared with the rest of the grazing group, warthogs (*P. africanus*), *E. quagga* and gemsbok (*O. gazella*). A Kruskal-Wallis test showed statistically significant differences in $\delta^{13}C$ (Kruskal-Wallis H (3) = 14.882, p = 0.002) and $\delta^{15}N$ values (Kruskal-Wallis H (3) = 15.615, p = 0.001) between the combined data set and *P. africanus*, *E. quagga* and *O. gazella* (see Figures 5.3 and 5.5).

The pairwise comparisons with adjusted p-values showed that there is a significant difference between the combined data set and *E*. *quagga* $\delta^{13}C$ values (p = 0.002) and $\delta^{15}N$ values (p = 0.007). There is also a significant difference between the *E*. *quagga* and *O*. *gazella* $\delta^{15}N$ values (p = 0.009). There is no significant difference between the combined data set and *O. gazella* $\delta^{13}C$ values (p = 0.445) and $\delta^{15}N$ values (p = 1.000). There were no statistically significant differences between the $\delta^{13}C$ and $\delta^{15}N$ values for waterbuck, wildebeest and warthogs. However, warthogs were not grouped with waterbuck and wildebeest because they are suids known to have varied omnivorous diets.

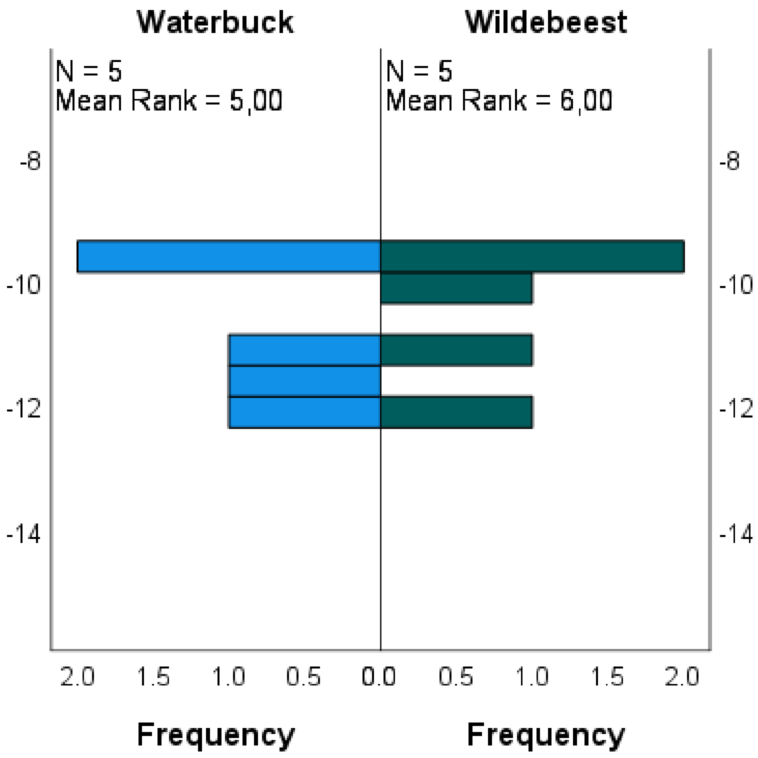


Figure 5.2: The distributions of δ^{13}C of waterbuck (*K. ellipsiprymnus*) and blue wildebeest (*C. taurinus*)

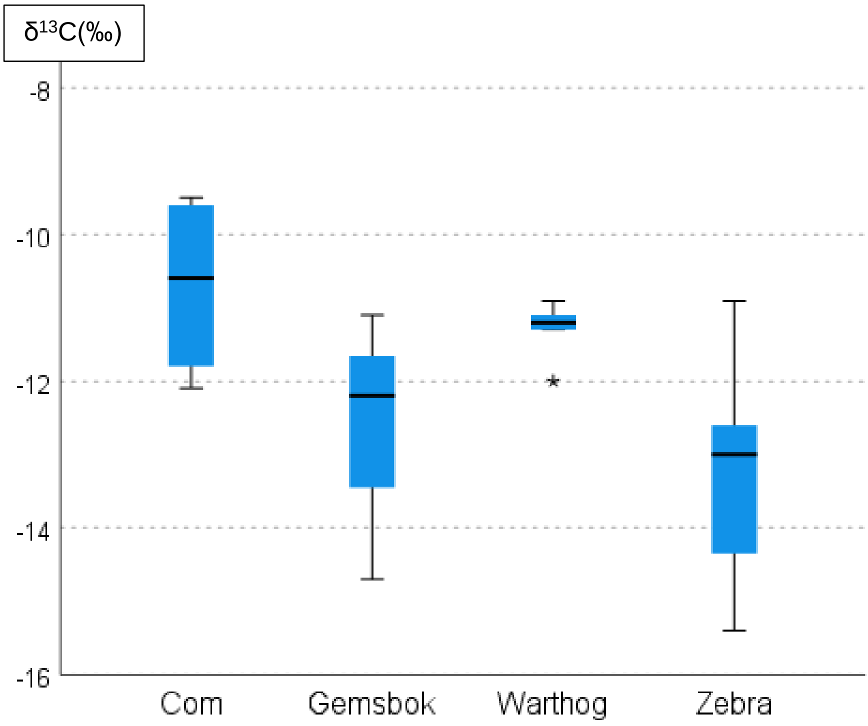


Figure 5.3: A box-and-whisker plot showing the distributions of δ^{13}C$_{collagen}$ values (‰) for a combined (Com) dataset (waterbuck and blue wildebeest) with gemsbok (*O. gazella*), warthogs (*P. africanus*) and plains zebra (*E. quagga*). The medians are indicated by bold horizontal lines. The boxes represent the interquartile ranges. The ranges of values that are not outliers are indicated by the whiskers. The star represents an extreme outlier (three times the interquartile range away from the median).

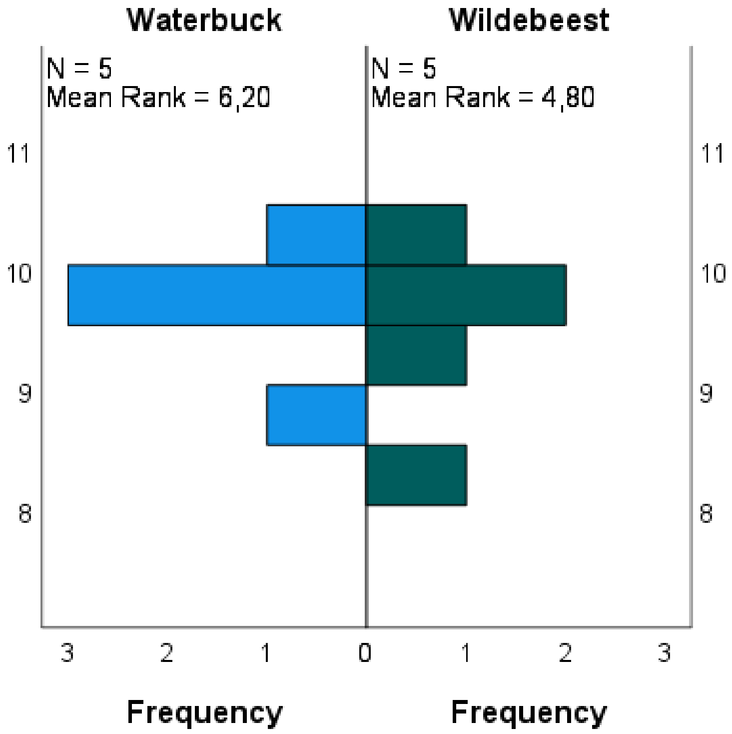


Figure 5.4: The distributions of δ^{15}N of waterbuck (*K. ellipsiprymnus*) and blue wildebeest (*C. taurinus*).

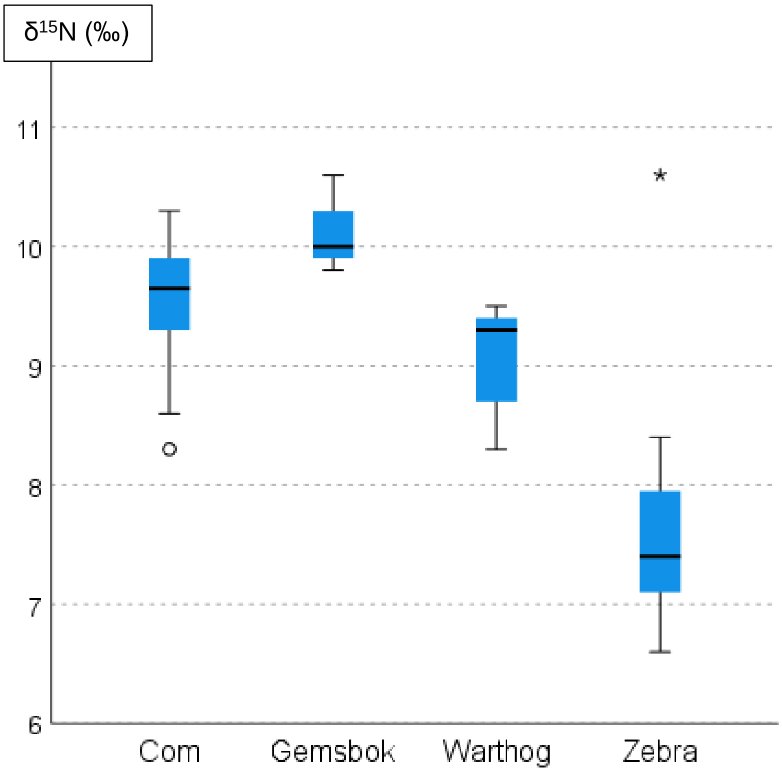


Figure 5.5: A box-and-whisker plot showing the distributions of δ^{15}N$_{\text{collagen}}$ values (‰) for a combined (Com) dataset (waterbuck and blue wildebeest) with gemsbok (*O. gazella*), warthogs (*P. africanus*) and plains zebra (*E. quagga*). The open circles represent outliers. See **Figure 5.3** above for a description of the boxplot format.

Within the browsing group, impala (*Aepyceros melampus*) have the most positive mean $\delta^{13}C$ value, with values that cluster towards the grazing group (i.e. intermediate diet). Bushbuck (*T*. *scriptus*), kudu (*T*. *strepsiceros*) and eland (*T*. *oryx*) have indistinguishable $\delta^{13}C$ values (Kruskal-Wallis H (2) = 0.386, p = 0.824) (Figure 5.6). Cape porcupines (*H*. *africaeaustralis*), chacma baboons (*P. ursinus*) and leopard tortoises (*S*. *pardalis*) also have similar $\delta^{13}C$ values (Kruskal-Wallis H (2) = 0.118, p = 0.943) (Figure 5.7). Although not “browsers”, these species consume C_3-based diets. The bushbuck, kudu and eland $\delta^{13}C$ values (combined) were compared with tortoises, impala, baboons and porcupines. A Kruskal-Wallis test showed statistically significant differences (Kruskal-Wallis H (4) = 15.460, p = 0.004) (Figure 5.6). The pairwise comparisons with adjusted p-values further showed that there is a significant difference between the $\delta^{13}C$ values of bushbuck, kudu and eland (combined) and *A. melampus* (p = 0.006). The bushbuck, kudu and eland $\delta^{13}C$ values (combined) were then compared with porcupines, baboons and tortoises (combined). A Mann-Whitney test showed statistically significant differences (Mann-Whitney Z = 154.5, p = 0.005) (Figure 5.8).

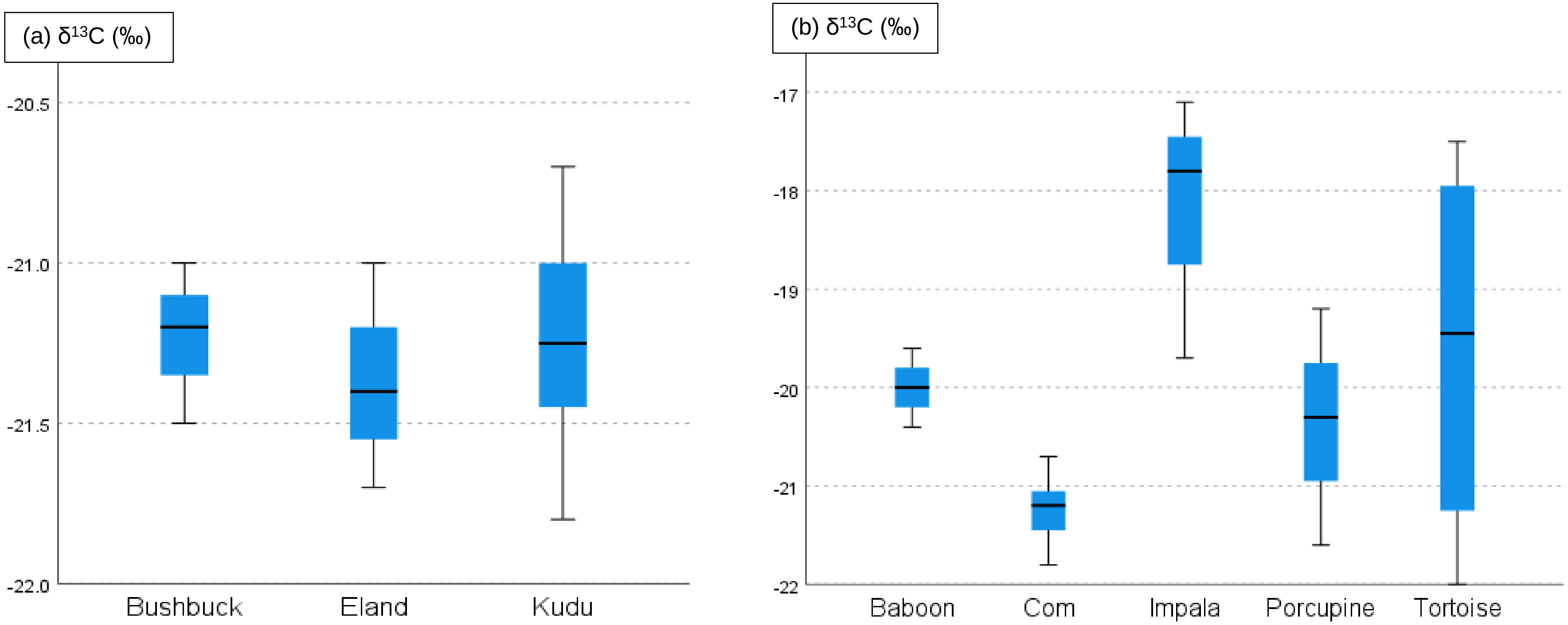


Figure 5.6: Box-and-whisker plots showing the distributions of $\delta^{13}C_{collagen}$ values (‰) for **a)** bushbuck (*T. scriptus*), eland (*T. oryx*) and greater kudu (*T. strepsiceros*) and **b)** a combined (Com) dataset (bushbuck, greater kudu and eland) with chacma baboons (*P. ursinus*), impala (*A. melampus*), Cape porcupines (*H. africaeaustralis*) and leopard tortoises (*S. pardalis*). See **Figure 5.3** for a description of the boxplot format.

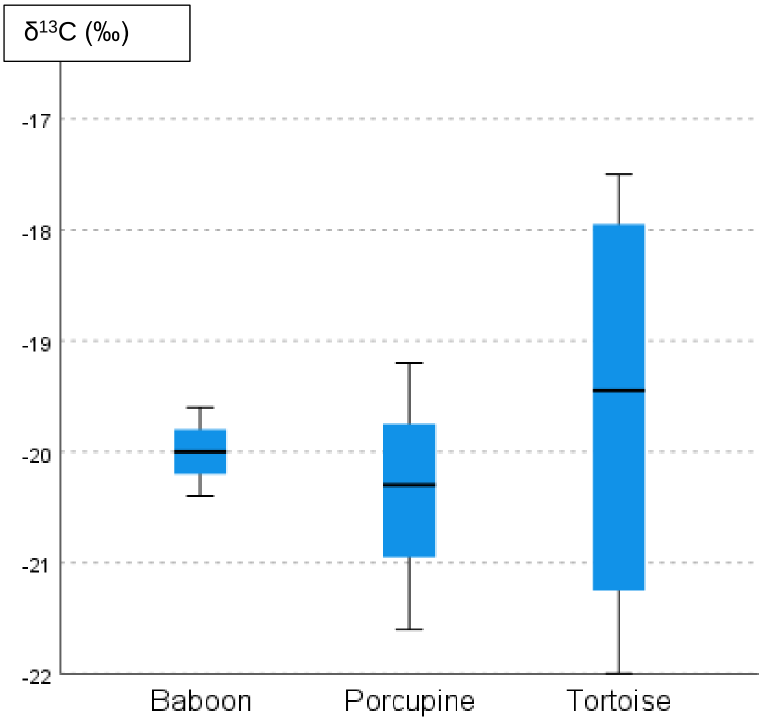


Figure 5.7: Box-and-whisker plots showing the distributions of δ^{13}C$_{collagen}$ values (‰) for chacma baboons (*P. ursinus*), Cape porcupines (*H. africaeaustralis*) and leopard tortoises (*S. pardalis*). See **Figure 5.3** for a description of the boxplot format.

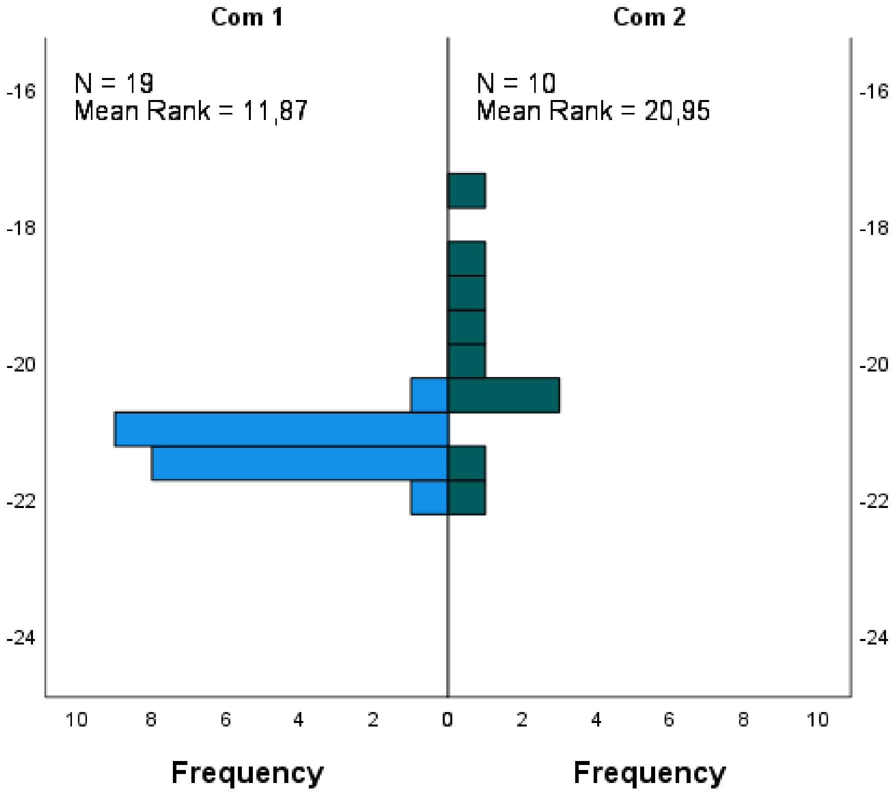


Figure 5.8: The distributions of δ^{13}C values for bushbuck, kudu and eland (Com 1) and porcupines, baboons and tortoises (Com 2).

Within the browsing group, leopard tortoises (*Stigmochelys pardalis*) have the highest mean $\delta^{15}N$ values, and Cape porcupines (*Hystrix africaeaustralis*) have the lowest (but see more detailed discussion of porcupines below). The $\delta^{15}N$ values for the four tortoises are very variable (range: 8.2 to 13.8‰), including the two most positive values in the entire dataset. The mean and standard deviation, therefore, do not characterize the tortoises well, and they will be excluded from the comparisons below. It is also important to note that the isotope ratios of the tortoises may not be directly comparable to those of the other animals in this study, given the fundamental differences between tortoise (i.e. reptile) and mammal physiology. Similar values might not indicate similar diets or adaptations. A Kruskal-Wallis test showed no significant differences in $\delta^{15}N$ for eland, impala and kudu (Kruskal-Wallis H (2) = 3.225, p = 0.199) (Figure 5.9). These values were combined and compared with bushbuck, baboons, porcupines and tortoises. The differences were not statistically significant (Kruskal-Wallis H (4) = 5.318, p = 0.256) (Figure 5.9). The pairwise comparisons further showed that there are no significant differences between the $\delta^{15}N$ values of the combined group and the other browsing species: bushbuck (unadjusted p-value = 0.100; adjusted p-value = 0.997), baboons (unadjusted p-value = 0.443; adjusted p-value = 1.000); porcupines (unadjusted p-value = 0.427; adjusted p-value = 1.000) and tortoises (unadjusted p-value = 0.411; adjusted p-value = 1.000). Although there are no statistically significant inter-species differences in $\delta^{15}N$ amongst the predominant browsers, the $\delta^{15}N$ values for eland and bushbuck are distinct and do not overlap. Due to the small sample size, this is not a sufficiently robust result to attempt to interpret.

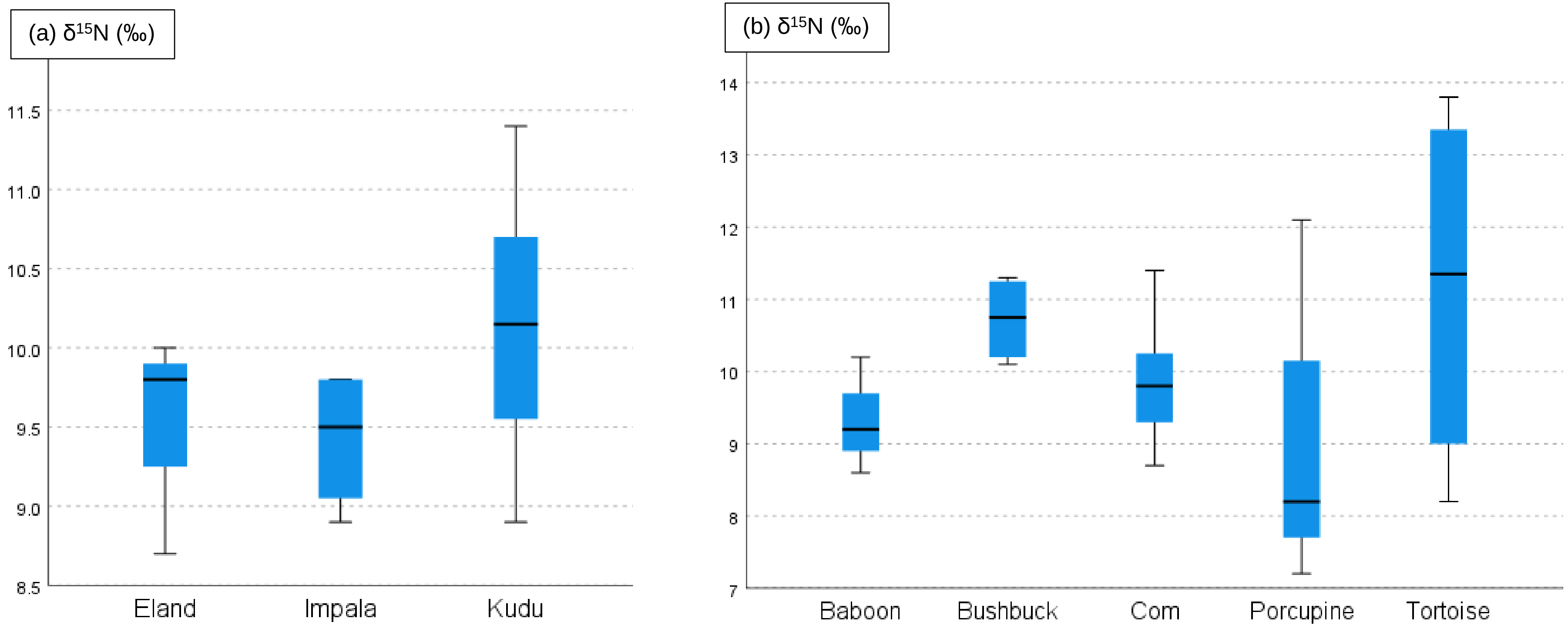


Figure 5.9: Box-and-whisker plots showing the distributions of $\delta^{15}N_{collagen}$ values (‰) for **a)** eland (*T. oryx*), impala (*A. melampus*) and greater kudu (*T. strepsiceros*) and **b)** a combined (Com) dataset (eland, impala and kudu) with chacma baboons (*P. ursinus*), bushbuck (*T. scriptus*), Cape porcupines (*H. africaeaustralis*) and leopard tortoises (*S. pardalis*). See **Figure 5.3** for a description of the boxplot format.

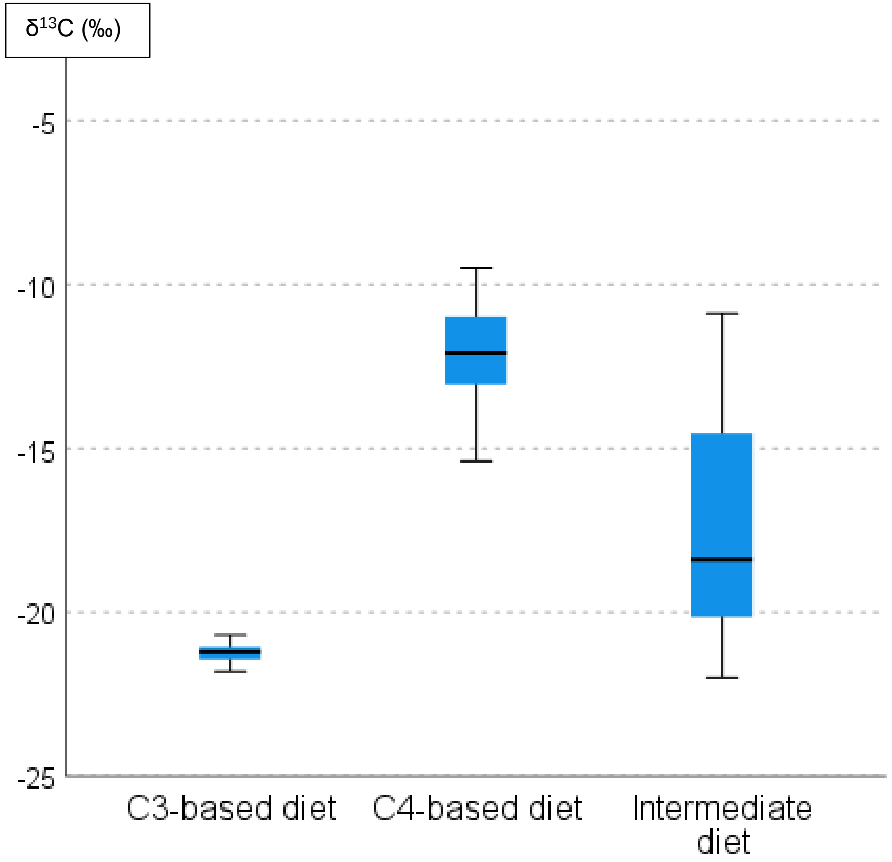


Figure 5.10: Box-and-whisker plots comparing the distributions of $\delta^{13}C_{collagen}$ values (‰) of species with a predominantly C_3-based diet (bushbuck, kudu, eland), species with a predominantly C_4-based diet (zebra, wildebeest, gemsbok, waterbuck) and intermediate feeders incorporating both C_3-based and C_4-based foods in varying proportions (warthog, tortoise, baboon, porcupine, impala). See **Figure 5.3** for a description of the boxplot format.

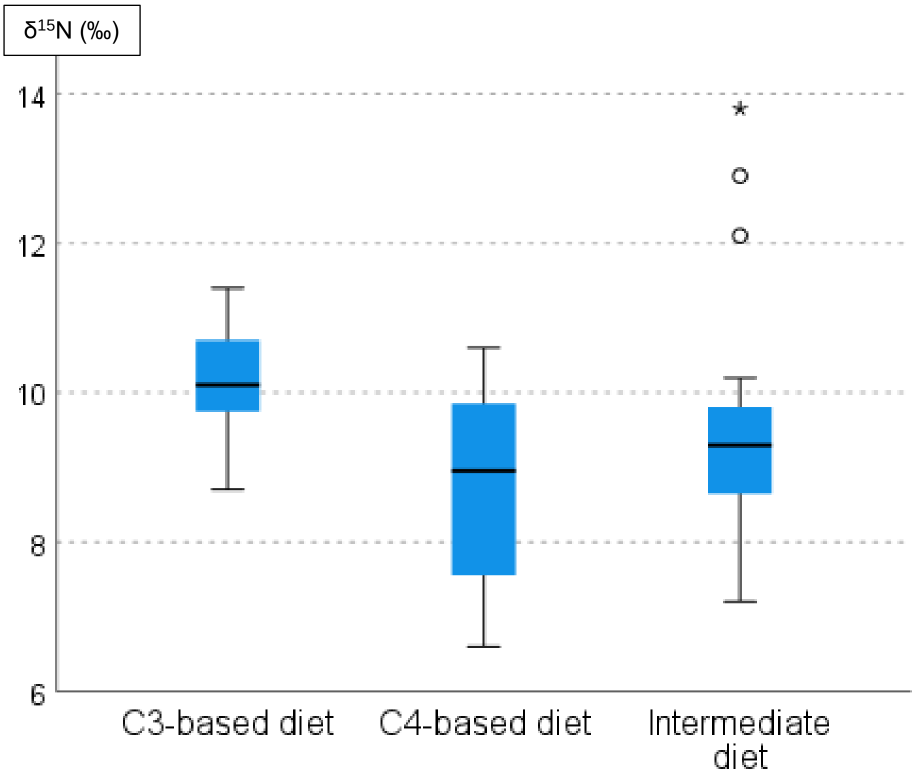


Figure 5.11: Box-and-whisker plots comparing the distributions of $\delta^{15}N_{collagen}$ values (‰) of species with a predominantly C_3-based diet (bushbuck, kudu, eland), species with a predominantly C_4-based diet (zebra, wildebeest, gemsbok, waterbuck) and intermediate feeders incorporating both C_3-based and C_4-based foods in varying proportions (warthog, tortoise, baboon, porcupine, impala). See **Figure 5.3** for a description of the boxplot format.

The species were further divided into three groups based on Figure 5.1 and the literature (section 3.2): species with a predominantly C_3-based diet (bushbuck, kudu, eland), species with a predominantly C_4-based diet (zebra, wildebeest, gemsbok, waterbuck) and mixed feeders incorporating both C_3-based and C_4-based foods in varying proportions (warthog, tortoise, baboon, porcupine, impala). A Kruskal-Wallis test showed significant differences in $\delta^{13}C$ values between the three groups (Kruskal-Wallis H (2) = 40.471, p = <0.001) (Figure 5.10). There are statistically significant differences in $\delta^{15}N$ values between the three groups (Kruskal-Wallis H (2) = 11.626, p = 0.003) (Figure 5.11).

Values for $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ for three *Hystrix africaeaustralis* quills are shown in Figures 5.11, 5.13 and Appendix 2. The $\delta^{13}C_{keratin}$ values are “raw” values, uncorrected for comparison with bone collagen. All samples have a mean $\delta^{13}C$ value of -21.38‰

and a mean $\delta^{15}N$ value of 8.16‰. The lowest $\delta^{13}C$ value recorded for all three individuals is -23.6‰ and the highest is -18.5‰. The lowest $\delta^{15}N$ value recorded across all three porcupine quills is 6.1‰, the highest is 12.7‰ (see Appendix 2). Porcupine quill 1 was divided into 42 sub-samples, each approximately 0.85 cm long. These yielded a mean $\delta^{13}C$ value of -23.04 ± 0.22‰ and a mean $\delta^{15}N$ of 11.92 ± 0.54‰. Porcupine quill 2 was divided into 134 sub-samples, each approximately 0.25 cm long. These yielded a mean $\delta^{13}C$ value of -20.58 ± 0.85‰ and a mean $\delta^{15}N$ of 7.96 ± 0.49‰. Porcupine quill 3 was divided into 107 sub-samples, each approximately 0.35 cm long. These yielded a mean $\delta^{13}C$ value of -21.73 ± 0.51‰ and a mean $\delta^{15}N$ of 6.95 ± 0.43‰. The distributions of $\delta^{13}C$ (Kruskal-Wallis H (2) = 174.538, p = 0.000) and $\delta^{15}N$ values (Kruskal-Wallis H (2) = 204.831, p = 0.000) are statistically different across all three porcupine quills (Figure 5.12). The pairwise comparisons with adjusted p-values further showed that there are statistically significant differences between each of the three porcupines ($\delta^{13}C$ and $\delta^{15}N$ values).

The three porcupine quills were cut into different sizes in order to investigate seasonality through different scales (P1 = 0.85 cm; P2 = 0.25 cm; P3 = 0.35 cm). A relationship was observed between the serial sample quill length and the mean $\delta^{13}C$ value (i.e. the smaller the piece, the higher the mean $\delta^{13}C$ value). However, there is no correlation observed between the quill length and mean $\delta^{15}N$ values. Therefore, there is no meaningful relationship between quill length and isotope values. For future studies, it is best to cut the quills into even smaller pieces (<0.25 cm) for maximum temporal resolution.

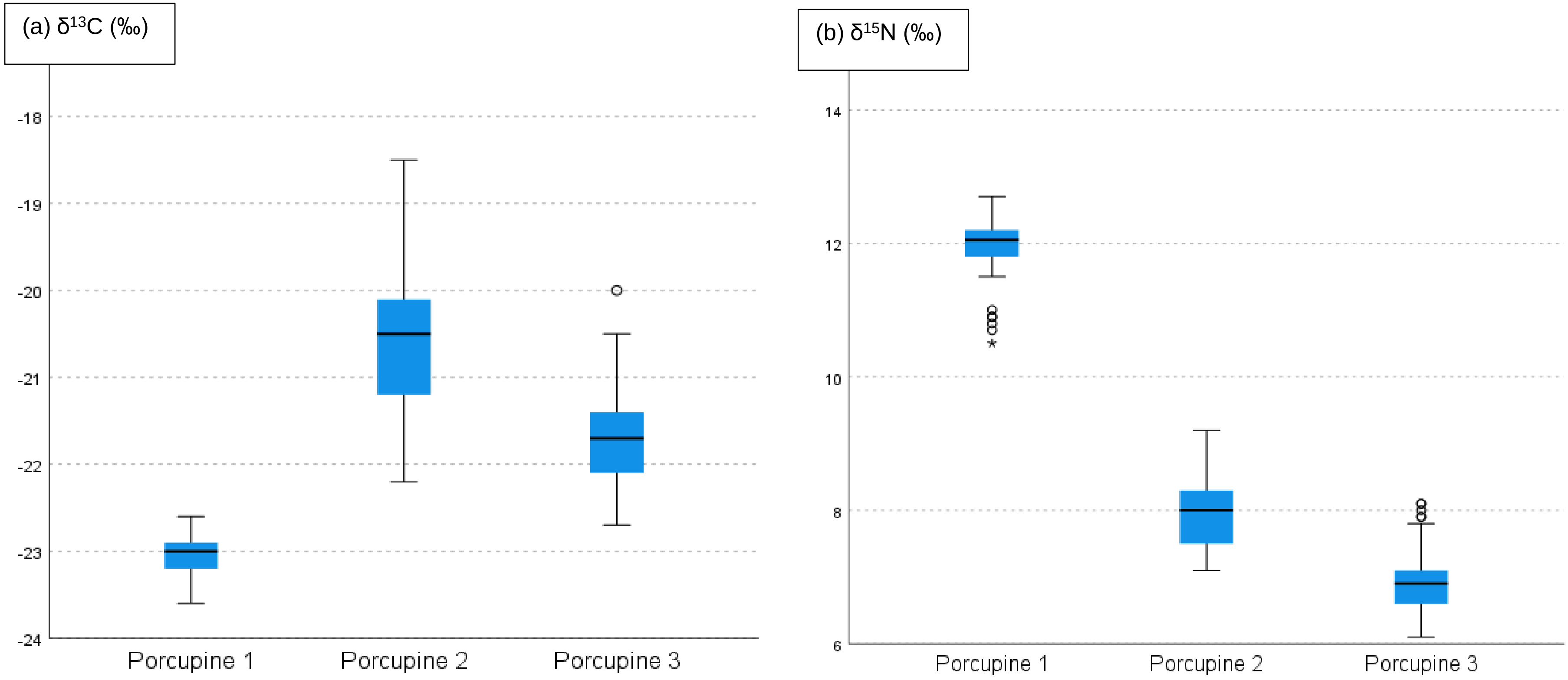


Figure 5.12: Box-and-whisker plots showing the distributions of **b)** δ^{15}N$_{\text{collagen}}$ values (‰) for Cape porcupine (*H. africaeaustralis*) quills from three different individuals (Porcupine 1, Porcupine 2, Porcupine 3). See **Figure 5.3** for a description of the boxplot format.

Table 5.3: Summary statistics for $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ for three porcupine quills.

	Sample Size	Length (cm)	Mean $\delta^{13}C$ (‰)	Median $\delta^{13}C$ (‰)	Std Dev $\delta^{13}C$ (‰)	Min $\delta^{13}C$ (‰)	Max $\delta^{13}C$ (‰)	Range $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	Median $\delta^{15}N$ (‰)	Std Dev $\delta^{15}N$ (‰)	Min $\delta^{15}N$ (‰)	Max $\delta^{15}N$ (‰)	Range $\delta^{15}N$ (‰)
Porcupine 1	42	0.85	-23.04	-23.0	0.22	-23.6	-22.6	1.0	11.92	12.0	0.54	10.5	12.7	2.2
Porcupine 2	134	0.25	-20.58	-20.5	0.85	-22.2	-18.5	3.7	7.96	8.0	0.49	7.1	9.2	2.1
Porcupine 3	107	0.35	-21.73	-21.7	0.51	-22.7	-20.0	2.7	6.95	6.9	0.43	6.1	8.1	2.0

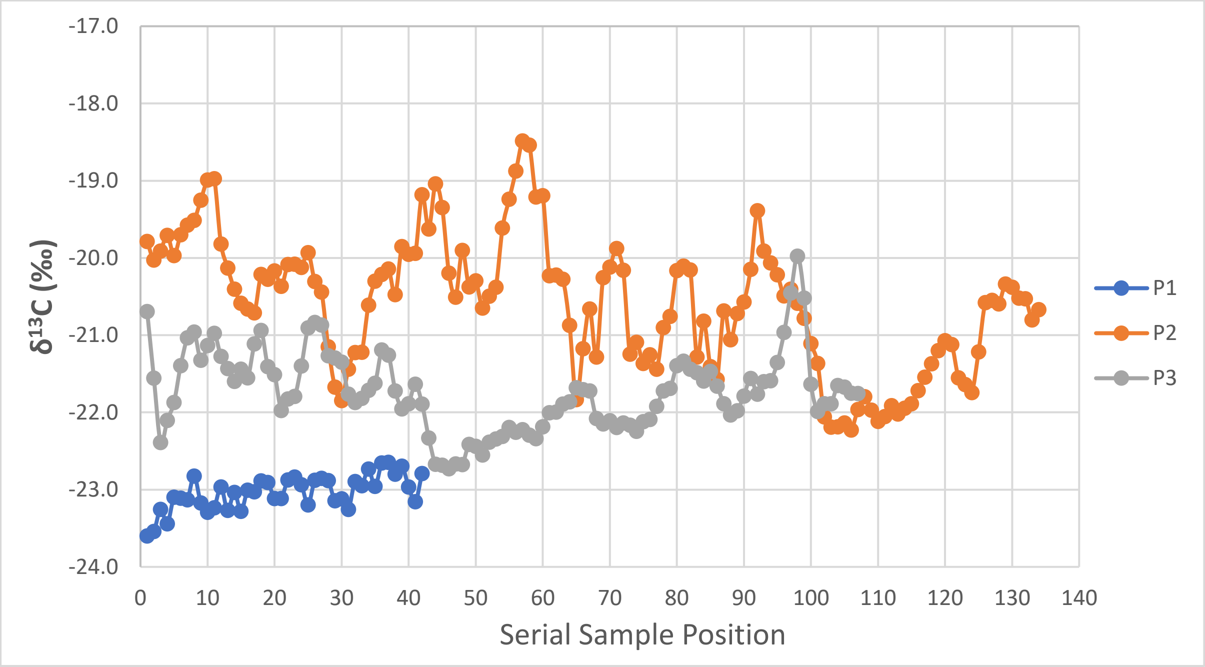


Figure 5.13: $\delta^{13}C_{keratin}$ (‰) values of serial samples of quills from three porcupines. Point 1 on the x-axis is the sample closest to the porcupine's body.

Figure 5.13 shows the δ^{13}C values of serial samples for three porcupines. Porcupine 1 yielded δ^{13}C values ranging from -23.6‰ to -22.6‰, Porcupine 2 from -22.2‰ to -18.5‰ and Porcupine 3 from -22.7‰ to -20.0‰. Porcupine 2 shows the largest range of 3.7‰, and Porcupine 1 the smallest (1.0‰). It is clear that porcupines prefer a predominantly C$_3$ diet, but there is considerable inter-individual variation. The substantial peaks and troughs observed for porcupines 2 and 3 are likely to reflect short-term or seasonal shifts in the choice of foods and/or in the isotopic ratios of these foods.

Figure 5.14 shows the δ^{15}N values of serial samples for three porcupines. Values for Porcupine 1 range from 10.5‰ to 12.7‰, for Porcupine 2 from 7.1‰ to 9.2‰ and for Porcupine 3 from 6.1‰ to 8.1‰. Each of the three porcupines displays a δ^{15}N range of approximately 2‰ (see Table 5.3 above). The record for Porcupine 1 does not show as many peaks and troughs as the other two quills because the resolution is lower. However, Porcupine 1 shows a steady decrease in δ^{15}N values from P1.1 to P1.42. Porcupines 2 and 3 do not show strong directional trends, nor do they show very strong patterns of peaks and troughs.

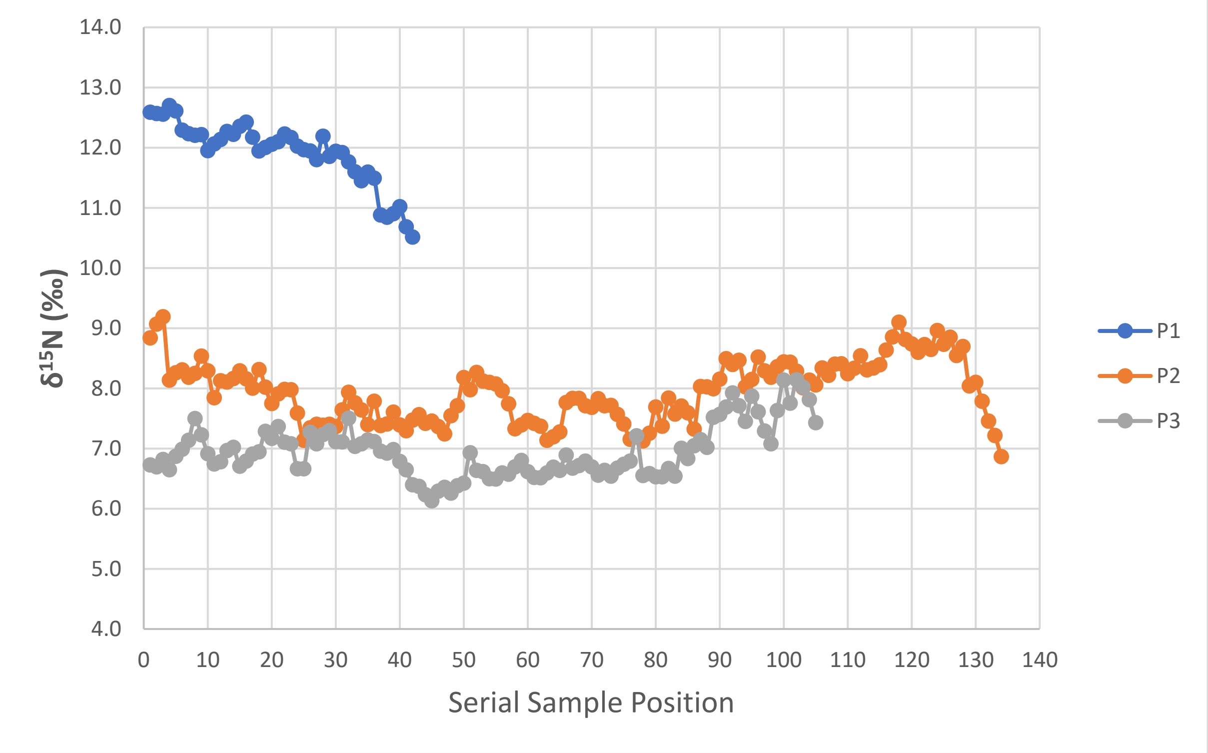


Figure 5.14: δ^{15}N$_{keratin}$ (‰) values of serial samples of quills from three porcupines. Point 1 on the x-axis is the sample closest to the porcupine's body.

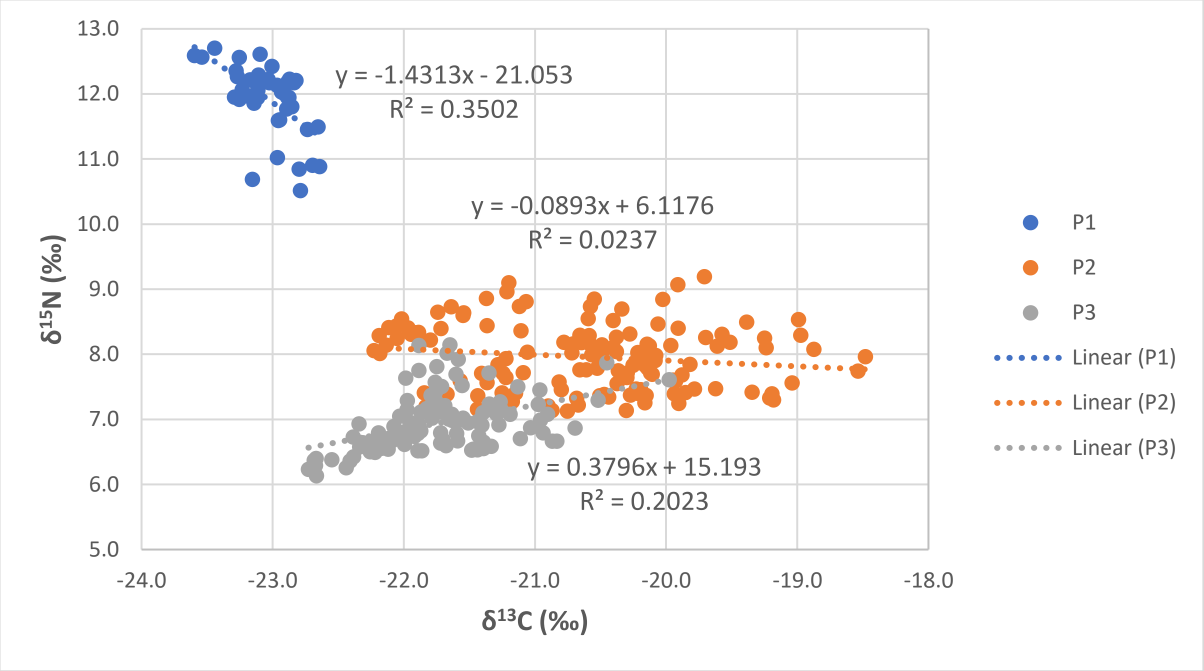


Figure 5.15: Scatter plot of $\delta^{13}C_{keratin}$ against $\delta^{15}N_{keratin}$ for three porcupine quills. The figure includes the linear regression equations and R^2 (coefficients of determination) for each quill.

Figure 5.15 shows the scatter plot of $\delta^{15}N_{keratin}$ against $\delta^{13}C_{keratin}$ for the three porcupine quills. For all three porcupines, the relationships between $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values are weak, with coefficients of determination ranging from 0.02 for porcupine 2 to 0.35 for porcupine 1. This shows that there are different factors driving variation in $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values. However, variation in the $\delta^{13}C$ values is not only about the proportions of C_3 and C_4 plants consumed. $\delta^{13}C$ values may vary depending on which part of the plant is consumed, and the choice of C_3 plants.

5.2. $\delta^{13}C$ and $\delta^{18}O$ of Tooth Enamel

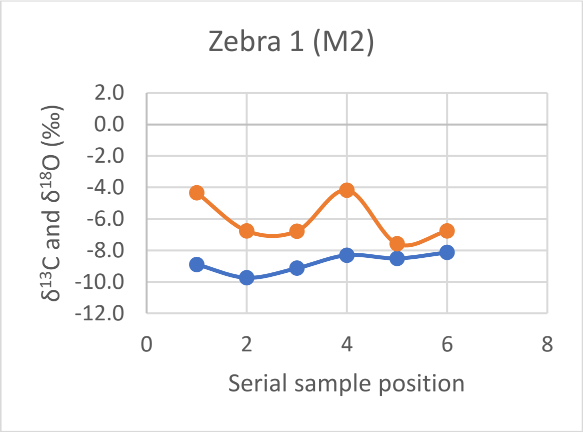
This section reports the $\delta^{13}C$ and $\delta^{18}O$ values of serial samples of tooth enamel from the second and third molars of nine plains zebra (*Equus quagga*) individuals. The $\delta^{13}C$ values for all samples ranged from -10.2‰ to -3.7‰ and the $\delta^{18}O$ values from -8.5‰ to 1.0‰ (n=107) (see Appendix 3). Table 5.4 shows the summary statistics for each individual. The intra-individual range in $\delta^{13}C$ values varies from 1.3‰ (zebra 6) to 5.1‰ (zebra 3), and that for $\delta^{18}O$ varies from 3.9‰ (zebra 1) to 8.1‰ (zebra 8). For seven animals, it was possible to sample both the second and third molars. In five out of the seven, the $\delta^{13}C_{enamel}$ values for the third molars (M3) were more negative than those for the second molars (M2).

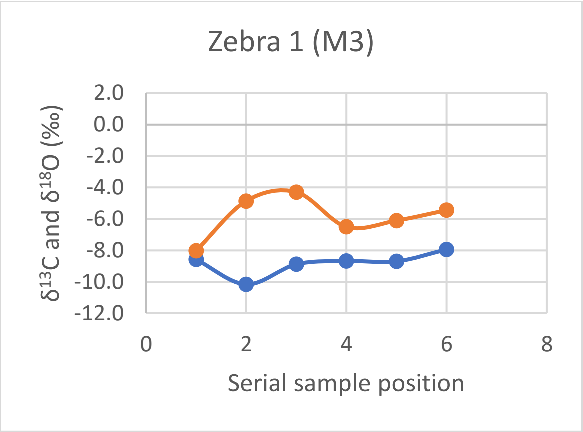
Table 5.4: Summary statistics for $\delta^{13}C_{enamel}$ and $\delta^{18}O_{enamel}$ for *Equus quagga* individuals.

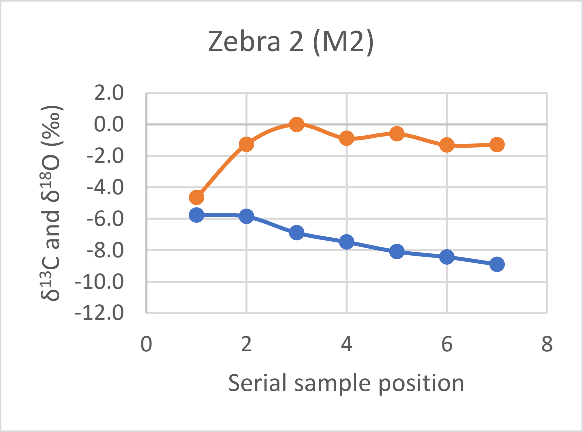
Individual	**Tooth**	**Mean $\delta^{13}C$ (‰)**	**Median $\delta^{13}C$ (‰)**	**Std Dev $\delta^{13}C$ (‰)**	**Range $\delta^{13}C$ (‰)**	**Overall Range $\delta^{13}C$ (‰)**	**Mean $\delta^{18}O$ (‰)**	**Median $\delta^{18}O$ (‰)**	**Std Dev $\delta^{18}O$ (‰)**	**Range $\delta^{18}O$ (‰)**	**Overall Range $\delta^{18}O$ (‰)**
Zebra 1	M2	-8.78	-8.7	0.60	1.6	2.2	-6.06	-6.8	1.44	3.4	3.9
	M3	-8.82	-8.7	0.73	2.2		-5.87	-5.8	1.32	3.7	
Zebra 2	M2	-7.35	-7.5	1.23	3.1	3.1	-1.43	-1.3	1.50	4.7	4.7
Zebra 3	M2	-6.22	-6.0	1.50	4.4	5.1	-3.40	-3.5	1.63	5.4	5.4
	M3	-7.51	-7.6	1.10	3.0		-3.53	-3.2	1.15	3.6	
Zebra 4	M2	-7.23	-7.2	0.62	1.7	2.6	-1.84	-2.1	2.13	6.1	6.5
	M3	-7.97	-8.2	0.91	2.6		-0.70	-1.1	0.97	2.6	
Zebra 5	M2	-5.38	-5.1	1.20	2.6	3.5	-2.82	-2.2	2.24	6.0	6.8
	M3	-7.25	-7.5	0.62	1.6		-1.41	-1.2	1.24	3.3	
Zebra 6	M2	-8.10	-8.2	0.37	1.1	1.3	-2.17	-2.1	1.23	3.7	5.1
	M3	-7.61	-7.6	0.24	0.8		-2.72	-2.3	1.48	3.8	
Zebra 7	M2	-5.82	-6.1	0.74	1.9	2.9	-2.66	-2.2	1.97	5.3	5.6
	M3	-7.04	-7.1	0.34	0.9		-2.75	-3.0	1.92	5.6	
Zebra 8	M2	-6.45	-6.7	1.12	3.3	3.3	-3.09	-1.8	3.05	8.1	8.1
Zebra 9	M2	-5.89	-5.8	0.63	1.8	3.4	-4.50	-4.7	1.95	5.2	7.0
	M3	-4.40	-4.5	0.58	1.6		-2.49	-1.8	2.05	6.0	

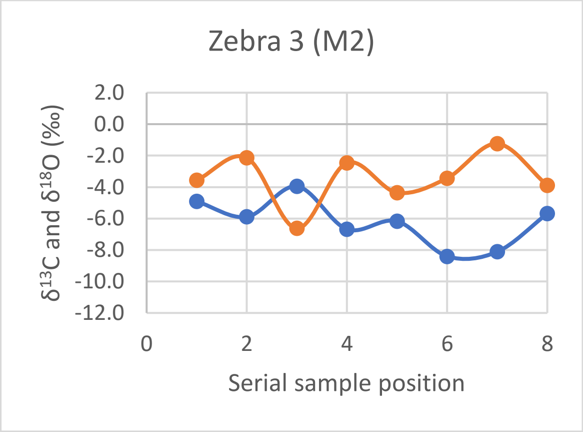
Figure 5.16 shows the $\delta^{13}C$ and $\delta^{18}O$ values of enamel serial samples in the second and third molars of nine *Equus quagga* individuals. There is a great deal of inter-individual variation. Some individuals display a clear pattern of increase or decrease across the series. For example, Zebra 2 M2 shows an overall decrease in $\delta^{13}C$ values. The intra-individual variation in $\delta^{13}C$ values ranges from -10.2‰ to -3.7‰, and in $\delta^{18}O$ values from -8.5‰ to 1.0‰. Although zebra are known as predominant grazers, the $\delta^{13}C$ values indicate that zebra from Mmabolela incorporated significant amounts of C_3 plants into their diet. The M2 is likely to provide information from approximately 7 to 17 months, and the M3 from ~21 to 32 months of age. Therefore, dietary and climatic records for *E. quagga* over approximately two years (i.e. two winters and two summers) can be traced.

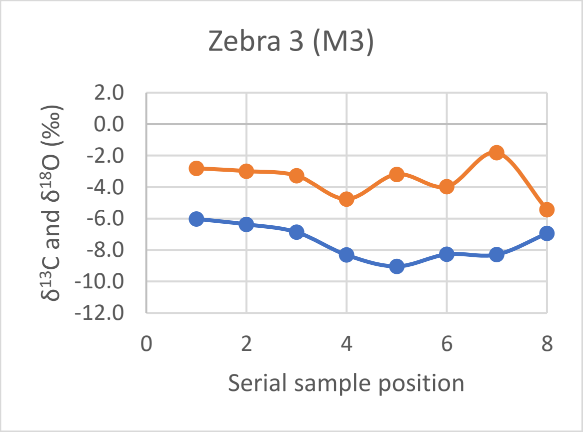
All zebra species breed throughout the year, but 85% of the births occur during rainy months (i.e. October to March) (Smuts, 1974). The gestation period is 336 to 390 days for *E. quagga* (Brown, 1936; Skinner and Smithers, 1990). The teeth record information at the beginning of the winter season (i.e. starting in approximately May for M2 and July for M3). More negative $\delta^{13}C$ values represent winter signals when grass quality and quantity are poor, and animals incorporate some C_3 resources into their diets, including lucerne supplied as supplementary feed. $\delta^{13}C$ values do indeed tend to decrease at the beginning of the sequence in most zebra, with the exception of Z9M2. In most animals (except Z1M3, Z2M2 and Z9M2), the $\delta^{18}O$ values also show a decline near the start of the sequence. Regular cyclical variations reflecting seasonality cannot easily be detected in these sequences because they are too short and variable. However, individuals Z1, Z3, Z4M3, Z6 and Z9 show clear cyclicity. Peaks and troughs represented by only one point are ignored (e.g. $\delta^{18}O$, Z3M2 point 3).

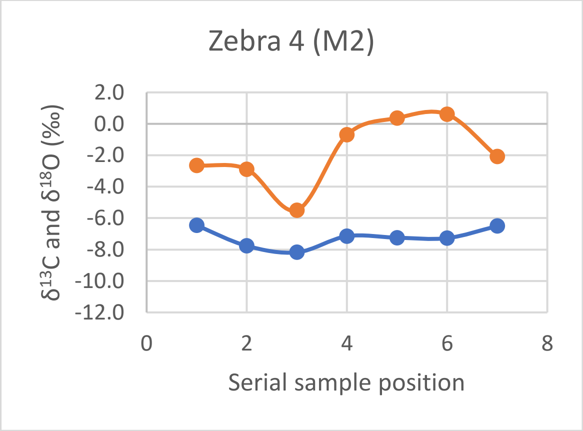

Zebra 1 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

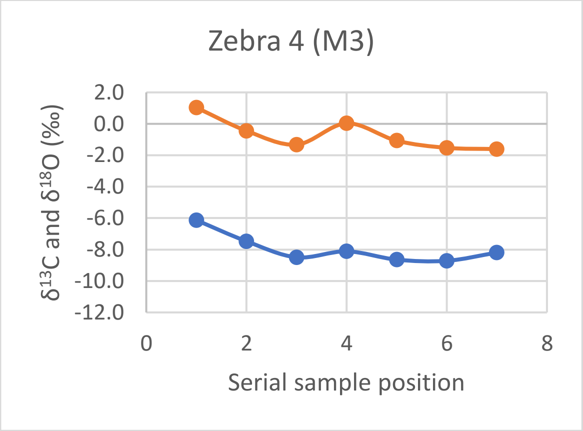

Zebra 1 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

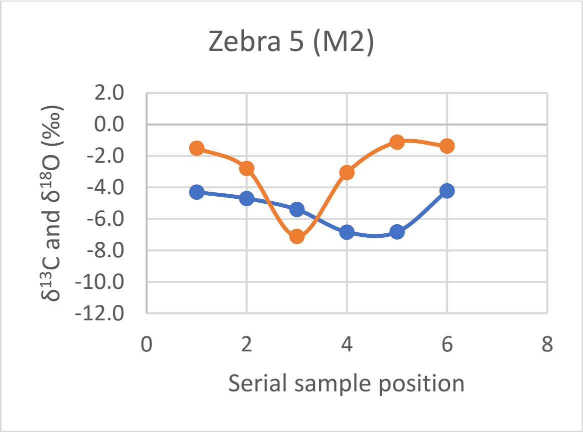

Zebra 2 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

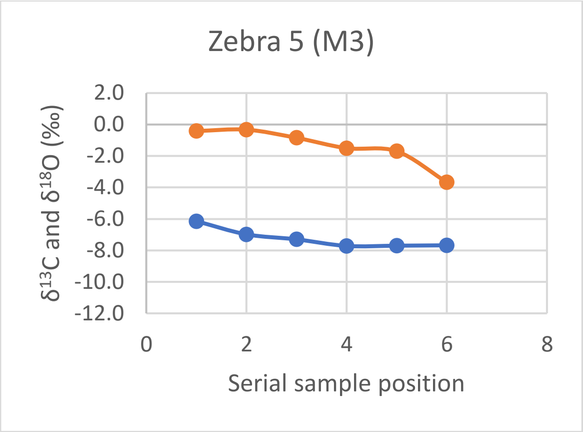

Zebra 3 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

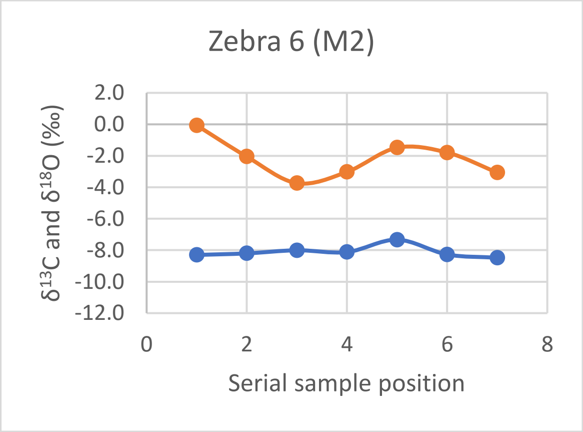

Zebra 3 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

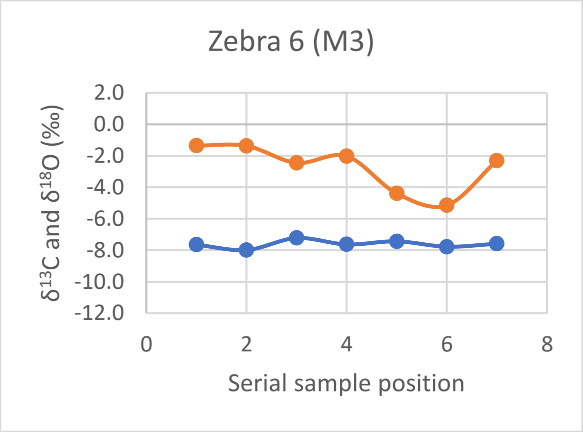

Zebra 4 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

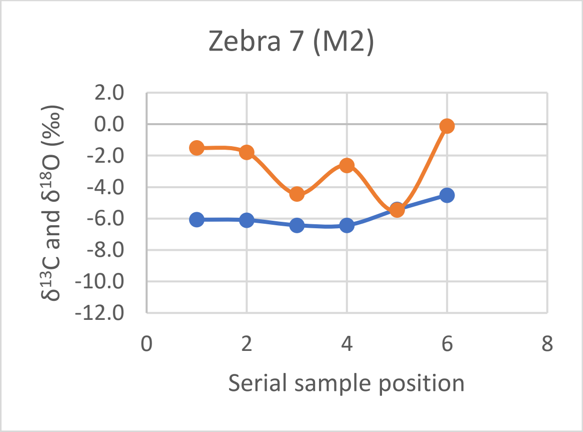

Zebra 4 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

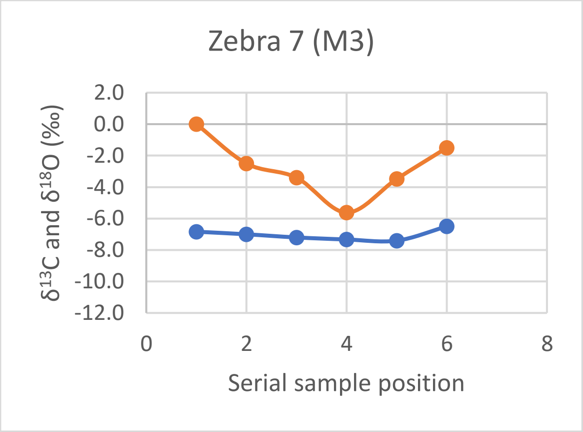

Zebra 5 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

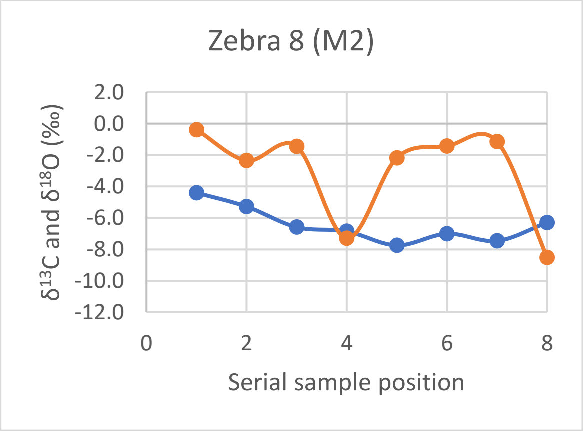

Zebra 5 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position


Zebra 6 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position


Zebra 6 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position


Zebra 7 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position


Zebra 7 (M3)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position


Zebra 8 (M2)
δ13C and δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0
2
4
6
8
Serial sample position

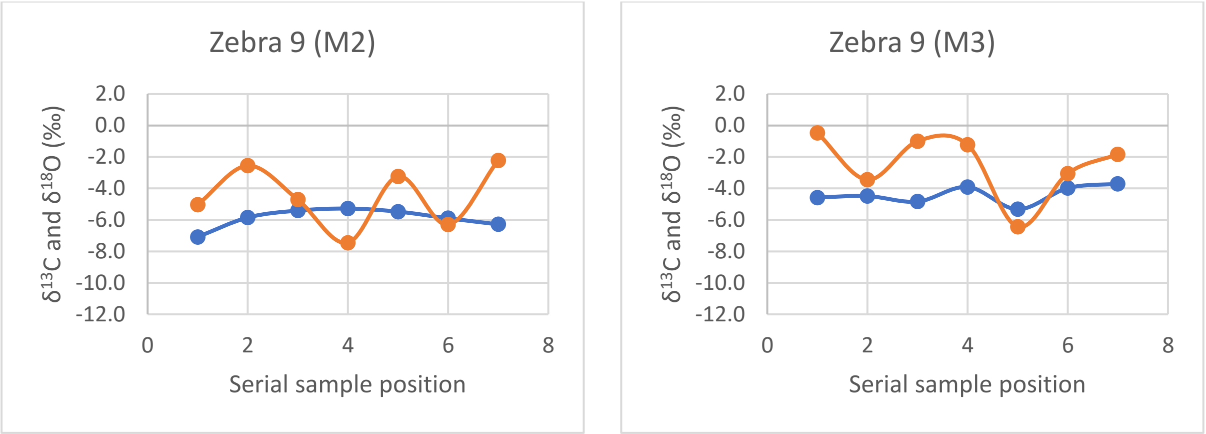
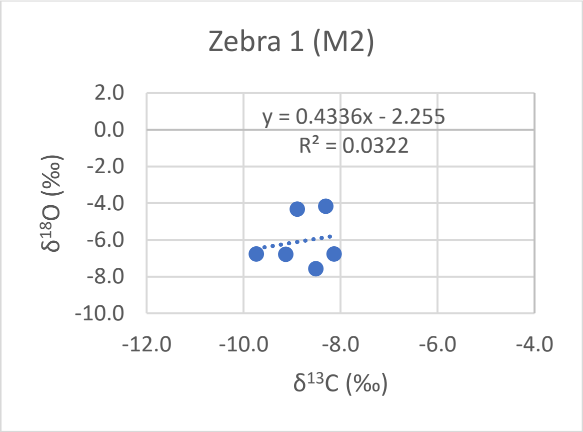
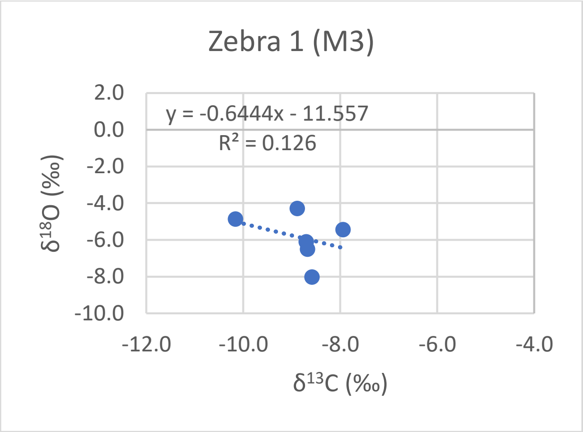
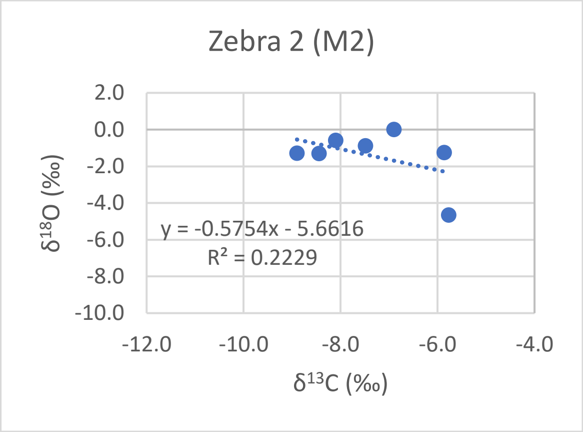


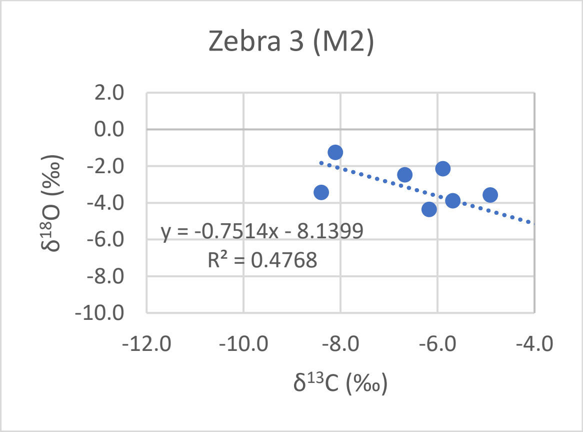
Figure 5.16: δ^{13}C (‰) and δ^{18}O (‰) values of enamel serial samples in nine *Equus quagga* individuals. 1 is the sample closest to the occlusal surface, and the last point represents the sample closest to the DEJ. δ^{13}C values are plotted in blue, and δ^{18}O values in orange.

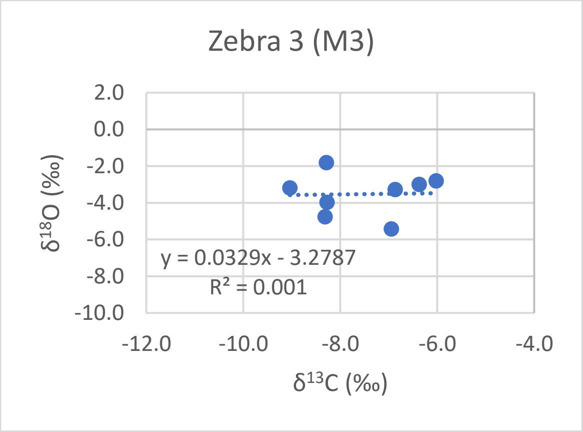
Figure 5.17 shows the correlations between the δ^{13}C and δ^{18}O values of enamel serial samples in nine *Equus quagga* individuals. R^2 values ranged from 0.001 to 0.756 across all individuals. Only zebra 4 (M3) showed a strong correlation (R^2 = 0.756) between δ^{13}C and δ^{18}O values. However, this outcome is mostly driven by a single point at the end of the distribution, without which the correlation is much weaker (R^2 = 0.3568). Zebra 7 (M2) shows the second strongest correlation between δ^{13}C and δ^{18}O values with an R^2 value of 0.5656. The generally weak correlations indicate that δ^{13}C and δ^{18}O values are controlled by different environmental factors. In zebra, δ^{13}C values depend mainly on the proportion of C_4 grass in the diet, while δ^{18}O depends on the oxygen isotope composition of drinking water. In most cases, there does not appear to be a direct relationship between these two variables.

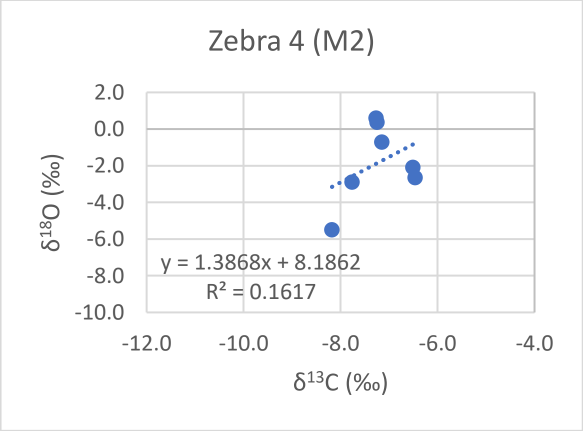

Zebra 1 (M2)
y = 0.4336x - 2.255
R² = 0.0322
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

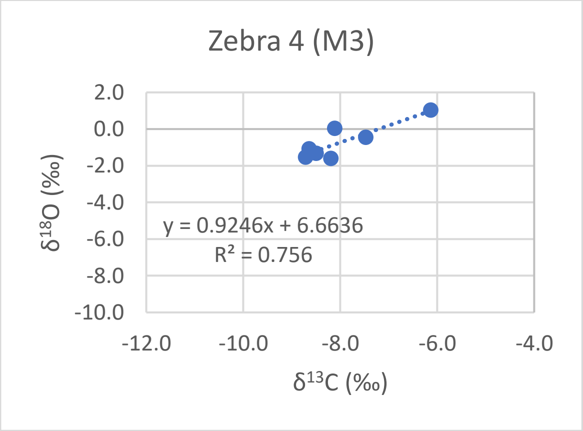

Zebra 1 (M3)
y = -0.6444x - 11.557
R² = 0.126
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

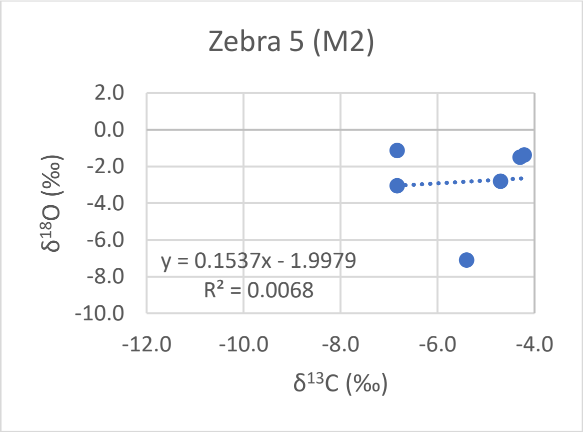

Zebra 2 (M2)
y = -0.5754x - 5.6616
R² = 0.2229
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

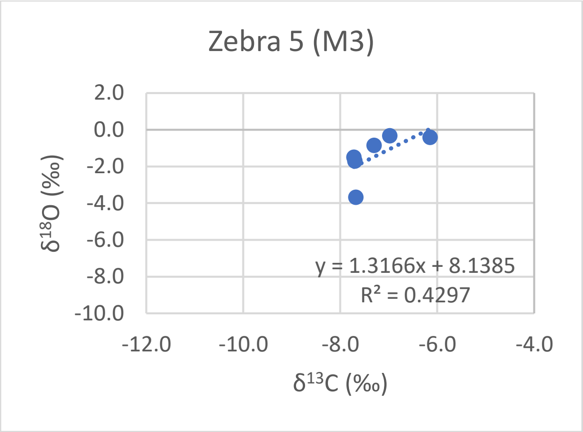

Zebra 3 (M2)
y = -0.7514x - 8.1399
R² = 0.4768
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

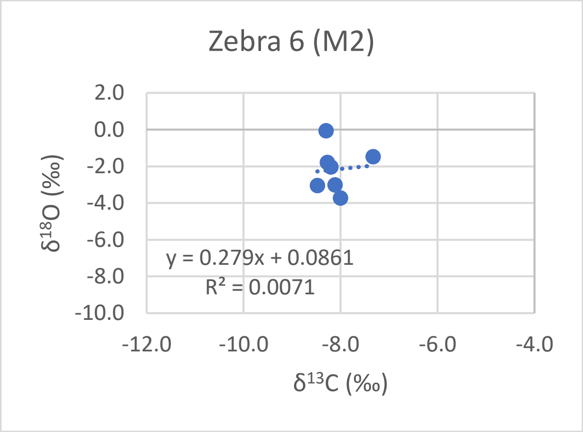

Zebra 3 (M3)
y = 0.0329x - 3.2787
R² = 0.001
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

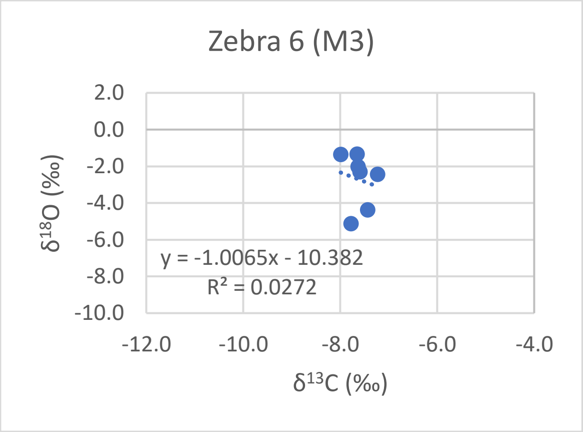

Zebra 4 (M2)
y = 1.3868x + 8.1862
R² = 0.1617
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

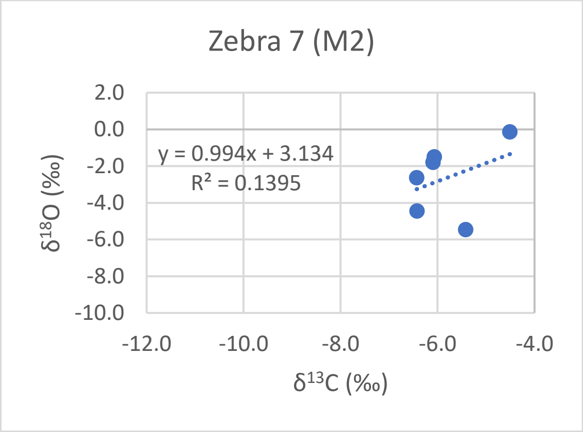

Zebra 4 (M3)
y = 0.9246x + 6.6636
R² = 0.756
δ18O (‰)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-10.0
-8.0
-6.0
-4.0
δ13C (‰)

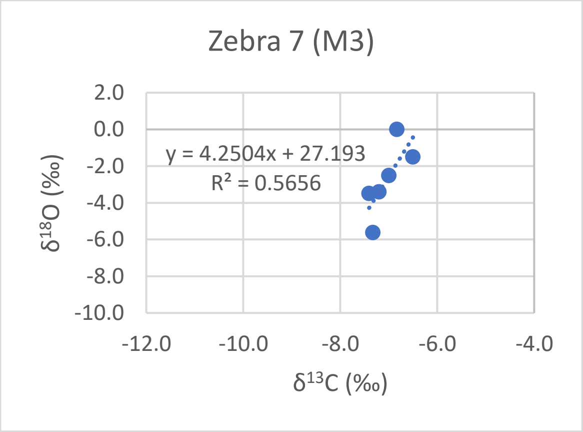

Zebra 5 (M2)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 0.1537x - 1.9979
R² = 0.0068
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)

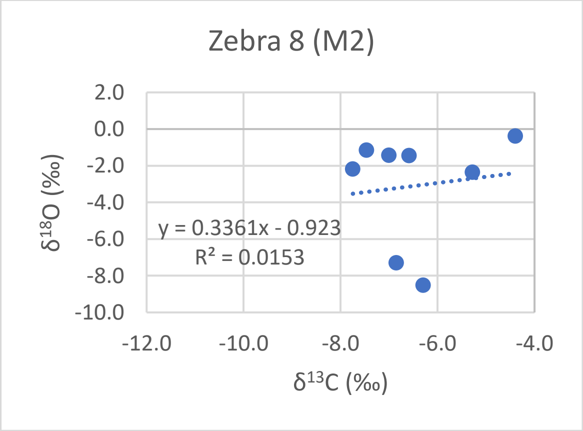

Zebra 5 (M3)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 1.3166x + 8.1385
R² = 0.4297
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)


Zebra 6 (M2)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 0.279x + 0.0861
R² = 0.0071
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)


Zebra 6 (M3)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = -1.0065x - 10.382
R² = 0.0272
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)


Zebra 7 (M2)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 0.994x + 3.134
R² = 0.1395
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)


Zebra 7 (M3)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 4.2504x + 27.193
R² = 0.5656
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)


Zebra 8 (M2)
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
$\delta^{18}O$ (‰)
y = 0.3361x - 0.923
R² = 0.0153
-12.0
-10.0
-8.0
-6.0
-4.0
$\delta^{13}C$ (‰)

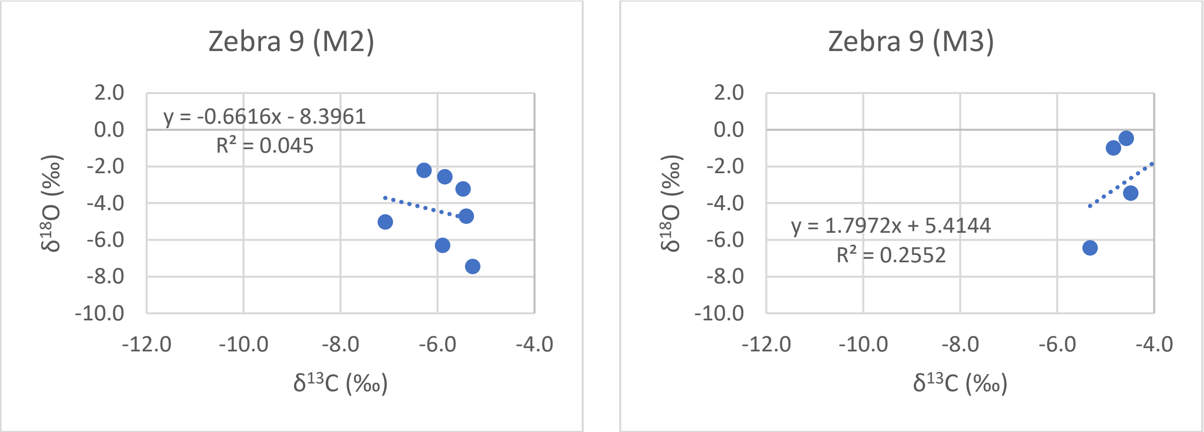


Figure 5.17: $\delta^{18}O$ plotted against $\delta^{13}C$ values for serial samples of enamel from nine *Equus quagga* individuals, with linear regression equations and R^2 (coefficients of determination).

In summary, the $\delta^{13}C$ values suggest that *E. quagga* had variable but mainly C_4 diets during the mineralisation of the M2 and M3. Since zebra are obligate drinkers, $\delta^{18}O_{\text{drinking water}}$ is likely to be a much more important determinant of $\delta^{18}O_{\text{enamel}}$ than possible variations in $\delta^{18}O$ of food consumed. These zebras drank from the Limpopo River when it was flowing, and (as mentioned in Chapter 2) from water pumped from boreholes when it was not. These different sources of drinking water probably complicate the patterning seen in $\delta^{18}O_{\text{enamel}}$ values. In the next chapter, the findings reported above will be considered in relation to previous studies of stable isotope patterning in African fauna.

Chapter 6: Discussion and Conclusion

The aim of this thesis is to document and try to interpret the isotopic ecology of multiple co-existing species of herbivores from a farm on the southern bank of the Limpopo River. This involves (i) investigating and interpreting isotopic similarities or differences between species (ii) exploring how different species within the categories of grazer, browser and mixed feeder relate to one another (iii) comparing these results with previous studies (Cerling *et al*., 2003; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a) (iv) investigating seasonal dietary shifts in the serial samples, and the factors that affect $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values over a seasonal cycle.

6.1. Isotopic Similarities and Differences Between Species

6.1.1. $\delta^{13}C_{bone\ collagen}$

$\delta^{13}C_{bone\ collagen}$ values indicate that bushbuck (*T. scriptus*), eland (*T. oryx*) and greater kudu (*T. strepsiceros*) are predominant browsers, as reported in previous studies (Cerling *et al*., 2003; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a). The pooled mean $\delta^{13}C$ value for the three species is -21.25 ± 0.30‰ (n=19) (Table 5.1); the distributions for the three species are not significantly different. This is potentially valuable information for palaeodietary studies since browsers have been shown to be useful environmental indicators. Luyt *et al*. (2019) demonstrate that $\delta^{13}C_{enamel}$ values for ungulate browsers track a number of environmental variables more closely than equivalent values for grazers and mixed feeders. Browsing species such as eland, kudu and bushbuck occur frequently in Pleistocene assemblages from the southern Cape and elsewhere (Klein, 1976; 1988). There are, however, no published studies of contemporary fauna that report $\delta^{13}C_{enamel}$ values for these three species from a single area. Chacma baboons (*P. ursinus*), Cape porcupines (*H. africaeaustralis*) and leopard tortoises (*S. pardalis*) also consumed diets based largely on C_3 plants.

Gemsbok (*O. gazella*), waterbuck (*K. ellipsiprymnus*), blue wildebeest (*C. taurinus*), warthogs (*P. africanus*) and plains zebra (*E. quagga*) have more positive $\delta^{13}C$ values reflecting a large proportion of C_4 graze. The mean $\delta^{13}C$ values for these five species range from -13.25‰ (for zebra) to -10.49‰ (for wildebeest). The most positive $\delta^{13}C$ value observed for an individual animal (wildebeest) is -9.5‰. These values suggest that these animals incorporated some C_3 plants into their diet. This is expected because animals were fed supplementary food such as lucerne (C_3) at Mmabolela during the dry winters. In the large South African study of Codron *et al*. (2007) in the Kruger National Park, where animals were not fed supplementary food in the dry season, mean values for faeces for *K. ellipsiprymnus* = -14.4‰; *C. taurinus* = -14.5‰; *P. africanus* = -14.2‰, which would equate to $\delta^{13}C_{collagen}$ of approximately -8.5‰. The implications of the $\delta^{13}C$ values of grazers will be discussed in more detail below (section 6.1.2).

In the area around Gqeberha (Eastern Cape), Potgieter and Kerley (2022) concluded that variation in grass availability led to zebra (*Equus quagga*) displaying substantial flexibility in terms of the proportion of grass in their diet (ranging from 66.8 to 83.5%). *E. quagga* from Mmabolela may also have incorporated some browse into their diet, and/or they may have consumed more of the lucerne than other grazers. Some studies have shown that southern African species consumed more C_3 plants than those from East Africa (see section 6.1.2). Therefore, location also plays a role in the variation of $\delta^{13}C$ values. Feeding strategy (bulk versus selective feeders, choice of plant parts) may contribute to inter-species differences within the categories of grazer and browser.

Impala (*A. melampus*), usually considered a mixed feeder, consumes a large proportion of browse in this environment (mean $\delta^{13}C$ of -18.10‰). Impala cluster closer to browsers than grazers, but they are at the most enriched end of the 'browser' group (i.e. impala have more positive $\delta^{13}C$ values compared with other species in the 'browser' group), indicating they are consuming some C_4 grass.

6.1.2. %C_4 Grass Consumption

A number of previous studies converted the $\delta^{13}C$ values into a percentage of C_4 grasses (%C_4) in the diet (Cerling *et al*., 2003; Codron *et al*., 2007; Sponheimer *et al*., 2003a). In order to compare the results from Mmabolela to previous studies, the formula below (from Codron, 2006) was used to convert the mean $\delta^{13}C$ values to percentage C_4 grass-intake (see Table 6.1):

$\%C_4 = (\delta^{13}C_{C3\ \text{plants}} + \Delta\delta^{13}C - \delta^{13}C_{\text{animal}}) / (\delta^{13}C_{C3\ \text{plants}} - \delta^{13}C_{C4\ \text{plants}}) \times 100$

Where '$\delta^{13}C_{C3}$ plants' is -28.77‰, the mean $\delta^{13}C$ value of plants that use the C_3 photosynthetic pathway, and '$\delta^{13}C_{C4}$ plants' is -12.90‰ (Cornwell *et al*., 2017). $\Delta\delta^{13}C$ is the isotopic enrichment factor for bone collagen compared with diet, assumed here to be +5.0‰ (Ambrose and DeNiro, 1986; Codron *et al*., 2018). Due to possible environmental influences on $\delta^{13}C$ values of C_3 plants, Sponheimer *et al*., (2003a) allowed for a 1.5‰ error in $\%C_4$ values.

Where the species of animals obtained from Mmabolela do not form part of previous studies, I will compare results with expectations about their diets, based on information reported in the Fauna and Ecology chapter. *H. africaeaustralis* were excluded from the calculations below because of additional uncertainties introduced by corrections of keratin to collagen $\delta^{13}C$ values.

According to results obtained for this thesis, *Aepyceros melampus* (impala) from Mmabolela consume 36% C_4 grass. Even though this value suggests an intermediate diet, it shows that this species prefers to browse. This is somewhat different from what was reported by Gagnon and Chew (2000), Sponheimer *et al*. (2003a) and Cerling *et al*. (2003), who classified impala as intermediate feeders that consume 45%, 51% and 52% C_4 grass respectively. Codron *et al*. (2007) classified impala as mixed-feeders that prefer grass ($\%C_4 = 60$). Skinner and Chimimba (2005) stated that C_4 grass intake for impala may vary depending on the season and locality. Pietersen *et al*. (1993) found that, in the eastern Limpopo Province, impala diet consists of 90% grass in the wet season and 65% in the dry season. Other factors leading to variation in $\delta^{13}C$ and $\%C_4$ in the same species include sex differences and variations in C_3 browse and C_4 graze availability across southern Africa (Sponheimer *et al*., 2003a). Therefore, it should be expected that diet might vary slightly between individuals of the same species.

Blue wildebeest (*C. taurinus*) $\%C_4$ values are similar to those reported in previous studies. This thesis reports a diet that consists of 84% C_4 grass for blue wildebeest. Gagnon and Chew (2000), Sponheimer *et al*. (2003a) and Codron *et al*. (2007) classified blue wildebeest as predominant grazers that consume 88%, 90% and 90% C_4 grass respectively. Cerling *et al*. (2003), in contrast, classified *C. taurinus* (in East Africa) as pure grazers ($\%C_4 = 100$).

Waterbuck (*K. ellipsiprymnus*) %C_4 values are also similar to those reported in previous studies. Waterbuck from Mmabolela eat 81% C_4 grass. Gagnon and Chew (2000), Cerling *et al*. (2003) and Codron *et al*. (2007) classified *K. ellipsiprymnus* as predominant grazers that consume 84%, 92% and 90% C_4 grass respectively. Sponheimer *et al*. (2003a), in contrast, classified waterbuck as pure grazers (%C_4 = 100). Skinner and Chimimba (2005) stated that waterbuck are primary grazers that occasionally add browse and fruits to their diet.

Gemsbok (*Oryx gazella*) %C_4 values are similar to those reported in previous studies. Gagnon and Chew (2000), Sponheimer *et al*. (2003a) and Cerling *et al*. (2003) classified gemsbok as predominant grazers that consume 75%, 81%, and 88% C_4 grass respectively. This thesis reports a diet that consists of 70% C_4 grass for gemsbok. In areas where grass cover is low, *O. gazella* thrive on a diet consisting of browse (Diekmann, 1980). In the Kgalagadi Transfrontier Park (Botswana and South Africa), gemsbok ate 89% grass during summer and 76% grass during winter (Knight, 1990).

Bushbuck (*T. scriptus*), eland (*T. oryx*) and greater kudu (*T. strepsiceros*) eat 15-16% C_4 grass at Mmabolela. For eland, this is rather more than the 8% reported by Sponheimer *et al*. (2003a) and 3% by Codron *et al*. (2007) for southern African eland. Cerling *et al*. (2003) reported that East African eland eat 18% C_4 grass. In contrast, Gagnon and Chew (2000) classified eland as mixed feeders (browser-grazer intermediates) (%C_4 = 50). *T. scriptus* from Mmabolela consume 16% C_4 grass, making them predominant browsers. Sponheimer *et al*. (2003a) and Cerling *et al*. (2003) found that bushbuck ate no C_4 grass, and Codron *et al*. (2007) found only 9%. Simpson (1974) stated that bushbuck are selective feeders, however, they are able to adjust their diet in unfavourable environments in order to survive.

There is a tendency for the grazing species studied in this thesis to display lower %C_4 values than those reported in previous studies (Cerling *et al*., 2003; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a). While the provision of some supplementary feed (lucerne) may be a contributing factor here, it may also be that in this relatively dry area, grazers consume more dicotyledonous plants. This shows that locality does have an effect on the proportion of C_4 grasses in the diet. The results from Mmabolela are consistent with those of Sponheimer *et al*. (2003a) and Codron *et al*. (2007) in finding that South African grazers tend to eat rather less C_4 grass than their East African counterparts. The results for predominant browsers, on the other hand, indicate greater consumption of C_4 grass than found in previous South African studies, and for bushbuck and kudu, greater than that reported by Cerling *et al*. (2003) for East Africa.

There is less comparative data for the remaining species: Chacma baboons (*Papio ursinus*, $\%C_4 = 24$), warthogs (*Phacochoerus africanus*, $\%C_4 = 78$), plains zebra (*Equus quagga*, $\%C_4 = 66$), leopard tortoise (*Stigmochelys pardalis*, $\%C_4 = 26$) and Cape porcupines (*Hystrix africaeaustralis*). With the exception of *E. quagga*, these results are largely as expected based on zoologists' understanding of the species' diets (section 3.2).

Results for *E*. *quagga* were surprising, because they were substantially different from previous studies classifying *E*. *quagga* as primary grazers consuming more than 90% C_4 grass (Codron *et al*., 2005a, 2007; Grubb, 1981; Gwynne and Bell, 1968; Steuer *et al*., 2014). *E. quagga* at Mmabolela ate only 66% C_4 grass. Skinner and Chimimba (2005) stated that these predominant grazers occasionally browse when forced into unusual environments. The low values observed in this thesis may reflect supplementary C_3 dietary input (lucerne) during the dry season (April to September) when grass coverage and quality are low. Some studies report that *E. quagga* may browse during dry periods (Grubb, 1981; Gwynne and Bell, 1968; Pienaar, 1963; Potgieter and Kerley, 2022). Potgieter and Kerley (2022) explored the question of the relative contributions of grass and forbs/browse to zebra diets. They reported a mean of only 75% grass, and at some sites and in some seasons, zebra avoided grass in favour of forbs. Zebra diets appear to be more flexible than previously realised. Nevertheless, the 66% grass intake reported in this study requires further investigation.

Table 6.1: Mean $\delta^{13}C$ values and estimated %C_4-intake from bone collagen of 11 species from Mmabolela Estates, compared with percentages of C_4 grass reported in previous studies. Hyphens indicate species not included in studies cited.

Species	**Common Name**	**Mean $\delta^{13}C$ (‰)**	**Std Dev (‰)**	**Sample Size**	**Mmabolela %C_4 in Diet**	**Gagnon and Chew (2000) %C_4 in Diet**	**Sponheimer *et al*. (2003a) %C_4 in Diet**	**Cerling *et al*. (2003) %C_4 in Diet**	**Codron *et al*. (2007) %C_4 in Diet**
Aepyceros melampus	Impala	-18.10	1.12	4	36	45	51	52	60
Connochaetes taurinus	Blue wildebeest	-10.49	1.20	5	84	88	90	100	90
Equus quagga	Plains zebra	-13.25	1.30	11	66	-	-	-	92
Kobus ellipsiprymnus	Waterbuck	-10.86	1.16	5	81	84	100	92	90
Oryx gazella	Gemsbok	-12.65	1.86	3	70	75	81	88	-
Papio ursinus	Chacma baboon	-20.01	0.40	3	24	-	-	-	-
Phacochoerus africanus	Warthog	-11.32	0.44	5	78	-	-	-	91
Stigmochelys pardalis	Leopard tortoise	-19.57	2.03	4	26	-	-	-	-
Taurotragus oryx	Common eland	-21.37	0.35	3	15	50	8	18	3
Tragelaphus scriptus	Bushbuck	-21.21	0.18	4	16	10	0	0	9
Tragelaphus strepsiceros	Greater kudu	-21.23	0.32	12	16	15	4	4	7

6.1.3. $\delta^{15}N_{\text{bone collagen}}$

Mean δ^{15}N values for the species analysed in this thesis ranged from 7.73‰ (n=11) for plains zebra (*E. quagga*) to 11.20‰ (n=4) for leopard tortoises (*S. pardalis*). However, as stated in the Results chapter, the mean and standard deviation do not characterize the tortoises well, because the δ^{15}N values for the four tortoises are very variable, and due to the differences in reptile and mammal physiology.

Among the bovids, species with a C_4-based diet do not have significantly different δ^{15}N values compared with species with a C_3-based diet. Some early papers on δ^{15}N values in animals reported that values of drought-tolerant (water-independent) species, which are mainly browsers (e.g. eland, bushbuck and kudu), are 2-4‰ higher than obligate drinkers (e.g. blue wildebeest and zebra), most of which are grazers (Ambrose and DeNiro, 1986; Sealy *et al*., 1987). The water-independent animals in this thesis include eland (mean δ^{15}N = 9.50‰), impala (9.42‰), leopard tortoises (11.20‰) and warthogs (9.03‰). The water-dependent animals consist of bushbuck (10.71‰), gemsbok (10.15‰), greater kudu (10.11‰), chacma baboons (9.33‰), waterbuck (9.64‰), blue wildebeest (9.44‰) and plains zebra (7.73‰). At Mmabolela, drinking water is readily available all year round, which likely diminishes any differences between water-dependent and water-independent species.

Zebra (n=11) have the lowest δ^{15}N values of all the grazing bovids, and compared with all bovids. In a study of multiple grazing species from South Africa, Codron *et al*. (2008b) also found that zebra had low δ^{15}N values. They point out that zebra are hindgut fermenters whereas bovids are foregut fermenters, which recycle urea to a greater extent than hindgut fermenters. Codron *et al*. (2008b) suggested that low δ^{15}N values in zebra may be due to a combination of factors including digestive metabolism and diet quality.

The δ^{15}N values for baboons, which are omnivores, are not significantly different from those for browsing herbivores (bushbuck, eland, impala and kudu) ($p = 1.000$). However, this is based on a small sample (3 baboons). There is also no significant difference in δ^{15}N values of grazing herbivores (combined) and baboons (Mann-Whitney $Z = 35.5$, $p = 0.624$). Codron *et al*. (2006) used δ^{13}C and δ^{15}N values to investigate the diet of chacma baboons at Waterberg and Kruger National Park. In both regions, baboons had lower faecal δ^{15}N values than other herbivore species (giraffe, zebra and impala). They hypothesised that baboons in these areas did not consume a significant amount of animal matter which would result in elevated $\delta^{15}N$ values. However, the consumption of insects does not necessarily lead to elevated $\delta^{15}N$ values. Smith *et al*. (2010) found that high $\delta^{15}N$ values of the Ganta (northern Liberia) chimpanzees did not result from consuming animal foods (termites), but rather from the environment they inhabited. Ganta chimpanzees live in tropical rainforests, where plants typically have higher $\delta^{15}N$ values than in temperate forests (Martinelli *et al*., 1999; Smith *et al*., 2010). Baboons from Mmabolela most likely also consumed largely plant-based diets, with similar $\delta^{15}N$ values to foods consumed by other browsing and grazing herbivores.

Low $\delta^{15}N$ values in animals may suggest the consumption of nitrogen-fixing (usually leguminous) plants. However, researchers need to be cautious when drawing such conclusions because some legumes (e.g. *Vachellia* spp. formerly *Acacia* and *Colophospermum mopane*) have relatively high $\delta^{15}N$ values (Codron *et al*., 2005b). Grass (C_4) is shallow-rooted while trees and shrubs (C_3) are usually deeper-rooted, as a result, these different plants access nitrogen differently. More positive $\delta^{15}N$ values are observed for deep-rooted plants than shallow-rooted ones. This is because deeper soils have usually undergone more denitrification which preferentially returns ^{14}N to the atmosphere, leaving the soil enriched in ^{15}N (Evans and Ehleringer, 1993; Kohzu *et al*., 2003; Nadelhoffer and Fry, 1988). Plants may reallocate nitrogen to different parts of the plant (e.g. stem, leaves, fruit) as the seasons change (Szpak, 2014). Therefore, the part of the plant consumed and time of year of consumption also contribute to variations in $\delta^{15}N$ values.

6.2. Indicators of Seasonality in Serial Samples

6.2.1. $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ for *Hystrix africaeaustralis*

$\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values for serially sampled Cape porcupines (*Hystrix africaeaustralis*) quills from three individuals (P1, P2 and P3) show that porcupines have very diverse diets. The $\delta^{13}C_{keratin}$ values for all samples analysed (range -23.6‰ to -18.5‰, n=283) reflect a predominantly C_3 diet. The $\delta^{15}N_{keratin}$ values for all three individuals range from 6.1‰ to 12.7‰ (n=283). When corrected for the difference between keratin and bone collagen, *H*. *africaeaustralis* have the lowest mean $\delta^{15}N$ value amongst C_3 feeders, and the second lowest across all species (only zebra are lower, see Figure 5.1).

There is significant inter-individual variation in both $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values. Porcupine 2 displayed higher $\delta^{13}C$ values (mean -20.58 ± 0.85‰) compared with P1 and P3 (means of -23.04 ± 0.22‰ and -21.73 ± 0.51‰ respectively). Porcupine 1 yielded $\delta^{13}C$ values ranging from -23.6‰ to -22.6‰, Porcupine 2 from -22.2‰ to -18.5‰ and Porcupine 3 from -22.7‰ to -20.0‰. Porcupine 1 displayed higher $\delta^{15}N$ values (mean 11.92 ± 0.54‰) compared with P2 and P3 (means of 7.96 ± 0.49‰ and 6.95 ± 0.43‰ respectively). $\delta^{15}N$ values for Porcupine 1 range from 10.5‰ to 12.7‰, for Porcupine 2 from 7.1‰ to 9.2‰ and for Porcupine 3 from 6.1‰ to 8.1‰. All three porcupines display similar ranges in $\delta^{15}N$ values of approximately 2‰ (see Table 5.3). However, ranges in $\delta^{13}C$ values are different (P1 = 1.0‰; P2 = 3.7‰; P3 = 2.7‰). This range encompasses much of the variation seen in this study.

Since the quills were found on different parts of the farm, they might be capturing different local habitats. Porcupines 2 and 3 may have eaten leguminous plants (Codron *et al*., 2005b) or shallow-rooted plants, which usually have lower $\delta^{15}N$ values (Kohzu *et al*., 2003). The high $\delta^{15}N$ values in Porcupine 1 may be caused by the consumption of plants with higher $\delta^{15}N$ values and perhaps also animal tissues. Porcupines are known to nibble on bones and the decaying flesh of animals (Duthie and Skinner, 1986; Pokines *et al*., 2017; Skinner and Chimimba, 2005).

The $\delta^{13}C$ and $\delta^{15}N$ patterns observed for P1 are too low-resolution to detect seasonality, although there is an overall trend towards less negative $\delta^{13}C$ and lower $\delta^{15}N$ values over the period of growth (Figures 5.10 and 5.11). The peaks and troughs observed for porcupines 2 and 3 reflect significant dietary shifts. The $\delta^{13}C$ values for P2 are similar for the first three troughs before a sharp increase in the pattern, the next three peaks occur at a similar level before a drop is observed. In contrast, patterns for $\delta^{15}N$ values do not show high variation. These results suggest that porcupines ate C_3 plants of various types and qualities at different times, however, the nitrogen isotope contents of the plants did not fluctuate as much.

As stated in section 3.2.3, there is little information on how long it takes for *Hystrix africaeaustralis* quills to form. Pigozzi (1988) reported how long it took for marked porcupine quills to fall off. Most of the marked quills were lost in 5-7 months, with only one left after 11 months. Taking this information into account, one can cautiously hypothesise that if porcupine quills take ~11 months to fall off, then they form within a year (i.e. if the growth period was longer, there would be more quills falling off than growing). Roze (2012) stated that the longest back quills (~100 mm) of North American porcupines take approximately 3.5 months to grow. The quills analysed in this thesis were approximately 300 mm long, so if they grew at the same rate, they would have taken almost a year to form. Based on both these studies, P1, P2 and P3 quills may represent a period of growth of approximately a year. Therefore the peaks and troughs observed in P2 and P3 most likely reflect short-term dietary variation, rather than clear wet/dry season signals. The reason for measuring porcupine quills was to assess how useful they might be as seasonal indicators. Given the amount of variation between the three quills and the short-term - but likely not seasonal - isotopic shifts, porcupine quills are not very good materials for tracking seasonality.

6.2.2. $\delta^{13}C_{enamel}$ and $\delta^{18}O_{enamel}$ for *Equus quagga*

Equid teeth are useful in serial sampling with the aim of detecting seasonal patterns due to their frequency in the fossil record and large crown height. $\delta^{13}C$ and $\delta^{18}O$ values of serial samples of tooth enamel from the M2 (second molar) and M3 (third molar) from nine plains zebra (*Equus quagga*) individuals in this study do appear to reflect seasonal variations. The enamel on equid second molars mineralises over a period of approximately 30 months (~7 to 37 months after birth) and that of the M3 over approximately 34 months (~21 to 55 months after birth) (Hoppe *et al*., 2004). However, in this thesis, only the parts of the teeth protruding into the mouth were sampled (i.e. approximately one-third of the entire tooth). Therefore, the M2 is likely to provide information from approximately 7 to 17 months, and the M3 from ~21 to 32 months of age. We are therefore able to track dietary and climatic information for *E. quagga* over about 25 months (approximately two winters and two summers), with a gap in the middle. Zebras 3 and 6 were juveniles because the apices of the M2 and M3 roots were open.

The $\delta^{13}C_{enamel}$ values for all samples ranged from -10.2‰ to -3.7‰ and $\delta^{18}O_{enamel}$ from -8.5‰ to 1.0‰ (n=107). The intra-individual ranges in $\delta^{13}C_{enamel}$ values vary from 1.3

(zebra 6) to 5.1‰ (zebra 3), and that for $\delta^{18}O$ varies from 3.9 (zebra 1) to 8.1‰ (zebra 8). There is substantial inter-individual variation in the $\delta^{18}O_{enamel}$ profiles. The mean $\delta^{13}C_{enamel}$ values for the M2s ranged from -8.78‰ to -5.38‰, and the $\delta^{18}O_{enamel}$ values from -6.06‰ to -1.43‰. The mean $\delta^{13}C_{enamel}$ values for the M3s ranged from -8.82‰ to -4.40‰, and the $\delta^{18}O_{enamel}$ values from -5.87‰ to -0.70‰. These indicate that these zebra individuals ate a mainly C_4 diet during the formation of the M2 and M3 teeth. As stated in section 5.2, in summer rainfall environments, young grazers are typically born in spring, so 7 months post-birth is likely to be early winter. These records (assuming no or negligible wear on the M2s) start at about 7 months of age.

Most individuals except Z9 show a trend towards more negative (more C_3) $\delta^{13}C_{enamel}$ values at the beginning of the M2 profiles (ignoring the 3rd point for Z3, a single more positive $\delta^{13}C$ value that may be anomalous). This may reflect a shift towards the consumption of more C_3 plants and/or supplementary feed during the dry winter season. Z3, Z4 and Z5 show clear seasonal patterns (i.e. cyclical variation) in $\delta^{13}C$ values. Some individuals (Z6) show almost no variation. Individuals Z2 and Z8 are hard to assess because there is only a single tooth from each. The fluctuations in $\delta^{18}O$ values are clearer, however, they are not necessarily very clearly patterned. Clear cyclical variation in $\delta^{18}O$ values is observed for Z1, Z3, Z4, Z5, Z6, Z7, Z8 and Z9.

There were no monthly precipitation records available for Mmabolela. Instead, there were average annual rainfall data measured in one of the farms in the Mmabolela conservancy from 1980 to 2019, and documentation of the intensity of the rain over a decade. There were only four reference materials ($\delta^{18}O_{groundwater}$) collected from areas around Mmabolela (Table 3.1). However, some studies showed that seasonality cannot be easily detected even with known parameters (e.g. vegetation distribution, animal ecology and rainfall data) due to errors (e.g. noisy data, complexity of isotopic values and animal physiology etc.) (Janzen *et al*., 2020; Norwood *et al*., 2023).

Samples collected from groundwater around Mmabolela in 2006 yielded $\delta^{18}O$ values that ranged from -34.53‰ to -33.20‰ (relative to VPDB) (Waterisotopes, 2023) (Table 3.1). There were no samples collected from precipitation and tap water. However, ground water can track precipitation $\delta^{18}O$ values (Bowen *et al*., 2011). At Mmabolela, the intra-individual $\delta^{18}O$ values ranged from -8.5‰ to 1.0‰. Therefore, these differences might suggest that the zebra from Mmabolela occupied a different habitat and received water from a different source. Higher $\delta^{18}O$ values indicate that zebra at Mmabolela were occasionally drinking from more evaporated water bodies such as seasonal pools and lakes (Barton *et al*., 1987; Janzen *et al*., 2020).

In summary, $\delta^{13}C$ values suggest that *E. quagga* had highly variable C_4-based diets during the mineralisation of the M2 and M3. $\delta^{18}O$ values are sensitive to different water sources, different parts of the plant consumed, and different environments. A few zebra individuals show clear seasonal patterns. However, it is difficult to detect seasonality, especially in short and highly variable sequences.

6.3. Conclusion

There have been many studies of the African savanna and the classification of its inhabitants along a browser-grazer continuum (e.g. Cerling *et al*., 2003; Codron *et al*., 2007; Gagnon and Chew, 2000; Skinner and Chimimba, 2005; Sponheimer *et al*., 2003a). Like Sponheimer *et al*. (2003a), this thesis found that $\%C_4$ values observed for species from Mmabolela (*A. melampus*, *C. taurinus*, *K. ellipsiprymnus*, *O. gazella* and *T. oryx*) tend to be lower than their East African counterparts (Cerling *et al*., 2003) and also somewhat lower than previous isotope-based studies in South Africa (Codron *et al*., 2007; Sponheimer *et al*., 2003a). It is difficult to know to what extent this is due to predominant grazers consuming more C_3 browse in this relatively dry environment, and to what extent it might be influenced by the provision of C_3 supplementary feed (lucerne) during the dry season. Interestingly, predominant browsers (*T.oryx*, *T. scriptus* and *T. strepsiceros*) consumed more C_4 grass (15-16%) than reported in most previous studies, with no detectable differences in $\delta^{13}C$ between the three species. There is no significant difference between $\delta^{15}N_{collagen}$ values of grazing and browsing bovids from Mmabolela, although zebra have lower values. Values for the small number of baboons (n=3) fall within the bovid range.

Isotopic analyses of incremental tissues (i.e. *H. africaeaustralis* quills and serial samples of *E. quagga* tooth enamel) were undertaken to assess their value as seasonal indicators in this environment. There is very substantial inter-individual variation in both $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values for *H. africaeaustralis*, with the $\delta^{13}C$ values reflecting a predominantly C_3 diet. $\delta^{13}C$ profiles reflect significant short-term variations in diet, although these appear to be on time scales of weeks or perhaps one or two months, and therefore probably do not record seasonality.

$\delta^{13}C_{enamel}$ and $\delta^{18}O_{enamel}$ profiles of the second and third molars of nine *E. quagga* individuals are highly variable and most show signs of seasonality. In most individuals, the first few $\delta^{13}C_{enamel}$ values starting from the occlusal surface of the M2 show a negative trend, as expected at the beginning of the dry season. $\delta^{18}O$ values in zebra reflect drinking water rather than leaf water. Zebra at Mmabolela drank from different water sources (i.e. Limpopo River and boreholes), making interpretation of $\delta^{18}O$ profiles difficult. Future studies should sample the entire heights of these teeth, which may make it easier to recognise patterning.

In summary, this thesis adds to our knowledge of $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ in different species of co-existing wild herbivores. It contributes data from an environment – on the banks of the Limpopo, in Limpopo Sweet Bushveld – rather different from those such as the Kruger Park, where most previous stable isotope work has been done. $\delta^{13}C_{enamel}$ and $\delta^{18}O_{enamel}$ profiles of zebra molars add to the growing evidence that extracting seasonal information from such proxies is challenging, and probably requires more sophisticated approaches.

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APPENDICES

Appendix 1: All isotopic data and quality control indicators for bone collagen.

UCT No.	Individual	Wt (mg)	Voltage (mv)	Area	%N	δ^{15}N (‰)	Std corrected δ^{15}N (‰)	Voltage (mv)	Area	%C	δ^{13}C (‰)	Std corrected δ^{13}C (‰)	C:N ratio (by wt)	C:N ratio elemental
24293	Kudu 1	0.445	4511	108.2	15.97	10.75	10.7	2781	103.9	43.91	-21.12	-21.1	2.7	3.2
24294	Kudu 2	0.401	4058	97.1	15.91	9.72	9.7	2513	93.7	43.98	-21.18	-21.1	2.8	3.2
24295	Kudu 3	0.445	4442	106.3	15.69	11.38	11.4	2727	101.7	42.99	-21.59	-21.5	2.7	3.2
24296	Kudu 4	0.355	3528	84.8	15.69	10.18	10.2	2181	81.3	43.08	-21.34	-21.3	2.7	3.2
24297	Kudu 5	0.431	4298	102.9	15.69	9.87	9.8	2659	99.1	43.24	-21.22	-21.2	2.8	3.2
24298	Kudu 6	0.393	3959	95.0	15.88	9.05	9.0	2428	90.5	43.32	-21.61	-21.6	2.7	3.2
24299	Kudu 7	0.443	4422	105.8	15.68	10.35	10.3	2724	101.5	43.11	-21.40	-21.4	2.7	3.2
24300	Kudu 8	0.422	4057	97.5	15.18	11.11	11.1	2634	98.3	43.82	-20.89	-20.8	2.9	3.4
24301	Kudu 9	0.396	3596	85.2	15.55	9.54	9.4	2462	93.1	43.41	-22.06	-21.4	2.8	3.3
24302	Kudu 10	0.409	4052	97.4	15.64	10.69	10.7	2541	94.9	43.64	-21.80	-21.8	2.8	3.3
24303	Kudu 11	0.406	4080	97.6	15.79	10.11	10.1	2509	93.4	43.30	-20.93	-20.9	2.7	3.2
24304	Kudu 12	0.430	4309	103.2	15.76	8.97	8.9	2692	100.3	43.90	-20.73	-20.7	2.8	3.2
24305	Eland 1	0.388	3458	81.9	15.26	8.80	8.7	2419	91.4	43.49	-21.69	-21.0	2.9	3.3
24306	Eland 2	0.383	3704	88.6	15.20	9.82	9.8	2368	88.0	43.21	-21.46	-21.4	2.8	3.3
24307	Eland 3	0.403	3641	86.0	15.42	10.12	10.0	2522	95.3	43.65	-22.42	-21.7	2.8	3.3
24308	Gemsbok 1	0.373	3622	86.6	15.25	10.65	10.6	2335	86.8	43.78	-11.00	-11.1	2.9	3.4
24309	Gemsbok 2	0.359	3527	84.4	15.44	9.85	9.8	2279	84.7	44.38	-12.16	-12.2	2.9	3.4
24310	Gemsbok 3	0.400	3604	85.1	15.39	10.15	10.0	2509	94.8	43.76	-15.29	-14.7	2.8	3.3
24311	Bushbuck 1	0.366	3654	87.1	15.64	11.32	11.3	2266	84.0	43.20	-21.23	-21.2	2.8	3.2
24312	Bushbuck 2	0.408	4073	96.6	15.56	10.09	10.1	2526	93.6	43.16	-21.51	-21.5	2.8	3.2
24313	Bushbuck 3	0.351	3484	83.0	15.54	11.20	11.2	2175	80.6	43.19	-21.08	-21.0	2.8	3.2

24314	Bushbuck 4	0.410	4092	96.8	15.51	10.30	10.3	2549	94.4	43.31	-21.20	-21.2	2.8	3.3
24315	Impala 1	0.405	4031	95.7	15.53	8.98	8.9	2503	92.7	43.06	-17.82	-17.8	2.8	3.2
24316	Impala 2	0.382	3865	91.5	15.73	9.22	9.2	2397	88.6	43.65	-17.83	-17.8	2.8	3.2
24317	Impala 3	0.445	4375	103.7	15.31	9.82	9.8	2847	105.6	44.63	-17.07	-17.1	2.9	3.4
24318	Impala 4	0.400	3640	85.7	15.48	9.96	9.8	2505	94.4	43.57	-20.36	-19.7	2.8	3.3
24319	Wildebeest 1	0.402	4009	95.0	15.52	9.64	9.6	2497	92.6	43.33	-9.96	-10.0	2.8	3.3
24320	Wildebeest 2	0.411	4078	96.9	15.48	8.33	8.3	2612	96.8	44.32	-11.27	-11.3	2.9	3.3
24321	Wildebeest 3	0.433	4314	102.3	15.52	10.34	10.3	2690	99.8	43.37	-9.41	-9.5	2.8	3.3
24322	Wildebeest 4	0.382	3813	90.6	15.59	9.70	9.7	2374	88.0	43.36	-12.10	-12.1	2.8	3.2
24323	Wildebeest 5	0.420	4297	101.9	15.95	9.37	9.3	2654	98.5	44.10	-9.39	-9.5	2.8	3.2
24324	Zebra 1	0.397	3593	84.8	15.46	7.93	7.8	2431	91.8	42.74	-13.62	-13.1	2.8	3.2
24325	Zebra 2	0.411	4061	96.3	15.40	8.15	8.1	2601	96.6	44.20	-12.65	-12.7	2.9	3.3
24326	Zebra 3	0.418	4171	99.0	15.55	7.47	7.4	2634	97.7	43.99	-12.95	-13.0	2.8	3.3
24327	Zebra 4	0.422	4210	99.9	15.55	7.35	7.3	2625	97.5	43.47	-14.45	-14.5	2.8	3.3
24328	Zebra 5	0.404	3957	94.1	15.31	7.75	7.7	2559	95.0	44.24	-12.79	-12.8	2.9	3.4
24329	Zebra 6	0.428	4296	102.1	15.67	6.90	6.8	2656	98.7	43.37	-14.30	-14.3	2.8	3.2
24330	Zebra 7	0.406	3985	94.8	15.33	6.95	6.9	2565	95.2	44.14	-14.37	-14.4	2.9	3.4
24331	Zebra 8	0.382	3806	90.6	15.59	7.49	7.4	2374	88.1	43.41	-12.07	-12.1	2.8	3.2
24332	Zebra 9	0.356	3521	83.9	15.49	8.48	8.4	2258	83.8	44.31	-12.46	-12.5	2.9	3.3
24333	Zebra 10	0.358	3521	84.0	15.42	6.73	6.6	2250	83.5	43.87	-15.40	-15.4	2.8	3.3
24334	Zebra 11	0.411	4131	98.4	15.73	10.63	10.6	2547	94.7	43.35	-10.80	-10.9	2.8	3.2
24335	Warthog 1	0.404	3936	93.9	15.27	8.77	8.7	2566	95.4	44.41	-11.03	-11.1	2.9	3.4
24336	Warthog 2	0.384	3759	89.8	15.37	8.36	8.3	2404	89.4	43.81	-11.18	-11.2	2.9	3.3
24337	Warthog 3	0.383	3887	92.7	15.89	9.44	9.4	2413	89.5	43.98	-12.00	-12.0	2.8	3.2
24338	Warthog 4	0.410	3986	95.0	15.23	9.50	9.5	2594	96.4	44.26	-10.83	-10.9	2.9	3.4
24339	Warthog 5	0.404	3978	94.9	15.43	9.32	9.3	2534	94.2	43.87	-11.27	-11.3	2.8	3.3
24340	Baboon 1	0.388	3804	91.1	15.43	10.25	10.2	2415	89.9	43.58	-20.46	-20.4	2.8	3.3
24341	Baboon 2	0.405	3997	95.7	15.53	9.21	9.2	2485	92.6	43.04	-19.65	-19.6	2.8	3.2

24342	Baboon 3	0.361	3571	85.8	15.62	8.64	8.6	2223	82.8	43.18	-20.01	-20.0	2.8	3.2
24343	Waterbuck 1	0.430	4238	101.6	15.52	8.61	8.6	2644	98.5	43.09	-9.56	-9.6	2.8	3.2
24344	Waterbuck 2	0.415	4132	98.8	15.64	10.28	10.3	2532	94.3	42.76	-11.14	-11.2	2.7	3.2
24345	Waterbuck 3	0.397	3917	93.8	15.53	9.68	9.6	2422	90.2	42.74	-11.98	-12.0	2.8	3.2
24346	Waterbuck 4	0.367	3630	86.7	15.52	9.82	9.8	2240	83.2	42.65	-9.55	-9.6	2.7	3.2
24347	Waterbuck 5	0.398	3928	94.1	15.54	9.96	9.9	2425	90.3	42.68	-11.74	-11.8	2.7	3.2
24351	Tortoise 1	0.375	3359	80.9	14.68	9.87	9.8	2242	83.6	42.05	-20.83	-20.5	2.9	3.3
24352	Tortoise 2	0.404	3701	89.1	14.99	12.90	12.9	2447	91.2	42.62	-17.78	-17.5	2.8	3.3
24353	Tortoise 3	0.436	3810	91.8	14.33	8.24	8.2	2601	97.2	42.07	-22.35	-22.0	2.9	3.4
24354	Tortoise 4	0.401	3595	86.4	14.66	13.79	13.8	2370	88.3	41.55	-18.71	-18.4	2.8	3.3

Appendix 2: δ^{13}C and δ^{15}N values and keratin quality control indicators for Hystrix africaeaustralis quills from three different porcupines (P1, P2 and P3). Serial sample P1.1 is the end of the quill closest to the porcupine's body. P1.42 is the end furthest away from the porcupine's body.

Sample	δ^{13}C (‰)	δ^{15}N (‰)	Wt %C	Wt %N	C/N elemental
P1.1	-23.6	12.6	47.7	14.9	3.7
P1.2	-23.5	12.6	48.1	15.4	3.6
P1.3	-23.3	12.6	47.6	15.2	3.6
P1.4	-23.4	12.7	47.3	15.2	3.6
P1.5	-23.1	12.6	47.0	15.1	3.6
P1.6	-23.1	12.3	47.8	15.5	3.6
P1.7	-23.1	12.2	46.9	15.1	3.6
P1.8	-22.8	12.2	47.0	15.1	3.6
P1.9	-23.2	12.2	47.0	15.2	3.6
P1.10	-23.3	12.0	47.1	15.1	3.6
P1.11	-23.2	12.1	47.1	15.2	3.6
P1.12	-23.0	12.1	48.0	15.5	3.6
P1.13	-23.3	12.3	46.1	14.9	3.6
P1.14	-23.0	12.2	46.0	14.9	3.6
P1.15	-23.3	12.4	46.0	15.0	3.6
P1.16	-23.0	12.4	46.4	15.0	3.6
P1.17	-23.0	12.2	45.8	14.8	3.6
P1.18	-22.9	11.9	46.2	14.9	3.6
P1.19	-22.9	12.0	46.8	15.2	3.6
P1.20	-23.1	12.1	46.7	15.1	3.6
P1.21	-23.1	12.1	46.9	15.1	3.6
P1.22	-22.9	12.2	46.9	15.3	3.6
P1.23	-22.8	12.2	46.7	15.1	3.6
P1.24	-22.9	12.0	46.6	15.1	3.6

Sample	δ^{13}C (‰)	δ^{15}N (‰)	Wt %C	Wt %N	C/N elemental
P2.1	-19.8	7.5	47.4	15.4	3.6
P2.2	-20.0	8.8	47.7	15.2	3.7
P2.3	-19.9	9.1	47.4	15.4	3.6
P2.4	-19.7	9.2	47.3	15.5	3.6
P2.5	-20.0	8.1	47.1	15.5	3.6
P2.6	-19.7	8.3	47.5	15.6	3.5
P2.7	-19.6	8.3	48.1	15.8	3.5
P2.8	-19.5	8.2	48.2	15.8	3.6
P2.9	-19.2	8.2	47.6	15.7	3.5
P2.10	-19.0	8.5	46.8	15.4	3.6
P2.11	-19.0	8.3	46.5	15.3	3.5
P2.12	-19.8	7.8	47.1	15.4	3.6
P2.13	-20.1	8.1	46.5	15.3	3.6
P2.14	-20.4	8.1	40.3	13.0	3.6
P2.15	-20.6	8.2	47.3	15.5	3.6
P2.16	-20.7	8.3	47.6	15.6	3.6
P2.17	-20.7	8.2	48.0	15.7	3.6
P2.18	-20.2	8.0	48.2	15.7	3.6
P2.19	-20.3	8.3	49.4	16.2	3.6
P2.20	-20.2	8.0	47.8	15.7	3.6
P2.21	-20.4	7.7	47.7	15.6	3.6
P2.22	-20.1	7.9	48.7	15.9	3.6
P2.23	-20.1	8.0	47.7	15.6	3.6
P2.24	-20.1	8.0	48.2	15.7	3.6

Sample	δ^{13}C (‰)	δ^{15}N (‰)	Wt %C	Wt %N	C/N elemental
P3.1	-20.7	6.9	48.5	15.4	3.7
P3.2	-21.6	7.0	48.1	15.2	3.7
P3.3	-22.4	6.7	47.9	15.2	3.7
P3.4	-22.1	6.7	47.6	15.4	3.6
P3.5	-21.9	6.8	47.6	15.5	3.6
P3.6	-21.4	6.6	48.0	15.4	3.6
P3.7	-21.0	6.9	47.6	15.5	3.6
P3.8	-21.0	7.0	47.8	15.6	3.6
P3.9	-21.3	7.1	47.5	15.5	3.6
P3.10	-21.1	7.5	47.5	15.4	3.6
P3.11	-21.0	7.2	47.3	15.3	3.6
P3.12	-21.3	6.9	47.1	15.4	3.6
P3.13	-21.4	6.7	48.2	15.8	3.6
P3.14	-21.6	6.8	47.7	15.5	3.6
P3.15	-21.4	7.0	47.2	15.5	3.6
P3.16	-21.5	7.0	47.3	15.5	3.6
P3.17	-21.1	6.7	47.1	15.4	3.6
P3.18	-20.9	6.8	47.6	15.5	3.6
P3.19	-21.4	6.9	46.8	15.2	3.6
P3.20	-21.5	6.9	47.2	15.5	3.6
P3.21	-22.0	7.3	47.1	15.4	3.6
P3.22	-21.8	7.2	47.2	15.4	3.6
P3.23	-21.8	7.4	46.7	15.2	3.6
P3.24	-21.4	7.1	46.9	15.4	3.6

P1.25	-23.2	12.0	46.5	15.0	3.6
P1.26	-22.9	11.9	46.6	15.2	3.6
P1.27	-22.9	11.8	45.9	14.9	3.6
P1.28	-22.9	12.2	45.8	14.9	3.6
P1.29	-23.1	11.9	46.1	15.0	3.6
P1.30	-23.1	11.9	45.9	15.0	3.6
P1.31	-23.3	11.9	45.6	14.8	3.6
P1.32	-22.9	11.8	46.2	15.2	3.6
P1.33	-22.9	11.6	46.3	15.2	3.6
P1.34	-22.7	11.5	46.4	15.1	3.6
P1.35	-23.0	11.6	46.0	15.1	3.6
P1.36	-22.7	11.5	46.4	15.2	3.6
P1.37	-22.6	10.9	46.0	14.9	3.6
P1.38	-22.8	10.8	46.6	15.2	3.6
P1.39	-22.7	10.9	46.2	15.1	3.6
P1.40	-23.0	11.0	47.1	15.3	3.6
P1.41	-23.2	10.7	46.5	15.1	3.6
P1.42	-22.8	10.5	45.8	14.8	3.6

P2.25	-19.9	7.6	47.7	15.6	3.6
P2.26	-20.3	7.1	47.2	15.3	3.6
P2.27	-20.4	7.3	47.4	15.3	3.6
P2.28	-21.1	7.4	46.9	15.2	3.6
P2.29	-21.7	7.4	46.8	15.2	3.6
P2.30	-21.8	7.4	46.8	15.1	3.6
P2.31	-21.4	7.4	46.7	15.1	3.6
P2.32	-21.2	7.6	47.1	15.2	3.6
P2.33	-21.2	7.9	46.7	15.2	3.6
P2.34	-20.6	7.8	46.9	15.1	3.6
P2.35	-20.3	7.6	46.5	14.9	3.7
P2.36	-20.2	7.4	47.0	15.0	3.6
P2.37	-20.1	7.8	46.4	15.0	3.6
P2.38	-20.5	7.4	46.8	15.0	3.6
P2.39	-19.9	7.4	46.4	14.8	3.7
P2.40	-20.0	7.6	46.3	15.0	3.6
P2.41	-19.9	7.4	46.4	15.0	3.6
P2.42	-19.2	7.3	46.1	14.9	3.6
P2.43	-19.6	7.5	46.3	15.0	3.6
P2.44	-19.0	7.6	46.1	14.9	3.6
P2.45	-19.3	7.4	46.8	15.1	3.6
P2.46	-20.2	7.5	47.0	15.1	3.6
P2.47	-20.5	7.4	46.9	15.1	3.6
P2.48	-19.9	7.2	46.8	15.1	3.6
P2.49	-20.4	7.5	46.8	15.1	3.6
P2.50	-20.3	7.7	47.0	15.2	3.6
P2.51	-20.6	8.2	46.7	15.2	3.6
P2.52	-20.5	8.0	46.7	15.1	3.6
P2.53	-20.4	8.3	46.8	15.2	3.6
P2.54	-19.6	8.1	46.6	15.2	3.6
P2.55	-19.2	8.1	46.4	15.2	3.6
P2.56	-18.9	8.1	46.5	15.2	3.6

P3.25	-20.9	7.1	46.8	15.2	3.6
P3.26	-20.8	6.7	46.1	15.0	3.6
P3.27	-20.9	6.7	51.4	16.8	3.6
P3.28	-21.3	7.3	53.6	17.5	3.6
P3.29	-21.3	7.1	47.4	15.4	3.6
P3.30	-21.3	7.2	46.8	15.4	3.5
P3.31	-21.8	7.3	46.7	15.3	3.6
P3.32	-21.9	7.1	46.5	15.3	3.6
P3.33	-21.8	7.1	46.5	15.2	3.6
P3.34	-21.7	7.5	46.4	15.1	3.6
P3.35	-21.6	7.0	47.2	15.4	3.6
P3.36	-21.2	7.1	46.5	15.2	3.6
P3.37	-21.3	7.1	46.9	15.4	3.6
P3.38	-21.7	7.1	46.1	15.2	3.6
P3.39	-22.0	7.0	46.6	15.3	3.6
P3.40	-21.9	6.9	46.3	15.3	3.5
P3.41	-21.6	7.0	46.7	15.3	3.6
P3.42	-21.9	6.8	46.7	15.3	3.6
P3.43	-22.3	6.6	46.5	15.2	3.6
P3.44	-22.7	6.4	46.1	15.2	3.5
P3.45	-22.7	6.4	46.6	15.3	3.5
P3.46	-22.7	6.2	46.5	15.3	3.6
P3.47	-22.7	6.1	46.4	15.2	3.6
P3.48	-22.7	6.3	46.1	15.2	3.6
P3.49	-22.4	6.4	47.1	15.5	3.5
P3.50	-22.4	6.3	46.7	15.4	3.5
P3.51	-22.6	6.4	46.9	15.4	3.6
P3.52	-22.4	6.4	46.9	15.4	3.5
P3.53	-22.3	6.9	47.5	15.5	3.6
P3.54	-22.3	6.6	47.2	15.4	3.6
P3.55	-22.2	6.6	47.2	15.5	3.6
P3.56	-22.3	6.5	46.7	15.4	3.5

P2.57	-18.5	8.0	46.7	15.2	3.6
P2.58	-18.5	7.7	46.5	15.1	3.6
P2.59	-19.2	7.3	46.6	15.2	3.6
P2.60	-19.2	7.4	46.5	15.2	3.6
P2.61	-20.2	7.5	46.5	15.1	3.6
P2.62	-20.2	7.4	46.5	15.2	3.6
P2.63	-20.3	7.4	46.6	15.2	3.6
P2.64	-20.9	7.1	46.5	15.2	3.6
P2.65	-21.8	7.2	46.6	15.2	3.6
P2.66	-21.2	7.3	46.0	15.0	3.6
P2.67	-20.7	7.8	46.5	15.1	3.6
P2.68	-21.3	7.8	46.2	15.1	3.6
P2.69	-20.3	7.8	46.0	15.0	3.6
P2.70	-20.1	7.7	46.8	15.1	3.6
P2.71	-19.9	7.7	47.0	15.2	3.6
P2.72	-20.2	7.8	47.0	15.3	3.6
P2.73	-21.2	7.7	46.5	15.1	3.6
P2.74	-21.1	7.7	46.6	15.2	3.6
P2.75	-21.4	7.6	46.5	15.2	3.6
P2.76	-21.3	7.4	46.6	15.2	3.6
P2.77	-21.4	7.2	46.6	15.2	3.6
P2.78	-20.9	7.2	46.8	15.2	3.6
P2.79	-20.8	7.1	46.5	15.1	3.6
P2.80	-20.2	7.3	46.2	15.1	3.6
P2.81	-20.1	7.7	46.7	15.3	3.6
P2.82	-20.2	7.4	46.6	15.2	3.6
P2.83	-21.3	7.8	46.7	15.2	3.6
P2.84	-20.8	7.6	46.5	15.1	3.6
P2.85	-21.4	7.7	46.4	15.1	3.6
P2.86	-21.6	7.6	46.7	15.2	3.6
P2.87	-20.7	7.3	46.7	15.2	3.6
P2.88	-21.1	8.0	46.6	15.2	3.6

P3.57	-22.2	6.5	47.1	15.6	3.5
P3.58	-22.3	6.6	47.0	15.6	3.5
P3.59	-22.3	6.6	46.7	15.5	3.5
P3.60	-22.2	6.7	46.9	15.5	3.5
P3.61	-22.0	6.8	46.9	15.4	3.6
P3.62	-22.0	6.6	46.7	15.4	3.5
P3.63	-21.9	6.5	46.8	15.5	3.5
P3.64	-21.9	6.5	46.2	15.3	3.5
P3.65	-21.7	6.6	46.4	15.5	3.5
P3.66	-21.7	6.7	46.8	15.6	3.5
P3.67	-21.7	6.6	46.3	15.4	3.5
P3.68	-22.1	6.9	47.4	15.7	3.5
P3.69	-22.1	6.7	46.8	15.6	3.5
P3.70	-22.1	6.7	46.4	15.4	3.5
P3.71	-22.2	6.8	46.5	15.5	3.5
P3.72	-22.1	6.7	46.5	15.6	3.5
P3.73	-22.2	6.6	46.4	15.4	3.5
P3.74	-22.2	6.6	46.2	15.4	3.5
P3.75	-22.1	6.5	46.5	15.5	3.5
P3.76	-22.1	6.7	46.2	15.5	3.5
P3.77	-21.9	6.7	46.5	15.5	3.5
P3.78	-21.7	6.8	47.0	15.8	3.5
P3.79	-21.7	7.2	46.4	15.7	3.5
P3.80	-21.4	6.6	46.5	15.3	3.5
P3.81	-21.3	6.6	46.4	15.4	3.5
P3.82	-21.4	6.5	46.5	15.3	3.5
P3.83	-21.5	6.5	46.4	15.3	3.5
P3.84	-21.6	6.7	46.2	15.5	3.5
P3.85	-21.5	6.5	47.5	15.6	3.6
P3.86	-21.7	7.0	47.1	15.5	3.5
P3.87	-21.9	6.8	47.2	15.4	3.6
P3.88	-22.0	7.0	46.5	15.3	3.6

P2.89	-20.7	8.0	47.0	15.3	3.6
P2.90	-20.6	8.0	46.9	15.3	3.6
P2.91	-20.1	8.2	61.9	20.2	3.6
P2.92	-19.4	8.5	46.8	15.1	3.6
P2.93	-19.9	8.4	46.6	15.2	3.6
P2.94	-20.1	8.5	46.7	15.2	3.6
P2.95	-20.2	8.0	46.6	15.2	3.6
P2.96	-20.5	8.1	46.2	15.0	3.6
P2.97	-20.4	8.5	46.6	15.2	3.6
P2.98	-20.6	8.3	50.0	16.3	3.6
P2.99	-20.8	8.2	46.9	15.3	3.6
P2.100	-21.1	8.4	46.8	15.2	3.6
P2.101	-21.4	8.4	46.5	15.1	3.6
P2.102	-22.1	8.4	46.7	15.2	3.6
P2.103	-22.2	8.3	46.7	15.2	3.6
P2.104	-22.2	8.0	46.6	15.2	3.6
P2.105	-22.1	8.1	46.6	15.2	3.6
P2.106	-22.2	8.1	46.5	15.1	3.6
P2.107	-22.0	8.3	46.7	15.2	3.6
P2.108	-21.8	8.2	46.3	15.2	3.6
P2.109	-22.0	8.4	46.6	15.2	3.6
P2.110	-22.1	8.4	45.4	14.8	3.6
P2.111	-22.1	8.2	46.1	15.1	3.6
P2.112	-21.9	8.3	46.1	15.0	3.6
P2.113	-22.0	8.5	46.4	15.2	3.6
P2.114	-21.9	8.3	46.4	15.1	3.6
P2.115	-21.9	8.3	46.7	15.2	3.6
P2.116	-21.7	8.4	47.8	15.4	3.6
P2.117	-21.5	8.6	46.6	15.2	3.6
P2.118	-21.4	8.9	46.2	15.1	3.6
P2.119	-21.2	9.1	46.3	15.1	3.6

P3.89	-22.0	7.1	46.9	15.3	3.6
P3.90	-21.8	7.0	43.0	14.1	3.6
P3.91	-21.6	7.5	46.3	15.2	3.5
P3.92	-21.8	7.6	46.4	15.3	3.5
P3.93	-21.6	7.7	45.8	14.9	3.6
P3.94	-21.6	7.9	46.0	15.4	3.5
P3.95	-21.4	7.7	46.3	15.3	3.5
P3.96	-21.0	7.5	46.2	15.1	3.6
P3.97	-20.5	7.9	46.0	15.2	3.5
P3.98	-20.0	7.6	46.2	15.2	3.5
P3.99	-20.5	7.3	46.3	15.1	3.6
P3.100	-21.6	7.1	46.2	15.1	3.6
P3.101	-22.0	7.6	45.9	15.1	3.5
P3.102	-21.9	8.1	46.3	15.2	3.5
P3.103	-21.9	7.8	46.2	15.3	3.5
P3.104	-21.6	8.1	46.6	15.3	3.5
P3.105	-21.7	8.0	46.7	15.5	3.5
P3.106	-21.7	7.8	45.9	15.2	3.5
P3.107	-21.8	7.4	45.7	15.0	3.5

P2.120	-21.1	8.8	46.3	15.1	3.6
P2.121	-21.1	8.7	46.9	15.3	3.6
P2.122	-21.5	8.6	46.3	15.1	3.6
P2.123	-21.6	8.7	46.7	15.2	3.6
P2.124	-21.7	8.6	46.7	15.3	3.6
P2.125	-21.2	9.0	47.4	15.4	3.6
P2.126	-20.6	8.7	46.7	15.3	3.6
P2.127	-20.5	8.8	47.0	15.3	3.6
P2.128	-20.6	8.5	47.1	15.4	3.6
P2.129	-20.3	8.7	46.8	15.3	3.6
P2.130	-20.4	8.0	46.0	15.1	3.6
P2.131	-20.5	8.1	46.7	15.3	3.6
P2.132	-20.5	7.8	46.3	15.1	3.6
P2.133	-20.8	7.5	46.9	15.2	3.6
P2.134	-20.7	7.2	46.5	15.1	3.6

Appendix 3: $\delta^{13}C$ and $\delta^{18}O$ values for serial samples of M2 and M3 tooth enamel in Equus quagga. Z1.1M2 indicates the sample closest to the occlusal surface for the second molar of zebra 1, and Z1.6M2 indicates the sample closest to the DEJ for the same tooth. There are 9 individuals in total. Zebras 2 and 8 have only the M2 tooth.

Individual	**Sample**	**$\delta^{13}C$ (‰)**	**$\delta^{18}O$ (‰)**
Zebra 1	Z1.1M2	-8.9	-4.3
	Z1.2M2	-9.7	-6.8
	Z1.3M2	-9.1	-6.8
	Z1.4M2	-8.3	-4.2
	Z1.5M2	-8.5	-7.6
	Z1.6M2	-8.1	-6.8
	Z1.1M3	-8.6	-8.0
	Z1.2M3	-10.2	-4.9
	Z1.3M3	-8.9	-4.3
	Z1.4M3	-8.7	-6.5
	Z1.5M3	-8.7	-6.1
	Z1.6M3	-7.9	-5.4
Zebra 2	Z 2.1M2	-5.8	-4.7
	Z 2.2M2	-5.9	-1.3
	Z 2.3M2	-6.9	0.0
	Z 2.4M2	-7.5	-0.9
	Z 2.5M2	-8.1	-0.6
	Z 2.6M2	-8.4	-1.3
	Z 2.7M2	-8.9	-1.3
Zebra 3	Z3.1M2	-4.9	-3.6
	Z3.2M2	-5.9	-2.1
	Z3.3M2	-4.0	-6.6
	Z3.4M2	-6.7	-2.5
	Z3.5M2	-6.2	-4.4
	Z3.6M2	-8.4	-3.4
	Z3.7M2	-8.1	-1.2
	Z3.8M2	-5.7	-3.9
	Z3.1M3	-6.0	-2.8
	Z3.2M3	-6.4	-3.0
	Z3.3M3	-6.9	-3.3
	Z3.4M3	-8.3	-4.8
	Z3.5M3	-9.0	-3.2
	Z3.6M3	-8.3	-4.0
	Z3.7M3	-8.3	-1.8
	Z3.8M3	-6.9	-5.4

Zebra 4	Z 4.1M2	-6.5	-2.6
	Z 4.2M2	-7.8	-2.9
	Z 4.3M2	-8.2	-5.5
	Z 4.4M2	-7.2	-0.7
	Z 4.5M2	-7.2	0.4
	Z 4.6M2	-7.3	0.6
	Z 4.7M2	-6.5	-2.1
	Z 4.1M3	-6.1	1.0
	Z 4.2M3	-7.5	-0.4
	Z 4.3M3	-8.5	-1.3
	Z 4.4M3	-8.1	0.0
	Z 4.5M3	-8.6	-1.1
	Z 4.6M3	-8.7	-1.5
	Z 4.7M3	-8.2	-1.6
Zebra 5	Z 5.1M2	-4.3	-1.5
	Z 5.2M2	-4.7	-2.8
	Z 5.3M2	-5.4	-7.1
	Z 5.4M2	-6.8	-3.1
	Z 5.5M2	-6.8	-1.1
	Z 5.6M2	-4.2	-1.4
	Z 5.1M3	-6.1	-0.4
	Z 5.2M3	-7.0	-0.3
	Z 5.3M3	-7.3	-0.8
	Z 5.4M3	-7.7	-1.5
	Z 5.5M3	-7.7	-1.7
	Z 5.6M3	-7.7	-3.7
Zebra 6	Z 6.1M2	-8.3	-0.1
	Z 6.2M2	-8.2	-2.1
	Z 6.3M2	-8.0	-3.7
	Z 6.4M2	-8.1	-3.0
	Z 6.5M2	-7.3	-1.5
	Z 6.6M2	-8.3	-1.8
	Z 6.7M2	-8.5	-3.1
	Z 6.1M3	-7.6	-1.4
	Z 6.2M3	-8.0	-1.4
	Z 6.3M3	-7.2	-2.5
	Z 6.4M3	-7.6	-2.0
	Z 6.5M3	-7.4	-4.4
	Z 6.6M3	-7.8	-5.1
	Z 6.7M3	-7.6	-2.3
Zebra 7	Z 7.1M2	-6.1	-1.5
	Z 7.2M2	-6.1	-1.8
	Z 7.3M2	-6.4	-4.4

	Z 7.4M2	-6.4	-2.6
	Z 7.5M2	-5.4	-5.5
	Z 7.6M2	-4.5	-0.1
	Z 7.1M3	-6.8	0.0
	Z 7.2M3	-7.0	-2.5
	Z 7.3M3	-7.2	-3.4
	Z 7.4M3	-7.3	-5.6
	Z 7.5M3	-7.4	-3.5
	Z 7.6M3	-6.5	-1.5
Zebra 8	Z 8.1M2	-4.4	-0.4
	Z 8.2M2	-5.3	-2.3
	Z 8.3M2	-6.6	-1.5
	Z 8.4M2	-6.9	-7.3
	Z 8.5M2	-7.7	-2.2
	Z 8.6M2	-7.0	-1.4
	Z 8.7M2	-7.5	-1.1
	Z 8.8M2	-6.3	-8.5
Zebra 9	Z9.1M2	-7.1	-5.0
	Z9.2M2	-5.8	-2.6
	Z9.3M2	-5.4	-4.7
	Z9.4M2	-5.3	-7.4
	Z9.5M2	-5.5	-3.2
	Z9.6M2	-5.9	-6.3
	Z9.7M2	-6.3	-2.2
	Z 9.1M3	-4.6	-0.5
	Z 9.2M3	-4.5	-3.4
	Z 9.3M3	-4.8	-1.0
	Z 9.4M3	-3.9	-1.2
	Z 9.5M3	-5.3	-6.4
	Z 9.6M3	-4.0	-3.0
	Z 9.7M3	-3.7	-1.8