Cape Peninsula University of Technology

THE DEVELOPMENT AND USE OF STABLE ISOTOPE ANALYSIS OF FELIDS' WHISKERS AS A TOOL TO STUDY THEIR FEEDING ECOLOGY

by

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Thesis submitted in fulfilment of the requirements for the degree

Master of Technology: Nature Conservation

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

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Cape Town

May 2017

DECLARATION

I, Ruwimbo Mutirwara, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Stable isotope analysis (SIA) of whiskers has been used to identify temporal feeding habits, intra-population diet variation, as well as individual dietary specialisation of marine and terrestrial carnivores. However, the potential of the method to disclose such dietary information for large wild felids has been little explored. The accurate interpretation of stable isotope ratios along serially sampled whiskers is hampered by lack of information on species-specific whisker growth rates, whisker growth patterns and whisker-diet trophic discrimination factors (TDFs). Whisker growth rate and growth pattern informs on the time period encapsulated in the analysed segment of a whisker, while whisker-diet TDFs are required to make correct deductions of the prey species consumed by a predator. The aim of this study was to develop and evaluate the technique of using stable carbon and nitrogen isotope analysis of felid whiskers to quantify the diet of wild felids and in particular, to identify diet variation among individuals. To achieve this, lion *Panthera leo* and leopard *Panthera pardus* whisker growth rate and growth pattern, and lion whisker-diet TDFs were measured, using captive individuals held at the National Zoological Gardens, Pretoria. The viability and applicability of the technique was then explored on six free-ranging leopards in Phinda Private Game Reserve (hereafter Phinda), northern KwaZulu-Natal (KZN) whose diets have been intensively studied using traditional methods.

Whisker growth rates and growth patterns were measured for four lions (three sub-adult females and one adult male) and an adult male leopard over 185 days using giraffe *Giraffa camelopardalis* meat as an endogenous biomarker to consecutively mark whiskers as they grew. The ^{13}C-depleted, C_3-derived giraffe meat with its characteristic isotopic signature could be discerned from the ^{13}C-enriched diet of C_4 grain-fed beef and chicken the felids were sustained on. Two whiskers were removed from each felid at the beginning of the experiment, and felids were fed the giraffe meat at four predetermined periods to mark the whiskers replacing the removed ones. The periods with low $\delta^{13}C$ values, identified following serial sectioning of the regrown whiskers at 1 mm intervals (and stable isotope analysis of these sections), were then correlated to specific giraffe meat feeding bouts and hence growth periods. Knowledge of the duration between giraffe meat feeding bouts enabled the calculation of whisker growth rate and determination of growth pattern. $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs were estimated for five lions whose diet remained consistent over multiple years. Whiskers removed from four lions at the beginning of the whisker growth experiment, a whisker removed from a female lion as part of a pilot study a year before the experiment and the diet (chicken and beef) samples collected during the experiment were analysed for

their isotopic ratios. These were used to calculate isotopic differences between lion whiskers and diet.

Whisker growth was estimated for the proximal 72 mm of whiskers, i.e. the last 76 days of the experiment, which covered the last three identifiable feeding bouts. Moreover, the male lion was excluded from whisker growth calculations as all his whiskers were broken at the end of the experiment. Results showed that whisker growth rates of three lionesses and the leopard were similar (range = 0.64 to 0.66 mm d^{-1}, mean = 0.65 ± 0.01 mm d^{-1}), despite species, age and sex differences. There was a decrease in whisker growth rates over time, suggesting non-linear whisker growth pattern. However, sample sizes of known growth intervals were too small for non-linear (von Bertalanffy) model fitting (n = 3 per individual). Consequently, linear and non-linear growth simulations were conducted and the effect of the two when reconstructing timeframes was compared. Simulations showed slight differences between the two growth patterns for the proximal ~50 mm of whiskers or the last 75 days of growth. However, beyond this length, as one sections more towards the whisker tip, growth pattern may have substantial effects on interpreting timeframes. $\delta^{13}C$ and $\delta^{15}N$ lion whisker-diet TDFs were also similar amongst individuals (range = 2.50 to 2.87 ‰, mean = 2.70 ± 0.12 ‰ for $\delta^{13}C$; range = 2.37 to 2.83 ‰, mean = 2.53 ± 0.08 ‰ for $\delta^{15}N$), irrespective of age and sex.

Sixty millimetres of a whisker from each Phinda leopard were incrementally sectioned at 2 mm intervals and analysed for their $\delta^{13}C$ and $\delta^{15}N$ values. Using the obtained felid whisker growth rate of 0.65 mm d^{-1}, this length of whisker presented an estimated 92 days of dietary information and approximately 18 kills (assuming leopards kill prey after every five days), making comparisons between diets of individuals possible. The 30 $\delta^{13}C$ values of each whisker were apportioned to those derived from browsers, intermediate feeders and grazers ascertained from isotopic ratios of hair and faeces of the Phinda leopards' primary prey species. Results showed that leopards predominantly consumed intermediate feeders, while grazers were less and browsers insignificantly consumed. This concurs with findings from traditional methods and demonstrates the reliability of SIA of whiskers to determine felid diets. Furthermore, the diets of leopards differed amongst individuals, despite similarities of individual proportional prey availability, suggesting individual prey preferences. Due to similarities in $\delta^{15}N$ values of C_3 and C_4 plants in Phinda, and of prey types (browsers, intermediate feeders and grazers), $\delta^{15}N$ could not provide information on leopard diets.

The study adds to the current narrow list of taxa with known whisker growth rates, growth patterns and whisker-diet TDFs, and this valuable knowledge can be used in future research to accurately interpret resource use in wild felids. Although SIA of whiskers could not

characterise the diets of Phinda leopards at species-level, the technique presented novel insights into their feeding ecology, especially, temporal intra-population diet variation, something that was previously unrecognised. This kind of information has important implications for conservation and management of the species, and possibly other felids whose populations and geographical ranges are diminishing globally.

ACKNOWLEDGEMENTS

I wish to thank:

- My supervisors: Dr. Frans Radloff, Dr. Daryl Codron and Dr. Guy Balme for their guidance, patience and outstanding support throughout the course of this study
    - Dr. Codron for his assistance with the data analyses and partly sponsoring my stay in Bloemfontein during my visits
    - Dr. Balme for providing whiskers and spatial data of six leopards at Phinda Private Game Reserve, and his assistance with home range mapping
- National Zoological Gardens of South Africa for granting permission to conduct the whisker growth rate experiment using their captive felids
- Pretoria Zoo staff: Prof. Antoinette Kotze for making the experiment a success, Dr. Adriaan Tordiffe for immobilising the animals, Sr. Marilise Meyer-Bouwer for her assistance during immobilisations and for providing necessary information on the study animals, Carol Thobela and the carnivore team (Nathaniel, Sonnyboy and Phanuel) for their assistance during feeding sessions. These people went out of their way to make sure everything was according to schedule
- Prof. Lourens Hoffman for providing the giraffe and processing the meat, which was fed to the felids during the whisker growth rate experiment
- Wildlife Act Zululand team for collecting meat, hair and faecal samples of leopard prey species from Phinda Private Game Reserve and the surrounding areas
- Craig and Tinumzi (former Nature Conservation students at Cape Peninsula University of Technology -CPUT) for their assistance with collection of hair and faecal samples of leopards' prey from Kube Yini and Kuleni Game Reserves, respectively
- Ian Newton and John Lanham at the Stable Light Isotope Laboratory, Department of Archaeology, University of Cape Town for their assistance with sample analyses and allowing me to use some of their equipment to process my samples
- Mrs Lisa Uys at the Department of Animal Sciences, Stellenbosch University, for her assistance with laboratory lipid-extraction of meat samples
- Mr Richardt J. Smith for assisting with Arcgis mapping and home range analyses
- The Codron family for the delicious dinners (thanks to the cook, Dr. Jacqueline Codron) and crazy laughter during my Bloemfontein visits
- The Chigodo family in Erasmuskloof, Pretoria for providing accommodation and transportation during the six months I conducted the whisker growth rate experiment at Pretoria Zoo

- My mum (Chipo Getrude Chigodo), sister (Rumbidzai Mutirwara), Prof. F.O. Shode and Prof. Kioko for their continued support and wise words of encouragement, especially when it seemed impossible to complete this project
- Above all, Almighty God for making this long journey a success despite the experienced trials and tribulations, and for proving His faithfulness to me as always

The financial assistance of CPUT, through the University Research Fund, and that of the National Research Foundation (NRF, Grant UID: 89370) towards this research is greatly acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the funders.

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GLOSSARY

Acronyms

ANOVA	Analysis of Variance
GIS	Geographic Information Systems
GME	Geospatial Modelling Environment
GPS	Global Positioning System
HSD	Honestly Significant Difference
IUCN	International Union for the Conservation of Nature
KDE	Kernel Density Estimation
KZN	KwaZulu-Natal
MELMs	Mixed Effects Linear Models
PEP	Phosphoenolpyruvate
RuBisCO	Ribulose-1.5-biphosphate carboxylase/oxygenase
SD	Standard Deviation
SEA*c*	Standard Ellipses Area (adjusted for small sample sizes)
SIA	Stable Isotope Analysis
SIAR	Stable Isotope Analysis in R
SIBER	Stable Isotope Bayesian Ellipses in R
SP.	Species (singular)
SPP.	Species (plural)
TDFs	Trophic Discrimination Factors
VBA	Visual Basic for Applications
VCAs	Variance Components Analysis
VHF	Very High Frequency

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Large mammalian carnivores are some of the world's most admired animals because of their charismatic nature, yet they are among the most threatened (Ripple *et al.* 2014). Their populations and geographical ranges have significantly declined due to persecution, trophy hunting and illegal hunting for skins and body parts, which are used as cultural regalia and traditional medicine, respectively (Ceballos & Ehrlich 2002; Hunter *et al.* 2013). In addition, human settlements have led to habitat loss and fragmentation, while human dependence on bush meat for animal protein has depleted prey for large carnivores (Ray *et al.* 2005; Morrison *et al.* 2007; Hunter 2011). These anthropogenic actions disrupt, and sometimes eliminate, the important roles carnivores play in maintaining biodiversity and ecosystem functioning.

1.1.1 The role of large carnivores in ecosystems

Large carnivores occupy the top position in food webs; therefore, they are responsible for shaping substantial components of an ecosystem (Ripple *et al.* 2014). Through predation, large carnivores control the numbers of herbivore populations, and thus reduce the occurrence of overgrazing and concomitant soil erosion (Berger *et al.* 2001; Sinclair *et al.* 2003; Beschta & Ripple 2006; Estes *et al.* 2011). They also regulate the abundance of meso-carnivores (e.g. black-backed jackal *Canis mesomelas* and brown hyaena *Hyaena brunnea*), thereby limiting predation pressure on smaller, more vulnerable prey species (Yarnell *et al.* 2013). Furthermore, large carnivores control intra-guild competition among similar prey species as their presence may cause prey to reduce their resource niches (Miller *et al.* 2001).

Large carnivores have the potential to indirectly deliver ecosystem services. For example, by limiting the numbers of herbivore prey and allowing plants to flourish, they augment carbon storage and mitigate climate change (Terborgh *et al.* 2001; Ripple & Beschta 2012). The iconic status of these predators also provide direct tourism-related economic benefits achieved when visitors pay to simply observe freely roaming animals in protected areas, a recreational activity commonly known as 'game viewing' (Ripple *et al.* 2014). All these ecological and economic benefits are obtained only if large carnivores are successfully conserved *in situ* and restored in areas where they have been extirpated. As a result, novel

and bold measures should be considered to prevent trophic downgrading or loss of carnivore species and their irreplaceable ecological function (Estes *et al.* 2011). Such actions include the need for more research on carnivore feeding ecology as this baseline information can aid in informed decision making for conserving large carnivores (Breuer 2005; Selvan *et al.* 2013; Ripple *et al.* 2014).

1.1.2 The importance of understanding the feeding ecology of large carnivores

A key part of understanding the ecology of an animal is the effect that its dietary preferences have on its growth, behaviour and reproduction (Mills 1992; Bailey 1993; Webster *et al.* 2002). The diets of mammalian carnivores are extremely complex as the number and diversity of prey species killed and eaten differ greatly across space and time (Bothma & Coertze 2004; Hayward *et al.* 2006). Knowledge on carnivores' feeding habits provides the primary basis for understanding their potential competitive interactions with sympatric species (Breuer 2005), impact(s) on prey species populations (Husseman *et al.* 2003; Owen-Smith 2008; Klare *et al.* 2011), seasonal variation in dietary composition (Begg *et al*. 2003) and the incidence of livestock predation (Marker *et al*. 2003). This important information often assists with the development of successful conservation management plans (Breuer 2005) and occasionally aids law enforcement (Korschgen 1971). The feeding ecology of large predators has been broadly explored using an array of traditional approaches, each with its unique challenges. In recent years, stable isotope analysis (SIA) has become a very useful technique, and is increasingly being applied to provide novel insights into the diets of animals at spatial, temporal and taxonomic scales that were previously unobtainable (Ruebenstein & Hobson 2004; Crawford *et al.* 2008).

1.2 Methods of obtaining dietary information from wild large carnivores

1.2.1 Traditional approaches

Obtaining accurate dietary information from wild large carnivores using traditional dietary analyses can be challenging. These predators often live at low densities, are nocturnal, wide-ranging, and difficult to locate and observe (Mills 1996; Avenant & Nel 2002; Bothma & Coertze 2004). As a result, considerable time and financial inputs are often required to obtain this information, especially from elusive individuals such as felids (Parng *et al.* 2014).

In southern Africa, the diets of large carnivores have been discerned using faecal analysis (e.g. Kruger *et al.* 1999; Marker *et al.* 2003; Ott *et al.* 2007; Rautenbach 2010; Davies-Mostert *et al.* 2010; Braczkowski *et al*. 2012; Mbizah *et al.* 2012), spoor tracking (e.g. Bothma & le Riche 1984; Mills 1984; Stander 1992; Pole *et al.* 2004), followings or

observations (e.g. le Roux & Skinner 1989; Funston *et al.* 1998; Mills *et al.* 2004; Balme *et al.* 2007; Hayward *et al.* 2009) and global positioning system (GPS) cluster analysis (e.g. Tambling *et al.* 2010; 2012; Martins *et al.* 2011; Frohlich *et al.* 2012; Pitman *et al.* 2012; 2013; 2014; Jooste *et al.* 2012; Davidson *et al.* 2013). Each study has revealed important dietary information on specific species and populations, but interpretation and applications are limited by the unique challenges and restrictions posed by the respective techniques.

Faecal analysis is a non-invasive and inexpensive approach widely used to determine diet and prey selection of large carnivores (Litvaitis 2000). However, the method provides only a snapshot of the animal's total diet as prey items are not equivalently represented by corresponding residue (Mukherjee *et al*. 1994) due to differences in digestibility (Darimont & Reimchen 2002). As a result, a large number of faecal samples are needed to assess the diet of large carnivores (Ott *et al.* 2007). Large prey are often under-represented, especially when bulk quantities of meat and fat are consumed (O'Gara 1986; Karanth & Sunquist 1995), and undigested prey items are frequently misidentified (Spaulding *et al.* 2000). In addition, one cannot differentiate samples into sex or age categories through morphological identification of prey remains in faeces (Litvaitis 2000). Recently, deoxyribonucleic acid analyses of faeces have been used to provide information on the sex of identified prey, but the approach is very expensive (Piggot & Taylor 2003; Waits & Paetkau 2005).

A couple of studies have used stomach contents analysis to provide detailed information on the diet of large terrestrial predators. The method can differentiate sex, age, physical condition and reproductive rates of the study animals (Litvaitis 2000; Zunna *et al.* 2009). However, this technique is seldom applied as large carnivores are hardly ever available in sufficient numbers to sacrifice (Smuts 1979; McInnis *et al.* 1983). Samples are usually limited to legal harvests by hunters or diseased animals (Litvaitis 2000). The technique over-estimates large prey items due to indigestibility or poor digestibility of their thicker skin, bone and cartilage (Smuts 1979; Landry & van Kruiningen 1979). Consequently, the contribution of small prey to the diet of an individual or species is frequently under-estimated. In some cases, stomachs are found empty, so a sample might not deliver any data (Smuts 1979). In a nutshell, the analysis of stomach contents is again a snapshot of an animal's total diet rather than a long-term perspective.

GPS cluster point analyses have enhanced our understanding of carnivore feeding patterns in remote mountainous areas (e.g. Martins *et al.* 2011, Pitman *et al.* 2012; 2014; Frohlich *et al.* 2012) and open to dense savanna habitats (e.g. Tambling *et al.* 2010; 2012; Davidson *et al.* 2013). Clustered GPS telemetry locations downloaded from satellite collars fitted to the study animals signify potential feeding sites, which are then inspected for prey remains

(Knopff *et al.* 2009; Martins *et al.* 2011). The method can be used to accurately estimate kill rates, to potentially provide information on the physical condition and sex of prey, and distinguish predation from scavenging events (Anderson & Lindzey 2003; Sand *et al.* 2005). Nonetheless, this method is biased towards large prey, and the value of GPS locations to identify smaller kills remains unclear (Knopff *et al*. 2009; Bacon *et al.* 2011). Moreover, telemetry technology is costly and satellite collars often miss locations, fall-off or stop transmitting whilst research is still in progress (Hebblewhite *et al.* 2007).

Spoor tracking, continuous followings, direct and opportunistic observations comprise a simple, basic procedure of directly monitoring carnivores with potentially little bias if surveys are conducted consistently (Litvaitis 2000). However, using trackers to follow and interpret the activities of large predators by spoor in sandy substrates, especially around carcasses, may result in misidentification of spoor (Stander 1997). Hence, incorrect dietary information can be assigned to individuals or species in question. Continuous followings may affect an animal's feeding behaviour and potential prey (Mills 1992), while opportunistic observations under-represent smaller prey as prey items are consumed quickly and are not easily observed (Mills 1992; Radloff & du Toit 2004). Furthermore, direct observations are mainly limited to species that utilise open habitats and forage during the day (Litvaitis 2000). Therefore, observations of secretive, solitary and nocturnal predators are not always feasible, especially on difficult terrain (Darimont & Reimchen 2002).

In general, all the discussed methods contribute to a better understanding of carnivore foraging ecology. Nonetheless, the methods are time consuming, labour intensive (Funston *et al.* 2001) and often fail to adequately determine temporal dietary patterns, as well as potential inter- and intra-population diet variations (Urton & Hobson 2005; Newsome *et al.* 2009; Robertson *et al.* 2013). Longitudinal diet records for individuals can be obtained through stable isotope profiles from the growth axes of incremental tissues such as whiskers, which provide dietary information over different time periods and temporal scales (Inger & Bearhop 2008).

1.2.2 Stable isotope analysis

SIA of carnivore tissues, in particular keratinous tissues (e.g. claws, hair and whiskers), has provided ecologists an alternative approach to obtain longitudinal diet records quickly and non-invasively (Inger & Bearhop 2008, Newsome *et al*. 2009). Because stable isotope ratios in consumer tissues reflect the portion of the diet actually assimilated and not ingested, the method can overcome some of the shortcomings associated with traditional dietary analyses (DeNiro & Epstein 1978; 1981; Gannes *et al.* 1998). The analysis of keratinous tissues

provides valuable additional insights into animals' diets, as they can record dietary information in a sequential manner (because growth is incremental and the tissue does not turn over), potentially providing temporal information on resource partitioning at all levels, from individual specialisation to entire populations (Urton & Hobson 2005; Inger & Bearhop 2008). Such knowledge is vital for the development of effective conservation management strategies of a particular species (Balme *et al.* 2007). In addition, SIA provides discrete information on elusive individuals, which are difficult to observe in their natural habitats (Newsome *et al.* 2010b).

1.3 What are stable isotopes?

Isotopes are atoms of a chemical element with the same number of protons and electrons, but a different number of neutrons (Sulzman 2007). Stable isotopes are those that are energetically stable and do not decay over geological time scales (Sulzman 2007). Many chemical elements have two or more naturally occurring stable isotopes, with different masses (Dawson & Siegwolf 2011). For example, carbon exists most commonly as carbon-12 (^{12}C) but has a heavier, less common carbon-13 (^{13}C) isotope. These isotopes react differently in many environmental and physiological processes due to their mass differences and accordingly, vary in their relative abundance across ecosystems (Gannes *et al.* 1998). In general, the lighter isotope has a higher vibration frequency; thus, it reacts faster and tends to form weaker bonds than the heavier isotope during chemical reactions. This leads to predictable changes in the heavy to light isotope ratios, a process known as isotopic fractionation (Dawson & Siegwolf 2011). These differences in isotopic ratios are recorded within animal and plant tissues, and are measured using an isotope ratio mass spectrometer (Kelly 2000; Ben-David & Flaherty 2012). Isotope ratios are quantified relative to a common international standard reference material and presented in delta (δ) notation in parts per thousand or per mil (‰) derived from the expression:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 * 1000 \qquad (1)$$

where X represents the stable isotope, R_{sample} and $R_{standard}$ denotes the ratio of the heavy to light isotopes in the sample and standard, respectively. Among the stable isotopes, the most useful as dietary tracers are the heavy isotopes of carbon (^{13}C) and nitrogen (^{15}N) (Peterson & Fry 1987; Gannes *et al*. 1997; Kelly 2000).

1.4 Application of stable carbon and nitrogen isotopes in animal ecology

SIA has become a useful, additional tool for gathering trophic and dietary information of animals in terrestrial and marine ecosystems (Wada *et al*. 1987; Kelly 2000; Inger & Bearhop 2008, Wolf *et al.* 2009). On land, in tropical and subtropical ecosystems, stable isotopes of carbon are often used to differentiate between animal diets made up of C_4 (composed of predominantly grass) and C_3 plants (trees, shrubs and herbs) (Vogel 1978; Ambrose & DeNiro 1986; Cerling & Harris 1999; Codron *et al.* 2005). These plants have different photosynthetic pathways; C_3 plants use the enzyme Ribulose-1.5-biphosphate carboxylase/oxygenase (RuBisCO) to fix carbon dioxide as part of the Calvin cycle, while C_4 plants fix carbon dioxide with phosphoenolpyruvate (PEP) carboxylase prior to its entry into the Calvin cycle (O'Leary 1988; Farquhar *et al.* 1989). C_3 plants fixate a lower number of the heavier carbon-13 isotope in their tissue per unit mass than C_4 grass, yielding depleted $\delta^{13}C$ values with a global median of -27 ‰ (range = -35 to -21 ‰) (Ehleringer 1991). In contrast, C_4 grasses fixate a higher number of the heavier carbon-13 isotope resulting in values of approximately -13 ‰ (range = -14 to -10 ‰) (Ehleringer 1991). This difference is due to the stronger discrimination against carbon-13 isotopes by the primary CO_2 fixing enzyme, RuBisCO, in C_3 plants, compared to the enzyme PEP carboxylase used by C_4 plants (Park & Epstein 1960; Farquhar *et al.* 1989). Hence, grass and browse (trees, shrubs and herbs) can be separated from each other based on the stable $^{13}C/^{12}C$ ratios of their tissues.

The utility of carbon isotopes in animal ecology relies on their distribution in foods and how they are incorporated into the tissues of consumers (Kelly 2000). Carbon isotope analysis can consequently be used with great success in subtropical areas to determine the proportion of C_4 grass versus C_3 browse in the diet of herbivores by determining the stable carbon isotope compositions of their body tissues (Vogel 1978; Cerling & Harris 1999). Animals do not significantly change the carbon isotopic composition of their food because of little discrimination (approximately 1 ‰) between a consumer's whole body and its diet (DeNiro & Epstein 1978). As a result, the carbon isotope signature of herbivores' analysed tissues reflects the source of carbon (C_3 or C_4) at the base of the food chain (DeNiro & Epstein 1978; McCutchan *et al.* 2003). Similarly, the proportion of grazing versus browsing prey consumed by predators can be determined by analysing the carbon isotope compositions of their tissues. Thus, 'consumers are what they eat' (Tykot 2004).

Terrestrial plants vary widely in $\delta^{15}N$ (range = -8 to 18 ‰) due to the differences in nitrogen composition of soils as a result of plant root depth, nitrogen assimilation influenced by mycorrhizal root associations, geology, soil type and climate (Virginia *et al.* 1989, Robinson 2001; Schmidt & Stewart 2003). Consequently, comparing stable $^{15}N/^{14}N$ ratios across food

webs can be difficult and problematic. Nitrogen isotopes are used to determine the trophic position, environmental condition and body condition of the consumer (Minagawa & Wada 1984; Hobson *et al.* 1993). The main source of nitrogen for most animals are amino acids derived from digested proteins (Eckert *et al.* 1988). When nitrogen is assimilated, the lighter nitrogen-14 isotope contained in ammonia, urea and uric acid is preferentially excreted causing a relative retention of the heavier nitrogen-15 isotope (Peterson & Fry 1987). Hence, consumers become enriched in nitrogen-15 relative to their diet (Ambrose & DeNiro 1986). This degree of enrichment is broadly predictable from one trophic level to the next, as nitrogen-15 abundances increase with an average of 3-5 ‰ upwards along different levels of the food chain (DeNiro & Epstein 1981; Minagawa & Wada 1984). As a result, animals higher up the food chain have more positive $\delta^{15}N$ values than animals lower in the food chain. In addition, nutritionally and water stressed animals show elevated $^{15}N/^{14}N$ ratios in their body tissues (Sealy *et al*. 1987; Ambrose 1991). Nutritionally stressed animals catabolise their body proteins, and water stressed individuals show greater fractionation of nitrogen isotopes in the production of concentrated nitrogenous waste (Ambrose & DeNiro 1986; Hobson *et al*. 1993).

The stable carbon and nitrogen isotope analysis of different animal tissues provides dietary information integrated over varying temporal and spatial scales, due to different tissue metabolic and turnover (length of time the molecules of a tissue are lost and replaced) rates (Tieszen *et al.* 1983; Hilderbrand *et al.* 1996). For example, tissues with higher metabolic and turnover rates such as blood and liver reflect diet assimilated the previous days or week, whereas muscle can provide dietary information for the past months (Tieszen *et al.* 1983; Vanderklift & Ponsard 2003). Bone has a very slow metabolic and turnover rate; hence, it can store dietary information over a much longer time period representing months to years (Dalerum & Angerbjorn 2005). Furthermore, inert incremental tissues (e.g. teeth, claws, hair and whiskers) in which no turnover takes place after deposition of molecules, preserve dietary information obtained from food ingested during their growth in a sequential manner along the growth axis (Hobson & Schell 1998; Ayliffe *et al.* 2004). Once the protein is incorporated, it remains biologically unchanged resulting in a long-term series of isotope data representing the period of growth (Hobson & Clark 1992; Hirons *et al.* 2001).

The correct and accurate application of stable carbon and nitrogen isotopes in animal ecology is influenced by two processes, i.e. (i) tissue-diet trophic discrimination (the difference between isotopic ratios of a consumer's bulk tissue and its diet as a result of metabolism) and (ii) tissue isotopic turnover (the length of time it takes for dietary isotopes to be assimilated into a consumer's tissue) (Gannes *et al.* 1997; Martinez del Rio *et al*. 2009; Alves-Stanley & Worthy 2009). Tissue-diet trophic discrimination factors (TDFs) and tissue

isotopic turnover rates have been estimated through controlled feeding experiments and were reported to vary among species, tissues and according to diet (Tieszen *et al.* 1983; Hobson *et al.* 1996; Parng *et al.* 2014; Webb *et al.* 2017). Therefore, at the least, approximate TDFs and turnover rates for a species can be used to account for the correct interpretation of isotopic ratios in consumer tissues (Caut *et al.* 2009).

1.4.1 Use of stable carbon and nitrogen isotopes in carnivore ecology

SIA of carbon and nitrogen has made a significant contribution to our comprehension of marine carnivores' feeding ecology (e.g. Best & Schell 1996; Hobson & Sease 1998; MacNeil *et al.* 2005, Cherel *et al.* 2007, Knoff *et al.* 2008) and is increasingly used to provide quantitative descriptions of terrestrial carnivore diets (see the overview below). Animal tissues such as blood, muscle, bone, hair, claws and whiskers, which provide information on diet representing different time scales have been analysed for their carbon and/or nitrogen ratios for a number of terrestrial carnivore species. The analysed tissues often provided important dietary insights that are difficult to obtain using traditional methods.

The overview below covers terrestrial carnivore dietary research that made use of SIA and highlights the unique contribution of this technique towards carnivore feeding ecology. Research papers are discussed according to whether the studies contributed towards knowledge of dietary variation within predator guilds or within populations of the same species. This is followed by a discussion of papers focusing on diet variation across seasons, years and space. There are also cases where the importance of a specific food type to a particular population of carnivores was investigated and these are discussed as such. Some studies have looked at the meat component consumed by omnivorous species such as bears, and these were included here as animal protein or carnivory plays an integral role in their diet.

1.4.1.1 Inter-predator guild diet variation

Codron *et al.* (2007) used stable carbon and nitrogen isotope analysis of hair and faeces of a range of mammalian carnivores to examine carnivore diets in the lowveld savanna habitats of South Africa, including Kruger National Park. Hair $\delta^{13}C$ values revealed that most carnivore species belong to the C_4 grass-based food web (range = -10.9 to -17.1 ‰), with lion *Panthera leo* having the highest values and genet *Genetta* species (sp.). the lowest in this range. Nitrogen isotope data differed between predators feeding on invertebrates and vertebrates, respectively. Invertebrate-feeders (black-backed jackal *Canis mesomelas*, genet and honey badger *Mellivora capensis*) had higher $\delta^{15}N$ values than expected compared to

vertebrate-feeders, signifying trophic level differences in $\delta^{15}N$ between carnivores feeding on either invertebrates or vertebrates. The diets of lion and spotted hyaena *Crocuta crocuta* were further examined using a mixing model programme, Isosource, which quantifies the proportional contributions of prey groups to the diets of consumers. Lion hair reflected a high consumption of large grazers in comparison to spotted hyaena, and this result agreed with predictions for the composition of lion diets in Kruger Park. SIA revealed differences in prey selection amongst predator guilds and their preferential selection for C_4-feeding herbivores.

1.4.1.2 Intra-population diet variation

SIA has proved to be a powerful tool in revealing intra-population diet variation and individual diet specialisation among carnivores, something that was little considered in the past mainly due to limitations of traditional methods in determining resource use (Bolnick *et al.* 2003). The lack of longitudinal diet records for individuals in question prevented studies that used traditional techniques to determine whether dietary differences between- and/or within-individuals were either persistent through time or short-term (Newsome *et al*. 2009).

Hobson *et al.* (2000) investigated trophic relationships among grizzly bears *Ursus arctos* and black bears *U. americanus* of the upper Columbia River basin, British Columbia. SIA of hair revealed that males of both species and female black bears had higher $\delta^{15}N$ values suggestive of utilisation of more animal protein, than female grizzly bears. However, black bear hair displayed much higher $\delta^{15}N$ values, which were possibly related to consumption of ants, albeit $\delta^{15}N$ values of ants and ungulates were indistinguishable, as ants are a more abundant and easily accessed resource than ungulate prey. The lower $\delta^{15}N$ values female grizzly bears may have been due to consumption of $\delta^{15}N$-depleted small mammals that occur in high altitude areas where the bears inhabited. As expected, bears that were caught close to human settlements had higher $\delta^{13}C$ and $\delta^{15}N$ values than the other bears, signifying a reliance on high quality anthropogenic food stuffs. Three nursing black bear cubs were a trophic level higher than their mothers, suggesting an exclusive dependence on their mothers' milk since maternal transfer of nutrients leads to isotopic enrichment in offspring. The study illustrated diet variation between two sympatric species and groups of individuals occupying the same area. It also points out that caution should be taken when interpreting results from SIA, especially when isotope values of prey species overlap. In cases like this, interpretations need to incorporate known behaviour and/or recognize limitations in stable isotope approaches.

Edwards *et al.* (2011) analysed hair and claws of grizzly bears in the Canadian Arctic and found high intra-population diet variation, as well as higher animal protein usage by males.

$\delta^{15}N$ values indicated that individual diets of bears ranged across three trophic levels resulting in a wide population niche width. The importance of plant foods in the diet of both males and females decreased with an increase in trophic position. They also found that the movement rate of females in search of food increased with an increase in trophic level as reflected by the high $\delta^{15}N$ values of females. Thus, variation in the diets of individuals was sex-related, driven by differences in resource usage among the bears.

Hopkins III *et al.* (2014) analysed hair of grizzly bears and found a significant difference in the $\delta^{15}N$ values of bears that were killed on or captured near the railway line, and those sampled away from the rail in Banff National Park, Canada. Rail-associated bears showed similar higher $\delta^{15}N$ values in both males and females, suggesting consumption of similar diets largely consisting of animal protein, in contrast to bears sampled away from the rail that had lower $\delta^{15}N$ values. These rail-associated bears might have consumed ants along the rail, which are a readily available source of animal protein with high nutritional value. Alternatively, they could have utilised prey that foraged along the rail or scavenged on train-killed animals. However, both groups of bears had similar low $\delta^{13}C$ values, suggesting a reliance on similar C_3 plant-derived diets. The study used stable carbon and nitrogen isotopes to identify at-risk, rail-based foraging behaviour of individual grizzly bears whose existence is threatened by the movement of trains in the Park.

Urton & Hobson (2005) examined trophic relationships between the grey wolf *Canis lupus* and 18 other mammalian species found in the Canadian boreal forest of central Saskatchewan. Wolves sampled from three different habitats within the study area had low guard hair $^{13}C/^{12}C$ ratios suggestive of foraging mainly on browsing prey species. There were differences in hair $\delta^{13}C$ and $\delta^{15}N$ values among populations in the three different environments, indicating variation in resource utilisation. Wolves in two habitats outside the protected area displayed more variable isotopic signatures than those within Prince Albert National Park. This discrepancy was possibly a result of differences in prey abundance and distribution within these habitats. Grey wolves were expected to be top predators in all habitats of this ecosystem. However, red foxes *Vulpes vulpes* and coyotes *Canis latrans* had more positive $\delta^{13}C$ and $\delta^{15}N$ values relative to their diet compared to wolves. These two species might have hunted or scavenged at or near open agricultural areas where prey tissues display high $^{15}N/^{14}N$ ratios due to the presence of commercial fertilizers, while wolves concentrated more in forested areas. SIA was able to reveal the previously unrecognized intra-population diet variation and trophic position of grey wolves, which are important for effective conservation management.

Yeakel *et al.* (2009) used stable carbon and nitrogen isotopes in bone collagen and hair to quantify individual dietary specialisation of two alleged man-eating ancient lions of Tsavo, Kenya. The isotopic signatures of these adult male lions were compared to those of modern Tsavo lions and Taita agropastoralists ascertained from analysis of skin and muscle tissues, and bone collagen, respectively. The consistent high $\delta^{13}C$ values displayed by modern Tsavo lions suggest that their diet was composed predominantly of grazers. The man-eating lions showed distinct isotope ratios; one had a diet similar to modern Tsavo lions, thus, the individual was heavily reliant on grazer prey. The other lion interchanged its diet between prey types i.e. grazers, browsers and mixed feeders. However, towards the end of its life, the latter individual obtained significant nutritional input from human consumption as indicated by intermediate $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios that matched the mixed diet of Taita agropastoralists, composed of C_4 corn and C_3 nitrogen-fixing legumes. The study evinced the behavioural plasticity and individually specialised diets of the Tsavo lion dyad, despite their socialisation.

Voigt *et al*. (2013) analysed the stable carbon isotope ratios of exhaled breath of central Namibian cheetahs *Acinonyx jubatus* to assign individuals to either a C_3 or C_4 food web of their habitat. $\delta^{13}C$ values of breath ranged widely, suggesting that cheetahs fed on a variety of isotopically distinct prey species. Two-thirds of the sampled individuals fed predominantly on grazers (cattle *Bos taurus*, hartebeest *Alcelaphus buselaphus*, gemsbok *Oryx gazella* and warthog *Phacochoerus africanus*), and a third fed predominantly on browsers (kudu *Tragelaphus strepsiceros)* and/or intermediate feeders (springbok *Antidorcas marsupialis*). These findings were in agreement with previous studies that investigated the diet of cheetahs using traditional methods such as faecal analysis. Hence, stable isotopes were able to reveal that the central Namibian cheetah population is composed of heterogeneous individuals, each utilizing a sub-component of habitats and prey available to the population.

Voigt *et al*. (2014) analysed blood, fur and muscle of Namibian cheetahs on farmland to investigate their prey preferences, consumption of livestock and/or trophy hunting species, as well as individual dietary specialisation. Their results showed that cheetahs of the same group were isotopically distinct from members of other groups, indicating that group members shared their prey. Solitary males and males in bachelor groups fed principally on grazers that had low $\delta^{15}N$ values such as hartebeest and warthog, whereas female cheetahs relied mostly on small browsing ungulates probably because of their smaller size compared to males. None of the cheetah groups fed predominantly on cattle and trophy species, which were all grazers with high $\delta^{15}N$ values. Although no individual had a unique isotopic signature that differed from the others, most of them showed a relatively high degree of

individual specialisation thought to be related to sex and individual hunting behavior. Stable isotopes showed that although Namibian cheetahs are persecuted for their alleged killing of livestock and big trophy game, these prey do not form an important part of their diet. A better understanding of the foraging ecology of cheetahs assists in raising awareness, particularly to farmers, and improves conservation management policies of the species in anthropogenic influenced landscapes.

Robertson *et al.* (2014) used the SIA of whiskers to quantify differences in resource use among individual Eurasian badgers *Meles meles* within shared group territories in Woodchester Park. They found a wide variance in badger $\delta^{13}C$ and $\delta^{15}N$ values, suggesting utilisation of diverse habitats and foods by individuals. Badgers within groups differed consistently in their isotopic signatures, signifying individual occupation of different foraging niches, even though they had access to the same resources. These differences between individuals persisted over a considerable period of time, suggesting long-term individual foraging specialisation; and they could not be linked to sex, age, social status and physiological differences. Thus, their study showed that stable carbon and nitrogen isotopes are able to reveal long-term individual diet specialisation within a territorial species.

Brickner *et al.* (2014) analysed the hair of black-footed ferrets *Mustela nigripes* to assess the degree to which individuals inhabiting Shirley Basin, Wyoming specialise on prairie dogs *Cynomys leucurus.* Prairie dogs were the most important single diet item to all ferrets, however, the degree to which individuals specialised on prairie dogs differed by age and sex. More than 70 % of the diet of both adult males and juveniles was composed mainly of prairie dogs. In contrast, female ferrets consumed a greater proportion of mid-sized mammals and mice, which were abundant within prairie dog colonies. Although female ferrets utilised small-bodied prey, they also appeared to hunt prairie dogs and provisioned them to their dependent young. The study used stable carbon and nitrogen isotopes to demonstrate dietary differences between age-sex groups and the importance of prairie dogs in the diet of ferrets. Such information can be useful for the conservation of ferrets, which are one of North America's endangered mammals. The recovery of this species will require not only the presence of prairie dogs, but also prairie dog colonies, which provide abundant, alternative prey for ferrets.

1.4.1.3 Seasonal diet variation

Stable carbon and nitrogen isotopes can be used to provide high-resolution, time-foraging (seasonal and/or annual) series of either individual animals or populations. Ben-David *et al.* (1997) used the SIA of muscle and blood to investigate seasonal and annual changes in the

diets of American martens *Martes americana* in response to the changing abundance of small rodents *Peromyscus keeni* and *Microtus longicaudus* on Chicagohof Island, Southeast Alaska. They found a high variability in the diets of individuals in autumn, with salmon carcasses from nearby streams constituting a large portion of their diet (as revealed by the high $\delta^{13}C$ and $\delta^{15}N$ values) during years of low rodent abundance. However, martens predominantly fed on small rodents in autumns when they were abundant, despite the availability of salmon carcasses. Individual specialisation was also detected where some martens principally fed on small rodents even in years of low abundance of this prey. However, in summer, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios suggested that squirrels, birds and berries were more preferable, despite the high numbers of small rodents, probably because these prey are encountered at higher rates and are easier to capture. SIA made it possible to highlight the influence of climatic conditions on prey abundance, which leads to changes in consumer foraging behavior.

Carlson *et al.* (2014) quantified the diet of American martens in northern Wisconsin, and investigated the role of prey in the recovery of the population. Stable carbon and nitrogen isotopes of hair collected from museum specimens and opportunistically from carcasses revealed that martens consume large quantities of shrews *Blarina brevicauda, Sorex cinereus* and white-tailed deer *Odocoileus virginianus* in autumn, while red-backed voles *Myodes gapperi* were the least preferred prey. Similar results were also obtained from faecal analysis. These findings were in contrast to the diets of martens reported in North America where voles were the dominant and shrews the least preferred prey item, irrespective of sampling approach or season. Little to no consumption of voles in northern Wisconsin may be owed to their low numbers in recovery areas and the continuous availability of the favoured alternative prey items. The use of SIA in harness with conventional methods (in this case faecal analysis) provided new knowledge on the feeding ecology of American martens. A strong reliance on less profitable (shrew) and higher risk (deer) diet could be a contributing factor to the delayed recovery of the endangered Wisconsin marten population.

Roth (2002) analysed hair of the Arctic fox *Alopex lagopus* near Cape Churchill, Manitoba and found temporal variability in their diet. Hair $^{13}C/^{12}C$ ratios varied seasonally; winter pelage (reflecting summer diet) displayed a more terrestrial C_3 herbivore diet (with low $\delta^{13}C$ values) when lemmings *Dicrostonyx richardsoni* were abundant during summer. The higher $\delta^{13}C$ values found in summer pelts indicated a higher consumption of marine-derived prey during winters with low lemming abundance and when the sea ice habitat is available. Hair stable carbon isotope data for the Arctic fox unveiled a crucial link between marine and terrestrial ecosystems, which might be disrupted by the continued decline of the sea ice

habitat caused by climatic changes, thereby jeopardising the survival of this carnivore species.

Milakovic & Parker (2011) quantified the proportional contribution of major prey items to the diets of five grey wolf packs in the largely undisturbed ecosystem of Besa Prophet, northern British Columbia. The low $\delta^{13}C$ and $\delta^{15}N$ values of analysed hair, muscle and blood suggest that these wolves foraged on prey feeding on C_3 terrestrial plants. Results revealed that the diet composition of the wolf packs varied with season, with all packs consuming large amounts of elk *Cervus elaphus* in winter. Stone's sheep *Ovis dalli stonei* and caribou *Rangifer tarandus* were important food sources in spring, and a combination of moose *Alces americanus* and elk in both autumn and summer for the western and central wolf packs. The diet of wolves as determined by stable isotopes was then compared with food habits ascertained from faecal samples during the summer period. Results from the two methods were similar, with high diet variability amongst and within packs between years of sample collection. The study showed that moose were not the principal prey item for wolves throughout the year as previously reported. It also evinced the importance of season-specific prey selection and the reliability of SIA in providing a clear understanding of complex ecosystem dietary dynamics.

Barker *et al*. (2015) used the stable nitrogen isotopes of forelimb hair to investigate individual trophic level of brown bears *Ursus arctos* and its relationship with their selection for Arctic ground squirrels *Urocitellus parryii* as prey in the Mackenzie Delta, Canada. Although Arctic ground squirrels were widely distributed and perennially consistent throughout the study area, no significant correlation between bears' trophic level ($\delta^{15}N$ value) and bears' selection for Arctic ground squirrels was found. Resource selection function models and telemetry bear activity site investigations carried out in the study support the findings that Arctic ground squirrels are not highly utilized by brown bears. Nonetheless, the trophic level of some female bears was positively correlated to their selection for Arctic ground squirrels in spring/early summer. This suggests that some individuals specialised on Arctic ground squirrels during early summer resulting in a quantifiable degree of ^{15}N enrichment gained from the nutrient-rich squirrels. However, the elevated $^{15}N/^{14}N$ ratios might have been a consequence of consumption of other available protein foods causing an inference of Arctic ground squirrel utilisation by brown bears. The use of stable nitrogen isotopes together with telemetry data provided insights that Arctic ground squirrels might not be as important a food source for Mackenzie Delta bears as previously reported.

1.4.1.4 Paleontological versus contemporary diet variation

Stable isotopes provide the unique opportunity to compare contemporary and paleontological predator samples' isotopic compositions to establish the change of diet over time. Hilderbrand *et al.* (1996) analysed bone of extinct European cave bears *Ursus spelaeus*, bone and hair of threatened grizzly bears *U. arctos horribilis*, and blood and hair of brown bears to determine the diets of living and extinct bears. Stable carbon and nitrogen isotopes revealed the omnivorous behaviour of cave bears; however, terrestrial meat contributed more to their diet. The high $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of grizzly bear museum specimens from coastal southeast Alaska and Columbia River Drainage highlighted historical salmon *Oncorhynchu*s species (spp.) utilisation by these bears. This was different to grizzlies that had no access to abundant salmon and relied on terrestrial food sources. Most brown bears whose habitats provided access to salmon fed upon this resource during the spawning season. However, a sub-population of brown bears survived entirely on terrestrial food sources as indicated by the lower $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of their tissues. Knowledge of the diet of cave bears can help discover the possible causes of their extinction, in this case the extinction of large herbivore prey might have contributed to their disappearance. Furthermore, because salmon were an important food source to grizzly bears historically, the recovery of grizzlies could be enhanced by recovering salmon populations and spawning areas in streams.

Fox-Dobbs *et al.* (2007) used SIA of bone collagen, muscle and hair to investigate variation in the foraging ecology of modern and ancient wolves of North America. The analysed muscle and hair of the Minnesota grey wolf population reflected individual foraging differences in summer and spring, possibly driven by temporary opportunistic foraging events. They also found, through analysis of bone collagen, that the Isle Royale grey wolves mainly consumed moose and beavers *Castor canadensis*, which are C_3 plant-eaters. This wolf population had a constant diet for the past 30 years, which supports observational records. In addition, Isle Royale wolves had lower intra-population diet variances as they are geographically isolated on the island with locally abundant, but low diversity of prey. To reconstruct the diets of the extinct dire wolves *Canis dirus*, bone samples of late Pleistocene La Brea tar pits museum specimens were analysed and compared to published isotope values for herbivores from the same tar pits as the dire wolves. It was concluded, from the obtained data, that large herbivores like bison *Bison antiquus*, camel *Camelus hesternus* and horse *Equus occidentalis* largely contributed to the wolves' diet.

1.4.1.5 Spatial dietary variation

Angerbjorn *et al*. (1994) used the stable carbon isotopes of bone collagen to infer spatial and individual variation in the diet of Arctic foxes from three localities; Iceland, west Greenland and Sweden. They compared the results with those from food remains (in dens) and faecal analysis obtained from the same individuals. Foxes from inland areas of Iceland and Sweden showed low $\delta^{13}C$ values indicative of terrestrial browser prey consumption. Individuals from coastal Iceland had a higher variance in $\delta^{13}C$ values than inland foxes, suggestive of utilisation of prey species from both marine and terrestrial environments by these individuals. However, faecal analysis and food remains indicated that the Icelandic foxes depended highly on marine resources. As expected, coastal Greenland foxes had higher $\delta^{13}C$ values signifying a large reliance on marine protein. The study showed the potential of stable carbon isotopes to disclose variation in the diet of Arctic foxes as a result of differences between habitats and individual selection of resources within habitats. It also proved the strength and reliability of the technique in providing information on the long-term diets of individuals, which is not easily attainable with the use of faecal analysis and food remains alone, especially when such specimens are collected over a short period of time.

Ehrich *et al.* (2015) monitored the composition of vertebrate prey species in the Arctic tundra through stable isotope analysis of Arctic fox fur. Winter fur isotopic signatures of foxes sampled from six sites in the Eurasian Arctic and Greenland reflected both spatial and temporal variability in the prey base of this predator. Marine resources were important in the diet of foxes at most sites, but they were used as an alternative on two high arctic sites, despite the close proximity of these areas to the coast. Foxes also utilised terrestrial prey differently amongst sites. In the high arctic ecosystems, foxes chiefly fed on lemmings *Dicrostonyx* and *Lemus* spp. when this resource was abundant as reflected by low $\delta^{13}C$ values. However, in years with low lemming numbers, foxes switched to a variety of terrestrial and marine substitutes. Individuals in areas devoid of small rodents and lemming cycles maintained a constant diet over multiple years. Some important shifts in the diet of Arctic foxes could not be detected due to overlap or unclear distinction of isotopic signatures of some prey species. SIA revealed several important aspects of prey availability in the Arctic tundra ecosystem whose ecological processes are currently disturbed by climate change. However, the technique should not be used entirely alone due to isotopic signature overlap of prey, but can be integrated as one of several key targets that would contribute in ecosystem-based monitoring programs.

Lavin *et al.* (2003) investigated the effects that habitat use and competition with coyotes had on the diets of red foxes in intensively farmed landscapes of Illinois. They analysed whole

blood, serum and hair, and found that rural foxes had higher $\delta^{13}C$ and $\delta^{15}N$ values than urban and farm foxes. This was possibly because of the displacement of rural foxes by coyotes from microhabitats that maintain lower trophic level species. As a result, rural foxes were forced to forage on higher trophic level prey, such as omnivorous insects, in comparison to urban foxes that ate mainly small herbivores. Moreover, rural foxes that utilised the agricultural habitat ate more C_4 plant consumers with fertiliser-enriched ^{15}N-compositions than rural coyotes and urban foxes. Hence, urban foxes had low $\delta^{13}C$ and $\delta^{15}N$ values indicative of their consumption of prey at lower trophic levels, such as the rabbit *Sylvilagus floridanus*, muskrat *Ondatra zibethicus* and groundhog *Marmota monax*, within a predominantly C_3 plant based food web. SIA made it possible to recognise how the diet of Illinois red foxes was influenced by both the environments they utilised and competition for resources with sympatric carnivore species.

Bodey *et al.* (2010) analysed whiskers and liver of the invasive American mink *Neovison vison* to understand their behaviour in response to an eradication programme on several islands of the Outer Hebrides, Scotland. They found that animals caught inland had low $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, suggesting a reliance on terrestrial food sources. As the project progressed, territories became vacant at the coast and were occupied by migrants from inland populations whose diet then changed from terrestrial to marine sources. Over successive years, individuals became more enriched in $\delta^{13}C$ and $\delta^{15}N$, despite of sex or habitat once occupied, signifying an increased reliance of the minks on marine protein. Stable carbon and nitrogen isotopes highlighted that the change in the feeding behaviour of minks appeared to be management related, as preferred marine resources became more available after the removal of conspecifics from the islands. Information of this kind potentially guide eradication programmes by highlighting focal areas and can improve management procedures for new habitats.

Magioli *et al.* (2014) evaluated the feeding patterns of pumas *Puma concolor* in the agricultural landscapes of South-eastern Brazil using the analysis of prey hair found in faeces. The $\delta^{13}C$ values indicated that pumas consumed prey from both forest remnants and agricultural areas. A higher proportion of C_3-feeding prey were taken from forested areas, while C_4-feeding prey (rodents *Hydrochoerus hydrochaeris*, *Sphiggurus villosus*, capuchin monkey *Sapajus nigritus* and tayra *Eira barbara*) were utilised where the agricultural matrix was predominant. However, some individuals preferentially and chiefly consumed C_4-feeding prey, demonstrating the importance of food resources from the agricultural landscape. Pumas had a wide range of $\delta^{15}N$ values, suggesting consumption of diverse prey species, and the higher $\delta^{15}N$ values of some individuals indicated their dependance on fertilizer

enriched foods from agricultural areas. Thus, SIA was useful in revealing the significance of the agricultural landscape for puma feeding ecology.

1.4.1.6 The importance of specific food items to some large carnivores

i) Salmon

Stable carbon and nitrogen isotope analysis has been widely used to examine the importance of salmon *Oncorhynchu*s spp. (a nutrient rich, marine food resource) to the diets of wolves and bears. Szepanski *et al.* (1999) analysed the bone collagen of Alexander Archipelago wolves *Canis lupus ligoni* in southeast Alaska to assess their extent of spawning Pacific salmon utilisation. Wolves' stable $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios reflected a broad range of terrestrial and marine signatures. Individuals inhabiting the coastal mainland showed highly varied and enriched isotopic signatures, signifying their utilisation of both terrestrial (in this case the Sitka black-tailed deer *Odocoileus hemionus sitkensis*) and marine prey, particularly salmon. Wolf populations found in interior habitats exhibited little isotopic variation and enriched $\delta^{13}C$ values that appear 'marine' similar to those of caribou, but low $\delta^{15}N$ values. The study showed that salmon – a readily available and easily accessible marine resource – may augment the diet of southeast Alaskan wolves, especially during seasonal fluctuations when the numbers of terrestrial ungulates are relatively low. Such feeding behaviour was previously unknown in wolves with the use of traditional methods alone, particularly faecal analysis, possibly due to the full digestion of salmon bones in faeces.

Similarly, Adams *et al.* (2010) demonstrated spatial variation in salmon use by inland grey wolves of Denali National Park and Preserve, Alaska using the analysis of bone collagen. Wolves in the north-western flats where there were more spawning salmon, but low ungulate availability, had higher $\delta^{15}N$ values indicative of high salmon consumption. In upland regions, wolves used little of the salmon due to restricted accessibility to spawning areas and the high abundance of ungulates. However, some wolves without salmon streams in the vicinity of their home ranges had high $\delta^{15}N$ values suggestive of salmon diet, probably because they trespassed on neighbouring packs' territories to obtain this lucrative food source. The availability of Pacific salmon might influence variability in wolf-ungulate interactions in this area as salmon biomass varies widely every year. These findings highlight the importance of Pacific salmon as a food source for wolves well beyond coastal areas, thereby providing a dynamic connection between inland wolf-ungulate communities and distant marine systems that was previously unrecognised.

Darimont & Reimchen (2002) analysed the guard hair of British Columbia grey wolves to investigate individual seasonal shifts in diet, i.e. from predominantly ungulates to marine resources – particularly salmon. They could show, using stable isotope analysis of base and tip hair segments, that there was little seasonal variation in wolf diet, and only five out of 19 wolves made use of salmon. These findings could not be obtained when whole guard hair isotope values were examined initially. The five individuals had a high inter-seasonal diet variation with relatively high $\delta^{13}C$ and $\delta^{15}N$ signatures assimilated during autumn when salmon, a more enriched marine resource, was abundant. Therefore, SIA of hair segments provides a true representation of food consumed by an individual during periods of hair growth and higher resolution data than the analysis of whole hair.

Ben-David *et al.* (2004) used the SIA of hair and blood to investigate the consumption of salmon by southeast Alaska brown bears. Nearly all males incorporated a larger proportion of salmon in their diet as indicated by the high $\delta^{15}N$ values of sampled tissues. However, females had highly variable $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios related to their reproductive status and access to salmon. Females with access to salmon consumed this marine protein especially when they had mated. Some females with cubs had lower $\delta^{15}N$ values due to the non-inclusion of salmon in their diet, while others consumed less salmon than they did when they were mated. This trend of female salmon-feeding habits was probably a response to the risk of infanticide since encounters between females with young offspring and non-parents are expected to be high in this densely bear populated area. Stable carbon and nitrogen isotopes were able to show how motherhood can influence the foraging strategies of female brown bears.

Mowat & Heard (2006) analysed the guard hair of grizzly bears from 81 populations across North America to describe patterns of marine- and terrestrial-derived meat in their diet. Results indicated that coastal grizzlies exhibited enriched $\delta^{13}C$ and $\delta^{15}N$ values derived from salmon, while interior populations occasionally fed on marine resources even in areas with relatively large salmon runs. Males largely consumed salmon, which probably contributed to their fitness and larger body sizes, than females. Grizzly bears in the Arctic and boreal areas where caribou and moose *Alces alces* were abundant, respectively, relied more on these terrestrial prey as shown by the low $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of their hair. The study highlights the importance of spawning salmon in the diet and growth of grizzly bears. Therefore, salmon conservation should be prioritised in areas with a high density of grizzly bears, such as North America, to ensure the continued existence of this carnivore.

Belant *et al*. (2006) used the SIA of red blood cells and claws to examine resource partitioning between two sympatric species, the brown bear and black bear, in south-central

Alaska. They estimated the contribution of salmon, terrestrial meat and plants to the diet of bears, and related the assimilated diet composition to body condition and reproduction. Brown bears consumed large proportions of salmon and less plants compared to black bears, regardless of black bears' access to salmon within their home ranges. This might be owed to the dominance of brown bears, thereby excluding black bears from preferred food sources. During periods of low spawning salmon numbers, black bears mainly fed on terrestrial food sources, while brown bears consumed little terrestrial meat. The size, body condition and reproductive performance of the two species were positively associated with salmon assimilated in their diet. Consequently, brown bears were larger in size, in good body condition and had a higher reproduction output than black bears. Stable isotopes provided insights into dietary differences between two sympatric bear species, and the influence of salmon consumption on the body condition and reproduction of these predators.

ii) Anthropogenic foods

Stable carbon and nitrogen isotopes have been used to highlight the reliance of some carnivore species on anthropogenic foods. Mizukami *et al.* (2005) examined the feeding habits of alpine and rural Asiatic black bears *Ursus thibetanus* in central Japan, and evaluated whether bears caught in cage traps caused nuisance activities (i.e. raiding cornfields and garbage disposal sites). They examined isotopic changes along the entire length of hair samples and showed that Alpine bears had similar low isotopic ratios suggestive of consistent consumption of C_3 plant-derived food items. In contrast, rural bears (including nuisance bears) had higher $\delta^{13}C$ and $\delta^{15}N$ hair values, which were similar to those of Japanese human hair and corn – a C_4 plant – indicative of feeding on anthropogenic food resources. They also found that not all captured bears caused nuisance activities as hair of some individuals displayed low $\delta^{13}C$ and $\delta^{15}N$ values. Thus, SIA proved useful in revealing the dependence of Asiatic black bears on anthropogenic foods and that bears should not be killed out of suspicion without any empirical evidence for damaging behaviour.

Bentzen *et al.* (2014) analysed the hair of grizzly bears in the oilfield region of Alaska's Arctic Coastal Plain to confirm their putative use of anthropogenic food waste. Bears conditioned to anthropogenic foods had higher $\delta^{13}C$ and $\delta^{15}N$ hair values, compared to those that fed on natural foods or prey. Elevated $\delta^{13}C$ values were to a larger extent a result of reliance on anthropogenic foods derived from C_4 plants i.e. corn or sugarcane in processed foods consumed by humans. In addition, the high $\delta^{13}C$ and $\delta^{15}N$ hair values of bears conditioned to the anthropogenic food sources were similar to human hair collected from the study area. The utilization of human foods by bear individuals depended on their age and reproductive status. Older bears largely consumed natural prey as displayed by their low hair $\delta^{13}C$ and

$\delta^{15}N$ values than younger individuals, while nursing females utilised anthropogenic foods more than non-parents. Stable isotopes were able to reveal the reliance of some grizzlies on food waste, which was influenced by the availability and easy accessibility of the resource due to the high concentration of human activities and lack of proper waste storage practices in the oilfield region.

Murray *et al*. (2015) used SIA of coyote hair to complement faecal analysis data acquired after conducting an investigation on the consumption of anthropogenic foods by urban relative to rural coyotes in Alberta, Canada. It was found that urban coyotes assimilated more human food material as indicated by their higher $^{13}C/^{12}C$ ratios than their rural counterparts who chiefly utilised natural prey. Both rural and urban coyotes that were not reported to pose any human conflict had similar $^{15}N/^{14}N$ ratios, suggesting consumption of similar amounts of protein. However, urban coyotes that displayed conflict-prone behavior took less animal protein in their diet, than other urban coyotes. The majority of these conflict-causing individuals exploited low-protein foods, i.e. compost remains, cultivated fruit and bird seed, because they were readily available and could be obtained with limited endurance. As a result, the health and body condition of these coyotes were poor. The use of SIA together with faecal analysis data highlighted the significance of processed anthropogenic foods to the survival of urban-adapted coyotes. Furthermore, the study showed that protein-deficient anthropogenic foods may be important contributors to human-coyote conflicts in Alberta.

Newsome *et al*. (2015) used SIA of whiskers in combination with radio-telemetry to assess the degree of anthropogenic resource consumption by a rapidly growing population of coyotes in Chicago. Coyotes were divided into three groups; resident (with home ranges in urban nature preserves), matrix (with a large portion of urban land in their home ranges) and transient (with relatively large home ranges and variable use of urban land). Most resident coyotes had relatively low $\delta^{13}C$ values, suggestive of predominant consumption of natural prey. However, half of the residents from two preserves utilised anthropogenic foods, despite having small proportions of urban land in their home ranges. These individuals also had higher intra-individual $\delta^{13}C$ variance resulting from switching between natural and anthropogenic prey. The majority of matrix coyotes consumed anthropogenic resources, yet a few had diets analogous to coyotes in nature preserves feeding on natural prey. Similarly, approximately half of transient coyotes demonstrated a large reliance on human foodstuffs. The use of SIA in tandem with radio-telemetry revealed the differential use of anthropogenic resources by an urban carnivore, related to individual movement strategies within the heterogeneous landscape of Chicago.

Newsome *et al.* (2010a) used SIA of whiskers to assess the utilisation of anthropogenic foods by the San Joaquin kit fox *Vulpes macrotis mutica* and compared the results with data from faecal analysis. Kit foxes living in urban areas extensively exploited C_4-derived anthropogenic foods (which contradict dietary data derived from faecal analysis), and had isotope values similar to urban human residents. Urban foxes had lower $^{15}N/^{14}N$ ratios than wild foxes, and these lower values were typical of many commercially produced products. Stable isotopes were thus used to show that urban foxes broadly exploit anthropogenic foods, something that faecal analysis could not reveal. This type of information is vital when evaluating the impacts of human-wildlife conflicts on animal populations and food web dynamics in urban environments.

Savory *et al.* (2014) assessed the importance of anthropogenic foods to the diets of red and arctic foxes and competition for food resources between the two species in Prudhoe Bay, Alaska. $\delta^{13}C$ and $\delta^{15}N$ values of fox hair, red blood cells, bone and muscle varied significantly between species and seasons. During late summer, both species heavily relied on lemmings, although red foxes utilised this prey more than arctic foxes. Anthropogenic foods, supplied by a landfill and dumpsters, made up a small portion of both species' diet as indicated by the elevated $\delta^{13}C$ values related to the substantial contribution of C_4 corn or sugarcane to commercially produced foods. Arctic foxes also consumed voles and/or shorebird and goose eggs to a higher degree, than red foxes. In late winter, use of lemmings decreased for both species, and red foxes utilised a higher proportion of anthropogenic food sources than arctic foxes. The analysis of bone collagen to determine lifetime diet of red foxes revealed results similar to their late winter diet, suggesting consistent use of anthropogenic foods over years. SIA highlighted the role of garbage disposal sites in providing reliable, easy to access food resources that can be used as an alternative by foxes, particularly in the absence of lemmings.

The studies examined clearly show that stable carbon and nitrogen isotope analysis has been widely used to gather dietary information of a variety of large-bodied terrestrial carnivore species. Some of the results obtained using the technique could not be acquired using traditional dietary analyses, and this demonstrates the usefulness of SIA to better our understanding of carnivore feeding ecology.

1.5 Advantages of stable isotope analysis

The use of naturally occurring stable isotopes as dietary indicators has some important advantages over traditional methods. The technique is quick and non-invasive as collection, processing and analysis of tissue samples can be carried out within a short period of time,

and tissue samples can be acquired without killing or endangering the health of study animals, although sedation might be required (Kelly & Finch 1998; Schoeninger *et al.* 1998; Urton & Hobson 2005). SIA provides information on assimilated nutrients rather than what has been recently ingested. As a result, the method can overcome some of the limitations associated with traditional dietary analyses, such as over- or under-representation of certain prey items taken by the consumer (Peterson & Fry 1987; Hobson & Clark 1992; Cherel *et al.* 2005). Furthermore, it offers insights into nutrients that are used for energy metabolism, growth and reproduction (Lavin *et al.* 2003).

Depending on the tissue(s) selected for analysis, e.g. blood, dung, hair and bone, which can be collected in a single sampling event, stable isotopes can facilitate reconstruction of animal diets over several time scales, from weeks to years (Tieszen *et al.* 1983; Bearhop *et al.* 2002; Inger & Bearhop 2008). Thus, SIA comparatively provides long-term, quantitative, time-related dietary information (Hobson & Clark 2002; McKechnie 2004), which requires intensive and extensive labour to obtain using traditional methods alone (Inger & Bearhop 2008). The analysis of sequentially-segmented keratinous tissues, such as claws, hair and whiskers, can reveal shifts in the feeding habits of an individual caused by changes in food availability or foranging opportunity (Bearhop *et al*. 2004). These tissues can also provide information from elusive stages of an animal's life history, which may otherwise remain unknown (Crawford *et al.* 2008; Radloff *et al.* 2012). SIA is powerful in determining diet variations between individuals of a population, and thus makes it possible to identify the resource niche of individuals (Bearhop *et al.* 2004; Robertson *et al.* 2014).

All these advantages make SIA a valuable tool that can provide more information on the diets of large carnivores. The benefits offered by the technique makes it useful especially in the study of elusive predators, such as felids, whose feeding habits can be challenging to observe in the wild (Parng *et al.* 2014). The use of SIA of keratinous tissues to obtain high-resolution data on carnivore diets has gained the attention of ecologists and is increasing. Although other keratinous tissues such as hair (e.g. Lavin *et al.* 2003; Magioli *et al.* 2014), claws (e.g. Edwards *et al.* 2010) and baleen plates (e.g. Hobson & Schell 1998) have been used to reconstruct the diets of large predators, whiskers have been advocated as a very useful material for study (Newsome *et al.* 2010a; Robertson *et al.* 2014).

1.6 The stable isotope analysis of whiskers

Whiskers chronologically archive the dietary history of individuals and the tissue is not remodelled over time (Darimont & Reimchen 2002; Rubenstein & Hobson 2004). Therefore, serial sub-sampling of whiskers can provide a series of long-term and shifts in foraging

habits (Hobson *et al.* 1996; Bearhop *et al.* 2003; Lee *et al.* 2005). The SIA of whiskers has been used with great success in aquatic mammals to infer temporal diet variations (e.g. Hall-Aspland *et al.* 2005; Newland *et al.* 2011), sex-related resource use (e.g. Lewis *et al.* 2006; Kernaleguen *et al.* 2011) and habitat use (e.g. Cherel *et al.* 2009) of individuals or species. In terrestrial carnivores, the technique has been used to examine the exploitation of anthropogenic foods by San Joaquin kit foxes (Newsome *et al.* 2010a) and coyotes (Newsome *et al*. 2015), and to assess the influence of human disturbances on the feeding behaviour of the invasive non-native American mink (Bodey *et al*. 2010).

SIA of carnivore whiskers can be used not only to identify a particular individual's diet over time, but also provides the opportunity to make accurate dietary comparisons of individuals within and between populations. It is particularly useful in determining whether a population is composed of specialists that all prey on the same limited number of species, individuals that either all take a wide range of prey species (Type A generalisation) or individuals that each specialise on a different, but narrow range of prey species, which together span a wide range (Type B generalisation) (Bearhop *et al.* 2004). For instance, Newsome *et al.* (2009) showed that individual California sea otters *Enhydra lutris nereis* had highly unique dietary patterns, which they maintained through time. Similarly, Robertson *et al.* (2014) also used whisker analyses and found long-term differences in resource utilisation among Eurasian badgers of the same group who had access to the same resources. These studies showed that individual dietary specialisation might be a common phenomenon in well known generalist-feeding species. It is therefore evident that SIA of whiskers can provide novel insights into the feeding ecology of carnivores and can be of great importance in the study of elusive carnivore species.

Although stable isotope measurements of carnivore whiskers can provide a deep understanding of an individual's resource preferences, the interpretation of patterns observed require knowledge of species-specific whisker growth rates, growth patterns and whisker-diet TDFs (Newsome *et al.* 2010b; Stricker *et al.* 2015). Whisker growth rates and growth patterns are required to assign the dietary record contained in a whisker of a specific length to the correct time period, while TDFs are necessary for the accurate identification of prey species consumed by a predator (Newland *et al.* 2011). Whisker growth rates and/or growth patterns have been measured for a number of marine carnivore species, particularly pinnipeds, including harbor seals *Phoca vitulina* (Hirons *et al.* 2001; Zhao & Schell 2004), leopard seals *Hydrurga leptonyx* (Hall-Aspland *et al.* 2005), southern elephant seals *Mirounga leonina* (Newland *et al.* 2011) and fur seals *Arctocephalus gazella* (Cherel *et al*. 2009; Kernaleguen *et al*. 2012). However, only a few terrestrial carnivores' whisker growth

data, i.e. stoats *Mustela erminea* (Spurr 2002) and Eurasian badgers (Robertson *et al.* 2013), have been documented. Similarly, stable carbon and nitrogen whisker-diet TDFs have been estimated for seals and sea otters, yet such information is lacking for terrestrial carnivore species. This makes it difficult to accurately determine the diets of terrestrial carnivores using the SIA of whiskers.

1.7 Research question

Can the stable carbon and nitrogen isotope analysis of felids' whiskers be used to determine their diet, and provide insights into individual-level dietary differences that are difficult to obtain using traditional dietary analyses?

1.8 Aims and objectives of the study

The overall aim of the study was to develop and evaluate the technique of using SIA of whiskers to quantify the diets of individual wild felids and to test for diet variation amongst the individuals. Currently, the technique is hampered by a lack of information on species-specific and even genus-specific whisker growth rates, growth patterns and whisker-diet TDFs. These gaps make the interpretation of whisker stable isotope values very difficult, especially when the timeframe represented by a whisker segment of a specific length is required (Newsome *et al.* 2010b; Tyrrell *et al.* 2013). Refinement of the technique might provide more insights into the foraging ecology of felids, in particular, temporal diet variations within- and between-individuals.

The study was divided into two components; firstly, large felid (lion and leopard) whisker growth rate and growth pattern, and lion whisker-diet TDFs were determined. Secondly, the feasibility and applicability of the technique was explored by measuring whisker isotopic compositions of free-ranging leopards in Phinda Private Game Reserve (hereafter Phinda), northern KwaZulu-Natal (KZN), South Africa to infer their diets.

The main objectives of the study were:

i) To estimate lion and leopard whisker growth rate and growth pattern; and lion whisker-diet TDFs
ii) To quantify the diet of individual free-ranging leopards in Phinda using stable carbon and nitrogen isotope analysis of their whiskers, and compare results with those obtained from traditional methods

iii) To evaluate the feasibility of using stable carbon and nitrogen isotope analysis of whiskers to determine individual prey preference and dietary niche variation amongst Phinda leopards

1.9 Overview of the thesis

This thesis is composed of five chapters of which chapters 2, 3 and 4 have been compiled as stand-alone manuscripts to facilitate publication in peer-reviewed journals.

Chapter 2 reports on the whisker growth rate and growth pattern measured over 185 days for four lions and one leopard held at the National Zoological Gardens, Pretoria. Two whiskers were removed from each individual and while regrowth was taking place, the animals were periodically fed giraffe *Giraffa camelopardalis* meat. The ^{13}C-depleted, C_3-derived giraffe meat acted as an endogenous biological marker as its characteristic isotopic signature could be discerned from the ^{13}C-enriched, C_4 grain-fed chicken and beef diet the animals were sustained on. Knowing the duration between giraffe meat feeding bouts, and being able to identify these feeding bouts isotopically along the whisker length enabled the calculation of felid whisker growth rate and growth pattern. The chapter also reports on $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs for the lion, estimated for five individuals whose diet remained consistent over multiple years.

In Chapter 3, SIA of felid whiskers is applied to six leopards in Phinda to quantify the diets of individuals and results are compared with those obtained from the same population using traditional methods. Sixty millimetres of a whisker from each individual were incrementally sampled and analysed for their $\delta^{13}C$ and $\delta^{15}N$ values. This length of whisker presented approximately 92 days of dietary information, which made comparisons between individuals' diets possible. The 30 $\delta^{13}C$ values of each whisker were apportioned to those derived from browser, intermediate feeder and grazer prey based on the isotope values of known prey species ascertained from the analysis of hair and faecal samples collected from the study area and surrounding properties. Factors that potentially influence the interpretation of the leopards' diet i.e. isotopic baseline, prey sources, isotopic turnover and diet-tissue discrimination were evaluated.

In Chapter 4, the feasibility of using SIA of leopards' whiskers to determine individual prey preferences and dietary niche separation amongst leopards is explored by comparing leopard prey use results obtained in Chapter 3 with the proportions of prey available within an individual's home range. The effects of prey availability on the determined leopard dietary niche differences were evaluated.

Chapter 5 summarises the main findings and limitations of the study, and conclusions drawn from evidence and reasoning. The chapter also provides recommendations for future stable isotope-based studies on large carnivores.

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CHAPTER 2

FELID WHISKER GROWTH RATE, GROWTH PATTERN AND ISOTOPE DISCRIMINATION FACTORS

2.1 Introduction

Stable isotope analysis (SIA) has become an important complementary tool to traditional methods for examining mammalian diets. The method can be used to investigate the foraging ecology of both marine and terrestrial animals, particularly trophic position, dietary preferences and habitat use (Kelly 2000; Crawford *et al.* 2008; Inger & Bearhop 2008; Ben-David & Flaherty 2012). The strength of the technique lies in its ability to reveal information on assimilated and not just ingested nutrients (Hobson & Clark 1992; Lavin *et al.* 2003; Cherel *et al.* 2005). It also eliminates some of the restrictions associated with traditional dietary analyses such as over- and under-representation of prey remains, failure to adequately determine temporal dietary patterns, as well as intra-population diet variation (Urton & Hobson 2005; Newsome *et al.* 2009; Robertson *et al.* 2013). SIA is particularly useful in the study of elusive species such as large felids whose feeding habits are expensive and time consuming to investigate with traditional methods alone (Parng *et al.* 2014).

The utility of stable carbon and nitrogen isotopes in dietary studies relies on the proportional distribution of the respective isotopes between different food types, and how they are incorporated into the tissues of consumers (Kelly 2000). It is based on the concept that isotope ratios assimilated in consumer tissues are directly related to those of diet and habitat(s) exploited by the respective individuals (DeNiro & Epstein 1978; 1981; Hobson & Clark 1992; Ben-David & Flaherty 2012). These isotopes are incorporated in a predictable manner which can be traced through analysis of both consumer and prey tissues (DeNiro & Epstein 1978; 1981; Crawford *et al*. 2008). Carbon isotopes in tissues of a consumer are directly related to the source (e.g. plants with either a C_3 or C_4 photosynthetic pathway) mainly because of little discrimination between an animal and its diet (DeNiro & Epstein 1978; McCutchan *et al.* 2003; Cherel & Hobson 2007). Hence, carbon isotopes can identify the source of primary production at the base of the food chain. Conversely, nitrogen abundances increase with each trophic step due to the preferential excretion of the lighter nitrogen-14 isotope (^{14}N) during assimilation (DeNiro & Epstein 1981; Minagawa & Wada 1984; Hobson *et al.* 1996). Therefore, consumers are usually enriched in nitrogen-15 (^{15}N) relative to their diet, and this predictable fractionation is used to determine the trophic level of a consumer (DeNiro & Epstein 1981; Peterson & Fry 1987).

Consumer tissues such as blood plasma, red blood cells, muscle and bone can be analysed to provide foraging and habitat use information representing different time windows (Hobson 1993). Information provided by these metabolically active tissues is dependent on the tissue turnover rate, which is the length of time a specific tissue is formed or synthesised (Hobson *et al.* 1996; Dalerum & Angerbjorn 2005). In contrast, inert keratinous tissues (i.e. baleen, claws, hair and whiskers) in which no turnover takes place, archive foraging habits of an individual at the time of their growth and this information remains unaltered over time (Mizutani *et al*. 1990; Hobson *et al.* 1996; Bearhop *et al.* 2003; Lee *et al.* 2005).

SIA of inert tissues has been used with great success to provide foraging information of carnivores such as bowhead whales (e.g. Hobson & Schell 1998; Lee *et al.* 2005), bears (e.g. Hobson *et al.* 2000; Bentzen *et al.* 2014), wolves (e.g. Darimont & Reimchen 2002; Urton & Hobson 2005), coyotes (e.g. Murray *et al*. 2015; Newsome *et al.* 2015) and foxes (e.g. Newsome *et al.* 2010a; Savory *et al.* 2014). However, the potential of the method to disclose unknown dietary information of large felids has been little explored. Most large felid species are under threat due to human-induced factors such as habitat loss and degradation, depletion of prey and direct persecution (Hunter 2011; Ripple *et al.* 2014). As a result, populations and geographical ranges of large felid species have diminished globally (Woodroffe 2000; Dalerum *et al.* 2008; Jacobson *et al.* 2016). This poses the need to thoroughly investigate their feeding habits to understand how their survival relates to other organisms and their physical environment. Dietary studies on wild felids have generally used traditional methods (e.g. faecal analysis, direct observations and spoor tracking), yet limitations inherent in these techniques makes it difficult to acquire detailed records on short-term feeding, as well as diet variations between individuals in a population (Lavin *et al.* 2003). To acquire such valuable information, isotope ratios in continuously growing inert keratinous tissues, specifically whiskers, can be analysed.

Whiskers, like any other body hair, grow incrementally but are thicker, stiffer and sometimes longer than pelage hair (Oliver 1966). In ungulate herbivores, tail hairs have a similar structure and are often used as an isotopic tissue of analysis when reconstructing the diets of animals (Codron *et al.* 2013). However, when inferring the feeding habits of large carnivores using SIA, whiskers are considered the best tissue of analysis as they potentially preserve more detailed dietary information than other body hairs (Newsome *et al.* 2010a; Robertson *et al.* 2014). Whiskers record the dietary history of individuals in a sequential manner (because growth is incremental and the tissue does not turnover) (Darimont & Reimchen 2002; Rubenstein & Hobson 2004), with the proximal root and distal tip representing recent and past information, respectively (Tyrrell *et al.* 2013). Once information

is recorded, the stable isotope signatures of whiskers remains biochemically unchanged over time, making it possible to acquire high-resolution dietary information through serial sampling of the tissue along the growth axis (Hobson *et al.* 1996; Newsome *et al.* 2010b). Serial sampling of whiskers can reveal isotopic diet variations within individuals and address important ecological questions point samples cannot (Zhao & Schell 2004; Newsome *et al.* 2009; Bodey *et al*. 2010).

To accurately interpret large carnivore whisker isotope series, knowledge of whisker growth rates and growth patterns, and how these vary with time and within populations, is required (Hall-Aspland *et al.* 2005; Tyrrell *et al.* 2013; Robertson *et al*. 2013). Understanding growth rates and growth patterns is necessary to inform the appropriate timeframe of the dietary history contained in a whisker (Newland *et al.* 2011). Whiskers, like other tissues, may grow either in a linear or non-linear pattern. In linear growth, segments of equal length represent an approximate equal time interval anywhere along the whisker (Hirons *et al.* 2001; Tyrrell *et al.* 2013). In non-linear growth (e.g. the asymptotic von Bertalanffy growth pattern), segments near the distal tip of the whisker likely represent higher temporal resolution than segments of equal length near the proximal root, as expected under typical non-linear growth patterns (von Bertalanffy 1938).

To date, studies on whisker growth rates have been conducted on several pinniped species (Hirons *et al.* 2001; Zhao & Schell 2004; Greaves *et al.* 2004; Hall-Aspland *et al.* 2005; Cherel *et al*. 2009; Newland *et al.* 2011; Kernaleguen *et al*. 2012; Rea *et al.* 2015) and the southern sea otter *Enhydra lutris nereis* (Tyrrell *et al.* 2013). However, only a few terrestrial species, i.e. laboratory rats *Rattus norvegicus* and mice *Mus domesticus* (Ibrahim & Wright 1975), stoats *Mustela erminea* (Spurr 2002) and Eurasian badgers *Meles meles* (Robertson *et al.* 2013), have been studied. Most whisker growth rate experiments have been conducted on small numbers of captive individuals (but see Cherel *et al*. 2009; Newland *et al.* 2011; Kernaleguen *et al*. 2012; Robertson *et al.* 2013; Rea *et al.* 2015). The experiments have used different approaches including ^{15}N-enriched glycine labelling (e.g. Hirons *et al.* 2001; Tyrrell *et al.* 2013) and Rhodamine B fluorescent biomarking (e.g. Spurr 2002; Robertson *et al.* 2013) to endogenously mark whiskers for growth rate calculations.

Terrestrial species whose whisker growth has been explored displayed varied growth rates and growth patterns, either linear or non-linear, followed by varying degrees of whisker retention and shedding. Whiskers of stoats and Eurasian badgers were reported to grow at a rate of 0.6 mm d^{-1} (Spurr 2002) and 0.43 mm d^{-1} (Robertson *et al.* 2013), respectively. In rodents, Ibrahim & Wright (1975) have shown that rat and mice whiskers grow at 1.5 mm d^{-1} and 1 mm d^{-1}, respectively, for four weeks, after which growth time slows for several days

before ceasing entirely. Apart from being species-specific, whisker and other hair growth rates can vary within populations due to factors including age, sex, disease and food deprivation (Wright 1965; Ibrahim & Wright 1975; Young & Oliver 1976). The method of whisker removal employed when conducting experiments can also affect growth rate. For example, plucking whiskers during the growth phase can lead to delays in the emergence of a new whisker (Ibrahim & Wright 1975).

The use of isotopic ratios in whiskers to reconstruct diets of large carnivores also requires knowledge of $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs, which is the isotopic difference between a consumer's bulk tissue (in this case whisker) and its diet (Newsome *et al.* 2010b; Stricker *et al.* 2015). TDFs are usually derived from captive feeding experiments and in a few cases, under well-constrained field conditions (Martinez del Rio *et al.* 2009; Newsome *et al.* 2010b). They are variable across species, individuals of the same species, tissues, ages, growth rates and diets (Robbins *et al.* 2010; Martinez del Rio & Carleton 2012; Webb *et al.* 2017). Studies where experimental determination of TDFs was not carried out commonly use surrogate values obtained from closely related species or consumer's whole body $\delta^{13}C$ and $\delta^{15}N$ TDFs of 1 and 3 ‰, respectively (DeNiro & Epstein 1978; 1981). Few studies have estimated TDFs for hair of terrestrial carnivore species, and in these cases pelage hair has mainly been studied (e.g. Roth & Hobson 2000; Parng *et al.* 2014; Montanari & Amato 2015). Experiments on whisker-diet TDFs have only been carried out for marine carnivores, revealing $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of 2.2 and 3.5 ‰ (Newsome *et al.* 2010b) and 2.8 and 5.5 ‰ (Tyrrell *et al.* 2013) for the southern sea otter, 3.3 and 3.7 ‰ for the Steller sea lion *Eumetopias jubatus* (Stricker *et al.* 2015), 3.5 and 2.8 ‰ for the elephant seal *Mirounga angustirostris* (Beltran *et al.* 2016), respectively. The lack of species-specific terrestrial carnivore whisker-diet TDFs makes it difficult to correctly interpret stable isotope patterns in whiskers when making quantitative diet statements.

In this study, whisker growth rate and growth pattern were measured for four captive lions *Panthera leo* and one leopard *Panthera pardus* with the use of giraffe *Giraffa camelopardalis* meat as an endogenous biomarker to consecutively mark whiskers as they grew. Furthermore, isotopic whisker-diet TDFs were estimated for the lion. Using the stable carbon and nitrogen isotope analysis of whiskers, the study aimed to (i) determine lion and leopard whisker growth rates (mm d^{-1}), (ii) determine lion and leopard whisker growth patterns, and (iii) estimate lion whisker-diet TDFs. The study adds to the current narrow list of taxa with known whisker growth rates, growth patterns and whisker-diet TDFs. This valuable knowledge can be used in future research using SIA of whiskers to quantify resource use in wild felids.

2.2 Materials and Methods

2.2.1 Whisker growth rate and growth pattern

2.2.1.1 Study animals

The growth of whiskers of four lions and one leopard was monitored in a controlled biomarker feeding experiment at the National Zoological Gardens (hereafter, Zoo), Pretoria, South Africa, for six months, from 3 June to 5 December 2014. Three female lions known by Zoo staff as Emma, Bianca and Tess, one male lion known as Boesman and one male leopard known as Diesel were available for the study. Table 2.1 provide details on individual weight, height and age.

Table 2.1: Weight, height and age of the study felids measured at the beginning of the experiment

Study animals	Weight (kgs)	Shoulder/Height (cm)	Age (years)
Emma	104.8	84	2.5
Bianca	111.5	80	2.5
Tess	120.1	89	2.6
Boesman	164.9	101	5
Diesel	113	82	4

2.2.1.2 Whisker harvesting and use of giraffe meat as a biomarker

Lions at the Zoo were reported to be fed 0.5 kg of chicken with feathers daily and 4 kg of beef every second day, while the leopard was fed 1.5 kg of chicken with feathers daily and 2 kg of beef every second day (M. Meyer-Bouwer pers. comm. 2014). To determine the isotopic nature of this food and consistency in feeding, the proximal 60 mm of a 130 mm whisker from a female lion (known as Amber) were sectioned at 2 mm intervals before the commencement of the experiment. The resultant 30 whisker sections were analysed for their carbon isotope values. The $\delta^{13}C$ values revealed a consistent C_4-derived signal with little variation (mean = 11.3 ‰; standard deviation (*SD*) = 0.40; range = -12.1 to -10.7 ‰). The consistency of the diet provided the opportunity to "isotopically" mark regrowing whiskers at predetermined intervals with ^{13}C-depleted, C_3 plant-derived giraffe meat sourced from a game farm in northern KwaZulu-Natal (KZN) to sufficiently alter whisker ^{13}C profiles. The identified periods with low $\delta^{13}C$ values would then be correlated to specific giraffe meat feeding bouts, and hence growth periods.

On the 3rd of June 2014, the study animals were intramuscularly administered a combination of zoletil and medetomidine following standard zoological protocols. The capture and handling protocol of these felids was approved by the Cape Peninsula University of Technology Ethics Committee (Ref. 01/2014) and the National Zoological Gardens Research Ethics and Scientific Committee (P14/03). The longest upper lip whisker available on both cheeks of an individual was plucked with its roots using steel forceps. The position of these whiskers was carefully recorded as the focus was on measuring the growth of the whiskers replacing those removed. The whiskers occurred in well defined rows; therefore, each whisker was allocated a unique identification number, Lx/y or Rx/y (Lyne *et al.* 1974). The prefixes L and R were used to refer to the left and right cheeks, respectively. x and y were row and column position, in sequence, numbered from the posterior end (Figure 2.1). The longest whisker (LD1 or RD1), determined after a whisker length profile was conducted on two individuals, was removed. Where this whisker was absent, whisker (LC1 or RC1) or (LE1 or RE1) was removed. Once the new whiskers were visible, felids were fed their first giraffe meat to mark the new whiskers with the C_3-depleted food.

Felids were fed the giraffe meat at four predetermined feeding bouts and each feeding bout comprised four consecutive days. The four giraffe meat feeding bouts took place 34 days (7–10 July), 105 days (16–19 September), 147 days (28–31 October) and 181 days (1–4 December 2014) after the initial whisker removal, respectively. On each of the four days of a feeding bout, the individual felids received 2.5 kg of giraffe meat. It was confirmed visually that the animals consumed the ration in total. Study animals were sedated a second time on the 5th of December 2014, 185 days after commencement of the experiment, to remove the regrown whiskers. During the second removal of whiskers, it was discovered that all of Boesman's whiskers had broken. Hence, available whiskers that were not plucked initially, but long enough to potentially provide some information were removed from this male lion at the end of the study.

Samples of giraffe, chicken and beef meat were collected at each feeding session for calculations of meat type average carbon and nitrogen delta-values. These were used to estimate whisker-diet TDFs. Meat samples were individually obtained from different meat packets and chunks. Six samples (two of each meat type) were collected during the first feeding bout and 36 samples (12 of each meat type) during the second, third and fourth feeding bouts. In total, 114 meat samples were collected during the experiment. The collected meat samples were kept frozen until arrival in the laboratory where they were selected and processed (see details below).

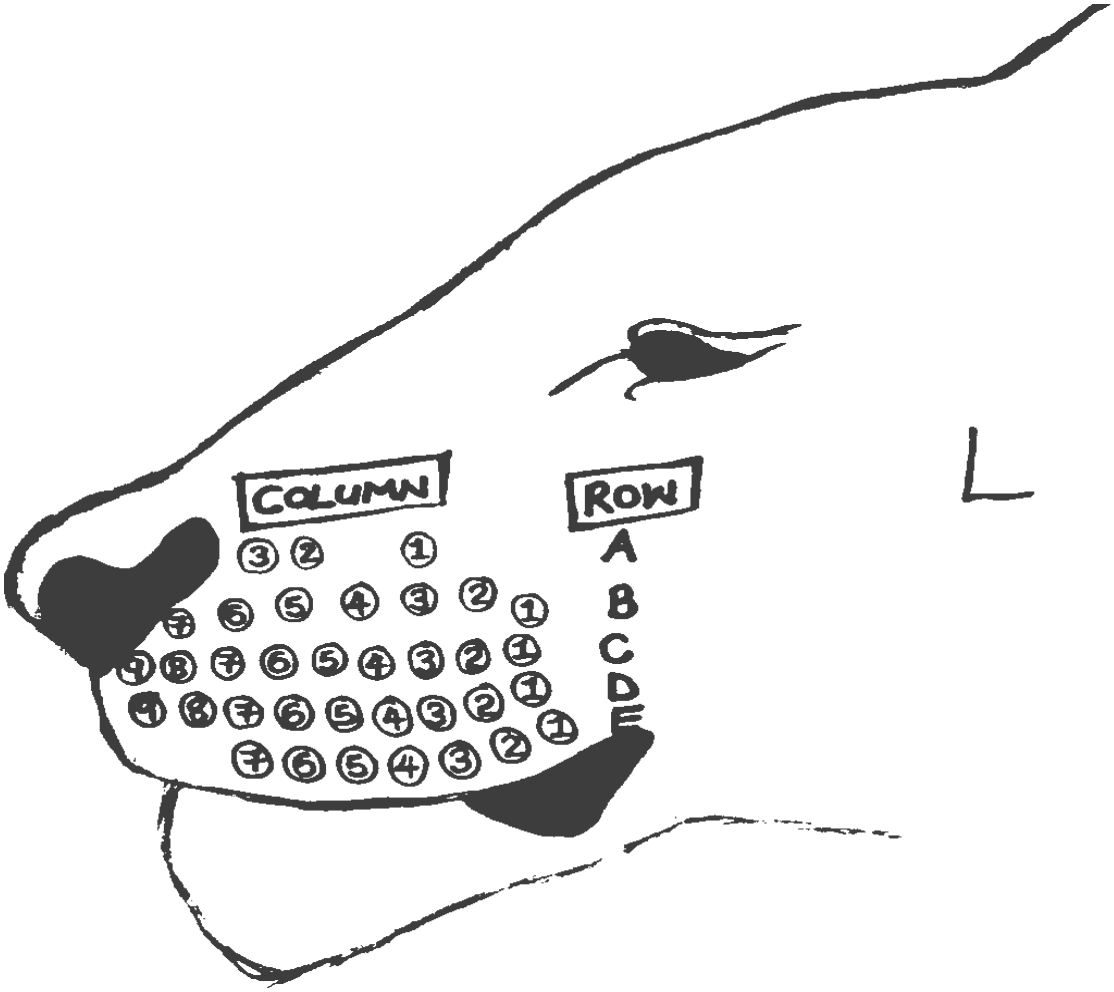

Figure 2.1: An illustration of well defined rows of felid whiskers and how each removed whisker was allocated an identification number

2.2.2 Whisker-diet trophic discrimination factors

Before the study commenced, the diet of the leopard (Diesel) was changed by reducing its food ration and altering its daily chicken and beef ratio as the individual was overweight (M. Meyer-Bouwer pers. comm. 2014). Due to this dietary change, stable carbon and nitrogen whisker-diet TDFs could only be determined for the lion. The whisker removed from a female lion (Amber) in June 2013 to assess the consistency of the diet fed to the Zoo felids (mentioned earlier), and those removed from three female lions (Emma, Bianca and Tess) and a male lion (Boesman) at the beginning of the growth rate experiment in June 2014 were used to estimate isotopic whisker-diet differences. Comparing the isotopic ratios of a whisker removed a year before the commencement of the experiment, and those of whiskers removed at the time of the experiment provided an opportunity to validate the consistency of the diet fed to the lions. The lion whiskers and the chicken and beef meat sampled at the

time of the experiment were analysed for their stable carbon and nitrogen ratios. These isotopic ratios were used to calculate isotopic differences between lion whiskers and diet.

2.2.3 Laboratory sample preparation

2.2.3.1 Growth rate experiment samples

The lengths of all the plucked whiskers were measured on a flat surface using a metal ruler and their masses were weighed on a microbalance. Individual whiskers were inspected for the presence of any club roots signifying cessation of growth (Fisher 1998). Five whiskers (one from each study animal) were washed in a 2:1 methanol: chloroform solution to remove surface contaminants, further cleaned in an ultrasonic bath (DG-600) using distilled water for five minutes and air dried. To obtain high-resolution dietary information, whiskers were sectioned at 1 mm intervals from the root towards the tip using a scalpel. Sections were weighed on an analytical balance (GH series), crimp sealed and packed in tin capsules for stable carbon and nitrogen isotope analysis. Precautionary measures, such as handling both samples and packaging material with forceps, were practised during the weighing process to prevent any sample contamination.

Forty-two of the 114 meat samples (14 of each meat type i.e. chicken, beef and giraffe) were randomly selected, homogenised to 2 g pieces, packed in small labelled plastic containers and placed in a freezer at -20 ˚C. These were then freeze-dried without extracting lipids at -55 ˚C for 48 hours. All dried samples were finely ground into homogenous powder using an analytical mill (A11 basic), weighed to 0.5 mg on an analytical balance and packed in tin capsules for stable carbon and nitrogen isotope analysis.

2.2.3.2 Whisker-diet discrimination samples

Lion whiskers were cleaned and weighed following the procedure described above. The proximal 60 mm of the whiskers of Bianca, Emma, Tess and Boesman (one from each individual), removed at the beginning of the growth rate experiment, were each cut into six 10 mm segments. From each of these six segments, the distal 2 mm were removed for analysis resulting in six whisker samples per individual. In total, 54 whisker samples including the 30 samples obtained from Amber for the “pilot” study (see section 2.2.1.2) were weighed, packed in tin capsules and sent for carbon and nitrogen isotope analysis.

Lipids are usually depleted in ^{13}C relative to the diet resulting in more negative $\delta^{13}C$ values (Tieszen *et al.* 1983). Therefore, the isotope compositions of 16 meat samples with and without lipids were compared. Eight beef and eight chicken samples were each cut into two,

and half of the samples were analysed with lipids as described earlier. The other half of the meat samples were lipid-extracted at the Stellenbosch University, Department of Animal Sciences, following the method of Lee *et al.* (1996). All the visible fat tissue was trimmed and samples were cut to 2 g pieces, individually weighed off into 205 x 30 mm extraction tubes with 20 mg of 2:1 chloroform: methanol solution. Meat samples were homogenised together with the extraction solvent for 10–20 seconds using a polytron mixer (Kinematica AG Homogeniser, PT-2500) set at 7–8 x 1 000 speed. To prevent sample contamination, the polytron was thoroughly cleaned between samples by removing residue sinews and tissue with tweezers, rinsed with water followed by chloroform/methanol solution then wipe clean. The meat and solution mixture were filtered through Whatman grade 1 filter paper into Erlenmeyer flasks. Meat sample residue was scraped from the filter paper into small labelled plastic containers and placed in a freezer at -20˚C. Samples were freeze-dried, ground, weighed and packed in tin capsules following the same procedure as the non-lipid extracted meat.

2.2.4 Stable isotope analysis

All samples were sent for carbon and nitrogen isotope analysis at the Stable Light Isotope Laboratory, Department of Archaeology, University of Cape Town. Samples were individually combusted in a Flash 2000 organic elemental analyser (Thermo Scientific, Bremen, Germany) and the resultant CO_2 and N_2 gases introduced into a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) via a continuous flow-through inlet system (Conflo IV). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are presented in delta (δ) notation in parts per thousand or per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 standards, respectively, derived from the expression:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 * 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard deviations of repeated measures of laboratory standards (Chocolate powder, Seal and Valine) were ≤ 0.3 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

2.2.5 Data analysis

2.2.5.1 Identification of feeding bouts along whisker lengths

Isotopic profiles of whiskers were plotted with whisker length (mm) on the x axis and $\delta^{13}C$ values on the y axis. These graphs were then visually inspected to identify the point, during whisker growth, that the giraffe meat feeding bouts took place. The point, along the whisker,

where carbon isotopes from the giraffe meat were incorporated was identified by sharp deviations toward low $\delta^{13}C$ values, representing the transition from ^{13}C-enriched, C_4-derived chicken and beef diet to experimental ^{13}C-depleted, C_3-derived giraffe diet. Four phases characterised by whisker segments of low $\delta^{13}C$ values illustrating the four feeding bouts were to be identified along each individual whisker.

2.2.5.2 Growth rate estimations

Whisker growth rate was investigated by two methods. First, the interval growth rate between two consecutive measurements was calculated (Δlength/Δtime) using the beginning of the C_3 peak at each feeding bout. This metric revealed the range of growth rates that occurred within an individual whisker at different phases during the experiment and made it possible to discern the whisker growth pattern (Greaves *et al.* 2004). It also revealed individual and mean felid whisker growth rates. Whisker growth rates were calculated for (i) whisker length grown for the entire duration of the experiment (185 days) and (ii) whisker length grown over the last 76 days of the experiment (from 109 to 185 days).

Second, the relationship between length of whiskers (mm) and growth rate (mm d^{-1}) with time (d) was modelled using mixed effects linear models (MELMs). MELMs are used for longitudinal data to quantify the relationship between a continuous dependent variable (fixed effect) and various predictor variables (random effects), while taking correlation errors into account (Peng & Lu 2012; Bates *et al.* 2014). The models can deal with missing values and naturally handle uneven spacing of repeated measures (West *et al.* 2014). MELMs were performed in R software, version 3.1.3 (R Core Team 2013) with add-on lmerTest package (Kuznetsova *et al.* 2013). The effect "individual" was included as a random effect to ensure that correct error terms were compared considering there were multiple measurements (i.e. non-independent measures) for each individual. However, only three empirical observations were available for each individual (experimental feeds 2, 3 and 4, from days 109 to 185). Therefore, the models were rerun including growth from day one of the experiment. Comparisons of growth rates (slopes) derived from the analysis of the two data sets were used to determine whether growth was linear or not. A significant decrease in growth rate between the latter and former models would indicate non-linear growth. Comparisons of whisker growth amongst individuals were based on 95 % confidence intervals of slopes and intercepts of the random effects. Despite the small sample size (three or "four" observations per individual), assumptions of normality and homogeneity of variance of the data were not violated, based on Shapiro-Wilk's *W* (Shapiro & Wilk 1965) and Levene's tests (Zar 2010), respectively. A significance level of 0.05 was used in all tests.

2.2.5.3 Growth pattern: linear versus non-linear

The MELMs used above assume that whisker growth in large felids is linear (apart from the comparison between models fitted to the exact obtained data and those with an assumed growth at day one). Many body tissues, including hair, are expected to exhibit a non-linear growth, generally slowing down until it reaches an asymptote after an initial phase of faster growth (Karkach 2006). A common physiological growth pattern is described by von Beratlanffy's (1938) model, although other non-linear growth patterns such as the Gompertz and logistic are also possible (Hernandez-Llamas & Ratkowsky 2004). The small sample obtained for the present study could not allow for fitting of non-linear growth models to the data due to over-parameterisation. Use of parameter values derived from linear growth models to reconstruct growth of whiskers harvested from free-ranging animals could be problematic. It is therefore necessary to consider (i) whether this approach would be appropriate for field studies and (ii) whether changes in growth patterns are such that linear growth can be assumed for certain portions of a whisker (i.e. a portion over which growth is presumably more-or-less constant). To address this issue, comparisons of simulated linear and non-linear (von Bertalanffy) whisker growth patterns were made. Linear growth was simulated using the following equation:

$$l_t = a + bt \quad (2)$$

where l_t is length (mm), a is the intercept (set at 0 assuming zero growth at zero days), b is growth rate and t is days. Growth rate (b) used was the slope of the MELM for millimetres (mm) over days (d). Non-linear growth was simulated using the following von Bertalanffy equation:

$$l_t = k\left[1 - e^{-b(t-to)}\right] \quad (3)$$

where l_t is length (mm), k is asymptotic length when growth rate is equal to zero, b is growth rate, t is days and t_0 is length at zero days (von Bertalanffy 1938). Parameters for the von Bertalanffy simulations were chosen by trial and error until the solution that most closely resembled the linear scenario was found. In this case, $b = 0.02$ and $k = 72$ (note that the value of k is equal to the number of days for which reliable growth rate data could be obtained from the whiskers measured in this study i.e. 105 days; see Results Figure 2.2). The trial and error solution was used because there was no obvious non-linear trend in observed data for the relevant whisker portions. Thus, no *a priori* assumptions about parameter values could be made.

After the establishment of the two hypothetical curves, the growth period represented in 72 mm (measured from root) of each curve was calculated and compared. Solving for growth rate or time represented within a 72 mm whisker, equation (2) gives:

$$t = (l_t - a)/b \quad (4)$$

and equation (3) gives:

$$t = \ln\left(1 - \frac{lt}{k}\right)/bt_0 \quad (5)$$

The two scenarios were compared in how they estimated whisker length grown after a specific time period (i.e. number of days represented within a 72 mm whisker). A dependent samples (paired) *t*-test was then used to compare time intervals between each simulated series, and hence determine whether the two curves differ significantly in expected growth rates. This procedure tests whether the application of linear versus non-linear growth models could be expected to have significant impacts on resolving growth rates and time periods represented in 72 mm of a felid whisker harvested in field. Simulations were carried out using the Visual Basic for Applications (VBA) editor of Microsoft Excel 365.

2.2.5.4 Lion whisker-diet trophic discrimination factors

The amount of chicken and beef consumed by the captive animals was converted to percentages, and the weighted mean delta-values were calculated. A two sample *t*-test, with Satterthwaite's (1946) approximation of degrees of freedom, performed in R, version 3.1.3 (R Core Team 2013), was used to determine whether the means of lipid- and non-lipid-extracted chicken and beef differed. TDFs were then calculated for each point along the whisker series, following Craig (1954):

$$\varepsilon^*_{\mathrm{WD}} = \left(\frac{1000+\delta^{13}C_{whisker}}{1000+\delta^{13}C_{diet}} - 1\right) * 1000 \quad (6)$$

where $\varepsilon^*_{\mathrm{WD}}$ is effectively the difference in delta-values of the whisker (W) and diet (D). Equation (6) is derived from geokinetics models, which capture the fact that isotope exchange between a source (diet) and sink (whisker) is non-linear or non-reversible. The asterisk denotes that isotopic equilibrium is not assumed. $\varepsilon^*_{\mathrm{WD}}$ values are sometimes, but not always, equivalent to the more commonly used Δ_{WD} ($= \delta^{13}C/\delta^{15}N_{consumer} - \delta^{13}C/\delta^{15}N_{diet}$) values. The former approach was followed here as it is not scale dependent (Cerling & Harris 1999).

To determine mean TDFs (i.e. mean $\varepsilon^*_{\mathrm{WD}}$), a randomisation procedure was used to account for biases such as pseudo-replication effects that could arise in simple calculations of

arithmetic means of data series, including both repeated measures and uneven sample sizes. For example, the ε^*_{WD} series for Amber was much longer ($n = 30$) than for the other individuals ($n = 24$). Thus, means and *SD*s from 1 000 bootstraps or random sub-samples of the data set (25 % of the sample size) were estimated. Resampling algorithms were coded using VBA editor of Microsoft Excel 365.

2.3 Results

2.3.1 Whisker growth rate experiment

2.3.1.1 Lengths and weights of individual whiskers

A total of 644 sections from the five felid whiskers removed at the end of the experiment were analysed (Appendix A). The lengths of the whiskers ranged from 72 to 140 mm, while their weights ranged from 24 to 32.3 mg. Table 2.2 provide detail on individual lengths and weights of the sampled whiskers, and the number of sections obtained from each whisker. As mentioned earlier, all of Boesman's whiskers were broken when the experiment was terminated; hence, the whisker length provided here was not the full length grown for the entire duration of the experiment.

Table 2.2: The lengths, weights and number of sections analysed from the five whiskers removed from the individual felids at the end of the study

Felid name	Whisker position	Length sampled (mm)	Weight (mg)	Number of sections
Bianca	LD1	128	22.5	130
Emma	LD1	140	29	144
Tess	RF1	135	24	142
Boesman	RC1	72	28	76
Diesel	LD1	138	32.3	152

2.3.1.2 Stable isotope compositions of the experimental diet

Table 2.3 provide mean (± *SD*) $\delta^{13}C$ and $\delta^{15}N$ values of the giraffe meat and Zoo chicken and beef samples (see Appendix B for the $\delta^{13}C$ and $\delta^{15}N$ values of meat samples). ^{13}C signatures of the giraffe meat were indicative of a C_3-based diet, while the Zoo chicken and beef were largely C_4-derived. The daily meat consumption of the five lions (including the pilot study individual, Amber) consisted of 20 % chicken and 80 % beef. The average $\delta^{13}C$ and

$\delta^{15}N$ values of lipid- and non-lipid-extracted chicken were not significantly different from each other (t = -0.07 for $\delta^{13}C$ and -0.13 for $\delta^{15}N$; $p > 0.05$). Similarly, the average $\delta^{13}C$ and $\delta^{15}N$ values of lipid- and non-lipid-extracted beef were not significantly different from each other (t = -1.02 for $\delta^{13}C$ and -0.11 for $\delta^{15}N$; $p > 0.05$). Mean (± *SD*) $\delta^{13}C$ and $\delta^{15}N$ values of the non-lipid-extracted diet (chicken and beef combined) were -14.3 ‰ ± 1.08 and 6.8 ‰ ± 1.29, while those of the lipid-extracted diet were -13.9 ‰ ± 0.92 and 6.8 ‰ ± 1.06, respectively.

Table 2.3: $\delta^{13}C$ and $\delta^{15}N$ values (mean ± *SD*) of the experimental diet

Diet	Treatment	Sample size (*n*)	$\delta^{13}C$ ± *SD* (‰)	$\delta^{15}N$ ± *SD* (‰)
Giraffe	Non-lipid extracted	14	-24.6 ± 0.29	5.7 ± 0.25
Chicken	Non-lipid extracted	14	-17.5 ± 0.47	2.4 ± 0.70
	Lipid extracted	8	-17.5 ± 0.48	2.4 ± 0.77
Beef	Non-lipid extracted	14	-13.5 ± 1.23	7.9 ± 1.44
	Lipid extracted	8	-13.0 ± 1.02	7.9 ± 1.14

2.3.1.3 Identification of feeding bouts

Preliminary plots of individual whisker series were inspected for sharp transitions from ^{13}C enriched C_4-derived chicken and beef diet to ^{13}C-depleted, C_3-derived giraffe diet. Four peaks with low $\delta^{13}C$ values representing the four feeding bouts were to be identified from each whisker. However, only the last three feeding bouts could be clearly recognised (Figure 2.2). The first feeding bout could not be detected as there was too much noise and uncertainty at the thinner part of whiskers probably because of rapid whisker growth during that phase (circled section in Figure 2.2). As a result, the first feeding bout, conducted 34 days after the commencement of the experiment, was excluded in whisker growth rate calculations to avoid bias. Thus, reliable and comparable data (i.e. 72 mm of whiskers from root) covering the three identifiable feeding bouts were used for further calculations (Figure 2.3 A-E).

The positions of the three giraffe meat feeding bouts, represented by low $\delta^{13}C$ values, were consistent for all three lionesses. Although Diesel (leopard) is a different species, his assimilation rate of the giraffe meat was similar to that of the lionesses. However, Boesman's rate of giraffe meat assimilation appeared to be slower than the other felids (Figure 2.2). Despite the obvious departure from "standard" whisker delta-values, the $\delta^{13}C$ values of individual whisker segments where felids were fed the experimental ^{13}C-depleted, C_3-based giraffe meat were higher than that of mean giraffe meat (i.e. -24.6 ‰) (Figure 2.3

A-E). For example, the mean carbon isotope signatures of all five felids for the second and third feeding bouts were -14.5 and -14.4 ‰, respectively. The last feeding bout had slightly lower mean $\delta^{13}C$ values (-16.7 ‰) than the first two feeding bouts, but still higher than the mean giraffe meat value.

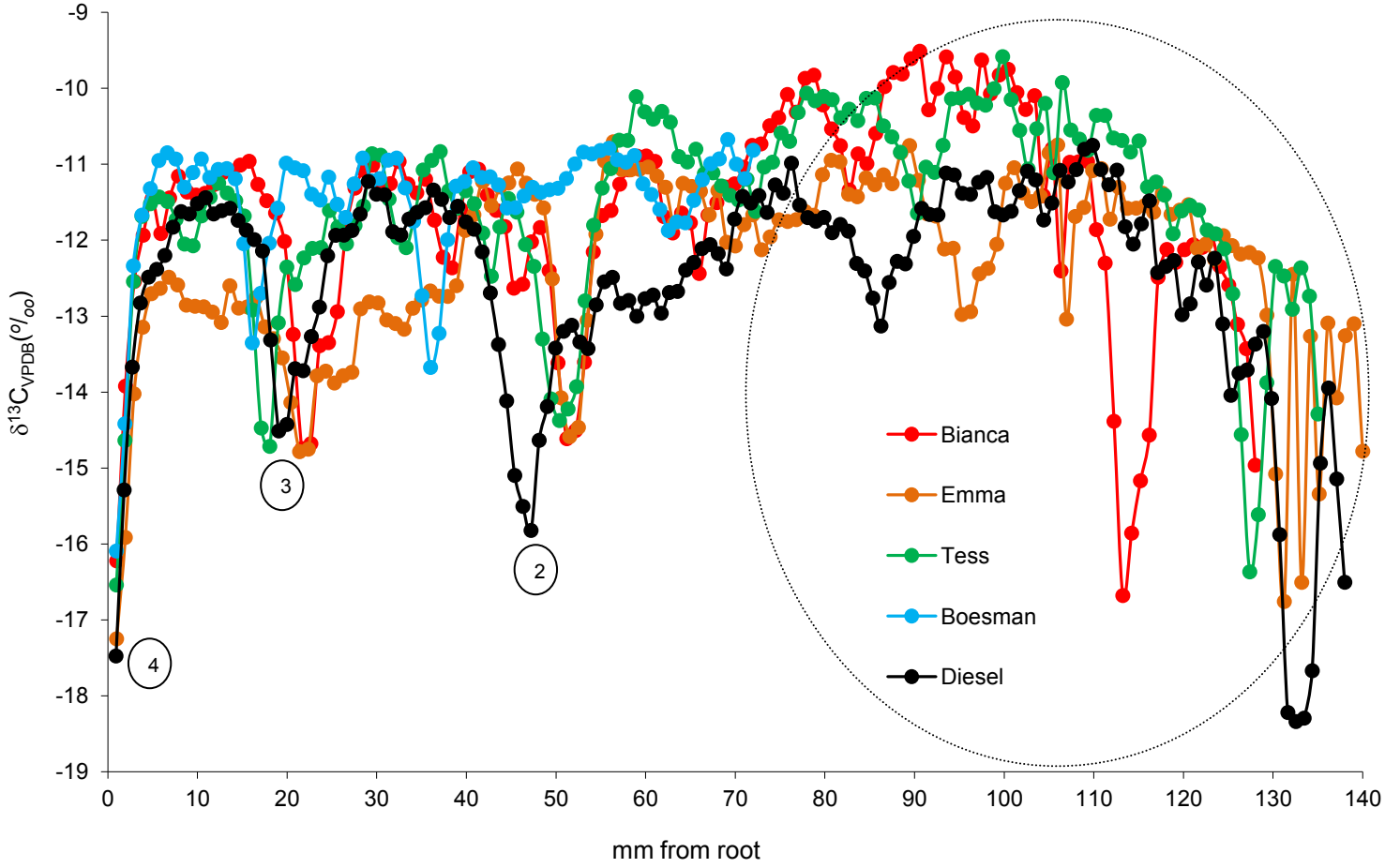

Figure 2.2: $\delta^{13}C$ values for whiskers of the study animals. The circled data includes the first feeding bout and were not used due to uncertainty. The numbered incisions of low $\delta^{13}C$ values represent the last three identifiable feeding bouts

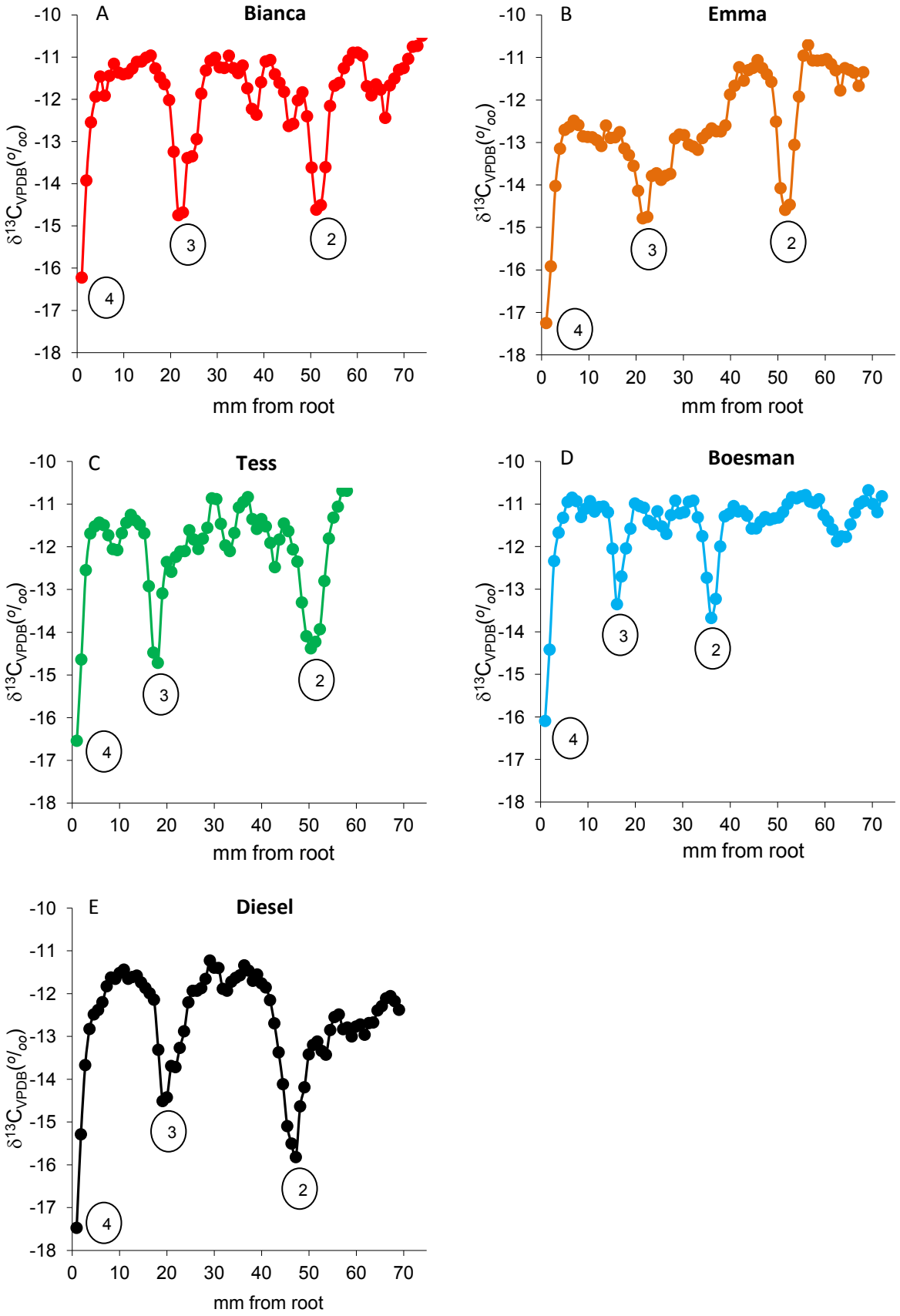

Figure 2.3 A-E: Whisker $\delta^{13}C$ values for the four lions (Bianca, Emma, Tess and Boesman) and leopard (Diesel). The three numbered phases with a sharp decrease of whisker $\delta^{13}C$ values represent the 2nd, 3rd and 4th feeding bouts where felids were fed the experimental C_3-derived giraffe meat

2.3.2 Whisker growth rates

The individual whisker ^{13}C series were used to estimate the growth rate (mm d^{-1}) because the exact dates of whisker removal at the beginning and end of the experiment were known, as well as the feeding dates. Boesman was not included in whisker growth rate calculations since the total length of the initially removed whisker and the one that was removed and analysed at the end of the study was not known. As a result, 288 (72 per individual) of the 644 samples provided usable data.

2.3.2.1 Whisker growth rates at different intervals

The Δlength/Δtime metric showed that whisker length increased with time (Figure 2.4 A & B), whereas growth slowed down (and differed between intervals) as the experiment progressed (Figure 2.5; Table 2.4). For all four felids, whisker growth was fastest the first 109 days of the experiment, although growth varied amongst individuals. During this period, Diesel's whisker grew faster than the other felids reaching a length of 91.70 mm, while Bianca had the slowest growing whisker measuring 77.78 mm. Whisker growth rates for this interval for the four felids ranged from 0.71 to 0.84 mm d^{-1}. From 109 to 151 days, individual whisker length grown ranged from 28.24 to 32.33 mm. Tess had a faster whisker growth (0.77 mm d^{-1}) than the other felids, while Diesel's whisker growth was the slowest (0.67 mm d^{-1}). Bianca and Emma had similar growth rates of 0.70 and 0.72 mm d^{-1}, respectively. The slowest whisker growth rates for all four felids were recorded the last 34 days of the experiment (from 151 to 185 days). During this period, Bianca and Emma had similar whisker growth rates (0.61 and 0.60 mm d^{-1}, respectively), while growth rates for Tess and Diesel were slower (0.50 and 0.53 mm d^{-1}, respectively). Individual whisker growth rates were similar for the total duration of the experiment (i.e. 185 days) as well as the period where detailed growth data was obtained (i.e. 76 days). Mean felid whisker growth rates were 0.73 ± 0.03 mm d^{-1} over 185 days and 0.65 ± 0.03 mm d^{-1} for the last 76 days of the experiment where the thicker section of whiskers was formed.

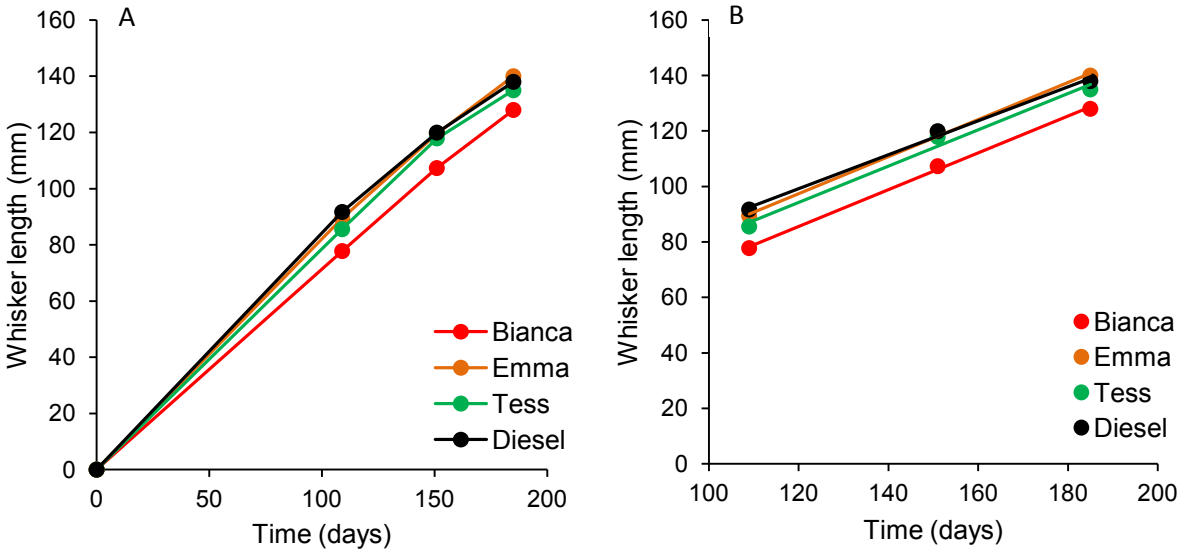

Figure 2.4 A & B: Whisker length grown over the entire duration of the experiment i.e. 185 days (A) and over the last 76 days of the experiment (B)

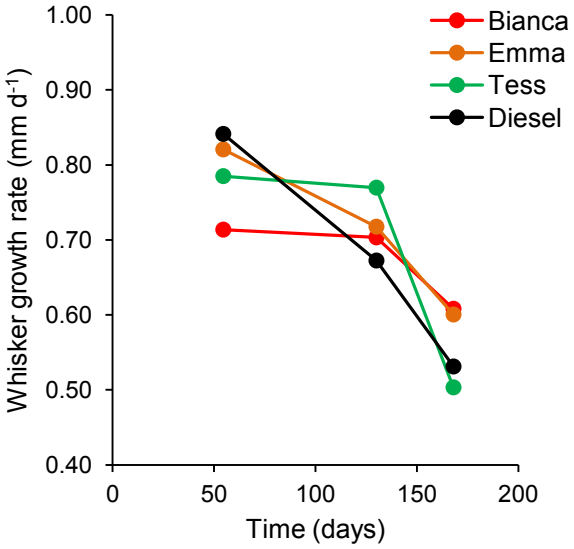

Figure 2.5: Calculated average whisker growth rates, plotted at the midpoint between giraffe meat feeding bouts, suggest non-linear growth pattern

Table 2.4: Whisker length grown between feeding bouts or intervals, interval growth rates and individual whisker growth rates for the four felids

Individual	Date	Activity	Days after initial removal	Day of expected C_3 feed peak	Days between giraffe meat feeding	Length from root (mm)	Length grown between C_3 feed peaks (mm)	Growth rate between C_3 feed peaks (mm d^{-1})	Individual growth rates over 185 days (mm d^{-1})	Individual growth rates for the last 76 days (mm d^{-1})
Bianca	3/06/2014	Whisker removal	0			0				
	16-19/09/2014	2nd feed	105-108	109	109	77.78	77.78	0.71		
	28-31/10/2014	3rd feed	147-150	151	42	107.32	29.54	0.70	0.69	0.66
	1-4/12/2014	4th feed	181-184	185	34	128.00	20.68	0.61		
Emma	3/06/2014	Whisker removal	0			0				
	16-19/09/2014	2nd feed	105-108	109	109	89.44	89.44	0.82		
	28-31/10/2014	3rd feed	147-150	151	42	119.58	30.14	0.72	0.76	0.67
	1-4/12/2014	4th feed	181-184	185	34	140.00	20.42	0.60		
Tess	3/06/2014	Whisker removal	0			0				
	16-19/09/2014	2nd feed	105-108	109	109	85.56	85.56	0.78		
	28-31/10/2014	3rd feed	147-150	151	42	117.89	32.33	0.77	0.73	0.65
	1-4/12/2014	4th feed	181-184	185	34	135.00	17.11	0.50		
Diesel	3/06/2014	Whisker removal	0			0				
	16-19/09/2014	2nd feed	105-108	109	109	91.70	91.70	0.84		
	28-31/10/2014	3rd feed	147-150	151	42	119.94	28.24	0.67	0.75	0.61
	1-4/12/2014	4th feed	181-184	185	34	138.00	18.06	0.53		
		Mean $\pm$ *SD*							0.73 $\pm$ 0.03	0.65 ± 0.03

2.3.2.2 Mixed effects linear models

Comparison of two models, one including empirical data only (last 76 days of the experiment, from 109 to 185 days), and another forcing the model through the origin (i.e. assuming zero growth at day zero, encompassing 185 days) differed in respective whisker growth rates (mean ± *SD* = 0.65 ± 0.01 and 0.74 ± 0.03 mm d^{-1}, respectively). Thus, whiskers grew faster at the beginning of the experiment, but growth slowed down as the experiment progressed. This result was consistent with a decline in interval growth rates through time (Table 2.4; Figure 2.5).

Whisker growth was broadly similar across individuals (range = 0.70 to 0.76 mm d^{-1} for 185 days, and 0.64 to 0.66 mm d^{-1} for the last 76 days of the experiment), despite the presence of two species and two sexes in the data (Table 2.5). However, some differences were evident, and the lack of significant differences between individuals (overlapping 95 % confidence intervals of growth rate estimates) may be due to low statistical power associated with the small sample size incorporated. Changes in growth rates through time may also have obscured individual-level effects. For instance, Emma and Diesel exhibited the highest whisker growth rates over 185 days (0.76 mm d^{-1}), but were the slowest-growing individuals over the last 76 days (0.60 mm d^{-1}). Bianca had the slowest growing whisker for the duration of the experiment (0.70 mm d^{-1}), however, in the last 76 days, her whisker grew faster than the other felids (0.66 mm d^{-1}).

Table 2.5: Individual whisker growth rates (mm d^{-1}) and confidence intervals (CI) for whisker length grown over 185 days and the last 76 days of the experiment

	185 days			76 days		
Felid	**Growth rate**	**-95 % CI**	**+95 % CI**	**Growth rate**	**-95 % CI**	**+95 % CI**
Bianca	0.70	0.66	0.74	0.66	0.62	0.70
Emma	0.76	0.72	0.80	0.64	0.60	0.68
Tess	0.74	0.70	0.78	0.65	0.61	0.69
Diesel	0.76	0.72	0.80	0.64	0.60	0.68
Mean ± *SD*		0.74 ± 0.03			0.65 ± 0.01	

2.3.3 Whisker growth patterns

The interval growth rates and the MELMs revealed that whisker growth decreased with time, suggesting a non-linear growth pattern (Figure 2.5). However, sample sizes of known growth intervals were too small for non-linear model fitting (n = 3 per individual). Thus, simulations

were used to compare effect of linear versus non-linear (von Bertalanffy) growth. Outcomes of these simulations indicate that the two types of growth patterns would appear similar for the proximal ~50 mm or last 75 days of growth (Figure 2.6 A). Until that point, as one sections more towards the whisker tip, the two types of growth trajectories would differ substantially. Therefore, reconstructing growth of free-ranging lion and leopard whiskers could be based on assumptions of linear growth with a reasonably high degree of confidence for the thicker proximal 50–60 mm. Most such reconstructions would, however, be aimed at inferring a represented time interval. As a result, more useful comparisons can be made by plotting time as a function of whisker length (Figure 2.6 B). Simulated time intervals for 105 days (the number of days for which 72 mm – measured from root – of reliable growth rate data could be obtained from whiskers of this study) did not differ significantly between the two hypothetical series ($t_{(34)} = -1.5$; $n = 36$; mean (± *SD*) = -7.72 ± 30.83; $p > 0.05$). Visually, however, it appears that slower growth under non-linear conditions may complicate timeframe reconstructions at whisker lengths that are above 60 mm as measured from the root (Figure 2.6 B).

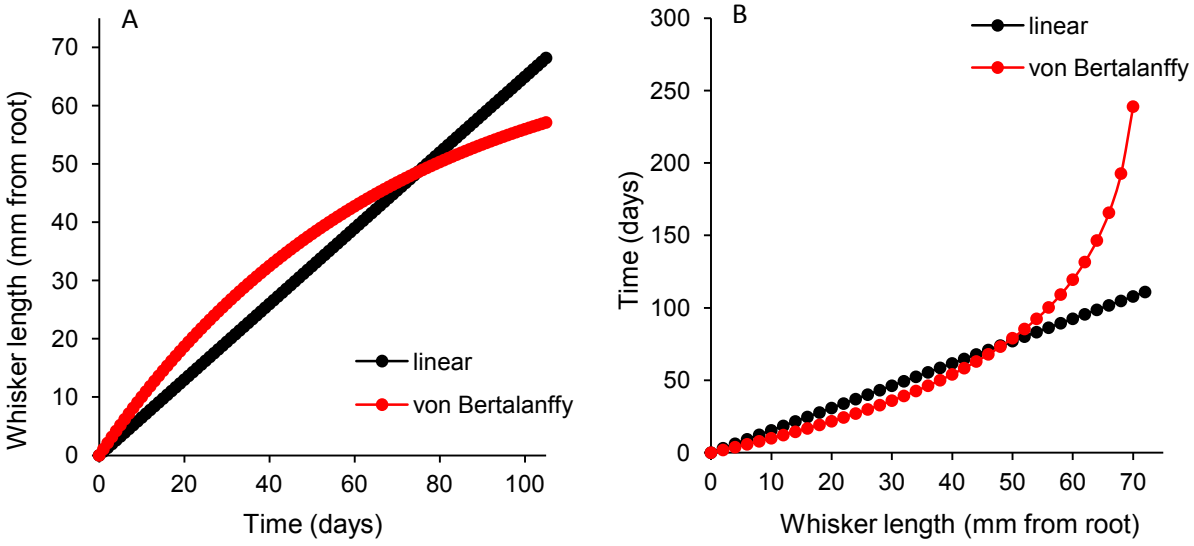

Figure 2.6 A & B: The differences in how the simulated linear and von Bertalanffy growth curves estimates whisker length grown over 105 days (A) and amount of time taken to reach a whisker length of 72 mm (B)

2.3.4 Lion whisker-diet trophic discrimination factors

Although the isotopic signatures of lipid- and non-lipid-extracted chicken and beef could not be distinguished (see section 2.3.1.2, Table 2.3), $\delta^{13}C$ and $\delta^{15}N$ values of lipid-extracted meat were used to calculate ε^*_{WD} (equation 6) for the lion in this study.

$\delta^{13}C$ and $\delta^{15}N$ values of whisker segments of the five lion whiskers (one removed for the pilot study in 2013 (Amber) and others removed at the beginning of the experiment in 2014) ranged from -10.6 to -12.7 ‰ and from 8.8 to 10.1 ‰, respectively (Figure 2.7 A & B; Appendix C). Whisker-diet TDFs (i.e. ε^*_{WD}) of these segments ranged from 1.2 to 3.4 ‰ for $\delta^{13}C$, and from 1.9 to 3.3 ‰ for $\delta^{15}N$ (Figure 2.7 C & D). Mean ε^*_{WD} values were similar amongst individuals, ranging from 2.5 to 2.9 ‰ for $\delta^{13}C$ and from 2.4 to 2.8 ‰ for $\delta^{15}N$. The bootstrapped means (± *SD*) across all individuals were 2.7 ± 0.12 ‰ for $\delta^{13}C$ and 2.5 ± 0.08 ‰ for $\delta^{15}N$ (Table 2.6).

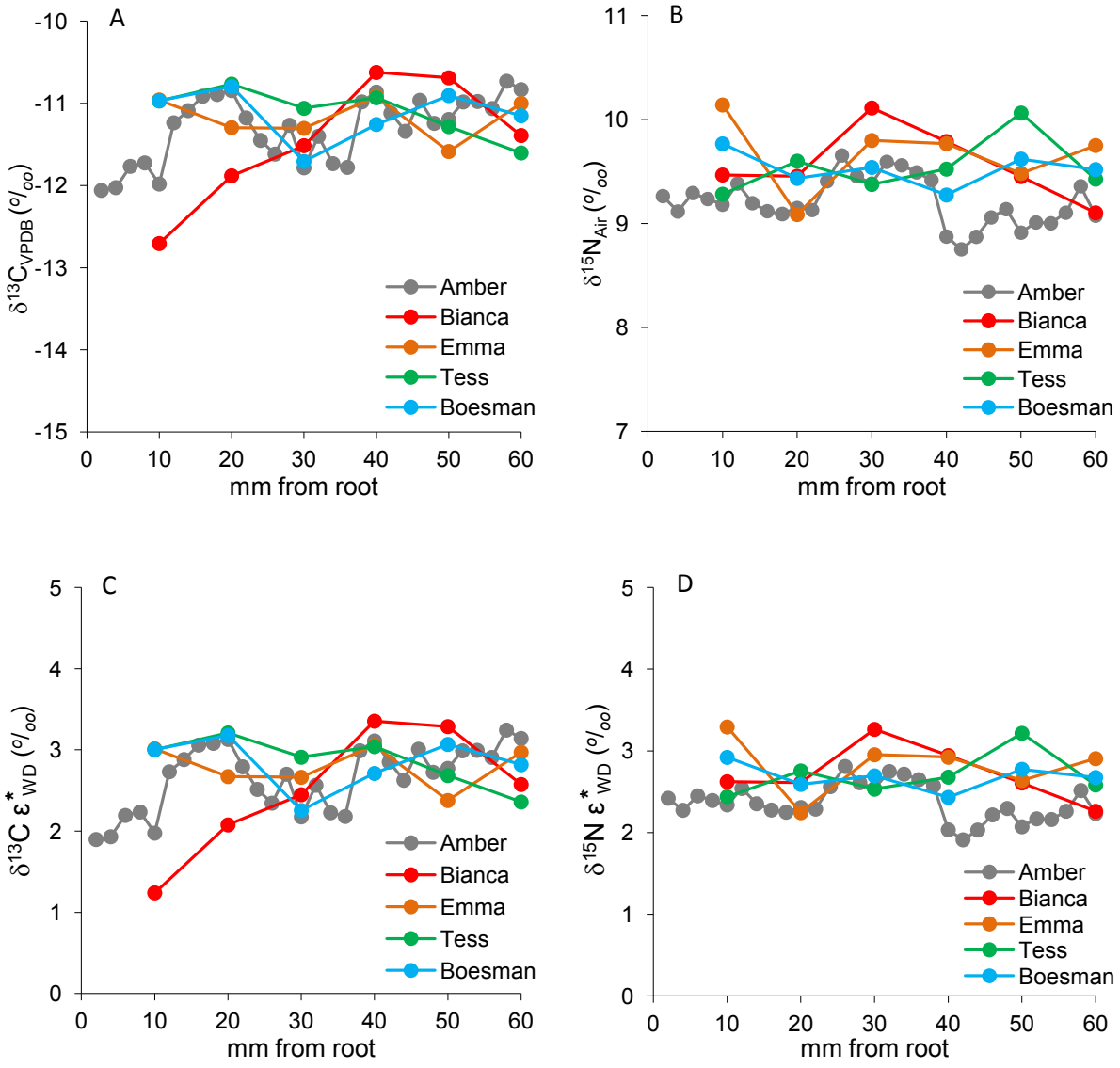

Figure 2.7 A-D: $\delta^{13}C$ and $\delta^{15}N$ values of the analysed whisker segments (A & B) used to calculate ε^*_{WD} (C & D) for the five lions

Table 2.6: Individual and bootsrapped mean $\delta^{13}C$ and $\delta^{15}N$ ε^{*}_{WD} values for the five lions

Individual	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Mean	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**
Amber	2.7	3.1	2.2	2.4	2.9	1.8
Bianca	2.5	3.0	2.0	2.7	3.3	2.2
Emma	2.8	3.3	2.3	2.8	3.4	2.3
Tess	2.9	3.3	2.4	2.7	3.2	2.2
Boesman	2.8	3.3	2.4	2.7	3.2	2.1
Bootstrapped Mean ± *SD*			2.7 ± 0.12			2.5 ± 0.08

2.4 Discussion

2.4.1 Whisker growth rates

The two analytical approaches used to estimate whisker growth rates i.e. Δlength/Δtime metric and MELMs showed similar results. Within individual growth rates revealed that whisker growth was faster towards the whisker tip (first 109 days of the experiment) and slower towards the root or base (last 76 days) (Table 2.4). This was probably because the thin whisker tip has smaller nuclear bodies of hair cells; therefore, little assimilated material is deposited towards the tip taking fewer days to grow than the thick basal section or whisker root (Teerink 2003). Whisker growth rates have been reported to vary with species, age, sex, reproductive status and food deprivation (Ibrahim & Wright 1975; Hirons *et al.* 2001). In this study, whisker growth rates of the adult male leopard (Diesel) and sub-adult female lions (Bianca, Emma and Tess) were similar. Moreover, the felids did not undergo nutritional stress during the experiment as they were maintained daily on diets that met their caloric requirements by the Zoo staff (M. Meyer-Bouwer pers. comm. 2014). During the experiment, Emma fell pregnant twice; her first litter was born on the 23rd of May and the second on the 8th of October 2014. Consequently, her whisker growth was expected to be slower than the other felids because nutrients would have been devoted to foetus growth (Fuller *et al.* 2004). However, this was not the case as her whisker growth was similar to individuals that were not mated. Thus, the aforementioned factors did not influence the measured felid whisker growth rates.

Results of this study concur with those of Ibrahim & Wright (1975) who found no significant differences between the growth rates of male and female rats and mice. On the other hand, they differ from findings of Kernaleguen *et al*. (2012) who showed that male fur seals have longer whiskers and therefore exhibit higher whisker growth rates than females. Other

studies reported different whisker growth amongst species and age groups. For instance, Hirons *et al*. (2001) showed that Steller sea lions and habor seals *Phoca vitulina* had different whisker growth characteristics. Their study, and that of Rea *et al.* (2015), also revealed that juvenile Steller sea lion whisker growth rates were twice those of adults.

The mean felid whisker growth rates of 0.74 mm d^{-1} (estimated from whisker lengths grown over 185 days) and 0.65 mm d^{-1} (estimated from whisker lengths grown over 76 days) were within the range of values for laboratory mice (0.3–1.0 mm d^{-1}), laboratory rats (0.6–1.5 mm d^{-1}), harbor seals (0.075–0.78 mm d^{-1}) and Steller sea lions (0.44–0.87 mm d^{-1}) (Ibrahim & Wright 1975; Zhao & Schell 2004; Rea *et al.* 2015). Both whisker growth rates were much higher than values for most pinniped species. For instance, whiskers of Steller sea lions were reported to grow at 0.05–0.07 mm d^{-1}, leopard seals *Hydrurga leptonyx* 0.08–0.1 mm d^{-1}, Antarctic fur seals *Arctocephalus gazella* 0.13 mm d^{-1} and grey seals *Halichoerus grypus* 0.24 mm d^{-1} (Hirons *et al.* 2001; Hall-Aspland *et al.* 2005; Cherel *et al.* 2009; Greaves *et al*. 2004). Felid whisker growth rates of this study were also higher than the measured whisker growth rates of sea otters and Eurasian badgers, i.e. 0.21 mm d^{-1} and 0.43 mm d^{-1}, respectively (Tyrrell *et al.* 2013; Robertson *et al.* 2013). However, the mean felid whisker grown rate of 0.65 mm d^{-1} was similar to that of stoats (0.60 mm d^{-1}, Spurr 2002).

The estimation of whisker growth rates in this study was however restricted by a number of factors. Typical of whisker growth studies, the sample available for the experiment was small and unbalanced (i.e. three lionesses, one male lion and one male leopard). The unavailability of Boesman's full whisker growth trajectory further reduced the sample size and made it impossible to ascertain the whisker growth rate of male lions. The scenario of finding broken whiskers during collection seem to be realistic and should be expected since Newland *et al*. (2011) also encountered a similar setback. Although the position of Boesman's second and third giraffe meat feeding bouts along the unbroken part of the whisker suggest a slower assimilation rate (and possibly whisker growth rate), compared with the smaller-bodied lionesses and the leopard (Figure 2.2), such a hypothesis could not be tested in this study due to the small sample size. Therefore, further experimentation on a larger sample is needed. The sample size of this study may also have limited the accurate evaluation of the influence of species, sex, age and reproduction on the measured whisker growth rates.

The second limitation was the indiscernibility of the first feeding bout contained towards the whisker tips (Figure 2.2). The lowest whisker tip segment mass was 0.003 mg, and from the results, it is evident that the samples were too small for reliable simultaneous analysis of ^{13}C and ^{15}N. As a result, individual whisker growth rates for the first growth period, i.e. the first 33

days of the experiment, could not be calculated. Stricker *et al.* (2015) experienced a similar challenge of increased uncertainties towards the whisker tips of both pup and adult seals. However, such error had a smaller effect on adult whiskers as the lengths necessary to achieve mass requirements and acquire comparable information were smaller. Studies using SIA of whiskers should therefore consider sectioning whiskers by weight rather than length in order to obtain reliable and comparable data. Thirdly, the fourth feeding bout revealed that whiskers were still in anagen (growing) phase when they were removed at the end of the experiment. This implies that the mean felid whisker growth rate presented in this study was calculated using incomplete growth trajectories of individuals as whiskers had not reached their asymptotic length, potentially affecting the measured growth rates. However, the lengths of the sampled felid whiskers ranged from 128 to 140 mm, which is close to the maximum length (i.e. 150 mm) encountered both in the field and at the Zoo to date. It is interesting that the $\delta^{13}C$ values for the fourth feeding bout were much lower than those for whisker segments of the other feeding bouts. The higher lipid content found in the root material, than in keratin, might have influenced the low carbon signatures of the root whisker segments since lipids are depleted in ^{13}C relative to proteins (DeNiro & Epstein 1977; West *et al.* 2004).

2.4.2 Whisker growth pattern

Whisker growth simulations suggest that growth pattern is negligible for a whisker length of ~50 mm (measured from the root) or the last 75 days of growth, assuming growth rates are similar to those found in this study, as both linear and non-linear von Bertalanffy growth simulations estimated time or whisker length indistinguishably (Figure 2.6 A & B). However, beyond this length (towards the whisker tip), growth pattern may have substantial effects on interpreting timeframes because either whisker growth starts to slow down if whiskers follow a von Bertalanffy growth pattern, or remains constant if growth is linear. These results imply that studies using SIA of whiskers to infer felid feeding ecology should not automatically assume that whisker growth is constant, but should consider the whisker length measured. Although whisker growth observed in this study suggested a non-linear growth pattern (Figure 2.5), further confirmation of this result was limited by the sample size that was too small ($n = 3$) for non-linear (von Bertalanffy) model fitting. Therefore, future investigations on whisker growth should endogenously mark whiskers for a longer period to acquire more data that provides all the parameters required for non-linear model fitting. Such might provide more detail on the growth pattern followed by felid whiskers.

Studies on rats, mice, Steller sea lions and sea otters found that whiskers of these species followed a linear growth pattern (Ibrahim & Wright 1975; Hirons *et al.* 2001; Tyrrell *et al.*

2013). This differs from experiments conducted on grey seals, leopard seals and Eurasian badgers that reported non-linear whisker growth in these species (Greaves *et al*. 2004; Hall-Aspland *et al.* 2005; Robertson *et al.* 2013). All these studies measured growth patterns from whiskers that had a mean length of > 70 mm, except for Eurasian badgers (mean = 45 mm).

2.4.3 Lion whisker-diet trophic discrimination factors

The lion $\delta^{13}C$ ε^*_{WD} values estimated in this study (2.5–2.9 ‰, Table 2.6) falls within the range (2.7–3.5 ‰) of previously reported TDFs for herbivore hair (e.g. Tieszen *et al.* 1983; Sponheimer *et al.* 2003), and were similar to hair-diet values for some terrestrial carnivores, i.e. the red fox *Vulpes vulpes* (2.6 ‰) (Roth & Hobson 2000) and Canada lynx *Lynx canadensis* (2.4 ‰) (Parng *et al.* 2014). The values also fall within the 2.2–3.9 ‰ range of whisker-diet TDFs reported for marine mammalian carnivore species including pinnipeds and sea otters (Hobson *et al.* 1996; Newsome *et al.* 2010b; Tyrrell *et al.* 2013; Stricker *et al.* 2015; Beltran *et al.* 2016). However, reported hair-diet $\delta^{13}C$ TDFs for some terrestrial felids were either much lower or higher than the mean lion value (2.7 ± 0.12 ‰) of this study. For example, hair-diet $\delta^{13}C$ differences for the African lion were 1.1 ± 0.2 ‰, mountain lion *Puma concolor* 4.7 ± 0.6 ‰, bobcat *Lynx rufus* 5.5 ± 0.5 ‰, (Parng *et al.* 2014), snow leopard *Uncia uncia* 6.0 ± 1.25 ‰ and tiger *Panthera tigris* 6.5 ± 0.54 ‰ (Montanari & Amato 2015). The estimated mean lion $\delta^{13}C$ ε^*_{WD} value was also higher than TDFs for commonly analysed mammalian tissues, i.e. muscle, liver and blood, in isotope ecology (e.g. Tieszen *et al.* 1983; Roth & Hobson 2000; Beltran *et al.* 2016). This was possibly because whiskers (and hair) are constructed from α-keratin, which is synthesised from non-essential amino acids glycine and serine, and these are naturally enriched in ^{13}C for some animals (Marshall *et al.* 1991; Hare *et al.* 1991).

The observed mean lion $\delta^{15}N$ ε^*_{WD} value of 2.5 ± 0.08 ‰ was similar to the ^{15}N trophic level difference obtained by Vanderklift & Ponsard (2003) of 2.5 ± 0.11 ‰. Lion $\delta^{15}N$ ε^*_{WD} values (2.4–2.8 ‰, Table 2.6) were also similar to whisker-diet TDFs for some pinnipeds (2.6–3.0 ‰, Hobson *et al.* 1996; Beltran *et al.* 2016), but lower than whisker $\delta^{15}N$ values of sea otters (5.5 ‰; Tyrrell *et al.* 2013) and hair values for some felid species, i.e. bobcats, mountain lions and the African lion (3.5–4.5 ‰, Parng *et al.* 2014). Furthermore, lion $\delta^{15}N$ ε^*_{WD} were lower than reported stepwise ^{15}N enrichment values of 3–4 ‰ across trophic levels in food webs (DeNiro & Epstein 1981; Minagawa & Wada 1984; Post 2002; Robbins *et al.* 2005). The values obtained in this study were however higher than $\delta^{15}N$ hair-diet TDFs reported for the tiger and snow leopard, i.e. -0.3 and 0.3 ‰, respectively (Montanari & Amato 2015). Although $\delta^{15}N$ TDFs can be affected by nutritional stress, dietary quality and quantity (Hobson *et al.* 1993; Vanderklift & Ponsard 2003; Robbins *et al.* 2005), these factors did not

influence the observed lion $\delta^{15}N$ TDFs as animals were well maintained by Pretoria Zoo staff and fed diets that met their caloric requirements.

TDFs were reported to vary between individuals of the same species due to differences in diet, age and sex (Martinez del Rio & Carleton 2012; Robbins *et al.* 2010). In this study, the sampled felids consumed the same diet in equal proportions, therefore, diet switching or alteration did not affect the obtained whisker-diet TDFs. Despite age and sex differences of the individual lions (i.e. the four female lions were sub-adults and the male was an adult, Table 2.1), $\delta^{13}C$ (2.5–2.9 ‰) and $\delta^{15}N$ (2.4–2.8 ‰) whisker-diet TDFs were similar amongst individual lions. Thus, these two factors seem not to have affected the accurate estimation of lion whisker-diet TDFs presented in this study. Other studies found corresponding results; for instance, Parng *et al*. (2014) showed similarities between fur-diet TDFs of male and female mountain lions (i.e. $\delta^{13}C$ and $\delta^{15}N$ TDFs for the female were 4.3 and 4.4 ‰, and those of the male were 5.1 and 4.6 ‰, respectively). Beltran *et al*. (2016) reported no significant variation between $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of two ringed seals *Pusa hispida* from different age and sex classes. However, in all cases, definitive deductions were limited by the small and unbalanced sample.

One of the important, yet controversial, stages of isotopic discrimination experiments is sample preparation, as there are different schools of thought on how samples should be prepared for analysis. The issue of lipid-extracting dietary sources has been addressed differently among ecologists over the years. Experiments on the estimation of tissue-diet TDFs are advised to first assess the lipid content of prey sources as automatic removal of lipids might result in unreliable estimates if animals consume lipid-rich diets (Newsome *et al.* 2010b; Wolf *et al.* 2015). In this study, the lipid content of the chicken and beef was not qualitatively and quantitatively assessed. Nevertheless, lipids were removed because they are usually depleted in ^{13}C relative to the diet, resulting in more negative $\delta^{13}C$ values (Tieszen *et al.* 1983; Post *et al.* 2007). Another major concern is the use of chloroform/methanol solution to extract lipids as the chemical can cause ^{15}N fractionation of 0.3-2.5 ‰ (Post *et al.* 2007; Montanari & Amato 2015). The similarities of the isotopic composition of lipid and non-lipid-extracted diets in the present study disqualify the possibility that whisker-diet TDFs obtained might be inaccurate due to treatment procedures. Indistinguishable isotopic signatures between lipid and non-lipid-extracted materials have also been reported by Caut *et al.* (2009) and Montanari & Amato (2015).

Studies on isotopic tissue-diet differences are often limited by sample size as zoological institutions where most experiments are carried out hold a small number of species and individuals, especially large carnivores. In this study, the available small and unbalanced

sample, i.e. five lions (four females and one male) with 30 data points from one individual (Amber) and six from the others, was used to estimate lion ε^*_{WD}. Another limitation was that the Zoo diet comprised two types of meat (chicken and beef) which were not isotopically homogeneous, although isotope compositions of both meat types (as well as the proportions of each meat eaten by experimental individuals) were consistent over time. It is possible that some individuals assimilated more of the chicken than beef or vice versa, which could influence the obtained values (Ambrose & Norr 1993). Furthermore, the feed was not only composed of muscle, but other tissues such as bone, skin, feathers and adipose tissue, which were also consumed by the lions. These have varying isotopic signatures that all contribute to the observed whisker isotope signatures (Tieszen *et al.* 1983; Dalerum & Angerbjorn 2005). However, the vast majority of the diet comprised muscle.

2.5 Conclusion

Whiskers are increasingly used in stable isotope-based dietary studies of free-ranging animals as the tissue provides an archive of dietary patterns recorded during the period of growth. This study contributed to resolving questions about the major limiting factors related to use of whiskers, i.e. whisker growth rates, whisker growth patterns and whisker-diet TDFs. The study showed that whisker growth rates and whisker-diet TDFs of large-bodied felids fall within the range of values previously reported for several marine and terrestrial mammals. However, $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs differed from hair values of some felid species. Age, sex and diet did not seem to influence $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs, and whisker growth differed only minimally between individuals. Further investigations need to be carried out on male lions using full growth trajectories for an understanding of their whisker growth and effects thereof. In addition, a larger and balanced sample is needed for more knowledge on life history influences on felid whisker growth rate. Results of this study also highlighted that whisker growth can be assumed to be linear for the proximal ~50-60 mm of whiskers, but the non-linear growth in whisker sections longer than 60 mm (measured from the root) may have substantial effects on interpreting timeframes.

The study did not directly measure the duration of whisker growth cycle, however, conclusions that it is longer than six months could be made as the whiskers were still growing. Thus, more research on the period of felid whisker growth cycle and shedding is needed. Long-term studies on turnover rates should be conducted to track the change in whisker-diet TDFs, along with any potential fluctuations in stable isotope ratios of the diet. It is important to study a variety of felid species and individuals in the future to explore the causes of such wide variation in felid hair TDFs as this might provide more insights into isotope discrimination. Despite the outlined limitations of this study, the average felid whisker

growth rate of 0.65 mm d^{-1} calculated for the proximal 72 mm of whiskers that are longer than 100 mm can be used in future studies with confidence. Moreover, the mean lion $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of 2.7 and 2.5 ‰, respectively, are probably the best appraisal for the species since they were estimated using more individuals ($n = 5$) whose chicken and beef diet was consistent over multiple years, compared to a previous study by Parng *et al.* (2014) where only one lion sustained on a premixed commercial diet consisting of various animal and plant sources, and supplements was used to measure hair-diet TDFs. The whisker growth rate and whisker-diet TDFs obtained in this study will be used in Chapter 3 to quantify the diets of wild individual leopards of a northern KZN population using stable isotope profiles of their whiskers.

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CHAPTER 3

THE USE OF STABLE ISOTOPE ANALYSIS OF WHISKERS TO DISCERN DIETS OF INDIVIDUAL FREE-RANGING LEOPARDS

3.1 Introduction

The African leopard *Panthera pardus pardus* is listed as 'Vulnerable' on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Stein *et al.* 2016). The species has vanished from approximately 48–67 % of its historic range, and populations continue to decline rapidly (Jacobson *et al.* 2016). The factors leading to the decline of leopards are similar to those affecting large carnivore species worldwide, such as habitat loss and fragmentation, depletion of natural prey and persecution (Henschel *et al.* 2011; Ripple *et al.* 2014). Leopards, like many other mammalian carnivores, have broad diets, feeding on a wide range of prey species that may differ across space and time (Hayward *et al.* 2006; Stein & Hayssen 2013). Knowledge of their feeding habits provides a foundation for understanding their potential impacts on preferred prey species and competition with other carnivores (Breuer 2005; Owen-Smith & Mills 2008). Such information is essential for the development of site- and species-specific conservation management plans (Breuer 2005).

Leopards are elusive, solitary predators with the widest geographic distribution of the wild cats (Myers 1986; Nowell & Jackson 1996), and in Africa, they are the most abundant large felid (Hunter *et al*. 2013). They occupy a broad spectrum of habitats; bushveld, woodlands, tropical rainforest, desert, remote mountain ranges and urban settlements (Nowell & Jackson 1996). Such success is mainly due to their highly adaptable hunting and feeding behaviour (Bertram 1999). Leopards are known to take prey of varying size, from arthropods (Fey 1964) to adult male eland *Tragelaphus oryx* (Kingdon 1977; de Ruiter & Berger 2001). The size of available prey species consumed by leopards in southern Africa differs greatly across habitats. For example, populations that persist in savanna environments usually feed on prey weighing 20-80 kg such as impala *Aepyceros melampus* and bushbuck *Tragelaphus sylvaticus* (Bailey 1993; Hayward *et al.* 2006; Balme *et al.* 2007). In forested habitats, smaller prey weighing 7–30 kg such as duikers (Bovidae: Cephalophini) are taken (Ray & Sunquist 2001). Leopards inhabiting mountainous areas, for instance in the Western Cape in South Africa, feed predominantly on small prey weighing 3–13 kg such as klipspringer *Oreotragus oreotragus* and rock hyrax *Procavia capensis* (Martins *et al*. 2011). Despite such variation, a meta-analysis of 33 studies on leopard feeding ecology from across their range revealed that leopards preferentially prey upon ungulate species within a weight range of 10-

40 kg, even if prey outside this weight range is more abundant (Hayward *et al.* 2006). In addition, African leopards occasionally appear to have individualised food preferences. For example, Fey (1964) recorded an individual that specialised on bush pigs *Potamochoerus larvatus*, despite the abundance of other prey species, and another that fed primarily on fish *Tilapia* species (spp.) in Lake Kariba, even though impala and grey duiker *Sylvicapra grimmia* were available. Bothma & le Richie (1984) recorded a leopard that preferred porcupines *Hystrix africaeaustralis*, regardless of the availability of ungulate prey in the Kalahari Gemsbok National Park.

In southern Africa (and indeed elsewhere), the feeding behaviour of leopards has been largely explored using traditional methods such as faecal analysis (e.g. Norton *et al.* 1986; Braczkowski *et al*. 2012; Pitman *et al.* 2014), direct observations (e.g. le Roux & Skinner 1989; de Ruiter & Berger 2001; Balme *et al.* 2007), global positioning cluster (GPS) analysis (e.g. Balme *et al.* 2007; Martins *et al.* 2011; Frohlich *et al.* 2012; Pitman *et al*. 2012; Pitman *et al*. 2014) and spoor tracking (e.g. Stander *et al.* 1997; Bothma & le Richie 1984; Mills 1984; Bothma & Coertze 2004). Traditional methods have contributed to our understanding of the feeding ecology of African leopards in their natural habitats. However, the long periods of time, high financial inputs and methodological hindrances inherent in these techniques makes it difficult to reliably capture the dietary diversity of cryptic, elusive species such as leopards (Newsome *et al.* 2009). Stable isotope analysis (SIA) of carnivore tissues provides another approach that can identify important feeding habits of large carnivores, and potentially overcome some of the limitations associated with traditional dietary analyses (Bearhop *et al.* 2004). The method can provide insights into temporal foraging habits of animals that can either be a result of seasonality or stochastic events (Ben-David *et al.* 1997; Roth 2002). It is also useful in revealing variation in resource utilisation among individuals of a population (Urton & Hobson 2005; Robertson *et al.* 2014), and this kind of information is very difficult to obtain using traditional methods alone, unless samples are collected over extended periods of time (Bearhop *et al.* 2004; Inger & Bearhop 2008).

The use of stable carbon and nitrogen isotopes in foraging studies is based on observations that the isotopic signatures of consumer tissues largely reflect the dietary items and habitats utilised over the period of tissue formation, with some further discrimination (Peterson & Fry 1987; Hobson & Schell 1998). In subtropical savannas, there is a bimodal distribution of carbon isotope ratios between C_3 (trees, shrubs and forbs) and C_4 (grass) photosynthesizing plants (Marshall *et al.* 2007). Hence, $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) is used to indicate the likely source of primary production (either C_3 or C_4 plants) because of little variation in $^{13}C/^{12}C$ ratios as carbon moves through food webs (Vogel 1978; DeNiro & Epstein 1978;

Cerling & Harris 1999). The isotopic distinction displayed by plants in these ecosystems creates a natural marker system that percolates up trophic levels through predation, and thus can be used to trace the diets of both herbivores and carnivores (Ben-David & Flaherty 2012). $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) is generally used to determine the trophic position of a consumer due to the predictable increase of nitrogen-15 abundances with 3–5 ‰ at each trophic step (DeNiro & Epstein 1981; Hobson & Clark 1992; Bearhop *et al.* 2002). Coupled $\delta^{13}C$ and $\delta^{15}N$ can, therefore, provide information on food web structure and proportional contributions of different dietary sources to consumers (Kelly 2000).

The analysis of different animal tissues (and excreta) such as blood, faeces, muscle and bone allows researchers to document dietary information integrated at different intervals of an individual's life i.e. from days to years (Tieszen *et al.* 1983; Hobson & Clark 1992; Vanderklift & Ponsard 2003; Dalerum & Angerbjorn 2005). In contrast, metabolically inert tissues such as teeth, claws, hair and whiskers record dietary information along the growth axis and increments do not turnover once accreted, providing a time-specific record of nutrient intake (Bearhop *et al.* 2003; Mizukami *et al*. 2005; Codron *et al.* 2012; 2013; Robertson *et al.* 2013). Whiskers, in particular, have been advocated as a useful tool in isotope studies (Newsome *et al.* 2010a; Robertson *et al.* 2014).

SIA of whiskers has been used successfully to explore the prevalence of seasonal dietary patterns (Hall-Aspland *et al.* 2005; Newland *et al.* 2011), individual diet specialisation (Newsome *et al.* 2009) and changes in habitat utilisation (Cherel *et al.* 2009) in marine carnivore species. The method has also been used to assess the foraging ecology of terrestrial carnivores, but only on a few species. For instance, it has revealed the dependence of foxes *Vulpes macrotis mutica* and coyotes *Canis latrans* on anthropogenic food sources (Newsome *et al.* 2010a; 2015), behavioural responses of the American mink *Neovison vison* to anthropogenic ecosystem disturbances (Bodey *et al.* 2010) and variation of resource use in a Eurasian badger *Meles meles* population (Robertson *et al.* 2014).

In this study, the feasibility of using SIA of whiskers to discern leopard diet was explored by analysing the stable carbon and nitrogen isotope ratios of whiskers obtained from six leopards in northern KwaZulu-Natal (KZN). The study objective was to determine the level of detail that SIA could provide with regards to prey type(s) consumed and dietary differences amongst individuals (if any). Using the felid whisker growth rate and whisker-diet trophic discrimination factors (TDFs) obtained in Chapter 2 of this thesis, and isotopic turnover parameters from Ayliffe *et al.* (2004), the objectives of the study were to (i) quantify the diets of individual leopards using a 60 mm series of their whiskers and compare results with those obtained from traditional methods, and (ii) evaluate the effect of isotopic baseline, prey

sources, isotopic turnover and tissue-diet discrimination on the interpretation of leopard diet. SIA of whiskers has not been applied to wild felids, and use of this technique on northern KZN leopards whose feeding ecology has been intensively studied through traditional approaches (Balme *et al.* 2007) might provide previously unrecognised dietary knowledge. Insights of leopards' foraging habits are important for their conservation and management, especially in an era where most large felid species are declining (Ripple *et al.* 2014; Jacobson *et al.* 2016).

3.2 Materials and Methods

3.2.1 Study area

The study took place in the 220 km^2 Phinda Private Game Reserve (hereafter Phinda) located in northern KZN, South Africa, at latitude 27˚44' to 27˚55' south and longitude 31˚12' to 32˚26' east (Figure 3.1). The reserve is situated in the southern Maputaland coastal plain region, which runs from the Umfolozi River north into Mozambique. The region is bounded by the Indian Ocean in the east and the Lebombo mountains in the west (Hunter 1998; Balme 2009). More than 95 % of the reserve lies beneath 100 m above sea level with the lowest altitude of 4 m and highest of 201 m above sea level where a part of the Lebombo mountains run through the reserve (Hunter 1998). The climate of the region is warm to hot, humid sub-tropical, with two distinct seasons; a warm, dry winter from April to September and a hot, humid, rainy summer from October to March (Schulze 1965). The average annual rainfall is approximately 600 mm, with mean minimum winter and maximum summer temperatures of 10 ˚C and 33 ˚C, respectively (Hunter 1998; Druce *et al.* 2006). The area experiences strong north-easterly and south-westerly winds in September, while June is generally considered the calmest month (Goodman 1981).

Phinda falls within the lowveld bioregion of the savanna biome, and the area consists of a broad spectrum of geological formations and soils that have given rise to a mosaic of savanna woodlands interspersed with grasslands and forests (Mucina & Rutherford 2006; van Rooyen & Morgan 2007). The vegetation units that occur in the reserve include Zululand lowveld, western Maputaland clay bushveld, western Maputaland sandy bushveld and northern Zululand sourveld dominated by *Vachellia* (formerly known as *Acacia*), *Terminalia*, *Combretum*, *Dichrostachys, Themeda* and *Eragrostis* spp. (Mucina & Rutherford 2006; Hunter *et al*. 2007).

Phinda is mainly used as a high-end ecotourism destination with seven lodges and 124 beds for tourists (Balme *et al.* 2009). Forty-four large mammal species (excluding bats and small

rodents) have been recorded in the reserve, including lion *Panthera leo*, leopard, cheetah *Acinonyx jubatus*, brown hyaena *Hyaena brunnea* and spotted hyaena *Crocuta crocuta* (Hunter *et al.* 2007). The area is bound by a 1.8 m high electric game fence; however, leopards are not constrained by the boundary fence as they are able to move between reserves or properties into neighbouring pastoral Zulu communities, livestock farms and commercial game ranches (Balme 2009).

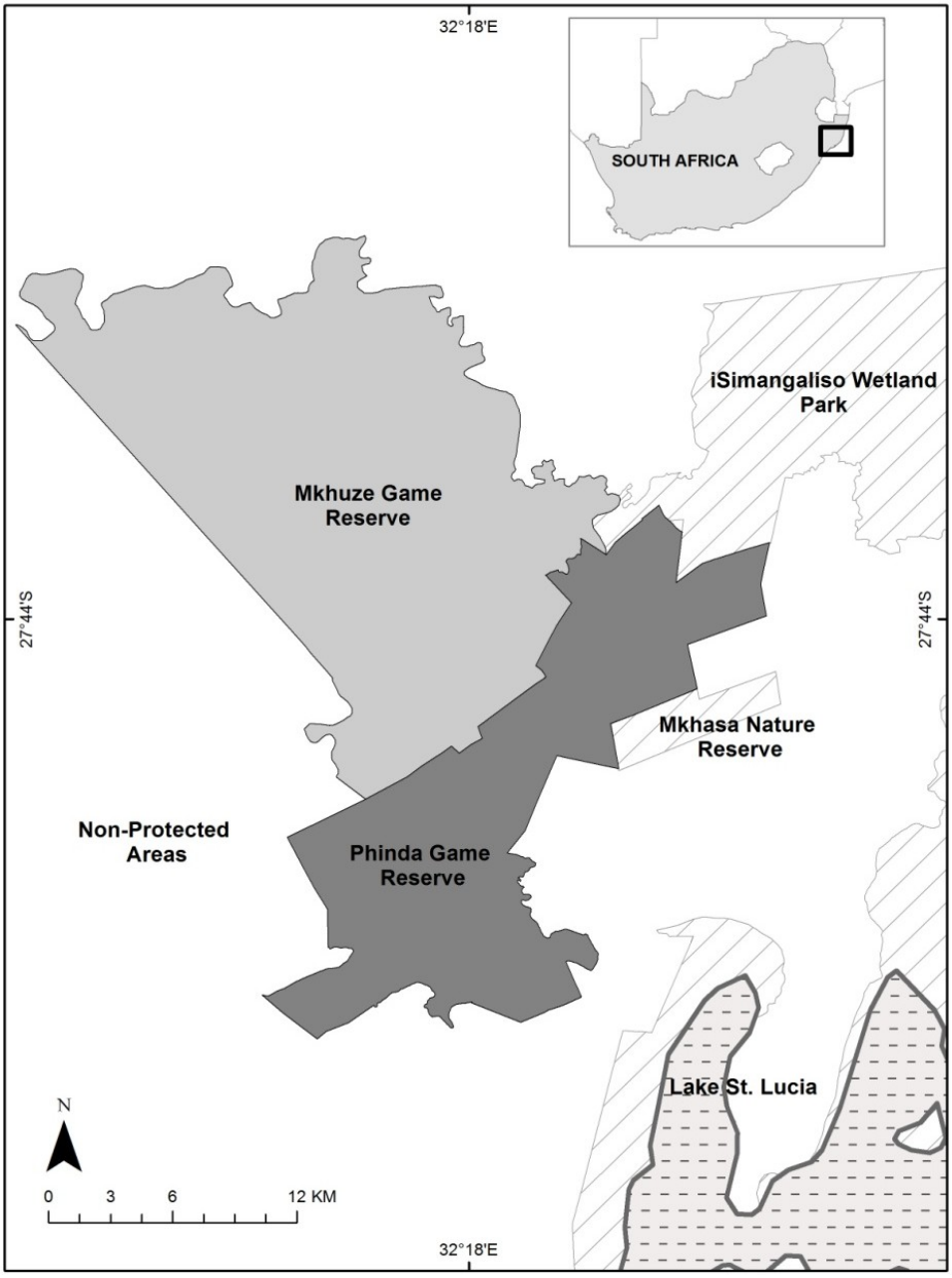


Figure 3.1: Map showing location of the study area and land-use types in the region. Non-protected areas consist of livestock farms, game farms and tribal authority land where leopards are exposed to legal hunting and persecution (Balme 2009)

3.2.2 Leopard sampling

An intensive study on the biology of leopards in Phinda was initiated in 2002 through Panthera's Munyawana Leopard Project to improve the conservation status of leopards in the northern KZN. Techniques such as GPS radio-telemetry and camera traps were used to monitor the leopard population dynamics and ecology (Balme *et al*. 2007; 2009). During the study, leopards were immobilised to fit and remove radio-collars according to the protocol explained in Balme *et al.* (2007). As part of the sampling protocol, the longest available whisker was removed from six adult individuals in 2011; five males (M60, M62, M70, M73, and M74) and one female (F08). The capture and handling of these leopards was approved by the Animal Ethics sub-committee of the University of KZN Ethics Committee (approval 051/12/Animal).

3.2.3 Prey sampling and identification

Tissue samples from the principal prey species of leopards were collected from Phinda and surrounding properties during 2014. These samples were used to determine patterns of prey selection for each leopard by comparing the isotopic signatures of leopard whiskers to those of prey. Scat samples and continuous follows have revealed that the primary prey species consumed by northern KZN leopards are nyala *Tragelaphus angasii,* impala, warthog *Phacochoerus africanus*, grey duiker, red duiker *Cephalophus natalensis* and reedbuck *Redunca arundinum* (Balme *et al.* 2007). Where possible, a minimum of five hair and five faecal samples were collected from different individuals of each of these species (excluding reedbuck) and other potential prey species i.e. blue wildebeest *Connochaetes taurinus*, kudu *Tragelaphus strepsiceros*, plains zebra *Equus quagga*, giraffe *Giraffa camelopardalis* and waterbuck *Kobus ellipsiprymnus*. Hair samples could only be obtained for four species (impala, nyala, kudu and blue wildebeest), either from carnivore kills encountered in the field or from carcasses made available by local hunters. Fresh faecal samples were collected opportunistically for all species, except kudu and nyala, and different faecal piles were taken to represent different individuals. All samples were enclosed in carefully labelled paper bags, and freshly collected faecal samples were frozen until laboratory processing.

3.2.4 Plant sampling

The use of SIA requires the average values of C_3 and C_4 plants specific to the area to be established for accurate analysis (Ben-David & Flaherty 2012). Therefore, representative samples from five common tree (browse) and five common grass (graze) species were collected from the general study area in July 2014. For browse, five samples of each of the following tree species were collected; marula *Sclerocarya birrea*, buffalo-thorn *Ziziphus*

mucronata, sickle bush *Dichrostachys cinerea*, fever tree *Vachellia xanthophloea* and scented-pod acacia *Vachellia nilotica.* Similarly, five samples of each of the following grass species were collected; red grass *Themeda triandra*, fan grass *Eustachys paspaloides*, couch grass *Cynodon dactylon*, broad-leaved turpentine *Cymbopogon excavatus* and cat's tail grass *Sporobolus pyramidalis*.

3.2.5 Sample preparation and stable isotope analysis

All samples were processed and prepared for SIA at the Cape Peninsula University of Technology. The six leopard whiskers (one from each individual) were washed in 2:1 methanol: chloroform solution to remove surface contaminants, including lipids, further cleaned in an ultrasonic bath (DG-600) with distilled water for five minutes and air dried. Whiskers were individually placed on a clean, flat surface to measure their length and then sectioned at 2 mm intervals using a scalpel. All whiskers were > 90 mm, but only the proximal 60 mm of whiskers were sampled (length in which reliable and comparable data can be obtained from felid whiskers; see Chapter 2), starting at the root working towards the tip, resulting in 30 sections per whisker. However, only 20 usable whisker sections could be obtained from leopard M70 due to the thinness of his whisker. A total of 170 whisker sections were each weighed to a minimum of 0.5 mg on an analytical balance (GH series), packed in tin capsules, crimp sealed and sent for stable carbon and nitrogen isotope analysis.

The same procedure used for cleaning leopard whiskers was followed to clean prey hair. Sections large enough for SIA (~0.5 mg) were cut from root to tip; and a total of 18 sub-samples were packed in tin capsules, crimp sealed and sent for stable carbon and nitrogen isotope analysis. Faecal and plant specimens were oven dried at 60°C for 24 hours and mill-ground through a 1 mm sieve into a homogenous powder (Codron *et al.* 2008). Sub-samples of 2 mg were then weighed and packed in tin capsules. In total, 30 plant samples (three of each species) and 44 faecal samples (of eight herbivore species) were sent for stable carbon and nitrogen isotope analysis.

All samples were sent for carbon and nitrogen isotope analysis at the Stable Isotope Laboratory, Department of Archaeology, University of Cape Town. Samples were individually combusted in a Flash 2000 organic elemental analyser (Thermo Scientific, Bremen, Germany) and the resultant CO_2 and N_2 gases introduced into a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) via a continuous flow-through inlet system (Conflo IV). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are presented in delta (δ) notation in parts

per thousand or per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 standards, respectively, derived from the expression:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 * 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard deviations of repeated measures of laboratory standards (Acacia, Sucrose, Lentil, Merck gel, Chocolate powder, Seal and Valine) were ≤ 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

3.2.6 Data analysis

3.2.6.1 Correction of leopard whisker series for isotope turnover

Leopard whisker $\delta^{13}C$ and $\delta^{15}N$ were corrected for attenuation effects that take place during isotope turnover using a three-pool turnover model initially parameterised for horses (Ayliffe *et al.* 2004). The model predicts that hair observed for dietary isotope changes has three exponential isotope pools; the first pool with a fast, second pool with an intermediate and third pool with a slow turnover rate. The protein complex (formed from amino acids) of the first pool corresponded to assimilated diet, while that of the other two pools was thought to be derived from catabolism of endogenous reserves released during tissue turnover (Ayliffe *et al.* 2004). The model is defined as;

$$\frac{\delta X_t - \delta X_{eq}}{\delta X_0 - \delta X_{eq}} = f_1 e^{-\lambda 1 t} + f_2 e^{-\lambda 2 t} + f_3 e^{-\lambda 3 t} \quad (2)$$

where δX_t is the isotopic value of a unique sub-sample representing a given point in time [*t* in days ($t_1^{0.5}(= 1.1)$; $t_2^{0.5}(= 4.1)$; $t_3^{0.5}(= 135)$]. δX_0 is the isotopic value at the start of the transition period and δX_{eq} is a model derived isotope value of the sample when the animal is at equilibrium with the diet. $f_1\,(= 0.4)$, $f_2\,(= 0.16)$ and $f_3\,(= 0.44)$ are the fractional contributions of each isotope pool represented by three independent constants [$\lambda_1\,(= 0.6301)$, $\lambda_2\,(= 0.1691)$ and $\lambda_3\,(= 0.0051)$] (Ayliffe *et al.* 2004). Although these parameter values were derived from experiments with horses, they are to date the most reliable turnover data available for large mammals (Cerling *et al.* 2007). To evaluate the significance of isotope turnover correction on leopard whiskers, whisker series that were corrected for turnover (turnover-corrected) were compared to those that were not corrected for turnover (raw) using pairwise *t*-tests for each individual, and the overall effect across all individuals was evaluated using Fisher's Global (combined probability) test (Fisher 1932). A significance level of 0.05 was used in both tests.

3.2.6.2 Adjustment of prey hair and faeces for isotope discrimination

The differences between isotopic compositions of prey hair and diet (C_3 or C_4 plants), and prey faeces and diet were adjusted using mammalian herbivore hair-diet and faeces-diet TDFs reported from controlled feeding studies (Table 3.1). Prey species were grouped into the main three herbivore feeding types based on literature. The red duiker, grey duiker and giraffe are browsers; impala and nyala are intermediate feeders; while blue wildebeest, zebra, waterbuck and warthog are grazers (e.g. Gagnon & Chew 2000; Sponheimer *et al.* 2003a; Skinner & Chimimba 2005). The discrimination-adjusted $\delta^{13}C$ and $\delta^{15}N$ values of hair and faeces were then compared across the three prey types. Because the two tissues showed similar prey type isotopic patterns, they were combined into one large and more robust dataset i.e. "muscle equivalents", since the bulk of mammalian carnivore diets are derived from prey muscle (Codron *et al.* 2007). Therefore, prey hair and faecal data were converted to muscle equivalents using the predicted values calculated from hair, faeces and muscle $\delta^{13}C$ and $\delta^{15}N$ TDFs that are displayed in Table 3.1. For example, the $\delta^{13}C$ values of herbivore faeces are 0.8 ‰ lower than the values of plants consumed, while herbivore muscle is 1.5 ‰ enriched in $\delta^{13}C$ relative to diet (Sponheimer *et al.* 2003b; 2006). To derive $\delta^{13}C$ values of herbivore muscle from faeces, 2.3 ‰ was added to prey faeces $\delta^{13}C$ values. This enabled comparisons between isotope signatures of leopards and those of prey in order to identify leopard diets.

Table 3.1: The stable carbon and nitrogen isotope discrimination factors of diet, hair, faeces and muscle for herbivores ([a]Cerling & Harris 1999; [b]Sutoh *et al*. 1987; [d]Sponheimer *et al.* 2003b; [e]Codron *et al.* 2005a; [f]Sponheimer *et al.* 2006; [g]Calculated using the listed diet-tissue differences)

Tissues	$\delta^{13}C_{VPDB}(^{o}/_{oo})$	$\delta^{15}N_{Air}(^{o}/_{oo})$
Diet-hair	+3.1[a]	+2.9[b]
Diet-faeces	-0.8[d,e]	+1.0[b]
Diet-muscle	+1.5[f]	+2.9[b]
Hair-muscle	-1.6[g]	0.0[g]
Faeces-muscle	+2.3[g]	+1.9[g]

To ensure the presumed prey types were isotopically distinct, the significant differences amongst mean muscle equivalent $\delta^{13}C$ and $\delta^{15}N$ values of grazers, intermediate feeders and browsers were assessed using one-way analysis of variance (ANOVA), and *post hoc* Tukey honestly significant difference (HSD) test (Tukey 1949). ANOVAs were run on ranked

(non-parametric) and non-ranked (parametric) data, and results obtained from the two tests were compared. Assumptions of normality and homoscedasticity of the data were tested using Shapiro-Wilk's *W* (Shapiro & Wilk 1965) and Levene's test (Zar 2010), respectively. All tests were performed in R software, version 3.1.3 (R Core Team 2013) and a significance level of 0.05 was used. Means are presented with standard deviation (± *SD*) as a measure of precision.

3.2.6.3 Adjustment of whisker series for isotopic discrimination

To date, whisker-diet TDFs have not been experimentally determined for the African leopard, and the most reliable values are those estimated for lions in Chapter 2. As a result, leopard whiskers were adjusted using $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of 2.70 and 2.53 ‰, respectively. These values were subtracted from $\delta^{13}C$ and $\delta^{15}N$ values of each whisker segment, which could then be compared directly to prey muscle equivalents.

3.2.6.4 Diet composition of leopards

The felid whisker growth rate of 0.65 mm d^{-1} enabled measurement of the time period represented in 60 mm of leopard whiskers. The length presented approximately 92 days and potentially 18 feeding events (assuming that leopards killed prey once every five days, Bothma & le Richie 1984), thereby providing a relatively long time period that could be used to trace and establish an individual's dietary history. Sectioning whiskers at 2 mm intervals therefore suggest the sampling of more or less a single feeding event.

The diet composition of the six Zululand leopards was quantified using a binning approach that involves assigning leopard whiskers to groups of C_3-, C_4- or intermediate feeding prey (Codron *et al.* 2016). The $\delta^{13}C$ values of individual leopard whiskers were grouped using $\delta^{13}C$ inter-quartile range of intermediate feeder prey muscle equivalents. $\delta^{13}C$ values that were above the upper quartile of intermediate feeders meant that a grazer was eaten, and those below the lower quartile of intermediate feeders meant that a browser was eaten. Whisker $\delta^{13}C$ values that fell between browser and grazer ranges were qualified as intermediate feeding prey. To evaluate the effects of isotope turnover on the determination of leopard diet, the frequency distributions of browsers, intermediate feeders and grazers in leopard diets were compared between whisker series that were adjusted for discrimination only, and those that were corrected for both turnover and discrimination. Comparisons between the two frequency distributions for each individual were made using Chi-square tests, with Yate's correction because counts were < 5 in many cells (Yates 1934), and the overall difference was tested using Fisher's Global test (using a significance level of 0.05 in both tests).

3.3 Results

3.3.1 Plant values of the study area

There was a clear separation between C_3 trees and C_4 grasses in the study area (Figure 3.2) and this made it possible to distinguish browsers from grazers. The $\delta^{13}C$ values of samples of common tree species (n = 15) ranged from -31.2 (fever tree) to -28.5 ‰ (sickle bush), and those of samples of common grass species (n = 15) ranged from -14.5 (couch grass) to -12.8 ‰ (red grass) (Appendix D). The mean $\delta^{13}C$ values of C_3 and C_4 plants in the study area were -29.6 ± 0.82 and -13.5 ± 0.82 ‰, respectively. Plant species had variable $\delta^{15}N$ values; C_3 plants ranged from -0.9 (sickle bush) to 7.8 ‰ (fever tree) with a mean of 3.5 ± 3.00 ‰, while C_4 plants ranged from -2.1 (fan grass) to 9.3 ‰ (couch grass) with a mean of 1.0 ± 3.82 ‰.

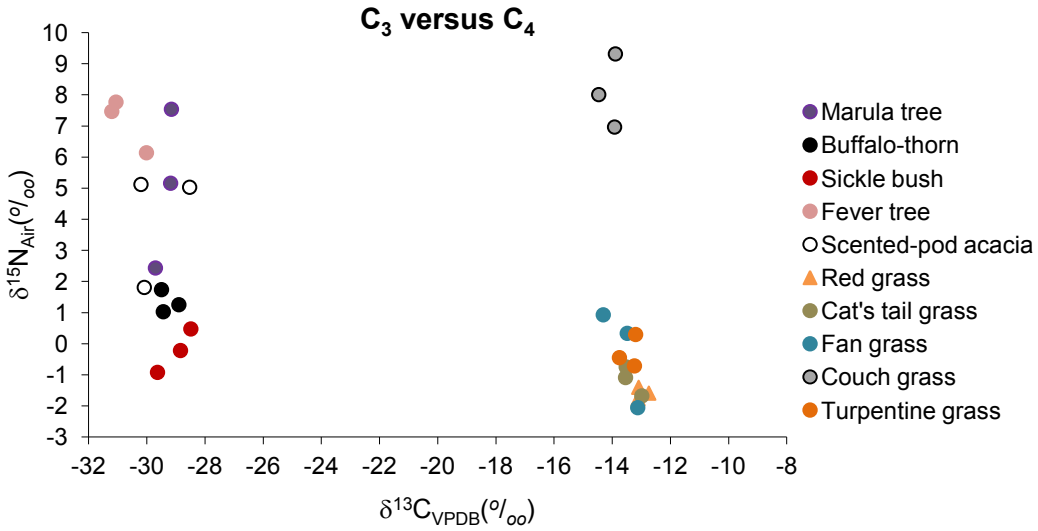


Figure 3.2: The $\delta^{13}C$ and $\delta^{15}N$ values of C_3 and C_4 plants collected from the study area

3.3.2 Prey tissues

3.3.2.1 Faeces

The ^{13}C signatures of prey faeces showed differences between the three prey types (Figure 3.3 A). Mean faecal $\delta^{13}C$ values were -28.4 ± 0.57 ‰ (range = -29.2 to -27.5 ‰; n = 16) for browsers, -21.8 ± 2.36 ‰ (range = -24.7 to -19.3 ‰; n = 9) for intermediate feeders and -14.5 ± 0.60 ‰ (range = -15.6 to -13.9 ‰; n = 19; Appendix E) for grazers. However, for ^{15}N, no clear differences were found across prey types. Faecal mean $\delta^{15}N$ values were 4.3 ± 1.58 ‰ (range = 1.5 to 7.1 ‰) for browsers, 4.2 ± 0.85 ‰ (range = 3.2 to 5.3 ‰) for intermediate feeders and 4.6 ± 0.55 ‰ (range = 3.9 to 5.8 ‰) for grazers.

3.3.2.2 Hair

The ^{13}C compositions of hair samples also showed differences between the three prey types (Figure 3.3 B). Browsers had mean hair $\delta^{13}C$ values of -23.2 ± 1.16 ‰ (range = -24.2 to -21. 9 ‰; $n = 3$), while those for intermediate feeders and grazers were -16.0 ± 3.54 ‰ (range = -22.2 to -10.9 ‰; $n = 10$) and -9.4 ± 0.22 ‰ (range = -9.8 to -9.2 ‰; $n = 5$; Appendix F), respectively. Like with faecal samples, the hair $\delta^{15}N$ values were similar amongst prey types. Mean $\delta^{15}N$ values were 5.7 ± 0.63 ‰ (range = 5.0 to 6.3 ‰) for browsers, 7.2 ± 0.60 ‰ (range = 6.2 to 7.9 ‰) for intermediate feeders and 6.3 ± 0.69 ‰ (range = 5.5 to 7.3 ‰) for grazers.

3.3.2.3 Muscle equivalents

The muscle equivalents $\delta^{13}C$ data from hair and faeces revealed a similar separation across prey types, and overlapped within browsers, intermediate feeders and grazers (Figure 3.3 C). Browsers had mean $\delta^{13}C$ values of -25.9 ± 0.81 ‰ (range = -26.9 to -23.5 ‰; $n = 19$), intermediate feeders -18.5 ± 3.11 ‰ (range = -23.8 to -12.5 ‰; $n = 19$) and grazers -12.0 ± 0.74 ‰ (range = -13.3 to -10.8 ‰; $n = 24$). The indistinguishable muscle equivalents $\delta^{15}N$ values were 6.1 ± 1.46 ‰ (range = 3.4 to 9.0 ‰) for browsers, 6.7 ± 0.90 ‰ (range = 5.1 to 7.9 ‰) for intermediate feeders and 6.5 ± 0.57 ‰ (range = 5.5 to 7.7 ‰) for grazers.

The relationship between diet and the observed muscle equivalent $\delta^{13}C$ signatures of prey evaluated using parametric one-way ANOVA revealed residuals that were not normally distributed (SW-$p < 0.05$), while residuals on ranked data were normally distributed (SW-$p = 0.47$). Conversely, the relationship between diet and $\delta^{15}N$ signatures of prey muscle equivalents showed residuals with normal distributions for the parametric test (SW-$p = 0.11$), similar to non-parametric results (SW-$p = 0.12$). Despite the non-normal distribution of the parametric data, residuals of both parametric and non-parametric statistics showed homogeneous variances for both $\delta^{13}C$ and $\delta^{15}N$ (Levene's $p = 1.00$), and the obtained *post hoc* Tukey HSD *p*-values for the two tests were similar. As a result, parametric results are presented here. There were significant differences in mean muscle equivalent $\delta^{13}C$ values of browsers, intermediate feeders and grazers ($F_{(2)} = 306.23$, $p < 0.05$; Tukey HSD $p < 0.05$). Like faecal and hair samples, mean muscle equivalent $\delta^{15}N$ values were similar across prey types ($F_{(2)} = 1.47$, $p = 0.24$; Tukey HSD $p = 0.22$–0.86).

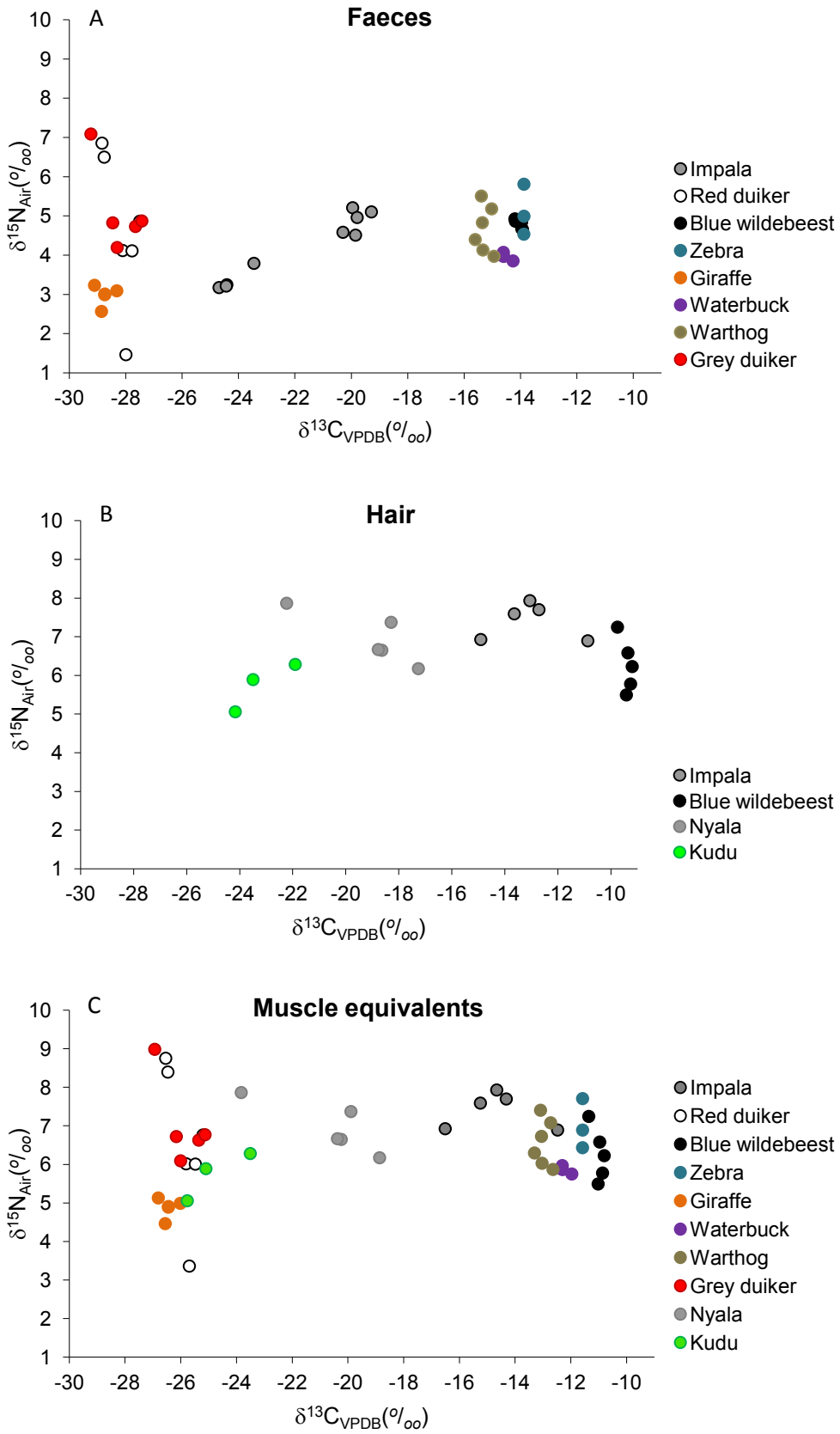
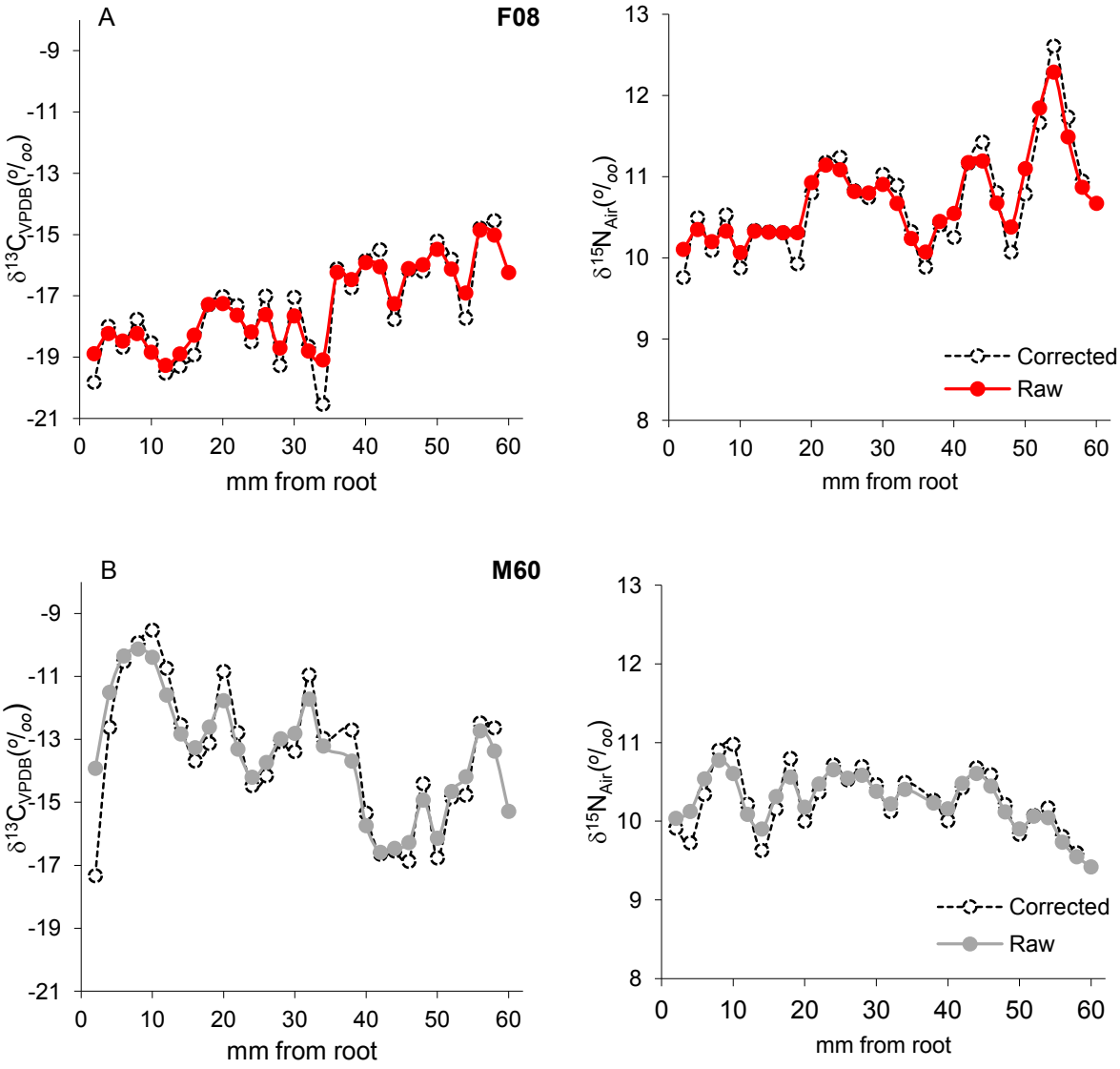
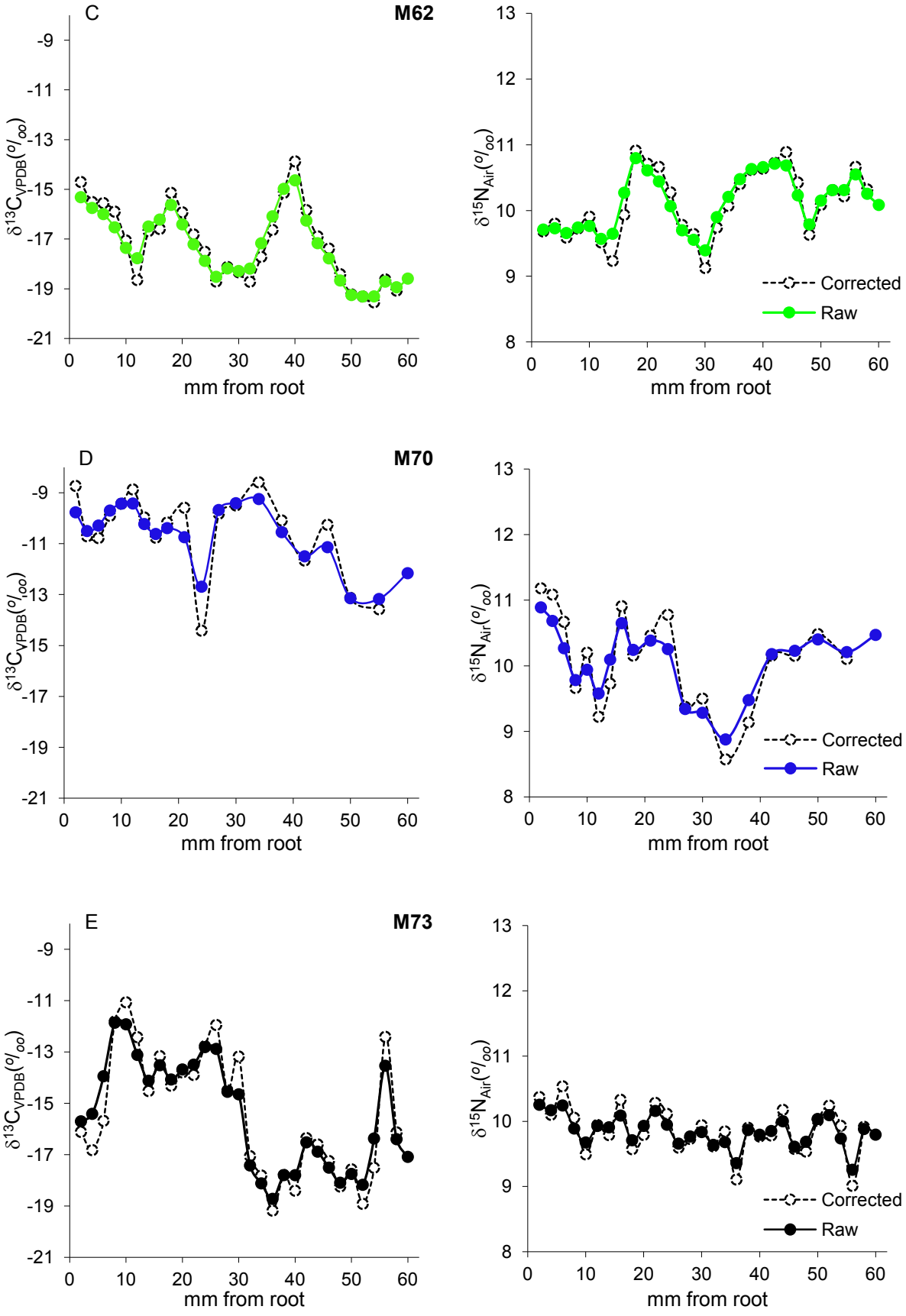


Figure 3.3 A-C: The $\delta^{13}C$ and $\delta^{15}N$ signatures of faeces (A), hair (B) and muscle equivalents (C) for the leopard prey species

3.3.3 Raw versus turnover-corrected leopard whisker series

There were slight effects of attenuation on the isotopic signatures of leopards' whisker series (Figure 3.4 A-F). Mean differences between raw and turnover-corrected whisker series were 0.1 ± 0.69 ‰ and 0.01 ± 0.19 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Subsequently, no significant differences were found between the two series for any individual pairwise *t*-tests (t = -0.58–0.70, p = 0.29–0.92 for $\delta^{13}C$; t = -0.03–0.53, p = 0.53–0.98 for $\delta^{15}N$) nor globally (Fisher's Global test $x^2_{(12)} = 6.36$, p = 0.90 for $\delta^{13}C$; $x^2_{(12)} = 4.01$, p = 0.98 for $\delta^{15}N$).




C
M62
D
M70
E
M73
δ13CVPDB(o/oo)
δ15NAir(o/oo)
mm from root
Corrected
Raw

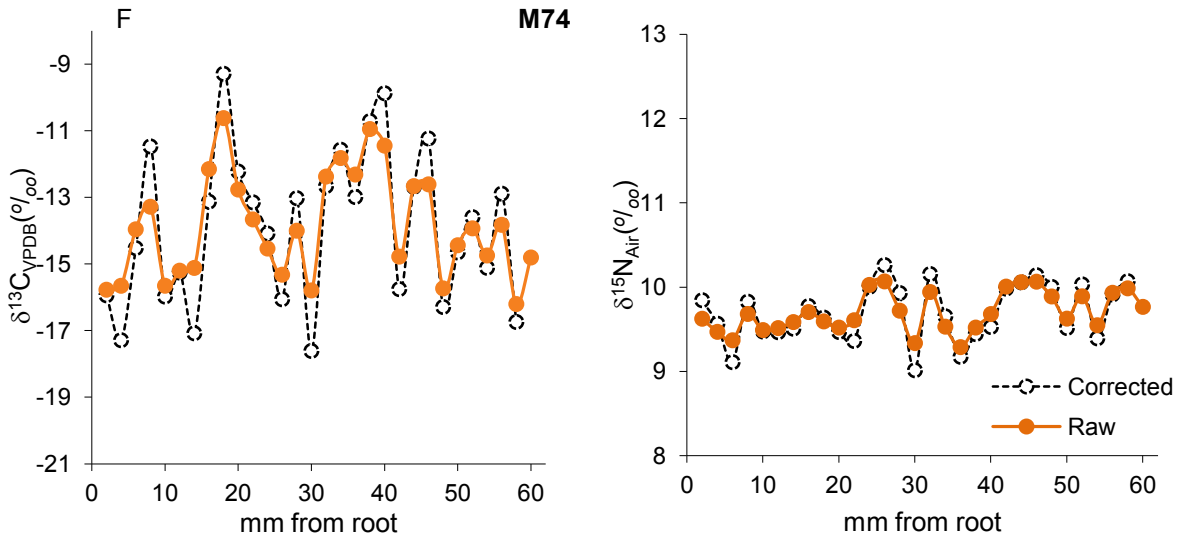


Figure 3.4 A-F: The differences between observed $\delta^{13}C$ and $\delta^{15}N$ whisker series of the six leopard individuals that were corrected for turnover (corrected) and those that were not corrected for turnover (raw)

3.3.4 The diet of leopards

The similarities between muscle equivalents $\delta^{15}N$ signatures of browsers, intermediate feeders and grazers suggest that nitrogen-15 could not provide information on the diet of leopards in Phinda. Therefore, only $\delta^{13}C$ signatures of the individually segmented leopard whiskers were used to quantify diets of Phinda leopards (Appendix G). Discrimination-adjusted $\delta^{13}C$ values for leopard individuals ranged from -22.0 to -11.9 ‰, and fell mostly within the range of $\delta^{13}C$ muscle equivalents of intermediate feeders (Figure 3.5 A). Intermediate feeders were the predominant prey type inferred for each leopard based on frequency distributions of browser-, intermediate feeder- and grazer-related $\delta^{13}C$ values in whisker isotope profiles (Figure 3.6 A). They appeared to constitute 100 % of the diet of leopards F08 and M62; and made up the vast majority of prey items utilised by individuals M60, M73 and M74 (75.86 %, 96.67 % and 86.67 %, respectively). However, only four of the 20 $\delta^{13}C$ values (20 %) of leopard M70's whisker indicated a diet of intermediate feeders. The remaining 16 $\delta^{13}C$ values (80 %) indicated a grazer diet, suggesting a strong reliance on this prey type by the individual. Leopards F08 and M62 did not include grazers in their diet.

The discrimination-adjusted data that was not corrected for turnover showed that none of the leopards incorporated browsers in their diet (Figures 3.5 A & 3.6 A). Conversely, $\delta^{13}C$ whisker series that were corrected for turnover suggest that 10.34 % (three observations) of leopard F08 and 3.45 % (one observation) of M62's diet comprised browsers (Figures 3.5 B & 3.6 B). Despite this, there were no significant differences in inferred frequency distributions

of the three prey types between discrimination-adjusted, but not turnover-corrected, and turnover-corrected + discrimination-adjusted whisker series [chi-square test with Yate's correction ($x^2_{(1)}$ = 0.00–1.48, p = 0.46–0.97); Fisher's combined probability test ($x^2_{(10)}$ = 4.01, p = 0.98)].

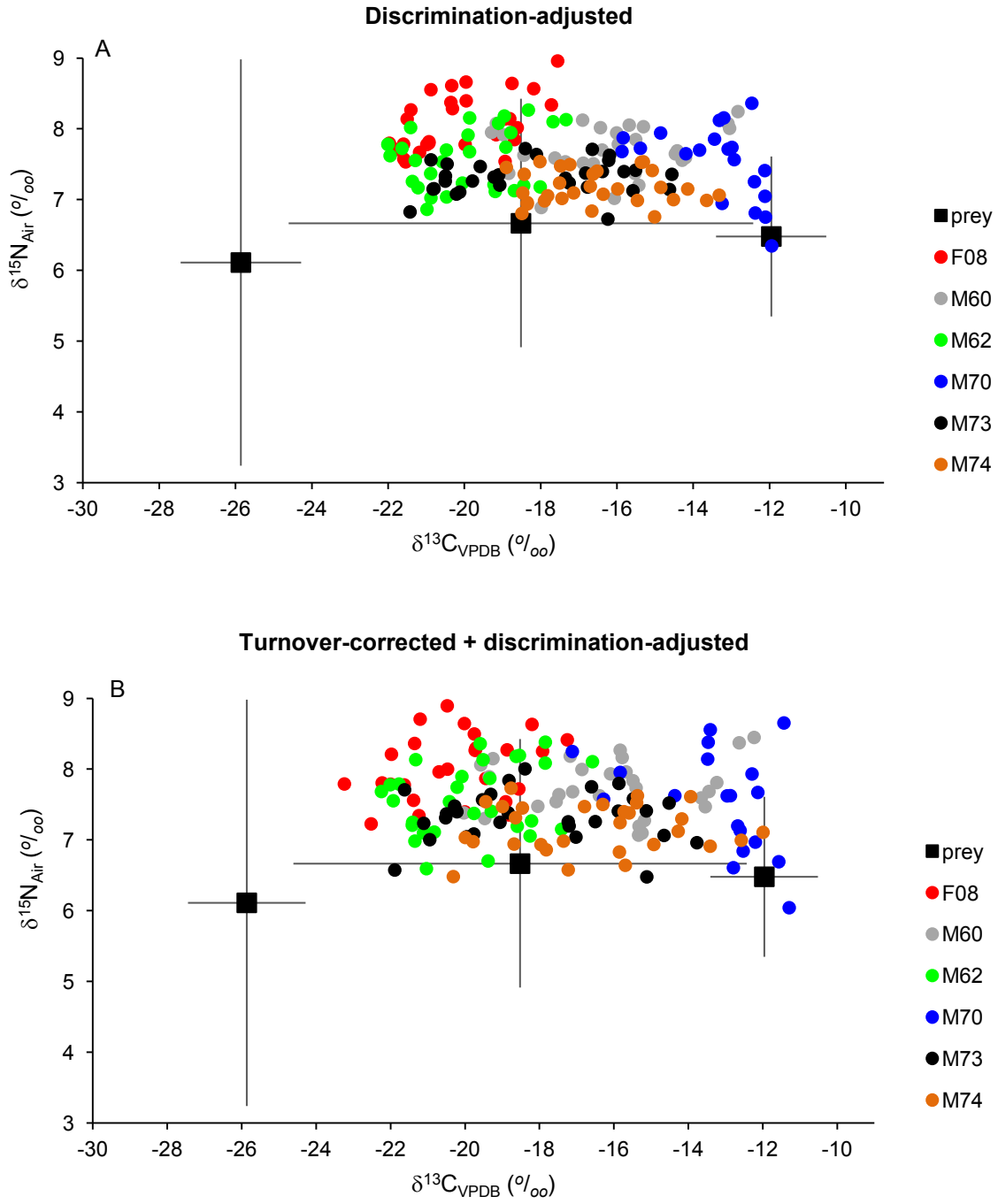


Figure 3.5: The mean (and ranges) $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions of prey muscle equivalents superimposed on leopards' discrimination-adjusted only (A) and turnover-corrected + discrimination-adjusted (B) whisker profiles to distinguish individual leopard diet

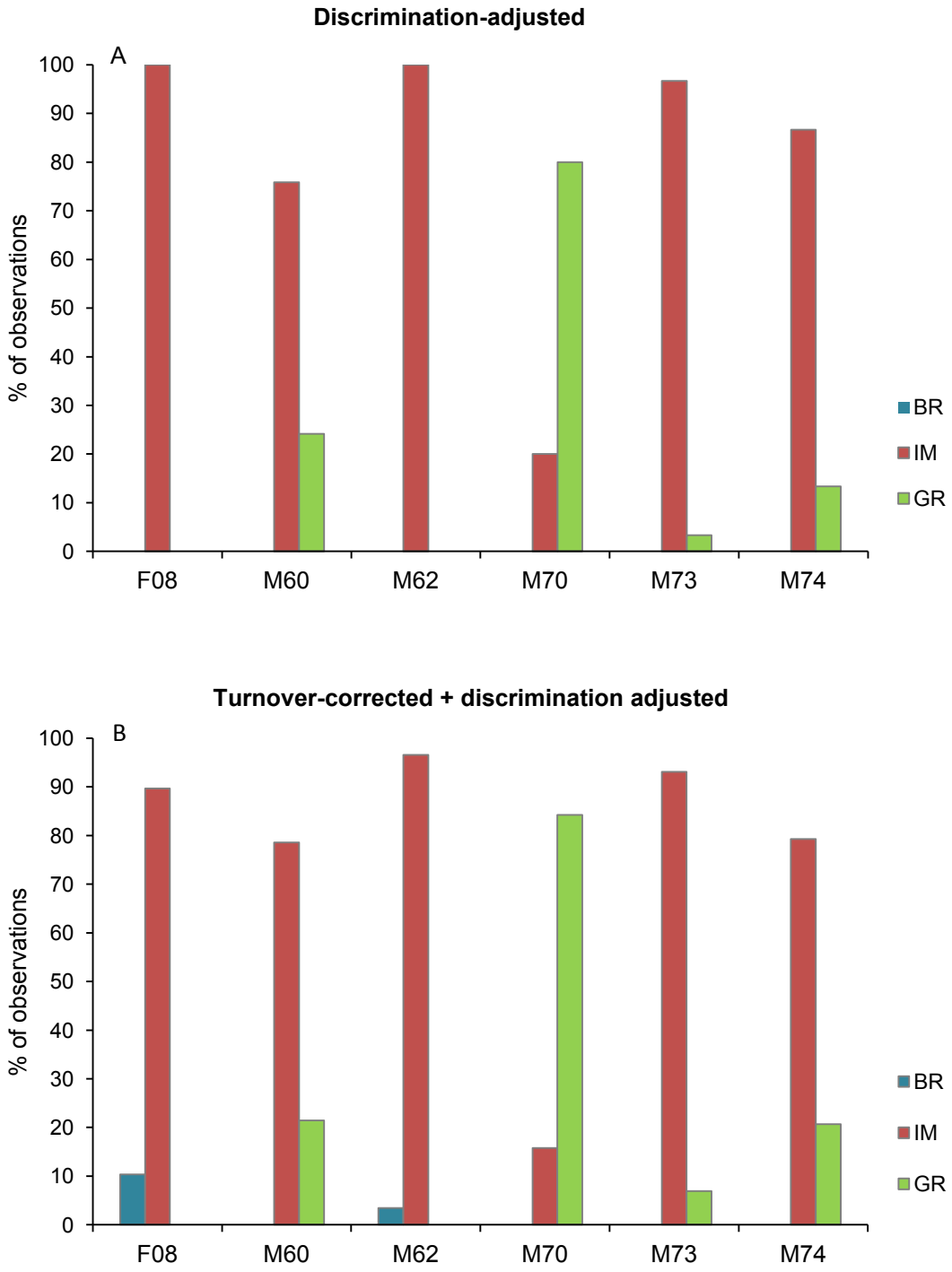


Figure 3.6: Percentage of browser (BR), intermediate feeder (IM) and grazer (GR) prey observed in the diets of the six leopards for discrimination-adjusted only (A) and turnover-corrected + discrimination-adjusted (B) whisker data

3.4 Disussion

3.4.1 Plant values of the study area

In the subtropical savanna of Phinda, there was a bimodal distribution of ^{13}C between C_3 browse (trees) and C_4 grass species that were potentially utilised by leopard prey (Figure 3.2). The mean $\delta^{13}C$ values for C_3 and C_4 plants (-29.6 and -13.5 ‰, respectively) from this study falls within the global range of $\delta^{13}C$ values for terrestrial C_3 and C_4 plants [mean = -27 ‰ (range = -35 to -21 ‰) and -13 ‰ (range = -14 to -10 ‰), respectively] (Ehleringer 1991; Marshall *et al.* 2007). The use of SIA requires the dietary items of interest to have isotopically distinct, non-overlapping signatures so that the contribution of each food item to the consumer's diet can be assessed (Gannes *et al*. 1998; Rosing *et al.* 1998). Therefore, the separate carbon delta-values displayed by plants collected from Phinda made it possible to use carbon-13 to discern the diet of both prey and predator in this ecosystem.

In contrast, $\delta^{15}N$ values of the sampled vegetation did not differ along a C_3/C_4 gradient. The $\delta^{15}N$ values for both plant groups varied widely and overlapped almost entirely, i.e. $\delta^{15}N$ values of C_3 and C_4 plant species ranged from 0.9 to 7.8 ‰ and from -2.1 to 9.3 ‰, respectively. A number of factors contribute to terrestrial plant $\delta^{15}N$ variation including soil, climate, plant physiology, nutrient and nitrogen cycling (Virginia & Dewilche 1982; Sealy *et al.* 1987; Ambrose 1991). Codron *et al.* (2005a; 2005b) also found similar $\delta^{15}N$ values amongst trees, forbs and grasses from different regions of the Kruger National Park and the Waterberg, South Africa, respectively. However, other studies, for instance, Swap *et al.* (2004) and Aranibar *et al.* (2008) found that C_3 plants of 21 selected sites in southern Africa and the Kalahari, respectively, had lower $\delta^{15}N$ values than C_4 grasses. The complexity of plant ^{15}N trends makes it difficult to identify the relative contribution of various plant functional groups on the basis of nitrogen isotope ratios (Kelly 2000).

3.4.2 Prey tissues

Faecal and hair analyses revealed a separation of prey types, i.e. browsers, intermediate feeders and grazers, along the ^{13}C axis, but not along the ^{15}N axis. Thus, in Phinda, ^{15}N differentiation across herbivore groups is not considered useful for resolving diets of leopards (and other apex predators). However, ^{13}C should differ depending on the proportions of browsers, intermediate feeders or grazers consumed. The study included two sources of prey samples, hair and faeces, which are known to have different isotope discriminations, and therefore different delta-value ranges. Moreover, the species composition in each sample was not identical. To overcome this, prey data were combined

by converting their respective delta-values to muscle equivalent values based on tissue discrimination values extracted from literature.

Faeces reflect short-term dietary intake, i.e. for several days (Tieszen *et al.* 1979; Sponheimer *et al*. 2003b), while hair integrates the isotopic signal of diet over extended periods of time (Schwertl *et al.* 2003; West *et al.* 2004). As a result, hair is potentially a more informative isotopic tissue than faeces (Wittmer *et al.* 2010). Despite this, the combined hair and faeces data revealed similar ^{13}C patterns across prey types and similar lack of ^{15}N variation along this gradient, with combined values for each prey type overlapping regardless of whether the data were derived from hair or faeces. Similarities between adjusted prey hair and faecal data indicate that both faeces and hair are reliable sources of dietary isotopic information, and thus can be used to characterise herbivore diets. Moreover, it indicates the reliability of the conversion method, hence, it should be considered a useful baseline for subsequent interpretation of carnivore diets. These findings concur with those of Wittmer *et al.* (2010) who used the stable isotope of Mongolian sheep hair and faeces to reconstruct C_3/C_4 ratios of vegetation consumed by the animals, and found that the two materials provided the same estimation. Codron *et al.* (2007) also found that prey types in the Kruger National Park and other lowveld savanna habitats of South Africa inferred from hair, faeces and muscle equivalents were similar.

3.4.3 Raw versus turnover-corrected leopard whisker series

The study also evaluated whether delayed isotopic turnover led to a significant reduction in the level of isotopic (and hence dietary) variation along leopard whisker profiles. Using a three-pool turnover model parameterised from a longer-term feeding experiment of horses (Ayliffe *et al.* 2004), no significant differences were found between whisker series that were corrected for turnover and those that were not. This may suggest that the time between each 2 mm segment is sufficient to allow for most isotopic turnover to occur, such that sampling leopard whiskers at this resolution gives a fairly accurate reflection of dietary isotope switching. Codron *et al.* (2013) found similar results when they modelled elephant tail hair using single- and three-pool isotope turnover models. However, in both studies, there were instances when $\delta^{13}C$ and $\delta^{15}N$ values of turnover-corrected data deviated from the observed values with ≥ 1 ‰, suggestive of an under-representation of animal diets when turnover effects are in process. These observations highlight the need for more accurate information about isotope turnover parameters for large mammalian carnivores, in this case leopards.

3.4.4 The diet of leopards

In this study, $\delta^{13}C$ whisker series revealed that intermediate feeders were the dominant prey type of leopards in Phinda (Figure 3.5 & 3.6). Discrimination-adjusted $\delta^{13}C$ whisker series showed that intermediate feeders comprised, on average, ~76 % of the leopards' diet. In some cases, i.e. individuals F08 and M62, intermediate feeders were the only prey type detectable in whisker $\delta^{13}C$ profiles (Figure 3.6 A). These findings concur with those of Balme *et al.* (2007) who investigated the feeding ecology of 27 leopards from the same leopard population using radio-telemetry and continuous follows. They found that intermediate feeders constituted 69 % of the leopards' diet. Other studies, including Smith (1978), Mills (1990), Radloff & du Toit (2004), Hayward *et al.* (2006) and Owen-Smith & Mills (2008) also found that leopards primarily prey on impala – an intermediate feeder – where available.

In the absence of the common intermediate feeders such as impala or nyala, leopards have been reported to prey mainly on smaller browsers such as grey duiker, red duiker, steenbok *Raphicerus campestris* and bushbuck (Stander *et al.* 1997; Hayward *et al.* 2006; Owen-Smith & Mills 2008; Stein & Heyssen 2013). However, whisker $\delta^{13}C$ profiles of the six Phinda leopards revealed very little evidence of browser consumption. Utilisation of browsers was evident in two individuals, F08 and M62, with 3/29 (10.34 %) and 1/29 (3.45 %) whisker $\delta^{13}C$ values, respectively, only when whisker series were "corrected" for isotope turnover and attenuation. Balme *et al.* (2007) also found a low, but more frequent browser utilisation by Phinda leopards (comprising 14 % of their diets overall) than inferred from the isotopic analyses presented in this study. It is possible that the small body size of browsers commonly taken by Phinda leopards meant that isotopic signals from this prey were effectively "swamped" by intake of larger-bodied intermediate feeders and grazers.

In contrast to browsers, grazers formed a recognisable component of Phinda leopards' diets. $\delta^{13}C$ series from four of the six individuals analysed provided evidence for utilisation of this prey, and in one case, M70, grazers made up ~80 % of the individual's diet. Similar results were obtained by Balme *et al.* (2007) who found that grazers comprised 17 % of the diet of Phinda leopards. Most ungulate grazers are large in size, and the average weight of potential grazer prey species observed in this study range from 45 kg (warthog) to ≥ 200 kg (zebra) (Stuart & Stuart 2000; Skinner & Chimimba 2005). Leopards likely hunted warthog frequently and reedbuck as leopards can efficiently kill prey weighing up to 70 kg (Norton *et al.* 1986; Stander *et al.* 1997; Mills & Harvey 2001). A meta-analysis of 33 studies also revealed that warthog is the fourth most commonly hunted prey of leopards (Hayward *et al.* 2006).

The specialised feeding behaviour of M70 suggests individual dietary preferences. Such individual dietary specialisation has been witnessed in Lake Kariba where a leopard fed predominantly on bush pigs and another that mainly consumed fish (Fey 1964), and in the Kalahari Gemsbok National Park where an individual was recorded to feed principally on porcupines (Bothma & le Richie 1984), despite the availability of more common prey.

The precise reconstruction of animals' diets using SIA is often challenging due to isotopic similarities across prey species within browsing and grazing groups (Gannes *et al.* 1998). In savanna ecosystems, the approach has been used with great success to distinguish the diets of consumers in terms of C_3, C_4 or intermediate prey proportions assimilated (e.g. Codron *et al*. 2007). Although the diets of Phinda leopards could not be resolved at species-level, the prey types utilised by the individuals were similar to findings of an early study that used traditional methods to assess leopard diet. The consistency between the results of this study and those of Balme *et al.* (2007) suggests that SIA of whiskers can provide a reliable estimation of leopard diet.

The quantification of leopard diet was, however, limited by some factors that are worth discussing. The assumption that each 2 mm whisker section represented a single feeding event might be a misrepresentation. It is possible that the mid-point between two feeding events was sectioned, and higher-resolution sampling (i.e. 1 mm increments) may have provided evidence for use of small-bodied browsers. Furthermore, an individual might not have consumed any prey for more than five days, yet every five day period was treated as a possible feeding event. Therefore, one cannot confidently prove that an exact feeding event was sampled at each interval. The study also assumed that the bulk of prey tissue ingested by the leopards was muscle. However, carnivores often ingest multiple body tissues of their prey with varying isotope signatures such as crushed bones and hair (Codron *et al.* 2007). Ingestion of bones might lead to an overestimation of assimilated C_4 or grazer prey observed in the leopard individuals due to the enriched $\delta^{13}C$ values of bone collagen and apatite relative to muscle (Tieszen *et al.* 1983; Ambrose & Norr 1993).

Another limitation to this study was the non-availability of leopard whisker-diet TDFs, necessitating the use of lion discrimination values to account for the isotopic difference between the diet of leopards and those of prey muscle. Tissue-diet isotopic discrimination was reported to vary among felid species due to different assimilation and metabolic efficiencies (Parng *et al.* 2014; Montanari & Amato 2015), therefore, use of lion values might have affected the accurate inference of leopard diet in this study. The lack of leopard tissue turnover rates that result in whisker formation probably influenced the presented findings as well. Similar to TDFs, isotopic tissue turnover has been reported to vary with the tissue,

species, growth rate and sex of individuals (Caut *et al.* 2009; Reich *et al.* 2008). Both species-specific tissue-diet TDFs and turnover rates are a major limitation to isotopic studies as experiments can be costly and time consuming (Crawford *et al.* 2008; Boecklen *et al.* 2011; Rosenblatt & Heithaus 2013). Thus, estimates for most species remain unquantified posing difficulties to make reliable inferences from stable isotopes. However, the consistency of the present study's results and those of Balme *et al.* (2007) suggest that the use of lion whisker-diet TDFs was probably realistic.

Lastly, the non-significant difference in $\delta^{15}N$ among prey species that were potentially consumed by the leopards meant that only carbon could be used to assess the diets of individual leopards. This made it impossible to use stable isotope mixing models to quantify the proportional contributions of prey species to leopard diet, which in general can improve the accuracy of diet estimation. Parnell *et al.* (2010) pointed out that Bayesian inferences will always attempt to fit a model, however, use of the model with only one isotope often under- or over-represents the contribution of certain prey species.

3.5 Conclusion

The analysis of carbon and nitrogen isotopic ratios in 60 mm of whiskers of six leopards in Phinda revealed that intermediate feeders were the dominant prey type consumed by all the leopards, while grazers were less and browsers insignificantly consumed. Despite the outlined limitations, the consistency between the results from this study and those derived using traditional approaches demonstrates the potential of whisker isotopic analyses to infer felid diets. Hence, whiskers could be used to make diet inferences for animals even with no observational history. The method also demonstrated strength in identifying individual foraging histories, something that is financially and time expensive to obtain with traditional approaches (Inger & Bearhop 2008). Knowledge of individual resource utilisation is essential for the development of effective conservation and management strategies of felid species, especially in an era where felid numbers are declining globally. Use of prey by the individual Phinda leopards will be investigated further in Chapter 4.

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CHAPTER 4

THE USE OF STABLE ISOTOPE ANALYSIS OF LEOPARD WHISKERS TO INFER DIETARY PREFERENCE AND NICHE SEPARATION AMONGST INDIVIDUALS

4.1 Introduction

The diets of animals have usually been described either at a species or population level with the implicit assumption that all individuals within the population use resources (i.e. habitats and prey) similarly (Feinsinger *et al.* 1981). Less consideration has been given to individual differences in foraging behaviour (Bolnick *et al*. 2003; Loudon *et al.* 2007, Araujo *et al.* 2011). In recent years, it is increasingly recognised that populations are often composed of individuals that actively select different prey from their shared environment (e.g. Bolnick *et al.* 2003; Araujo *et al.* 2007a; Newsome *et al.* 2009; Cryan *et al.* 2012; Robertson *et al.* 2014). Such intra-population niche variation is commonly a by-product of sexual dimorphism, morphological differences, ontogenetic niche shifts and microhabitat use (Skulason & Smith 1995; Durell 2000; Temeles *et al.* 2009; Radloff *et al.* 2012). However, there are cases where individuals display substantial variation in foraging behaviour that is not attributed to these factors, but to 'individual specialisation' (Robertson *et al.* 2014). Such behaviour, in which individuals use only a sub-component of the population's overall available resources, has been recorded in many invertebrate and vertebrate taxa, and may be more widespread than previously thought (Bolnick *et al.* 2003; 2007; Araujo *et al.* 2007a). Intra-population niche variation has profound implications for ecological (e.g. population dynamics and interspecific competition) and evolutionary processes (e.g. speciation and adaptation) (Bolnick *et al.* 2003; Dall *et al*. 2012; Violle *et al.* 2012). Therefore, identifying and understanding its role in population ecology can improve species conservation strategies.

The realisation that ecological traits vary among individuals has often been underappreciated due to limitations posed by the use of traditional dietary analyses (Bolnick *et al.* 2003). Most studies have relied on direct observations of feeding events (e.g. West 1986), radio-telemetry (e.g. Bourke *et al.* 1997; Begg *et al.* 2003) or stomach content sampling (e.g. Bryan & Larkin 1972; Robinson *et al.* 1993; Warburton *et al*. 1998; Novak & Tinker 2015) to infer the dietary niche of individuals. However, studies seldom extend over multiple seasons or years, during which prey abundance can vary markedly (Bolnick *et al.* 2002; 2003). To account for such variation, dietary information should be collected from individuals over protracted periods (Newsome *et al.* 2009). A plethora of investigations on intra-population niche variation have used stable isotope analysis (SIA), particularly the

measurement of carbon and nitrogen isotope ratios in consumer tissues, to examine temporal consistency in the diets of individuals (e.g. Fry *et al.* 1978; Angerbjorn *et al.* 1994; Gu *et al.* 1997; Araujo *et al.* 2007b; Newsome *et al*. 2009; Vander Zanden *et al.* 2010; Robertson *et al*. 2014).

Stable isotopes of carbon ($\delta^{13}C$ or $^{13}C/^{12}C$) have been successfully used in subtropical environments to distinguish between herbivore species or populations that rely on ^{13}C-depleted C_3 vegetation (browsers), and those that predominantly utilise ^{13}C-enriched C_4 grasses (grazers) through space and time (Vogel 1978; Cerling & Harris 1999; Sponheimer *et al.* 2003; Codron & Codron 2008; Codron *et al.* 2016; Radloff *et al.* 2013). Similarly, the proportion of browsing, grazing and intermediate feeding prey consumed by carnivores is reflected in the carbon isotope variance of their tissues (Newsome *et al.* 2009). The carbon isotope signatures of carnivore tissues can also reflect the availability of prey within habitats. For example, they may show higher levels of browser and grazer prey intake in densely and sparsely wooded habitats, respectively (Angerbjorn *et al.* 1994; Codron *et al*. 2007). Nitrogen stable isotopes ($\delta^{15}N$ or $^{15}N/^{14}N$), on the other hand, signify the trophic position of a consumer because of the predictable enrichment of ^{15}N along the food chain (Minagawa & Wada 1984; Post 2002). Therefore, animals occupying higher trophic levels exhibit higher $^{15}N/^{14}N$ ratios, although these can be affected by rainfall, nutritional stress and ecophysiology (Sealy *et al.* 1987; Ambrose 1991).

The use of SIA to infer individual dietary niche requires individuals to produce an incrementally growing, metabolically inert tissue that can be serially sub-sampled to provide a longitudinal archive of dietary change (Gannes *et al.* 1998; Bearhop *et al.* 2004; Newsome *et al.* 2009). Inert tissues (i.e. teeth, scutes, baleen, horns, feathers, hair and whiskers) have been used to validate within- and between-individual diet variation and specialisation across taxa including coyotes, badgers, elephants, sea turtles and sea otters (Newsome *et al.* 2009; 2015; Vander Zanden *et al.* 2010; Codron *et al.* 2012; Robertson *et al*. 2014), but has never been applied to large felids. Whiskers, like any other hair, are an informative isotopic substrate for quantifying intra-population diet variation, as well as individual diet specialisation in both marine and terrestrial carnivores (e.g. Newsome *et al.* 2009; 2010; 2015a, 2015b; Robertson *et al.* 2014; 2015). The tissue grows from the basal epidermis, and constantly accumulates dietary isotopes with the oldest information at the distal tip of the whisker (Tyrrell *et al*. 2013). Successive sampling of whisker micro-layers can reveal ^{13}C and ^{15}N isotopic variation, which is a reflection of the variation in isotopic composition of diet consumed (Hobson *et al.* 1996; Newsome *et al.* 2009). Moreover, isotopic information integrated in whiskers remains constant over time, hence, the method is not subject to

stochastic sampling as are other traditional approaches, and is potentially more reliable at understanding intra-population diet variation (Rubenstein & Hobson 2004; Araujo *et al.* 2007b).

In this study, the feasibility of using SIA of leopards' *Panthera pardus pardus* whiskers was explored to determine whether it can provide a better understanding of intra-population diet variation. Serial SIA of whiskers was applied to six leopards in northern KwaZulu-Natal (KZN) to investigate individual prey preference and dietary niche separation. To achieve this, the $\delta^{13}C$ and $\delta^{15}N$ values of each 2mm sample along 60 mm of leopards' whiskers were compared to the prey available within the individual's home range. By quantifying local prey availability at a home range scale, it can be established whether individuals are selecting specific prey species or using the prey base in accordance to its availability (Angerbjorn *et al.* 1994, Ben-David *et al*. 1997).

4.2 Materials and methods

4.2.1 Study area

The study leopards were individuals of a resident population in Phinda Private Game Reserve (hereafter, Phinda). The reserve is situated in the Maputaland region of northern KZN, South Africa (27˚44'–27˚55' S and 31˚12'–32˚26'E), and covers an area of 220 km^2 (Balme 2009). Chapter 3 of this thesis, section 3.2.1, provides a detailed description of the study area, i.e. topography, climate and fauna. Only the vegetation types of the reserve will be discussed in detail here.

Phinda comprises a mosaic of different savanna woodlands and forests. Hunter (1998) classified the reserve into nine distinct vegetation or habitat types (Figure 4.1). Below is a brief description of the nine vegetation types according to Hunter (1998).

i) Closed mixed bushveld: The vegetation comprise a dense woodland, with an approximate distance of 10 m or less between neighbouring trees that are taller than 6 m. It is dominated by *Vachellia tortilis*, *V. nilotica, V. grandicornata, Senegalia senegal, Spirostachys africana* and *Schotia brachypetala*. *Eragrostis* and *Aristida* species (spp.) commonly occur in the poorly developed grass understorey.

ii) Open mixed bushveld: The same description and species composition of the closed mixed bushveld applies to this vegetation type. However, neighbouring trees above 6 m in height are 10 m or more away from each other.

iii) Closed red sand bushveld: It consists of 6–10 m tall woodland and scattered thickets; with a less than 10 m approximate distance between neighbouring trees that are taller than 6 m. The vegetation is dominated by *Senegalia burkei, Combretum molle, Sclerocarya caffra, Ziziphus mucronata, Albizia versicolor* and *Terminalia sericea*. The herbaceous layer is sparse and common species include *Aristida* spp., *Eragrostis* spp., *Panicum maximum* and *Pogonarthria squarrosa*.

iv) Open red sand bushveld: The same description and species composition of the closed red sand bushveld applies to this vegetation type, except that neighbouring trees above 6 m in height are 10 m or more distanced from each other.

v) Dry mountain bushveld: It is an open woodland that occurs on rocky soils, usually at altitudes that are 100 m or more above sea level. It is dominated by *Combretum apiculatum*, *Senegalia nigrescens, Heteropogon contortus*, *Themeda triandra*, *Cymbopogon excavatus* and *Aloe marlothii*.

vi) Palmveld: The vegetation principally consists of *Hyphaene natalensis* in open, diverse grassland that is dominated by *Themeda*, *Eragrostis*, *Aristida* and *Perotis* spp. Common associated trees include *Phoenix reclinata*, *Dichrostachys cinerea* and *Strychnos madagascariensis*.

vii) Grasslands: The reserve is home to two types of grasslands that are grouped into a single unit. Both have tall tussocks, but one occurs on seasonally overflowing floodplains and species such as *Phragmites australis*, *Echinochloa pyramidalis*, *Erichloa* and *Sorghum* spp. are common. The other type of grasslands is found in areas that were formerly affected by intensive cultivation, and they are dominated by *Aristida*, *Themeda*, *Tristachya* and *Paspalum* spp.

viii) Sand forest: Endemic to the Maputaland region, the critically endangered sand forest comprise dense thickets of 6–15 m (although high canopies reaching to 25 m occur), a well developed shrub layer and a non-existence herbaceous layer (Mucina & Rutherford 2006). Important tree species include *Newtonia hilderbrandtii*, *Cleistanthus schlechteri* and *Croton gratissimus*. The common shrubs include *Croton pseudopulchelus* and *Pteleopsis myrtifolia*.

ix) Riparian woodland: This well developed woodland occurs adjacent the two main rivers flowing in the reserve, the Munyawana and Mzinene. The main woody species include *Vachellia xanthophloea*, *V. robusta*, *Spirostachys africana* and *Trichilia emetica*. The dense shrub layer is dominated by *Senegalia schweinfurthii*, *Azima tetracantha* and the invasive *Chromolaena odorata*.

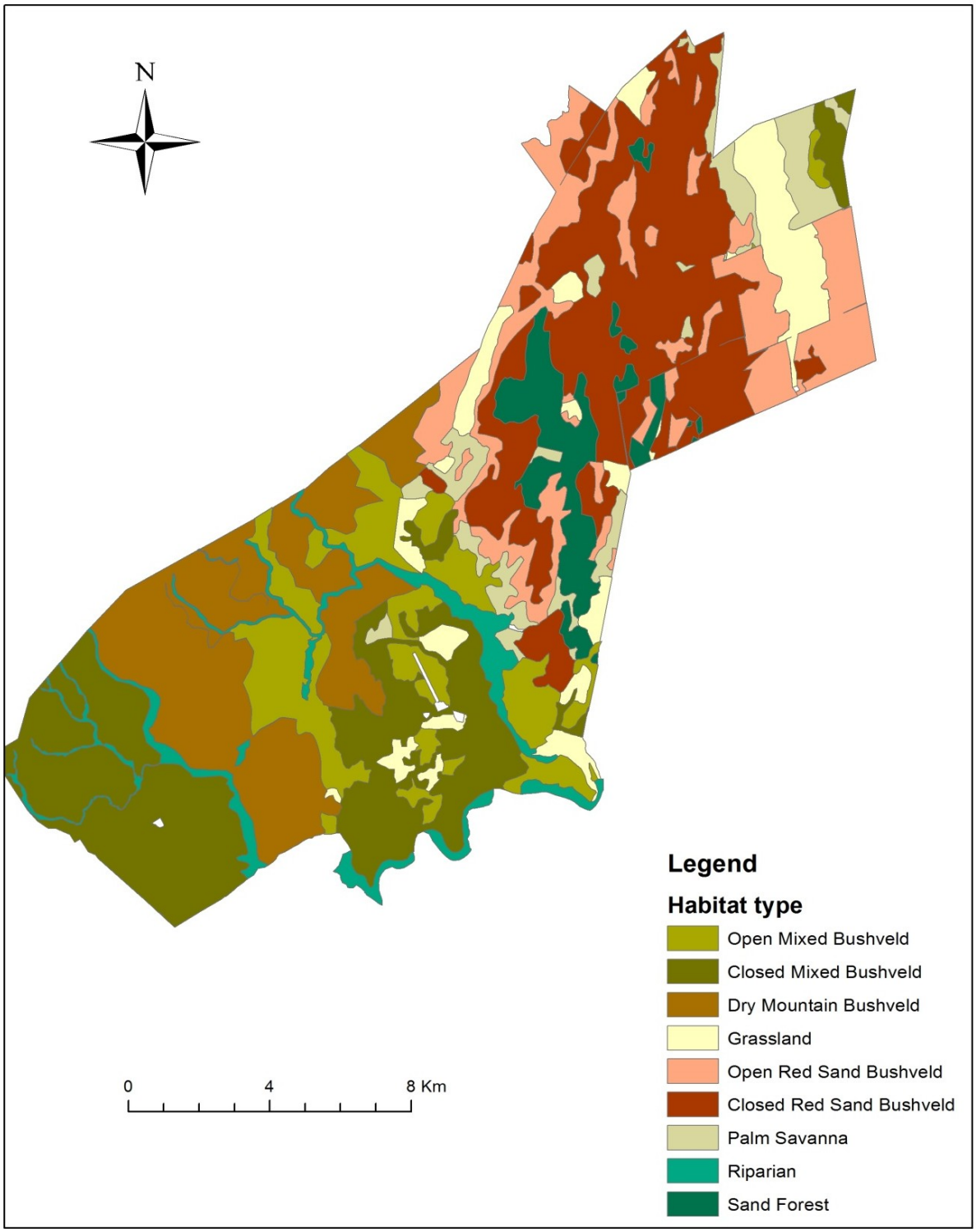

Figure 4.1: Map showing the nine distinct vegetation (habitat) types of Phinda

4.2.2 Dietary niche differentiation

The discrimination-adjusted and turnover-corrected $\delta^{13}C$ and $\delta^{15}N$ values of the six Phinda leopard whiskers presented in Chapter 3 were used to determine dietary niche differences amongst individuals. Whiskers were removed (one from each animal) in 2011 as part of

Panthera's Munyawana Leopard Project, and these were made available for this study. The whiskers were cut into 2 mm increments over 60 mm starting from the root, corresponding to approximately 92 days of growth. Table 4.1 provides detail on leopard identification, sex, age and date of whisker removal. The capture and handling protocol of the leopards were approved by the Animal Ethics sub-committee of the University of KZN Ethics Committee (approval 051/12/Animal).

Table 4.1: Sex, age and date of whisker removal for the six study leopards

Individual	Sex	Age (years)	Date of whisker removal
F08	Female	10.8	02/04/2011
M60	Male	7.1	06/06/2011
M62	Male	4.1	09/08/2011
M70	Male	1.6	15/08/2011
M73	Male	2.6	14/06/2011
M74	Male	3	24/08/2011

4.2.3 Prey preference

A prey item is considered as preferred when it is consumed more frequently than it is available (Ivlev 1961; Jacobs 1974). Therefore, knowledge of prey use and prey availability/abundance is required to determine an individual's prey preference (Hayward & Kerley 2005; Hayward *et al.* 2006). As Phinda leopard prey cannot be accurately identified at the species-level based on ^{13}C data (Chapter 3), prey preference was investigated with regard to prey types (i.e. browser, intermediate feeder and grazer).

4.2.3.1 Prey use

The amount of browser, intermediate feeder and grazer prey utilised by the leopards was estimated by grouping discrimination-adjusted and turnover-corrected $\delta^{13}C$ values of each individual's whisker into the three prey types (Table 4.2; Chapter 3).

Table 4.2: The quantity of browsers, intermediate feeders and grazers consumed by the six leopards

Individual	% Browsers	% Intermediate feeders	% Grazers
F08	10.30	89.70	0.00
M60	0.00	78.57	21.43
M62	3.45	96.55	0.00
M70	0.00	15.79	84.21
M73	0.00	93.10	6.90
M74	0.00	79.31	20.69

4.2.3.2 Prey availability

To quantify the prey available to each leopard, the core home ranges of individuals were first determined using very high frequency (VHF) collar data. The area covered by the different vegetation types within each leopard's home range was then calculated, and used in conjunction with the available habitat prey density data to estimate an individual's prey availability.

An animal's home range or utilisation distribution is defined as the area moved by an individual during its daily activities, including hunting, mating and caring for the young, at a specified time period (Burt 1943; Harris *et al*. 1990). In this study, information on the home range and vegetation types utilised by the individual leopards was obtained through radio-telemetry as explained by Balme *et al.* (2007). Leopards were immobilised using a combination of tiletamine and zolazepam (at 3.5 kg/mg), and fitted with VHF radio collars weighing approximately 250 g. Collared individuals were followed and located at least once daily, and their location (latitude and longitude) to the nearest 50 m was recorded using a handheld global positioning system (GPS) receiver or by radio-triangulation when close approach was not possible. GPS locations were downloaded to a computer, and used to calculate home ranges of the six Phinda leopards. Spatial data were collected over different time periods for the individuals (Table 4.3); however, all the available GPS locations for each individual were used in home range calculations.

Table 4.3: The time periods of the recorded GPS locations for the six leopards

Individual	Time period
F08	03/02/2003 – 02/09/2012
M60	29/10/2008 – 27/06/2012
M62	08/11/2010 – 23/07/2012
M70	14/03/2011 – 03/06/2012
M73	16/06/2011 – 29/11/2011
M74	31/07/2011 – 15/11/2011

Six prey species (i.e. nyala *Tragelaphus angasii,* impala *Aepyceros melampus*, warthog *Phacochoerus africanus*, grey duiker *Sylvicapra grimmia*, red duiker *Cephalophus natalensis* and reedbuck *Redunca arundinum*) accounted for 86 % of the diet of Phinda leopards (Balme *et al*. 2007). The densities of these species within each of the respective vegetation types were extracted from Balme *et al.* (2007) (Table 4.4). These values were estimated using road strip sampling whereby two separate transect routes totalling 70 km, which passed through all the reserve's vegetation types, were driven at least three times over 6-8 days twice a year (in wet and dry seasons) from 2002 to 2005. Estimated density was calculated as the number of animals counted of each species divided by the total distance driven in each habitat type, while accounting for variable limits (i.e. effective sampling widths) of the different species (Balme *et al.* 2007).

Table 4.4: The mean estimated density (animals/km^2) per vegetation type of the six primary prey species fed on by leopards at Phinda (Balme *et al*. 2007)

Vegetation type	Grey duiker	Impala	Nyala	Red duiker	Reedbuck	Warthog
Closed mixed bushveld	0.00	15.66	36.41	2.77	0.00	5.19
Open mixed bushveld	1.17	29.05	5.44	0.00	0.00	6.27
Closed red sand bushveld	0.74	3.94	30.29	3.93	0.00	0.34
Open red sand bushveld	3.12	27.00	15.47	0.00	0.21	5.35
Dry mountain bushveld	1.12	6.19	3.10	0.00	0.00	0.82
Palmveld	1.08	0.00	3.51	0.00	2.42	3.69
Grassland	0.54	10.21	2.64	0.00	0.56	4.26
Sand forest	0.00	0.00	30.59	14.88	0.00	0.46
Riparian woodland	0.00	3.82	60.08	7.67	0.00	0.00

4.2.4 Data analysis

4.2.4.1 Leopard home ranges

The home ranges of the individual leopards were estimated by means of kernel density estimation (KDE) approach, which uses the density of locations within each grid cell to describe the frequency or amount of time an individual spends in an area (Worton 1989; Kenward *et al.* 2001). All available downloaded GPS locations for the six leopards were projected to the UTM coordinate system (WGS 1984 UTM Zone 35S) for use in ArcView GIS, version 10.3 (ESRI 2014) and Geospatial Modelling Environment (GME), version 0.7.4 (Beyer 2014) that runs in R programme language, version 3.1.3 (R Core team 2013). Thereafter, the locations for each leopard individual were displayed in ArcMap, exported as a shapefile, and clipped to the study area boundary layer. The bivariate '*kde*' function of the GME and plug-in algorithm for bandwidth estimation were used to determine the possible extent of each leopard's home range (Beyer 2014). Home ranges for individual KDE rasters were created as 90 % utilisation polygons using the '*isopleth*' function in GME. Ninety percent utilisation is considered a more accurate estimator of an animal's total range size as it represents the area that an animal spends the majority of its time in (Borger *et al.* 2006). Home range sizes and the area (km^2) of each habitat type within an individual's home range were calculated in ArcView GIS.

4.2.4.2 Prey availability

Prey availability for each individual leopard was calculated by multiplying the area of each vegetation type within an individual's home range by the mean density for each prey species within each vegetation type (as presented in Table 4.4). Prey species were then grouped into the main three herbivore feeding types, i.e. grazers (reedbuck and warthog), intermediate feeders (nyala and impala) and browsers (red duiker and grey duiker) using the dietary description in Skinner & Chimimba (2005). The proportion of grazers, intermediate feeders and browsers available to each leopard was then quantified.

4.2.4.3 Prey preference

Individual leopard prey preferences were assessed using the Jacobs' selection index, which is one of the indices that minimise biases associated with small prey sample size, rare food items and non-linearity in proportional use of prey over time (Jacobs 1974; Hayward *et al.* 2006). The index is defined as;

$$D = \frac{r-p}{r+p-2rp} \quad (1)$$

where r is the proportion that a respective prey type (browser, intermediate feeder and grazer) makes up of the total number of kills (determined by the $\delta^{13}C$ value of each 2 mm whisker segment) and p is the proportional availability of the respective prey type within an individual's home range (Jacobs 1974). The resulting value ranges from 1 (indicating exclusive feeding on a given prey) to -1 (indicating total avoidance of a prey) (Jacobs 1974). Values between 0.2 and -0.2 denote that the prey was consumed proportionally to what was available, while values between 0.2 and 1 indicate preference and those between -0.2 and -1 indicate avoidance (Hayward *et al.* 2011).

4.2.4.4 Dietary niche differentiation

Mixed effects linear models (MELMs) were used to compare leopard isotopic profiles across individuals, and to test for the effects of prey availability on these. For the models, $\delta^{13}C$ and $\delta^{15}N$ values were dependent variables, respectively. "Individual" was included as a random effect, and "prey availability" was classified as either "high browsers/low grazers" (> 10 % of available prey were browsers, and < 10 % were grazers) or "low browsers/high grazers" (< 10 % of available prey were browsers, and > 10 % were grazers). Proportions of available prey that were intermediate feeders were high and did not vary across habitats (~80 to 85 % in all cases, Figure 4.3). Thus, further resolution of differences in available prey were not possible, at least in the context of this isotope-based study for which diets are only resolved at the scale of browser/grazer/intermediate feeder. Individual was treated as a random effect to ensure appropriate error terms were compared in the analysis where repeated (non-independent) measurements were taken for each individual. Variance components analyses (VCAs) were used to quantify the percentage explained by within- (the overall error term) versus between-individual differences in diet (Newsome *et al.* 2009; Vander Zanden *et al.* 2010; Codron *et al.* 2016). MELMs and VCAs were performed in R software, version 3.1.3 (R Core Team 2013) with add-on nlme (Pinheiro *et al.* 2016) and ape (Paradis *et al.* 2004) packages. Assumptions of normality and homoscedasticity of the data were not violated based on Shapiro-Wilk's *W* (Shapiro & Wilk 1965) and Levene's (Zar 2010) tests, respectively. A significance level of 0.05 was used in all tests.

The isotopic niche breadth of each leopard was defined using Stable Isotope Bayesian Ellipses in R (SIBER, using R version 3.1.3) implemented in the Stable Isotope Analysis in R (SIAR) package (Jackson *et al.* 2011). Individual isotopic breadths were measured as the standard ellipses area, adjusted for small sample sizes (SEA*c*), in $\delta^{13}C$ and $\delta^{15}N$ bi-space. This approach provides information about the core aspects of an individual's niche, and is less sensitive to outliers and small sample sizes (n < 30, Syvaranta *et al.* 2013). Ellipse areas of individuals, and the degree of overlap, were compared by Monte Carlo comparisons

of Bayesian posterior distributions of estimated SEA*c*, over 10^4 iterations, using a significance level of 0.05. Note that niche overlap estimates were standardised to 1, where a value of 0 indicated no overlap, and 1 indicated complete overlap between pairs of individuals.

4.3 Results

4.3.1 Leopards' home ranges and prey availability

The home ranges of the respective leopards are displayed in Figure 4.2 and the representation of the respective vegetation types within each home range is summarised in Table 4.5. Individual M60 had the largest home range size (~95.63 km^2, approximately 44 % of the reserve's total area), followed by M74 whose home range measured 66.42 km^2. The estimated home ranges of F08, M62 and M73 were 46.83 km^2, 35.68 km^2 and 17.24 km^2, respectively. M70 had the smallest home range of 15.33 km^2. The closed red sand bushveld dominated the home ranges of M60, F08 and M62, while the closed mixed bushveld was the major vegetation type found in home ranges of M73 and M74. M70 was mostly made use of the open red sand bushveld. The home ranges of M62, M73 and M74 included the dry mountain bushveld, but M74 had a larger area of this vegetation type (17.23 km^2) in his total home range. F08 and M62 had higher percentages of the sand forest in their ranges, than the other felids, i.e. 18.51 and 12.36 %, respectively. Although the home ranges of all six leopards included portions of grassland, M70 had a much larger proportion (34.18 %) of this vegetation type within his small home range.

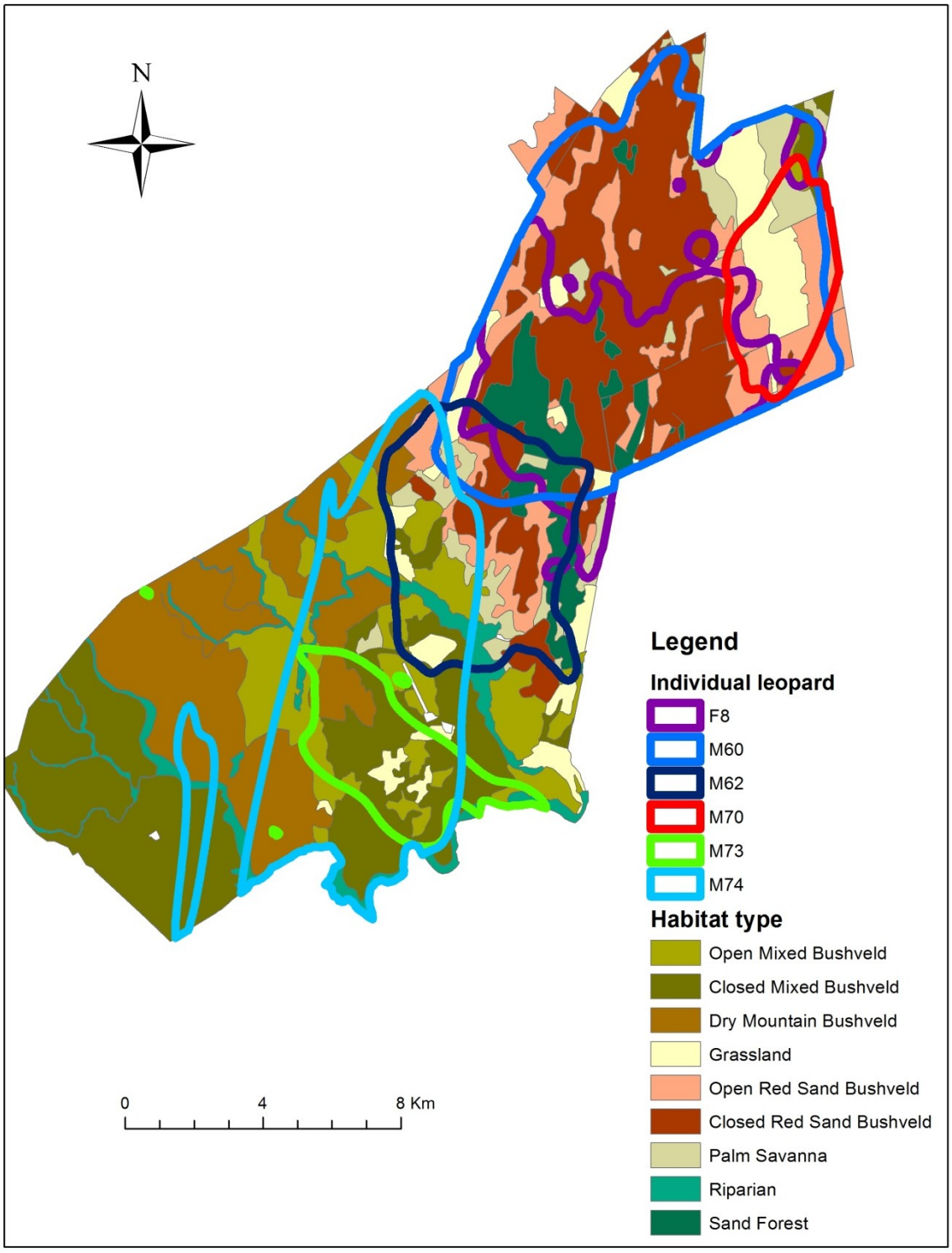

Figure 4.2: Vegetation map of the study area showing the home ranges, estimated as 90 % isopleths, of the six leopards

Table 4.5: The size/area of each vegetation type within each leopard's home range and the total home range size, measured in km^2

Leopard ID	Closed mixed bushveld	Open mixed bushveld	Closed red sand bushveld	Open red sand bushveld	Dry mountain bushveld	Palm veld	Grassland	Sand forest	Riparian Woodland	Home range size
F08	1.01	0.43	25.07	8.72	0.00	1.39	1.55	8.67	0.00	46.83
M60	1.14	0.53	45.38	20.93	0.00	6.98	11.55	9.11	0.00	95.63
M62	2.43	4.32	7.85	6.36	0.99	4.80	2.53	4.41	2.00	35.68
M70	0.39	0.36	0.51	7.25	0.00	1.58	5.24	0.00	0.00	15.33
M73	9.66	3.21	0.00	0.00	2.62	0.00	1.25	0.00	0.50	17.24
M74	20.84	15.71	1.03	2.28	17.23	1.89	3.72	0.00	3.72	66.42

Intermediate feeders were the dominant prey available to the six leopards; they comprised 81.34 % of prey in F08's, 81.51 % in M60's, 78.61 % in M62's, 79.56 % in M70's, 85.50 % in M73's and 84.58 % in M74's home range (Figure 4.3). The proportion of grazers available to the leopards was generally low (mean = 8.88 ± 3.56 %), although M70 had marginally more grazers available in his home range (14.70 %) than other individuals. Availability of browsers was also low for leopards (mean = 9.27 ± 4.47 %), but slightly greater in the home ranges of F08 (14.30 %), M60 (11.81 %) and M62 (13.68 %).

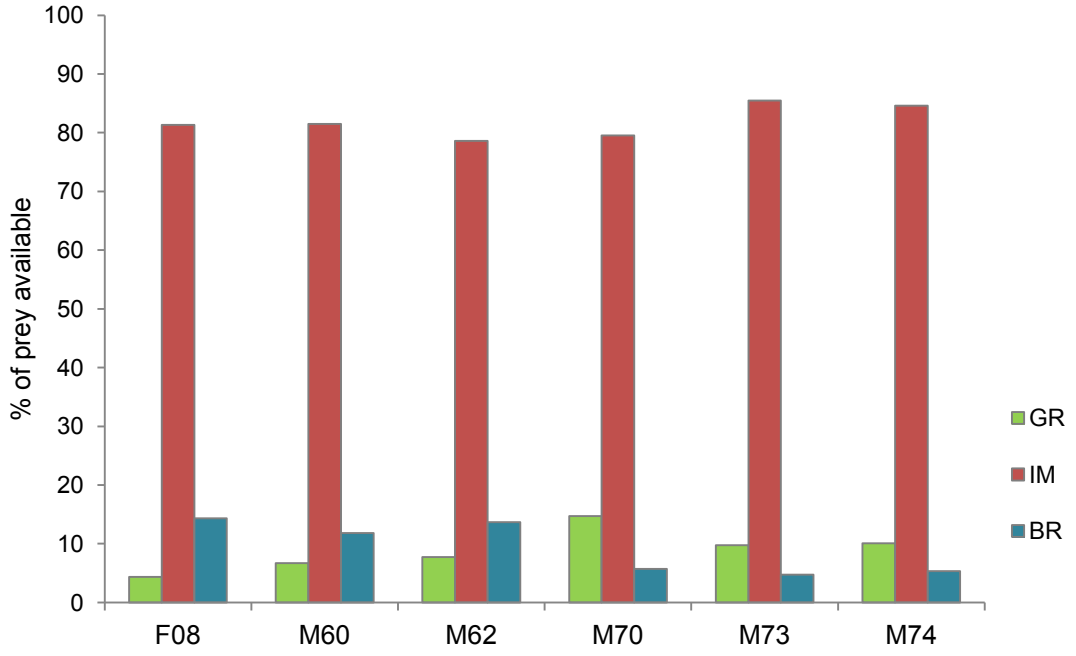

Figure 4.3: Percentage of browser (BR), intermediate feeder (IM) and grazer prey available in the home ranges of the six leopards

4.3.2 Prey preferences

Intermediate feeders were preferred by leopards F08, M73 and especially M62 (Jacobs' index = 0.77, Figure 4.4). In contrast, M60 and M74 selected intermediate feeders in accordance to their abundance, while M70 avoided intermediate feeders (Jacobs' index = -0.91). Grazers were preferred by M60 and M74 (Jacobs' index = 0.58 and 0.40, respectively), but particularly by M70 (Jacobs' index = 0.94). M73 utilised grazers in proportion to their availability in his home range, while F08 and M62 totally avoided grazers (Jacob's index = -1). Four leopards (M60, M70, M73 and M74) completely avoided browsers (Jacob's index = -1), while M62 avoided browsers to a lesser extent (Jacobs' index = -0.63). F08 was the only leopard that fed on browsers in accordance with their availability.

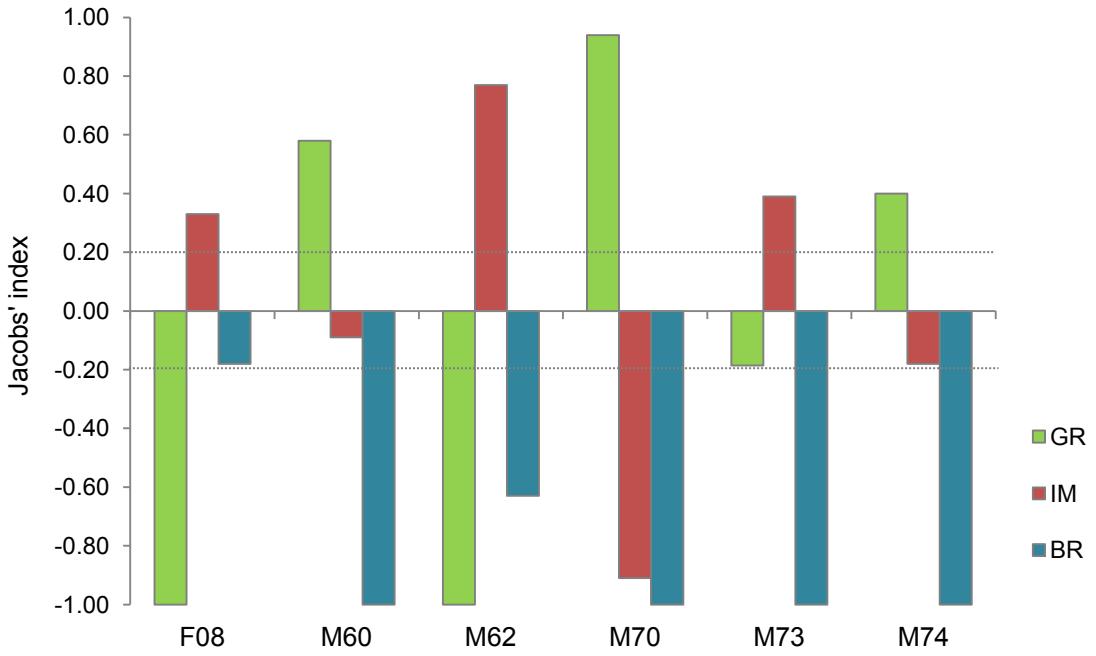

Figure 4.4: Prey, i.e. grazers (GR), intermediate feeders (IM) and browsers (BR), preferences of the six Phinda leopards based on the Jacobs' index. Values greater than 0.2 and less than -0.2 indicate preference and avoidance, respectively (dashed lines).

4.3.3 Dietary niche differences amongst leopards

Individual $\delta^{13}C$ niche ranges showed differences between individuals, whereas $\delta^{15}N$ niche ranges overlapped considerably (Figure 4.5). VCAs revealed that between-individual differences accounts for 67 % of the total variance in $\delta^{13}C$ data, but only 29 % of the variance in $\delta^{15}N$ data. Overall, stable carbon isotope analysis indicated differences in diet between individuals. Moreover, differences in prey availability had no significant effect on leopard $\delta^{13}C$ values ($F_{(1)} = 1.99$, $p = 0.23$) because there were little differences in proportions of prey available to the individuals (Figure 4.3). The lack of variation in $\delta^{15}N$ data across individuals is likely because of the low variation in $\delta^{15}N$ across prey types in Phinda (see Chapter 3). Not surprisingly, prey availability also did not influence leopard $\delta^{15}N$ values ($F_{(1)} = 5.48$, $p = 0.08$).

Isotopic niche breadths, (measured as the standard ellipse areas, SEA*c*), were similar for all individuals (range = 1.70 to 2.34, pairwise Monte Carlo comparisons $p = 0.10$–0.90, Figure 4.5). Isotopic niche overlap between individuals, on the other hand, was generally low with a maximum of 0.40 between M73 and M74 (Table 4.6). This was consistent with the high rate of inter-individual differences in diet inferred from the VCAs (at least for $\delta^{13}C$ values).

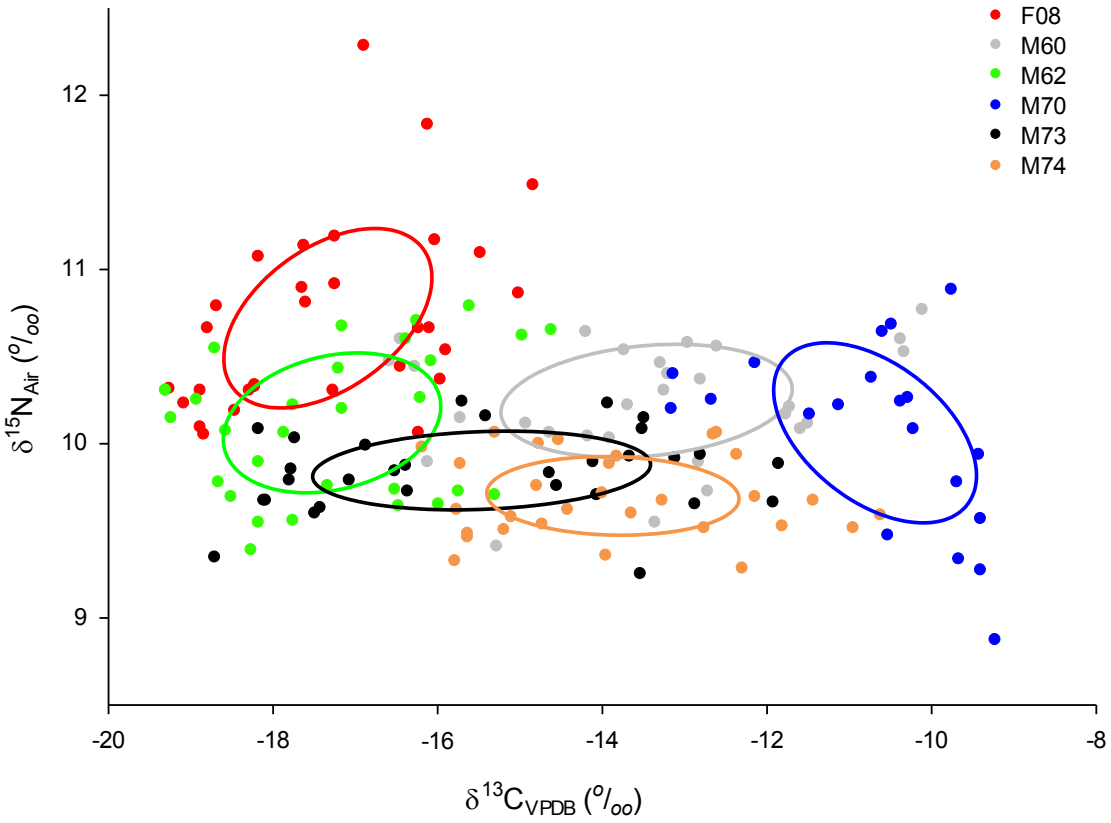

Figure 4.5: Isotopic variability amongst the six Phinda leopards, measured as standard ellipse areas (SEA*c*), in carbon and nitrogen bi-space

Table 4.6: The magnitude of isotopic niche overlap of the six leopards (the non-symmetrical values indicate overlap between two leopards compared as columns and rows)

			Overlap			
Individual	F08	M60	M62	M70	M73	M74
F08	1.00	0.00	0.21	0.00	0.00	0.00
M60	0.00	1.00	0.00	0.04	0.11	0.01
M62	0.23	0.00	1.00	0.00	0.17	0.00
M70	0.00	0.05	0.00	1.00	0.00	0.00
M73	0.00	0.13	0.19	0.00	1.00	0.30
M74	0.00	0.02	0.00	0.00	0.40	1.00

4.4 Discussion

The use of SIA of whiskers to determine the dietary preference and niche of Phinda leopards provided unique dietary information by clearly showing differences amongst individuals, at

least in $\delta^{13}C$. Although the main prey species eaten and habitats preferred by this leopard population were determined by Balme *et al.* (2007) using continuous follows and radio-telemetry, dietary knowledge at the individual level remained unknown.

In this study, proportional prey availability was similar across all home ranges, with high numbers of intermediate feeders, and low availability of browsers and grazers (Figure 4.3). This was irrespective of the home range size and habitats traversed by the individual leopards (Figure 4.2; Table 4.5). Interestingly, $\delta^{13}C$ of whisker series showed that there was a high (67 %) between-individual niche variation, indicating differences in diet amongst individuals, despite the similarity in prey availability. Thus, the dietary differences displayed by the leopards reflect individual foraging traits rather than differences in prey availability. For example; M70 used grazers disproportionally more than the other leopards, while only F08 consumed browsers in accordance to their availability and all other individuals avoided browsers (Figure 4.4). On the other hand, the low within-individual variation of $\delta^{13}C$ whisker series suggest that the spatial and foraging habits of individual leopards were consistent over the estimated 92 days of foraging reflected in 60 mm of whiskers (Chapter 2).

Dietary studies conducted, using similar SIA methods, on a variety of mammalian and non-mammalian carnivore, omnivore and herbivore species such as elephant seals *Mirounga leonine* (Huckstadt *et al.* 2011), sea otters *Enhydra lutris nereis* (Newsome *et al.* 2009), coyotes *Canis latrans* (Newsome *et al*. 2015a), sea turtles *Coretta coretta* (Vander Zanden *et al*. 2010), bats *Eptesicus fuscus* (Cryan *et al*. 2012) and badgers *Meles meles* (Robertson *et al*. 2014) also found that the diets of individuals mostly differed between- rather than within-individuals, in terms of $\delta^{13}C$. In contrast, fur seals *Arctocephalus gazella* and elephants *Loxodonta africana* displayed greater within-individual variation in $\delta^{13}C$ indicative of periodic foraging shifts (Cherel *et al.* 2009; Codron *et al*. 2012).

$\delta^{15}N$ whisker series showed a low (29 %) between-individual variation, possibly influenced by the similarities of $\delta^{15}N$ values between C_3 and C_4 plants of the study area, and of the three prey types (see Chapter 3). This supports the notion expressed in Chapter 3 that nitrogen-15 could not provide dietary insight on Phinda leopards, and the same likely applies to other large predator species within the ecosystem. $\delta^{15}N$ whisker data of elephant seals and badgers also showed a low between-individual variation, i.e. 36 and 30 %, respectively, likely associated with similarities of $\delta^{15}N$ values of prey within a location (Huckstadt *et al.* 2011; Robertson *et al*. 2014).

The diets of the six Phinda leopards generally reflected individual prey preferences (Figure 4.4). Three individuals (i.e. F08, M62 and M73) preferred intermediate feeders (in this case

nyala and impala), which concurs with findings of Hayward *et al.* (2006) who, through a meta-analysis of 33 studies, showed that impala is the most preferred prey species of the leopard in areas where the prey is abundant. On the other hand, leopards M60, M70 and M74 showed preference for grazers (presumably warthog and/or reedbuck), in spite of their low proportional availability across home ranges (i.e. < 15 %, Figure 4.3). The very high affinity for grazers shown by M70 is worth noting. The feeding behaviour of M70 was perhaps associated with functional trade-offs or the realised profitability (i.e. nutritional value and easy to capture) of exploiting grazers (Svanback & Bolnick 2005). Therefore, the individual may have learned special hunting and handling skills to efficiently capture and process the prey (Robinson & Wilson 1998). In addition, the dietary preference of M70 might have been influenced by age-specific spatial resource partitioning, which limited intra-specific competition with the adult male M60, since the young leopard's (less than 1.6 years at the time of whisker growth) home range was held within M60's home range (Figure 4.2).

Other studies that used SIA to determine the feeding habits of individuals also found individual dietary preferences. For instance, Yeakel *et al.* (2009) analysed tail hair of two co-operative Tsavo lions *Panthera leo* and disclosed that one individual preferred to hunt on humans for a period of three months. Newsome *et al*. (2015a) showed, using whisker analyses, incidences whereby some coyote individuals specialised on either anthropogenic foodstuffs or natural prey, despite having small portions of habitats that supported the two resources in their home ranges. On the contrary, young southern elephant seals were reported to be more generalist feeders (Huckstadt *et al.* 2011).

The avoidance of browsers by Phinda leopards (except F08) was not surprising, as a previous study showed that leopards preferred hunting in habitats with intermediate cover levels where prey is much easier to catch (Balme *et al.* 2007). Although M60 and M62 had proportions of browser availability similar to F08, the avoidance of browsers by these two individuals can likely be attributed to the reduced probability of encountering prey in dense vegetation (i.e. closed red sand bushveld, closed mixed bushveld, riparian woodland and sand forest), the main habitat of duikers (Balme *et al.* 2007). In contrast, Hayward *et al.* (2006) reported that browsers, particularly species within the weight range of 10–40 kg, are a preferred prey item of the leopard.

The isotopic niche breadths (SEA*c*) of all leopards were similar, albeit individuals showed differences in diet, suggesting consumption of similar prey diversity. All individuals utilised two of the three prey types, either browsers and intermediate feeders or grazers and intermediate feeders, and each leopard predominantly made use of one prey group (Table 4.2; Chapter 3 Figure 3.6 A). Thus, individuals were equally generalist or equally specialist

feeders of the three prey types (Bolnick *et al.* 2007). The similarity of the niche breadths also suggest that none of the studied leopard individuals was particularly stressed or ecologically unfit, since marginally foraging individuals would be expected to have wider or narrower niche breadths (Araujo *et al.* 2011). This study also revealed a low magnitude of isotopic niche overlap between individuals, except for leopards M73 and M74, indicating that individuals utilised different proportions of the available prey.

This study showed the potential for SIA of whiskers to distinguish dietary specialisation amongst individual leopards, a behaviour not yet documented for the species but which has been described for a wide variety of other taxa (e.g. Bolnick *et al*. 2003; Robertson *et al*. 2014). To acquire such novel information in an easy and non-invasive manner, as it is with using the technique, is almost impossible with the use of traditional methods alone. Analysis of 60 mm of a whisker presents 92 days of dietary information and approximately 18 kills (assuming the longest time taken by leopards to kill a prey was after every five days, Bothma & le Richie 1984). Obtaining such amount of data without continuously following leopards in their natural habitat for months, even years, to record what they eat renders whisker isotopic analyses an advantage over traditional analyses. The successful reconstruction of carnivore diets, particularly patterns of individual niche variation, using SIA of whiskers can be achieved when whisker growth rate of the genus or species in question is known, prey items are isotopically distinct, the diversity of prey is small and when data on habitat usage and prey availability is available, as shown by the present and previous studies (e.g. Newsome *et al.* 2015a; Robertson *et al.* 2015). Although the isotopic approach used in this study does not typically reveal utilisation of prey at fine taxonomic levels (Gannes *et al.* 1998), it is a useful proxy for characterising inter-individual diet variation for large predators, especially for elusive species such as felids that are difficult to observe in the wild.

The inference of individual dietary niche variation was, however, limited by the small and uneven leopard sample size available for the study, i.e. five males and a female, therefore, the degree of dietary variability may be under-represented. Leopard whiskers were not harvested at the same time (Table 4.1) making it difficult to identify the time period or season represented by the dietary information contained in the analysed whiskers. Furthermore, the available spatial data for each individual used to calculate home range sizes were collected over different timeframes (Table 4.3). As a result, the leopard home range sizes presented here might not be a true reflection of the individuals' movements at the exact time of whisker growth. However, each leopard was utilising at least a part of its home range while the analysed whiskers were growing. The dietary data of each leopard only reflected a period of more or less three months; hence, the deduced variability may not be a reflection of long-term feeding patterns. Lumping of ecologically and isotopically convergent prey into three

prey types (i.e. browsers, intermediate feeders and grazers) might have led to underestimation of individual dietary preferences or specialisation, which is more conspicuous in situations where animals have diverse prey (Darimont *et al.* 2009).

4.5 Conclusion

In this study, the utility of SIA of whiskers in tandem with telemetry provided individual-level dietary information that is crucial for developing a clear understanding of ecosystem processes and function, especially for top predators that are the focus of conservation interest. Whisker isotopic analyses offered novel insights into leopard foraging patterns of individual leopards that were not apparent from traditional methods. Dietary variation among leopards has important implications for management and conservation of the species, and possibly other large felids. Indeed, the use of universal management plans compiled from average resources and general behaviours of the species may either harm individual specialists or have unforeseen consequences for the preferred prey population. Future studies conducted using the technique described in this study should consider harvesting matured whiskers from animals during initial GPS collar fitting and again when collars are removed to obtain at least six months of dietary information, which can be used to validate long-term individual niche variation.

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions and recommendations

The aim of this study was to develop and evaluate the technique of using stable carbon and nitrogen isotope analysis of whiskers to quantify the diets of large wild felids and in particular, to determine dietary differences amongst individuals. To achieve this, large felid (lion *Panthera leo* and leopard *Panthera pardus*) whisker growth rate and growth pattern were determined over a period of 185 days using an endogenous biomarker approach, and lion whisker-diet trophic discrimination factors (TDFs) were also measured; using captive individuals held at the National Zoological Gardens, Pretoria. The feasibility and applicability of the technique was then explored by analysis of isotopic profiles along whisker growth axes of six free-ranging leopards in Phinda Private Game Reserve (hereafter Phinda), northern KwaZulu-Natal (KZN), whose feeding habits have been intensively studied using traditional methods (Balme *et al.* 2007).

Results showed that whisker growth rates of three sub-adult lionesses and an adult male leopard estimated for the last 76 days of the experiment (period for which reliable and comparable growth data – contained in proximal 72 mm of whiskers – were obtained) did not differ substantially (range = 0.64 to 0.66 mm d^{-1}), despite age, sex and species differences. Thus, leopards appear to have whisker growth characteristics similar to those of lions; however, further experimentation is needed on a larger sample. Felid whisker growth rates obtained in this study were similar to those measured for some terrestrial mammals, i.e. laboratory mice *Mus domesticus* and rats *Rattus norvegicus* (0.3–1.0 mm d^{-1} and 0.6–1.5 mm d^{-1}, respectively, Ibrahim & Wright 1975) and stoats *Mustela ermine* (0.60 mm d^{-1}, Spurr 2002), but higher than whisker growth rates of Eurasian badgers *Meles meles* (0.43 mm d^{-1}, Robertson *et al.* 2013). The study also showed that growth pattern, i.e. linear or non-linear growth, is of little consequence for reconstructing time periods represented within a whisker length of ~50–60 mm (measured from the root). However, it is recognised that beyond this length, in particular towards the whisker tip where faster growth phases are likely to be represented, growth pattern may have substantial effects on interpreting timeframes. These results imply that studies using SIA of whiskers to infer felid feeding ecology should consider the whisker length measured to correctly interpret timeframes of diet in whiskers.

$\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs estimated for five lions were similar amongst individuals (range = 2.5 to 2.9 ‰ for $\delta^{13}C$; and 2.4 to 2.8 ‰ for $\delta^{15}N$), irrespective of age and sex. $\delta^{13}C$

whisker-diet TDFs obtained in this study were similar to hair-diet values reported for some terrestrial carnivores, i.e. Canada lynx *Lynx canadensis* (2.4 ‰, Parng *et al.* 2014) and red fox *Vulpes vulpes* (2.6 ‰, Roth & Hobson), and hair/whisker-diet values estimated for seals and sea otters (range = 2.4 to 2.8 ‰, Hobson *et al.* 1996; Tyrell *et al.* 2013; Beltran *et al.* 2016). However, $\delta^{15}N$ whisker-diet TDFs were either higher or lower than hair-diet values for most felid species, e.g. bobcats *Lynx rufus* (4.1 ‰, Parng *et al*. 2014) and tigers *Panthera tigris* (-2.6 ‰, Montanari & Amato 2015).

The estimation of whisker growth rates, growth pattern and whisker-diet TDFs was, however, restricted by the small and unbalanced (three sub-adult lionesses and one adult male leopard) sample available for the experiment. Experimentation on a larger and balanced sample is therefore needed for more knowledge on life history influences on felid whisker growth. Moreover, one study animal – a male lion – was excluded from whisker growth calculations as all his whiskers were broken at the end of the experiment. Therefore, further investigations should be carried out on male lions using full whisker growth trajectories to understand their whisker growth and effects thereof. Research on the period of felid whisker growth cycle and shedding, which seems to be longer than 185 days as whiskers were still growing when the experiment was terminated, is also needed. To acquire more detail on the growth pattern(s) of felid whiskers, future studies should endogenously mark whiskers for a longer period to obtain more data that provides higher resolution continuous sequence that would be required for non-linear model fitting. Long-term experiments on whisker isotopic-turnover rates are required to track the change in whisker-diet TDFs, along with any potential fluctuations in stable isotope incorporation rates. It is also important to study a variety of felid species and individuals to explore the causes of a wide variation in felid whisker/hair TDFs reported by other studies (e.g. Parng *et al.* 2014; Montanari & Amato 2015).

The stable isotope analysis (SIA) of 60 mm of whiskers of six leopards in Phinda suggested that intermediate feeders (e.g. impala and nyala) were the dominant prey type consumed, while grazers (e.g. warthog) were less and browsers (e.g. red and grey duiker) insignificantly consumed. These results are consistent with those derived using traditional dietary analyses (Balme *et al.* 2007), thereby demonstrating the reliability of whisker isotopic analyses to infer felid diets. Additionally, SIA of whiskers identified dietary differences amongst the individual leopards, which reflected individual prey preferences rather than differences in prey availability. Dietary differences among individuals has been reported for other carnivores (e.g. Newsome *et al.* 2009; 2015; Robertson *et al.* 2014) and identification of such behaviour in leopards is essential for the development of effective conservation and management strategies that are not only compiled from average resource use and general behaviours of the species, but also individual feeding habits. Using the felid whisker growth rate of 0.65

mm d^{-1}, analysis of 60 mm of whiskers presented 92 days of dietary information and potentially 18 kills (if leopards killed prey after every five days, Bothma & le Richie 1984) that was acquired in a quick and non-invasive manner. Obtaining data over this extended time scale using traditional methods alone can be challenging as protracted periods of time and high financial inputs are required.

The quantification of leopard diet was also limited by the available small and unbalanced sample. The unavailability of leopard whisker-diet TDFs and leopard whisker turnover rates prompted the use of parameters derived from studies of lions (Chapter 2) and horses (Ayliffe *et al.* 2004), respectively. Therefore, experimental evidence from leopards is needed for more accurate deductions of their diet in free-ranging conditions. In this study, $\delta^{15}N$ values of C_3 and C_4 plants, and those of potential prey species consumed by the leopards were indistinguishable. As a result, only carbon could be used to assess the diets of individual leopards. An improved understanding of $\delta^{15}N$ abundances in plants is thus required. Furthermore, periodic harvesting and analysis of whiskers from individual leopards should be considered to validate long-term individual diet variation.

The study contributed to resolving questions about the major limiting factors related to use of whiskers for isotope-based studies of African mammalian carnivores. New data and insights about whisker growth rates, whisker growth patterns and whisker-diet TDFs have been provided. The average felid whisker growth rate of 0.65 mm d^{-1}, calculated for the proximal 72 mm of whiskers that are longer than 100 mm, can be used in future studies to assign the dietary information contained in analysed felid whiskers to the correct time period with confidence, at least for species with similar body size and are genetically related to lion and leopard. Moreover, the mean lion $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of 2.7 and 2.5 ‰, respectively, are probably the best appraisal for the species to date as they were measured using more individuals ($n = 5$) whose diet was remained consistent over multiple years, compared to previous experiments. Although SIA of whiskers could not resolve diets of Phinda leopards at species-level, it offered novel insights into prey types (browsers, intermediate feeders and grazers) consumed and temporal dietary differences amongst individuals. The refined technique can be used on felid species across the globe to improve our understanding of their feeding ecology, and improve conservation management efforts of these threatened carnivores (Ripple *et al.* 2014).

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Appendix A: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of the analysed sections from the five felid whiskers removed at the end of the Zoo experiment

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1462	Emma	1	0.4	8.6	9.0	25.6	-17.2	3.0
FR 1463	Emma	2	0.4	11.2	9.0	32.2	-15.9	2.9
FR 1464	Emma	3	0.4	11.1	9.0	31.6	-14.0	2.8
FR 1465	Emma	4	0.4	12.3	8.8	34.9	-13.1	2.8
FR 1466	Emma	5	0.6	8.4	8.6	23.7	-12.7	2.8
FR 1467	Emma	6	0.5	9.7	8.5	27.4	-12.6	2.8
FR 1468	Emma	7	0.5	10.1	8.4	28.7	-12.5	2.8
FR 1469	Emma	8	0.4	14.9	8.3	42.4	-12.6	2.8
FR 1470	Emma	9	0.4	10.8	8.2	30.6	-12.9	2.8
FR 1471	Emma	10	0.5	11.5	8.3	32.9	-12.9	2.9
FR 1472	Emma	11	0.5	11.3	8.5	32.3	-12.9	2.9
FR 1473	Emma	12	0.5	11.6	8.4	33.2	-12.9	2.9
FR 1474	Emma	13	0.4	13.5	8.4	38.6	-13.1	2.9
FR 1475	Emma	14	0.5	11.8	8.4	33.6	-12.6	2.9
FR 1476	Emma	15	0.4	14.1	8.3	40.1	-12.9	2.8
FR 1477	Emma	16	0.4	13.4	8.2	38.4	-12.9	2.9
FR 1478	Emma	17	0.5	11.0	8.2	31.5	-12.8	2.9
FR 1479	Emma	18	0.5	11.9	8.1	33.9	-13.1	2.9
FR 1480	Emma	19	0.4	14.2	8.1	40.6	-13.3	2.9
FR 1481	Emma	20	0.4	12.5	8.3	35.9	-13.5	2.9
FR 1482	Emma	21	0.5	10.5	8.4	30.2	-14.1	2.9
FR 1483	Emma	22	0.4	12.1	8.8	34.6	-14.8	2.9
FR 1484	Emma	23	0.5	10.4	9.0	29.9	-14.7	2.9
FR 1485	Emma	24	0.4	13.1	8.9	37.5	-13.8	2.9
FR 1486	Emma	25	0.4	12.0	8.5	34.6	-13.7	2.9
FR 1487	Emma	26	0.4	12.4	8.2	35.4	-13.9	2.9
FR 1488	Emma	27	0.4	13.0	8.3	37.5	-13.8	2.9
FR 1489	Emma	28	0.3	14.8	8.3	42.4	-13.7	2.9
FR 1490	Emma	29	0.5	10.9	8.7	31.0	-12.9	2.9
FR 1491	Emma	30	0.5	8.7	8.5	24.9	-12.8	2.9
FR 1492	Emma	31	0.4	11.2	8.5	32.3	-12.8	2.9
FR 1493	Emma	32	0.4	11.6	8.5	33.5	-13.0	2.9
FR 1494	Emma	33	0.3	12.8	8.4	37.0	-13.1	2.9
FR 1495	Emma	34	0.3	14.9	8.4	44.0	-13.2	2.9
FR 1496	Emma	35	0.4	11.4	8.4	33.3	-12.9	2.9
FR 1497	Emma	36	0.4	8.3	8.5	24.2	-12.8	2.9
FR 1498	Emma	37	0.4	11.8	8.7	34.5	-12.7	2.9
FR 1499	Emma	38	0.4	13.5	8.8	39.5	-12.7	2.9
FR 1500	Emma	39	0.4	9.0	8.9	26.4	-12.7	2.9
FR 1501	Emma	40	0.3	14.9	9.2	43.8	-12.6	2.9
FR 1502	Emma	41	0.3	13.0	9.8	38.2	-11.9	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1503	Emma	42	0.4	9.4	9.7	27.6	-11.7	2.9
FR 1504	Emma	43	0.4	11.1	9.7	32.2	-11.2	2.9
FR 1505	Emma	44	0.3	14.9	10.0	43.2	-11.5	2.9
FR 1506	Emma	45	0.4	9.9	10.4	28.7	-11.3	2.9
FR 1507	Emma	46	0.3	11.7	10.6	33.6	-11.2	2.9
FR 1508	Emma	47	0.4	9.5	10.6	27.4	-11.1	2.9
FR 1509	Emma	48	0.4	8.4	10.4	24.1	-11.2	2.9
FR 1510	Emma	49	0.4	11.9	10.2	33.8	-11.4	2.8
FR 1511	Emma	50	0.4	8.3	10.3	23.8	-11.6	2.9
FR 1512	Emma	51	0.3	9.5	10.3	27.3	-12.5	2.9
FR 1513	Emma	52	0.4	7.8	10.0	22.2	-14.1	2.8
FR 1514	Emma	53	0.3	9.1	9.9	26.2	-14.6	2.9
FR 1515	Emma	54	0.3	11.8	10.2	33.6	-14.5	2.8
FR 1516	Emma	55	0.2	11.8	10.7	33.8	-13.1	2.9
FR 1517	Emma	56	0.3	8.8	10.8	25.3	-11.9	2.9
FR 1518	Emma	57	0.2	13.9	10.6	39.7	-11.0	2.9
FR 1519	Emma	58	0.3	8.7	10.6	24.9	-10.7	2.9
FR 1520	Emma	59	0.3	7.7	10.3	22.0	-11.1	2.9
FR 1521	Emma	60	0.3	7.5	10.5	21.5	-11.1	2.9
FR 1522	Emma	61	0.2	10.6	10.6	30.4	-11.1	2.9
FR 1523	Emma	62	0.2	11.3	10.5	33.0	-11.0	2.9
FR 1524	Emma	63	0.2	9.9	10.7	28.5	-11.1	2.9
FR 1525	Emma	64	0.2	12.4	10.4	35.4	-11.3	2.9
FR 1526	Emma	65	0.2	12.3	9.8	34.8	-11.8	2.8
FR 1527	Emma	66	0.135	15.9	9.8	44.6	-11.2	2.8
FR 1528	Emma	67	0.111	15.8	9.8	44.5	-11.3	2.8
FR 1529	Emma	68	0.118	15.9	9.6	44.5	-11.4	2.8
FR 1530	Emma	69	0.1	16.0	9.5	45.7	-11.7	2.9
FR 1531	Emma	70	0.105	16.3	9.7	45.9	-11.3	2.8
FR 1532	Emma	71	0.072	15.5	9.9	44.0	-10.9	2.8
FR 1533	Emma	72	0.081	16.1	10.0	44.1	-11.0	2.7
FR 1534	Emma	73	0.078	15.7	10.1	43.4	-10.7	2.8
FR 1535	Emma	74	0.095	16.1	10.1	44.0	-10.5	2.7
FR 1536	Emma	75	0.07	15.5	9.9	45.2	-11.0	2.9
FR 1537	Emma	76	0.07	15.8	9.8	43.6	-10.9	2.8
FR 1538	Emma	77	0.085	16.1	9.9	44.5	-10.6	2.8
FR 1539	Emma	78	0.077	15.7	10.0	42.9	-10.6	2.7
FR 1540	Emma	79	0.086	15.7	10.0	43.1	-10.6	2.7
FR 1541	Emma	80	0.09	15.7	10.1	44.1	-10.5	2.8
FR 1542	Emma	81	0.08	14.7	10.2	42.8	-10.6	2.9
FR 1543	Emma	82	0.077	15.2	10.1	43.1	-10.0	2.8
FR 1544	Emma	83	0.074	13.9	10.1	40.0	-9.8	2.9
FR 1545	Emma	84	0.08	15.5	10.2	43.4	-9.8	2.8

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1546	Emma	85	0.06	14.3	9.8	41.2	-10.3	2.9
FR 1547	Emma	86	0.05	16.5	9.5	46.5	-10.3	2.8
FR 1548	Emma	87	0.08	15.3	9.6	42.6	-10.0	2.8
FR 1549	Emma	88	0.078	15.3	9.9	42.9	-10.1	2.8
FR 1550	Emma	89	0.065	15.2	9.6	42.6	-10.0	2.8
FR 1551	Emma	90	0.052	14.6	10.2	41.9	-10.1	2.9
FR 1552	Emma	91	0.06	15.1	10.8	42.7	-9.7	2.8
FR 1553	Emma	92	0.075	15.2	11.4	42.6	-9.6	2.8
FR 1554	Emma	93	0.07	15.3	11.0	43.1	-10.1	2.8
FR 1555	Emma	94	0.045	14.3	9.8	41.5	-10.5	2.9
FR 1556	Emma	95	0.047	15.3	9.3	43.9	-10.6	2.9
FR 1557	Emma	96	0.044	14.6	8.6	42.8	-11.0	2.9
FR 1558	Emma	97	0.052	15.2	9.0	43.7	-11.0	2.9
FR 1559	Emma	98	0.045	14.2	8.6	41.9	-11.9	3.0
FR 1560	Emma	99	0.055	15.3	8.5	43.5	-11.9	2.8
FR 1561	Emma	100	0.06	15.1	9.1	42.3	-11.4	2.8
FR 1562	Emma	101	0.06	15.2	9.3	43.1	-11.3	2.8
FR 1563	Emma	102	0.07	15.3	9.4	41.9	-11.0	2.7
FR 1564	Emma	103	0.075	16.0	9.7	44.8	-10.1	2.8
FR 1565	Emma	104	0.063	15.3	9.7	42.8	-9.9	2.8
FR 1566	Emma	105	0.05	14.7	10.1	41.7	-10.2	2.8
FR 1567	Emma	106	0.055	15.1	11.3	42.4	-10.4	2.8
FR 1568	Emma	107	0.057	14.8	10.6	43.3	-10.3	2.9
FR 1569	Emma	108	0.056	14.8	9.7	42.1	-9.7	2.8
FR 1570	Emma	109	0.065	15.3	9.1	42.8	-9.6	2.8
FR 1571	Emma	110	0.06	14.8	8.8	47.1	-12.0	3.2
FR 1572	Emma	111	0.05	15.5	8.7	43.7	-10.6	2.8
FR 1573	Emma	112	0.056	15.6	9.4	43.2	-10.4	2.8
FR 1574	Emma	113	0.05	15.5	9.4	43.1	-9.9	2.8
FR 1575	Emma	114	0.05	14.9	9.0	42.1	-9.9	2.8
FR 1576	Emma	115	0.05	8.7	9.7	25.5	-10.6	2.9
FR 1577	Emma	116	0.05	14.6	9.3	41.9	-10.2	2.9
FR 1578	Emma	117	0.05	15.1	8.5	43.0	-10.5	2.8
FR 1579	Emma	118	0.042	15.7	9.9	44.4	-10.5	2.8
FR 1580	Emma	119	0.04	15.0	9.1	43.7	-10.4	2.9
FR 1581	Emma	120	0.043	15.9	9.4	45.4	-10.5	2.9
FR 1582	Emma	121	0.04	15.2	9.1	43.5	-10.2	2.9
FR 1583	Emma	122	0.04	15.0	9.6	43.2	-10.5	2.9
FR 1584	Emma	123	0.042	15.5	9.3	45.0	-10.5	2.9
FR 1585	Emma	124	0.035	17.9	9.2	52.8	-10.4	2.9
FR 1586	Emma	125	0.035	15.1	10.4	43.4	-11.0	2.9
FR 1587	Emma	126	0.036	15.7	10.5	45.4	-11.0	2.9
FR 1588	Emma	127	0.035	12.1	10.0	35.6	-11.2	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1589	Emma	128	0.035	14.2	9.7	42.7	-10.8	3.0
FR 1590	Emma	129	0.035	15.6	10.2	44.7	-11.0	2.9
FR 1591	Emma	130	0.03	15.6	9.6	45.5	-11.1	2.9
FR 1592	Emma	131	0.026	17.5	9.5	51.0	-11.1	2.9
FR 1593	Emma	132	0.03	14.8	10.2	43.6	-11.1	2.9
FR 1594	Emma	133	0.022	17.1	9.7	51.3	-11.9	3.0
FR 1595	Emma	134	0.022	17.0	9.7	51.1	-14.2	3.0
FR 1596	Emma	135	0.02	20.0	9.1	59.5	-16.0	3.0
FR 1597	Emma	136	0.022	19.9	9.9	57.9	-11.4	2.9
FR 1598	Emma	137	0.017	19.0	9.0	59.1	-15.7	3.1
FR 1599	Emma	138	0.018	16.6	8.6	50.9	-12.2	3.1
FR 1600	Emma	139	0.013	22.6	9.2	70.3	-14.4	3.1
FR 1601	Emma	140	0.011	22.4	9.1	69.9	-12.1	3.1
FR 1602	Emma	141	0.012	13.5	10.2	47.4	-13.1	3.5
FR 1603	Emma	142	0.01	22.3	9.7	71.5	-12.2	3.2
FR 1604	Emma	143	0.007	29.4	10.4	95.2	-12.1	3.2
FR 1605	Emma	144	0.005	21.3	9.8	77.2	-13.8	3.6
FR 1606	Bianca	1	0.3	11.1	9.3	32.1	-16.2	2.9
FR 1607	Bianca	2	0.4	12.1	9.1	34.5	-13.9	2.8
FR 1608	Bianca	3	0.3	15.5	9.2	43.8	-12.5	2.8
FR 1609	Bianca	4	0.4	11.7	9.6	33.3	-11.9	2.8
FR 1610	Bianca	5	0.4	14.1	9.6	40.0	-11.5	2.8
FR 1611	Bianca	6	0.4	10.3	9.9	30.0	-11.9	2.9
FR 1612	Bianca	7	0.3	12.7	10.0	36.2	-11.4	2.9
FR 1613	Bianca	8	0.4	10.9	9.8	31.2	-11.2	2.9
FR 1614	Bianca	9	0.4	11.4	9.8	32.9	-11.4	2.9
FR 1615	Bianca	10	0.4	11.7	9.8	33.2	-11.4	2.8
FR 1616	Bianca	11	0.4	11.6	9.9	33.4	-11.4	2.9
FR 1617	Bianca	12	0.4	11.5	9.9	32.9	-11.3	2.9
FR 1618	Bianca	13	0.4	11.7	10.0	33.7	-11.1	2.9
FR 1619	Bianca	14	0.4	10.2	10.2	29.5	-11.1	2.9
FR 1620	Bianca	15	0.3	13.5	10.2	38.6	-11.0	2.9
FR 1621	Bianca	16	0.3	13.8	10.2	39.5	-11.0	2.9
FR 1622	Bianca	17	0.4	10.5	10.5	29.9	-11.3	2.9
FR 1623	Bianca	18	0.4	8.4	10.5	24.1	-11.5	2.9
FR 1624	Bianca	19	0.4	11.5	10.8	32.6	-11.6	2.8
FR 1625	Bianca	20	0.3	12.8	10.7	36.5	-12.0	2.9
FR 1626	Bianca	21	0.3	16.9	10.1	48.4	-13.2	2.9
FR 1627	Bianca	22	0.4	10.3	10.0	29.3	-14.7	2.9
FR 1628	Bianca	23	0.3	11.7	9.8	33.6	-14.7	2.9
FR 1629	Bianca	24	0.3	14.0	10.1	40.4	-13.4	2.9
FR 1630	Bianca	25	0.3	13.9	10.4	39.6	-13.3	2.9
FR 1631	Bianca	26	0.3	13.2	9.9	37.8	-12.9	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1632	Bianca	27	0.3	13.8	9.9	39.4	-11.9	2.9
FR 1633	Bianca	28	0.3	14.6	9.9	41.6	-11.3	2.9
FR 1634	Bianca	29	0.4	9.5	9.7	27.3	-11.1	2.9
FR 1635	Bianca	30	0.4	10.5	9.5	30.1	-11.0	2.9
FR 1636	Bianca	31	0.3	12.3	9.6	35.1	-11.2	2.9
FR 1637	Bianca	32	0.3	11.0	9.6	31.6	-11.3	2.9
FR 1638	Bianca	33	0.4	9.7	9.8	27.9	-11.0	2.9
FR 1639	Bianca	34	0.3	10.3	9.7	29.6	-11.3	2.9
FR 1640	Bianca	35	0.4	10.2	9.6	29.1	-11.4	2.9
FR 1641	Bianca	36	0.3	13.9	9.7	40.0	-11.2	2.9
FR 1642	Bianca	37	0.2	17.4	9.3	50.1	-11.7	2.9
FR 1643	Bianca	38	0.3	11.0	9.0	31.8	-12.2	2.9
FR 1644	Bianca	39	0.3	9.9	8.7	28.3	-12.4	2.9
FR 1645	Bianca	40	0.2	12.1	8.9	34.7	-11.6	2.9
FR 1646	Bianca	41	0.3	11.1	9.1	31.7	-11.1	2.9
FR 1647	Bianca	42	0.2	16.3	9.3	46.6	-11.1	2.9
FR 1648	Bianca	43	0.3	11.4	9.9	32.6	-11.4	2.9
FR 1649	Bianca	44	0.2	14.4	10.2	41.7	-11.6	2.9
FR 1650	Bianca	45	0.3	10.0	10.3	28.6	-11.8	2.9
FR 1651	Bianca	46	0.3	10.5	10.0	29.9	-12.6	2.9
FR 1652	Bianca	47	0.3	10.8	9.8	30.9	-12.6	2.8
FR 1653	Bianca	48	0.3	9.1	10.0	26.3	-12.0	2.9
FR 1654	Bianca	49	0.3	9.7	10.2	27.9	-11.8	2.9
FR 1655	Bianca	50	0.2	10.8	10.1	31.2	-12.4	2.9
FR 1656	Bianca	51	0.2	12.8	10.0	37.0	-13.6	2.9
FR 1657	Bianca	52	0.2	12.8	10.0	37.1	-14.6	2.9
FR 1658	Bianca	53	0.3	7.6	10.1	22.1	-14.5	2.9
FR 1659	Bianca	54	0.2	11.9	10.6	34.7	-13.6	2.9
FR 1660	Bianca	55	0.2	11.7	10.8	34.2	-12.2	2.9
FR 1661	Bianca	56	0.2	12.0	10.0	35.0	-11.7	2.9
FR 1662	Bianca	57	0.2	11.1	10.1	32.6	-11.6	2.9
FR 1663	Bianca	58	0.162	11.4	10.3	32.9	-11.3	2.9
FR 1664	Bianca	59	0.132	15.3	10.1	43.6	-11.1	2.9
FR 1665	Bianca	60	0.138	15.1	10.0	43.6	-10.9	2.9
FR 1666	Bianca	61	0.14	15.1	9.8	43.1	-10.9	2.9
FR 1667	Bianca	62	0.16	15.1	9.7	42.7	-11.0	2.8
FR 1668	Bianca	63	0.152	15.5	9.2	43.7	-11.7	2.8
FR 1669	Bianca	64	0.135	15.3	8.9	43.2	-11.9	2.8
FR 1670	Bianca	65	0.135	15.7	9.1	44.9	-11.6	2.8
FR 1671	Bianca	66	0.127	16.0	9.4	44.9	-11.8	2.8
FR 1672	Bianca	67	0.114	15.4	9.0	43.3	-12.4	2.8
FR 1673	Bianca	68	0.112	15.8	9.3	44.8	-11.7	2.8
FR 1674	Bianca	69	0.1	15.2	9.1	43.5	-11.5	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1675	Bianca	70	0.11	15.7	9.3	44.8	-11.3	2.9
FR 1676	Bianca	71	0.118	14.9	9.5	42.7	-11.3	2.9
FR 1677	Bianca	72	0.112	14.9	9.7	42.4	-11.0	2.8
FR 1678	Bianca	73	0.135	15.5	9.6	43.5	-10.8	2.8
FR 1679	Bianca	74	0.095	15.4	9.4	44.0	-10.7	2.9
FR 1680	Bianca	75	0.102	15.9	9.0	45.0	-10.5	2.8
FR 1681	Bianca	76	0.1	15.6	8.9	44.6	-10.4	2.9
FR 1682	Bianca	77	0.087	16.1	9.3	44.7	-10.1	2.8
FR 1683	Bianca	78	0.08	15.2	9.3	42.7	-10.3	2.8
FR 1684	Bianca	79	0.084	15.9	9.8	44.2	-9.9	2.8
FR 1685	Bianca	80	0.074	15.9	9.9	44.2	-9.8	2.8
FR 1686	Bianca	81	0.068	16.2	9.2	44.9	-10.2	2.8
FR 1687	Bianca	82	0.07	16.7	9.1	46.5	-10.5	2.8
FR 1688	Bianca	83	0.082	16.4	9.2	45.6	-10.8	2.8
FR 1689	Bianca	84	0.085	16.8	9.0	46.6	-11.3	2.8
FR 1690	Bianca	85	0.085	16.2	9.1	44.7	-10.9	2.8
FR 1691	Bianca	86	0.07	15.6	8.9	43.4	-11.0	2.8
FR 1692	Bianca	87	0.06	16.7	9.3	46.9	-10.6	2.8
FR 1693	Bianca	88	0.077	16.7	9.4	46.5	-10.0	2.8
FR 1694	Bianca	89	0.074	16.2	9.6	44.9	-9.8	2.8
FR 1695	Bianca	90	0.07	15.8	9.1	44.0	-9.8	2.8
FR 1696	Bianca	91	0.075	14.7	9.4	41.1	-9.6	2.8
FR 1697	Bianca	92	0.065	17.7	9.0	49.2	-9.5	2.8
FR 1698	Bianca	93	0.068	17.2	8.9	48.1	-10.3	2.8
FR 1699	Bianca	94	0.063	15.3	9.2	43.1	-10.0	2.8
FR 1700	Bianca	95	0.06	15.6	9.2	44.0	-9.6	2.8
FR 1701	Bianca	96	0.061	16.1	9.2	45.8	-9.8	2.8
FR 1702	Bianca	97	0.063	16.1	8.9	46.0	-10.4	2.9
FR 1703	Bianca	98	0.05	13.6	9.2	39.1	-10.5	2.9
FR 1704	Bianca	99	0.056	18.0	9.2	51.6	-9.6	2.9
FR 1705	Bianca	100	0.055	11.1	9.2	32.9	-10.1	3.0
FR 1706	Bianca	101	0.055	13.0	9.5	37.7	-9.8	2.9
FR 1707	Bianca	102	0.046	15.7	9.5	45.5	-9.7	2.9
FR 1708	Bianca	103	0.048	16.0	9.9	45.8	-10.1	2.9
FR 1709	Bianca	104	0.045	15.6	9.6	45.2	-10.3	2.9
FR 1710	Bianca	105	0.045	16.7	10.3	47.9	-10.1	2.9
FR 1711	Bianca	106	0.033	15.2	9.9	46.8	-11.4	3.1
FR 1712	Bianca	107	0.038	14.8	10.3	43.2	-10.8	2.9
FR 1713	Bianca	108	0.031	16.3	9.7	53.6	-12.4	3.3
FR 1714	Bianca	109	0.035	14.5	9.4	43.7	-11.0	3.0
FR 1715	Bianca	110	0.035	15.8	9.7	46.6	-11.0	2.9
FR 1716	Bianca	111	0.04	16.1	9.8	46.6	-11.0	2.9
FR 1717	Bianca	112	0.032	14.9	9.4	45.4	-11.9	3.0

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1718	Bianca	113	0.03	15.3	8.8	45.5	-12.3	3.0
FR 1719	Bianca	114	0.028	14.0	9.0	43.4	-14.4	3.1
FR 1720	Bianca	115	0.024	13.3	9.2	43.6	-16.7	3.3
FR 1721	Bianca	116	0.023	15.4	9.2	48.0	-15.9	3.1
FR 1722	Bianca	117	0.021	15.0	9.3	47.2	-15.2	3.1
FR 1723	Bianca	118	0.016	17.0	8.6	53.4	-14.6	3.1
FR 1724	Bianca	119	0.022	16.5	8.5	51.5	-12.5	3.1
FR 1725	Bianca	120	0.02	14.6	8.5	47.0	-12.1	3.2
FR 1726	Bianca	121	0.02	13.0	8.6	41.7	-12.3	3.2
FR 1727	Bianca	122	0.014	16.4	8.8	54.2	-12.1	3.3
FR 1728	Bianca	123	0.017	15.1	10.0	48.9	-12.1	3.2
FR 1729	Bianca	124	0.015	15.9	9.7	51.7	-12.1	3.2
FR 1730	Bianca	125	0.015	17.5	10.7	57.7	-11.9	3.3
FR 1731	Bianca	126	0.013	13.7	10.0	47.7	-12.3	3.5
FR 1732	Bianca	127	0.011	15.3	11.0	53.1	-12.6	3.5
FR 1733	Bianca	128	0.009	13.7	10.8	49.2	-13.1	3.6
FR 1734	Bianca	129	0.006	17.2	10.0	65.5	-13.4	3.8
FR 1735	Bianca	130	0.003	25.5	11.4	105.7	-15.0	4.2
FR 1736	Diesel	1	0.2	31.2	8.7	88.3	-17.5	2.8
FR 1737	Diesel	2	0.3	18.6	8.7	52.1	-15.4	2.8
FR 1738	Diesel	3	0.4	17.8	8.8	49.9	-13.7	2.8
FR 1739	Diesel	4	0.5	14.7	8.6	41.0	-12.9	2.8
FR 1740	Diesel	5	0.5	13.6	8.5	37.9	-12.6	2.8
FR 1741	Diesel	6	0.5	13.7	8.5	38.3	-12.5	2.8
FR 1742	Diesel	7	0.5	14.7	8.6	41.1	-12.3	2.8
FR 1743	Diesel	8	0.5	14.5	8.6	40.3	-11.9	2.8
FR 1744	Diesel	9	0.5	13.2	8.9	36.8	-11.7	2.8
FR 1745	Diesel	10	0.5	14.0	8.8	39.1	-11.7	2.8
FR 1746	Diesel	11	0.5	12.8	8.7	36.0	-11.6	2.8
FR 1747	Diesel	12	0.5	16.7	8.7	46.7	-11.5	2.8
FR 1748	Diesel	13	0.5	12.9	8.8	36.1	-11.6	2.8
FR 1749	Diesel	14	0.5	12.9	8.9	36.0	-11.6	2.8
FR 1750	Diesel	15	0.4	13.0	9.2	36.3	-11.5	2.8
FR 1751	Diesel	16	0.4	14.0	9.1	38.9	-11.7	2.8
FR 1752	Diesel	17	0.4	11.6	9.2	32.4	-11.8	2.8
FR 1753	Diesel	18	0.4	11.9	9.2	33.1	-11.9	2.8
FR 1754	Diesel	19	0.4	16.8	9.4	46.9	-12.1	2.8
FR 1755	Diesel	20	0.5	10.9	9.3	30.5	-13.3	2.8
FR 1756	Diesel	21	0.5	7.7	9.2	21.5	-14.5	2.8
FR 1757	Diesel	22	0.5	13.8	9.2	38.7	-14.4	2.8
FR 1758	Diesel	23	0.6	13.5	9.3	37.8	-13.6	2.8
FR 1759	Diesel	24	0.5	16.8	9.5	46.9	-13.7	2.8
FR 1760	Diesel	25	0.6	13.8	9.1	38.6	-13.2	2.8

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1761	Diesel	26	0.6	13.3	9.1	37.3	-12.8	2.8
FR 1762	Diesel	27	0.5	14.9	9.2	41.8	-12.2	2.8
FR 1763	Diesel	28	0.6	12.5	9.2	35.1	-11.9	2.8
FR 1764	Diesel	29	0.5	11.8	8.9	33.4	-11.9	2.8
FR 1765	Diesel	30	0.5	12.0	8.8	34.0	-11.8	2.8
FR 1766	Diesel	31	0.5	13.1	8.9	37.0	-11.6	2.8
FR 1767	Diesel	32	0.5	13.3	9.0	37.6	-11.2	2.8
FR 1768	Diesel	33	0.4	12.2	8.9	35.0	-11.3	2.9
FR 1769	Diesel	34	0.4	16.5	8.8	47.1	-11.4	2.9
FR 1770	Diesel	35	0.4	13.0	8.8	37.3	-11.8	2.9
FR 1771	Diesel	36	0.4	13.1	8.7	37.3	-11.9	2.9
FR 1772	Diesel	37	0.4	16.4	9.0	47.0	-11.7	2.9
FR 1773	Diesel	38	0.4	13.6	9.2	39.2	-11.6	2.9
FR 1774	Diesel	39	0.4	14.9	8.7	42.7	-11.5	2.9
FR 1775	Diesel	40	0.4	14.3	8.8	41.1	-11.3	2.9
FR 1776	Diesel	41	0.4	12.8	8.7	37.0	-11.4	2.9
FR 1777	Diesel	42	0.3	16.9	8.8	49.3	-11.7	2.9
FR 1778	Diesel	43	0.3	16.3	9.3	47.7	-11.5	2.9
FR 1779	Diesel	44	0.4	13.7	9.2	39.8	-11.7	2.9
FR 1780	Diesel	45	0.4	11.2	9.4	32.9	-11.8	2.9
FR 1781	Diesel	46	0.3	15.5	9.2	45.3	-12.1	2.9
FR 1782	Diesel	47	0.3	14.8	8.7	43.4	-12.6	2.9
FR 1783	Diesel	48	0.3	13.0	8.5	38.4	-13.3	3.0
FR 1784	Diesel	49	0.4	11.7	8.9	34.6	-14.1	3.0
FR 1785	Diesel	50	0.3	14.1	8.9	42.1	-15.1	3.0
FR 1786	Diesel	51	0.3	12.5	8.8	37.5	-15.5	3.0
FR 1787	Diesel	52	0.3	12.5	8.7	37.2	-15.8	3.0
FR 1788	Diesel	53	0.3	13.1	8.6	38.8	-14.6	2.9
FR 1789	Diesel	54	0.4	8.7	8.5	26.1	-14.1	3.0
FR 1790	Diesel	55	0.4	10.0	9.0	29.8	-13.4	3.0
FR 1791	Diesel	56	0.3	11.6	9.3	35.0	-13.2	3.0
FR 1792	Diesel	57	0.3	11.2	9.6	33.5	-13.1	3.0
FR 1793	Diesel	58	0.3	12.2	9.0	36.4	-13.3	3.0
FR 1794	Diesel	59	0.3	8.2	8.9	24.8	-13.4	3.0
FR 1795	Diesel	60	0.4	7.6	8.9	22.9	-12.8	3.0
FR 1796	Diesel	61	0.3	10.2	9.0	30.5	-12.5	3.0
FR 1797	Diesel	62	0.3	8.4	8.7	25.7	-12.4	3.1
FR 1798	Diesel	63	0.2	15.9	8.5	47.7	-12.8	3.0
FR 1799	Diesel	64	0.3	8.9	8.4	27.1	-12.7	3.0
FR 1800	Diesel	65	0.2	10.8	8.2	33.5	-13.0	3.1
FR 1801	Diesel	66	0.3	8.0	8.3	24.0	-12.7	3.0
FR 1802	Diesel	67	0.2	12.0	8.5	35.8	-12.7	3.0
FR 1803	Diesel	68	0.2	11.5	8.3	35.0	-12.9	3.0

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1804	Diesel	69	0.2	12.5	8.3	37.7	-12.6	3.0
FR 1805	Diesel	70	0.2	11.6	8.4	34.6	-12.6	3.0
FR 1806	Diesel	71	0.13	14.5	8.5	42.1	-12.4	2.9
FR 1807	Diesel	72	0.139	15.2	8.5	44.3	-12.3	2.9
FR 1808	Diesel	73	0.147	15.4	8.6	45.0	-12.1	2.9
FR 1809	Diesel	74	0.12	14.5	8.8	42.4	-12.1	2.9
FR 1810	Diesel	75	0.137	15.0	8.6	44.0	-12.2	2.9
FR 1811	Diesel	76	0.115	14.3	8.3	42.2	-12.4	3.0
FR 1812	Diesel	77	0.086	10.5	8.9	44.6	-11.7	4.3
FR 1813	Diesel	78	0.105	8.6	9.2	43.9	-11.4	5.1
FR 1814	Diesel	79	0.082	11.0	9.2	42.7	-11.5	3.9
FR 1815	Diesel	80	0.108	8.3	8.9	43.0	-11.4	5.2
FR 1816	Diesel	81	0.075	12.0	8.8	44.0	-11.6	3.7
FR 1817	Diesel	82	0.09	10.0	9.2	44.2	-11.3	4.4
FR 1818	Diesel	83	0.094	9.6	9.2	45.1	-11.4	4.7
FR 1819	Diesel	84	0.088	10.2	9.3	43.1	-11.0	4.2
FR 1820	Diesel	85	0.076	11.8	8.8	44.2	-11.5	3.7
FR 1821	Diesel	86	0.069	13.1	8.6	43.4	-11.7	3.3
FR 1822	Diesel	87	0.069	13.1	8.4	45.5	-11.7	3.5
FR 1823	Diesel	88	0.064	14.1	8.4	45.2	-11.7	3.2
FR 1824	Diesel	89	0.071	12.7	8.5	43.9	-11.9	3.5
FR 1825	Diesel	90	0.087	10.4	8.8	43.7	-11.8	4.2
FR 1826	Diesel	91	0.074	12.2	8.7	44.7	-11.9	3.7
FR 1827	Diesel	92	0.065	13.9	8.1	44.4	-12.3	3.2
FR 1828	Diesel	93	0.064	14.1	7.8	46.5	-12.4	3.3
FR 1829	Diesel	94	0.062	14.5	7.4	44.6	-12.8	3.1
FR 1830	Diesel	95	0.062	14.5	7.2	45.2	-13.1	3.1
FR 1831	Diesel	96	0.058	15.5	7.5	44.5	-12.6	2.9
FR 1832	Diesel	97	0.058	15.5	7.8	46.5	-12.3	3.0
FR 1833	Diesel	98	0.055	16.4	7.9	43.1	-12.3	2.6
FR 1834	Diesel	99	0.05	18.0	7.8	44.4	-11.9	2.5
FR 1835	Diesel	100	0.053	17.0	7.5	45.3	-11.6	2.7
FR 1836	Diesel	101	0.057	15.8	8.1	46.3	-11.7	2.9
FR 1837	Diesel	102	0.057	15.8	8.2	44.3	-11.7	2.8
FR 1838	Diesel	103	0.052	17.3	7.9	47.9	-11.1	2.8
FR 1839	Diesel	104	0.056	16.1	8.2	45.0	-11.1	2.8
FR 1840	Diesel	105	0.053	17.0	8.0	44.6	-11.4	2.6
FR 1841	Diesel	106	0.048	18.8	8.1	46.8	-11.4	2.5
FR 1842	Diesel	107	0.04	22.5	7.9	44.6	-11.2	2.0
FR 1843	Diesel	108	0.048	18.8	7.7	44.6	-11.2	2.4
FR 1844	Diesel	109	0.05	18.0	7.8	46.6	-11.6	2.6
FR 1845	Diesel	110	0.048	18.8	7.9	44.9	-11.7	2.4
FR 1846	Diesel	111	0.042	21.4	7.8	45.6	-11.6	2.1

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1847	Diesel	112	0.045	20.0	7.9	46.5	-11.3	2.3
FR 1848	Diesel	113	0.045	20.0	8.2	42.9	-11.1	2.1
FR 1849	Diesel	114	0.038	23.7	7.9	44.8	-11.2	1.9
FR 1850	Diesel	115	0.037	24.3	7.8	46.3	-11.7	1.9
FR 1851	Diesel	116	0.039	23.1	7.8	47.9	-11.5	2.1
FR 1852	Diesel	117	0.042	21.4	7.9	45.6	-11.1	2.1
FR 1853	Diesel	118	0.034	26.5	7.9	44.0	-11.2	1.7
FR 1854	Diesel	119	0.039	23.1	7.9	43.0	-11.1	1.9
FR 1855	Diesel	120	0.041	22.0	7.8	43.2	-10.8	2.0
FR 1856	Diesel	121	0.041	22.0	7.4	45.4	-10.7	2.1
FR 1857	Diesel	122	0.039	23.1	7.5	47.1	-11.1	2.0
FR 1858	Diesel	123	0.035	25.7	7.6	42.0	-11.3	1.6
FR 1859	Diesel	124	0.04	22.5	7.9	43.4	-11.1	1.9
FR 1860	Diesel	125	0.037	24.3	7.9	42.5	-11.8	1.7
FR 1861	Diesel	126	0.032	28.1	8.1	43.2	-12.0	1.5
FR 1862	Diesel	127	0.032	28.1	7.7	43.1	-11.8	1.5
FR 1863	Diesel	128	0.036	25.0	7.8	45.4	-11.5	1.8
FR 1864	Diesel	129	0.033	27.3	8.4	45.2	-12.4	1.7
FR 1865	Diesel	130	0.032	28.1	9.0	43.5	-12.3	1.5
FR 1866	Diesel	131	0.031	29.1	9.1	45.5	-12.3	1.6
FR 1867	Diesel	132	0.032	28.1	8.4	50.6	-13.0	1.8
FR 1868	Diesel	133	0.03	30.0	8.6	46.7	-12.8	1.6
FR 1869	Diesel	134	0.03	30.0	9.1	47.9	-12.3	1.6
FR 1870	Diesel	135	0.035	25.7	8.7	45.0	-12.6	1.8
FR 1871	Diesel	136	0.033	27.3	8.8	43.6	-12.2	1.6
FR 1872	Diesel	137	0.03	30.0	8.2	42.2	-13.1	1.4
FR 1873	Diesel	138	0.028	32.2	8.0	43.0	-14.0	1.3
FR 1874	Diesel	139	0.026	34.6	8.0	47.3	-13.7	1.4
FR 1875	Diesel	140	0.019	47.4	8.2	54.8	-13.7	1.2
FR 1876	Diesel	141	0.016	56.3	8.0	64.2	-13.4	1.1
FR 1877	Diesel	142	0.015	60.0	8.1	65.4	-13.2	1.1
FR 1878	Diesel	143	0.015	60.0	8.6	55.9	-14.1	0.9
FR 1879	Diesel	144	0.019	47.4	8.5	49.3	-15.9	1.0
FR 1880	Diesel	145	0.019	47.4	8.3	46.8	-18.2	1.0
FR 1881	Diesel	146	0.018	50.0	8.0	52.2	-18.3	1.0
FR 1882	Diesel	147	0.016	56.3	8.7	47.4	-18.3	0.8
FR 1883	Diesel	148	0.011	81.9	7.9	60.6	-17.7	0.7
FR 1884	Diesel	149	0.011	81.9	8.0	64.7	-14.9	0.8
FR 1885	Diesel	150	0.009	100.1	7.7	57.0	-13.9	0.6
FR 1886	Diesel	151	0.009	100.1	8.8	54.2	-15.1	0.5
FR 1887	Diesel	152	0.007	128.7	8.1	53.7	-16.5	0.4
FR 1888	Tess	1	0.3	8.6	9.2	25.7	-16.5	3.0
FR 1889	Tess	2	0.3	18.6	9.2	53.4	-14.6	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1890	Tess	3	0.5	11.6	9.2	33.3	-12.5	2.9
FR 1891	Tess	4	0.5	13.3	9.3	38.0	-11.7	2.9
FR 1892	Tess	5	0.4	15.4	9.4	43.9	-11.5	2.9
FR 1893	Tess	6	0.5	14.0	9.7	40.0	-11.4	2.9
FR 1894	Tess	7	0.4	16.1	9.8	46.2	-11.5	2.9
FR 1895	Tess	8	0.4	14.8	9.8	42.3	-11.7	2.9
FR 1896	Tess	9	0.4	13.6	9.8	39.0	-12.0	2.9
FR 1897	Tess	10	0.4	16.3	9.8	46.8	-12.1	2.9
FR 1898	Tess	11	0.3	18.3	9.4	52.5	-11.7	2.9
FR 1899	Tess	12	0.4	15.4	9.6	44.2	-11.4	2.9
FR 1900	Tess	13	0.4	16.1	9.6	46.1	-11.2	2.9
FR 1901	Tess	14	0.4	15.6	9.7	45.0	-11.4	2.9
FR 1902	Tess	15	0.5	10.8	9.7	31.2	-11.5	2.9
FR 1903	Tess	16	0.5	11.7	9.9	33.4	-11.7	2.9
FR 1904	Tess	17	0.4	15.1	9.8	43.1	-12.9	2.8
FR 1905	Tess	18	0.4	15.2	9.3	43.0	-14.4	2.8
FR 1906	Tess	19	0.4	11.5	9.1	32.5	-14.7	2.8
FR 1907	Tess	20	0.5	9.6	9.2	27.3	-13.1	2.8
FR 1908	Tess	21	0.5	8.0	9.3	22.8	-12.3	2.8
FR 1909	Tess	22	0.3	10.5	8.8	30.0	-12.6	2.8
FR 1910	Tess	23	0.3	13.6	8.9	38.6	-12.2	2.8
FR 1911	Tess	24	0.3	13.1	8.9	37.3	-12.1	2.9
FR 1912	Tess	25	0.3	11.8	8.9	33.7	-12.1	2.9
FR 1913	Tess	26	0.3	11.8	9.1	33.6	-11.6	2.8
FR 1914	Tess	27	0.3	12.0	9.1	34.0	-11.8	2.8
FR 1915	Tess	28	0.2	13.5	8.7	38.4	-12.0	2.8
FR 1916	Tess	29	0.18	15.6	8.9	44.0	-11.8	2.8
FR 1917	Tess	30	0.188	15.5	8.8	43.9	-11.5	2.8
FR 1918	Tess	31	0.18	15.3	8.9	44.3	-10.9	2.9
FR 1919	Tess	32	0.181	15.2	9.0	43.3	-10.9	2.8
FR 1920	Tess	33	0.2	15.6	8.9	44.1	-11.5	2.8
FR 1921	Tess	34	0.18	14.8	8.9	42.2	-12.0	2.8
FR 1922	Tess	35	0.155	15.6	8.7	44.5	-12.1	2.9
FR 1923	Tess	36	0.16	15.5	8.9	44.2	-11.7	2.8
FR 1924	Tess	37	0.147	15.6	9.1	44.6	-11.1	2.9
FR 1925	Tess	38	0.16	15.5	9.2	44.0	-10.9	2.8
FR 1926	Tess	39	0.16	15.3	9.3	43.9	-10.8	2.9
FR 1927	Tess	40	0.145	14.7	9.6	42.1	-11.3	2.9
FR 1928	Tess	41	0.142	15.0	9.9	43.4	-11.6	2.9
FR 1929	Tess	42	0.136	15.2	10.2	44.1	-11.3	2.9
FR 1930	Tess	43	0.127	14.9	10.5	43.0	-11.5	2.9
FR 1931	Tess	44	0.125	15.4	10.2	44.3	-11.9	2.9
FR 1932	Tess	45	0.142	15.2	9.9	44.1	-12.5	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1933	Tess	46	0.135	15.1	9.9	43.2	-11.8	2.9
FR 1934	Tess	47	0.13	15.1	10.0	43.1	-11.4	2.9
FR 1935	Tess	48	0.118	15.2	9.9	43.1	-11.6	2.8
FR 1936	Tess	49	0.116	15.1	9.7	43.2	-12.1	2.9
FR 1937	Tess	50	0.128	15.1	9.8	42.8	-12.3	2.8
FR 1938	Tess	51	0.125	15.0	9.8	43.0	-13.3	2.9
FR 1939	Tess	52	0.118	15.2	9.7	43.4	-14.1	2.8
FR 1940	Tess	53	0.1	14.8	9.6	43.0	-14.4	2.9
FR 1941	Tess	54	0.1	15.2	9.7	43.5	-14.2	2.9
FR 1942	Tess	55	0.108	15.4	9.7	43.5	-13.9	2.8
FR 1943	Tess	56	0.13	15.4	9.9	43.8	-12.8	2.8
FR 1944	Tess	57	0.105	15.5	9.9	44.1	-11.8	2.9
FR 1945	Tess	58	0.1	14.4	10.3	41.3	-11.3	2.9
FR 1946	Tess	59	0.095	15.7	10.3	44.5	-11.1	2.8
FR 1947	Tess	60	0.1	16.0	10.4	45.5	-10.7	2.8
FR 1948	Tess	61	0.1	15.2	10.4	43.2	-10.7	2.8
FR 1949	Tess	62	0.094	16.4	10.7	45.5	-10.1	2.8
FR 1950	Tess	63	0.094	17.0	10.6	47.4	-10.3	2.8
FR 1951	Tess	64	0.09	15.9	10.5	44.3	-10.4	2.8
FR 1952	Tess	65	0.092	16.2	10.4	45.1	-10.3	2.8
FR 1953	Tess	66	0.09	16.5	10.3	46.5	-10.4	2.8
FR 1954	Tess	67	0.095	16.7	9.8	46.5	-10.9	2.8
FR 1955	Tess	68	0.092	16.7	9.7	46.6	-11.0	2.8
FR 1956	Tess	69	0.1	15.7	9.8	43.9	-10.8	2.8
FR 1957	Tess	70	0.083	15.4	9.7	43.4	-11.1	2.8
FR 1958	Tess	71	0.085	15.7	9.8	44.3	-11.1	2.8
FR 1959	Tess	72	0.075	16.4	9.6	46.6	-11.3	2.8
FR 1960	Tess	73	0.082	15.2	9.5	43.3	-11.4	2.8
FR 1961	Tess	74	0.09	16.1	9.5	45.1	-11.5	2.8
FR 1962	Tess	75	0.09	14.8	9.6	41.2	-11.2	2.8
FR 1963	Tess	76	0.07	15.5	9.1	44.2	-11.6	2.9
FR 1964	Tess	77	0.075	17.9	9.2	50.1	-11.0	2.8
FR 1965	Tess	78	0.07	16.4	9.6	46.4	-11.0	2.8
FR 1966	Tess	79	0.085	15.4	9.5	43.7	-10.6	2.8
FR 1967	Tess	80	0.073	15.2	9.6	43.1	-10.7	2.8
FR 1968	Tess	81	0.075	16.1	9.5	45.6	-10.3	2.8
FR 1969	Tess	82	0.056	17.8	9.2	50.5	-10.1	2.8
FR 1970	Tess	83	0.063	16.7	9.2	47.2	-10.2	2.8
FR 1971	Tess	84	0.056	16.6	9.2	47.4	-10.1	2.8
FR 1972	Tess	85	0.065	15.5	9.3	44.1	-10.1	2.9
FR 1973	Tess	86	0.06	15.4	9.1	44.0	-10.4	2.9
FR 1974	Tess	87	0.062	15.4	9.5	43.9	-10.3	2.9
FR 1975	Tess	88	0.06	15.3	9.5	44.3	-10.4	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 1976	Tess	89	0.067	15.5	9.1	44.2	-10.1	2.8
FR 1977	Tess	90	0.06	15.7	8.8	45.0	-10.1	2.9
FR 1978	Tess	91	0.055	15.7	9.2	45.1	-10.5	2.9
FR 1979	Tess	92	0.055	16.1	9.1	46.9	-10.6	2.9
FR 1980	Tess	93	0.05	15.5	9.0	44.8	-10.8	2.9
FR 1981	Tess	94	0.055	15.7	8.6	45.2	-11.2	2.9
FR 1982	Tess	95	0.047	18.0	8.4	51.5	-11.6	2.9
FR 1983	Tess	96	0.045	16.6	8.7	47.8	-11.0	2.9
FR 1984	Tess	97	0.048	15.9	8.8	46.3	-11.1	2.9
FR 1985	Tess	98	0.046	16.3	9.0	46.9	-10.7	2.9
FR 1986	Tess	99	0.046	17.0	9.2	49.2	-10.1	2.9
FR 1987	Tess	100	0.04	15.5	9.0	45.6	-10.1	2.9
FR 1988	Tess	101	0.042	14.9	9.2	43.5	-10.1	2.9
FR 1989	Tess	102	0.04	15.5	8.6	45.9	-10.2	3.0
FR 1990	Tess	103	0.038	15.5	8.8	45.9	-10.2	3.0
FR 1991	Tess	104	0.04	15.8	8.7	46.3	-10.0	2.9
FR 1992	Tess	105	0.042	18.1	8.6	53.2	-9.6	2.9
FR 1993	Tess	106	0.04	14.8	8.4	43.9	-10.1	3.0
FR 1994	Tess	107	0.045	15.4	8.5	45.6	-10.6	3.0
FR 1995	Tess	108	0.038	15.5	8.6	46.7	-11.1	3.0
FR 1996	Tess	109	0.035	15.1	8.7	45.3	-10.5	3.0
FR 1997	Tess	110	0.034	15.1	8.8	46.0	-10.2	3.0
FR 1998	Tess	111	0.036	15.0	8.6	48.1	-11.1	3.2
FR 1999	Tess	112	0.04	15.7	9.0	46.7	-9.9	3.0
FR 2000	Tess	113	0.036	15.1	8.8	45.4	-10.5	3.0
FR 2001	Tess	114	0.034	15.1	8.6	46.4	-10.7	3.1
FR 2002	Tess	115	0.03	14.6	8.7	45.3	-10.8	3.1
FR 2003	Tess	116	0.03	15.6	9.3	47.2	-10.4	3.0
FR 2004	Tess	117	0.03	15.4	8.8	47.3	-10.4	3.1
FR 2005	Tess	118	0.028	15.0	9.1	46.9	-10.7	3.1
FR 2006	Tess	119	0.032	14.2	9.2	43.7	-10.7	3.1
FR 2007	Tess	120	0.027	14.6	8.7	45.9	-10.8	3.2
FR 2008	Tess	121	0.028	16.2	8.6	49.5	-10.7	3.1
FR 2009	Tess	122	0.02	17.4	9.4	54.0	-11.3	3.1
FR 2010	Tess	123	0.025	15.0	9.1	46.3	-11.2	3.1
FR 2011	Tess	124	0.027	14.3	9.8	43.6	-11.4	3.0
FR 2012	Tess	125	0.024	16.1	9.6	49.6	-11.9	3.1
FR 2013	Tess	126	0.02	17.5	8.7	54.3	-11.6	3.1
FR 2014	Tess	127	0.025	15.7	9.1	47.5	-11.5	3.0
FR 2015	Tess	128	0.025	14.6	9.4	44.9	-11.6	3.1
FR 2016	Tess	129	0.022	14.1	9.4	43.4	-11.9	3.1
FR 2017	Tess	130	0.02	15.3	8.9	48.1	-11.9	3.2
FR 2018	Tess	131	0.022	14.8	8.3	45.8	-12.1	3.1

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 2019	Tess	132	0.02	16.6	8.7	52.5	-12.7	3.2
FR 2020	Tess	133	0.018	16.3	8.6	50.9	-14.6	3.1
FR 2021	Tess	134	0.019	14.9	8.8	47.6	-16.4	3.2
FR 2022	Tess	135	0.018	14.2	8.7	46.9	-15.6	3.3
FR 2023	Tess	136	0.015	16.4	8.7	55.0	-13.9	3.3
FR 2024	Tess	137	0.015	20.6	8.4	65.1	-12.3	3.2
FR 2025	Tess	138	0.014	18.9	9.0	60.6	-12.5	3.2
FR 2026	Tess	139	0.011	20.2	8.4	66.1	-12.9	3.3
FR 2027	Tess	140	0.011	19.6	11.0	63.8	-12.4	3.3
FR 2028	Tess	141	0.01	17.9	10.5	60.3	-12.7	3.4
FR 2029	Tess	142	0.005	22.5	10.6	85.2	-14.3	3.8
FR 2143	Boesman	1	0.3	10.3	9.2	30.5	-16.2	3.0
FR 2144	Boesman	2	0.4	12.4	9.1	35.5	-14.6	2.9
FR 2145	Boesman	3	0.5	12.6	9.2	36.0	-12.5	2.9
FR 2146	Boesman	4	0.5	14.7	9.4	42.0	-11.8	2.9
FR 2147	Boesman	5	0.5	14.0	9.7	39.9	-11.5	2.9
FR 2148	Boesman	6	0.5	15.8	9.5	45.0	-11.1	2.8
FR 2149	Boesman	7	0.5	14.3	9.7	40.8	-11.0	2.9
FR 2150	Boesman	8	0.7	13.2	9.7	38.0	-11.1	2.9
FR 2151	Boesman	9	0.6	11.9	9.9	33.9	-11.5	2.9
FR 2152	Boesman	10	0.6	14.0	9.9	40.0	-11.3	2.9
FR 2153	Boesman	11	0.6	11.8	10.0	33.8	-11.1	2.9
FR 2154	Boesman	12	0.6	12.7	10.1	35.9	-11.3	2.8
FR 2155	Boesman	13	0.7	9.6	10.0	27.1	-11.2	2.8
FR 2156	Boesman	14	0.6	13.6	10.1	38.6	-11.2	2.8
FR 2157	Boesman	15	0.5	12.5	10.2	35.6	-11.4	2.8
FR 2158	Boesman	16	0.6	13.1	10.0	37.2	-12.2	2.8
FR 2159	Boesman	17	0.6	13.2	9.8	37.3	-13.5	2.8
FR 2160	Boesman	18	0.7	11.8	9.7	33.5	-12.9	2.8
FR 2161	Boesman	19	0.5	17.0	9.4	48.3	-12.2	2.8
FR 2162	Boesman	20	0.5	17.1	9.4	48.4	-11.7	2.8
FR 2163	Boesman	21	0.5	14.8	9.4	42.1	-11.2	2.8
FR 2164	Boesman	22	0.6	12.4	9.8	34.9	-11.2	2.8
FR 2165	Boesman	23	0.5	14.2	9.7	40.2	-11.3	2.8
FR 2166	Boesman	24	0.5	15.7	9.7	44.6	-11.6	2.8
FR 2167	Boesman	25	0.5	13.9	9.6	39.6	-11.6	2.8
FR 2168	Boesman	26	0.5	16.1	9.6	45.9	-11.3	2.8
FR 2169	Boesman	27	0.5	13.0	9.5	37.1	-11.7	2.9
FR 2170	Boesman	28	0.6	12.5	9.2	35.4	-11.9	2.8
FR 2171	Boesman	29	0.5	11.7	9.2	33.3	-11.4	2.8
FR 2172	Boesman	30	0.4	17.0	9.4	48.2	-11.1	2.8
FR 2173	Boesman	31	0.5	11.7	9.7	33.2	-11.4	2.8
FR 2174	Boesman	32	0.4	14.4	10.1	40.8	-11.4	2.8

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N (‰)	% C	Standard corrected δ13C (‰)	C:N ratio elemental
FR 2175	Boesman	33	0.4	14.5	10.3	41.1	-11.1	2.8
FR 2176	Boesman	34	0.4	14.9	10.3	42.6	-11.1	2.9
FR 2177	Boesman	35	0.5	11.4	10.2	32.6	-11.5	2.9
FR 2178	Boesman	36	0.4	12.3	10.1	35.3	-11.9	2.9
FR 2179	Boesman	37	0.5	13.0	10.1	37.0	-12.9	2.8
FR 2180	Boesman	38	0.4	13.1	9.9	37.3	-13.8	2.8
FR 2181	Boesman	39	0.4	14.8	10.1	42.2	-13.4	2.9
FR 2182	Boesman	40	0.4	13.6	10.2	38.7	-12.2	2.9
FR 2183	Boesman	41	0.4	14.1	10.3	40.4	-11.5	2.9
FR 2184	Boesman	42	0.3	16.7	10.4	47.9	-11.0	2.9
FR 2185	Boesman	43	0.3	17.2	10.8	49.5	-10.8	2.9
FR 2186	Boesman	44	0.3	16.9	10.8	48.3	-10.9	2.9
FR 2187	Boesman	45	0.3	17.5	10.4	49.9	-10.9	2.9
FR 2188	Boesman	46	0.3	17.2	10.0	49.1	-11.0	2.9
FR 2189	Boesman	47	0.243	14.9	9.9	42.9	-11.4	2.9
FR 2190	Boesman	48	0.26	15.4	9.8	44.0	-11.3	2.9
FR 2191	Boesman	49	0.258	14.9	9.7	43.0	-11.2	2.9
FR 2192	Boesman	50	0.3	14.8	9.7	42.3	-11.1	2.9
FR 2193	Boesman	51	0.24	14.6	9.7	42.3	-11.1	2.9
FR 2194	Boesman	52	0.24	15.0	9.8	43.0	-11.1	2.9
FR 2195	Boesman	53	0.232	15.0	9.7	43.0	-11.1	2.9
FR 2196	Boesman	54	0.227	14.7	9.8	42.3	-11.0	2.9
FR 2197	Boesman	55	0.22	14.7	9.7	42.3	-10.8	2.9
FR 2198	Boesman	56	0.205	15.1	9.8	43.8	-10.6	2.9
FR 2199	Boesman	57	0.17	18.8	9.6	54.0	-10.6	2.9
FR 2200	Boesman	58	0.19	15.9	9.4	46.1	-10.6	2.9
FR 2201	Boesman	59	0.2	17.4	9.5	51.1	-10.6	2.9
FR 2202	Boesman	60	0.194	14.8	9.5	43.2	-10.7	2.9
FR 2203	Boesman	61	0.182	14.8	9.5	43.5	-10.7	2.9
FR 2204	Boesman	62	0.17	14.9	9.5	44.0	-10.7	3.0
FR 2205	Boesman	63	0.19	15.6	9.7	46.2	-11.0	3.0
FR 2206	Boesman	64	0.174	14.8	9.7	43.7	-11.2	3.0
FR 2207	Boesman	65	0.167	14.7	9.4	43.4	-11.4	3.0
FR 2208	Boesman	66	0.168	14.6	8.9	43.3	-11.7	3.0
FR 2209	Boesman	67	0.17	15.6	9.1	46.3	-11.5	3.0
FR 2210	Boesman	68	0.176	15.8	9.1	46.2	-11.6	2.9
FR 2211	Boesman	69	0.175	14.5	9.2	43.6	-11.3	3.0
FR 2212	Boesman	70	0.161	14.7	9.3	43.4	-11.0	3.0
FR 2213	Boesman	71	0.168	15.2	9.7	45.0	-10.8	3.0
FR 2214	Boesman	72	0.144	14.2	9.6	42.5	-10.7	3.0
FR 2215	Boesman	73	0.13	12.5	9.0	37.2	-10.4	3.0
FR 2216	Boesman	74	0.154	15.8	8.8	46.5	-10.8	2.9
FR 2217	Boesman	75	0.157	14.5	8.8	42.9	-11.0	3.0
FR 2218	Boesman	76	0.142	15.4	9.2	45.2	-10.6	2.9

Appendix B: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of the analysed non-lipid extracted and lipid-extracted meat samples used to calculate whisker-diet TDFs

NON-LIPID EXTRACTED							
Sample ID	**Meat type**	**Weight (mg)**	**% N**	**Standard corrected δ15N**	**% C**	**Standard corrected δ13C**	**C:N ratio elemental**
FR2077	Giraffe	0.6	8.7	5.7	28.1	-24.5	3.2
FR2078	Beef	0.6	14.6	6.2	46.6	-12.1	3.2
FR2079	Chicken	0.6	18.1	1.4	57.3	-18.0	3.2
FR2088	Chicken	0.5	16.8	1.5	54.0	-18.2	3.2
FR2089	Beef	0.5	14.6	6.3	47.3	-12.5	3.2
FR2090	Giraffe	0.5	10.9	6.2	37.3	-25.0	3.4
FR2091	Beef	0.5	14.2	9.1	46.3	-15.4	3.3
FR2092	Beef	0.6	12.9	9.0	43.6	-14.8	3.4
FR2093	Beef	0.5	16.2	9.4	54.5	-14.1	3.4
FR2094	Beef	0.5	15.8	9.8	55.9	-14.3	3.5
FR2095	Giraffe	0.5	4.6	5.6	15.6	-25.0	3.4
FR2096	Giraffe	0.6	17.1	5.8	56.1	-24.5	3.3
FR2097	Giraffe	0.6	12.9	6.1	42.3	-24.6	3.3
FR2098	Giraffe	0.5	12.2	5.7	41.3	-25.0	3.4
FR2099	Chicken	0.6	5.6	3.1	20.4	-18.2	3.7
FR2100	Chicken	0.5	7.3	2.6	25.9	-17.8	3.5
FR2101	Chicken	0.5	28.2	2.6	92.2	-17.2	3.3
FR2102	Chicken	0.5	13.7	3.3	48.5	-17.0	3.5
FR2103	Giraffe	0.5	6.1	5.8	21.0	-25.0	3.4
FR2104	Giraffe	0.5	7.9	5.3	25.7	-24.5	3.3
FR2105	Giraffe	0.5	10.3	5.5	34.6	-24.7	3.4
FR2106	Giraffe	0.5	13.5	5.8	45.3	-24.7	3.4
FR2107	Chicken	0.5	15.9	2.5	51.4	-17.0	3.2
FR2108	Chicken	0.5	9.8	3.2	36.5	-17.5	3.7
FR2109	Chicken	0.5	11.3	2.2	37.4	-17.7	3.3
FR2110	Chicken	0.6	23.5	3.4	77.6	-16.5	3.3
FR2111	Beef	0.6	13.9	5.3	44.9	-15.1	3.2
FR2112	Beef	0.5	13.7	6.9	42.2	-11.2	3.1
FR2113	Beef	0.5	7.3	7.8	25.0	-12.7	3.4
FR2114	Beef	0.5	12.8	8.6	42.6	-12.5	3.3
FR2115	Beef	0.6	12.6	7.1	42.8	-13.5	3.4
FR2116	Beef	0.6	16.1	7.5	53.3	-13.4	3.3
FR2117	Beef	0.5	10.0	7.1	33.3	-13.5	3.3
FR2118	Beef	0.6	12.5	9.8	42.9	-14.3	3.4
FR2119	Chicken	0.6	15.3	2.0	49.5	-17.3	3.2
FR2120	Chicken	0.5	15.5	1.9	51.6	-17.4	3.3
FR2121	Chicken	0.5	14.7	1.8	48.3	-17.8	3.3
FR2122	Chicken	0.5	15.0	1.7	50.1	-17.8	3.3
FR2123	Giraffe	0.5	14.7	5.4	48.1	-24.0	3.3
FR2124	Giraffe	0.6	5.6	5.9	19.1	-24.4	3.4

Sample ID	Meat type	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR2125	Giraffe	0.6	12.5	5.7	42.7	-24.4	3.4
FR2126	Giraffe	0.5	11.1	5.9	37.8	-24.4	3.4
			LIPID-EXCTRACTED				
FR2492	Beef	0.5	11.7	6.5	36.8	-11.9	3.1
FR2493	Chicken	0.6	10.8	1.6	33.5	-17.8	3.1
FR2494	Chicken	0.6	9.1	1.7	28.0	-17.6	3.1
FR2495	Beef	0.5	22.6	6.4	71.4	-11.8	3.2
FR2496	Beef	0.5	14.3	9.3	44.9	-14.8	3.1
FR2497	Beef	0.6	7.5	9.4	24.3	-13.4	3.2
FR2498	Chicken	0.6	30.9	2.7	96.2	-17.4	3.1
FR2499	Chicken	0.6	9.9	3.5	31.3	-16.7	3.2
FR2500	Chicken	0.5	14.5	2.5	44.7	-17.6	3.1
FR2501	Chicken	0.5	11.6	3.5	35.7	-17.0	3.1
FR2502	Beef	0.5	9.2	8.1	30.4	-12.7	3.3
FR2503	Beef	0.6	9.1	8.7	29.1	-12.3	3.2
FR2504	Beef	0.5	21.0	7.4	67.8	-13.6	3.2
FR2505	Beef	0.5	7.5	7.6	23.7	-13.7	3.1
FR2506	Chicken	0.5	13.2	2.2	41.0	-17.8	3.1
FR2507	Chicken	0.5	14.1	1.7	43.1	-18.2	3.1

Appendix C: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of the analysed whisker sections from the five Zoo lions used to calculate whisker-diet TDFs

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 722	Amber	1	**1.0**	10.0	9.3	29.1	-12.1	2.9
FR 723	Amber	2	1.0	11.8	9.1	33.6	-12.0	2.9
FR 724	Amber	3	1.1	11.0	9.3	31.4	-11.8	2.9
FR 725	Amber	4	1.1	10.2	9.2	29.4	-11.7	2.9
FR 726	Amber	5	1.1	10.7	9.2	30.6	-12.0	2.9
FR 727	Amber	6	1.0	10.9	9.4	31.3	-11.2	2.9
FR 728	Amber	7	1.0	9.5	9.2	27.2	-11.1	2.9
FR 729	Amber	8	1.0	9.2	9.1	26.4	-10.9	2.9
FR 730	Amber	9	0.9	11.8	9.1	33.8	-10.9	2.9
FR 731	Amber	10	0.9	9.6	9.2	27.1	-10.8	2.8
FR 732	Amber	11	0.9	11.9	9.1	34.3	-11.2	2.9
FR 733	Amber	12	0.9	11.3	9.4	32.5	-11.5	2.9
FR 734	Amber	13	0.8	13.7	9.7	39.4	-11.6	2.9
FR 735	Amber	14	0.8	12.2	9.5	34.8	-11.3	2.9
FR 736	Amber	15	0.8	11.1	9.4	32.0	-11.8	2.9
FR 737	Amber	16	0.8	12.2	9.6	34.8	-11.4	2.9
FR 738	Amber	17	0.7	13.4	9.6	38.2	-11.7	2.8
FR 739	Amber	18	0.7	13.0	9.5	37.1	-11.8	2.9
FR 740	Amber	19	0.7	11.8	9.4	33.5	-11.0	2.8
FR 741	Amber	20	0.6	13.6	8.9	38.6	-10.9	2.8
FR 742	Amber	21	0.6	11.6	8.8	32.9	-11.1	2.8
FR 743	Amber	22	0.6	13.2	8.9	37.5	-11.3	2.8
FR 744	Amber	23	0.6	11.5	9.1	32.6	-11.0	2.8
FR 745	Amber	24	0.6	11.8	9.1	33.6	-11.2	2.8
FR 746	Amber	25	0.5	12.4	8.9	35.3	-11.2	2.8
FR 747	Amber	26	0.5	12.7	9.0	36.0	-11.0	2.8
FR 748	Amber	27	0.5	11.1	9.0	31.4	-11.0	2.8
FR 749	Amber	28	0.5	12.5	9.1	35.2	-11.1	2.8
FR 750	Amber	29	0.6	17.8	9.4	50.2	-10.7	2.8
FR 751	Amber	30	0.7	14.8	9.1	41.8	-10.8	2.8
FR 2462	Bianca	1	1.3	14.3	9.5	41.4	-10.6	2.89
FR 2463	Bianca	2	1.1	13.2	9.5	37.8	-10.7	2.87
FR 2464	Bianca	3	1.1	13.1	10.1	37.4	-11.4	2.87
FR 2465	Bianca	4	0.9	11.9	9.8	34.1	-12.7	2.9
FR 2466	Bianca	5	0.8	9.5	9.5	27.3	-11.9	2.9
FR 2467	Bianca	6	0.6	8.8	9.1	25.4	-11.5	2.9
FR 2468	Emma	1	0.9	12.4	10.1	35.6	-11.0	2.9
FR 2469	Emma	2	0.6	14.7	9.1	42.3	-11.3	2.9
FR 2470	Emma	3	0.4	18.5	9.8	52.7	-11.3	2.8

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 2472	Emma	5	0.4	13.6	9.5	38.5	-11.6	2.8
FR 2473	Emma	6	0.3	11.7	9.8	33.2	-11.0	2.8
FR 2474	Tess	1	0.9	17.6	9.3	50.3	-11.0	2.9
FR 2475	Tess	2	1.0	13.8	9.6	39.3	-10.8	2.8
FR 2476	Tess	3	0.8	15.6	9.4	43.9	-11.1	2.8
FR 2477	Tess	4	0.6	15.7	9.5	43.5	-10.9	2.8
FR 2478	Tess	5	0.5	16.3	10.1	45.6	-11.3	2.8
FR 2479	Tess	6	0.3	17.5	9.4	48.8	-11.6	2.8
FR 2480	Boesman	1	1.5	14.9	9.8	42.4	-11.0	2.9
FR 2481	Boesman	2	1.1	14.1	9.4	40.1	-10.8	2.8
FR 2482	Boesman	3	0.9	12.4	9.5	35.4	-11.7	2.8
FR 2483	Boesman	4	0.7	14.6	9.3	41.3	-11.3	2.8
FR 2484	Boesman	5	0.5	15.7	9.6	44.4	-10.9	2.8
FR 2485	Boesman	6	0.5	14.4	9.5	40.7	-11.2	2.8
FR 2486	Diesel	1	1.5	14.2	8.6	40.7	-10.9	2.9
FR 2487	Diesel	2	1.3	14.2	7.9	40.7	-11.5	2.9
FR 2488	Diesel	3	1.3	14.3	8.9	41.0	-11.5	2.9
FR 2489	Diesel	4	1.1	12.7	8.6	38.8	-11.5	3.1
FR 2490	Diesel	5	0.7	12.0	8.3	34.5	-13.4	2.9
FR 2491	Diesel	6	0.6	14.2	8.4	40.8	-12.5	2.9

Appendix D: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of the analysed common C_3 and C_4 plant species from the study area

Sample ID	Species	Common name	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 1234	*Sclerocarya birrea*	Marula tree	1.9	1.4	5.2	56.2	-29.2	39.6
FR 1235	*Sclerocarya birrea*	Marula tree	2.0	1.2	7.5	41.0	-29.2	35.2
FR 1236	*Sclerocarya birrea*	Marula tree	2.0	1.3	2.4	46.3	-29.7	35.3
FR 1237	*Ziziphus mucronata*	Buffalo-thorn tree	2.1	2.0	1.0	42.7	-29.4	21.6
FR 1238	*Ziziphus mucronata*	Buffalo-thorn tree	2.0	2.1	1.7	38.9	-29.5	18.6
FR 1239	*Ziziphus mucronata*	Buffalo-thorn tree	2.0	2.2	1.3	43.9	-28.9	20.4
FR 1240	*Dichrostachys cinerea*	Sickle bush	1.9	1.6	0.5	42.7	-28.5	27.5
FR 1241	*Dichrostachys cinerea*	Sickle bush	2.0	1.5	-0.2	37.5	-28.8	25.5
FR 1242	*Dichrostachys cinerea*	Sickle bush	2.0	1.4	-0.9	46.4	-29.6	32.7
FR 1243	*Vachellia xanthophloea*	Fever tree	2.1	2.3	7.5	38.2	-31.2	16.6
FR 1244	*Vachellia xanthophloea*	Fever tree	2.1	2.3	7.8	39.4	-31.1	17.4
FR 1245	*Vachellia xanthophloea*	Fever tree	2.0	3.0	6.1	44.0	-30.0	14.7
FR 1246	*Vachellia nilotica*	Scented-pod acacia	2.0	2.1	1.8	47.4	-30.1	22.2
FR 1247	*Vachellia nilotica*	Scented-pod acacia	2.1	2.1	5.0	42.7	-28.5	20.0
FR 1248	*Vachellia nilotica*	Scented-pod acacia	2.0	3.0	5.1	51.3	-30.2	17.2
FR 1249	*Themeda triandra*	Red grass	2.0	0.8	-1.4	37.9	-13.1	49.4
FR 1250	*Themeda triandra*	Red grass	1.9	0.5	-1.8	34.6	-13.1	63.7
FR 1251	*Themeda triandra*	Red grass	2.0	0.6	-1.6	36.5	-12.7	56.3
FR 1252	*Sporobolus pyramidalus*	Cat's tail grass	2.0	0.6	-0.7	43.2	-13.5	75.0
FR 1253	*Sporobolus pyramidalus*	Cat's tail grass	2.0	0.5	-1.1	41.2	-13.5	78.8
FR 1254	*Sporobolus pyramidalus*	Cat's tail grass	2.1	0.4	-1.7	38.6	-13.0	93.9
FR 1255	*Eustachys paspaloides*	Fan grass	2.0	1.1	0.9	52.9	-14.3	46.7
FR 1256	*Eustachys paspaloides*	Fan grass	2.1	0.6	-2.1	40.2	-13.1	68.0
FR 1257	*Eustachys paspaloides*	Fan grass	2.0	0.6	0.3	39.9	-13.5	65.3
FR 1258	*Cynodon dactylon*	Couch grass	2.0	1.1	7.0	34.4	-13.9	30.1
FR 1259	*Cynodon dactylon*	Couch grass	2.0	1.0	8.0	32.1	-14.5	33.3
FR 1260	*Cynodon dactylon*	Couch grass	2.0	0.9	9.3	40.2	-13.9	44.0

Sample ID	Species	Common name	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 1261	*Cymbopogon excavatus*	Broad-leaved turpentine	2.0	0.9	0.3	39.9	-13.2	45.1
FR 1262	*Cymbopogon excavatus*	Broad-leaved turpentine	2.1	0.7	-0.4	42.9	-13.8	62.8
FR 1263	*Cymbopogon excavatus*	Broad-leaved turpentine	1.9	0.5	-0.7	36.9	-13.2	76.5

Appendix E: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of analysed faecal samples for the leopard primary prey collected from Phinda and the surrounding areas

Sample ID	Species	Common name	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 1147	*Connochaetes taurinus*	Wildebeest	2.0	1.2	4.8	39.0	-14.0	33.7
FR 1148	*Connochaetes taurinus*	Wildebeest	2.1	1.0	4.7	32.9	-14.0	34.3
FR 1149	*Connochaetes taurinus*	Wildebeest	2.0	1.1	4.7	39.2	-13.9	35.7
FR 1150	*Connochaetes taurinus*	Wildebeest	2.0	1.3	4.9	39.6	-14.2	29.4
FR 1151	*Connochaetes taurinus*	Wildebeest	2.1	1.2	4.9	40.5	-14.2	33.7
FR 1152	*Connochaetes taurinus*	Wildebeest	2.0	1.2	4.9	41.9	-14.0	35.6
FR 1153	*Equus quagga*	Zebra	2.1	1.6	5.0	46.1	-13.9	28.8
FR 1154	*Equus quagga*	Zebra	2.0	0.9	4.5	36.3	-13.9	39.2
FR 1155	*Equus quagga*	Zebra	2.0	0.9	5.8	32.1	-13.9	34.7
FR 1156	*Aepyceros melampus*	Impala	2.0	1.3	5.2	30.0	-20.0	23.8
FR 1157	*Aepyceros melampus*	Impala	2.1	1.9	4.5	44.8	-19.8	23.6
FR 1158	*Aepyceros melampus*	Impala	2.1	2.0	5.0	43.6	-19.8	22.0
FR 1159	*Aepyceros melampus*	Impala	1.9	1.9	4.6	40.7	-20.3	21.2
FR 1160	*Aepyceros melampus*	Impala	1.9	2.0	5.1	41.4	-19.3	20.6
FR 1264	*Cephalophus natalensis*	Red duiker	2.1	2.0	4.1	38.3	-28.1	19.0
FR 1265	*Cephalophus natalensis*	Red duiker	2.1	2.2	4.1	41.6	-27.8	19.3
FR 1266	*Cephalophus natalensis*	Red duiker	2.0	2.3	1.5	48.2	-28.0	20.8
FR 1267	*Cephalophus natalensis*	Red duiker	2.0	2.1	6.5	45.4	-28.8	21.6
FR 1268	*Cephalophus natalensis*	Red duiker	2.0	3.4	4.9	48.3	-27.5	14.3
FR 1269	*Cephalophus natalensis*	Red duiker	2.0	2.3	6.9	43.0	-28.8	19.0
FR 1270	*Sylvicapra grimmia*	Common duiker	2.1	2.5	4.8	44.2	-28.5	17.7
FR 1271	*Sylvicapra grimmia*	Common duiker	1.9	2.3	4.2	37.7	-28.3	16.5
FR 1272	*Sylvicapra grimmia*	Common duiker	2.0	2.7	4.7	37.4	-27.7	13.8
FR 1273	*Sylvicapra grimmia*	Common duiker	2.0	1.8	7.1	36.0	-29.2	19.9
FR 1274	*Sylvicapra grimmia*	Common duiker	2.0	2.7	4.9	39.7	-27.4	14.6
FR 1275	*Giraffa camelopardalis*	Giraffe	2.1	2.7	2.6	46.2	-28.9	17.1
FR 1276	*Giraffa camelopardalis*	Giraffe	1.9	2.5	3.0	43.9	-28.7	17.9
FR 1277	*Giraffa camelopardalis*	Giraffe	2.0	2.7	3.0	50.2	-28.7	18.3

Sample ID	**Species**	**Common name**	**Weight (mg)**	**% N**	**Standard corrected δ15N**	**% C**	**Standard corrected δ13C**	**C:N ratio elemental**
FR 1278	*Giraffa camelopardalis*	Giraffe	2.0	2.4	3.2	44.8	-29.1	19.0
FR 1279	*Giraffa camelopardalis*	Giraffe	2.1	2.5	3.1	40.9	-28.3	16.4
FR 1280	*Aepyceros melampus*	Impala	2.1	2.2	3.2	44.0	-24.7	20.4
FR 1281	*Aepyceros melampus*	Impala	2.1	2.1	3.2	42.2	-24.4	19.8
FR 1282	*Aepyceros melampus*	Impala	2.0	1.9	3.8	42.6	-23.5	22.3
FR 1283	*Aepyceros melampus*	Impala	2.0	2.3	3.2	45.2	-24.4	19.4
FR 1288	*Kobus ellipsiprymnus*	Waterbuck	2.0	0.9	4.0	35.9	-14.7	39.2
FR 1289	*Kobus ellipsiprymnus*	Waterbuck	2.1	0.9	4.0	35.3	-14.6	39.8
FR 1290	*Kobus ellipsiprymnus*	Waterbuck	2.0	1.0	3.9	39.8	-14.3	41.2
FR 1291	*Kobus ellipsiprymnus*	Waterbuck	2.0	1.0	4.1	35.7	-14.6	34.2
FR 2317	*Phacochoerus africanus*	Warthog	2.1	1.2	5.2	32.8	-15.0	26.2
FR 2318	*Phacochoerus africanus*	Warthog	2.1	1.2	5.5	26.1	-15.4	21.9
FR 2319	*Phacochoerus africanus*	Warthog	2.0	1.5	4.4	34.4	-15.6	23.7
FR 2320	*Phacochoerus africanus*	Warthog	2.0	1.3	4.8	34.9	-15.4	26.3
FR 2321	*Phacochoerus africanus*	Warthog	2.0	1.6	4.0	34.5	-14.9	22.1
FR 2322	*Phacochoerus africanus*	Warthog	2.0	1.4	4.1	34.8	-15.3	24.8

Appendix F: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of analysed hair samples for the leopard primary prey collected from Phinda and the surrounding areas

Sample ID	Species	Common name	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 1161	*Connochaetes taurinus*	Wildebeest	0.5	15.2	7.2	44.6	-9.8	2.9
FR 1162	*Connochaetes taurinus*	Wildebeest	0.6	7.7	5.5	22.6	-9.4	2.9
FR 1163	*Connochaetes taurinus*	Wildebeest	0.5	7.6	6.2	22.6	-9.2	3.0
FR 1164	*Connochaetes taurinus*	Wildebeest	0.5	17.4	6.6	52.2	-9.4	3.0
FR 1165	*Connochaetes taurinus*	Wildebeest	0.5	9.4	5.8	28.2	-9.3	3.0
FR 1166	*Tragelaphus angasii*	Nyala	0.5	9.3	6.6	28.1	-18.6	3.0
FR 1167	*Tragelaphus angasii*	Nyala	0.5	3.3	6.7	10.0	-18.8	3.0
FR 1168	*Tragelaphus angasii*	Nyala	0.5	5.6	7.9	16.7	-22.2	3.0
FR 1169	*Tragelaphus angasii*	Nyala	0.6	4.8	7.4	14.3	-18.3	3.0
FR 1170	*Tragelaphus angasii*	Nyala	0.5	12.3	6.2	36.6	-17.3	3.0
FR 1171	*Aepyceros melampus*	Impala	0.5	11.7	7.6	36.4	-13.6	3.1
FR 1172	*Aepyceros melampus*	Impala	0.6	14.2	6.9	43.2	-10.9	3.0
FR 1173	*Aepyceros melampus*	Impala	0.6	14.1	6.9	41.9	-14.9	3.0
FR 1174	*Aepyceros melampus*	Impala	0.5	17.1	7.7	51.1	-12.7	3.0
FR 1175	*Aepyceros melampus*	Impala	0.5	16.6	7.9	50.6	-13.1	3.0
FR 1176	*Tragelaphus strepsiceros*	Kudu	0.5	24.3	5.1	74.1	-24.2	3.1
FR 1177	*Tragelaphus strepsiceros*	Kudu	0.6	16.4	5.9	50.3	-23.5	3.1
FR 1178	*Tragelaphus strepsiceros*	Kudu	0.5	14.1	6.3	42.9	-21.9	3.0

Appendix G: $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N ratio values of the analysed sections for the six leopard whiskers collected from Phinda

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 277	M70	1	0.5	15.3	10.9	43.9	-9.8	2.9
FR 278	M70	2	0.5	14.0	10.7	40.3	-10.5	2.9
FR 279	M70	3	0.5	14.0	10.3	40.2	-10.3	2.9
FR 280	M70	4	0.5	11.7	9.8	33.5	-9.7	2.9
FR 281	M70	5	0.5	11.6	9.9	33.3	-9.4	2.9
FR 282	M70	6	0.5	10.5	9.6	30.4	-9.4	2.9
FR 283	M70	7	0.5	11.4	10.1	32.6	-10.2	2.9
FR 284	M70	8	0.5	10.4	10.7	29.9	-10.6	2.9
FR 285	M70	9	0.5	11.7	10.2	33.7	-10.4	2.9
FR 286	M70	10	0.7	7.9	10.4	22.7	-10.7	2.9
FR 287	M70	11	0.7	10.7	10.3	30.9	-12.7	2.9
FR 288	M70	12	0.5	12.2	9.3	35.2	-9.7	2.9
FR 289	M70	13	0.5	14.2	9.3	40.9	-9.4	2.9
FR 290	M70	14	0.5	12.0	8.9	34.5	-9.2	2.9
FR 291	M70	15	0.5	11.7	9.5	34.0	-10.5	2.9
FR 292	M70	16	0.5	9.7	10.2	28.1	-11.5	2.9
FR 293	M70	17	0.5	12.5	10.2	36.1	-11.1	2.9
FR 294	M70	18	0.5	15.1	10.4	43.9	-13.1	2.9
FR 295	M70	19	0.5	10.8	10.2	31.3	-13.2	2.9
FR 296	M70	20	0.5	10.9	10.5	31.6	-12.2	2.9
FR 297	M73	1	1.2	11.1	10.3	31.6	-15.7	2.9
FR 298	M73	2	1.2	13.4	10.2	38.5	-15.4	2.9
FR 299	M73	3	1.1	13.3	10.2	38.1	-13.9	2.9
FR 300	M73	4	1.1	12.1	9.9	34.7	-11.9	2.9
FR 301	M73	5	1.1	12.0	9.7	34.2	-11.9	2.9
FR 302	M73	6	1.1	11.4	9.9	32.8	-13.1	2.9
FR 303	M73	7	1.1	13.0	9.9	37.5	-14.1	2.9
FR 304	M73	8	1	15.0	10.1	43.6	-13.5	2.9
FR 305	M73	9	1	12.9	9.7	37.5	-14.1	2.9
FR 306	M73	10	1	12.3	9.9	35.8	-13.7	2.9
FR 307	M73	11	1	10.9	10.2	31.4	-13.5	2.9
FR 308	M73	12	0.9	12.5	9.9	36.3	-12.8	2.9
FR 309	M73	13	0.9	13.7	9.7	39.9	-12.9	2.9
FR 310	M73	14	0.9	12.3	9.8	35.6	-14.6	2.9
FR 311	M73	15	0.8	14.0	9.8	41.0	-14.7	2.9
FR 312	M73	16	0.8	13.8	9.6	40.5	-17.4	2.9
FR 313	M73	17	0.8	13.1	9.7	38.3	-18.1	2.9
FR 314	M73	18	0.8	12.2	9.4	35.4	-18.7	2.9
FR 315	M73	19	0.8	13.9	9.9	40.4	-17.8	2.9
FR 316	M73	20	0.8	10.3	9.8	29.8	-17.8	2.9
FR 317	M73	21	0.7	13.1	9.8	38.0	-16.5	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 319	M73	23	0.6	12.4	9.6	35.8	-17.5	2.9
FR 320	M73	24	0.6	12.6	9.7	36.4	-18.1	2.9
FR 321	M73	25	0.6	11.0	10.0	32.0	-17.8	2.9
FR 322	M73	26	0.6	11.5	10.1	33.6	-18.2	2.9
FR 323	M73	27	0.5	13.8	9.7	40.1	-16.4	2.9
FR 324	M73	28	0.5	11.4	9.3	33.1	-13.5	2.9
FR 325	M73	29	0.5	12.8	9.9	36.9	-16.4	2.9
FR 326	M73	30	0.5	13.7	9.8	39.9	-17.1	2.9
FR 327	M74	1	1.6	15.1	9.6	43.0	-15.8	2.8
FR 328	M74	2	1.6	15.1	9.5	43.0	-15.7	2.8
FR 329	M74	3	1.6	14.2	9.4	40.4	-14.0	2.8
FR 330	M74	4	1.6	14.9	9.7	42.5	-13.3	2.9
FR 331	M74	5	1.5	13.3	9.5	38.0	-15.7	2.8
FR 332	M74	6	1.5	13.2	9.5	37.5	-15.2	2.9
FR 333	M74	7	1.5	12.8	9.6	36.5	-15.1	2.8
FR 334	M74	8	1.5	13.6	9.7	38.7	-12.2	2.9
FR 335	M74	9	1.4	12.2	9.6	34.9	-10.6	2.9
FR 336	M74	10	1.4	11.6	9.5	33.2	-12.8	2.9
FR 337	M74	11	1.3	11.8	9.6	33.7	-13.7	2.9
FR 338	M74	12	1.2	14.4	10.0	41.1	-14.5	2.9
FR 339	M74	13	1.2	13.8	10.1	39.5	-15.3	2.9
FR 340	M74	14	1.1	15.5	9.7	44.2	-14.0	2.9
FR 341	M74	15	1.1	14.5	9.3	41.5	-15.8	2.9
FR 342	M74	16	1.1	14.7	9.9	42.1	-12.4	2.9
FR 343	M74	17	1.1	14.0	9.5	40.2	-11.8	2.9
FR 344	M74	18	1.1	11.4	9.3	32.8	-12.3	2.9
FR 345	M74	19	1.0	13.1	9.5	37.5	-11.0	2.9
FR 346	M74	20	1.0	11.7	9.7	33.5	-11.4	2.9
FR 347	M74	21	1.0	10.3	10.0	29.7	-14.8	2.9
FR 348	M74	22	0.9	10.3	10.1	29.5	-12.7	2.9
FR 349	M74	23	0.9	11.1	10.1	31.9	-12.6	2.9
FR 350	M74	24	0.8	11.5	9.9	33.0	-15.7	2.9
FR 351	M74	25	0.8	11.3	9.6	32.4	-14.4	2.9
FR 352	M74	26	0.8	11.2	9.9	32.1	-13.9	2.9
FR 353	M74	27	0.7	10.9	9.5	31.3	-14.7	2.9
FR 354	M74	28	0.7	9.8	9.9	28.1	-13.8	2.9
FR 355	M74	29	0.7	9.6	10.0	27.6	-16.2	2.9
FR 356	M74	30	0.6	10.0	9.8	28.6	-14.8	2.9
FR 357	F08	1	1.2	11.5	10.1	34.0	-18.9	3.0
FR 358	F08	2	1.6	14.5	10.3	42.2	-18.2	2.9
FR 359	F08	3	1.6	12.6	10.2	36.7	-18.5	2.9
FR 360	F08	4	1.6	14.2	10.3	41.3	-18.2	2.9
FR 361	F08	5	1.6	13.4	10.1	38.9	-18.8	2.9
FR 362	F08	6	1.6	13.5	10.3	39.1	-19.3	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 363	F08	7	1.5	14.0	10.3	40.3	-18.9	2.9
FR 364	F08	8	1.5	14.6	10.3	42.2	-18.3	2.9
FR 365	F08	9	1.5	13.3	10.3	38.8	-17.3	2.9
FR 366	F08	10	1.5	13.9	10.9	40.2	-17.3	2.9
FR 367	F08	11	1.4	14.0	11.1	40.1	-17.6	2.9
FR 368	F08	12	1.4	14.5	11.1	41.9	-18.2	2.9
FR 369	F08	13	1.3	12.6	10.8	36.5	-17.6	2.9
FR 370	F08	14	1.3	12.8	10.8	36.9	-18.7	2.9
FR 371	F08	15	1.3	12.2	10.9	35.5	-17.7	2.9
FR 372	F08	16	1.2	10.3	10.7	29.9	-18.8	2.9
FR 373	F08	17	1.2	12.3	10.2	35.5	-19.1	2.9
FR 374	F08	18	1.2	12.2	10.1	35.1	-16.2	2.9
FR 375	F08	19	1.2	11.9	10.4	34.4	-16.5	2.9
FR 376	F08	20	1.2	11.9	10.5	34.4	-15.9	2.9
FR 377	F08	21	1.1	11.6	11.2	33.4	-16.1	2.9
FR 378	F08	22	1.1	10.6	11.2	30.8	-17.3	2.9
FR 379	F08	23	1	12.5	10.7	36.2	-16.1	2.9
FR 380	F08	24	1	10.9	10.4	31.6	-16.0	2.9
FR 381	F08	25	1	10.0	11.1	28.8	-15.5	2.9
FR 382	F08	26	0.9	9.6	11.8	27.7	-16.1	2.9
FR 383	F08	27	0.9	9.3	12.3	26.8	-16.9	2.9
FR 384	F08	28	0.8	10.2	11.5	29.4	-14.9	2.9
FR 385	F08	29	0.8	8.8	10.9	25.6	-15.0	2.9
FR 386	F08	30	0.8	7.5	10.7	21.7	-16.2	2.9
FR 387	M62	1	2.3	14.4	9.7	41.3	-15.3	2.9
FR 388	M62	2	2.3	15.0	9.7	42.7	-15.7	2.9
FR 389	M62	3	2.1	14.7	9.7	41.8	-16.0	2.8
FR 390	M62	4	2.1	16.9	9.7	48.0	-16.5	2.8
FR 391	M62	5	2.1	16.8	9.8	47.9	-17.4	2.9
FR 392	M62	6	2.1	16.2	9.6	46.2	-17.8	2.9
FR 393	M62	7	2.1	15.4	9.6	43.8	-16.5	2.8
FR 394	M62	8	2	15.3	10.3	43.6	-16.2	2.8
FR 395	M62	9	2	16.5	10.8	47.2	-15.6	2.9
FR 396	M62	10	2	15.9	10.6	45.6	-16.4	2.9
FR 397	M62	11	2	14.7	10.4	42.1	-17.2	2.9
FR 398	M62	12	1.8	17.2	10.1	49.6	-17.9	2.9
FR 399	M62	13	1.8	16.8	9.7	48.3	-18.5	2.9
FR 400	M62	14	1.8	15.7	9.6	45.1	-18.2	2.9
FR 401	M62	15	1.8	14.1	9.4	40.4	-18.3	2.9
FR 402	M62	16	1.7	16.0	9.9	46.2	-18.2	2.9
FR 403	M62	17	1.7	15.4	10.2	44.2	-17.2	2.9
FR 404	M62	18	1.5	16.1	10.5	46.3	-16.1	2.9
FR 405	M62	19	1.5	16.4	10.6	47.3	-15.0	2.9
FR 406	M62	20	1.4	13.3	10.7	38.2	-14.6	2.9

Sample ID	Individual	Increment No.	Weight (mg)	% N	Standard corrected δ15N	% C	Standard corrected δ13C	C:N ratio elemental
FR 407	M62	21	1.4	14.5	10.7	41.9	-16.3	2.9
FR 408	M62	22	1.3	14.6	10.7	42.3	-17.2	2.9
FR 409	M62	23	1.2	17.4	10.2	50.5	-17.8	2.9
FR 410	M62	24	1.2	14.6	9.8	42.2	-18.7	2.9
FR 411	M62	25	1.0	14.9	10.2	42.9	-19.3	2.9
FR 412	M62	26	1.0	14.9	10.3	43.1	-19.3	2.9
FR 413	M62	27	0.9	16.6	10.3	48.0	-19.3	2.9
FR 414	M62	28	0.9	14.7	10.5	42.5	-18.7	2.9
FR 415	M62	29	0.9	14.0	10.3	40.5	-18.9	2.9
FR 416	M62	30	0.8	17.3	10.1	49.9	-18.6	2.9
FR 417	M60	1	1.3	14.7	10.0	42.3	-13.9	2.9
FR 418	M60	2	1.3	16.4	10.1	46.9	-11.5	2.9
FR 419	M60	3	1.3	13.6	10.5	38.9	-10.3	2.9
FR 420	M60	4	1.3	14.6	10.8	41.7	-10.1	2.9
FR 421	M60	5	1.2	16.1	10.6	45.9	-10.4	2.9
FR 422	M60	6	1.2	15.4	10.1	44.1	-11.6	2.9
FR 423	M60	7	1.2	14.3	9.9	40.8	-12.8	2.9
FR 424	M60	8	1.1	18.2	10.3	52.1	-13.3	2.9
FR 425	M60	9	1.1	18.9	10.6	54.1	-12.6	2.9
FR 426	M60	10	1.1	17.1	10.2	49.2	-11.8	2.9
FR 427	M60	11	1.1	18.9	10.5	54.1	-13.3	2.9
FR 428	M60	12	1.0	20.3	10.7	57.8	-14.2	2.9
FR 429	M60	13	1.0	20.2	10.5	57.7	-13.7	2.9
FR 430	M60	14	1.0	20.8	10.6	59.6	-13.0	2.9
FR 431	M60	15	1.0	18.5	10.4	53.1	-12.8	2.9
FR 432	M60	16	0.9	23.3	10.2	66.6	-11.7	2.9
FR 433	M60	17	0.9	20.6	10.4	58.8	-13.2	2.9
FR434	M60	NO PEAK	NO PEAK	NO PEAK	NO PEAK	NO PEAK	NO PEAK	NO PEAK
FR 435	M60	18	0.8	22.8	10.2	65.3	-13.7	2.9
FR 436	M60	19	0.8	24.4	10.2	70.2	-15.7	2.9
FR 437	M60	20	0.8	22.2	10.5	63.8	-16.6	2.9
FR 438	M60	21	0.8	21.3	10.6	60.9	-16.5	2.9
FR 439	M60	22	0.7	23.4	10.4	66.9	-16.3	2.9
FR 440	M60	23	0.7	41.6	10.1	120.3	-14.9	2.9
FR 441	M60	24	0.7	20.2	9.9	58.0	-16.1	2.9
FR 442	M60	25	0.7	20.6	10.1	59.0	-14.7	2.9
FR 443	M60	26	0.7	18.9	10.0	54.1	-14.2	2.9
FR 444	M60	27	0.7	20.5	9.7	58.7	-12.7	2.9
FR 445	M60	28	0.6	21.7	9.5	62.1	-13.4	2.9
FR 446	M60	29	0.6	20.1	9.4	57.3	-15.3	2.9