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The Ecological and Evolutionary Significance of Browsing and Grazing in Savanna Ungulates

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DECLARATION

I declare that this work is my own, unless indicated by author citations, and has not been submitted before for any other degree at any other university.

Signed by candidate

DEDICATION

For Ethan…

"Freedom is right over there, on the other side of those fences we built all by ourselves"

Daniel Quinn (Ishmael, 1992, Bantam)

ABSTRACT

The species richness and abundance of African savanna ungulates are often explained using nutritional models for niche separation and evolutionary diversification. One hypothesis, the diet-type model, predicts niche differentiation based on a browser/grazer dichotomy, while another, the diet-quality model, hypothesizes ecological diversity along a body size gradient. I test these contrasting hypotheses amongst 19 free-ranging species from the semi-arid savannas of Kruger National Park and Hans Merensky Nature Reserve, South Africa. I use stable carbon and nitrogen isotope, percent nitrogen, and percent fibre data from faeces and vegetation to document variations in diet and diet quality at a variety of high-resolution spatial and temporal scales, and integrate these variations in statistical models for testing hypotheses.

Results suggest that a dichotomous scheme for the diversity of African ungulates oversimplifies the full extent of niche complexity within the group. Diet is continuous and more variable than is generally accepted. Herbivores select foods to maintain optimal nutritional uptake, either by maximizing diet quality (protein/fibre ratios) or minimizing plant secondary compound intake. However, browse and grass foods offer similar energy yields, thus it is unlikely that ungulates diversified along a browser/grazer continuum to benefit from the nutritional value of either of these two food types. Body size constrains high quality food selection, especially during limiting periods (e.g. dry seasons) when larger-bodied species avoid competition with smaller animals by consuming poorer quality foods. The exceptional species richness of African ungulates appears to be a functional derivative of evolutionary diversification into different body size classes, in terms of both mass and height, radiating into different nutritional niches.

ACKNOWLEDGEMENTS

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Carl Morrow and Jo Crane provided access to their facilities for fibre analyses, and I thank Carl in particular for patiently waiting to use *his* instruments while I laboured on and on. Nick Lindenberg guided me in the use of GIS software for developing the maps presented in chapters 2, 3, and 5. Processing of samples, i.e. drying and grinding, during the final year of fieldwork was carried out at the Agricultural Research Council, Nelspruit, with assistance from Elsabe Aylward, Elijah Masinga, and his team.

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I conclude by thanking those people who have aided my endeavors on a more personal level. My parents, brother Grant, sister Keshia, in-laws (both the Johannesburg- and Cape Town-based Nolls), and cousins Linda and Janice Codron have been a source of inspiration, or at least competitive rivalry compelling me to finish this work. Yasmin Rahman and Karim Sponheimer gave up large portions of their vacation time to assist with fieldwork. I owe what remains of my common sense to Andi Dorfan and Ivor Polo – that we have remained friends throughout my phases of research-based depression and resultant unintelligible ramblings is a great testament to their loyalty and reliability. On that note, I thank Martha the ever-reliable 20-year-old Honda Ballade, for moving me 2 000 km between the Kruger Park and Cape Town on several occasions during the past four years, and her unfailing willingness to accompany me on several trips to the field.

My final thanks go to my wife Jacqui, for all she has done to assist with my research, and for consistently reminding me of life's priorities no matter what the circumstances. My hope is that, on completion of my thesis, I will be able to return even a fraction of her support as she ploughs through her own.

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CHAPTER 1

Introduction

Ungulates (hoofed-mammals) are a prominent and characteristic feature of the African savanna. Dietary diversity across the various taxa, and the array of ecological niches occupied by each, make them an integral component of community interactions that maintain savanna ecosystems. Ungulates, and evolutionary turnover within the group, are well represented in the fossil record, making them suitable for reconstructing palaeoenvironmental conditions, for establishing first and last appearances that have both chronological and evolutionary significance, and for testing contrasting evolutionary theories (Vrba, 1974, 1980, 1992, 1995; Cerling *et al.*, 1997a, 1997b; Kappelman *et al.*, 1997; Spencer, 1997; Reed, 1997; Sponheimer and Lee-Thorp, 2003a). Ungulates are equally significant to modern wildlife ecologists. The conservation of Africa's remaining wildlife refuges relies heavily on understanding the ungulate component of the ecosystem, with particular reference to provision of resources, determination of carrying capacity, and inferring top-down effects of herbivory on landscapes (Owen-Smith, 1996; van de Koppel and Prins, 1998; de Mazancourt *et al.*, 1999; Bodenstein *et al.*, 2000; Scholes *et al.*, 2003).

The two ungulate orders represented in this thesis (Perissodactyla and Artiodactyla) comprise some 200 species worldwide. Species-richness is greatest on the African continent, with the majority of species concentrated in the savanna biome. Hence, much attention has been paid to ecological and evolutionary adaptations of ungulates in relation to habitat heterogeneity across the savanna (Jarman, 1974;

McNaughton and Georgiadis, 1986; Vrba, 1992; Owen-Smith, 1997; Illius and O'Connor, 2000; du Toit, 2003).

Hypotheses for ungulate species diversity, niche compartmentalization, and macroevolution have been based largely on diet and nutritional ecology (Bell, 1970; Hofmann and Stewart, 1972; Jarman, 1974; Janis, 1976; Demment and van Soest, 1985; Hofmann, 1989; Duncan *et al.*, 1990; Gordon and Illius, 1994, 1996; Owen-Smith, 1997; Perez-Barberia *et al.*, 2004). Ungulate diets are most often studied along a browser/grazer continuum, with browse referring to trees, shrubs, and forbs, and graze being primarily grasses (Jarman, 1974; Hofmann and Stewart, 1972; Hofmann, 1989; Owen-Smith, 1997; du Toit, 2003). In general, browse foods are rich in cell solubles, whereas grasses have a higher cell wall:cell soluble ratio, forming the basis for most distinctions of ungulate dietary preferences.

There are two conflicting hypotheses for ruminant diversity, which can ultimately be extended for all ungulate herbivores. These are the browser/grazer and body size hypotheses. The browser/grazer (diet-type) model is based on differences in gut morphology, and assumed digestive physiology, between three guilds: browsers or concentrate selectors, grazers or roughage feeders, and mixed browser/grazers (Hofmann and Stewart, 1972; Hofmann, 1989). Specific predictions are that the larger volume of the rumen of grazers/roughage feeders, together with increased microbial activity and slower passage rate, allows for higher intake and longer digestion of fibrous foods. In browsers/concentrate selectors, a shorter passage rate leads to increased caecal digestion and rapid absorption of available cell solubles. The body size (or diet-quality) model, often termed the Bell-Jarman principle, is based on the concept that metabolic requirements scale negatively with body mass ($M^{-0.75}$). Predictions are that smaller animals should require foods with high energy yields, while larger species can subsist on bulk intake of poorer quality forages (Bell, 1970; Jarman, 1974; Demment and van Soest, 1985). The diet-type and diet-quality hypotheses are, however, not totally exclusive. The browser-grazer dichotomy is partially consistent with body size scaling, in that many small-bodied ungulates feed on supposedly higher quality browse foods, and larger species are often bulk grazers (Demment and van Soest, 1985; Gagnon and Chew, 2000; Cerling *et al.*, 2003). Nevertheless, exceptions are known, and Hofmann (1989) denies a relationship between dietary adaptation and body size apart from his prediction that smaller animals should eat concentrate foods (browse) and large species graze.

Bodmer (1990) first challenged Hofmann's (1989) browser, grazer, and mixed-feeder scheme, suggesting that the frugivorous diets of certain taxa necessitated that diet be classified along a frugivore-browser-grazer continuum. From a synthesis of available literature on African bovid diets, Gagnon and Chew (2000) recognized six dietary strategies for this group of ungulates alone: frugivores, browsers, generalists (fruit/browse/grass feeders), browser-grazer intermediates, variable grazers, and obligate grazers. Cerling *et al.* (2003) recognized five dietary groupings based on carbon isotope data for East African bovids: hyperbrowsers, browsers, mixed-feeders, grazers, and hypergrazers.

Several studies of ungulate digestive physiology have tested the efficacy of the diet-type and diet-quality models. Significantly, it has been argued that the relative digestive efficiencies of browsers and grazers are, in fact, similar, hence browsers and grazers may not differ in functional terms, other than with respect to the type of food they eat (Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995; Illius, 1997). Rather, these studies supported a consistent relationship between body size and digestive function. However, Ditchkoff (2000) and Clauss and Lechner-Doll (2001) claim that available data are insufficient to adequately test Hofmann's (1989) proposal. For example, Gordon and Illius (1994, 1996) relied primarily on data provided by Foose (1982), which were from experiments designed for examining broader differences between ruminant and nonruminant guilds.

Oral and dental morphology has also been related to feeding style, for example incisor arcade breadth, increased enamel thickness, hypsodonty, and increased molar row volume appear to be adaptations to deal with abrasive, coarse foods such as grasses (Fortelius, 1985; Owen-Smith, 1985; Janis, 1988; Janis and Erhardt, 1988). However, grasses are not necessarily more coarse than browse, and consequently many browsing or mixed-feeding species have similar dental adaptations compared with grazers (see Janis, 1988). Interestingly, Perez-Barberia and Gordon (2001) found no relationship between feeding style and oral morphology, but showed that hypsodonty does correlate with body size.

The persistence of debate is attributable to a number of causes. First, despite extensive research on ungulate feeding behaviour, the diets of several taxa remain unresolved, or in some cases contentious (Skinner and Smithers, 1990; du Toit, 1993; Gagnon and Chew, 2000; Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a). Second, testing of models has hitherto been constrained by the number of species for which reliable information is available. Indeed, a major constraint to current understanding of ruminant digestive function is that our knowledge is based largely on two *domesticated* species, i.e.

cattle and sheep (Hofmann, 1989). Third, renewed interpretation of the nutritional value of different forage types has brought conventional assumptions into contention. Forage quality is often assumed a function of cell soluble:cell wall ratios (Jarman, 1974; Demment and van Soest, 1985). However, this may be misleading, because interactions between different cell wall components (hemicellulose, cellulose, and lignin) are more important predictors of digestibility than overall fibre content (Robbins, 1993; van Soest, 1994; Illius, 1997; Owen-Smith, 1997; Meissner *et al.*, 1999). In addition, plant secondary compounds and insoluble nutrients bound within cell walls also influence metabolisable energy yields. Finally, herbivore diets may vary considerably across habitats and time periods (Owen-Smith, 1988, 1997; du Toit, 2003). Mixed-feeders in particular are able to switch back-and-forth between grazing and browsing, depending on seasonal and/or spatial distribution of resources (Owen-Smith, 1997; Hofmann, 1989; du Toit, 2003). Even taxa classified as browsers or grazers may alternate their diets under certain conditions (e.g. Wilson and Hirst, 1977; Owen-Smith and Cooper, 1989; Gagnon and Chew, 2000). Temporal variations in diet are further emphasized in deep time: browsing is generally regarded as an ancestral character state, dating back to the Palaeocene, while transitions to grazing occurred more recently in the Oligocene and through the Miocene and Neogene (Janis 1976; Janis *et al.*, 1994, 2000; Perez-Barberia *et al.*, 2001a).

For the most part, spatio-temporal variations, which form a major theme of this thesis, have been poorly documented, and even neglected from contemporary models of herbivory. Studies that focus on a species' diet in a particular place and time are not necessarily comparable with studies carried out over a different scale, and using different methodologies, therefore synthesis of dietary information from varied sources is problematic (Owen-Smith, 1988; Gagnon and Chew, 2000). Owen-Smith (1997) concluded that refining concepts about the relationship between diet and morphological adaptation requires studies that rigorously address intraspecific variation.

1.1. The Fossil Record and Ungulate Evolution

Perissodactyls and artiodactyls both originated in the Palaeocene of the Early Tertiary, during the rapid diversification of mammals that followed mass extinction of the large terrestrial amniotes. Perissodactyls were the dominant ungulates for most of the Eocene, but declined towards the end of the Eocene and into the Oligocene (Janis, 1976). During the Oligocene, it was the artiodactyls that showed significant diversification. By the end of the Miocene, all major groups of ungulates surviving in Africa today are represented in the fossil record; equids and rhinocerotids amongst perissodactyls, and suids, hippopotamids, giraffids, and bovids amongst artiodactyls (Janis, 1976; Churcher, 1978; Churcher and Richardson, 1978; Cooke and Wilkinson, 1978; Coryndon, 1978; Gentry, 1978; Hooijer, 1978).

In Africa, a significant number of taxa have persisted from Plio-Pleistocene times to the present, despite mass extinctions of large mammals in Eurasia and the Americas between 10 and 12 ka (Bigalke, 1978; Martin and Klein, 1984). In fact, all genera and most species discussed in this thesis had already appeared by the end of the Pliocene (Figure 1.1). The two genera of rhinoceros surviving in Africa, *Diceros* and *Ceratotherium*, both originated during the mid to late Miocene (Hooijer, 1978). *Equus*, the only surviving African equid, first appeared in Africa during the Pliocene, likely as an

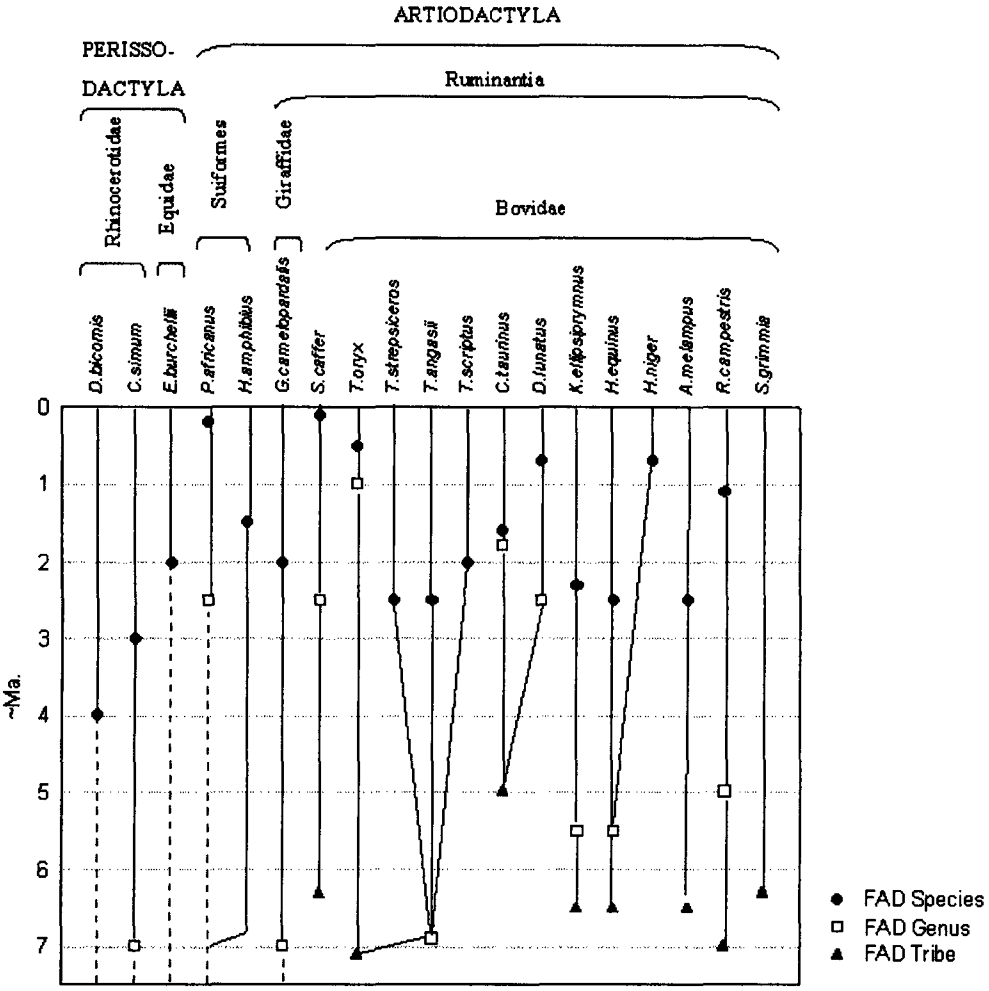

Figure 1.1. Species-timeline for ungulates included in this study, based on data in Churcher (1978); Churcher and Richardson (1978); Cooke and Wilkinson (1978); Coryndon (1978); Hooijer (1978); Vrba (1995); and Behrensmeyer *et al.* (1997). Dashed lines denote ancestry pre-dating 7 Ma. FAD = First Appearance Date.

immigrant from Eurasia (Churcher and Richardson, 1978). The suid *Phacochoerus*, as well as the other modern genera *Potamochoerus* and *Hylochoerus*, appear in the African fossil record between ca. 2 and 3 Ma, although extinct taxa such as *Notochoerus* date back at least to the Miocene (Cooke and Wilkinson, 1978; Behrensmeyer *et al.*, 1997). Early members of the Hippopotamidae such as *Hexaprotodon* date back to the Late Miocene of East Africa (Boisserie *et al.*, 2005), but the modern species *Hippopotamus amphibius*, which is poorly represented in the fossil record, appeared only in the early to mid Pleistocene (Coryndon, 1978; Behrensmeyer *et al.*, 1997). The giraffe genus *Giraffa* dates as far back as the Late Miocene (Churcher, 1978). Bovids originated during the mid Miocene, ca. 17 to 18 Ma, although of the eight bovid tribes represented in this thesis, seven appeared only in the late Miocene (6 to 7 Ma), while the Alcelaphini emerged slightly later at ca. 5 Ma (Vrba, 1995).

The ungulate fossil record reveals two interesting phenomena. First, the stagnation of diversification amongst perissodactyls co-incided with the rise of the artiodactyls through the Oligocene, prompting belief that the two groups occupy similar ecological niches, with artiodactyls commonly accepted as holding a competitive advantage mainly because of their (presumed) more efficient digestive systems (Janis, 1976; but see Duncan *et al.*, 1990). This trend continued through the Miocene and Plio-Pleistocene; the artiodactyls continued to diversify, particularly the bovids, while the number of perissodactyl genera and species declined. Second, the evolutionary history of mammals is characterized by phases of rapid turnover, in response to climatic and other changes to the physical environment (Vrba, 1992, 1995; Cerling *et al.*, 1997a, 2005; deMenocal, 2004). For example, numerous contemporary bovid and other ungulate species first appear in the fossil record between 2.8 and 2.5 Ma, following a phase of apparent global cooling (Vrba, 1992, 1995). However, sampling gaps and preservational/taphonomic bias may hamper this interpretation. Behrensmeyer *et al.* (1997), for example, argued that the most significant faunal turnover in East Africa occurred at ~2.5 Ma and continued through to 1.8 Ma. In any case, interpretations of controlling mechanisms for evolutionary turnover based on fossil evidence are limited to observations of contemporary events (deMenocal, 2004) that may in fact present one or more environmental autocorrelations. Understanding the how and why of patterns evident in the fossil record is only achievable through inquiry into the patterns of ecological variation evinced by modern descendants of ancient forms.

1.2. Objectives

This thesis tests the hypothesis that the functional diversity of ungulates can be simplified according to a dichotomous niche compartmentalization that recognizes grazers as distinct from browsers (Hofmann and Stewart, 1972; Hofmann, 1989). The converse hypothesis is that ungulates achieve ecological niche separation through variations in the relative nutritional value of the foods they consume (and by implication, body size), despite differences in the primary feeding preferences of browsers and grazers (Bell, 1970; Jarman, 1974; Demment and van Soest, 1985). Testing of these hypotheses is based on the following key questions addressed here:

1. How reliable are current dietary predictions in the literature?
2. Do dietary predictions for African ungulates persist when spatial and temporal variations are accounted for?

3. How do different savanna plant foods compare in nutritional quality, and do the same patterns persist across space and time?
4. To what extent do the diets of different species vary at different spatial and seasonal scales?
5. What factors can be said to underlie food choice in African ungulates, and hence what causative effects were likely responsible for evolutionary transitions amongst ungulate diets?
6. Do differences in the respective ecological adaptations of browsers and grazers extend beyond the obvious difference that they consume different types of foods?
7. Does body size play a role in separating ungulate ecological niches?

The thesis deals with hitherto poorly-documented spatial, and particularly seasonal, variations in diet and nutritional ecology of free-ranging taxa. The study focuses on 19 ungulate species from the semi-arid savannas of the Kruger National Park and Hans Merensky Nature Reserve, South Africa.

Stable light isotope ecology provides a uniform technique for qualifying and quantifying feeding ecology across a range of taxa, habitats, and time frames (reviewed in Post, 2002; Dalerum and Angerbjorn, 2005; Thompson *et al.*, 2005). Results of this thesis are based primarily on stable carbon isotope analysis, which for African savanna herbivores provide empirical reference points for proportions of C_3 (browse) and C_4 (grass) consumption (Vogel, 1978; Lee-Thorp and van der Merwe, 1987; Cerling and Harris, 1999). Thus, while the method largely excludes information about plant species and plant parts utilized, it is an ideal tool for testing hypotheses about browsing and grazing[1]. Moreover, results are readily reproduced, so that information can be extracted for a variety of taxonomic, spatial, and temporal scales exceeding that which can be addressed by time-consuming field observations and analyses of gut content. This technique therefore facilitates regional and seasonal control for proposed models. Stable carbon isotope studies of fossil fauna have also provided empirical information about palaeodiet (Lee-Thorp *et al.*, 1989a, 1994; Quade *et al.*, 1995; Bocherens *et al.*, 1996, 1999; Cerling *et al.*, 1997b, 1999; Koch, 1998; MacFadden, 1998; Sponheimer and Lee-Thorp, 1999, 2003b), overcoming assumptions associated with traditional approaches such as taxonomic uniformitarianism (see Sponheimer *et al.*, 1999). More rigorous studies of stable isotope distributions within modern plant-mammal ecosystems are required to help refine interpretations of data from fossil materials. For example, accurate palaeoecological studies require differentiation between diet- and climate/environment-based variations in animal carbon isotope compositions (van Klinken *et al.*, 1994; Heaton, 1999; Codron, J. *et al.*, 2005). This thesis combines stable carbon isotope data with stable nitrogen isotope data, percent nitrogen, and fibre concentrations to facilitate interpretation of stable isotope and feeding ecology in modern savanna systems in terms of overall nutritional ecology.

1.3.Thesis Outline

In the following chapter, I provide a description of the climate and landscape heterogeneity of the study areas and outline the principles of stable isotope ecology. Thereafter, components of the data are presented in a series of chapters, each addressing

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[1] A possible caveat is that C_4 grasses contain a higher proportion of mesophyll and are hence less digestible than C_3 grasses (Heckathorn *et al.*, 1999). However, the latter are virtually absent from subtropical Africa.

specific question (s) identified as prerequisite for achieving the primary objective. In Chapter 3, stable isotope data from faeces of Kruger Park ungulates are used to test the reliability of expectations for diet based on results of field studies and previous carbon isotope studies of diet, and whether the predictions persist when spatio-temporal variations are accounted for. Chapter 4 presents nutritional data (percent nitrogen and concentrations of fibre components) for plants from the South African savanna, addressing questions about the relative nutritional value of different forage types and the extent to which diet quality varies across space and time. This is a significant chapter for further interpretation of ungulate diets, since nutrient uptake has been considered the most important aspect of dietary adaptation (see Illius, 1997). Implications for the nutritional ecology of taxa with different types of diets are discussed in Chapter 4. Chapters 5 to 7 make use of isotope-based records of dietary variations, incorporating a variety of spatio-temporal scales, to test competing models of herbivory. In Chapter 5, I test how the feeding responses of different species to environmental changes through the seasonal cycle vary from each other, based on data representing very high resolutions, i.e. monthly intervals. These data are presented in conjunction with isotope and nutritional data from local vegetation to predict patterns of food selection in savanna systems. In Chapter 6, I explore environmental correlates of browse and grass selection in mixed-feeding impala *Aepyceros melampus*, using the impala model to test the mechanism (s) for evolutionary transitions from browsing to grazing. In Chapter 7, I combine isotope evidence with faecal protein and fibre content to test predictions for the functional significance of diet-type (%grass consumed), diet-quality, and body size. The final chapter is a synthesis of this study, and includes remarks about the implications for both palaeoecology and conservation biology.

Raw data are attached as a digital (CD-ROM) appendix in Microsoft Excel format. It is important to note that the data used in different chapters overlap in several instances, although the portions used are never totally repeated. For example, Chapter 3 presents a summary of all available faecal stable isotope data, Chapter 5 uses data only from materials collected during the final year of the study, and Chapter 7 focuses on data primarily from a single region (the Northern Basalt Plains) of Kruger Park. Several spreadsheets are presented within the appendix, each containing the data used for a specific chapter.

CHAPTER 2

Approach to the Study

In this chapter, I provide a description of the study areas. I review briefly the major landscape and climatic variations of the study regions, as well as climate data for the study period (June 2002 to May 2005). I also provide background information on the principles and practical applications of stable light isotope ecology.

2.1. Study Areas

2.1.1. Kruger National Park

Since its inception over 100 years ago, the world-renowned Kruger National Park has evolved into an important hub for the development of savanna conservation biology research and the development of wildlife management in southern Africa (Mabunda *et al.*, 2003). Today, the Park supports a rich abundance of indigenous mammal species (147), including 32 ungulates (Pienaar *et al.* 1987). The spatial mosaic of landscapes in the region offer resident herbivores a wide range of contrasting habitats differing in available resources. The physical characteristics of the various ecotypes have been well-studied (reviewed in Venter *et al.*, 2003). Thus, Kruger Park is particularly well-suited to studies of ecosystem heterogeneity and the dynamics of plant-mammal interactions.

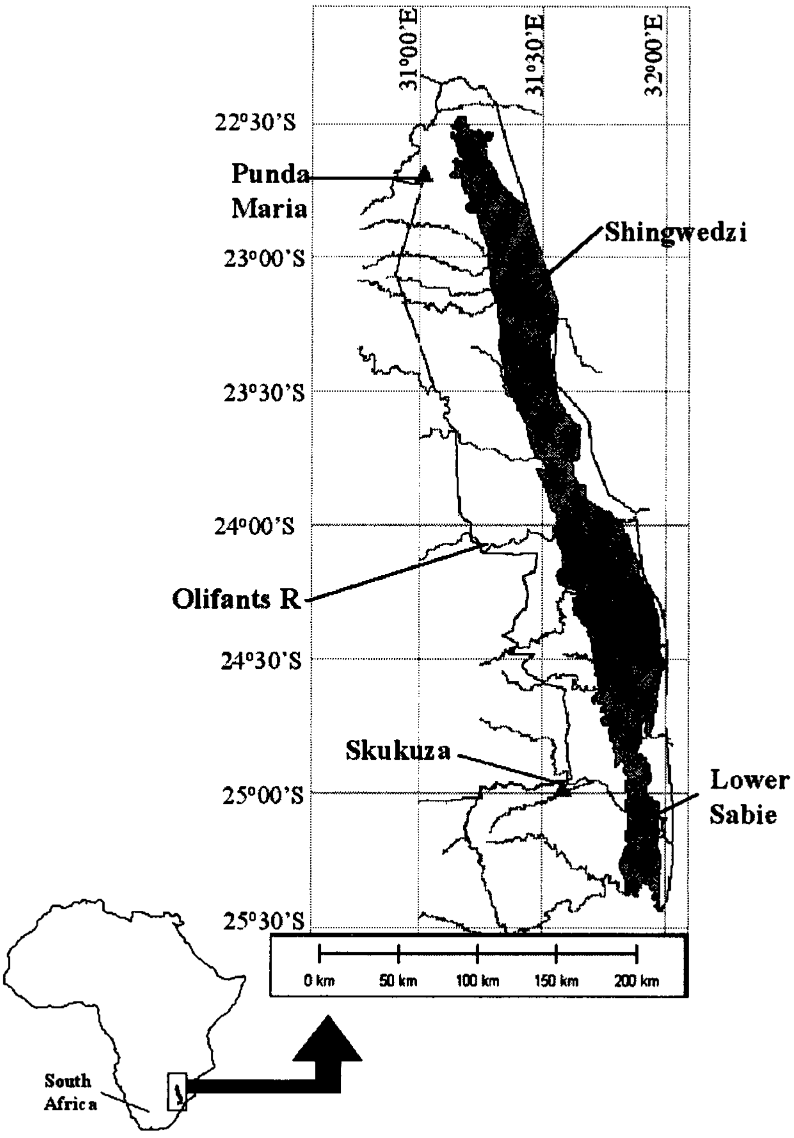

Figure 2.1. Map of Kruger National Park, showing west-east geological gradient between granites (clear region) and basalts (shaded region). The Olifants River separates northern and southern regions. Triangles show weather stations from where climate data was derived for this study. The inset shows the geographic location of the Park in Africa.

The Kruger Park is a large (~ 2 000 000 ha) semi-arid savanna, situated in the northeast of South Africa between the latitudes 22°20' and 25°32' S, and the longitudes 30°53' and 32°02' E, forming part of the "lowveld" savanna between the Drakensberg escarpment to the west and Mozambique to the east (Figure 2.1). Seven major river systems traverse the Park, with the Crocodile River forming the southern boundary, and the Limpopo River the northern boundary. The region lies at ~300 m above sea level, on average, although this varies from 900 m in the southwest to 200 m in the east. Mean annual rainfall varies between 500 and 700 mm in the south to between 300 and 500 mm in the north (Venter *et al.*, 2003). Rainfall is highly seasonal, falling mainly during the austral summer between October and March, while April to November are dry (Figure 2.2). Over decadal scales, an 18 to 20 year cycle prevails, with nine or 10 years of above-average rainfall proceeded by nine or 10 years below-average rainfall (Tyson and Dyer, 1975; Gertenbach, 1980; Venter *et al.*, 2003).

The geological succession of Kruger Park is divided longitudinally into granitic substrates forming relatively nutrient-poor sandy soils in the west, and nutrient-rich basalt-derived clay soils in the east (Venter *et al.*, 2003). The northern regions are dominated by mopane *Colophospermum mopane* "shrubveld" and woodland, while fine-leaved *Acacia* and *Combretum* spp. trees abound in the south. Thus, for broader ecological purposes, the Park can be simplistically divided into northern and southern granite and basalt landscapes, respectively (Grant *et al.*, 2002). In this thesis, the densely wooded, partially mountainous region around Punda Maria in the far north is treated as a fifth landscape. A summary of the physical characteristics of each of the five major landscapes referred to throughout this thesis is presented in Table 2.1.

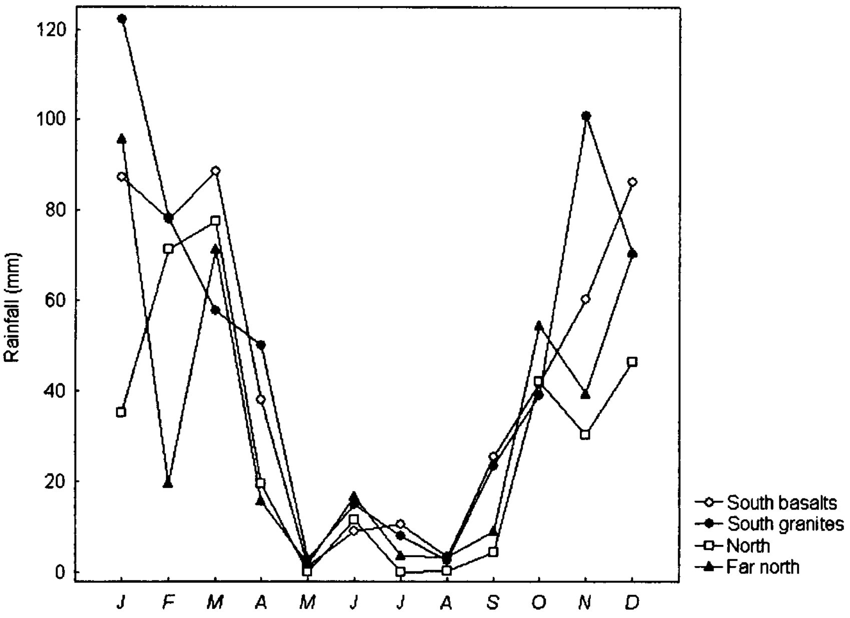

Figure 2.2. Partitioning of rain through the seasonal cycle at four weather stations in Kruger National Park representing the monthly means from June 2002 and May 2005. Rainfall is recorded from four weather stations (see Figure 2.1) - Lower Sabie (south basalts), Skukuza weather station (south granites), Shingwedzi (north), and Punda Maria (far north) weather stations (data from the South African Weather Bureau and Nick Zambatis, Scientific Services, Kruger Park).

2.1.2. Key Landscapes

A focal landscape for this thesis is the Northern Basalt Plains (NBP) of Kruger Park. The NBP form a distinctive vegetational corridor in the northeast of Kruger Park supporting the Park's surviving roan antelope *Hippotragus equinus* and tsessebe *Damaliscus lunatus* populations, making the region important to local conservation objectives (Grant *et al.*, 2002). The NBP are underlain by basalt-derived clay soils supporting mopane *Colophospermum mopane* "shrubveld" on grassland. The area is interspersed by a network of seasonal rivers draining into 'dambo'-like grasslands, and an ecotone area that is considered an important source of forage for bulk grazers (Grant *et al.*, 2002). For comparative purposes, data for the NBP are examined alongside data from the southern *Acacia/Combretum*-dominated savannas, the Punda Maria (PM) landscape of Kruger, and from the nearby Hans Merensky Nature Reserve (HM). The PM and HM landscapes are in sharp contrast with the NBP, but have some similarities making them suitable for comparison (e.g. *C. mopane* savanna is present within all three regions). PM is situated on the sandveld of the far northern Kruger Park, and represents one of the most densely wooded regions of the Park, characterized by broad-leaved savanna woodland on undulating plains and hillslopes (Venter *et al.*, 2003). HM is a reserve of ~ 5 000 ha situated ~100 km to the west of Kruger Park in the Tzaneen District of the Limpopo Province (23°39' S, 30°40' E), with the Letaba River forming its northern boundary. Vegetation is predominantly *C. mopane* woodland savanna on a relatively flat topography (428 to 542 m above sea level). Underlying geology is uniformly granite, although the landscape is broken by a doleritic dyke on the eastern boundary (information from the Limpopo Parks and Tourism Board).

The vegetation of the NBP and PM is classified as "Transvaal Sweet Bushveld", while Hans Merensky is classified as "Transvaal Mixed Bushveld" (Tainton, 1999). The woody vegetation of the NBP is dominated by the shrub mopane *C. mopane* morph, and common grasses include *Bothriochloa radicans, Cenchrus ciliaris, Panicum coloratum, Themeda triandra* and *Urochloa mossambicensis* (Venter *et al.*, 2003). Woody vegetation at PM includes *Burkea africana, Combretum apiculatum,* and *Terminalia sericea*, while *C. mopane* is present in low-lying areas (Venter *et al.*, 2003). Common grasses at PM include *Digitaria eriantha, Enneapogon cenchroides, P. maximum, T. triandra,* and *U. mossambicensis*. On HM, the tree morph of *C. mopane* dominates amongst woody vegetation, although *C. apiculatum* dominates in certain patches. Common grass species include *Aristida congesta, D. eriantha, Elionurus muticus, P. maximum, Schmidtia pappophoroides,* and *T. triandra*. Brackish patches, supporting poor stands of palatable grasses, are a common feature on HM (see Wilson and Hirst, 1975).

Comparisons between the NBP, PM, and HM are based on data from samples collected at monthly intervals during the final year of the study (June 2004 to May 2005; see Chapters 4 and 5). I provide a brief account of the climate data for this period (data from the South African Weather Bureau and Nick Zambatis, Scientific Services, Kruger Park). The study period forms part of a current "dry-cycle" of the long-term (18 to 20 year) oscillations. Rainfall during the study period (June 2004 to May 2005: NBP 229 mm, PM 268 mm, HM 272 mm) was more than 200 mm lower than the long-term annual mean for all three regions (NBP 458 mm, PM 557 mm, HM 558 mm) (Figure 2.3a). Rainfall followed the predicted trend for the temperate summer rainfall region of the interior of South Africa, falling mainly between October and March (Figure 2.3b). Daily maximum temperatures varied from 30 to 35°C in the summer and from 25 to 30°C in winter, although temperatures at HM were slightly lower than NBP and PM in Kruger Park (Figure 2.3c).

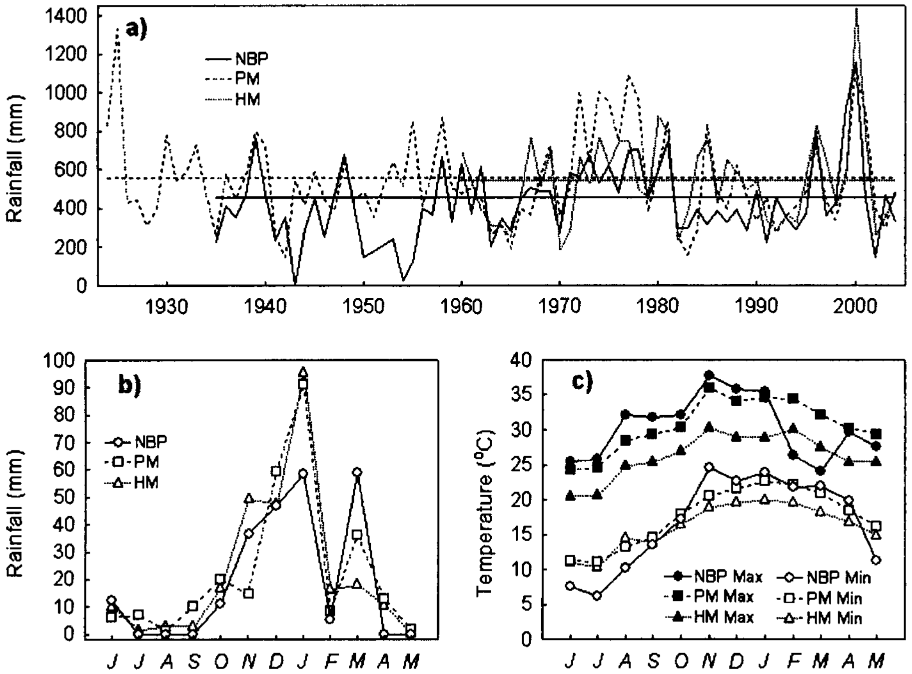

Figure 2.3. Climate data for the Northern Basalt Plains (NBP), Punda Maria (PM), and Hans Merensky Nature Reserve (HM), showing a) long-term oscillations in annual rainfall compared with the long-term mean (horizontal lines), b) seasonal partitioning of rainfall for the period (June 2004 to May 2005), and c) monthly maximum and minimum temperatures for the study period. Data supplied by the South African Weather Bureau and Nick Zambatis.

2.2. Background to Stable Light Isotope Ecology

Stable isotope ecology is an empirical natural abundance tool for studying diet and other ecological parameters of organisms, populations, and communities. The technique is based on differences in stable isotope ratios of different biological materials. By convention, these ratios are measured with reference to international standards (Vienna PeeDee Belemnite, or VPDB, for stable carbon isotope ratios and atmospheric N_2 for stable nitrogen isotope ratios), expressed in parts per mil (*‰*) using the delta (δ) notation, derived from the equation:

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

where R = the isotope ratio of element R (^{x}R / ^{y}R).

This study makes use of stable carbon and stable nitrogen isotope ratios, and the abbreviations $\delta^{13}C$ and $\delta^{15}N$ denote the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of the material, with respect to the relevant standards (VPDB and Air), respectively.

2.2.1 Stable Carbon Isotope Ecology

Application of stable carbon isotope studies to ecology is based on anatomical and physiological differences in plants at the base of the food chain. Plants that photosynthesize using the C_3 pathway discriminate more strongly against ^{13}C than do plants following the C_4 pathway. The difference is due primarily to differential isotope discriminations during photosynthetic fixation of CO_2 by the enzymes *ribulose bisphosphate carboxylase* (Rubisco) and *phosphoenolpyruvate carboxylase* (PEP) in C_3 and C_4 plants, respectively, the theoretical basis of which is extensively reviewed elsewhere (Vogel, 1980; Farquhar *et al.*, 1982, 1989; O'Leary, 1988, 1993). The result is a consistent bimodal distribution of $\delta^{13}C$ values amongst higher plants: C_3 plants have consistently lower $\delta^{13}C$ values, mean ~ -27.0‰, than do C_4 plants, mean ~ -12.5‰ (Smith and Epstein, 1971; Vogel, 1978). Animal tissues record the carbon isotope composition of the basic plant diet, hence $\delta^{13}C$ values of herbivore tissues reliably reflect the proportions of C_3 to C_4 plants consumed (Vogel, 1978; Lee-Thorp and van der Merwe, 1987; Cerling and Harris, 1999). In the African savanna, almost all dicotyledonous trees, shrubs, and forbs are C_3, while grasses are predominantly C_4 (Vogel *et al.*, 1978; Cerling and Harris, 1999). Savanna herbivore $\delta^{13}C$ values are therefore reliable indicators of the relative proportions in which browse and grass foods are consumed.

Stable carbon isotope ecology has been widely applied to study the diets of mammals, both in modern (Ambrose and DeNiro, 1986; van der Merwe *et al.*, 1988; Tieszen *et al.*, 1989; Koch *et al.*, 1991, 1995; Schoeninger *et al.*, 1997, 1999; Cerling *et al.* 2003; Sponheimer *et al.*, 2003a; Cerling and Viehl, 2004; Urton and Hobson, 2005; Codron *et al.*, 2006) and palaeoecological contexts (Lee-Thorp *et al.*, 1989a, 1994; Quade *et al.*, 1995; Bocherens *et al.*, 1996, 1999; Cerling *et al.*, 1997b, 1999; Koch, 1998; MacFadden, 1998; Sponheimer and Lee-Thorp, 1999, 2003b). There are, however, two complicating factors, that may either constrain or enhance interpretations, depending on the level of quality control that can be applied. The first is that the carbon isotope composition of C_3 and C_4 plants may vary (although their respective $\delta^{13}C$ ranges never overlap), the second is related to the biological nature of the material used.

Environmental Variations in Plant $\delta^{13}C$

It is well documented that the carbon isotope composition of C_3 plants varies in response to changing environmental conditions, because stomatal diffusion and photosynthetic carboxylation rates vary with differing levels of atmospheric CO_2, and across plants with different physiologies (Farquhar *et al.*, 1982, 1989; Tieszen, 1991; Ehleringer, 1993; Vogel, 1993; Stewart *et al.*, 1995; Heaton, 1999). It is thus necessary to distinguish environmental effects (on plants) from dietary effects as the cause of isotope differences between animals. For instance, C_3 plants are expected to display lower $\delta^{13}C$ values in areas with higher rainfall or water availability, and dense canopy cover (van der Merwe and Medina, 1989, 1991; Tieszen, 1991; Stewart *et al.*, 1995). In extreme cases, C_3 plant $\delta^{13}C$ may vary widely, from -37‰ in dense tropical rainforests (Farquhar *et al.*, 1982; van der Merwe and Medina 1989, 1991), to –20 in deserts (Ehleringer and Cooper, 1988; Ehleringer, 1993). However, this variation is considerably smaller within a single biome, such as the savanna (~2‰ variation; see Codron, J. *et al.*, 2005).

C_4 plants are typically less variable, because carbon is fixed early on during photosynthesis, limiting further fractionation (Vogel, 1980; O'Leary, 1988). It has been shown that C_4 plants following different photosynthetic sub-pathways have significantly different mean $\delta^{13}C$ values, albeit these differences are subtle, only about 1‰ (Hattersley, 1982; Cerling and Harris, 1999). Cerling and Harris (1999) reported a mean $\delta^{13}C$ value of –11.8‰ for NADP-ME, and slightly more depleted values (-12.8‰) for PCK and NAD-ME grasses from East Africa. Overall variation within a semi-arid savanna such as South Africa's Kruger National Park is reportedly 2‰ for C_3 plants and 1‰ for C_4 plants (Codron, J. *et al.*, 2005). Codron, J. *et al.* (2005) further argued that isotope variation should be even smaller at the herbivore level, since animals eat a variety of plants, averaging out isotope differences that occur amongst the vegetation of any one region. Codron *et al.* (2005a) showed that applying data for local C_3 and C_4 vegetation to dietary models significantly improves precision with which diet composition can be predicted from animal $\delta^{13}C$; on this basis, proportions of C_3:C_4 intake can be calculated to within ±5%.

Information Archived in Body Tissues and Faeces

Stable isotope analysis of different tissues provides information over different time scales, depending on growth and turnover rates (Tieszen *et al.*, 1983; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Sponheimer *et al.*, 2003b). Teeth are formed early on in life, therefore carbon isotope data from teeth provide dietary information for the first few years of life (unless the teeth are continually growing as in elephant ivory). Bone undergoes continual remodeling and turnover throughout life, and carbon isotope data from this tissue represent a near-lifetime dietary average. Softer tissues such as hair record much shorter-term information, over a few months to years. An additional advantage for using incremental tissues such as teeth and hair is that isotope profiles taken along the growth lines can be used to extract temporal dietary shifts within an individual (Koch, 1989; Balasse *et al.*, 2001; Cerling and Viehl, 2004; Cerling *et al.*, 2004a, 2006). Tissues also differ in the isotope discrimination that takes place during synthesis. Bone and dentine collagen is 4.5 to 6.0‰ enriched in ^{13}C compared to the diet (although this fraction may be smaller in micromammals), tooth enamel carbonate of herbivores is 12.5 to 14‰ enriched, and hair is 3.2‰ enriched (Vogel, 1978; Jones *et al.*, 1981; Nakamura *et al.*, 1982; Lee-Thorp *et al.*, 1989b; Ambrose and Norr, 1993; Cerling and Harris, 1999; Sponheimer *et al.*, 2003b; Passey *et al.*, 2005).

Traditionally, stable isotope studies of diet have relied on the use of bones and teeth (Ambrose and DeNiro, 1986; Koch *et al.*, 1995; Cormie and Schwarcz, 1996; Smith *et al.*, 2002; Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a). The carbon isotope composition of herbivore faeces is also consistent with that of the diet (Tieszen *et al.*, 1979; Jones *et al.*, 1981; van der Merwe *et al.*, 1988; Sponheimer *et al.*, 2003b; Codron *et al.*, 2005a, 2005b). Despite that faeces represent mostly the undigested portion of the diet, there is a consistent diet-faeces isotope offset of –0.9‰, regardless of whether the diet is predominantly browse or grass (Sponheimer *et al.*, 2003b; Codron *et al.*, 2005a). In other words, any differential digestibility between browse and grass has little effect on the isotope composition of faeces, hence faeces are a reliable proxy for diet studies.

I have opted to use faeces as sample material for this thesis, for several reasons. In contrast to hard tissues, faeces are not subject to extended turnover periods of several months or years, but represent the diet over the last few days prior to deposition (Jones *et al.* 1981; Tieszen *et al.*, 1983; Sponheimer *et al.* 2003b). This allows for high-resolution temporal records of diet, provided collections are made on an appropriate scale. Unlike body tissues, collection of faecal material does not require that animals are dead, nor that they be manipulated or slaughtered, as is necessary when gut contents are analyzed. Faeces are also relatively easy to obtain, such that numerous samples can be collected and analyzed to yield a sufficiently large database that is suited for rigorous statistical testing, minimizing the effects of random variation and pseudo-replication of data.

2.2.2. Stable Nitrogen Isotope Ecology

Combined analysis of stable carbon and nitrogen isotope ratios can reveal inter-specific ecological differentiation beyond C_3/C_4 distinctions (Ambrose and DeNiro, 1986; Cormie and Schwarcz, 1996; Hobson *et al.*, 1996; Schoeninger *et al.*, 1997, 1999; Post, 2002; Urton and Hobson, 2005). However, insights are constrained because the relationship between diet and consumer tissue nitrogen isotope composition is influenced by a complex combination of factors including nutritional stress, water stress, climate, ecophysiological adaptation, and even age (Ambrose and DeNiro, 1986; Sealy *et al.*, 1987; Ambrose, 1991, 2000; Sponheimer *et al.*, 2003c, 2003d; Robbins *et al.*, 2005).

At the base of the food chain, plant and (soil) $\delta^{15}N$ values reflect the complexity of the nitrogen cycle. Both soil and plant $\delta^{15}N$ may vary with factors including soil type and $\delta^{15}N$ value, climate, nitrogen assimilation, and nitrogen availability (Heaton, 1987; Handley and Raven, 1992; Handley *et al.*, 1994; Muzuka, 1999; Robinson, 2001; Schmidt and Stewart, 2003). For example, biological fixation of nitrogen tends to decrease plant and soil $\delta^{15}N$, although several studies have shown that nitrogen-fixing plants are not always ^{15}N-depleted relative to non-fixers (Handley *et* al., 1994; Schmidt and Stewart, 2003; Codron, J. *et al.*, 2005). Plants (and soils) are also expected to display higher $\delta^{15}N$ values in arid, sandy, and sodic environments (Heaton, 1987; Muzuka, 1999; Schwarcz *et al.*, 1999), but in Kruger Park at least, these patterns are not consistent (Codron, J. *et al.*, 2005).

Further isotope discriminations occur in animals feeding on these plants. Several studies have shown a stepwise enrichment of ^{15}N by ~2 to 4‰ along increasing trophic levels (Schoeninger and DeNiro, 1984; Sealy *et al.*, 1987; Ambrose, 1991). However, nitrogen isotope variation within trophic levels may exceed differences between trophic levels (Sponheimer *et al.*, 2003c, 2003d). The urea mass-balance hypothesis for nitrogen isotope discrimination (Ambrose, 1991) holds that increased loss of ^{15}N-depleted urea leads to raised body tissue δ^{15}N values. Within the paradigm of this model, higher δ^{15}N values in herbivores have been ascribed to increases in protein intake and nutritional stress, aridity and water stress, and age (Ambrose and DeNiro, 1986; Sealy *et al.*, 1987; Schoeninger *et al.*, 1997, 1999; Ambrose, 2000; Sponheimer *et al.*, 2003c; Robbins *et al.*, 2005). Sponheimer *et al.* (2003c) argued that these patterns do not persist by necessity, because the urea-mass balance model does not account for nitrogen loss via faeces. Hence, patterns of ^{15}N distribution amongst herbivore faeces may in fact be imperative for clarifying controlling mechanisms of ^{15}N-abundances in herbivores. This thesis does explore potential insights from combined carbon and nitrogen isotope analysis, although I rely primarily on evidence from carbon isotope ratios for differential C_3 and C_4 plant food use.

Table 2.1. Description of Kruger Park landscape types included in this study, based on Land Systems described in Venter *et al.* (2003) and landscapes of Gertenbach (1983).

Region	Land System	Annual rainfall	Topography	Vegetation	Common trees	Common grasses
Southern granites	Skukuza	500 to 750 mm	Slightly undulating plains	Broadleaved bushveld and sandy soils on uplands, fine-leaved *Acacia* spp. and clay soils on bottomlands	*Combretum apiculatum*, *C. zeyheri*, *Terminalia sericea*, and *Acacia nigrescens*	*Digitaria eriantha*, *Panicum maximum*, *Pogonarthria squarrosa*, *Schmidtia pappophoroides*, *Themeda triandra*, *Urochloa mosambicensis*
Southern basalts	Satara	500 to 650 mm	Flat plains	Fine-leaved *Acacia* savannas on clay soils	*Acacia nigrescens*, *Sclerocarya birrea*, *Dichrostachys cinerea*	*Bothriochloa radicans*, *Cenchrus ciliaris*, *Digitaria eriantha*, *Eragrostis superba*, *Heteropogon contortus*, *Panicum coloratum*, *P. maximum*, *Sporobolus nitens*, *Themeda triandra*, *Urochloa mosambicensis*
Northern granites	Phalaborwa	450 to 600 mm	Slightly to strongly undulating plains	Broadleaved *Colophospermum mopane* bushveld on sandy soils	*Colophospermum mopane*	*Andropogon gayanus*, *Bothriochloa radicans*, *Brachiaria nigropedata*, *Cymbopogon plurinodis*, *Digitaria eriantha*, *Eragrostis rigidior*, *Heteropogon contortus*, *Panicum coloratum*, *P. maximum*, *Themeda triandra*, *Urochloa mosambicensis*
Northern basalts	Letaba	450 to 600 mm	Flat plains	Broadleaved *C. mopane* bushveld on clay soils	*Colophospermum mopane*	*Aristida* spp., *Bothriochloa radicans*, *Cenchrus ciliaris*, *Digitaria eriantha*, *Heteropogon contortus*, *Panicum coloratum*, *Setaria woodii*, *Themeda triandra*, *Urochloa mosambicensis*
Punda Maria	Pafuri	400 to 650 mm	Slightly to strongly undulating plains	Broad-leaved bushveld, *Colophospermum mopane* present in low-lying areas	*Colophospermum mopane*, *Combretum apiculatum*, *Burkea africana*, *Terminalia sericea*, *Pseudolachnostylis maprouneifolia*, *Kirkia acuminata*, *Commiphora glandulosa*	*Digitaria eriantha*, *Enneapogon cenchroides*, *Panicum maximum*, *Schmidtia pappophoroides*, and *Urochloa mosambicensis* on plains; *Andropogon gayanus*, *Digitaria eriantha*, *Panicum maximum*, *Setaria sphacelata*, *Themeda triandra* on mountain slopes

CHAPTER 3

Diets of Savanna Ungulates from Stable Isotope Composition of Faeces

In this chapter, I test whether dietary predictions for African ungulates in the literature, based primarily on field observations, are consistent with stable isotope evidence from faeces, and whether previous predictions persist when spatio-temporal variations are accounted for. Results are based on analysis of carbon and nitrogen isotope compositions of faeces of 19 ungulate species collected over three years from various regions and seasons of the Kruger National Park, South Africa. Overall proportions of C_3 (dicot) and C_4 (grass) inputs (derived from faecal carbon isotope ratios) into diet are compared with information from field studies, and with previous carbon isotope studies of diet. I also explore the use of a dual-isotope mixing model to partition dietary inputs of multiple C_3 and C_4 food sources. Cluster analyses of inter-group differences, incorporating spatial and seasonal dietary variation are used to assess dietary niche separation along the browser/grazer continuum. Lastly, the chapter prevents a test of previous models of nitrogen isotope abundances amongst ungulates using multivariate analyses of ecophysiological, climatic, and dietary predictors.

3.1. Introduction

The dietary niches of ungulates are most often explained along a browser/grazer continuum, and models of herbivore diversity and niche compartmentalization rely largely on information about diet at this scale (Jarman, 1974; Hofmann, 1989; Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995; Owen-Smith, 1997). Assessments of feeding style commonly distinguish between three guilds: browsers, grazers, and mixed-feeders (Hofmann and Stewart, 1972; Hofmann, 1989; van Wieren, 1996; Owen-Smith, 1997; Brashares *et al.*, 2000; Perez-Barberia *et al.* 2001b; du Toit, 2003). However, there is substantial disagreement about whether this system is sufficient to explain the full extent of dietary diversity amongst ungulates. For instance, it has been proposed that frugivory should be treated as separate from browsing and grazing, and that distinction should be made between obligate and variable grazers (Bodmer, 1990; Gagnon and Chew, 2000; Cerling *et al.*, 2003).

Numerous field studies, carried out across a range of African environments, have documented proportions of browse and grass consumed by ungulates (e.g. Owaga, 1975; Wilson and Hirst, 1977; Dunham, 1980; Grobler, 1981; Buys, 1990; Meissner *et al.*, 1996; Watson and Owen-Smith, 2000). In a major synthesis of available information, Gagnon and Chew (2000) reviewed the relevant literature on African bovid feeding ecology to test previously proposed diet classification systems. Their study was, however, constrained by a lack of quality control for the data used, because the available literature on bovid diets is often disparate and contentious; they considered available literature to provide reliable data for only 42 of the 78 bovid species they examined. Gagnon and Chew (2000) suggested that disparate dietary classification schemes are an artifact of the lack of uniformity amongst methodological approaches to diet. The incompatibility of studies isolated in space and time also limits broad-scale assessment of diet, in that dietary variations are difficult to quantify (Owen-Smith, 1988, 1997). Perhaps the most lucid example of this problem can be seen in the literature regarding the diet of a species not included in this thesis, that of the African elephant *Loxodonta africana*. Opinions vary as to whether this species should be regarded as a browser or a grazer (Guy, 1976; Norton-Griffiths, 1979; Jachmann & Bell, 1985; Dublin, 1995; Tangley, 1997). This contention most likely arises due to the isolation of studies in space and time. For example, while elephants in Queen Elizabeth National Park, Uganda, may have diets comprised of 90% grass (Wing and Buss, 1970), in Hwange National Park, Zimbabwe, they feed almost entirely on woody plants (Williamson, 1975).

Previous carbon isotope comparisons with field studies for bovid diets in East and southern Africa have revealed significant discrepancies with predictions of Gagnon and Chew (2000) for several taxa (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a). These studies were based on analysis of hard tissues, such as hair, bone collagen, and tooth enamel, integrating data over relatively broad spatio-temporal scales. Here, I use stable carbon isotope data from faeces collected over multiple high-resolution spatial and temporal scales from Kruger Park to test dietary predictions for African ungulates along a browser/grazer (or C_3/C_4) continuum, and whether these predictions persist when dietary variations are accounted for. I also test previous models for ^{15}N-abundance variations amongst mammals, in terms of adaptations to diet type, diet quality, adaptations to water stress, digestive physiology, and rainfall.

3.2. Methods

Faeces were collected from June 2002 to May 2005, biannually during the first two years, and monthly from February 2004 to May 2005, throughout Kruger Park. Only fresh faeces were collected, i.e. damp or wet specimens, to ensure that these represented the appropriate season, and that they were free of contaminants such as fungi, soil, or insects (see Wrench *et al.*, 1996). Each dung pile encountered was assumed to represent a different individual of the relevant species. Faeces that are readily identifiable, and relatively easy to obtain, such as those of buffalo *Syncerus caffer* and square-lipped (white) rhinoceros *Ceratotherium simum*, were sampled opportunistically, i.e. as and when these were encountered in the field. Collection of samples which are more difficult to identify and/or harder to locate in the field, e.g. kudu *Tragelaphus strepsiceros* and nyala *T. angasii* were made by locating animals from a vehicle and then following on foot.

Samples were oven-dried at 60°C for 24 hours, and mill-ground into a homogenous powder through a 1mm sieve. Powdered samples were individually combusted in an automated Elemental Analyzer (Carlo Erba, Milan), and the resultant CO_2 and N_2 gases introduced to a Finnigan MAT 252 or DELTA XP Mass Spectrometer via a continuous flow-through inlet system (Conflo). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are expressed in the delta (δ) notation in parts per mil (‰) relative to the VPDB and Air standards, respectively. Standard deviations of repeated measurements of internal plant, protein, and chocolate standards were less than 0.1‰ for $\delta^{13}C$, and 0.3‰ for $\delta^{15}N$. This method also provided the percent nitrogen (%N), by weight, for each sample.

To compare carbon isotope data with data from field studies, faecal $\delta^{13}C$ values were converted to estimates of % grass (C_4) intake, using a dual-endpoint mixing model (e.g. Vander Zanden and Rasmussen, 1999; Post, 2002; Sponheimer *et al.*, 2003a; Codron *et al.*, 2005a):

$$(\delta^{13}C_{C3}\text{ plants} + \Delta\delta^{13}C - \delta^{13}C_{\text{animal}}) / (\delta^{13}C_{C3}\text{ plants} - \delta^{13}C_{C4}\text{ plants}),$$

where $\Delta\delta^{13}C$ is the magnitude of isotopic discrimination from diet to faeces, assumed here to be –0.9‰ (Sponheimer *et al.*, 2003b; Codron *et al.*, 2005a). Previous studies employing a similar approach have relied on global plant mean $\delta^{13}C$ values (-27.0 and –12.5‰ for C_3 and C_4 plants, respectively). Here, I used regionally and seasonally specific endpoint values based on data for Kruger Park vegetation (Codron, J. *et al.*, 2005; see Table 3.1). For example, mean $\delta^{13}C$ values of vegetation for northern basalts during the dry season (April to September) were –26.0 and –12.7‰ for C_3 and C_4 plants, respectively, and these values were assigned as endpoints for faeces collected from this region during the dry season. Similarly, wet season (October to March) means for C_3 and C_4 northern basalt plants were –25.9 and –12.1‰, respectively, defining the endpoint values for wet season faeces from this region.

Comparative data for field studies were taken from Gagnon and Chew's (2000) review for bovids, and Owen-Smith (1988) and Skinner and Smithers (1990) for non-bovid taxa represented here. Reported dietary contributions of dicots and fruits (e.g. Gagnon and Chew, 2000) were combined to represent total percent C_3 in the diet (see Sponheimer *et al.*, 2003a). Monocots were assumed to represent C_4 intake, although some aquatic monocots (e.g. reeds and sedges) in Kruger Park may be either C_3 or C_4 (Codron, J. *et al.*, 2005). Nevertheless, reeds and sedges are likely to make a significant contribution to the diets of only a few grazing species used in this study (i.e. buffalo, blue wildebeest *Connochaetes taurinus*, waterbuck *Kobus ellipsiprymnus* and hippopotamus *Hippopotamus amphibius*).

In a similar comparison between carbon isotope and field studies, Sponheimer *et al.* (2003a) allowed for a ~15% error in isotope-based derivations of C_4 contributions to diet, because of potential environmental influences on the carbon isotope composition of C_3 plants (Ehleringer and Cooper 1988; Tieszen, 1991; van der Merwe and Medina 1991), and because specimens from that study spanned the twentieth century during which there has been considerable ^{13}C-depletion of atmospheric CO_2 (e.g. Friedli *et* al., 1986; Marino and McElroy, 1991; Arens *et al.*, 2000). Use of regionally specific plant data to derive percent dietary C_4 enhances the accuracy of calculations (Codron *et al.* 2005a), hence results for Kruger Park faeces were considered to differ significantly from those reported in the literature if the difference between two data points was 10% or more.

3.3. Results

Percent C_4 (grass) intake of Kruger Park ungulates, as reflected by faecal $\delta^{13}C$, was similar to expectations derived from field studies for Burchell's zebra *Equus burchellii* (92% C_4; $n = 166$), the two rhinoceros species *C. simum* (90% C_4; $n = 29$) and *Diceros bicornis* (9% C_4; $n = 9$), hippopotamus (91% C_4, $n = 26$), warthog *Phacochoerus africanus* (91% C_4, $n = 62$), and giraffe *Giraffa camelopardalis* (5% C_4, $n = 174$) (Table 3.2). It is amongst the 13 bovid species that carbon isotope data often conflicted with data from field observations. Six of the 13 species analyzed have faecal $\delta^{13}C$ values depicting C_4 contributions to diet differing by more than 10% from those predicted by Gagnon and Chew (2000). These are buffalo (88% C_4, $n = 319$), eland *Taurotragus oryx* (3% C_4, $n = 5$), roan antelope *Hippotragus equinus* (96% C_4, $n = 86$), sable antelope *H. niger* (98% C_4, $n = 122$), impala *Aepyceros melampus* (60% C_4, $n = 606$), and steenbok *Raphicerus campestris* (7% C_4, $n = 26$). Gagnon and Chew's (2000) review predicts diets of 78, 50, 50, 45, and 34% grass for these species, respectively. By contrast, Sponheimer *et al.* (2003a) observed such differences in only three out of 27 species analyzed, due in part to their more conservative conversion of animal $\delta^{13}C$ to estimated dietary $\%C_4$ in the absence of temporally and regionally specific vegetational baseline data. In comparison with previous carbon isotope studies of bovid diets, faeces from Kruger Park differ with evidence from hard tissues of specimens from across the subcontinent (Sponheimer *et al.*, 2003a) in two species (nyala 23% C_4, $n = 102$; and grey duiker *Sylvicapra grimmia* 15% C_4, $n = 14$), and with East African conspecifics (Cerling *et al.*, 2003) in five out of 12 cases (blue wildebeest 90% C_4, $n = 151$, as well as buffalo, eland, steenbok, and grey duiker).

Mean faecal $\delta^{15}N$ values ranged from 3.0 ± 0.6‰ ($n = 5$) in eland and 3.2 ± 1.2‰ ($n = 122$) in sable antelope to 5.8 ± 2.1‰ ($n = 606$) in impala and 6.0 ± 0.3‰ ($n = 7$) in bushbuck *Tragelaphus scriptus* (Table 3.2). Analysis of variance (ANOVA) revealed significant differences in mean $\delta^{15}N$ between groups with different dietary and ecophysiological adaptations ($F_{7, 2194} = 32.561$; $P < 0.001$; Figure 3.1). However, the data show a significant dry-to-wet season increase in $\delta^{15}N$ ($F_{1, 2200} = 75.815$; $P < 0.0001$), indicating that inter-specific differences should be assessed within these respective seasons. Browsers and mixed-feeders have higher faecal $\delta^{15}N$ values than grazers during the dry season ($P < 0.0001$). However, while faecal $\delta^{15}N$ of mixed-feeders and grazers increased from the dry to the wet season ($P < 0.0001$), browser values did not change ($P = 0.99$). The net effect is that the wet season mean for mixed-feeders was higher than that of both browsers and grazers ($P < 0.0001$). Faeces of ruminants have a higher mean $\delta^{15}N$ than non-ruminants during both seasons ($P < 0.01$). Species were further categorized as either drought-tolerant or water-dependant, according to descriptions in Skinner and Smithers (1990). Drought tolerant herbivores had higher faecal $\delta^{15}N$ in the dry season ($P < 0.0001$), but in the wet season, higher values were recorded for water dependant species ($P < 0.0001$).

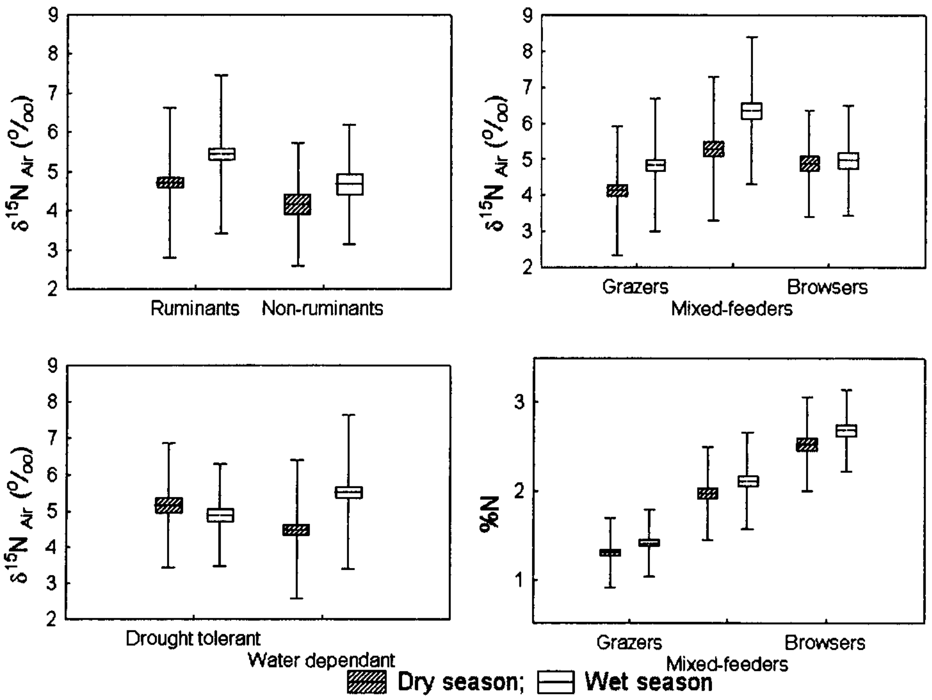

Figure 3.1. Comparison of faecal $\delta^{15}N$ and faecal %N amongst different guilds of Kruger Park ungulates. The boxes depict the 95% confidence limits for the means (horizontal bars), and the vertical bars denote ±1 standard deviation.

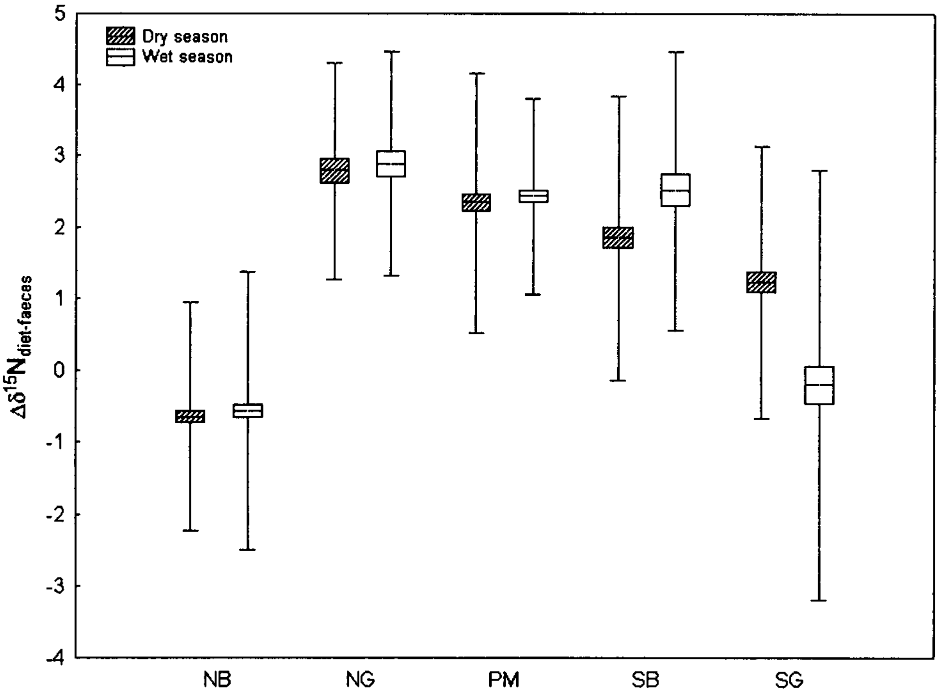

Figure 3.2. Differences between $\delta^{15}N$ of diet (calculated from proportions of C_3 and C_4 foods consumed and reported $\delta^{15}N$ values of local C_3 and C_4 vegetation) and faeces, across different regions and seasons in Kruger Park. The boxes represent standard error, and the vertical bars ±1 standard deviation from the mean (horizontal bars).

Percent N of faeces is expected to provide an indication of crude protein intake (Holecheck *et al.*, 1982; Leslie and Starkey, 1985; Grant *et al.*, 2000). Faecal %N was lowest in sable (mean = 1.0 ± 0.2%, n = 122) and roan antelope (mean = 1.1 ± 0.3%, n = 86), and highest in kudu (mean = 2.8 ± 0.4%, n = 145) (Table 3.2). Despite a dry-to-wet season increase in faecal %N ($P < 0.05$), faecal %N of grazers is consistently lower than that of browsers during both seasons ($P < 0.0001$), while faecal %N of mixed-feeders is intermediate ($P < 0.0001$; Figure 3.2). These results agree with predictions for savanna vegetation, in that grasses generally have a lower crude protein (and hence %N) content than woody plants, and crude protein content of savanna plants increases during the wet/growing season (Boutton *et al.*, 1988; Hofmann, 1989; Owen-Smith, 1997; Meissner *et al.*, 1999).

3.4. Discussion

3.4.1. Comparison with Predictions from Field Studies

Stable carbon isotope evidence from faeces for mean percent dietary C_4 differs from predictions from field studies (reviewed in Gagnon and Chew, 2000) in six bovid species (Table 3.2). The largest discrepancies are observed for eland and steenbok. Carbon isotope data from Kruger Park faeces suggest that both these species consume much less grass (<10% in both cases) than predicted by Gagnon and Chew (2000) (~50 and 35%, respectively). While my data are supported only by 5 faecal samples in the case of eland, Sponheimer *et al.* (2003a) reported similar disagreement with Gagnon and Chew (2000) for both eland and steenbok. Carbon isotope data for the same species in East Africa showed that they consume more grass (~18% of bulk intake) than their southern African counterparts (Cerling *et al.* 2003). Nevertheless, even the East African data portray that C_4 foods make smaller contributions to the diets of both eland and steenbok than predicted from field studies (e.g. Lamprey, 1963; Hofmann and Stewart, 1972; Gagnon and Chew, 2000). Carbon isotope evidence from faeces of eland from the Waterberg savanna, South Africa, also suggests a browse-based diet (Codron, *et al.*

2005b). Similarly, a field study of eland in the Eastern Cape of South Africa showed that they eat very little grass (Watson and Owen-Smith 2000). Buys (1990) reported that eland diets in the S. A. Lombard Nature Reserve in the western Limpopo Province comprised only between 25 and 65% woody browse, depending on seasonal availability of resources, but the balance consisted of both non-woody forbs as well as grasses For steenbok, du Toit (1993) reported a predominantly browse-based diet in populations from Kruger Park. In sum, it may be that some populations of eland eat significant amounts of grass (Lamprey, 1963; Buys, 1990), but current evidence indicates that both eland and steenbok consume less grass than is widely believed.

Amongst grazing taxa, three species included in this study yielded results for mean %C_4-intake differing from literature predictions: buffalo, roan antelope, and sable antelope. According to available literature on these species' diets, grass comprises between 78 and 85% of bulk intake, the balance being a mix of various C_3 based-foods (Lamprey, 1963; Child and Wilson, 1964; Wilson and Hirst, 1977; Owen-Smith, 1997; Gagnon and Chew, 2000). Wilson and Hirst (1977) reported that in other savanna areas of South Africa, as much as 59% of dry season forage time of roan antelope was dedicated to browsing. Faecal $\delta^{13}C$ values, however, show that roan and sable antelope do not consume sufficient amounts of C_3 foods to influence the overall isotopic composition of their diets, having diets ranging between 95 and 100% C_4. This result is not restricted to Kruger Park populations alone. Carbon isotope data from body tissues of southern and East African representatives of roan and sable (Cerling *et al.* 2003; Sponheimer *et al.* 2003a) agree closely with results from Kruger, suggesting that these taxa are near exclusive grazers across many southern and East African savannas.

Some grazers also differ in mean $\delta^{13}C$ and $\%C_4$ intake compared with isotope data from East Africa (Table 3.2). In particular, Cerling *et al.* (2003) reported diets of 100% grass in buffalo and wildebeest, while results for Kruger Park and indeed all of southern Africa (Sponheimer *et al.*, 2003a) are consistent with >10% C_3 contributions to diet in these species. Cerling and Harris (1999) proposed that tooth enamel $\delta^{13}C$ of grazers from xeric East African environments, where NADP-ME grasses predominated, was distinct from those of grazers from mesic environments dominated by NAD-ME and PCK grasses. It is possible that differences between Kruger Park and East African grazers are related to differential utilization of grasses following alternative C_4 photosynthetic pathways. For example, the lower $\delta^{13}C$ values for Kruger Park buffalo and wildebeest compared with pure grazers from this region, such as roan and sable antelope, may reflect greater reliance on NAD and PCK grasses by the former. However, the isotopic difference between these grazer groups is ~1.5‰, larger than reported differences (~0.5‰) between grass subtypes within Kruger Park (Codron, J. *et al.*, 2005), and indeed elsewhere (see Hattersley, 1982; Cerling and Harris, 1999). Thus the most plausible explanation is that buffalo and wildebeest in Kruger Park do indeed utilize small, but significant, amounts of C_3 dicots, whereas their conspecifics inhabiting the wide open grasslands of East Africa do not. Some portion of the C_3 component of buffalo and wildebeest diets may in fact be derived from reeds and sedges (which in Kruger Park are predominantly C_3; Stock *et* al., 2004; Codron, J. *et al.*, 2005), as noted from field studies (Skinner and Smithers, 1990), but it is difficult to distinguish C_3-dicot from C_3-monocot feeding from carbon isotope data alone.

The other species included in this study that displayed discordant results compared with field studies is the impala (60% C_4 compared with 45% predicted by Gagnon and Chew, 2000). However, impala are a notoriously enigmatic mixed browser/grazer species (Dunham, 1980; Skinner and Smithers, 1990; Meissner *et al.* 1996; Wronski 2002). Indeed, impala diets actually varied between ~37 and 74% C_4 across Kruger Park regions and seasons. Similarly, a previous study based on faecal carbon isotope data showed that impala diets in Kruger Park also differ between northern (~40% C_4 grass) and southern (~60% grass) populations (Sponheimer *et al.*, 2003e). Comparable variations have also been reported by Vogel (1978) based on carbon isotope data from impala bone collagen from other South African savannas. Thus the difference between results of the current study and field observations can be considered an artifact of spatio-temporal dietary flux in the species. Similarly, faecal $\delta^{13}C$ values for nyala reflect a diet comprising ~20% C_4 grass, similar to the predictions of Gagnon and Chew (2000), but lower than the 33% reported by Sponheimer *et al.* (2003a). However, nyala in Kruger Park vary their diet from ~14 to 29% C_4 foods from the dry to the wet season. Dietary variation in this species is further emphasized by a recent carbon isotope study of nyala in Hluhluwe-Umfolozi Park, Kwazulu-Natal, South Africa; faecal $\delta^{13}C$ values from this savanna region reflect very high grass intake, over 50% throughout the year (Botha and Stock, 2005).

For the grey duiker, Kruger Park $\delta^{13}C$ data portray a ~15% C_4 diet, as opposed to a pure C_3 diet inferred from $\delta^{13}C$ values of hard tissues for both southern and East African representatives (Lee-Thorp, 1989; Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a). According to Skinner and Smithers (1990), grey duiker are almost exclusively browsers, and very seldom eat any grass. On the other hand, Gagnon and Chew (2000) predicted a 12% grass diet in the species. In Kruger Park, duiker diets appear to vary between 5 and 30% C_4 grass from the dry to the wet season, a range encompassing that of all previous estimates (see Skinner and Smithers, 1990; Gagnon and Chew, 2000; Sponheimer *et al.*, 2003a). Grey duiker do, on occasion, eat animal matter (Skinner and Smithers, 1990), thus the C_4 component of their diet may represent consumption of C_4 animal matter. However, faunivory is unlikely to account for as much as 10% or more of their diets. Notably, faecal $\delta^{15}N$ of duikers are amongst the lowest of all browsing taxa (see Table 2), exactly the opposite of what one might expect had animal matter made significant contributions to diet (see Sealy *et al.*, 1987). All duikers (Cephalophini), including the savanna species *S. grimmia*, also consume varying quantities of fruit (Skinner and Smithers, 1990; Owen-Smith, 1997; Gagnon and Chew, 2000). It has been shown that fruits are 1 to 2‰ enriched in ^{13}C compared to C_3 foliage in the South African savanna environments of the Kruger Park and the Waterberg (Codron *et al.*, 2005b; Codron, J. *et al.*, 2005), as well as in the Ituri forest, Democratic Republic of Congo (Cerling *et al.*, 2004b). Therefore, some of the ^{13}C-enrichment of Kruger Park duiker faeces may reflect utilization of fruits, although the wet season mean for this species (-23.4 ± 3.7, $n = 6$) is certainly consistent with at least some grass intake. These data confirm that the grey duiker, while predominantly a browser, does feed on grass to some extent, at least in some regions and seasons.

3.4.2. Partitioning Multiple Food Sources

While carbon isotope evidence offers a rigorous and empirical approach to total C_3 and C_4 intake, these data alone cannot distinguish between foods such as leaves, fruit, sedges, etc. In the current study, I was sometimes forced to make *post hoc* inferences, for instance questions of fruit intake by duikers, and consumption of different grass types, as well as reeds and sedges, amongst grazers. A variety of C_3 (e.g. leaves, fruits, flowers, sedges) and C_4 (NADP-ME, PCK, NAD-ME grasses) plant foods are in fact isotopically distinguishable from one another, either in terms of $\delta^{13}C$, $\delta^{15}N$, or both (Codron, J. *et al.*, 2005; Table 3.1). Linear models based on *n* isotopes (as used estimating C_3:C_4 intake) cannot resolve dietary inputs of more than $n + 1$ sources, because they yield more mathematical unknowns than equations (Post, 2002; Phillips and Gregg, 2003). Dual-isotope multiple-source mixing models, on the other hand, can potentially reveal inputs of several sources (e.g. Post, 2002; Phillips and Gregg, 2003; Phillips *et al.*, 2005; Urton and Hobson, 2005). A common formula for resolving feasible contributions of three potential sources from two isotopes is

$$\delta^1 m = f_a \delta^1{}_a + f_b \delta^1{}_b + f_c \delta^1{}_c,$$

$$\delta^2 m = f_a \delta^2{}_a + f_b \delta^2{}_b + f_c \delta^2{}_c,$$

$$1 = f_a + f_b + f_c;$$

where δ^1 and δ^2 are the respective ratios of the two isotopes (C and N), δm is the isotopic value of the diet mix (i.e. value of the material corrected for diet-tissue discrimination), and f represents unknown proportions in which sources a, b, and c are consumed (Phillips and Gregg, 2003; Phillips *et al.*, 2005).

Here, I attempt to partition contributions of multiple food sources to diet, using the Isosource model of Phillips and Gregg (2003), which is an iterative mixing model that considers all possible solutions to the means of consumers and source endpoints. The model also allows the user to account for analytical error by adjusting isotopic increment and tolerance values. However, it requires appropriate diet-tissue discrimination factors, and while herbivore faeces are consistently 0.9‰ (± 0.4) lower in $\delta^{13}C$ than the diet (Sponheimer *et al.*, 2003b; Codron *et al.*, 2003a), little is known about nitrogen isotopic discriminations between diet and faeces. Limited controlled feeding-experiments suggest positive nitrogen isotopic discriminations, but reported differences between diet and faeces vary widely, i.e. from 0.4 to 3.0‰ across cattle, pigs, and llamas (Steele and Daniel, 1978; Sutoh *et al.*, 1987; Sponheimer *et al.*, 2003d). Nitrogen isotope discriminations may also vary in response to a multitude of factors including dietary protein, water stress, digestive physiology, climate, and age (Sealy *et al.*, 1987; Ambrose, 1991, 2000; Sponheimer *et al.*, 2003c; Robbins *et al.*, 2005; and see section 2.4.4 below). Because of this variation, endemic discrimination factors had to be estimated for Kruger Park ungulates. I estimated dietary $\delta^{15}N$ based on regional and seasonal differences in $\delta^{15}N$ values of Kruger Park vegetation (Codron, J. *et al.*, 2005; Table 3.1) and proportions of C_3:C_4 foods consumed (Table 3.2), using the formula

$$[100 / (\%C_{4\ \mathrm{diet}} \times \delta^{15}N_{C4\ \mathrm{plants}})] + [100 / (\%C_{3\ \mathrm{diet}} \times \delta^{15}N_{C3\ \mathrm{plants}})]$$

This method presents a somewhat circular means for resolving diet, because discrimination factors ($\delta^{15}N_{\mathrm{intake}}$ - $\delta^{15}N_{\mathrm{faeces}}$) are based on total C_3 and C_4 inputs inferred from carbon isotopic data. Nevertheless, the aim here was to resolve dietary inputs of different types of C_3 and C_4 foods that were not incorporated into calculations of %C_4-intake, thus the method is expected to be adequate for the purpose of this study.

Nitrogen isotope differences between inferred intake and faeces varied widely between season and region, ranging from 2.0 to 3.0‰ in most cases (ANOVA $F_{9, 2192} = 141.042$, $P < 0.001$; Figure 3.2). Within the different regions and seasons, discriminations also varied widely (SD 1.4 to 3.0‰), attributable to observed inter-specific differences (Table 3.2). Thus, nitrogen isotope diet-faeces discrimination factors used in the Isosource model were separated by species differences for each region and season, e.g. northern granite dry season discrimination for giraffe was 1.8‰, while that for buffalo was 3.7‰.

Endpoint values for multiple sources were taken from Kruger Park plant data, separating isotopically distinguishable C_3 and C_4 sources (Codron, J. *et al.*, 2005; see Table 3.1). For example, tree foliage and forbs differ in $\delta^{13}C$ compared with fruits and flowers (the former being enriched in ^{13}C), and NAD-ME and PCK grasses are ^{13}C-depleted compared with NADP-ME grasses (~0.5‰; see also Hattersley, 1982; Cerling and Harris, 1999). $\delta^{15}N$ differences exist between NAD/PCK and NADP grasses (~1.5 to 2‰), and between reeds and sedges compared with tree leaves, forbs, and grasses (~2.0 to 2.5‰). Tree leaves and forbs from Kruger Park are completely indistinguishable based on both carbon and nitrogen isotopic compositions, and are hence treated here as a single group.

For taxa classed as browsers or mixed-feeders, I assessed inputs of tree leaves and forbs, fruits/flowers, and grass. For grazers, I tested intake of four potential food sources, i.e. NAD/PCK grasses, NADP grasses, dicot (tree and forb) leaves, and reeds/sedges. The latter group incorporated only data for C_3 reeds and sedges, since C_4 representatives of these growth forms are rare in Kruger Park (12% and 25%, respectively; see Stock *et al.*, 2004; Codron, J. *et al.*, 2005). To control for spatio-temporal variations in diet, the Isosource model was run for each species, separated by landscape and season. Regional and seasonal results of the models were then combined for each species, and thereafter reduced to 25th - 75th percentile ranges. Isotopic increment and tolerance ranges were adjusted to 1‰ for the former and between 0.1 and 0.5‰ for the latter.

Model output generally agreed with interpretations based on $\delta^{13}C$ values alone, and with predictions from the literature (Figure 3.3; Table 3.2). Most browsers are shown to consume negligible amounts of grass (<10%), although the model suggests that steenbok and grey duiker eat between 0 and 13%, and 1 and 29% grass, respectively (Figure 3.3a). Mixed-feeding nyala and impala have diets of 11 to 26% grass and 48 to 71% grass, respectively. Consumption of fruit/flowers is revealed as significant (i.e. >10%) for giraffe (0 to 13%), eland (0 to 19%), nyala (10 to 57%), kudu (0 to 22%), impala (0 to 22%), steenbok (0 to 14%), and duiker (0 to 10%). Giraffe are well known to consume fruit and flowers in varying proportions when they are available (Hall-Martin, 1974; Owen-Smith, 2004, pers. comm.). Gagnon and Chew (2000) reported mean fruit intake for eland, nyala, kudu, impala, steenbok, and duiker of 5, 30, 30, 10, 5, and 5%, respectively. The model shows that grasses contribute between about 80 and 100% of total food intake by grazers, the majority of this appears to comprise NAD-ME/PCK grasses (~ 43 to 83%), whereas NADP-ME grasses make up only between 14 and 48% of the diet of all species (Figure 3.3b). Grazer species that are shown to eat significant proportions of dicots (tree leaves and/or forbs) are zebra (5 to 11%), warthog (7 to 12%), buffalo (12 to 17%), and wildebeest (6 to 13%). Interestingly, no grazers appear to consume significant (>10%) proportions of reeds and sedges, likely because faecal $\delta^{15}N$ values are far lower than means for reeds and sedges that have higher $\delta^{15}N$ than any other plants from Kruger Park. Most field studies tend to agree with this finding for taxa such as hippopotamus, buffalo, wildebeest, and waterbuck (e.g. Owen-Smith, 1988; Skinner and Smithers, 1990).

The Isosource model depicted slightly higher fruit intake amongst eland, nyala, and impala than predicted. One likely source of error here arises from uncertainty in diet-faeces nitrogen isotope discriminations. To test this, the model was re-run for these taxa using alternate values for $\delta^{15}N$ diet-faeces differences (±1.0‰ of inferred values). The effect of these adjustments was that, in most cases, values fell outside the range of potential dietary source values (plant data) for each region. Thus the isotope discrimination factors used in the model appear to be sufficient for predicting dietary values. Discrepancies between expected and modeled fruit intake appear to arise merely because model output is in terms of feasible intake ranges rather than means. Intake of food sources not accounted for here may also present a constraint. For example, eland faecal $\delta^{15}N$ is lower (mean = 3.0 ± 0.6‰; Table 3.2) than that of all other taxa included here, possibly reflecting consumption of ^{15}N-depleted foods such as underground storage organs and roots, that are known to make substantial contributions to the diet of this species (Skinner and Smithers, 1990), and which are heavily ^{15}N-depleted at least in the Waterberg savanna of South Africa (mean = -2.8 ± 1.5‰, *n* = 2; Codron *et al.*, 2005b).

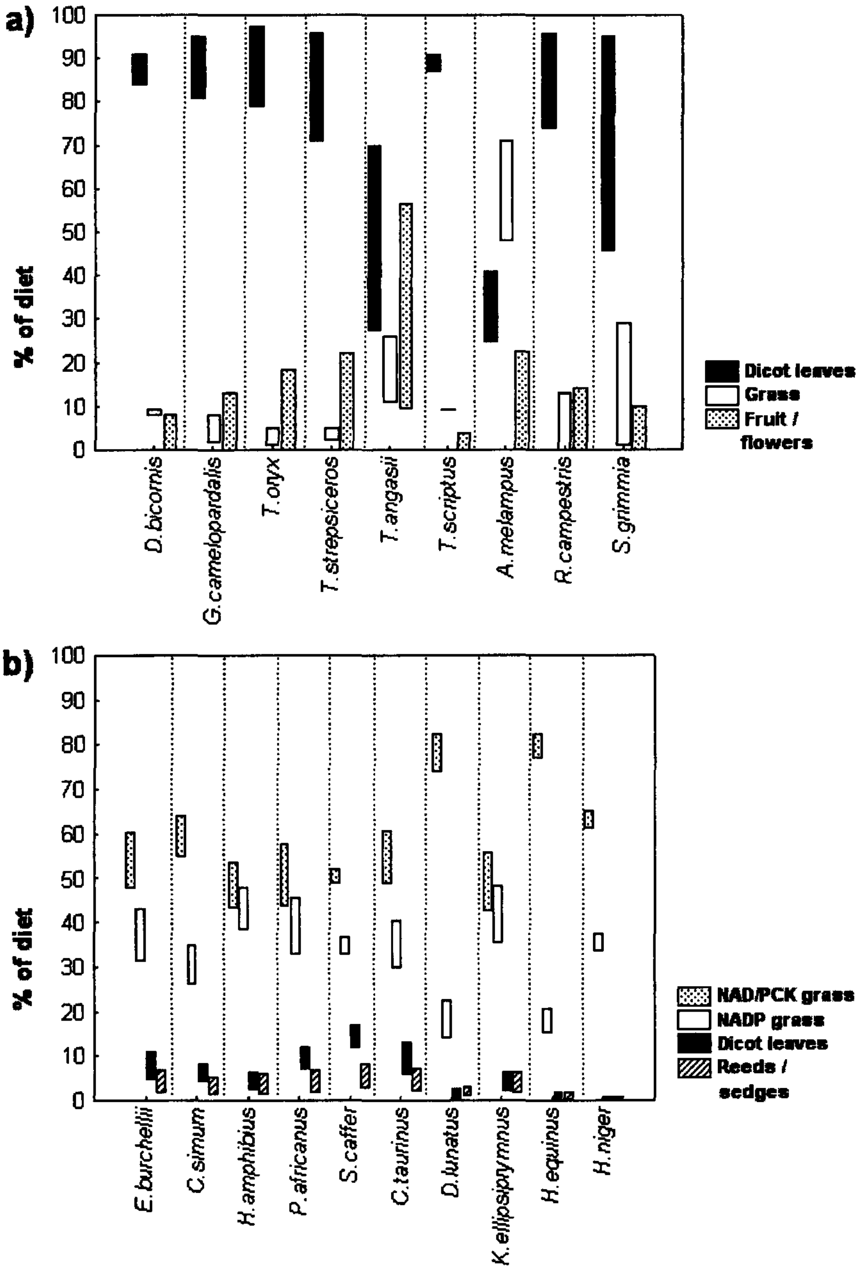

Figure 3.3. Feasible contributions of multiple C_3 and C_4 food sources to the diets of browsers/mixed-feeders (a), edaphic/wetland grazers (b), and grazers (c) from Kruger Park, based on the Isosource multiple mixing model of Phillips and Gregg (2003). The columns depict the 25th to 75th percentiles of dietary inputs across different regions and seasons. Dicots are represented by tree leaves and forbs.

Despite potential limitations, the dual-isotope multiple-source model yielded useful insights into diet that extend beyond the C_3:C_4 (or browser/grazer) continuum. For example, partitioning of feasible intake ranges of leaves, fruit, and grasses by grey duikers confirm that fruit-eating alone cannot account for the relatively ^{13}C-enriched wet season faecal values for this species, and that they do indeed consume a small proportion of grasses. Amongst grazers, different species could not be separated based on dietary inputs of NAD/PCK and NADP-ME grass type, supporting the earlier *post hoc* assertion that carbon isotope differences between southern and East African grazers are likely due to C_3 consumption by the former rather than utilization of different grass types. It is also interesting that all Kruger Park grazers appear to prefer NAD/PCK to NADP grass sources, although this is not necessarily surprising given the taxonomic distribution of these sub-types. The NAD and PCK sub-pathways are more common amongst chloridoids, whereas NADP is more prevalent amongst the andropogonoids (Sage *et al.*, 1999). In turn, chloridoid grass species are widely considered to be more palatable than andropogons. Even within grass genera that are represented by all C_4 sub-pathways, proportions of less-digestible mesophyll are higher in NADP than NAD/PCK species (Wilson and Hattersley, 1989). Recent field studies of buffalo and roan antelope feeding ecology in Kruger Park (Macandza *et* al., 2004; Knoop, 2004) found that they avoid species such as *Themeda triandra*, an NADP-ME grass expected to be highly palatable, at least in some savannas of South Africa. Knoop (2004) further showed that roan in Kruger favour *Sporobolus* spp., which may be either NAD-ME or PCK (see Sage *et al.*, 1999).

3.4.3. Ecological Niche Separation Based on Diet Strategy

Dietary niches of Kruger Park ungulates were determined using inter-group cluster analyses based on the Euclidean distance measure. To account for spatio-temporal variations, I incorporated the 25th – 75th percentile intake ranges for each species across all regions and seasons into the analyses. I repeated the cluster analysis using first the data for $\%C_4$ intake (Figure 3.4a), and then data for multiple source intake revealed by Isosource (Figure 3.4b). These analyses revealed, as expected, a strong dichotomy between browsing (group 1) and grazing (group 3) taxa, with mixed browser/grazers (group 2) appearing as out-groups of either of the primary clusters. However, there are more than three discrete groups in the tree diagrams, identifiable according to relative reliance on different food types, although some groups are monospecific because of the limited number of species included in this study ($n = 19$). Even though the two schemes are similar, it is necessary here first to consider the differences that exist between them.

Group 1 is represented by species with predominantly browse-based (C_3) diets (Figure 3.4a), although Isosource revealed two browser clusters, namely pure folivores (group 1a) and browsers that include significant amounts (>10%) of fruits and flowers (and possibly other foods not represented here) into their diets (group 1b). Group 2 represents mixed-feeding taxa, which are separate from browsers and grazers in both schemes. There appear to be two mixed-feeder groups that can be defined as mixed-feeders preferring browse (group 2b) and mixed-feeders preferring grass (group 2c). Interestingly, the grey duiker appears as a browser in Figure 3.4a, but as a mixed browser/grazer when combined evidence from carbon and nitrogen isotopes are considered (Figure 3.4b), likely because this species clearly consumes a substantial amount of grass (up to 29% of bulk, and likely some proportion of fruit as well) in Kruger Park. Nevertheless, carbon isotope evidence alone advocates that this species is at least partially distinct from pure browsers, confirming its status as a more generalist feeder. Group 3 are grazing taxa, which separate into variable (group 3a) and obligate (group 3b) grazers. However, four species (white rhino, hippopotamus, waterbuck, and tsessebe *Damaliscus lunatus*) are evinced as variable grazers through carbon isotope data alone, but are obligate grazers according to dual-isotopic evidence. The disparity here suggests that shifts in carbon isotope values for these taxa more likely reflect dietary switches between NAD/PCK and NADP grasses (and possibly some utilization of C_3 reeds and/or sedges), rather than shifts between C_3 and C_4 food use. Thus, these taxa are probably more correctly defined as obligate grazers, or at least obligate monocot-feeders, an interpretation that agrees with results of the majority of observational studies (Child *et al.*, 1972; Joubert and Bronkhorst, 1977; Owen-Smith, 1988; Skinner and Smithers, 1990).

This dietary categorization of Kruger Park ungulates differs from previous models in several important ways. While there is a clear distinction between browsing and grazing guilds, as predicted by Hofmann and Stewart (1972), species within these groups exhibit a range of dietary preferences. Moreover, mixed-feeding taxa do not form a third group, but are present in both the browser and grazer clusters. Within the browser group, there appears to be a continuum of increasing reliance on alternative food resources, i.e. pure folivores; predominantly folivorous taxa that utilize small proportions of fruit; and taxa relying on a mix of leaves and fruit/flowers as well as grasses. Bodmer (1990) and Gagnon and Chew (2000) both recognized frugivory as a separate feeding style from browsing and grazing. No such distinction is evident in the current study, although these data do not include the only frugivorous taxa identified by previous authors, i.e. duikers of the genus *Cephalophus*. Nevertheless, the increased reliance on fruit within the browser guild is accompanied by increased utilization of grasses, and not in the other direction, as predicted by Bodmer (1990). Yet, the same might not be true of forest-dwelling species such as *Cephalophus*. Similarly, while Gagnon and Chew (2000) recognized frugivory as distinct from browsing, their analysis suggested that the continuum from frugivore-browser-grazer is not necessarily linear. Thus, it remains unclear whether frugivory exists as an extension of browsing or mixed-feeding character states. For example, the non-linearity of the continuum forced Gagnon and Chew (2000) to recognize two different mixed-feeder guilds, i.e. generalists (browser/grazer/frugivores) and mixed browser/grazers. While the isotopic evidence agrees strongly with data of Gagnon and Chew (2000) in this regard, further evidence for *Cephalophus* diets are required to fully test the connection between frugivory and the browser/grazer continuum.

Amongst grazing taxa, there appear to be two discrete groups, i.e. variable (consume small, but significant amounts of dicots) and obligate grazers. Gagnon and Chew (2000) and Cerling *et al.* (2003) recognized similar sub-categories. However, these two studies differed in terms of the species composition of these groups. For example, Gagnon and Chew (2000) included red hartebeest, roan antelope, and sable antelope as variable grazers, although carbon isotope data (this study; Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a) indicate that these species are more consistent grazers than the literature predicts. Cerling *et al.* (2003), by contrast, classified blue wildebeest (and red hartebeest) as a prominent hypergrazer species. Carbon isotope data for the Kruger Park wildebeest population suggest some intake of C_3 foods (~10%), as do isotope data for specimens from across the sub-continent (Sponheimer *et al.*, 2003a). Given these disparities, which are likely related to geographical differences in diet (e.g. between wildebeest from southern and East Africa), distinction between variable and obligate grazers is at best site-specific, rather than a characteristic feature of certain taxa, and possibly even redundant.

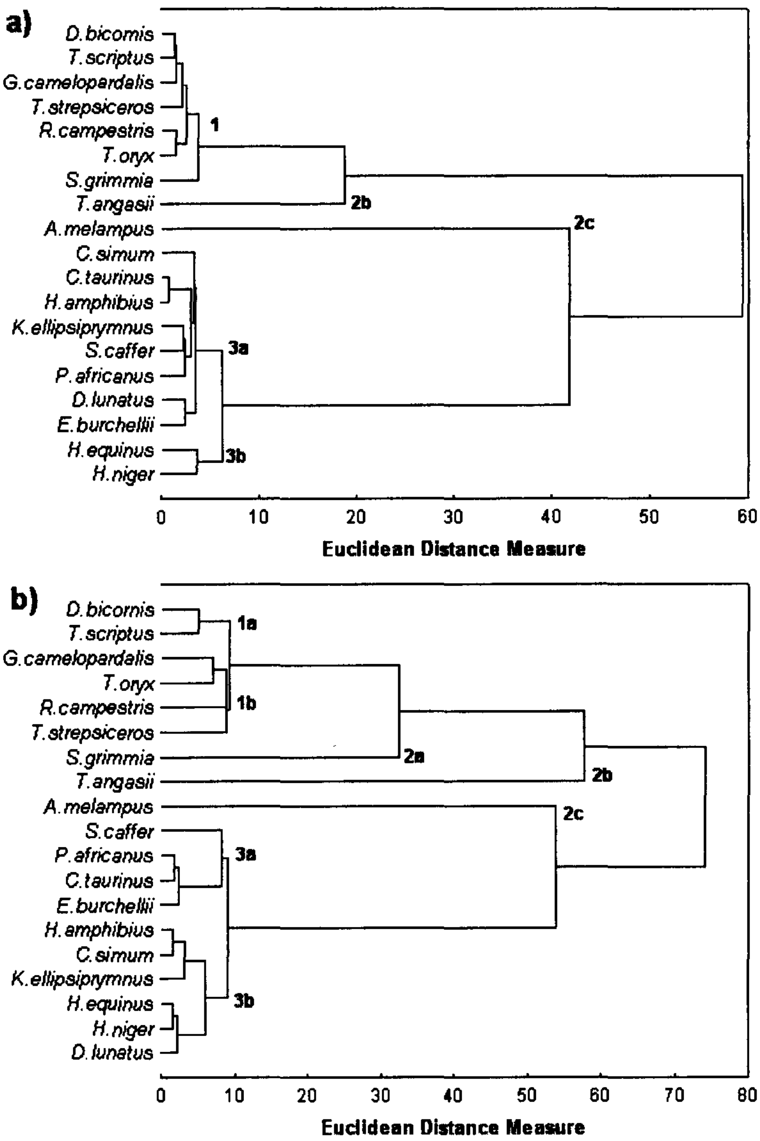

Figure 3.4. Diet-niche differentiation amongst Kruger Pak ungulates based on stable isotope evidence from faeces. The tree diagram represents a cluster analysis of Euclidean Distance inter-group measures, based on a) variations (25th to 75th percentiles) in total C_3 and C_4 food intake in diet and b) 25th to 75th percentile dietary input ranges of dicots (tree leaves and forbs), fruits, and monocots (grasses, and C_3 reeds and sedges) as revealed by the Isosource model. Numbers 1 to 3 represent clusters distinguished by reliance on differential proportions of the two plant food groups.

3.4.4. Predictions for Faecal $\delta^{15}N$

It is generally agreed that trends in mammalian ^{15}N-abundances are poorly understood (see Sponheimer *et al.*, 2003c; Robbins *et al.*, 2005). The current study presents a clear example of the problem, in that I was forced to estimate diet-faeces discrimination factors for local ungulate populations. While these estimations yielded satisfactory results, there is little doubt that the accuracy of inferred nitrogen isotope intake would be greatly improved through enhanced understandings of variations in ^{15}N-partitioning within different species across different environments. I provide here a brief account of variations in faecal $\delta^{15}N$ amongst Kruger Park ungulates.

Predictions for $\delta^{15}N$ variation amongst mammalian herbivores rely largely on the nitrogen isotope urea mass-balance model, derived from studies of ^{15}N-abundances in body tissues such as bone collagen and hair (Ambrose and DeNiro, 1986; Ambrose, 1991). Faeces can be expected to display similar trends to body tissues, because both materials consist of nitrogen that remains in the body pool following excretion of ^{15}N-depleted urea. The urea mass-balance model proposes that factors leading to raised kidney osmolality, and hence increased loss of ^{15}N-depleted urea, result in higher body tissue $\delta^{15}N$ (Ambrose, 1991). Specific predictions are i) drought tolerant species should have higher $\delta^{15}N$ values than obligate drinkers, ii) browsers should have higher $\delta^{15}N$ than grazers, and iii) $\delta^{15}N$ should increase with increased dietary protein. Sealy *et al.* (1987) proposed a further scenario, that ruminants should have higher $\delta^{15}N$ values than non-ruminants, due to more intensive recycling of urea and resorption of gut microbes, effectively adding additional trophic levels to the system. Sponheimer *et al.* (2003c, 2003d) showed from controlled feeding experiments that $\delta^{15}N$ increases with protein intake, although this relationship appears to be more functionally related to the biological nature of dietary proteins, i.e. the fraction of absorbed protein that is retained (see Robbins *et al.*, 2005).

Of the ecophysiological parameters considered here, only differences between ruminants and non-ruminants persist (Figure 3.1); predicted differences between species with different feeding styles (browsers, grazers, and mixed-feeders) and adaptations to water stress (water-dependant or drought-tolerant) are not consistent across different seasons. However, dry season data agrees with expectations of Ambrose's (1991) model for browsers versus grazers, and for drought-tolerant versus water dependant species. These patterns clearly illustrate that environmental controls for ^{15}N-abundances in savanna herbivores are multifactorial, and that predicted patterns may be specific to alternate geographical and temporal scales.

In an attempt to resolve this complexity, I used a multiple linear regression model (MLRM) to test the presumed environmental predictors of mammalian ^{15}N-abundances. Differences between feeding styles were accounted for using %C_4-intake data obtained from carbon isotope analysis, and protein intake using %N data (Table 3.2). Data for Kruger Park vegetation also revealed large geographical and seasonal differences (~ 4‰) that might be expected to influence herbivore $\delta^{15}N$ (Codron, J. *et al.*, 2005; see also Heaton *et al.*, 1986). For example, plants growing on southern granites were found to be ^{15}N-enriched compared with those on granites, whereas the opposite was found amongst plants from northern granites and basalts (see Table 3.1). To control for these variations, I incorporated estimated $\delta^{15}N$ of the diet, based on $\delta^{15}N$ of local C_3 and C_4 plants and the fractions in which these groups were consumed (see section 2.4.3), into the model.

Mammal $\delta^{15}N$ also reportedly varies in response to rainfall and aridity (Heaton *et al.*, 1986; Sealy *et al.*, 1987), thus mean seasonal rainfall for each region during the study period was included (data from the South African Weather Bureau and Nick Zambatis). Finally, to account, at least in part, for ecophysiological differences, the variable "species" was inserted as a categorical predictor.

The MLRM shows, after removal of residual outliers (σ-level ± 3), that faecal %N is the most important predictor of faecal $\delta^{15}N$ (beta = 0.4755, $r^2 = 0.5541$, $P < 0.0001$; Table 3.3). It is not surprising then that the model also reveals a strong correlation between $\delta^{15}N$ and %C_4-intake ($r^2 = 0.5717$, $P < 0.01$), since faecal %N is higher in C_3-browsers than C_4-grazers. Rainfall and $\delta^{15}N$ of available vegetation are significant correlates of faecal $\delta^{15}N$ ($P < 0.0001$ and $P < 0.01$, respectively), but the correlations are weak in both cases ($r^2 = 0.0724$ and 0.1052, respectively). Species differences also appear to be a poor predictor of faecal $\delta^{15}N$ ($r^2 = 0.1253$, $P < 0.01$), although this may be due to the lack of a suitable continuous predictor for ecophysiological adaptation.

Based on the results of the MLRM, interspecific differences in faecal $\delta^{15}N$ were examined using a simple linear regression between mean faecal $\delta^{15}N$ and %N. Interestingly, this analysis revealed two strongly correlated regression curves, with the two groups representing water-dependant and drought-tolerant species, respectively (Figure 3.5). The regression suggests that 65% of inter-specific variation in faecal $\delta^{15}N$ of drought-tolerant herbivores can be ascribed to faecal %N, and hence dietary protein levels ($r^2 = 0.6491$, $P < 0.05$). This effect is even stronger for water-dependant species, faecal %N accounting for 79% of inter-species variation ($r^2 = 0.7920$, $P < 0.001$). In both cases, the correlations persisted for wet and dry seasons. Thus it appears that animals with differing adaptations to water stress do indeed discriminate against ^{15}N in different ways (see Ambrose, 1991), but these differences are only discernable if differing levels of protein uptake are controlled. A strong correlation has also been reported between blood ^{15}N-discrimination and protein *quality* uptake in both mammals and birds (Robbins *et al.*, 2005). Hence, even though faecal δ^{15}N and %N are likely influenced by the presence of non-dietary proteins and undigested food remains (Robbins, 1993; Sponheimer *et al.*, 2003d), there is little reason to assume that the relationships revealed here would not persist in body tissues as well.

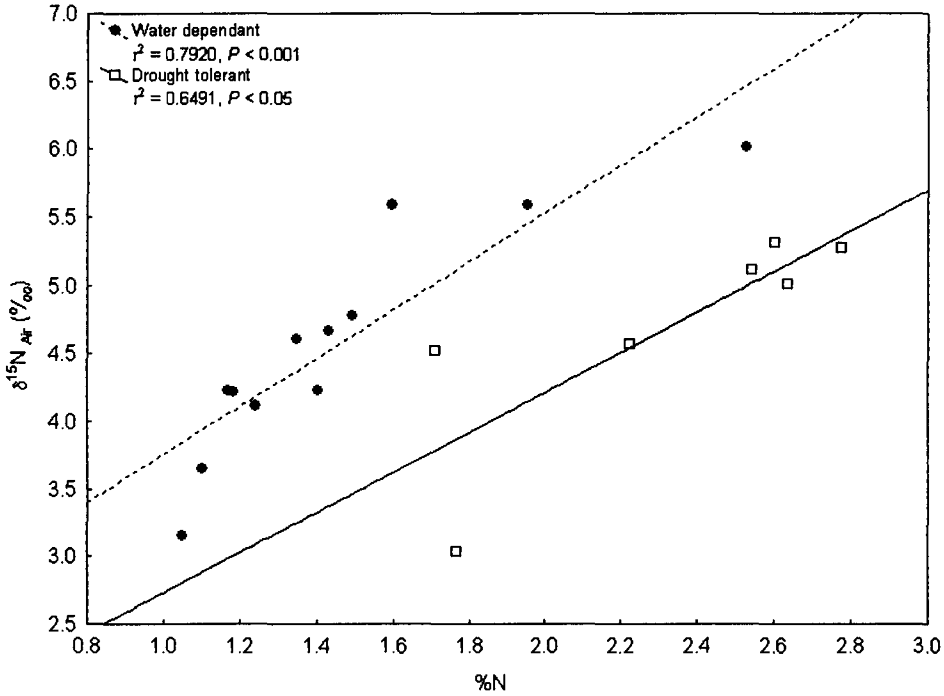

Figure 3.5. Regression curves for faecal δ^{15}N with %N of water dependant and drought tolerant species, respectively. Symbols in the graph depict the means for each species after removal of residual outliers revealed by the MLRM.

3.5. Conclusion

There is incongruence between results of stable isotope and field studies for the diets of several African savanna ungulate species. In some cases, this is likely due to variation in the quality of available observational data. Lack of consistent and uniform data can have a profound impact on general assessments of feeding ecology. For example, eland are widely quoted as mixed-feeders preferring browse (Hofmann and Stewart, 1972; Buys, 1990; Skinner and Smithers, 1990; Owen-Smith, 1997). While some eland populations may include higher proportions of grass into their diets, such as those in East African grasslands (Lamprey, 1963), available isotopic evidence indicates that they are predominantly browsers rather than true mixed-feeders in more woodland/grassland savannas. Gut morphology of the steenbok suggests a mixed browse/grass diet (Hofmann and Stewart, 1972), yet carbon isotope data, as well as field observations (du Toit, 1993), confirm that this species is almost exclusively a browser in many savanna systems.

A further caveat is the hitherto poorly-documented phenomenon of spatio-temporal variability amongst taxa. Perez-Barberia *et al.* (2004) advocated that ungulate diet studies should endeavor to treat feeding style as a continuous, rather than discrete, variable. Even Hofmann (1989), in his landmark description of a browser/grazer dichotomy, recognized that feeding style evolved on a continuous scale, and hence will likely always be so. In this broad assessment of ungulate feeding habits, I was able to incorporate variations across space and time in testing dietary predictions. These analyses, and transpiring discrepancies with previous models, show that there can be no unambiguous classification scheme for diet that is consistent for all environments.

Taxonomic, spatial, and temporal variations present serious limitations to dietary classification systems, hence distinctions between apparently "discrete" feeding categories are unlikely to provide unequivocal interpretations of ungulate dietary ecology.

While models presented here do control for dietary variations, I have not attempted to document variance between specific regional and seasonal scales. This begs an important question for future redress: to what extent would the overall analysis have differed if results for particular regions and/or seasons were treated separately? What this study has shown, however, is that stable isotope ecology is a powerful tool for documenting dietary continuity. Further, more rigorous exploitation of this potential can help overcome some of the limitations of previous studies, and assist with providing refined answers to questions of herbivore feeding ecology.

Table 3.1. Stable carbon and nitrogen isotope data for Kruger Park vegetation used for dietary source endpoint values (from Codron, J. *et al.*, 2005). The data are separated into plant groups that are isotopically distinguishable ($P < 0.001$). C_4 reeds and sedges are omitted since these are rare in Kruger Park.

			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
Form	**Region**	***n***	**Mean**	**SD**	**Mean**	**SD**	***n***	**Mean**	**SD**	**Mean**	**SD**
		Dry Season					**Wet Season**				
Dicots											
C_3 Tree leaves and forbs	Northern basalts	13	-26.0	0.7	5.9	1.0	18	-25.9	1.1	5.5	2.0
	Northern granites	27	-26.2	0.8	2.8	1.0	32	-26.5	1.2	3.2	0.9
	Punda Maria	17	-26.6	1.0	2.8	2.0	28	-26.6	3.3	3.1	2.9
	Southern basalts	133	-26.6	2.1	2.6	2.2	103	-26.6	1.3	3.5	1.5
	Southern granites	103	-26.1	1.4	4.7	2.6	96	-26.7	1.1	5.7	2.5
Fruit/ flowers		41	-24.8	1.3	3.9	2.6	5	-25.3	1.0	3.0	1.7
Monocots											
C_4 Grass											
All grass	Northern basalts	16	-12.7	0.7	4.6	1.3	17	-12.1	0.8	5.7	1.6
NADP							7	-11.8	0.8	5.2	1.1
NAD/PCK		16	-12.7	0.7	4.6	1.3	10	-12.3	0.8	6.1	1.9
All grass	Northern granites	37	-12.1	1.6	1.5	0.9	32	-12.0	0.7	3.3	1.7
NADP		18	-11.4	2.0	1.1	0.6	15	-11.8	0.5	2.1	1.0
NAD/PCK		19	-12.8	0.6	1.9	0.9	17	-12.3	0.9	4.3	1.6
All grass	Punda Maria	19	-12.2	0.2	1.9	1.3	20	-12.0	0.9	2.5	1.6
NADP		1	-11.8	0.0	0.8	0.0	6	-10.9	0.4	2.8	2.5
NAD/PCK		18	-12.2	0.2	2.0	1.3	14	-12.5	0.6	2.3	1.0
All grass	Southern basalts	87	-11.2	1.3	1.3	1.4	86	-12.5	0.7	2.7	1.3
NADP		39	-10.9	1.2	1.0	1.3	36	-12.1	0.5	2.1	1.0
NAD/PCK		48	-11.5	1.3	1.6	1.4	50	-12.8	0.6	3.2	1.4
All grass	Southern granites	71	-11.7	1.0	4.0	1.9	60	-12.5	0.7	6.0	2.3
NADP		12	-11.3	1.0	2.7	1.7	10	-12.1	1.0	4.2	2.1
NAD/PCK		59	-11.8	1.0	4.3	1.8	50	-12.6	0.6	6.3	2.2
C_3 Reeds and Sedges		40	-26.5	1.8	5.9	1.6	34	-26.4	1.2	6.8	2.0

n = number of samples
SD = standard deviation

Table 3.2. Mean $\delta^{13}C$, $\delta^{15}N$, and %N from faeces of 19 ungulate species from Kruger National Park (KNP). Comparative data for southern Africa is from Sponheimer *et al.* (2003a), and for East Africa from Cerling *et al.* (2003), based on carbon isotopic composition of hair, bone, and tooth enamel. Data from field studies are from Gagnon and Chew (2000) for bovids, and Owen-Smith (1988) and Skinner and Smithers (1990) for other taxa.

		KNP faeces						%C_4 in Diet			
		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		%N					
Taxon	***n***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**KNP**	**S.Afr.**	**E.Afr.**	**Field studies**
Perissodactyla											
Equidae											
Equus burchellii	166	-14.1	1.0	4.3	1.5	1.2	0.4	92			90
Rhinocerotidae											
Diceros bicornis	9	-26.1	0.4	4.6	0.8	1.3	0.3	9			5
Ceratotherium simum	29	-14.0	1.4	4.2	1.3	1.3	0.3	90			98
Artiodactyla											
Suiformes											
Hippopotamus amphibius	26	-13.8	1.6	4.3	2.1	1.2	0.3	91			95
Phacochoerus africanus	62	-14.2	1.2	4.7	1.6	1.7	0.4	91			95
Ruminantia											
Giraffidae											
Giraffa camelopardalis	177	-26.4	0.8	4.8	1.7	2.6	0.5	5			0
Bovidae											
Bovini											
Syncerus caffer	319	-14.7	1.1	4.7	1.8	1.5	0.3	88	88	100	78
Tragelaphini											
Taurotragus oryx	5	-26.6	1.0	3.0	0.6	2.6	0.8	3	8	18	50
Tragelaphus strepsiceros	145	-26.4	0.8	5.2	1.2	2.8	0.4	7	4	4	15
Tragelaphus angasii	102	-24.2	3.7	5.6	1.6	2.6	0.6	23	33		20
Tragelaphus scriptus	7	-26.3	1.6	6.0	0.3	2.5	0.3	9	0	0	10
Alcelaphini											
Connochaetes taurinus	151	-14.5	1.1	5.2	2.2	1.4	0.4	90	90	100	88
Damaliscus lunatus	55	-13.8	1.0	4.1	1.2	1.5	0.4	94	100	100	95
Reduncini											
Kobus ellipsiprymnus	95	-14.4	1.2	5.4	2.5	1.5	0.4	90	100	92	84
Hippotragini											
Hippotragus equinus	86	-13.5	1.1	3.6	0.9	1.1	0.3	96	91	100	85
Hippotragus niger	122	-13.0	0.8	3.2	1.2	1.0	0.2	98	100	94	85
Aepycerotini											
Aepyceros melampus	606	-18.8	2.9	5.8	2.1	2.0	0.5	60	51	52	45
Neotragini											
Raphicerus campestris	26	-26.4	2.1	4.4	1.3	2.3	0.5	7	10	18	34
Cephalophini											
Sylvicapra grimmia	14	-25.1	2.9	4.9	1.4	2.6	0.5	15	2	0	12

n = number of samples
SD = standard deviation

Table 3.3. Summary of the multiple linear regression model for environmental and ecological predictors of faecal δ^{15}N, after removal of residuals outliers (ϙ-level ± 3).

Predictor of d^{15}N	**Beta**	**Partial correlation**	**r^2**	***P***
%N	0.48	0.33	0.56	<0.0001
Rainfall	0.13	0.09	0.59	<0.0001
%C_4 in Diet	0.11	0.12	0.08	<0.0001
d^{15}N Diet/plants	0.05	0.05	0.06	<0.05
Species	0.01	0.01	0.22	0.74

CHAPTER 4

Nutritional Content of Savanna Plants: Seasonal, Landscape, and Patch Scale Variations in Food Quality for Herbivores

The objective of this chapter is to assess the nutritional value of different plant foods available to ungulates foraging across different savanna landscape and microhabitat/patch scales, and throughout the seasonal cycle. I present crude protein (%N) as well as cell wall component (NDF, ADF, and ADL) data for various food groups from semi-arid savannas of the Kruger National Park and Hans Merensky Nature Reserve, South Africa. These habitats include the *Acacia/Combretum*-dominated southern Kruger Park, the open grasslands in the Northern Basalt Plains of Kruger National Park, the *Colophospermum mopane*-dominated woodlands of Hans Merensky Nature Reserve, and the densely wooded far northern Punda Maria region of Kruger Park. I investigate changes in food quality across multiple scales, and discuss how these are likely to influence observed patterns of morphological and behavioural adaptations of ungulates to their diets.

4.1. Introduction

Plant nutritional composition and diet quality are regarded as key drivers of herbivore performance, niche compartmentalization, and evolutionary adaptation. Oral and dental morphology, gut anatomy, digestive physiology, body size, activity time, and social behaviour have all been related to intake and processing of food types differing in cell soluble and/or cell wall content; amongst ungulates these refer principally to differences between browse (dicots) and grass forages (Gwynne and Bell, 1968; Bell, 1970; Hofmann and Stewart, 1972; Jarman, 1974; McNaughton and Georgiadis, 1986; Hofmann, 1989; Janis, 1988; Owen-Smith, 1997; Mysterud, 1998; Brashares *et al.*, 2000). However, the widespread assumption that diet quality increases with increasing cell soluble:cell wall ratios (or protein:fibre ratios), which are higher in browse than in grass, but this may be misleading. Interactions between various structural and molecular components of plants are more important determinants of overall digestibility and nutritional value than *in situ* concentrations of each (e.g. Illius, 1997; Owen-Smith, 1997; Meissner *et al.*, 1999). Hence, different food types such as browse and grass do not necessarily differ in metabolisable energy yields, at least not in easily predictable ways.

From a herbivore's perspective, plants are composed of two basic components: cell solubles and cell wall (Demment and van Soest, 1985; Hofmann, 1989; Robbins, 1993; van Soest, 1994; Meissner *et al.*, 1999). Cell solubles are biomolecules that are freely available for digestion, while the energy bound within structural carbohydrates (cell wall) is only available through more intensive mechanical breakdown and microbial fermentation. Protein is considered the most limiting nutrient, hence plant crude protein content (determined by percent nitrogen) is a common measure of food quality. Some proportion of the nitrogen in plants is, however, unavailable for digestion, in the form of acid detergent insoluble nitrogen (ADIN) bound within the cell wall (van Soest, 1994; Meissner *et al.*, 1999). Plant cell walls comprise three fractions: neutral-detergent fibre (NDF; hemicellulose, cellulose, and lignin), acid-detergent fibre (ADF; cellulose and lignin), and acid-detergent lignin (ADL). These structural carbohydrates vary in their potential to be broken down by microbial fermentation in the digestive tract. Hemicellulose is largely digestible (80 to 100%), cellulose digestibility may vary between ~30 and 80%, while lignin is virtually indigestible (van Soest, 1994; Meissner *et al.*, 1999). Numerous studies have employed assays of %N, NDF, ADF, and ADL to assess diet quality, nutritional stress, and to qualify diet selection within optimal foraging paradigms (Erasmus *et al.*, 1978; Murray, 1993; Owen-Smith, 1994; Meissner *et al.*, 1996; Dörgeloh *et al.*, 1998; Bodenstein *et al.*, 2000; Grant *et al.*, 2000; Molloy and Hart, 2002; Watson and Owen-Smith, 2002).

Browse forages have higher protein:fibre ratios than grass, and are hence commonly considered to be of superior quality (Bell, 1970; Jarman, 1974; Demment and van Soest, 1985; Hofmann, 1989; Gagnon and Chew, 2000). However, the higher lignin and insoluble protein content of dicotyledonous plant leaves may result in browse foods being similar in overall digestibility, or even less so, compared to grasses (Gordon and Illius, 1996; Illius, 1997; Owen-Smith, 1997; Meissner *et al.*, 1999). In addition, higher concentrations of insoluble nitrogen and digestion-inhibiting secondary compounds such as condensed tannins further reduce browse quality (Cooper and Owen-Smith 1985; Robbins *et al.*, 1987a; Hay and van Hoven, 1988; Owen-Smith 1993; van Soest, 1994; Illius, 1997). Fruits are also generally regarded as high quality foods, commonly defining the seemingly high-quality diets of small-bodied concentrate selectors such as duikers (Cephalophini). Yet, fruits often have a low digestibility index, since they may contain as much as 60-70% fibre (Demment and van Soest, 1985; Barton *et al.* 1993; Molloy and Hart, 2002).

Spatio-temporal variations in plant nutritional composition have also been reported. Macronutrients, including crude protein, are associated with underlying geology and rainfall; leaching of soil nutrients in sandy, high rainfall regions ("sourveld") results in lower N content of local vegetation, while clay soils and low rainfall regimes ("sweetveld") support forage of higher nutrient content (Acocks, 1988; Ellery *et* al., 1995; Tainton, 1999). During the dry season, sandy habitats are particularly nutrient-poor, whereas clay-based soils tend to retain nutrients through the seasonal cycle. Fibrous constituents appear to respond to temperature and rainfall patterns. High temperatures generally increase growth rate, fibre content (especially lignin) and hence decrease dry matter digestibility, although in dry conditions growth rates and fibre content decline (Minson, 1990; Ellery *et al.*, 1995; Meissner *et al.*, 1999). Few studies have attempted to document inter- and intra-regional differences intensively, and spatio-temporal comparisons are usually restricted to collation of data from different studies, representing diverse time scales.

Here, I document spatial and seasonal changes in the nutritional content of various food types available to herbivores foraging in savanna landscapes. Results are based on measurement of %N, ADIN (acid detergent insoluble nitrogen), NDF, ADF, and ADL of plants collected at monthly intervals over one year from different habitats within the Kruger Park and Hans Merensky Nature Reserve, South Africa. I compare differences in the nutritional content and forage value of trees and grass across different landscape and habitat scales throughout the seasonal cycle, and make further comparisons with other food groups, including forbs, fruit, and sedges. I discuss the implications of these findings for predictions of ungulate feeding adaptation and ecological diversity between trophic guilds.

4.2. Methods

4.2.1 Materials

The data used for this study are from vegetation samples collected at monthly intervals from June 2004 to May 2005, although the majority of vegetation samples were collected in July 2004 (representing the dry season) and January 2005 (wet season). Circular sampling transects, ~ 10 m in diameter, were established in Kruger National Park on northern and southern granites and basalts, respectively, at Punda Maria, and on Hans Merensky Nature Reserve, to represent a diversity of microhabitat types (Figure 4.1). Samples of tree foliage and grasses (leaves and stems) were collected to represent the most prominent species occurring within each transect. Three to five specimens of each species were obtained per site, and pooled for analysis. Other samples collected for comparison included forbs, fruit, and sedges, obtained at random localities within the study areas when available. All forbs collected were small woody shrublets. Fruits represented both pods and fleshy drupes; in the case of drupes bearing large kernels, such as *Strychnos madagascariensis* and *Sclerocarya birrea*, only the fleshy parts were retained for analysis.

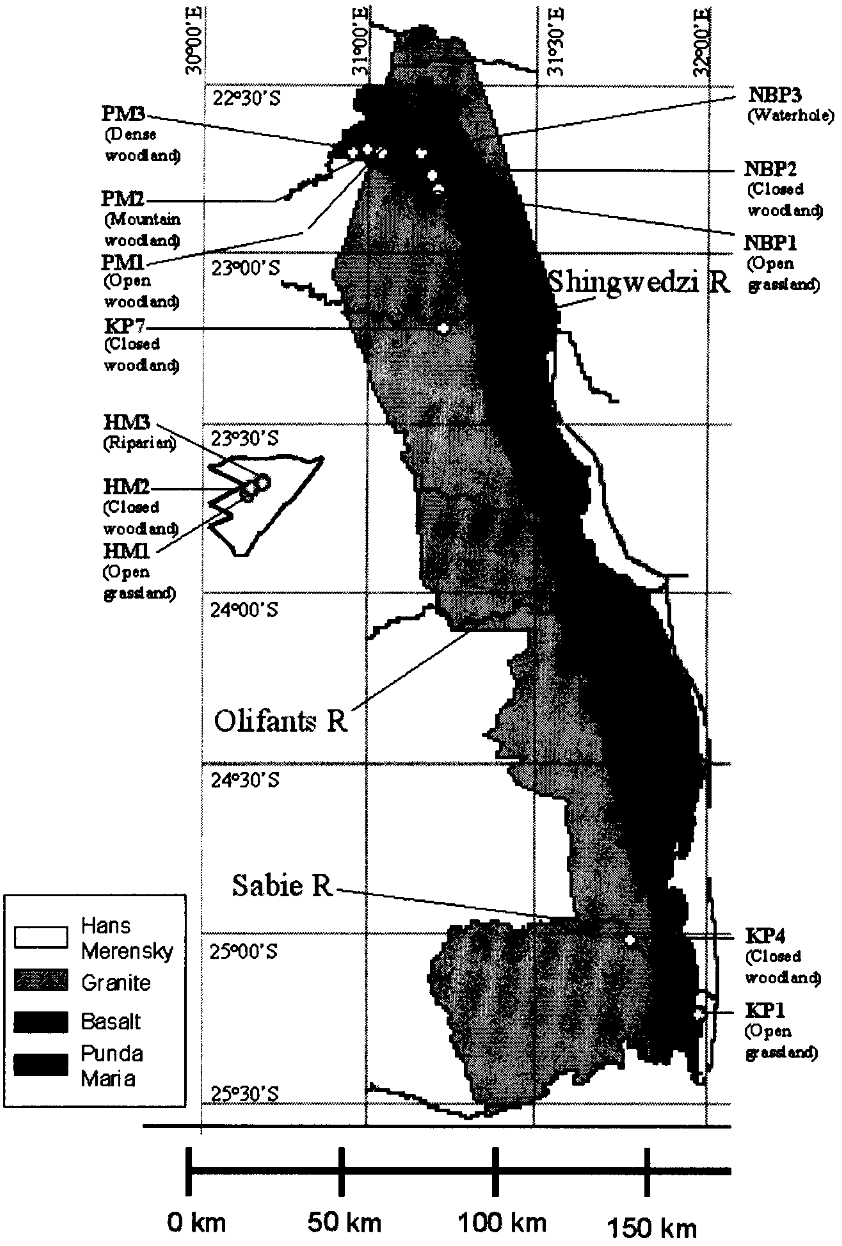

Figure 4.1. Simplified geological map of Kruger National Park and outline of Hans Merensky Nature Reserve, showing the longitudinal granite/basalt division of Kruger, and location and number allocated to vegetation sampling sites (white circles) with visual descriptions of site-type in parentheses.

4.2.2. Analytical Techniques

Plant samples were oven-dried at 60°C for 24 hours, and mill-ground into a homogenous powder through a 1mm sieve. Data for %N is available from stable light isotope ratio mass spectrometry (Chapter 2). Cell wall constituents were determined by exposure to neutral detergent and acid detergent solutions in an ANKOM 220 Fiber Analyzer, yielding the NDF and ADF fractions, respectively (van Soest and Wine, 1967). Lignin (ADL) was determined by exposure of the ADF fraction to 72% (by weight) H_2SO_4. NDF, ADF, and ADL fractions are presented in terms of organic matter (OM), correcting for ash residue following furnace combustion at 525°C.

One of the main difficulties with interpreting these data arises in assessing the interactions between the variables to quantify potential digestibility. Ideally, testing differential digestibilities requires *in vivo* measurements, but controlled-feeding experiments with the variety of diets used here, representing similar spatio-temporal resolutions, are clearly impractical, especially for the diversity of ungulates found in African savannas. Thus, I use several equations obtained from some previous studies, derived from relationships between plant nutritional content and *in vivo* digestibility.

Firstly, I use the common equation for determining cell wall digestibility (CWD) of forage fed to domestic livestock from ADF content:

$$\text{CWD} = 88.9 - (0.779 * \text{ADF}) \quad \text{.......... equation (1)}$$

I also use lignin:NDF ratios as a crude indicator of potential digestibility (e.g. Perez-Barberia *et al.*, 2004); the higher this ratio the less digestible the plant:

$$\text{ADL} / \text{NDF} \quad \text{.......... equation (2)}$$

More accurate determinations of potential cell wall digestibility can be drawn from the regression relationship between plant NDF and ADL content with *in vivo* digestibility. Mould and Robbins (1982) provided the following equations based on digestive capacities of elk (*Cervus elaphus nelsoni*) and white-tailed deer (*Odocoileus virginianus*) fed a variety of browse and grass food types:

$\mathrm{CWD\ (Elk)} = (1.11\mathrm{NDS} - 21.88) + \mathrm{NDF}[(176.92 - 40.50\log_e \mathrm{A})/100]$.....equation (3)

$\mathrm{CWD\ (Deer)} = (1.06\mathrm{NDS} - 18.06) + \mathrm{NDF}[(161.39 - 36.95\log_e \mathrm{A})/100]$...equation (4)

where NDS represents neutral detergent solubles (100-%NDF) and A lignin + cutin (ash).

Equations 1 through 4 only represent the fraction of the plant cell wall that is potentially digestible by herbivores. Hence, I use the term cell wall digestibility (CWD) to define these indices, rather than dry matter digestibility (DMD). Here, DMD is taken to represent the overall nutritional value of plant foods, taking into account CWD as well as protein availability. Robbins *et al.* (1987a, 1987b) provide the following equation for calculating DMD, from regressions based on *in vivo* digestibilities of white-tailed (*O. virginianus*) and mule deer (*O. hemionus*):

$\mathrm{DMD} = (0.9231e^{-0.0451\mathrm{A}} - 0.03\mathrm{B})(\mathrm{NDF}) + [(-16.03 + 1.02\mathrm{NDS}) - 2.8\mathrm{P}]$....equation (5)

where B is biogenic silica content (assumed here to be zero; see also Hanley *et al.*, 1992), and P represents protein availability based on protein content (here %N) and reductions due to the effects of tannins (not measured here, see discussion). Comparing outcomes of the variety of calibrations mentioned are used as a guideline for interpreting potential digestibility of different food types.

Analysis of %N of ADF fractions yielded ADIN for a subset ($n = 29$) of samples, chosen to represent tree foliage and grass for different regions and seasons, as well as fruits, forbs, and sedges. It is unclear using this method whether the measured ADIN represents tannin-bound nitrogen released during oven-drying, or fibre-bound protein. However, these two fractions are functionally similar in that they are both unavailable for digestion, hence the ADIN data here provides a useful guide to quantities of non-dietary proteins in different plant foods.

4.3. Results

4.3.1. Nutritional Composition of Forage

Percent N, NDF, ADF, and ADL varied widely between different tree and grass species (Table 4.1). Percent N of tree foliage varied from 1.4% in *Euclea divinorum* to 3.1% in *Philonoptera violacea.* Grass %N ranged from 0.6% in *Sporobolus africanus* to 1.3% in *Bothriochloa* sp. and *Panicum maximum.* Fibrous components of tree foliage ranged in NDF from 21.32% (*Vitex* sp.) to 59.43% (*P. violoacea*), ADF from 13.24% (*Vitex sp.*) to 40.65% (*P. violacea*), and ADL from 5.68% (*Combretum apiculatum*) to 19.74% (*P. violacea*). Grass ranges were narrower, NDF varying from 60.72% (*Bothriochloa* spp.) to 78.46% (*S. africanus*), ADF from 29.39% (*Bothriochloa* spp.) to 39.02% (*S. africanus*), and ADL from 3.32% (*Panicum coloratum*) to 5.27% (*Bothriochloa* sp.). Data presented here are consistent with available literature. For instance, relatively high %N has previously been reported for *P. violacea* (= *Lonchocarpus capassa*; du Toit, 2003), and %N and fibre data for grasses are generally similar to previously reported values for grass forages in the Timbavati, on the western boundary of Kruger Park (see Meissner *et al.*, 1996; Bodenstein *et al.*, 2000). Species differences, however, were too small to create significant overlap between the nutritional content of tree foliage and grass; tree foliage maintained a higher N and ADL content and lower NDF and ADF than grasses (ANOVA and Tukey's HSD *post hoc* test: $P < 0.0001$ for all variables).

Tree fruits, contrary to general expectations, had similar %N compared with leaves ($P = 0.82$), but higher mean NDF ($P < 0.05$) and ADF ($P < 0.01$) (Table 4.1). Demment and van Soest (1985) suggested that fruit hulls have a higher fibrous component than the fleshy portions. In the current study, fruits enclosed in pods were not consistently more fibrous than fleshy drupes ($P = 0.99$ for %N and NDF, $P = 0.92$ for ADF, $P = 0.83$ for ADL). Notably, *Rhoicissus tomentosa* and *S. madagascariensis*, which are both often eaten by ungulates and other mammals (e.g. Skinner and Smithers, 1990; van Wyk *et al.*, 2000), consist of >50% NDF, and *R. tomentosa* is very highly lignified (25.98% ADL).

Percent N of forbs was similar to that of tree foliage ($P = 0.81$), although there appeared to be a seasonal effect; mean N content of forbs shifted from 1.3 ± 0.3% ($n = 14$) in the dry season to 2.5 ± 0.7% ($n = 17$) in the wet, and consequently had lower %N than tree foliage during the dry season ($P < 0.0001$). Forbs had higher NDF and ADF content than trees ($P < 0.001$), and in this instance seasonal changes did not account for the difference. Sedge %N, NDF, ADF, and ADL values were similar to those for grasses ($P = 0.99$, 0.25, 0.53, and 0.80, respectively).

ADIN for selected representative trees, fruit, grass, forbs, and sedges ($n = 29$) conformed to expectations, with tree leaves (mean = 1.13 ± 0.26, $n = 10$) having considerably higher insoluble nitrogen content than grasses (mean = 0.32 ± 0.13, $n = 13$) (Table 4.2). Forb ADIN was intermediate between that of trees and grass (mean = 0.68 ±

0.39, $n = 2$), as were values for fruit (mean = 0.56 ± 0.15, $n = 2$). Sedge ADIN was as low as that of grass (mean = 0.37 ± 0.04, $n = 2$).

4.3.2. Calibrations of Overall Plant Food Quality

Calculations of cell wall (CWD) and dry matter digestibility (DMD) provide an indication of differences in overall nutritional value of different food groups. For CWD calculated from the relationship between ADF and dry matter digestibility of domestic animals (equation 1), only tree foliage differed significantly from other groups ($P < 0.01$), having the highest CWD due to their relatively low ADF content (Figure 4.2). However, while ADF is a commonly used indicator of forage quality, digestibility indices based on this variable alone can be misleading because ADL (and total lignin) may have a more substantial influence on potential digestibility of plant foods (e.g. Jung *et al.*, 1997; Meissner et al., 1999). The two CWD indices based on plant NDF and ADL content (equations 3 and 4), derived from regressions of Mould and Robbins (1982) for *in vivo* dry matter digestibility of deer elk (*C. e. nelsoni*) and white-tailed deer (*O. virginianus*), respectively, showed that grasses and more especially sedges had far higher potential CWDs than all dicotyledonous plant types ($P < 0.01$). The higher CWD of the monocots is in accord with general expectations, owing to the higher NDF and lower ADL content of these food types (Owen-Smith, 1997; Meissner *et al.*, 1999). Based on these indices, large differences in CWD (> 10%) were also recorded between fruits (both drupes and pods) and leaves, although these differences are not statistically significant ($P > 0.09$ for all cases).

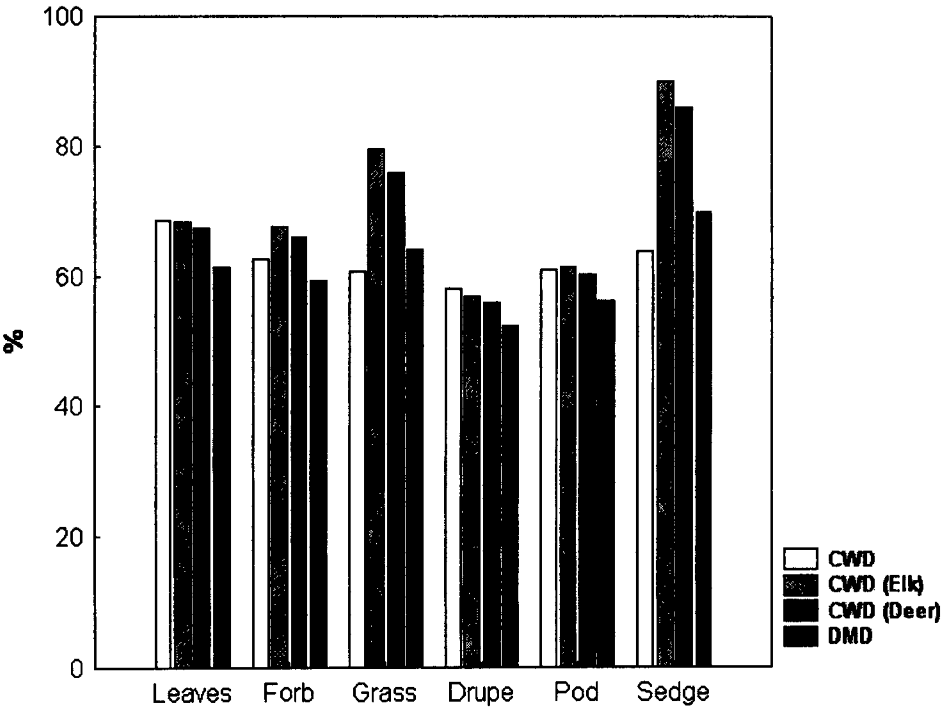

Figure 4.2. Relative nutritional value of different plant food types, comparing results from four calibrations to calculate digestibility (cell wall, CWD, and dry matter, DMD). CWD was determined from ADF content, CWD (Elk) and CWD (Deer) are based on NDF and ADL content using *in vivo* regressions presented in Mould and Robbins (1982), and DMD using regressions presented in Robbins *et al.* (1987a, 1987b) to control for variations in %N.

ADL/NDF ratios show a similar pattern to calculations of CWD, with monocots having a higher fraction of potentially digestible cell wall than dicots ($P < 0.0001$), although in this instance pods had a significantly higher ADL/NDF ratio ($P < 0.01$) than foliage and therefore a lower digestibility. The indices used here therefore appear to be consistent and reliable indicators of CWD. However, these calculations are not necessarily true representations of overall plant food quality, as the equations do not account for variations in cell solubles, most importantly plant crude protein content (%N).

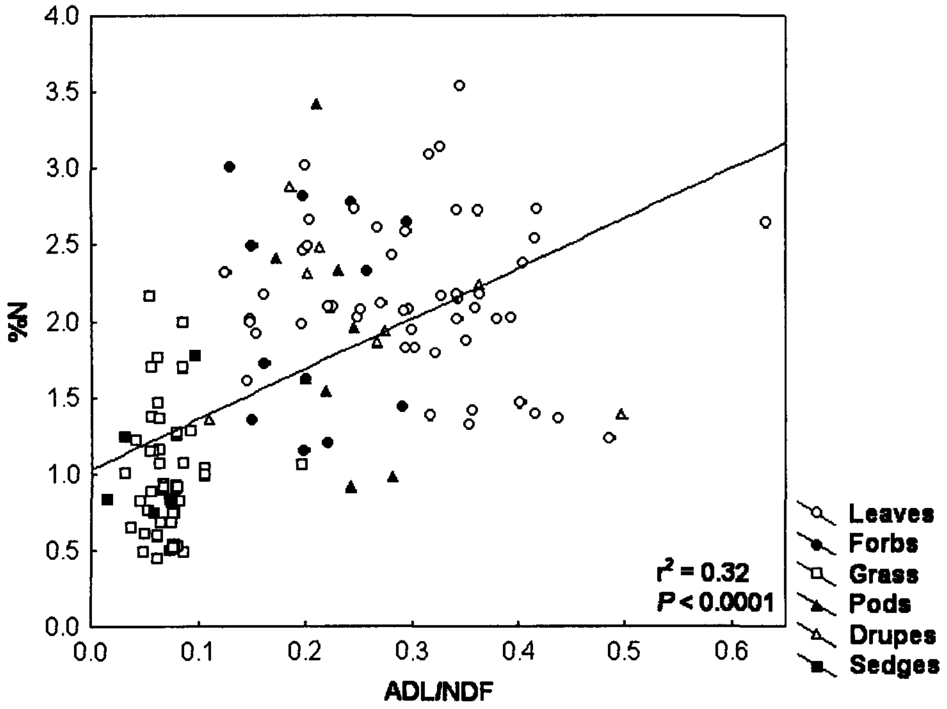

Figure 4.3. Trend of increasing %N across different plant food groups with decreasing cell wall digestibility (expressed as ADL/NDF ratios). Similar correlations were observed for %N compared with all other calibrations of potential cell wall digestibility.

Simple linear regression analysis revealed a trend of increasing %N across plant groups with decreasing fractions of potentially digestible cell wall (ADL/NDF ratios; $r^2 = 0.32$, $P < 0.0001$; Figure 4.3). A similar, though weaker, correlation exists between %N an calculated CWD ($r^2 = 0.12$, $P < 0.0001$ for the equation derived from digestibility achieved by elk; $r^2 = 0.10$, $P < 0.001$ for that of deer). Hence, plant food quality is better expressed by both potential digestibility of the cell wall and %N. The most appropriate equation available for quantifying this interaction is that derived from deer feeding trials, provided in Robbins *et al.* (1987a, 1987b; equation 5). This calibration revealed no difference in the DMD of tree foliage and grass ($P = 0.32$). In other words, despite that tree foliage contains more %N than grasses, the higher fraction of potentially digestible cell wall of the latter means that metabolizable energy yields of the two major plant food groups utilized by ungulates may in fact be similar.

There were, however, substantial inter-specific variations in CWD and DMD. Figure 4.4 shows that foliage of *C. apiculatum* and *Grewia* spp. seem to have higher CWD than other species (lower ADL/NDF ratios), whereas *E. divinorum* and *Dichrostachys cinerea* have the lowest. *Panicum coloratum* had the lowest ADL/NDF ratios of grasses, and *Bothriochloa* spp. the highest. The DMD index showed much smaller inter-specific variations, with leaves of almost all trees and all grasses falling within the range of ~60 to 70%, hence the finding that tree foliage and grass have similar DMD persisted amongst most taxa. Only *P. violacea* amongst trees was outside this range. Estimated DMD of fruits showed no overall difference between fleshy drupes and pods ($P = 0.94$), as was the case for %N and fibre data in general. However, inter-specific variation amongst fruits was larger than that observed for tree foliage and grasses; most had a slightly lower DMD than values derived for foliage and grass (between ~40 and 60%), with *Afzelia quanzensis* having an estimated DMD of only 23.09% owing to the high NDF (85.20%) and ADL (23.92%) content of these pods.

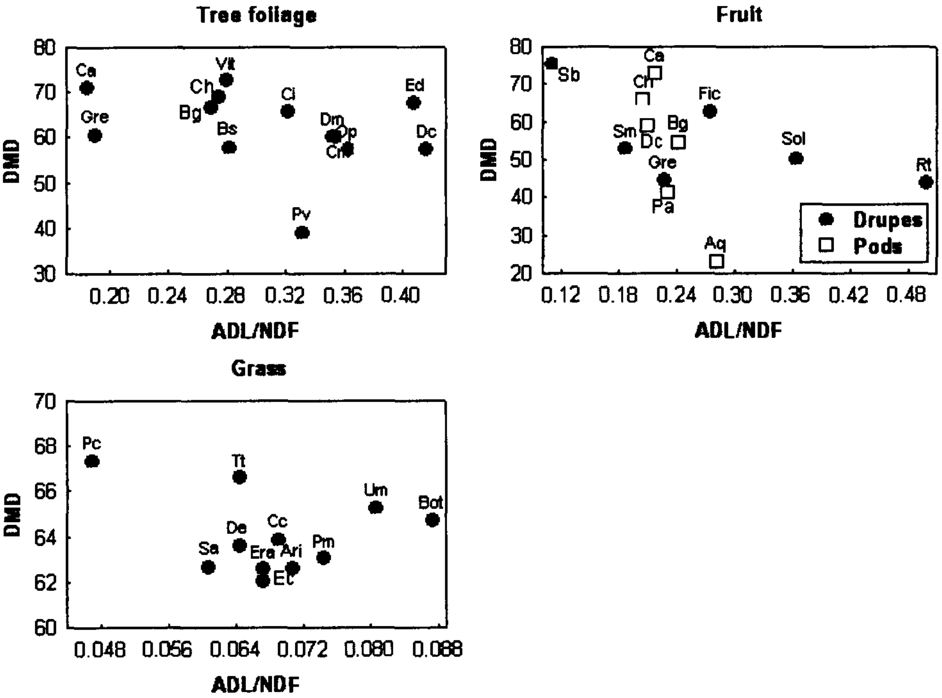

Figure 4.4. Species differences in potential cell wall digestibility (based on ADL/NDF ratios) and dry matter digestibility (DMD, calculated from *in vivo* regressions for deer presented in Robbins *et al.* 1987a, 1987b). Key: *Aq – Afzelia quanzensis, Ari – Aristida spp., Bg – Bauhinia galpini, Bs – Bolusanthus speciosus, Bot – Bothriochloa spp., Ca – Combretum apiculatum, Cc – Cenchrus ciliaris, Ch – C. hereroense, Ci – C. imberbe, Cm – Colophospermum mopane, Dc – Dichrostachys cinerea, De – Digitaria eriantha, Dm – Diospyros mespiliformis, Ec – Eragrostis curvula, Era – Eragrostis spp., Ed – Euclea divinorum, Fi – Ficus sp., Gr – Grewia spp., Op – Ozoroa paniculosa, Pa – Peltophorum africanum, Pc – Panicum coloratum, Pm – Panicum maximum, Pv – Philonoptera violacea, Rt – Rhoicissus tomentosa, Sa – Sporobolus africanus, Sb – Sclerocarya birrea, Sm – Strychnos madagascariensis, Sol – Solanum sp., Tt – Themeda triandra, Um – Urochloa mosambicensis, Vit – Vitex sp.*

4.3.3. Seasonal and Regional Changes

Regional and seasonal differences in plant nutritional content were compared between dry (April to September, principally June) and wet (October to March, principally January) seasons and across the six major landscapes included here (southern and northern granites and basalts, Punda Maria, and Hans Merensky). In general, differences between regions and seasons for the study areas were relatively small or negligible (generally < 1% for %N, and < 10% for NDF, ADF, and ADL). This pattern of low spatio-temporal variation was observed at the species level (Figures 4.5 and 4.6), as well as within broader food groups of tree foliage and forbs (browse) and grass (Figure 4.7). Amongst two tree taxa represented across a variety of environmental conditions (*C. mopane* and *Combretum* spp.), neither showed significant seasonal and regional differences in %N, NDF, ADF, or ADL ($P > 0.10$ in all cases; Figure 4.5). Similarly, two grass species *P. maximum* and *Urochloa mossambicensis* showed no differences across space or between seasons ($P > 0.13$ for all cases; Figure 4.6).

Combining tree foliage into a broader plant food group showed no significant seasonal effect on %N (Figure 4.7; $P = 0.38$), but NDF, ADF, and ADL all increased from the dry to the wet season ($P < 0.05$). Grasses, by contrast, increased in %N from the dry to the wet season ($P < 0.001$) and decreased in NDF and ADF ($P < 0.0001$), but grass ADL remained constant through the seasonal cycle ($P = 0.44$). As a result, potential CWD and DMD (based on all five indices used here) of tree foliage decreased from the dry to wet season ($P < 0.05$) while that of grasses remained constant ($P > 0.34$). Thus, while tree foliage and grass generally differed in calculated CWD (but not in DMD;

Figure 4.2), the difference was only significant in the wet season ($P < 0.05$). Indeed, dry season data even imply lower DMD in tree foliage compared to grass ($P = 0.93$).

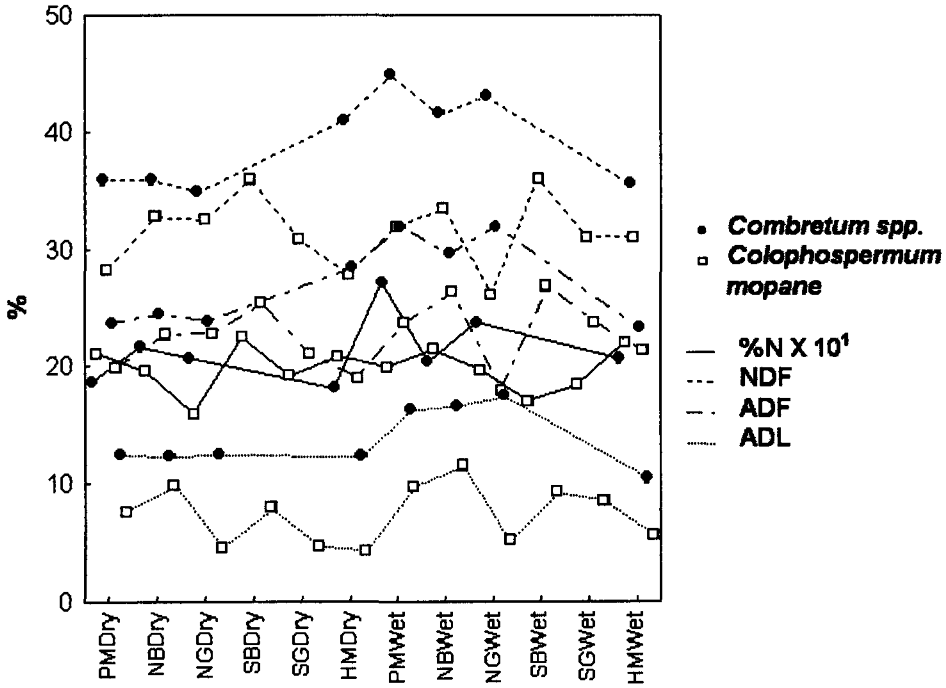

Figure 4.5. Seasonal (dry versus wet) and regional differences in %N, NDF, ADF, and ADL of the foliage of two tree types, *Combretum* spp. and *Colophospermum mopane*. PM = Punda Maria, NB = northern basalts, NG = northern granites, SB = southern basalts, SG = southern granites, HM = Hans Merensky.

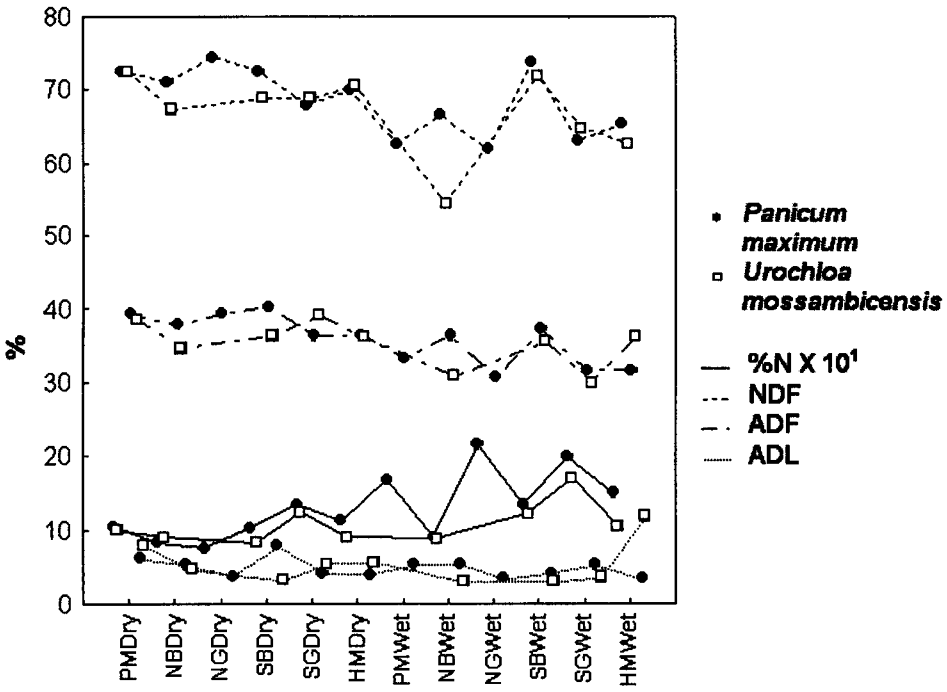

Figure 4.6. Seasonal (dry versus wet) and regional differences in %N, NDF, ADF, and ADL of two grasses, *Panicum maximum* and *Urochloa mosambicensis*. PM = Punda Maria, NB = northern basalts, NG = northern granites, SB = southern basalts, SG = southern granites, HM = Hans Merensky.

There were no differences in foliar %N, NDF, or ADF between the six landscapes ($P > 0.51$), but ADL was significantly higher on northern basalts and lower at Hans Merensky than in other regions ($P < 0.05$). Spatial differences in foliar ADL seemed, however, to be influenced by patch-scale (collection site) differences in tree data, i.e. NBP3 (northern basalt waterhole site) trees had higher and HM3 (Hans Merensky riparian site) lower ADL compared with data for other sites ($P < 0.05$). Accordingly, spatial differences in calculated cell wall and dry matter digestibility appeared to be more

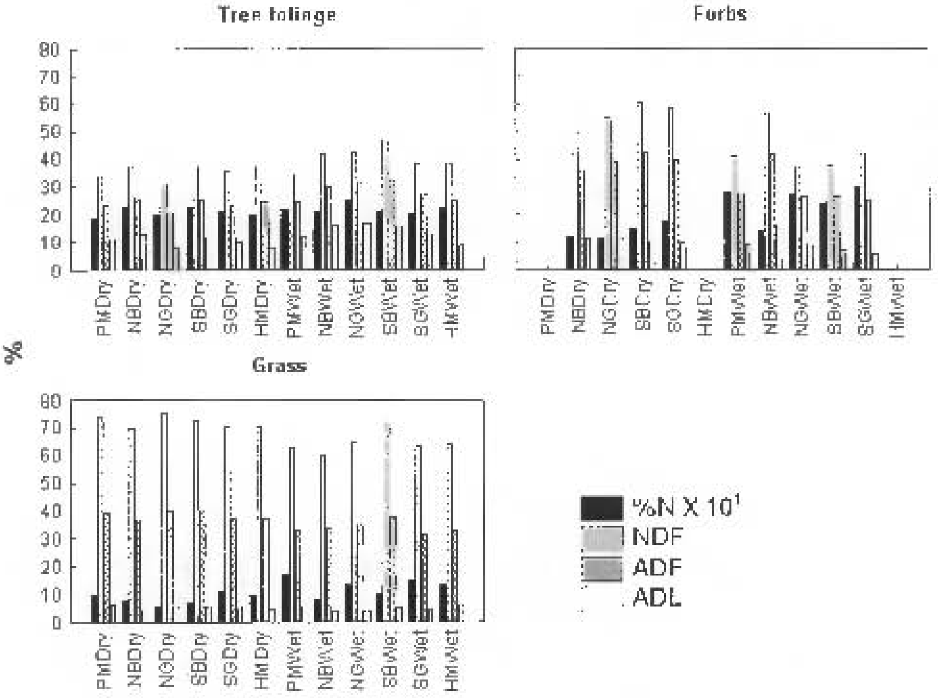

Figure 4.7. Seasonal (dry versus wet) and regional differences in %N (presented x 10^1 for scale), NDF, ADF, and ADL of tree foliage (all species), woody forbs, and grasses (all species). PM = Punda Maria, NB = northern basalts, NG = northern granites, SB = southern basalts, SG = southern granites, HM = Hans Merensky.

important at microhabitat/patch (collection site) scales than across landscapes. Notably, there were no landscape differences in dry matter digestibility based on any of the five indices used here ($P > 0.34$), but all measures of DMD (except CWD based only on variations in ADF, equation 1) suggest that tree leaf quality was lowest at the waterhole site (NBP3, $P < 0.01$) and highest at the riparian site (lower ADL/NDF ratio at HM3, $P < 0.01$). Grass fibres were also similar across all landscapes ($P = 0.12$), but grass %N was lower on northern basalts and higher on southern granites compared with other regions (*P*

< 0.05). Similarly, grass %N was the only variable to differ significantly at the microhabitat scale; grasses at NBP1 (northern basalts open grassland site) had lower %N and grasses from HM3 (riparian) higher %N than grasses from other sites ($P < 0.05$).

4.4. Discussion

The browser/grazer dichotomy is the most widely-accepted classification scheme for ecological differentiation amongst African ruminants, and ungulates in general (Hofmann and Stewart, 1972; Jarman, 1974; McNaughton and Georgiadis, 1986; Hofmann, 1989; Owen-Smith, 1997; du Toit, 2003). However, considerable debate has emerged during the past decade or so as to the reliability of this model (Illius and Gordon, 1992; Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995; Ditchkoff, 2000; Clauss and Lechner-Doll, 2001; Clauss *et al.*, 2002; Perez-Barberia *et al.*, 2004). The primary component of the browser/grazer scheme is that distinctive gut morphologies between browsers and grazers allow them to process soluble-rich browse and high-fibre grass foods in different ways in order to obtain sufficient energy yields from the diet (Hofmann and Stewart, 1972; Hofmann, 1989).

Data presented in this chapter show that the potential digestibility of plant cell walls is higher amongst all grass species compared with browse species, due mainly to the higher NDF and lower ADL of the former. Although the difference was dampened during the wet season, due to an increase in browse (especially tree foliage) and decrease in grass CWD and DMD, this may be because of the unusually low rainfall of the study period leading to deviant environmental conditions especially during the dry season. Indeed, other studies have shown that tree nutritional quality remains relatively static through the seasonal cycle, whereas grass quality decreases significantly during the dry season (Owen-Smith, 1997). However, the same pattern occurred for southern granite plants, an area which received over 700 mm of rain during the study period. Most authors generally agree that the higher lignin content of woody plants leads to decreased digestibility of browse compared with grass (Owen-Smith, 1997; Illius, 1997; Meissner *et al.*, 1999). The question this raises is whether grazers are indeed adapted to exploit the higher fibre-digestion potential of a grass diet, or whether differential fibre-digestion (if any) is achieved based on the nutritional content of the food alone?

Several studies have argued for similarities in the digestive efficiency of browsers and grazers (Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995; Illius, 1997). Gordon and Illius (1996) go so far as to state that the nutritional composition of a herbivore's diet more heavily influences performance than any morpho-physiological adaptation. In other words, the higher fibre digestion efficiency expected and sometimes reported for grazers is perhaps due only to the higher fraction of potentially digestible cell wall in their food. One study of morphological variations across ruminant digestive tracts in fact revealed no relationship between gut anatomy and feeding style after body size and phylogenetic affiliations were controlled for (Perez-Barberia *et al.*, 2001).

Perez-Barberia *et al.* (2004), by contrast, reported increasing NDF digestibilities (NDFD) across ungulate species with increasing levels of grass in the natural diet, even after the effects of diet quality (lignin:NDF ratios) and body size were accounted for. However, these authors acknowledged some drawbacks to their study, including reliance on published data for species' NDFD and natural levels of grass intake without accounting for spatio-temporal variations in grass intake *and* NDFD. For example, many ungulates show dietary switches throughout the seasonal cycle, and across different habitats. Further, animals may also achieve different digestibilities at different times of the year (e.g. Meissner *et al.*, 1996; Bodenstein *et al.*, 2000). Others have argued that selective retention of larger particles in the rumen of grazers compared with browsers allows them to achieve higher fibre-digestibilities (Ditchkoff, 2000; Clauss and Lechner-Doll, 2001; Clauss *et al.*, 2002). Browsers have been proposed to elicit enhanced ruminal bypass of larger particles to facilitate rapid availability and digestion of cell solubles, although these studies did not control for differences in particle size that may exist between the two food types.

More cannot be said of this debate here, although it may be telling that these data show no differences in estimated DMD (and hence metabolizable energy yields) of browse and grass foods. If this is the case, then we can expect considerable evolutionary investment in gut morphologies that are specific to either browsing or grazing to be unlikely. Nonetheless, this finding is based only on *in vivo* digestibility regressions obtained for two species of deer (Robbins *et al.*, 1987a, 1987b). Although Hanley *et al.* (1992) tested these regressions against *in vivo* trials for a wide range of forages, and found strong similarities between *in vivo* and predicted DMD, it is possible that the relationship between actual and predicted DMDs may vary in taxa other than deer, especially amongst African non-ruminants and bovids. More data on digestive capacities for a wide range of African ungulates, utilizing a variety of diets, are required before we can accurately test differential metabolizable energy yields of browse and grass.

Another constraint to this study is the lack of data for variations in plant secondary compound content. While a number of studies have shown that some grasses do contain tannins, as well as other secondary compounds including cyanogens and flavonoids that reduce forage quality (e.g. Georgiadis and McNaughton, 1988; du Toit *et al.*, 1991; Chesselet *et al.*, 1992; Ndakidemi and Dakora, 2003), these generally occur in larger proportions in woody plants. Hofmann (1989) proposed that a major difference between grazers and browsers is that the latter have relatively larger parotid glands and hence salivary flow, a possible adaptation to neutralize the toxic effects of secondary compounds in the diet. By contrast, Robbins *et al.* (1995) observed that kudu *Tragelaphus strepsiceros*, a model browser species amongst savanna ungulates, do not have particularly enlarged parotid glands. Clauss and Lechner-Doll (2001) contend that the absence of this adaptation within only a single browser species is insufficient to refute Hofmann's (1989) proposal (they also noted that kudu are the only browser documented to have experienced mortalities due to a tannin-rich diet). Whatever the case, compounds such as tannins are expected to reduce protein availability and, ultimately, DMD (Robbins *et al.*, 1987a; Hanley *et al.*, 1992). Indeed, whereas the regressions presented in Mould and Robbins (1982) were for animals fed a wide range of diets, including browse and grass foods, the calculations from Robbins *et al.* (1987a, 1987b) were derived from animals fed tannin-rich diets. Thus, while calculations for CWD used here reliably predict differential potential digestibilities for a variety of food types, calculations of DMD likely overestimate the nutritional quality of browse foods compared to grass. The implication here is that metabolizable energy yields may in fact be lower in browse than in grass foods (see also Illius 1997).

Another result of interest is that fruits (both fleshy drupes and pods), so often deemed to provide high quality foods to herbivores, often have very high NDF and ADL content, and hence likely a lower digestibility than browse and grass. Obviously, the data presented here excludes non-structural carbohydrates (such as sugars) in fruits that may increase overall value of the food. Nevertheless, many fruits consist of a high proportion of structural cell wall, especially the NDF fraction, which can be as high as 70% (or 80% in the case of *A. quanzensis* pods). Not surprisingly, predominantly frugivorous duikers *Cephalophus* spp. appear to have unusually high retention times and achieve high NDF digestibilities given their small body size (Wenninger and Shipley, 2000; Shipley and Felicetti, 2002; Perez-Barberia *et al.*, 2004; see also Sponheimer *et al.*, 2003a).

While seasonal comparisons may have been confounded by the extremely low rainfall for the study period, it remains that regional differences were relatively small. This result is somewhat surprising, since the data represent plants from a variety of landscapes, differing in geological substrate, rainfall (which was not unusually low in all regions, i.e. southern granites and southern basalts with > 700 and 400 mm rain, respectively), temperature, and vegetational composition, all factors that might be expected to influence plant growth rates and nutritional composition (e.g. Minson, 1990; Meissner *et al.*, 1999). The lack of major landscape differences could not be ascribed to different species compositions within each region. Amongst grasses, species compositions collected from each landscape were quite different. For tree foliage, ADL was highest on the northern basalts, and lowest at Hans Merensky, but these regions shared common tree species, especially samples for *C. mopane*, whereas this species is absent from sites with intermediate foliar ADL such in the southern parts of Kruger Park.

Differences in plant food quality, especially amongst woody plants, did however appear to be more pronounced at the microhabitat/patch scale. For example, lower CWD and DMD of foliage occurred at the waterhole site on the northern basalts whereas trees from the riparian site on Hans Merensky had the highest potential digestibility. Unfortunately, these microhabitats are each only represented by a single site, thus these data cannot confirm whether the same pattern would persist for similar habitats in other regions. Nonetheless, the fact that spatial differences in browse quality showed a patch-scale distribution may be particularly relevant to future studies of herbivory in the savanna. It is at this scale that optimal foraging models based on field studies (especially amongst browsing kudu) predict that ungulates select foods and exhibit dietary variation (Owen-Smith and Novellie, 1982; Owen-Smith and Cooper, 1989). Hence, patch-scale differences in plant species composition are likely to have an even greater impact for ungulate nutritional ecology than inter-habitat or inter-seasonal changes.

4.5. Conclusion

Data presented here provide substantial evidence for variations in diet quality that can be expected for ungulates feeding on different food types, at different spatial and temporal scales. The implications for feeding ecology imply that further tests of dietary variations in ungulates are necessary to fully understand morpho-physiological and behavioural differences across taxa. What is evident is that traditional assumptions about food quality can be deceptive, such as conceptions that tree foliage and fruits offer greater nutritional value than grasses. On the basis of interactions between protein content and various fibrous components, overall nutritional differences between different food types are small or non-existent. Feeding preferences of ungulates need to be assessed in terms of overall nutritive value of the diet, rather than simplified notions of protein:fibre ratios of foods consumed.

Table 4.1. Species, growth form, and anatomic differences in crude protein (%N) and cell wall constituents of plants from Kruger National Park and the Hans Merensky Nature Reserve.

			%N		%NDF		%ADF		%ADL	
Food group	**Species**	***n***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Tree foliage	*Bauhinia galpini*	2	2.3	0.7	32.59	3.81	23.19	3.63	8.84	2.17
	Bolusanthus speciosus	2	2.6	0.0	42.18	13.97	26.54	7.53	11.99	4.75
	Colophospermum mopane	25	2.1	0.4	38.64	5.04	26.79	4.46	13.84	3.81
	Combretum apiculatum	6	2.0	0.3	30.24	3.03	21.49	3.09	5.68	2.76
	Combretum hereroense	6	2.0	0.1	29.79	3.32	21.32	3.02	8.24	2.62
	Combretum imberbe	2	2.0	0.1	33.07	0.40	24.53	2.51	10.67	1.13
	Dichrostachys cinerea	2	2.6	0.1	37.85	0.50	26.74	0.71	15.80	0.25
	Diospyros mespiliformis	2	2.1	0.1	38.43	1.05	28.76	1.28	13.55	0.92
	Euclea divinorum	6	1.4	0.1	30.03	1.68	22.16	1.16	12.24	1.13
	Grewia sp.	8	2.4	0.5	46.32	4.55	28.25	3.78	8.88	1.98
	Ozoroa paniculosa	4	2.0	0.5	40.76	12.26	28.92	13.76	16.17	13.43
	Philonoptera violacea	4	3.1	0.3	59.43	6.66	40.65	4.45	19.74	2.58
	Vitex sp.	1	2.4		21.32		13.24		5.96	
	Average	**70**	**2.1**	**0.5**	**38.10**	**9.06**	**26.24**	**6.45**	**12.07**	**5.30**
Fruit (pods)	*Afzelia quanzensis*	1	1.0		85.20		65.46		23.92	
	Bauhinia galpini	1	0.9		55.73		39.83		13.47	
	Combretum apiculatum	1	1.5		27.72		20.79		6.06	
	Combretum hereroense	3	2.0	0.4	38.96	3.95	27.94	2.15	7.92	0.61
	Dichrostachys cinerea	1	3.4		43.16		27.60		9.11	
	Peltophorum africanum	1	2.3		67.88		50.47		15.61	
	Average	**8**	**1.9**	**0.8**	**49.57**	**19.01**	**36.00**	**15.11**	**11.49**	**5.97**
Fruit (drupes)	*Ficus sp.*	1	1.9		39.83		29.37		10.96	
	Grewia sp.	3	2.2	0.3	64.94	10.17	48.70	7.26	14.55	0.73
	Rhoicissus tomentosa	1	1.4		52.16		44.05		25.98	
	Sclerocarya birrea	1	1.3		29.44		23.26		3.23	
	Solanum sp.	1	2.2		48.37		39.94		17.56	
	Strychnos madagascariensis	1	2.9		56.40		33.99		10.53	
	Average	**8**	**2.0**	**0.5**	**52.63**	**14.12**	**39.59**	**10.53**	**13.99**	**6.50**
Woody forbs	*Solanum* sp.	3	2.3	0.6	52.36	8.45	37.49	6.38	10.89	2.16
	Unidentified spp.	28	2.0	0.9	47.94	12.92	33.39	9.71	9.75	3.96
	Average	**31**	**2.0**	**0.8**	**48.37**	**12.52**	**33.78**	**9.44**	**9.86**	**3.82**
Grasses	*Aristida sp.*	3	0.7	0.2	74.13	1.04	41.30	3.08	5.23	0.34
	Bothriochloa sp.	2	1.3	0.0	60.72	7.11	29.39	3.47	5.27	0.06
	Cenchrus ciliaris	5	0.7	0.1	71.62	3.11	39.31	2.90	4.97	1.28
	Digitaria eriantha	2	0.7	0.3	74.24	0.83	40.73	2.83	4.78	1.69
	Eragrostis curvula	4	0.8	0.4	74.92	7.09	40.91	4.75	5.12	1.57
	Eragrostis sp.	3	1.0	0.2	73.48	3.01	38.35	1.34	4.95	0.53
	Panicum coloratum	2	1.0	0.0	69.42	5.02	34.47	1.70	3.32	1.71
	Panicum maximum	24	1.3	0.5	68.10	5.36	35.95	3.86	5.07	1.49
	Sporobolus africanus	1	0.6		78.46		39.02		4.78	
	Themeda triandra	8	0.8	0.3	66.18	6.62	35.42	3.56	4.30	1.28
	Urochloa mossambicensis	17	1.0	0.3	63.97	8.65	34.26	3.96	5.09	3.49
	Average	**71**	**1.0**	**0.4**	**68.16**	**7.08**	**36.28**	**4.27**	**4.93**	**2.03**
Sedges	*Cyperus sp.*	4	0.8	0.0	65.94	5.10	35.85	4.83	3.04	2.54
	Unidentified sp. 1	2	1.2	0.0	66.94	3.24	31.18	1.19	2.10	0.09
	Unidentified sp. 2	2	1.8	1.2	37.06	9.66	18.98	6.19	3.53	0.83
	Unidentified sp. 3	2	0.7	0.3	68.01	3.67	38.93	1.41	4.05	1.14
	All sedges	**10**	**1.1**	**0.6**	**60.78**	**13.36**	**32.16**	**8.21**	**3.15**	**1.68**

n = number of samples; SD = standard deviation

Table 4.2. Regional and seasonal comparison of crude protein (%N) and insoluble protein (ADIN) content of semi-arid savanna plant growth forms and plant parts. NB = Northern Basalt Plains, PM = Punda Maria, HM = Hans Merensky Nature Reserve. Sample numbers (*n*) are displayed in the format *x*, *y*, where *x* represents the total number of specimens collected, and *y* the number of pooled samples analyzed.

				%N		ADIN		
Growth form	**Season**	**Region**	***n***	**Mean**	**SD**	**Mean**	**SD**	**AvailN**
Tree leaves	Dry	NBP	5, 1	1.71		1.07		1.56
		PM	5, 2	2.07	0.07	1.19	0.33	1.92
		HM	6, 2	2.26	0.37	1.17	0.17	2.15
		All regions	*16, 5*	*2.07*	*0.29*	*1.16*	*0.19*	*1.94*
	Wet	NBP	5, 1	1.90		1.51		1.67
		PM	5, 2	2.10	0.11	1.21	0.20	1.95
		HM	6, 2	2.33	0.35	0.79	0.07	2.24
		All regions	*16, 5*	*2.15*	*0.26*	*1.10*	*0.33*	*2.01*
	All tree leaves		**32, 10**	**2.11**	**0.27**	**1.13**	**0.26**	**1.98**
Tree fruits	Wet	PM	2, 2	1.63	0.41	0.56	0.15	1.54
Forb leaves / stems	Dry	NBP	1, 1	0.93		0.41		0.77
	Wet	PM	1, 1	2.32		0.95		2.21
	All forbs		**2, 2**	**1.62**	**0.99**	**0.68**	**0.39**	**1.49**
Grass	Dry	NBP	15,3	0.56	0.11	0.20	0.04	0.42
		PM	10, 2	0.94	0.50	0.29	0.19	0.83
		HM	10, 2	0.98	0.52	0.36	0.00	0.82
		All regions	*35, 7*	*0.79*	*0.37*	*0.27*	*0.11*	*0.65*
	Wet	NBP	15, 3	0.70	0.17	0.34	0.14	0.54
		PM	5, 1	1.73		0.43		1.65
		HM	10, 2	1.76	0.41	0.41	0.23	1.69
		All regions	*30, 6*	*1.23*	*0.61*	*0.38*	*0.14*	*1.11*
	All grasses		**65, 13**	**0.99**	**0.53**	**0.32**	**0.13**	**0.86**
C_3 sedge	Wet	NBP	1, 1	0.83		0.34		0.69
C_4 sedge		PM	1, 1	0.92		0.39		0.86
	All sedges		**2, 2**	**0.88**	**0.06**	**0.37**	**0.04**	**0.78**

SD = standard deviation

CHAPTER 5

Feeding Responses of Ungulates to Environmental Changes Through the Seasonal Cycle

In this chapter, I examine high-resolution dietary variations of ungulates in the context of environmental changes through the seasonal cycle, using stable isotope composition of faeces of 11 ungulate species from three semi-arid savannas in the northeast of South Africa. I test whether diet shifts and the mechanisms underlying them are similar for all species with similar diets, and whether constraints to dietary variation persist across various spatial and temporal scales. I am primarily interested in dietary changes from month-to-month, and how these correspond with environmental variables including rainfall and changes in the nutritional composition of available vegetation.

5.1. Introduction

The evolution and ecological diversity of ungulate herbivores in the African savanna biome is functionally related to environmental heterogeneity at various patch, habitat and temporal scales (Vrba, 1992; du Toit and Cumming, 1999; du Toit, 2003). Dietary variation across these scales defines the complexity of plant-mammal interactions, and is thus crucial for understanding patterns of population and community ecology (Illius and O'Connor, 2000; du Toit, 2003). From a conservation perspective, spatio-temporal variations in diet can reveal local trends about impacts of herbivory on vegetation (*cf.* Caughley, 1976; Cumming *et al.*, 1997), and coupled with long-term records, also provide insight into animal responses to global change (Brown *et al.*, 2001).

Explanations for ecological variation amongst ungulates stem primarily from the hypothesis that rainfall and primary productivity control population demographics (Coe *et al.*, 1976). Hence, ungulate biomass and feeding preferences are often assumed to be directly proportional to vegetational composition, i.e. proportions of woody plants:grass in the landscape, in both modern (Scholes *et al.*, 2003) and palaeoecological contexts (Vrba, 1974, 1975; Kappelman *et al.*, 1997; Bobe *et al.*, 2002). While this may be true in a very broad sense, there are numerous instances in which browser and grazer densities do not reflect corresponding variations in vegetational composition of landscapes (e.g. Hirst, 1975). Optimal foraging models provide an alternative approach, taking into account food availability as well as other factors that regulate food use, such as traveling distance, encounter rate, time-risk/benefit assessment, plant palatability and nutritional value (Owen-Smith and Novellie, 1982). Constraints to optimal foraging appear to be elastic (Owen-Smith, 1994), thus a univariate mechanism for dietary variation (e.g. primary production) is unlikely to persist across multiple taxonomic, spatial, and temporal scales.

Optimal foraging has been studied extensively in kudu *Tragelaphus strepsiceros* (Owen-Smith, 1979, 1994; Owen-Smith and Cooper, 1989), and less so in eland *Taurotragus oryx* (Watson and Owen-Smith, 2000, 2002) and impala *Aepyceros melampus* (Wronski, 2002). For the majority of African ungulates, dietary variations and the mechanisms that underlie them are poorly-documented, because the time-consuming nature of traditional approaches to diet (i.e. field observations, and analysis of faeces and gut content) imposes considerable restrictions to the scale of information that can be obtained. Although a number of studies have yielded invaluable insights into dietary changes throughout the seasonal cycle (e.g. Dunham, 1980; Owen-Smith and Cooper, 1989; du Toit, 1993; Bodenstein *et al.*, 2000), they are limited in the number of taxa observed simultaneously. These studies, isolated in space and time, are seldom comparable, since results may differ because of the scales of variation that cannot be addressed (Owen-Smith, 1988).

Here, I use stable carbon and nitrogen isotope data from ungulate faeces collected at monthly intervals over one year to test dietary flexibility in response to changes in the environment throughout the seasonal cycle. Changes in diet are matched with monthly rainfall and shifts in the nutritional content of available vegetation (%N and cell wall constituents) over the same time period (data presented in Chapter 4).

5.2. Methods

The study was carried out in the three savanna landscapes of South Africa, i.e. the open grassland Northern Basalt Plains (NBP) and the densely wooded Punda Maria (PM) regions of the Kruger National Park, and the woodland savanna of Hans Merensky Nature Reserve (HM). I use data from and fresh faeces collected on a monthly basis from June 2004 to May 2005. Whereas vegetation baseline data used in Chapter 3 were based on wet and dry season data for Kruger Park from Codron, J. *et al.* (2005), here I present new data for plant isotope compositions at the monthly scale. These data are incorporated as endpoint values into mixing models for dual- (C_3/C_4) and multiple-source (leaves, fruit/flowers, NADP-ME, NAD/PCK grasses, sedges) intake, according to procedures described in Chapter 3, repeating each model per animal species per month.

Collection of plants followed the protocol described in Chapter 4, except that samples were not pooled for stable isotope analysis. Faecal collections were focused on species for which samples were likely to be readily available during each field trip, with the aim of obtaining 10 samples for each species in each region per month. Targeted species on the NBP were Burchell's zebra *Equus burchellii*, giraffe *Giraffa camelopardalis*, buffalo *Syncerus caffer*, blue wildebeest *Connochaetes taurinus*, tsessebe *Damaliscus lunatus*, roan antelope *Hippotragus equinus*, and impala. The majority of roan antelope and tsessebe samples were collected by Kruger Park ground staff. Warthog *Phacochoerus africanus* and waterbuck *Kobus ellipsiprymnus* faeces were sampled continually for only six months (December 2004 to May 2005), prior to which sightings of these species had been too scarce for regular collections to be made. Nevertheless, the time period represented here is sufficient for meaningful interpretations of seasonality. At PM, faeces of buffalo, kudu, nyala *Tragelaphus angasii*, and impala were collected. Three species (giraffe, blue wildebeest, and impala) representing a browser, grazer, and mixed-feeder, respectively, were sampled at HM. Samples were analyzed for stable $\delta^{13}C$, $\delta^{15}N$, and %N following the procedure for stable light isotope ratio mass spectrometry described in Chapter 3.

For the purpose of this study, it is convenient to separate species into browsing, grazing, and mixed-feeding groups, despite the likelihood that feeding style is more continuous than generally accepted (Perez-Barberia *et al.*, 2004; Chapter 3). This procedure was also necessary to test the reliability of separating ungulates into these trophic guilds. Dietary categorizations followed those widely disseminated in the literature (e.g. Hofmann and Stewart, 1972; Skinner and Smithers, 1990; Gagnon and Chew, 2000). Statistical hypothesis testing was carried out using analysis of variance (ANOVA) and Tukey's *post hoc* test to compare differences between means (P-level 0.05). For statistical comparison across the seasons, data were categorized as either dry (April to September) or wet (October to March). I used multiple linear regression models (MLRMs) of log-transformed data (in order to normalize distributions) to test relationships between diet (%C_4-intake) and potential ecological correlates (monthly changes in rainfall and nutritional composition of available vegetation; data presented in Chapter 4).

5.3. Results

5.3.1. Diet Shifts through the Seasonal Cycle

Stable isotope data for vegetation collected from the NBP, PM, and HM, used here as isotopic baselines for available vegetation within these habitats, are presented in Table 5.1. Data for faeces are presented in Table 5.2, showing the clear distinction between faecal $\delta^{13}C$ of C_3-browsers (mean = -26.3 ± 0.7‰, $n = 322$), C_4-grazers (mean = -14.6 ± 1.0‰, $n = 720$), and mixed C_3/C_4-feeders (mean = -19.4 ± 3.7‰, $n = 462$) ($F_{2,1501} = 3272.319$, $P < 0.0001$). By contrast, faecal $\delta^{15}N$ of browsers (mean = 4.9 ± 1.5‰) and grazers (mean = 4.7 ± 1.7‰) is similar ($P = 0.46$), but mixed-feeders have slightly higher mean $\delta^{15}N$ values (mean = 5.2 ± 1.9‰; $F = 13.405$, $P < 0.01$). These patterns parallel current and previously published data for Kruger Park and other savanna plants, in that while $\delta^{13}C$ values are bimodally distributed amongst C_3 and C_4 photosynthesizing groups,

$\delta^{15}N$ values do not always differ between the two groups (Vogel *et al.*, 1978; Handley *et al.*, 1994; Cerling and Harris, 1999; Codron, J. *et al.*, 2005).

Regional and seasonal (dry-to-wet) differences in faecal $\delta^{13}C$ and $\delta^{15}N$ are significant for browsers ($\delta^{13}C$: $F_{5,316} = 11.659$, $P < 0.0001$; $\delta^{15}N$: $F = 7.249$, $P < 0.0001$), grazers ($\delta^{13}C$: $F_{5,714} = 8.141$, $P < 0.0001$; $\delta^{15}N$: $F = 17.532$, $P < 0.0001$), and mixed-feeders ($\delta^{13}C$: $F_{5,456} = 13.409$, $P < 0.0001$; $\delta^{15}N$: $F = 29.062$, $P < 0.0001$). The relationship between isotopic signatures of animals and the actual diet may be obscured by more subtle shifts in the isotopic composition of plants through space and time (e.g. van Klinken *et al.*, 1994; Heaton, 1999). Although variations in $\delta^{13}C$ of plants from these savanna regions are relatively small (~1.0 to 1.5‰; Table 5.1), plant $\delta^{15}N$ is far more variable, differing by up to 3‰ or more across different regions and seasons (see also Codron, J. *et al.*, 2005). The isotope-source mixing models used here control for variations in the isotopic composition of available vegetation, and I consider the implications of variation amongst plants for interpreting these results.

Corresponding shifts in plant and faecal $\delta^{13}C$, and the effects these have on calculations of dietary %C_4, are displayed in Figures 5.1 to 5.3. Monthly changes in faecal $\delta^{13}C$ generally mirror changes in $\delta^{13}C$ of available vegetation, especially amongst trees/browsers and grass/grazers. For example, giraffe on the NBP show a continual increase in $\delta^{13}C$ from October through until the early dry season (April/May), corresponding closely with an increase in $\delta^{13}C$ of tree foliage during this time (Figure 5.1). Previously, Codron, J. *et al.* (2005) reported relative ^{13}C-depletion of Kruger Park vegetation during the wet season, although the difference between dry and wet season data was much smaller (< 0.5‰) than observed here at the monthly scale (~2.0‰). The large increase in plant ^{13}C-content through the wet season in the current study is probably due to the particularly low rainfall for the 2004/2005 seasonal cycle (more than 200 mm less than the long-term mean) leading to reduced discrimination against ^{13}C during photosynthesis (e.g. Ehleringer and Cooper, 1988; Stewart *et al.*, 1995). The implications for diet are particularly evident in this instance; controlling for variations in the carbon isotope composition of available plant foods shows that the increase in giraffe faecal δ^{13}C of ~1.5‰, which might otherwise have been interpreted to reflect a decrease in C_3 foliage intake, is merely tracking isotopic changes in plants rather than changes in diet. Grazer faeces also track changes in the carbon isotopic composition of available grasses (Figure 5.3), including decreases in plant and faecal δ^{13}C towards the end of the dry season (August/September) and increases in the late dry season (January to March), at least on the NBP. Again, these patterns reveal that increases in faecal δ^{13}C do not necessarily reflect increases in C_4-intake, and similarly that decreases in faecal δ^{13}C (during the dry season) do not necessarily reflect C_3 vegetation in the diet. Rather, changes in faecal data are often related to changes in the isotopic composition of local vegetation.

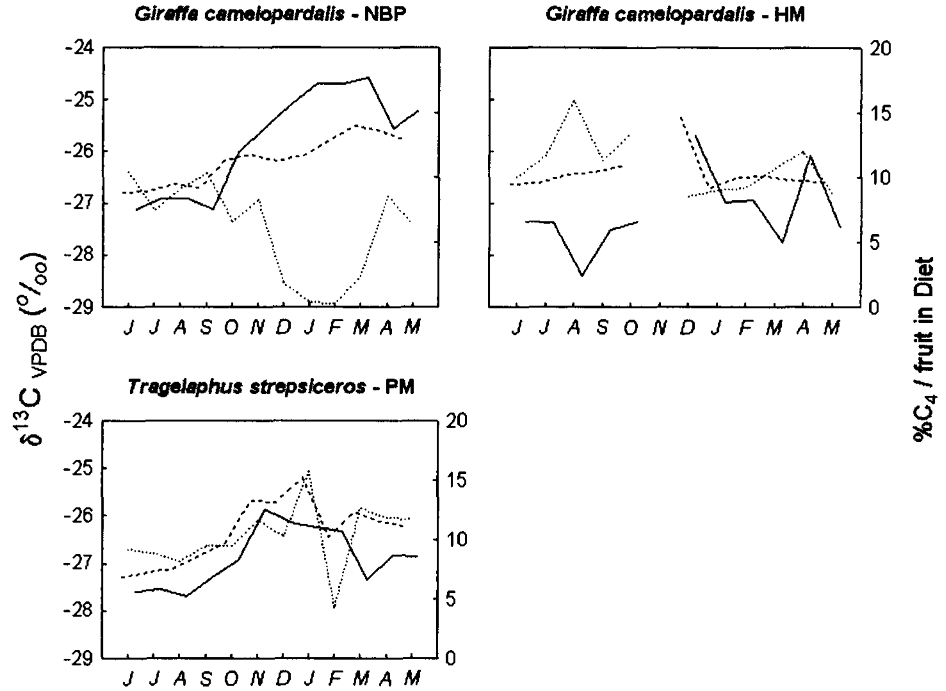

Figure 5.1. Corresponding shifts in mean $\delta^{13}C$ of tree foliage and browser faeces (left y-axis) in comparison with monthly changes in %C_4 (grass) intake (right y-axis). Higher levels of C_4-intake in browsers may be partially obscured by intake of ^{13}C-enriched fruits and/or flowers, hence these included in the right y-axis label.

Key: —— $\delta^{13}C_{Trees}$; ---- $\delta^{13}C_{Faeces}$; ········ %C_4/fruit in Diet.

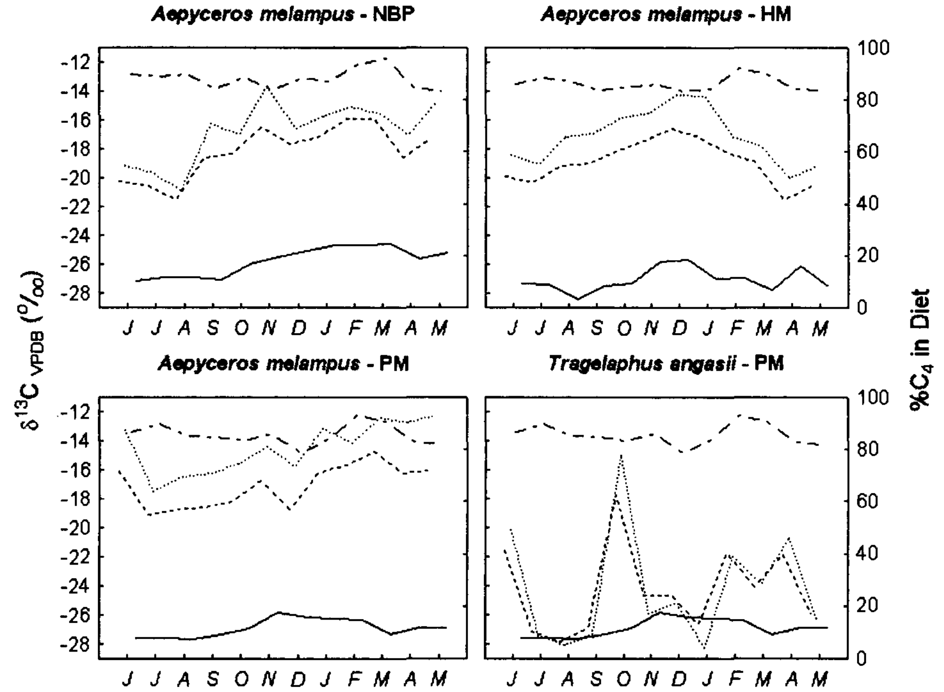

Figure 5.2. Corresponding shifts in mean $\delta^{13}C$ of tree foliage, grasses, and mixed-feeder faeces (left y-axis) in comparison with monthly changes in $\%C_4$ (grass) intake (right y-axis).

Key: ____ $\delta^{13}C_{Trees}$; ----- $\delta^{13}C_{Grass}$; ---- $\delta^{13}C_{Faeces}$; .... $\%C_4$ in Diet.

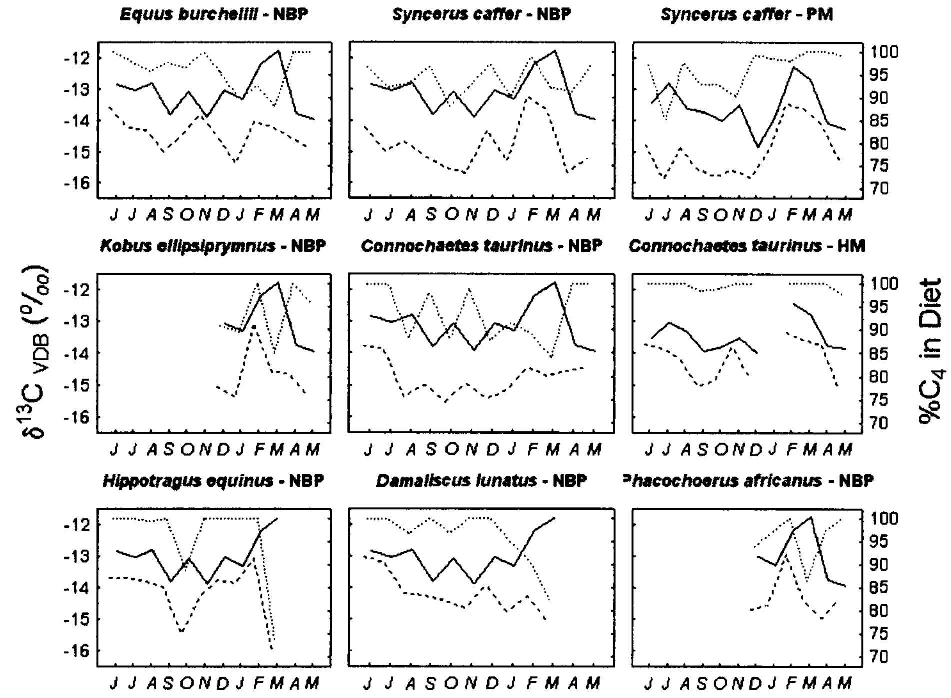

Figure 5.3. Corresponding shifts in mean $\delta^{13}C$ of grasses and grazer faeces (left y-axis) in comparison with monthly changes in $\%C_4$ (grass) intake (right y-axis).

Key: —— $\delta^{13}C_{Grass}$; ---- $\delta^{13}C_{Faeces}$; .... $\%C_4$ in Diet.

The consistency in patterns of monthly changes amongst plants compared with browser and grazer faeces exemplifies the fidelity with which faecal $\delta^{13}C$ records the carbon isotopic composition of the diet. However, these patterns re-iterate the need for baseline isotopic control (in this case plant data from appropriate space-and-time scales) for accurate determinations of diet (see also Post, 2002; Codron *et al.*, 2005a), a feature absent from the majority of previous carbon isotope studies. In this context, spatio-temporal variations in diet are more reliably inferred from derived calculations of $\%C_4$

(grass) intake, based on models that incorporate spatial and temporal variations in plant isotopic compositions, rather than changes in faecal $\delta^{13}C$.

Analysis of %C_4-intake revealed that browsers consume little or no grass (generally < 10% of the diet). One caveat here is that giraffe faeces are ~1.0 to 1.5‰ higher during August and September than in most other months (Figure 5.1), which could be interpreted as indicating significant levels (~10% or more) of grass during these late dry season months. A similar isotopic enrichment is observed for kudu faeces collected during the middle-to-late wet season (January and March). While kudu diets may comprise up to ~10% grass in the wet season (Owen-Smith and Copper, 1989), giraffe diets in the lowveld savanna are expected to be almost entirely browse-based (Hall-Martin, 1974). The most likely source for this apparent discordance is that the late dry season data for giraffe reflect use of fruit and/or flowers, both of which are ~1.0 to 1.5‰ enriched in ^{13}C compared to leaves of C_3 plants, at least in some South African savannas including this study region (Codron *et al.*, 2005b; Codron, J. *et al.*, 2005; this study). Fruits and flowers may form an important component of giraffe diets, especially flowers of *Acacia nigrescens* that appear in South African lowveld savannas during August and September (Hall-Martin, 1974; Owen-Smith, 2004, pers. comm.). Indeed, combined evidence from faecal $\delta^{13}C$ and $\delta^{15}N$, based on the multiple-source mixing model (Isosource), suggests a higher intake of fruit and/or flowers by giraffe during the dry season (Table 5.3). Similarly, faecal $\delta^{13}C$ for kudu that reflect increased grass consumption during the wet season may also be influenced by an increase in fruit-intake at this time.

Mixed-feeders show substantial spatio-temporal changes in faecal $\delta^{13}C$ (up to 6‰ throughout the seasonal cycle), and hence %C_4 grass consumption (Figure 5.2). Impala at PM eat more grass than their counterparts on the NBP and at PM ($F_{2, 357} = 29.683$, $P < 0.0001$), although their diets also differ between dry and wet season ($F_{1, 358} = 31.518$, $P < 0.0001$). However, while impala diets on the NBP and at HM change from ~50 to 60% grass in the dry season to >70% in the wet ($P < 0.0001$), at PM they maintain a diet of ~70 to 80% grass across both seasons ($P = 0.99$). These patterns persist even when variations in $\delta^{15}N$ and the potential effects of fruit consumption are accounted for using the dual-isotope model (Isosource; Table 5.3). Nyala also vary their diets between the seasons, eating more grass (~30%) in the wet season overall compared with ~20% in the dry ($F_{1, 100} = 4.062$, $P < 0.05$). Monthly records for this species however, reveal punctuated diet switches, rather than gradual seasonal changes, including a shift from an almost pure browse-based diet in the dry season to almost 80% grass-intake in October. Thereafter, nyala return to a browse-dominated diet until the late wet season (February to April) when they consume between ~20 and 40% grass. This pattern for nyala, which was not observed across broader dry-to-wet season comparisons, exemplifies the importance of high-resolution studies to properly understand ungulate diets and dietary variation.

Variations in grazer diets are, predictably, restricted to subtle shifts at the C_4 end of the spectrum (Figure 5.3). The only species for which dry-to-wet season changes in faecal $\delta^{13}C$ are significant is the wildebeest, which appears to use some C_3-based foods (~10% of bulk) in the dry season ($F_{1, 203} = 16.015$, $P < 0.01$). Interestingly, wildebeest are also the only grazers that show regional differences in diet ($F = 40.730$, $P < 0.0001$), i.e. faecal data reflect some C_3-intake by this species on the NBP but not at HM. All grazer species nevertheless show some fluctuations in diet at various stages of the seasonal cycle, despite the lack of broader dry-to-wet season differences. The most obvious shifts occur at the end of the wet season (March), when all grazers on the NBP appear to switch from almost pure grass-based diets to include between 10 and 20% C_3 foods. At PM, buffalo also include ~10 to 15% C_3 foods during the middle of the dry season (July).

A potential complicating factor for interpreting grazer diets from stable isotope data is that enzyme-controlled photosynthetic discrimination against ^{13}C differs slightly between NADP-ME, NAD-ME, and PCK C_4 grass sub-types, leading to small differences in $\delta^{13}C$ (Hattersley, 1982; Cerling and Harris, 1999). Here, NAD-ME, PCK, and NADP-ME groups were identified based on lists in Sage *et al.* (1999), and Aliscioni *et al.* (2003) for *Panicum* spp. *Sporobolus* spp. grasses are excluded from the NAD/PCK and NADP sub-analysis, because this genus is represented across all sub-types (Sage *et al.* 1999). In general, NAD and PCK grasses are ~1.0 to 1.5‰ depleted in ^{13}C relative to NADP species. Although the difference is smaller (~0.5‰) within the current study areas, $\delta^{15}N$ values vary substantially (up to 2‰, or more) between these groups, being notably lower in the NADP compared to the NAD/PCK group (Table 5.1; see also Codron, J. *et al.*, 2005). Incorporating these differences in the multiple source mixing model suggested that some of the variation in grazer faecal $\delta^{13}C$ can be ascribed to consumption of different grass sub-types, and not necessarily C_3 foods (Table 5.3). On this basis, only wildebeest and buffalo amongst the grazing species represented here can be said with certainty to consume significant amounts (>10%) of C_3-based foods at certain times of the year. Thus the dietary change in March for other NBP grazers more likely represents a switch to more ^{13}C-depleted NAD/PCK grasses, although small contributions of C_3 foods cannot be ruled out.

Results presented in Table 5.3 suggest that all grazers consume more NAD/PCK than NADP grasses, implying a preference for generally more palatable chloridoids above less palatable andropogonoids (see Sage *et al.*, 1999; Chapter 3). Grazers appear to switch to more equal amounts of both grass types during the dry season, although this observation might be an artefact of the smaller isotopic differences between NAD/PCK and NADP grasses in the dry period (Table 5.1). Nonetheless, δ^{15}N differences between the grass sub-types are maintained during the dry season at HM, and here the represented grazer (wildebeest) shows a markedly higher intake of NADP grass than in the dry season. In fact, grazer faeces from the dry season for all three regions have lower δ^{15}N values than in the wet ($F_{5,\ 714} = 17.532$, $P < 0.0001$), further implying an increased reliance on ^{15}N-depleted NADP grasses during the dry period.

5.3.2. Changes in Diet Quality

Faecal %N of browsers (mean = 2.7 ± 0.5%) is higher than that of grazers (mean = 1.4 ± 0.3%), with values for mixed-feeders being intermediate (mean = 2.0 ± 1.5%; $F_{2,\ 1501} = 927.399$, $P < 0.0001$; Table 5.2). Similarly, crude protein content (%N) of C_3 dicots is expected to be higher than that of C_4 grass (Robbins, 1993; van Soest, 1994; Chapter 4). Regional and seasonal comparisons revealed that amongst browsers, faecal %N is highest at PM, at least during the wet season ($P < 0.05$), although no regional differences in browser faeces were recorded for the dry season (Figure 5.4). This finding contrasts with %N data for trees from these regions, which reveal higher values for the NBP compared with other regions (Figure 5.5). However, faecal %N, especially amongst browsers, may be unusually elevated due to protein-precipitation by tannins in the diet (Arman *et al.*, 1975; Robbins *et al.*, 1987a), hence the disparity between regional trends in plants and faeces is not altogether surprising. The implication is that browsers at PM perhaps consume higher proportions of secondary compounds than those on the NBP and at HM, but further data is required to test this possibility. Nevertheless, the higher %N of PM browser faeces during the wet season is consistent with the suggestion that savanna trees generally have higher tannin content in the wet season than in the dry (Styles and Skinner, 2000), although further evidence for this phenomenon is required.

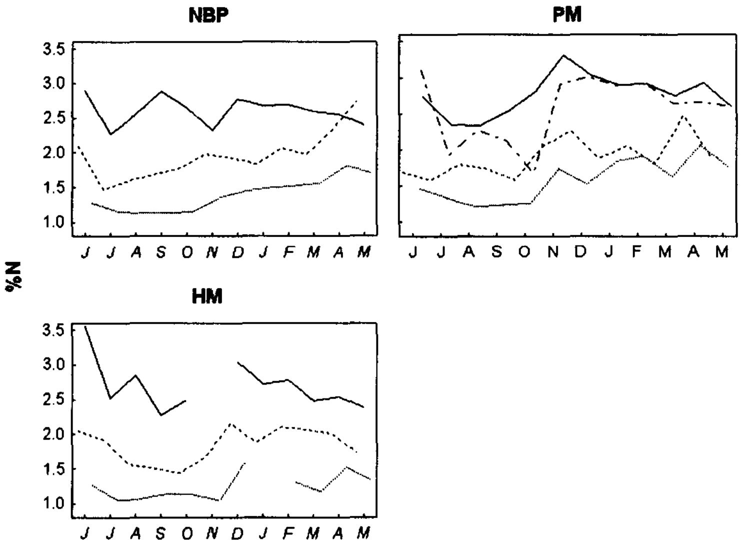

Figure 5.4. Monthly changes in faecal %N of browsers, mixed-feeders, and grazers from the NBP, PM, and HM. Key: ___ Browsers; ---- Mixed-feeder (*Aepyceros melampus*); _._._ Mixed-feeder (*Tragelaphus angasii*); .... Grazers.

Amongst grazers, no dry-to-wet season changes in faecal %N are evident within any of the three regions (*P* = 0.99 at NBP and HM, 0.55 at PM). There are regional differences since faeces from PM have higher N content than faeces from the other two regions (*P* < 0.0001; Figure 5.4). These patterns parallel available data for grasses, which show no seasonal changes in %N, but higher values are evident at PM (Figure 5.5).

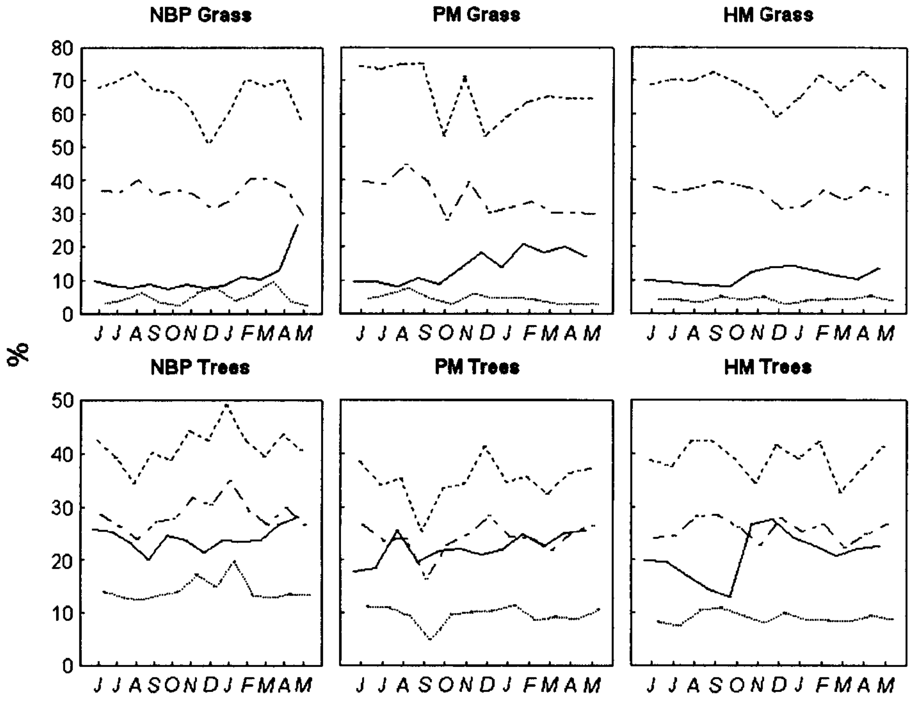

Figure 5.5. Monthly changes in nutritional composition of available vegetation (grass and tree foliage) on the NBP, PM, and HM.

Key: —— %N (x 10^1 for scale); ---- NDF; -·-·- ADF; ········ ADL.

The two mixed-feeder species, impala and nyala, differ from each other in that faecal %N of impala does not vary across space and time ($P = 0.15$), but that of nyala does ($P < 0.0001$; Figure 5.4). Thus, impala seem to maintain consistent quality diets, at least in terms of crude protein intake and excretion, despite large variations in proportions of browse:grass consumed. By contrast, nyala have higher faecal N content in the wet season than in the dry, but the seasonal change in nyala %N data are in the opposite direction than might be expected, i.e. increased faecal %N in the wet season corresponds to an increase in grass intake (Figure 5.6), despite that Kruger Park grasses are consistently lower in %N than available woody vegetation.

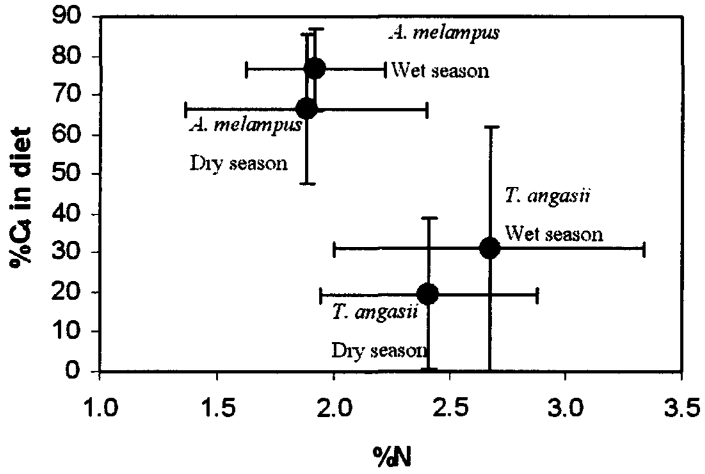

Figure 5.6. Bivariate plot showing different seasonal changes in grass consumption and faecal %N for two mixed-feeders, nyala and impala.. Symbols in the graph depict the means, and the bars ± 1 standard deviation.

5.3.3. Environmental Correlates of Food Selection

Multiple linear regression models (MLRMs) were used to test for significant correlations between %C_4 (grass) intake and environmental correlates (plant nutritional content and monthly rainfall) for diet (Table 5.4). A few species show positive results; giraffe diets varied with rainfall (Beta = 0.56, $P < 0.05$), impala with %N of grass in surrounding vegetational landscapes (Beta = 0.69, $P < 0.01$), and wildebeest with the overall nutritional content of available grasses, especially grass ADL content (Beta = 0.70, $P < 0.01$). More detailed accounts of diet, such as inclusions of plant parts (e.g. leaf sheaths or stems of grasses) and plant species utilized, might improve linear testing between correlates of diet quality and food choice. In addition, the exceptionally dry year during which this study was carried places some limitations for testing responses to rainfall and primary production.

Figure 5.7 shows the most significant correlations revealed by the MLRMs. The graph reveals decreasing %C_3-foliage consumption by giraffe with increasing rainfall ($r^2 = 0.10$, $P < 0.05$; but only when a one-month lag time was fitted to the model). In this case, the diet shift is likely due to an increase in consumption of relatively ^{13}C-enriched *Acacia nigrescens* flowers as these became available towards the end of the dry season (see Figure 5.1 and section 5.3.1.). Impala are shown to consume a greater proportion of C_4 grasses in regions and/or seasons in which grasses have a higher N content ($r^2 = 0.24$, $P < 0.01$). Wildebeest and buffalo are the only two grazer species that can be shown with certainty to vary their diets to include some C_3-based browse foods during the seasonal cycle, i.e. isotope shifts in wildebeest and buffalo faeces are larger than can be ascribed to switches between NAD/PCK- and NADP-feeding alone. Thus, it is not altogether surprising that regressions between %C4-intake, rainfall, and forage quality of available vegetation show significant correlations only for wildebeest amongst grazers (although the same cannot be said for buffalo). Wildebeest appear to shift away from a pure grass diet when forage quality of available grasses drops, primarily because of increases in indigestible ADL content ($r^2 = 0.24$, $P < 0.05$), but also due to decreases in grass %N and overall DMD (see Table 5.4).

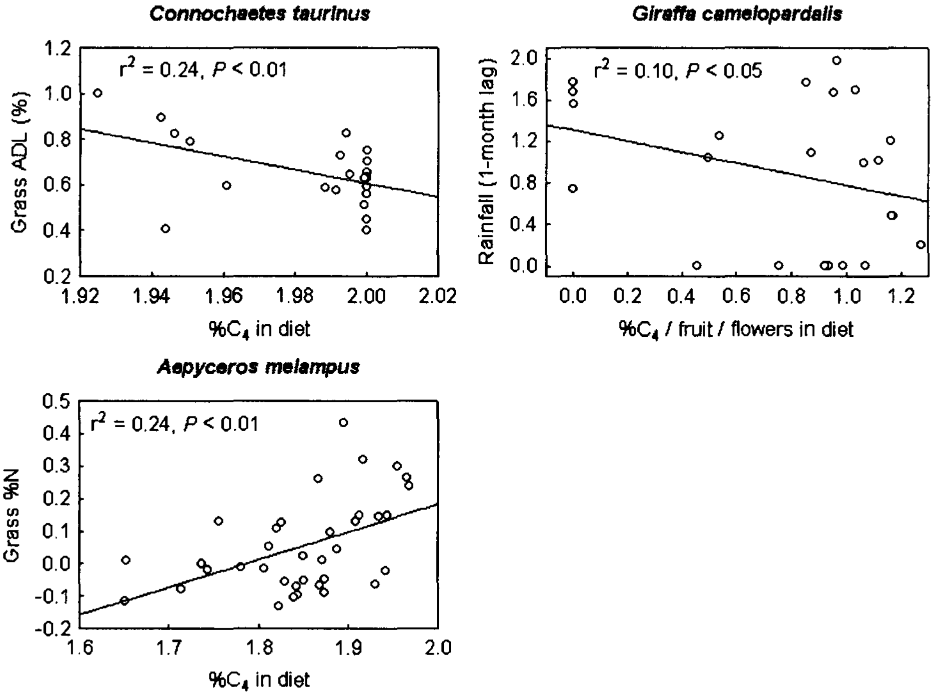

Figure 5.7. Linear relationships between %C4-grass (or fruit in the case of browsers) intake and dietary correlates for cases where significant correlations were revealed by MLRMs. Symbols depict the monthly and regional means for each species (log-transformed).

5.4. Discussion

Data presented here reveal variations in the foraging strategies of browsers, grazers, and mixed-feeders, providing valuable insights into mechanisms underlying food selection. The diets of browsers vary mainly in terms of proportions of leaves, fruits, and flowers consumed. That dietary variations for giraffe varied linearly with rainfall (Figure 5.7) is a reflection of diet shifts that occur when favoured fruits and/or flowers become available (such as *Acacia nigrescens* during the months of August and September). Inclusion of fruits/flowers when these are available might represent adaptive selection for higher quality food sources in variable environments. However, many fruits (including many edible species) do not necessarily provide herbivores with a higher quality food source, because these are often not only highly fibrous (up to 70% NDF), but many also have a relatively high lignin (ADL) content (~10 to 20%) (e.g. Barton *et al.*, 1993; Molloy and Hart, 2002; Chapter 4). On the other hand, browsers are faced with the challenges imposed by chemical defense systems of woody plants, particularly the presence of condensed tannins that act as antifeedants and reduce digestibility (Cooper and Owen-Smith, 1985; Robbins *et al.*, 1987a; Hay and van Hoven, 1988). Thus, browsers may be forced to consume a wide variety of foods to limit the digestion-inhibiting effects of these compounds (Freeland and Janzen, 1974; Cooper and Owen-Smith, 1985; Dearing *et al.*, 2000). Yet, fruits are also rich in secondary compounds (Owen-Smith, 1997), hence it is likely that browsers use these foods simply to maximize the number of *different types* of secondary compounds that are ingested. Provided they can maintain a diverse diet, browsers should be able to neutralize the toxic effects of a tannin-rich diet. Indeed, many browsers have enlarged salivary glands that are expected to be a morpho-physiological feature for neutralizing secondary compounds (Hofmann, 1989; but see Robbins *et al.*, 1995 for kudu).

Mixed-feeder species differ from one another in their respective responses to environmental heterogeneity. Impala show a distinct pattern of increasing grass consumption in areas and/or seasons where grasses have a higher nutritional value, at least in terms of crude protein content (%N). At PM their diets are predominantly grass-based (>70%) throughout the seasonal cycle, despite that this area is one of the most densely wooded regions of Kruger Park (and certainly offers a greater diversity of woody species than the open grasslands of the NBP) (see Venter *et al.*, 2003). Thus, impala do not eat browse and grass in proportions related to tree:grass leaf ratios in the surrounding environment. The PM data also suggest that food selection is not based on grass %N alone. The high wet season N content of browsing kudu from this region imply higher tannin content in available woody vegetation than in the other landscapes, a factor that might also induce impala to seek alternative food sources such as grass. Nyala at PM, by contrast, only consume grasses at intermittent periods of the wet season; during the dry season they prefer a more browse-dominated diet. It is likely that nyala, like the browsing kudu and giraffe, forage on tannin-rich browse alternatives at certain times of the year.

Differences in feeding responses between mixed-feeders are not limited to impala and nyala alone. Carbon isotope data for another prominent mixed-feeder, the elephant, show that their diets in the northern regions of Kruger Park do not change seasonally, in terms of the proportion of browse to grass consumed (Codron, J. *et al.*, 2006). This is quite different compared to the foraging responses of impala on the NBP, which increase grass intake from the dry to the wet season by ~20%.

All grazers appear to be constrained by the same factors, evinced most clearly by the diet shift to less grass (i.e. lower faecal $\delta^{13}C$ with no corresponding shift in grass data) displayed by the NBP grazers in March, while all species' diets remained relatively static across the remainder of the year. The simultaneous switch in March occurred in all species despite the fact that the diversity of taxa represented here include short- and tall-grass feeders, hindgut and foregut fermenters, and a variety of body sizes, which all might be expected to elicit differential responses to environmental heterogeneity (e.g. Demment and van Soest, 1985; Skinner and Smithers, 1990; Gordon and Illius, 1996; du Toit, 2003). It is unclear from these data alone whether the diet shift in March reflects the point of switching from NAD/PCK to a more NADP grass diet, or to increased use of woody plants and/or forbs. However, the Isosource model suggests different foraging responses amongst buffalo and wildebeest compared with other grazers, hence not all grazing taxa should be regarded as ecologically identical.

Grazer faecal %N also increased during the late wet season (March to April), which again suggests either higher protein browse foods in their diets, or simply increases in %N of NBP grasses at the same time. This shift in diet quality at the end of the wet season is nevertheless opposite to what is normally expected for savanna grasslands and grazers, in that grasses tend to decrease in N content through the dry season (Boutton *et al.*, 1988; Acocks, 1988; Tainton, 1999; Bodenstein *et al.*, 2000). This implies that the nutritional content of available foods, rather than primary production of the grass layer (at least in the biomass sense), underlie patterns of food choice. Indeed, the late rains of this seasonal cycle (January and March, see Chapter 4) would be expected to have improved biomass production of the NBP grass layer (Coe *et al.*, 1976; Tainton, 1999), such that the observed diet shifts (either towards browse-based foods or seemingly less-preferred NADP grasses) contrast the predictions of the primary productivity hypotheses for dietary variation. For example, wildebeest show a decrease in %C_4 intake with decreasing quality of available grasses, particularly in terms of lignin content (Figure 5.7).

Primary production appears to only influence food choice if resources are limiting, such as during the dry season when herbivores seek out alternative food sources. This agrees with the widespread interpretation that periods of resource limitation have been a fundamental aspect of ungulate evolution (e.g. Vrba, 1992; du Toit, 2003). For example, large body size allows many herbivore species to subsist on relatively poor quality foods (Demment and van Soest, 1985; Owen-Smith, 1988), hence increases in body size have been postulated as an evolutionary response to dry season feeding, avoiding competition with smaller animals for high quality forages (Gordon and Illius, 1996). Under less limiting conditions, niche differentiation and resultant phases of allopatry (see Vrba, 1992) are unlikely to occur, since all species should be able to maintain optimal nutritional uptake and/or minimize ingesting lethal doses of secondary compounds.

5.5. Conclusion

Variations in constraints to food selection by ungulates limit current understanding of the functional significance of herbivory in savanna ecosystems. While selection for higher quality foods appears to prevail throughout, the mechanisms that underlie this selection differ widely across species, even within similar feeding guilds. While single-species studies have provided useful insights, future research should aim to develop methods for multi-species investigations. Isotopic tools offer one alternative, but further multivariate studies are badly needed in order to account for factors such as plant defense systems, physiological constraints, predation risk, etc. over a range of taxonomic, spatial, and temporal scales. Combining multiple species studies with multivariate approaches is necessary to refine optimal foraging models that can explain dietary diversity across the browser/grazer continuum.

Table 5.1. Stable carbon and nitrogen isotope composition of vegetation on the Northern Basalt Plains (NBP) and Punda Maria (PM), Kruger National Park, and Hans Merensky Nature Reserve (HM), collected at monthly intervals from June 2004 to May 2005. The dry season is represented from April to September, and the wet from October to March. *Sporobolus* spp. grasses are excluded from the NAD/PCK and NADP sub-analysis, because this genus is represented in all sub-types (Sage *et al.* 1999).

			δ^{13}C (‰)		δ^{15}N (‰)			δ^{13}C (‰)		δ^{15}N (‰)	
Form	**Region**	***n***	**Mean**	**SD**	**Mean**	**SD**	***n***	**Mean**	**SD**	**Mean**	**SD**
		Dry Season					**Wet Season**				
Dicots											
C_3 Tree leaves	NBP	24	-25.2	1.2	2.9	1.0	24	-26.6	1.0	4.7	2.7
	PM	36	-26.4	1.0	3.6	1.8	36	-27.2	1.0	3.5	1.6
	HM	33	-26.6	1.3	3.3	1.4	36	-27.2	1.8	2.6	1.5
Fruit							10	-25.7	1.4	3.6	1.2
Monocots											
C_4 Grass											
All grass	NBP	48	-13.4	1.0	3.0	2.2	45	-13.0	1.0	2.2	2.0
NADP		18	-13.0	0.6	2.6	2.4	15	-13.3	1.0	2.3	1.7
NAD/PCK		30	-13.7	1.1	3.2	2.2	30	-12.9	1.0	2.1	2.1
All grass	PM	35	-13.7	0.8	3.0	1.7	31	-13.4	1.2	3.2	1.5
NADP		5	-13.5	0.9	3.0	2.3	5	-12.8	1.1	3.3	1.6
NAD/PCK		24	-13.6	0.7	3.4	1.4	23	-13.3	0.9	3.2	1.6
All grass	HM	36	-13.5	0.6	1.9	1.7	36	-13.2	0.8	2.2	1.7
NADP		6	-13.4	0.7	1.9	1.2	6	-13.4	0.5	1.3	1.6
NAD/PCK		30	-13.5	0.6	1.9	1.8	30	-13.2	0.8	2.4	1.7
C_3 Reeds and Sedges							6	-26.1	1.3	4.9	1.8
C_4 Reeds and Sedges							4	-12.5	0.9	6.4	1.3

n = number of samples
SD = standard deviation

Table 5.2. Stable carbon and nitrogen isotope composition, and percent nitrogen, of faeces from the Northern Plains (NBP), Punda Maria (PM), and Hans Merensky (HM), collected at monthly intervals from June 2004 to May 2005. The dry season is represented from April to September, and the wet from October to March.

				$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		%N	
Species	**Region**	**Season**	***n***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Browsers									
Giraffa camelopardalis	NBP	Dry	53	-26.3	0.7	5.2	1.6	2.6	0.4
		Wet	54	-26.0	0.6	4.6	2.0	2.6	0.3
	HM	Dry	58	-26.5	0.4	4.3	1.2	2.7	0.9
		Wet	50	-26.3	0.9	4.4	0.7	2.7	0.3
Tragelaphus strepsiceros	PM	Dry	48	-26.7	0.6	5.4	1.4	2.6	0.4
		Wet	59	-25.9	0.6	5.3	1.0	3.0	0.3
Mixed-feeders									
Aepyceros melampus	NBP	Dry	60	-19.5	2.4	4.7	2.3	2.0	0.6
		Wet	60	-16.9	1.5	7.4	2.0	1.9	0.2
	PM	Dry	60	-17.5	2.0	4.5	1.6	1.9	0.5
		Wet	60	-16.7	1.6	5.2	1.3	1.9	0.3
	HM	Dry	60	-20.1	2.3	4.1	1.1	1.8	0.5
		Wet	60	-17.7	1.2	5.1	1.1	1.9	0.3
Tragelaphus angasii	PM	Dry	43	-25.4	3.2	6.5	1.9	2.4	0.5
		Wet	59	-23.2	3.8	5.0	1.0	2.7	0.7
Grazers									
Connochaetes taurinus	NBP	Dry	50	-14.4	1.0	4.4	2.2	1.5	0.4
		Wet	45	-15.1	0.8	5.8	1.9	1.4	0.2
	HM	Dry	60	-14.3	0.9	3.7	1.0	1.2	0.2
		Wet	50	-14.1	0.8	4.3	1.4	1.3	0.2
Damaliscus lunatus	NBP	Dry	11	-13.6	0.6	4.7	0.7	1.2	0.2
		Wet	29	-14.5	0.7	4.0	1.5	1.5	0.4
Equus burchellii	NBP	Dry	48	-14.4	0.7	4.2	1.4	1.3	0.4
		Wet	60	-14.4	0.9	4.5	0.9	1.2	0.2
Hippotragus equinus	NBP	Dry	10	-13.8	0.3	3.8	1.3	1.0	0.1
		Wet	20	-14.3	1.5	3.9	1.0	1.4	0.3
Kobus ellipsiprymnus	NBP	Dry	20	-15.0	1.4	6.0	1.0	1.9	0.4
		Wet	39	-14.5	1.1	6.8	1.9	1.5	0.2
Phacochoerus africanus	NBP	Dry	11	-14.9	1.1	4.6	1.3	2.0	0.2
		Wet	29	-14.4	1.1	4.5	1.3	1.6	0.2
Syncerus caffer	NBP	Dry	59	-15.0	0.7	4.3	1.0	1.4	0.2
		Wet	59	-14.6	1.1	5.0	1.2	1.5	0.2
	PM	Dry	60	-15.1	0.8	4.5	1.9	1.5	0.4
		Wet	60	-14.9	1.2	6.0	1.3	1.7	0.3

n = number of samples

SD = standard deviation

Table 5.3. Overall dry-to-wet season differences in diets of ungulates from the Northern Plains (NBP), Punda Maria (PM), and Hans Merensky (HM), as determined using the Isosource (Phillips and Gregg, 2003) dual-isotope three-source mixing model. Foliage represents leaves of both C_3 trees and forbs, since the two are isotopically indistinguishable.

		Diet source (25th - 75th percentiles of range, in %)					
Species	**Region**	**Dry season**			**Wet season**		
Browsers/ Mixed-feeders		Foliage	Fruit	Grass	Foliage	Fruit	Grass
Giraffa camelopardalis	NBP	71-80	8-17	1-3	77-92	8-10	2-8
	HM	78-89	5-16	6-7	91-94	3-7	2-3
Tragelaphus strepsiceros	PM	68-84	2-22	1-3	59-72	14-41	12-15
Aepyceros melampus	NBP	11-35	11-23	53-57	18-19	0-2	80-81
	PM	7-18	7-19	74-75	6-18	6-21	68-79
	HM	24-37	9-23	54-55	17-22	3-9	75-76
Tragelaphus angasii	PM	23-65	21-37	11-13	29-52	14-41	32-35
Grazers		NAD/PCK	NADP	Foliage	NAD/PCK	NADP	Foliage
Connochaetes taurinus	NBP	24-72	25-73	1-5	73-86	6-18	7-11
	HM	2-4	93-95	3-4	73-92	7-27	0-1
Damaliscus lunatus	NBP	0-20	100	0	83-93	2-11	4-7
Equus burchellii	NBP	24-72	25-73	1-5	86-93	2-9	3-6
Hippotragus equinus	NBP	23-68	32-77	0-1	86-94	2-9	3-6
Kobus ellipsiprymnus	NBP	25-70	23-68	5-9	83-93	2-11	4-7
Phacochoerus africanus	NBP	24-72	22-69	4-8	86-93	2-9	3-6
Syncerus caffer	NBP	25-70	23-68	5-9	81-92	3-13	4-7
	PM	22-69	24-70	4-11	82-92	5-16	3-4

Table 5.4. Partial correlations between food intake and possible ecological predictors of food selection (nutritional content of available vegetation and rainfall), revealed from multiple linear regression models. CWD and DMD denote potential cell wall and dry matter digestibilities, respectively, based on regressions of Robbins *et al.* (1987a, 1987b). Significant correlations ($P < 0.05$) are marked with an asterisk (*).

								Rainfall	
Species	**Food**	**%N**	**NDF**	**ADF**	**ADL**	**CWD**	**DMD**	**Per month**	**1-month lag**
Browsers/Mixed-feeders									
Giaffe camelopardalis	Tree foliage	-0.18	-0.32	0.45	-0.25	0.19	-0.26	-0.44	-0.56 *
Tragelaphus strepsiceros	Tree foliage	0.34	-0.02	0.08	-0.10	-0.08	-0.14	0.85	-0.72
Aepyceros melampus	Tree foliage	-0.14	0.11	0.26	-0.23	-0.20	0.14	0.32	0.21
	Grass	0.69 *	0.09	0.15	-0.38	-0.16	-0.12		
Tragelaphus angasii	Tree foliage	0.56	0.52	0.30	-0.39	-0.46	0.62	0.43	-0.54
	Grass	-0.32	-0.40	0.44	0.08	0.37	-0.38		
Grazers									
Connochaetes taurinus	Grass	0.56 *	0.06	-0.48	0.70 *	0.18	0.51 *	0.28	-0.46
Damaliscus lunatus	Grass	0.53	0.61	0.27	0.92	-0.48	0.88	0.03	-0.79
Equus burchellii	Grass	0.69	0.35	0.01	0.46	-0.34	0.68	-0.36	-0.60
Hippotragus equinus	Grass	0.31	-0.46	0.67	0.86	0.55	0.34	-0.58	-0.40
Kobus ellipsiprymnus	Grass	0.79	-0.80	0.81	-0.84	-0.25	0.10	-0.92	0.85
Phacochoerus africanus	Grass	1.00	-1.00	1.00	-1.00	0.09	0.72	-0.56	0.35
Syncerus caffer	Grass	0.31	0.13	-0.11	0.10	-0.12	0.19	-0.20	-0.10

CHAPTER 6

Evolutionary Transitions from Browsing to Grazing in African Ungulates: An Interpretation from Spatio-temporal Dietary Variations of Impala

In the previous chapter, I examined seasonal dietary changes amongst a variety of ungulate species. The results of Chapter 5 suggest that, while different species may respond differently to seasonal environmental changes, diet switching is most likely a strategy to maintain optimum nutrient uptake. In this chapter, I further explore the theme of dietary variation amongst mixed-feeding impala *Aepyceros melampus*, using data from faeces collected over a multitude of spatial, seasonal, and annual scales within Kruger Park. I examine environmental correlates for diet switches in the species, and discuss the results in terms of evolutionary transitions between browsing and grazing states.

6.1. Introduction

The most significant feature of ungulate evolutionary history and ecological diversity is the distribution of feeding styles along a browser/grazer continuum (Hofmann and Stewart, 1972; Jarman, 1974; Demment and van Soest, 1985; McNaughton and Georgiadis, 1986; Hofmann, 1989; Gordon and Illius, 1996; Owen-Smith, 1997; Gagnon and Chew, 2000; du Toit, 2003). In functional terms, this continuum is expected to break down into three guilds, i.e. browsers, grazers, and mixed-feeders (Hofmann and Stewart, 1972; Hofmann, 1989; du Toit, 2003). Evidence from the fossil record indicates that browsing is a primitive character state, while grazing evolved later on in geological time, initially during the Oligocene and accelerating through the Miocene (Janis, 1982; Janis *et al.*, 2000), and particularly the Neogene alongside the proliferation of C_4 grasslands (Cerling *et al.*, 1997a, 2005). Differences in feeding style have been related to several morpho-physiological and behavioural adaptations, including molar morphology, mouth morphology, stomach anatomy, and activity time (Janis, 1988; Janis and Ehrhardt, 1988; Hofmann, 1989; Mysterud, 1998; Janis *et al.*, 2000; Perez-Barberia and Gordon, 2001). However, the mechanisms regulating just how evolutionary transitions between browsing and grazing occurred are not clear.

Models of diet along the browser-grazer continuum may be used to understand this important transition (e.g. Perez-Barberia *et al.*, 2001a). One hypothesis predicts that differences in the anatomy of the alimentary canal enable browsers and grazers to maximize energy absorption from the different food types on which they subsist (Hofmann, 1989). Conversely, models of digesta kinetics and retention time suggest that browsers and grazers do not differ in overall digestive efficiency, but that digestive efficiency scales allometrically with body size (Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995).

Mixed-feeders display diet breadth extending across the browser-grazer spectrum, representing a transitional state between the two extremes (Hofmann, 1989; Perez-Barberia *et al.*, 2001a). Understanding ecological correlates of dietary variation in mixed-feeders may therefore highlight mechanisms underlying dietary transitions. Amongst modern African species, impala *Aepyceros melampus* have perhaps the most flexible diets, ranging from almost pure browse to nearly exclusive grass intake, depending on local habitat conditions, season, and even the sex of the animal (Dunham, 1980, 1982;

Monro, 1980; van Rooyen and Skinner, 1989; Skinner and Smithers, 1990; Meissner *et al.*, 1996; Wronski, 2002). Accordingly, impala gut morphology is extraordinarily elastic, shifting from an apparently browse-adapted morphology in the dry season, entailing decreased blood flow to the ruminal epithelium and ruminal papillae, to a more grazer-like form in the wet (Hofmann and Stewart, 1972; Hofmann, 1989).

As I have shown throughout this thesis, stable carbon isotope analysis of faeces allows rapid tracking of shifts in proportions of browse (C_3 plants) and grass (C_4 plants) consumed. For mixed-feeders such as impala, this technique can be particularly useful. For example, preliminary carbon isotope data from impala faeces and hair from Kruger Park showed that populations in the southern regions of the Park, in areas of fine-leaved *Acacia* and *Combretum* savanna, eat more grass than their counterparts inhabiting the northern regions, where broad-leaved *Colophospermum mopane* savanna abound (Sponheimer *et al.*, 2003e). The spatial mosaic of ecotypes found within Kruger Park in fact incorporate an even wider array of habitat types (Venter *et al.*, 2003). The extreme seasonality of the rainfall cycle adds a temporal dimension to the heterogeneity of the landscape. Thus, given the diet breadth expected for impala, broad-scale comparisons between southern and northern Kruger populations probably oversimplify the full range of variation that exists in this heterogeneous savanna environment. Faecal carbon isotope data presented in Sponheimer *et al.* (2003e) also represent only one season of one year (dry season/June 2002).

The aim of this chapter is to test ecological correlates for browsing/grazing transitions in ungulates, using mixed-feeding impala as a model taxon. To discern drivers of diet switching across the maximum scale of dietary variation in the species, I use stable carbon isotope evidence from faeces representing a multitude of high-resolution spatial and temporal scales within the Kruger Park. Carbon isotope evidence for changes in browsing and grazing are compared with climate records (monthly rainfall), and with well-documented spatial heterogeneity in geological substrate and vegetational landscapes within the Park (reviewed in Venter *et al.*, 2003). I also compare diet shifts with changes in diet quality, based on faecal percent nitrogen.

6.2. Methods

For this chapter, I use data from impala faeces collected from Kruger Park throughout the three-year study period, i.e. from June 2002 to May 2005, biannually during the first two years, and at monthly intervals from February 2004 to January 2005 (continuing until May 2005 on northern basalts). Monthly collections at Punda Maria were carried out from June 2004 to May 2005. These samples also represent the five major sampling landscapes within the Park, described in Chapter 2 (northern basalts and granites, southern basalts and granites, and Punda Maria) (Figure 6.1). Briefly, the northern regions of Kruger are dominated by broad-leaved *Colophospermum mopane* savanna, while the south is predominantly fine-leaved *Acacia* and *Combretum savanna* (Venter *et al.*, 2003). The relatively nutrient-rich basalts in the east give rise to open grasslands, while the sandy, granite-based landscapes in the west support woodland savannas. Punda Maria is a heavily wooded landscape in the far north of the Park. For high-resolution spatial analysis, latitude and longitude co-ordinates were recorded for each faecal sample using a Global Positioning System (GPS; Figure 6.1), and visual descriptions of surroundings were also documented for this scale (e.g. open grassland, woodland, sodic patch, riparian zone, waterhole). Collection sites that often yielded an abundance of impala faecal samples and/or latrines were revisited during consecutive field trips.

Analysis of faecal $\delta^{13}C$, $\delta^{15}N$, and %N followed the procedure described in Chapter 3, and $\delta^{13}C$ values were converted to estimates of %C_4-intake using the dual-endpoint mixing model described therein. Isotope baseline data for local vegetation was obtained from Codron, J. *et al.* (2005). For the sake of completeness, I report results of nitrogen isotope analysis alongside carbon isotope data (and estimated %C_4 grass intake), but interpretation of the former are beyond the scope of this chapter, as I am interested only in switches between browsing and grazing. Except for month-to-month comparisons, distinction was made between wet and dry season data. Significant differences between groups were tested using analysis of variance (ANOVA) and Tukey's HSD *post hoc* test for differences between means (P-level 0.05), after normal distributions were confirmed using the Shapiro-Wilks test ($\delta^{13}C$ SW-W = 0.98, $P <$ 0.0001; $\delta^{15}N$ SW-W = 0.99, $P < 0.01$; and %N SW-W = 0.94, $P < 0.0001$). The relationship between diet changes and environmental/ecological heterogeneity (monthly rainfall, diet quality from faecal %N, latitude) are examined using linear regression analyses of log-transformed data.

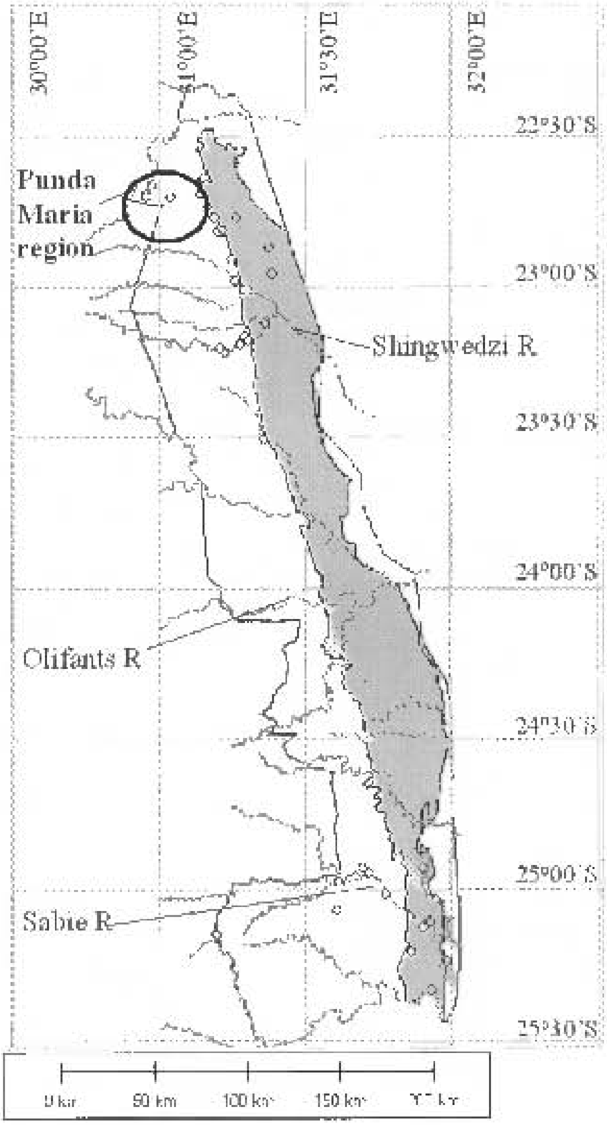

Figure 6.1. Map of Kruger National Park showing impala faecal sampling localities (white circles). The white region represents granites and the shaded eastern basaltic soils. The major river systems are displayed, including the Olifants River separating northern *Colophospermum mopane* dominated landscapes from southern *Acacia* and *Combretum* landscapes, as well as the Shingwedzi and Sabie river systems.

6.3. Results

6.3.1. Carbon Isotope Evidence for Dietary Variation

Faecal $\delta^{13}C$ of Kruger Park impala for the three-year study period indicate that northern and southern populations have similar diets (~56% C_4-intake in both regions; $F_{1, 479} = 0.087$, $P = 0.77$). Thus, the broad-scale north-south dietary difference reported by Sponheimer *et al.* (2003e) for faeces collected during a single dry season (June 2002) did not persist. By contrast, there is a complex array of significant differences in grass intake across landscapes, seasons, and from year-to-year (Table 6.1; Figure 6.2; $F_{29, 576} = 20.893$, $P < 0.0001$).

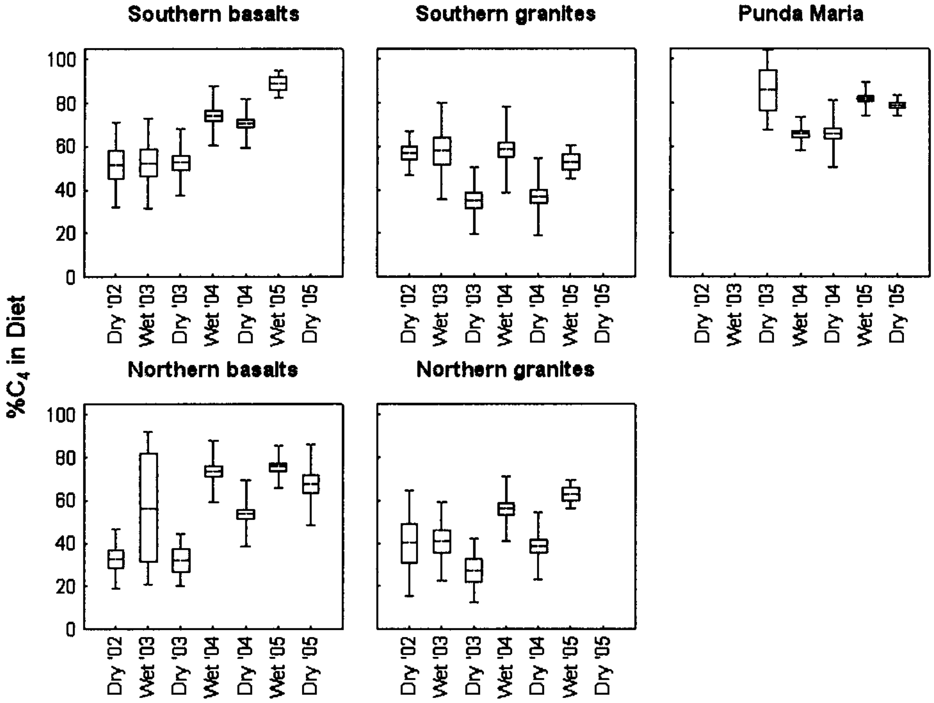

Figure 6.2. Seasonal and annual changes in $\%C_4$ consumed by impala from different landscapes in Kruger Park. The boxes in the graph show standard error, and the whiskers standard deviation from the mean (horizontal bars).

Impala diets ranged from ~37 to 74% in grass content between the five major landscapes (changing by ~20 to 30% between wet and dry seasons), and variance in grass consumption at the annual scale was between ~27 and 89%. At the landscape scale, grass intake is generally higher on basalts than on granites ($P < 0.0001$), but maximum grass intake (~72% of bulk) occurs at Punda Maria in the far north ($P < 0.0001$; Table 6.1). However, landscape differences are larger during the dry season (~30%) than the wet (~15%), due to dry-to-wet season increases in grass consumption in three regions ($P < 0.001$) (no seasonal shifts occur on southern basalts ($P = 0.99$) or at Punda Maria ($P = 1.00$)). Moreover, year-to-year differences (up to ~40%) often exceed the magnitude of overall regional and seasonal differences (Figure 6.2). Significant annual diet changes are noted for southern granites and basalts, northern basalts, and Punda Maria across dry seasons, and for southern basalts and Punda Maria between consecutive wet seasons ($P < 0.01$).

Even larger variations in proportions of C_3 and C_4 consumption are evinced from month-to-month comparisons of samples collected during the final year of fieldwork (Figure 6.3). At this scale, impala diets are shown to fluctuate between ~16 and 89% C_4 grass throughout the seasonal cycle. Monthly shifts in diet are significant for northern granites and basalts, and southern granites, with impala diets in these landscapes changing from more than 60% grass during the wet season to less than 40% in the dry ($P < 0.0001$). The shift away from a grass-rich diet to include more browse occurs at the onset of the dry season (April), and continues through the dry season reaching a C_4-minimum during the driest months (May, June, July). Sharp grazing peaks (when diet comprises 80 to 90% grass) are noted during March and December on southern granites and February on northern basalts.

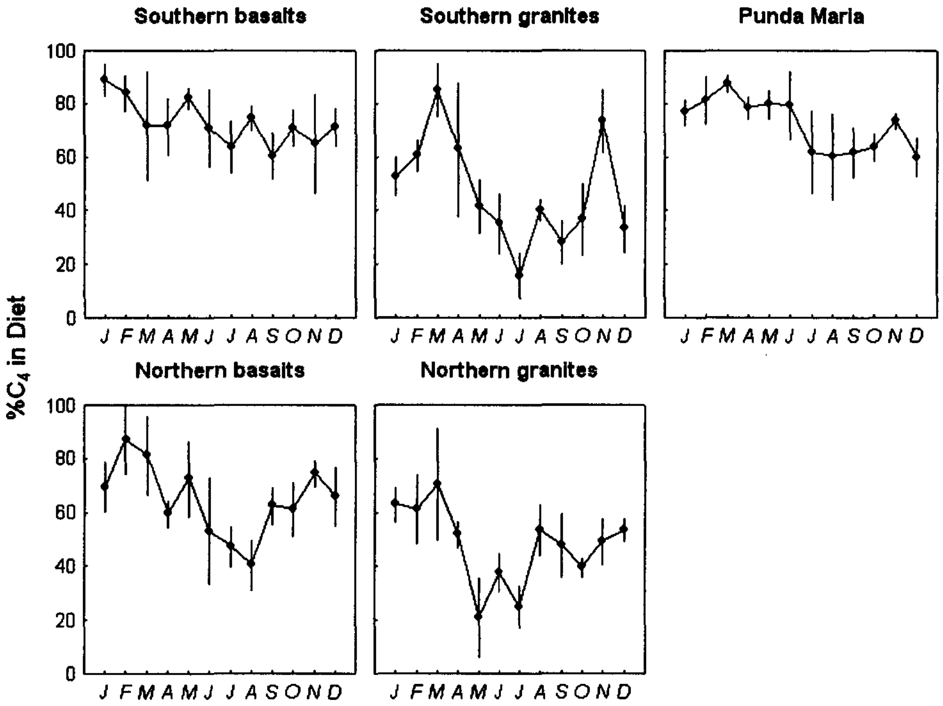

Figure 6.3. Monthly changes in Kruger Park impala diets for the period February 2004 to January 2005 (June 2004 to May 2005 for Punda Maria). The symbols in the graph represent means, and the bars show standard deviation.

Monthly data also portray more detailed seasonal patterns for southern basalts and Punda Maria that are not discernible through broader dry/wet season comparisons (Figure 6.3). On southern basalts, impala diets generally comprise between ~60 and 70% C_4 grass, but during the wet season months of January and February, and the dry season month of May, grass intake is greater than 80%. Punda Maria data evince significantly lower grass intake for the period of June to December 2004 compared to the period of

January to May 2005 ($P < 0.05$). In this case, however, the difference can be attributed to the sudden switch to increased grass consumption that occurred between December 2004 and January 2005 ($P < 0.001$; Figure 6.2), rather than to changes along a seasonal gradient.

6.3.2. Changes Beyond the Granite-Basalt Gradient

While useful for ecological studies at a very broad scale, the granite-basalt distinction of Kruger Park landscapes oversimplifies the geological and associated vegetational complexity of the region (see Venter, 1986; Venter *et al.*, 2003). For further resolution of spatial heterogeneity in diet, $\%C_4$ intake was assessed along a west-to-east gradient based on GPS locality. To control for animal movement between feeding and defaecation sites, samples collected within 10 km^2 were treated as having the same GPS point, based on reported home range areas of ~5 km^2 in this species (Skinner and Smithers, 1990; Owen-Smith, 2005, pers. comm.). Although territorial males may have much larger home ranges (up to 80 km^2), the vast majority of individual specimens were collected from defaecation sites of breeding herds, hence a 10 km^2 spatial separation is considered sufficient for these analyses. Latitude and longitude co-ordinates were converted to decimal degree points using the equation

$$dd.ff = dd + mm/60 + ss/3600,$$

where dd represents degrees east or south (^{o}), mm minutes ('), and ss seconds (").

Linear regression analyses revealed that impala vary their diets along a latitudinal gradient (Figure 6.4), at least during the dry seasons when spatial differences in diet are more pronounced than in wet seasons (see Table 6.1). The effect differs, however, between impala from the northern and southern regions of Kruger Park, respectively. Impala from the south increase the amount of grass in their diets from west-to-east (Figure 6.4a; $r^2 = 0.22$, $P < 0.0001$), consistent with the finding that basalt-based grassland landscapes in the east generally support a more grass-dominated diet than do granites in the west. By contrast, impala from northern regions decrease their grass intake from west-to-east (Figure 6.4b; $r^2 = 0.15$, $P < 0.0001$), although this is result is skewed by the unusually high grass intake by impala at Punda Maria. The latitudinal effect for the north disappears when Punda Maria is excluded from the analysis (Figure 6.4b; $r^2 = 0.01$, $P = 0.78$).

These analyses also showed changes in diet at higher-resolution microhabitat/patch scales beyond broader differences between granitic and basaltic landscapes. Samples representing non-riparian woodland habitats (based on visual descriptions of sampling locality) lie above the regression lines, and depict significantly higher grass intake than other habitat types (~76 to 56% in the south and north, respectively; $P < 0.0001$). Samples from sodic patches also show elevated grass intake compared with other habitat-types, at least in the south (~66%; $P < 0.0001$), but in the north sodic patch samples do not differ significantly from the regression line (~38%; $P = 0.14$). Samples from riparian habitats reflect diets comprising only ~36 and 21% C_4 grass in the south and north, respectively, falling below the regression line ($P < 0.001$ in the south and $P < 0.01$ in the north), implying a higher proportion of C_3-browse intake in riverine areas. Even in the wet season, proportions of grass-intake in riparian zones are lower than in other habitats (~44% C_4; $P < 0.001$). This finding is in agreement with field studies of impala, in that impala are expected to obtain much of their browse foods from fallen leaf litter in riparian areas (Cooper and Owen-Smith, 1985).

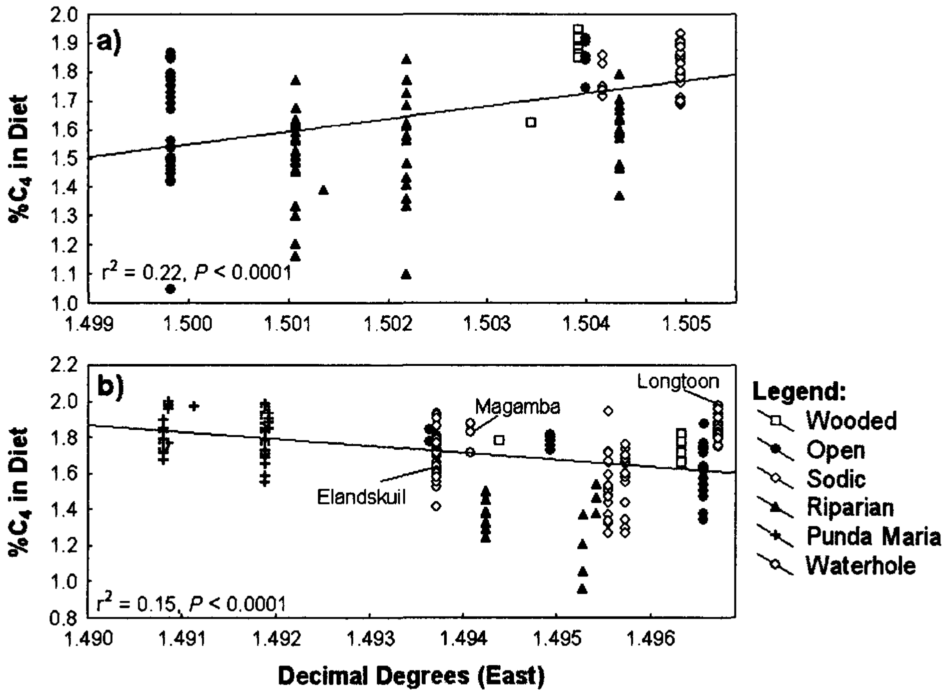

Figure 6.4. Spatial differences in dry season %C4 grass intake by impala along a west-to-east gradient within a) southern and b) northern Kruger Park landscapes (log-transformed data). The graphs show significant correlations between diet, geology, and other landscape effects. Elandskuil, Magamba, and Longtoon are the three northern Kruger artificial watering points included in the study.

In the north, a number of specimens were also collected around artificial watering points, adding a further dimension to spatial comparisons for this region. Impala faeces collected around the Longtoon Dam (22°52'18.4"S, 31°23'5.5"E) reflect grass-rich diets relative to other northern habitats (~68% C_4; $P < 0.001$). By contrast, faeces from the Elandskuil waterhole (22°44’9.1”S, 31°10’6.0”E) do not portray particularly high grass intake (~52% C_4; $P = 0.99$).

6.3.3. Diet Quality

Diet quality, as evinced from faecal N content, seems to vary minimally in this species. Mean %N of impala faeces varies between 1.8 ± 0.5 and 2.3 ± 0.6% (Table 6.1), intermediate between reported faecal N content of grazers (1.2 to 1.8‰) and browsers (2.3 to 2.9‰) from Kruger Park (Sponheimer *et al.*, 2003e; Codron, J. *et al.*, 2006; Chapter 5). No differences are evident between mean %N of impala faeces from different landscapes of Kruger Park, or between dry and wet seasons within each landscape (*P*-values ranged between 0.15 and 0.99). The only exception is faeces from southern granites, which during the dry season have slightly higher values than faeces from northern landscapes ($F_{9,596} = 5.173$; $P < 0.05$).

6.4. Discussion

Several studies of impala elsewhere in Africa have indicated that they prefer grass above browse, and will eat more grass when palatable grasses are available in abundance (Dunham, 1980; Monro, 1980; Attwell and Bhika, 1985). Impala rumen anatomy suggests they are more suited to a grass-rich diet (Hofmann and Stewart, 1972). By contrast, impala in Namibia seemed to prefer feeding on browse plants (Gaerdes, 1965). The disparity is likely because many field studies on impala diets were carried out in extreme and/or marginal environments. The current study incorporates data from a variety of landscapes within a semi-arid savanna, and are hence sufficiently comparable to test browse/grass diet preferences.

In Kruger Park, granitic substrates form sandy soils that support wooded savanna habitats, while the more clayey soils derived from basalts are associated with more open savannas and grassland (Venter, 1990; Venter *et al.*, 2003). Thus, the finding that impala on basalts generally include more grass in their diets than those on granite landscapes (Table 6.1) is consistent with the prediction that they eat a higher proportion of grass in areas where tree:grass leaf ratios are lower. However, the basalt-granite dietary difference was *not* consistent throughout the study. For example, impala on southern basalts had similar diets to those on southern granites during 2002 and 2003 (~50% C_4 intake), exemplifying that annual changes may be as significant for diet studies as are seasonal variations. Impala in the densely wooded region of Punda Maria ate more grass than impala in all other (more open) landscapes. Higher-resolution spatial analysis of diet shifts along a west-to-east gradient reveals further apparent anomalies at the microhabitat scale, even when the effects of geological substrate are accounted for. The graphs in Figure 6.4 suggest that impala in woodland habitats eat even more grass than in open grasslands, and impala foraging in riparian zones seem to prefer a browse-rich diet (only ~20 to 35% C_4), despite the fact that watercourses support an abundance of both browse-foods and grass (Young, 1972; Dunham, 1994).

Grass production is controlled by rainfall patterns throughout the seasonal cycle, with maximum growth, and elevated nutritional value, generally occurring during the rainy season (Acocks, 1988; Tainton, 1999). Accordingly, herbivores respond to changes in rainfall and primary productivity (Coe *et al.*, 1976), thus one might expect a sharp switch to higher grass intake as soon as fresh, nutritious grass becomes available in the growing (wet) season. Indeed, this appears to be the case in several savannas across Africa (Monro, 1980; Dunham, 1980; Meissner *et al.*, 1996; Wronski, 2002). However, dry-to-wet season increases in grass consumption amongst Kruger Park impala were only apparent for three out of the five principal landscapes included (Tale 5.2). Nevertheless, temporal shifts in diet were evident within all landscapes at different time scales, i.e. from year-to-year and at monthly resolutions (Figures 6.2 and 6.3). To control for this variation, I performed linear regression analysis between mean grass intake and rainfall for all months, years, and landscapes. The regression shows no significant correlation between variations in %C_4-intake and rainfall ($r^2 = 0.03$, $P = 0.14$; Figure 6.5a). Assuming that rainfall offers a reasonable prediction for grass production (Coe *et al.*, 1976; Acocks, 1988; Meissner *et al.*, 1999; Tainton, 1999), this result shows that dietary complexity amongst impala cannot be ascribed simply to variations in grass availability. The relationship improves slightly, but remains insignificant, when a lag time between rainfall and grass regeneration (one month) is fitted to the regression model ($r^2 = 0.09$, $P = 0.08$).

Based on these data, the hypothesis that impala consume grass in direct proportion to its availability must be rejected. Several studies have found that impala are selective feeders, choosing foods from the surrounding environment to optimize nutritional uptake (Monro, 1980; Attwell and Bhika, 1985; Meissner *et al.*, 1996; Wronski, 2002; Chapter 5). Protein (and therefore nitrogen) is considered the most limiting nutrient for savanna ungulates (Jarman, 1974; Owen-Smith and Novellie, 1982), hence diet selectivity in impala is best characterized based on protein uptake, discernible (to some degree) from faecal N content. Faecal %N for Kruger Park impala shows few differences across landscapes and seasons (Table 6.1). Regression analysis also showed no significant correlation between faecal %N and variations in $\%C_4$ intake ($r^2 = 0.00$, $P = 0.59$; Figure 6.5b), despite considerable regional and temporal differences in proportions of browse:grass eaten. This result is somewhat surprising since woody plants have consistently higher N content than grasses (Hofmann, 1989; Robbins, 1993; van Soest, 1994; Meissner *et al.*, 1999; Chapter 4). In addition, impala faecal %N did not scale linearly with rainfall (Figure 6.5c; $r^2 = 0.00$, $P = 0.59$). The implication is that impala maintain a similar diet quality in all conditions, regardless of the type of food they eat. Similarly, both Meissner *et al.* (1996) and Sponheimer *et al.* (2003e) concluded that impala diet quality is maintained in all environments.

The observation that impala eat more grass in sodic areas, even when broader landscape and geological differences are considered, provides further evidence for optimal nutritional selection. Sodic patches generally support stands of palatable and relatively nutritious grasses, which are often over-utilized by herbivores (Venter *et al.*, 2003). Unpublished data (Lee-Thorp and Sponheimer) for Kruger Park grasses are consistent with this trend, i.e. sodic-site grasses have a higher %N than grasses from other habitats. In the north, the sodic sampling site was heavily overgrazed and almost devoid of grasses (personal observation). Artificial waterholes are also expected to promote growth of nutritious grass species (Grant *et al.*, 2002). Indeed, impala faeces from the Longtoon Dam reflected relatively high grass intake (~68%). The Elandskuil waterhole, however, did not appear to promote grazing, likely because this waterhole was closed during the course of this study as part of implementation of the Park's management policy for promoting habitat heterogeneity (see Gaylard *et al.*, 2003).

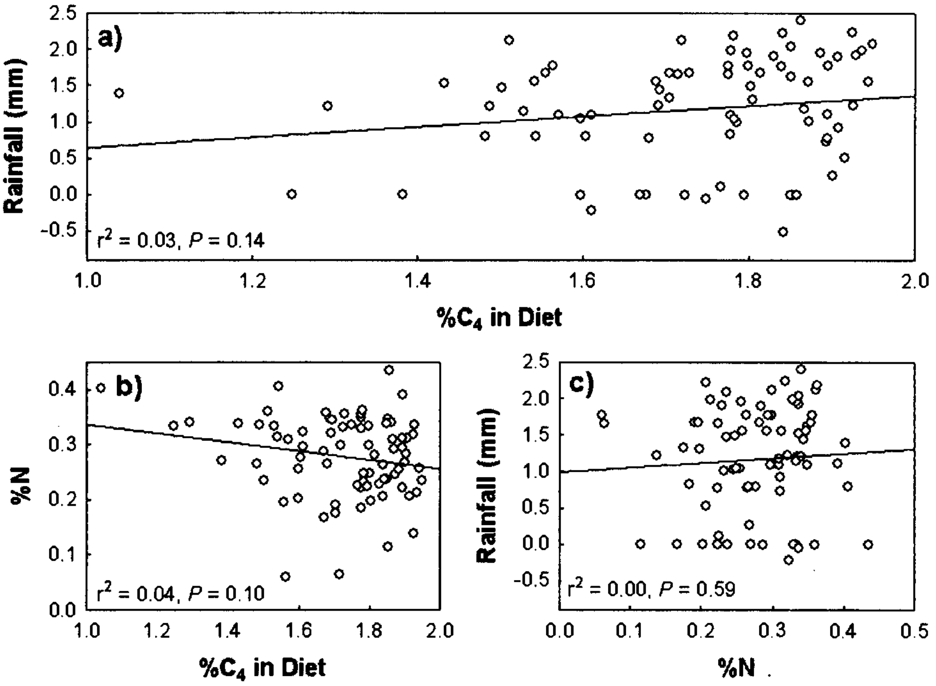

Figure 6.5. Linear regressions revealing lack of significant correlations between diet (%C_4-intake) and diet quality (faecal %N) with rainfall amongst Kruger Park impala. Symbols show the means for every month, year, and landscape included in the study (log-transformed data).

Faecal %N of herbivores feeding on woody plants is often elevated because of condensed tannins in the diet that reduce protein digestibility, resulting in precipitation of protein in faeces (Robbins *et al.*, 1987a). The lack of negative correlation between faecal %N and %C_4 grass (which has a much lower tannin content than browse-based foods) in the diet of impala (Figure 6.5b) may thus indicate that optimal foraging also allows them to avoid intake of harmful secondary compounds. Indeed, many herbivores avoid foods with high concentrations of secondary compounds (Cooper and Owen-Smith, 1985; du Toit, 2003; Lindroth and Dearing, 2005). This behaviour possibly explains why impala prefer grass to browse in the densely wooded regions around Punda Maria. Similar data for browsing kudu *Tragelaphus strepsiceros* at Punda Maria revealed higher faecal %N in this region compared to other savanna landscapes (Chapter 5). Thus, while kudu browsing at Punda Maria perhaps cannot avoid relatively tannin-rich diets, impala do so by switching to an alternative food source, i.e. grass.

Plants and their mammalian predators have long evolved in parallel, plants having adopted many physical and chemical defense mechanisms, and herbivores an array of behavioural, morphological, and physiological adaptations to overcome plant defenses (Owen-Smith and Novellie, 1982; Cooper and Owen-Smith, 1985, 1986; Georgiadis and McNaughton, 1988; Illius, 1997; Lindroth and Dearing, 2005). Thus, herbivores foraging in the past were likely faced with similar challenges as they are today, so that patterns observed for impala offer a glimpse into driving mechanisms for transitions between browsing and grazing in ungulate evolutionary history. Previously, Perez-Barberia *et al.* (2001a), using a maximum likelihood model for diet and habitat trait evolution based on reported data for numerous ungulate species, suggested a three-step transition from browsing to grazing. Their model implied that primitive browsers initially adopted a mixed-feeding diet within closed, wooded habitats. Thereafter, ungulates changed their habitat preference for more open environs, and in the third phase, adopted a grazing diet. Results for Kruger Park impala are to some degree consistent with this interpretation;

impala in all habitats consume a mixed-diet, and notably their diets in the heavily wooded regions of Punda Maria are particularly grass-rich. Even in the riparian zones, where browsing is at a maximum, grasses consistently make up more than 20% of their diets.

Diet quality is a fundamental aspect of herbivore feeding ecology. The nutritive value of foods consumed may be an even more important predictor of herbivore performance than perceived morpho-physiological adaptations (Gordon and Illius, 1994, 1996). Diet switches between browsing and grazing amongst impala do not result in differences in diet quality, which is not altogether surprising because metabolisable energy yields of browse and grass may be similar (Illius, 1997; Owen-Smith, 1997; Chapter 4). But, the fundamental mechanism for diet shifts appears to be selection for optimum protein uptake, possibly in conjunction with maintaining dietary flexibility to avoid excessive intake of plant secondary compounds (Meissner *et al.*, 1996; Wronski, 2002; this study). Similar optimal foraging has been documented previously for browsing kudu (Owen-Smith and Novellie, 1982; Owen-Smith, 1994) and eland *Taurotragus oryx* (Watson and Owen-Smith, 2000). For impala, optimal foraging is not achieved through preferential feeding on either browse or grass, but food choice appears to be determined by the relative benefits of these food types across various feeding-patch scales. It is likely that dietary transitions are regulated by microhabitat/patch scale differences in food quality, especially during limiting periods (e.g. the dry season), agreeing with interpretations based on %N and data for fibre content of plants from this region (Chapter 4). If this is true, morpho-physiological adaptations to diet are likely only of secondary importance to ecological diversification, and probably occurred in an evolutionary sense only after new diets had been adopted. This might explain why differential digestive anatomies of browsers and grazers do not necessarily reflect different abilities to maximize digestion of either high-protein (browse) or high-fibre (grass) foods (see Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995).

A potential drawback to this negative feedback interpretation is whether impala are indeed representative of diet switches in the past (and present) across other ungulate taxonomic and ecological guilds. One other species that shows a similar discordance between diet and habitat composition occurs in the African elephant *Loxodonta africana*. Carbon isotope evidence for elephant diets in Kruger Park have shown that they eat more grass in the broad-leaved, more wooded mopane landscapes of the north than in the less-wooded southern regions (Codron, J. *et al.*, 2006). The pattern that emerges across both mixed-feeding species, as well as from more subtle changes in the diets of grazers and browsers (see Chapter 5), is that diet quality is the primary environmental driver of transitional feeding.

Notably, transitions from browsing to grazing as evinced from the fossil record differ widely amongst different lineages in terms of the timing and duration of the change (Janis *et al.*, 2000; Cerling *et al.*, 2005; Segalen *et al.*, in press). For instance, the precursors of equids originated as browsers, but evidence from dental morphology confirms that by 10 Ma, the equids had already adopted a predominantly grazing diet (feeding on C_3 grass) (Janis *et al.*, 1994). Carbon isotope data from fossil tooth enamel from the Turkana Basin, Kenya, have revealed rapid transitions to C_4 grass-dominated diets during the Late Miocene in equids and proboscideans, but more gradual changes amongst suids (Cerling *et al.*, 2005). Mixed-feeder phases have also been recorded for several bovids and other ungulate taxa from more recent South African Plio-Pleistocene sites (Segalen *et al.*, in press), implying that the timing of Neogene dietary transitions was not a uniform event across the African continent. Based on results of the current study, I suggest that equivocal patterns of diet shifts observed across different taxa and landscapes should be assessed in terms of changes in plant nutritional value forcing ungulates feeding in dynamic palaeolandscapes to adopt alternate feeding strategies. Such changes may have included decreases in primary productivity and lower nutritional value of available foods (especially C_3 plants) mediated by declining atmospheric CO_2 levels (Janis *et al.*, 2000; Lindroth and Dearing, 2005). The latter may have had significant impacts on metabolic costs for plants to produce herbivore defense chemicals (Lindroth and Dearing, 2005). Given that plant tannin content appears to be an important driver of ungulate diet switches (Owen-Smith and Cooper, 1989; Owen-Smith, 1993; du Toit, 2003), it is quite possible that transitions to grazing occurred largely as animals replaced their relatively tannin-rich browse diets with a much less limiting food source in terms of secondary compound concentrations.

6.5. Conclusion

Dietary variations amongst mixed-feeders such as impala occur not only across different landscape and seasonal scales, but also annually or sub-annually. This shows that studies of dietary variation should look beyond traditional spatio-temporal differences, but also over the longer-term. In this study, data for impala span a three-year period; doubtless, further insights would be gained at larger decadal or even centennial scales. Carbon isotope ecology is shown here to be an extremely powerful tool for examining high-resolution diet shifts, but with appropriate historical materials such as bones and teeth, insights can also be gained over longer-term periods.

One limitation to this study is the use of faecal %N alone as a proxy for diet quality. While the evidence appears to have implications for other aspects of diet quality, such as intake of protein-precipitating secondary compounds, such interpretations are tentative. Diet quality and selection for optimum nutritional quality foods is likely the primary mechanism for niche differentiation amongst ungulate herbivores. To fully understand the ecological and palaeoecological implications of diet switching in both the present and the past, future studies will need to find ways to address variations not only in the types of foods consumed, but also in terms of diet quality across multiple spatio-temporal scales.

Table 6.1. Regional and seasonal mean δ^{13}C, δ^{15}N, and %N of impala faeces from Kruger Park. For this broad-scale comparison, the Park was divided into southern and northern basalt and granite landscapes, respectively, and the Punda Maria region. The dry season is represented by samples collected between April and September, while samples from October to March represent the wet season.

			δ^{13}C (‰)			δ^{15}N (‰)		%N	
Region	**Season**	***n***	**Mean**	**SD**	**%C4 in Diet**	**Mean**	**SD**	**Mean**	**SD**
Southern basalts	Dry	61	-18.0	2.6	62	4.2	1.7	1.8	0.5
	Wet	45	-17.6	2.6	71	5.9	1.9	2.0	0.5
Southern granites	Dry	67	-21.3	2.5	40	6.7	1.7	2.0	0.4
	Wet	50	-19.4	2.8	58	8.1	2.2	2.3	0.6
Punda Maria	Dry	65	-17.3	2.1	71	4.4	1.6	1.8	0.5
	Wet	60	-16.7	1.6	74	5.2	1.3	1.9	0.3
Northern basalts	Dry	88	-19.8	2.6	53	4.8	2.0	1.9	0.5
	Wet	78	-16.6	1.9	74	7.2	2.0	1.9	0.3
Northern granites	Dry	44	-21.9	2.5	37	5.6	1.6	2.1	0.5
	Wet	48	-19.8	2.4	53	6.7	1.5	2.0	0.4

n = number of samples
SD = standard deviation

CHAPTER 7

The Functional Significance of Diet Type and Diet Quality for Ungulate Ecological Diversity

In this chapter, I test the conflicting models of i) feeding style (browser/grazer/mixed-feeder) and ii) body size, for ecological niche separation amongst ungulates. I combine stable isotope data from faeces of Kruger Park ungulates, reflecting variations in percent C_4 grass intake (as well as more subtle variations in quantities of different types of C_3 and C_4 foods consumed) with nutritional data (%N, NDF, ADF, and ADL). I use a series of linear regression analyses to explore the relationships between diet type, diet quality, and body size.

7.1. Introduction

Numerous models have been proposed that describe ecological niche differentiation amongst ungulate herbivores from a variety of dietary and nutritional guilds (Gwynne and Bell, 1968; Bell, 1970; Hofmann and Stewart, 1972; Jarman, 1974; Janis, 1976; Demment and van Soest, 1985; Duncan *et al.*, 1990). The most widely-accepted scheme is one that distinguishes browsers from grazers, based on differences in the type of foods they eat and dichotomous digestive adaptations to deal with either food type, i.e. the diet-type model (Hofmann, 1989). This scheme has been challenged on the basis of compartmental models of digesta flow that revealed little or no difference between browsers and grazers in terms of digesta retention time and overall digestive efficiency (Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995). Dietary distinction may also be obscured by the effect of body size; a number of studies have reported allometric scaling between body size, digestive function, and diet quality, i.e. the diet-quality model (Bell 1970; Jarman, 1974; Demment and van Soest 1985; Owen-Smith, 1988; Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995; Gagnon and Chew, 2000). Gordon and Illius (1994, 1996) even suggest that body size, rather than the browser/grazer continuum, is in fact the primary determinant of niche diversity.

Although recent tests for various aspects of these models have entailed novel statistical approaches (e.g. Illius and Gordon, 1992; Brashares *et al.*, 2000; Gagnon and Chew, 2000; Peres-Barberia *et al.*, 2004), models are based on dietary and ecological data available from published field studies, which do not capture the full extent of inter- and intra-specific dietary variation that occurs in nature. Collating data from numerous studies, carried out over non-comparable spatial and temporal scales, does not always produce desirable results because accounts of diet and ecological niche differentiation are likely to be site- and/or season-specific (Owen-Smith, 1988; see also Chapters 3 and 5). Information about digestive physiology, on the other hand, is available mostly from controlled-feeding experiments (but see Meissner *et al.*, 1996; Bodenstein *et al.*, 2000). While controlled studies yield valuable data about digestive parameters such as mean retention time and passage rate, insights are limited because artificial diets seldom represent the natural food intake of a species, and the animals are not exposed to the same array of environmental stimuli they experience in free-ranging conditions.

Stable carbon isotope ecology as a technique for recording and accounting for dietary variations over multiple taxonomic, spatial, and temporal scales has been thoroughly researched throughout this thesis. Faecal %N provides a useful measure for diet quality (Holecheck *et al.*, 1982; Leslie and Starkey, 1985; Wrench *et al.*, 1997; Grant *et al.*, 2000). Fibre content (%NDF, ADF, and ADL) of faeces have also been used as proxies for diet quality (e.g. Erasmus *et al.*, 1978; Dörgeloh *et al.*, 1998), although it is difficult to tease out whether data reflect differential digestive efficiencies amongst animals, or differences in the quality of foods consumed. In this chapter I combine stable carbon isotope evidence for diet with data for %N and fibre content of faeces to test the effects of feeding style and diet quality on niche separation in free-ranging conditions. Results are based on faecal specimens representing dietary variation across numerous temporal scales (seasonal and monthly) from the Kruger National Park, South Africa.

7.2. Methods

7.2.1. Materials and Methods

This study is based primarily on faeces collected from the northern basalts (NB) of Kruger Park. Data for taxa not represented on the NB were taken from other regions of the Park where available, including northern granites (NG) southern basalts (SB), southern granites (SG), and Punda Maria in the far North of Kruger (see Table 7.1). Of these, fifty-four specimens were selected for detergent fibre analysis, chosen to incorporate dry and wet season material, taking two dry and two wet season specimens for each species where possible. In cases where available sample was too small for analysis (<0.5 g), several individual samples representing the same species, region, and season were pooled (see Table 7.2).

Analysis of faecal $\delta^{13}C$ and %N followed procedures described in Chapter 3, and %C_4 grass intake was calculated using the dual-endpoint mixing model (based on plant data from Codron, J. *et al.*, 2005 *and* Chapter 5 for monthly samples from northern basalts and Punda Maria). Analysis of faecal fibres followed the procedure described in Chapter 4 for plants. Briefly, dried, powdered samples were analyzed for %N by combustion in an automated Elemental Analyzer (Carlo Erba, Milan) and introducing the resultant N_2 gas to a Finnigan MAT 252 or DELTA XP Mass Spectrometer via a continuous flow-through inlet system (Conflo). Exposure to neutral detergent and acid detergent solutions in an ANKOM 220 Fiber Analyzer yielded NDF and ADF fractions, respectively, and ADL was determined by exposure of the ADF fraction to 72% (by weight) H_2SO_4. All fractions are presented in terms of organic matter (OM), correcting for ash residue following furnace combustion at 525°C. Despite relatively small sample sizes for faecal fibre analysis, these data are sufficient for this preliminary study across a broad suite of taxa with diets ranging across the browser/grazer continuum. In any case, variations between two samples representing a particular species within each season are relatively small, less than 5% for NDF, ADF, and ADL, with few exceptions. Thus single faecal samples seem to provide a suitable representation of average excretion in the field, and data are unlikely to vary substantially with multiple collections and analyses, although it remains that these data should only be considered preliminary. Nevertheless, a 5% error may significantly influence diet quality and digestibility. Hence, comparisons are restricted to correlations between isotopic indices for diet and faecal indices for diet quality rather than making inter-specific comparisons (except for *post hoc* interpretations for species with substantially deviant values, e.g. giraffe *Giraffa camelopardalis*).

7.2.2. Data Analysis

Although feeding style appears to be continuous, rather than categorical (Perez-Barberia *et al.*, 2004; this thesis), I test differences between means of species listed as browsers, grazers, and mixed-feeders, using analysis of variance (ANOVA). This categorization is necessary because one objective of the study is to properly test Hofmann's (1989) categorical model of feeding style. All data were log-transformed for linear regression analyses, to determine correlative interactions between variables. Analysis of variance also revealed no differences between foregut and hindgut fermenters for carbon isotope, %N, and fibre data ($P > 0.09$ for all cases; Tables 7.1 and 7.2). Thus, despite the fact that these groups differ widely in digestive physiology (e.g. Janis, 1976; Duncan *et al.*, 1990), foregut and hindgut species are treated equally.

7.3. Results and Discussion

7.3.1. Relationships Between C_4-grass intake, Faecal %N, and Body Size

Proposed effects of body size on diet and nutritional physiology of ungulates have been discussed by a number of authors (Bell 1970; Jarman, 1974; Demment and van Soest 1985; Owen-Smith, 1988; Gordon and Illius, 1994, 1996). In principle, smaller-bodied animals consume higher quality foods than do larger species, in order to maintain higher metabolic rates. Amongst African Bovidae, a positive relationship between body mass and grass (monocot) intake has previously been proposed, suggesting that larger species (usually grazers) consume poorer quality (lower protein:fibre ratio) foods than smaller species (Jarman, 1974; Gagnon and Chew, 2000; Cerling *et al.*, 2003). Carbon isotope data for southern African bovids, by contrast, showed no significant correlation between body mass and grass intake (Sponheimer *et al.* 2003a).

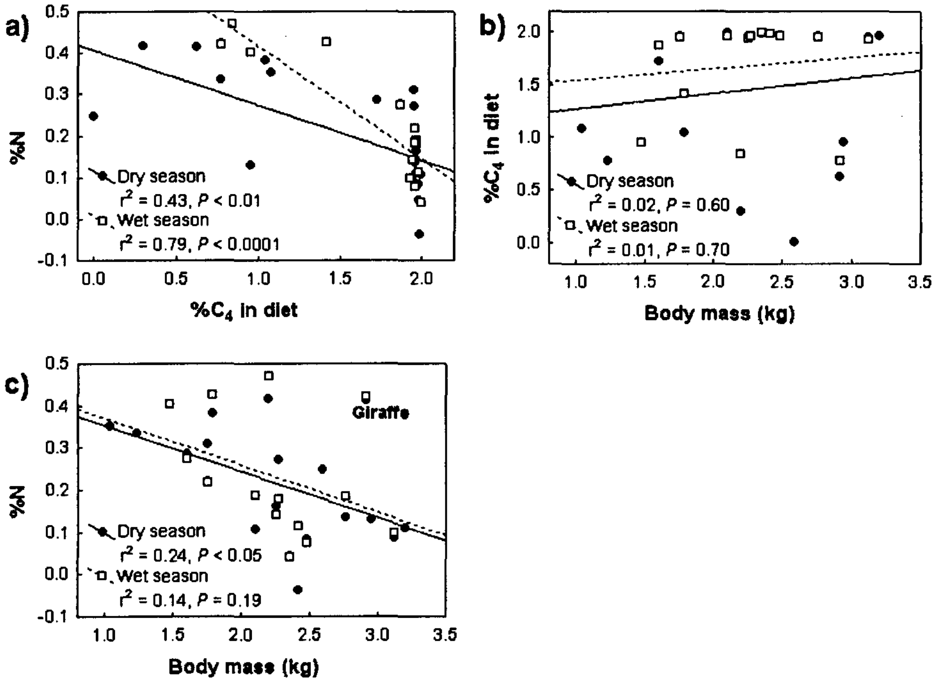

Figure 7.1. Relationship between diet type, diet quality, and body size amongst Kruger Park ungulates, based on faecal $\delta^{13}C$ (reflecting %C_4-intake), %N, and mean body mass of females reported in Skinner and Smithers (1990) and Estes (1991). Symbols depict seasonal means for individual species (all data log-transformed).

In the current study, proportions of C_3 (browse):C_4 (grass) foods consumed by Kruger Park ungulates are available from carbon isotope data of faeces (Table 7.1). Faecal %N is a reflection of crude protein intake, providing one indication of diet quality (Erasmus *et al.*, 1978; Holecheck *et al.*, 1982; Leslie and Starkey, 1985; Wrench *et al.*, 1997; Grant *et al.*, 2000). Faecal N content ranged from 0.9 ± 0.2% (*n* = 47) for roan antelope *Hippotragus equinus* in the dry season to 3.0 ± 0.3% (*n* = 59) for kudu *Tragelaphus strepsiceros* in the wet season (Table 7.1). Despite this variation, there is no significant effect of season on faecal %N (ANOVA $F_{1,\ 1267} = 3.033$, $P = 0.08$). Low seasonal variation in %N is likely due to particularly low rainfall during the study period. Indeed, %N of local vegetation also showed no significant seasonal changes during the same time period (see Chapter 4). Overall, species normally classified as browsers, grazers, and mixed-feeders have significantly different faecal %N ($F_{2,\ 1266} = 941.568$, $P < 0.001$), with browsers having the highest values and grazers the lowest. This parallels expectations for higher %N of savanna browse foods compared to grass (e.g. Hofmann, 1989; Robbins, 1993; van Soest, 1994; Owen-Smith, 1997; Meissner *et al.*, 1999; Chapter 4). Accordingly, faecal %N increases linearly with decreasing C_4 grass intake (Figure 7.1a; $r^2 = 0.43$, $P < 0.01$ for the dry season; $r^2 = 0.79$, $P < 0.0001$ for the wet).

Body size was taken from the mean body mass of females, reported in Skinner and Smithers (1990) and Estes (1991). Linear regression analysis of body mass and %C_4-intake derived from faecal carbon isotope data reveals no significant relationship between body size and the amount of grass consumed in both the dry ($r^2 = 0.02$, $P = 0.60$) and the wet season ($r^2 = 0.01$, $P = 0.70$; Figure 7.1b). The lack of significant relationship persists even when only bovids are included in the analysis ($r^2 = 0.05$, $P = 0.50$ and $r^2 = 0.25$, $P = 0.14$, for the dry and wet season, respectively), which parallels findings of Sponheimer *et al.* (2003a) for bovids from across the subcontinent. As noted by Sponheimer *et al.* (2003a), the main reason for this result is the presence of large-bodied tragelaphines such as eland *Taurotragus oryx* and kudu at the C_3 end of the browser/grazer spectrum. In the current study, large-bodied browsers such as giraffe and black rhinoceros *Diceros bicornis* further diminish predictions based on body size. The positive correlations between body mass and grass intake reported by Gagnon and Chew (2000) and Cerling *et al.* (2003) appear to be because those studies incorporated data for numerous small-bodied browsers, especially *Cephalophus* spp. duikers. There are sufficient large-bodied browser species amongst African ungulates to reject the hypothesis for a relationship between body size and grass consumption. The aggregation of closely-related taxa at the browsing end of the continuum (e.g. Gagnon and Chew, 2000) is therefore more likely due to phylogenetic retention of character traits (*cf.* Perez-Barberia *et al.*, 2004) rather than preference for browse-based foods amongst small taxa.

Given the lower metabolic requirements per unit body mass (Demment and van Soest, 1985; Illius and Gordon, 1992; Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995), and the conventional assumption that grass is of lower nutritional quality, the poor correlation between body mass and grass intake is perhaps unexpected. However, as shown in this thesis, diet quality cannot necessarily be interpreted on the basis of browse:grass intake; woody plants are more lignified and contain a higher proportion of digestion-inhibiting phenols and other secondary compounds than do grasses (Gordon and Illius 1996; Illius 1997; Owen-Smith, 1997; Meissner *et al.* 1999; Chapter 4). Hence, metabolisable energy yields of browse foods compared to grasses do not necessarily differ according to protein:fibre ratios, as is often assumed. A more appropriate approach is to examine changes in faecal %N and hence diet quality across different body sizes. Linear regression analysis shows a significant correlation between faecal %N and body size, but only during the dry season (Figure 7.1c; $r^2 = 0.24$, $P < 0.05$). The negative slope of the regression agrees with predictions that larger animals subsist on poorer quality foods than smaller-bodied species (e.g. Owen-Smith, 1988; Gordon and Illius, 1996), and the trend persists regardless of whether the diet is browse- or grass-based. Previously, Gordon and Illius (1996) hypothesized that the longer digesta retention times of large-bodied species, resulting in a greater potential to digest dietary fibre, allows them to avoid competition with smaller species by consuming poorer quality foods in the dry season. They reasoned that increased body size evolved as an adaptation to exploit a wider diversity of resources during periods of limitation, an assertion supported by data presented here. During less limiting periods (i.e. the wet season), species of all sizes appear to consume a similar range of diet qualities, in the presence of an abundance of high quality forages. Indeed, Figure 7.1c shows that during the wet season, faecal %N does not correlate with body size ($r^2 = 0.14$, $P = 0.19$).

Some caution should be observed when interpreting faecal %N as a direct measure of diet quality. The relationship between dietary and faecal %N, especially amongst browsers, is complicated by protein-precipitating effects of phenolic compounds in the diet, and nitrogenous contributions to faeces from sloughed endogenous materials and gut microbes (Arman *et al.*, 1975; Robbins *et al.*, 1987a; van Soest 1994). For example, giraffe faecal %N was consistently higher than might be expected for its large size (Fig. 6.1c), possibly a result of high tannin intake on a browse-based diet. Browsing kudu, too, had relatively high faecal %N, but values for this species did not deviate from the %N / body mass regression curve to the same extent as did giraffe. Removal of giraffe from the analysis not only strengthened the correlation between faecal %N and body size during the dry season ($r^2 = 0.42$, $P < 0.01$), but also revealed a pattern of decreasing diet quality (faecal %N) with increasing body size even in the wet season (r2 = 0.38, $P < 0.05$). Thus, differential diet quality-niche occupation between small- and large-bodied species may persist even during less limiting periods (wet season). Given the extremely low rainfall for the study period, however, it is possible that ungulates experienced some level of resource limitation during both seasons, resulting in diet quality-driven niche differentiations (*sensu* Gordon and Illius, 1996) throughout the seasonal cycle.

7.3.2. Faecal Fibre Content, C_4-intake, and Body Size

Results of analysis of faeces fibre content (NDF, ADF, and ADL) are presented in Table 7.2. One-way ANOVA's showed that faecal NDF does not differ between browsing (mean = 57.98 ± 8.75%, $n = 13$), mixed-feeding (mean = 59.07 ± 8.60%, $n = 8$), and grazing (mean = 57.61 ± 5.30%, $n = 33$) groups ($F_{2, 51} = 0.153$, $P = 0.86$). ADF of browser faeces (mean = 43.58 ± 8.12%) is higher than that of mixed-feeders (mean = 38.53 ± 3.47%), which are in turn higher than grazers (mean = 29.86 ± 4.18%) (F = 33.964, $P < 0.0001$). Mean ADL is also highest in browser faeces (mean = 25.07 ± 7.91%), lowest in grazers (mean = 7.25 ± 3.49%), and intermediate for mixed-feeders (mean = 17.67 ± 4.48%) (F = 62.840, $P < 0.0001$). There is no significant effect of season on these data (ANOVA $F_{1, 52} = 2.895$, $P = 0.09$ for NDF; F = 0.347, $P = 0.56$ for ADF; and F = 0.796, $P = 0.38$ for ADL), paralleling the lack of significant seasonal changes in fibre content of local vegetation collected over the same time period (see Chapter 4). Therefore, in the analyses that follow, wet and dry season data are treated together.

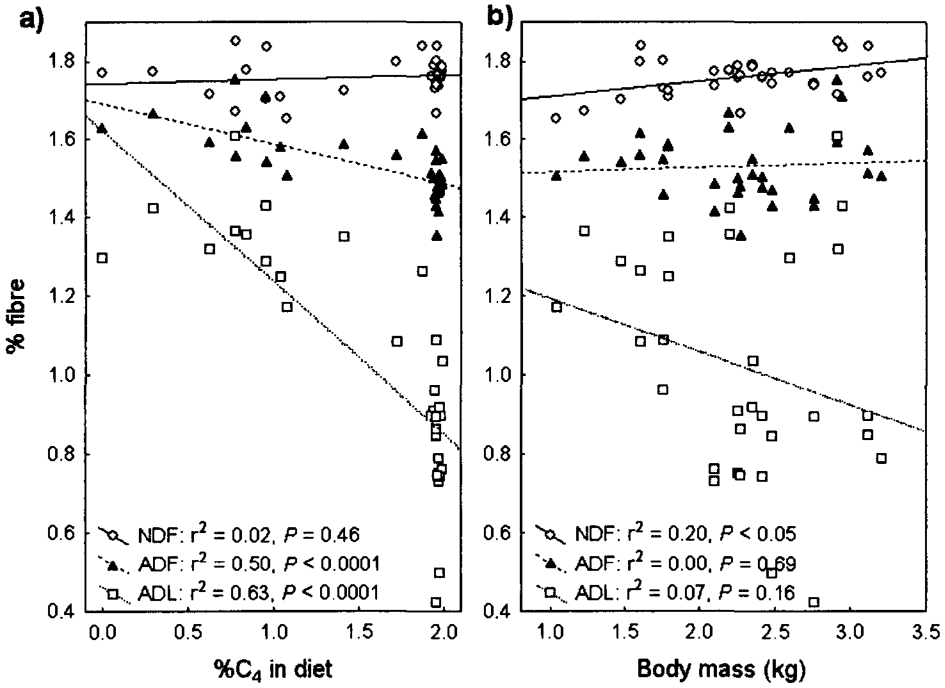

Figure 7.2. Correlations of faecal fibres with a) %C_4-intake (from faecal $\delta^{13}C$), and b) body size amongst Kruger Park ungulates. Symbols depict seasonal means for individual species (all data, including means for body mass are log-transformed).

Linear regression analysis shows that faecal NDF does not vary with grass intake ($r^2 = 0.02$; $P = 0.46$), but ADF and ADL decrease with increasing C_4 (grass) in the diet (Figure 7.2a; $r^2 = 0.50$, $P < 0.0001$ and $r^2 = 0.63$, $P < 0.0001$, respectively). Lower faecal ADL with lower grass intake confirms that digestibility of lignin is negligible amongst ungulate herbivores, since grass has a much lower lignin content than woody plants (Demment and van Soest, 1985; Robbins, 1993; van Soest, 1994; Meissner *et al.*, 1999; Chapter 4). By contrast, the results for faecal NDF and ADF do not reflect trends in vegetation, because savanna grasses have far higher NDF and ADF contents than trees and forbs. The increased NDF and ADF loss via faeces in browsers is almost certainly owing to the reduced potential digestibility of cell walls (based on higher ADL and lower NDF content) of browse foods (see Illius, 1997; Owen-Smith, 1997; Chapter 4). It is unclear from these data alone whether similarities in faecal NDF across the browser-grazer spectrum represent the higher fractions of digestible fibre in grass cell walls compared to woody plants, a higher fibre-digestion efficiency amongst grazers, or both.

Two recent studies have advocated higher fibre-digestion efficiency amongst grazers compared with browsers. Clauss *et al.* (2002) studied the distribution of particle sizes amongst faeces of ruminants, and found that classified browsers generally had larger particle sizes in their faeces than mixed-feeders and grazers. Their study concurs with predictions that the rumen anatomy of grazers allows them to achieve greater retention of larger particles, increasing ruminal fermentation of digesta (Hofmann, 1989; Ditchkoff, 2000; Clauss and Lechner-Doll, 2001). Perez-Barberia *et al.* (2004) examined patterns of NDF digestibility (NDFD) amongst ruminants from data reported from controlled-feeding experiments. They implemented a residual maximum-likelihood model (REML) that accounted for phylogenetic affiliations, as well as variations in body size and diet quality (lignin:NDF ratios), and found that NDFD increased with increasing grass in the natural diet. However, Clauss *et al.* (2002) were unable to control for differences in particle sizes precipitated by different food types, and Perez-Barberia *et al.* (2004) relied on published data for natural diets that do not account for intra-specific variations (e.g. Chapter 3). There is also contention about the content of the natural diets of some of the species used by Perez-Barberia *et al.* (2004). Eland, for example, are traditionally listed as mixed browser/grazers (e.g. Hofmann and Stewart, 1972; Skinner and Smithers, 1990; Perez-Barberia *et al.*, 2004), but recent field and carbon isotope studies suggests that they are primarily browsers in many South African savannas (Watson and Owen-Smith, 2000; Sponheimer *et al.*, 2003a; Codron *et al.*, 2005b; Chapter 3). Similarly, roan antelope are expected to consume between 10 and 15% C_3 browse foods (Owen-Smith, 1997; Gagnon and Chew, 2000; Perez-Barberia *et al.*, 2004), but carbon isotope data reflect almost pure C_4 diets in various southern and East African habitats (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003a; Chapter 3). Digestive efficiency may also vary considerably through time. In the Timbavati, a region bordering the western boundary of Kruger Park, Burchell's zebra *Equus burchellii* and blue wildebeest *Connochaetes taurinus* showed changes in NDFD ranging from ~35 to 50% and ~40 to 55%, respectively, throughout the seasonal cycle (Bodenstein *et al.*, 2000).

There is a trend of increasing faecal NDF with increasing body mass (Figure 7.2b; $r^2 = 0.20$, $P < 0.05$). This result reinforces assertions that body size significantly influences digestive efficiency (Foose, 1982; Owen-Smith, 1988; Gordon and Illius, 1994, 1996; Robbins *et al.*, 1995). The lack of correlation between feeding style and NDF is contrary to Hofmann's (1989) predictions for a browser/grazer dichotomy; even though faecal NDF differed between species such as giraffe and similar-sized grazes, the lack of correlation between faecal NDF and diet across all body size classes suggests that such differences are due to body size rather than feeding style. Perez-Barberia *et al.* (2004) discerned no relationship between NDFD and body mass, which they ascribed to the fact that small frugivorous species *Cephalophus monticola* and *Tragulus javanicus* achieve unusually long retention times relative to their small size (see also Shipley and Felicetti, 2003). However, the resultant high NDFD of these animals is perhaps a reflection of the fact that many fruits contain up to 70% NDF (Barton *et al.*, 1993; Molloy and Hart, 2002; Chapter 4), impressing that diet quality is an important determinant of niche differentiation.

Neither Perez-Barberia *et al.* (2004) nor Clauss *et al.* (2002) controlled for the effects of differential retention time in the gut. Excluding duikers, the lower quality diets of larger-bodied species almost certainly necessitate improved digestive efficiencies, which may be achieved through longer retention times. For example, Illius and Gordon (1992) reported particularly strong correlations between mean retention time and body size for both ruminants (r^2 = 0.97) and hindgut fermenters (r^2 = 0.94), regardless of dietary preferences. Further data for retention time of different food types across a wide range of species are needed to refine the diet quality hypothesis.

7.3.3. Implications for Niche Differentiation

To test the combined effects of diet-type and diet quality on niche partitioning, I performed an inter-group cluster analysis, based on Euclidean distances, using carbon isotope reflections of diet type (%C_4 intake), and faecal proxies for diet quality (%N, NDF, ADF, and ADL). The cluster analysis shows that while browsers (group 1), mixed-feeders (group 2), and grazers (group 3) partition into discrete groups, ecological differentiation occurs at other scales within this continuum (Figure 7.3). This finding parallels results based on combined carbon and nitrogen isotope evidence (Chapter 3), except in that instance dietary niches were identifiable based on relative inputs of C_3 and C_4 foods (as well as more subtle changes in dietary inputs of leaves, fruit/flowers, NADP-

ME grasses, NAD/PCK grasses, and sedges). Here, ecological partitioning is based largely on diet quality.

The cluster analysis suggests that species with similar diets and/or nutritional uptake (at least based on faecal evidence for diet quality) occupy distinct nutritional niches. Within each guild, body size appears to be crucial for competition avoidance. There are two groups of browser, representing larger-bodied (> 100 kg, group 1a) and smaller species (< 100 kg, group 1b), respectively. Niche partitioning amongst browsers are based not only on body mass, but on height as well. For example, the four species within group 1a, giraffe, black rhinoceros, kudu, and eland, all belong to a different height class and therefore forage at different canopy levels (Skinner and Smithers, 1990). This finding is consistent with a proposed three-dimensional mechanism for ecological separation amongst browsers (Fritz *et al.*, 2002; du Toit, 2003), and further enhances predictions that body size places fundamental constraints on herbivore feeding ecology. Smaller browsers (group 1b) appear to occupy different feeding niches in terms of the types of foods they eat; nyala *Tragelaphus angasii* and grey duiker *Sylvicapra grimmia* both eat significant proportions of grass in Kruger Park and elsewhere in Africa (Gagnon and Chew, 2000; Chapter 3), whereas steenbok *Raphicerus campestris* and bushbuck *Tragelaphus scriptus* are almost exclusive browsers in this region (Chapter 3; this study).

The trophic groups revealed by this cluster analysis support, to some degree, findings of Fritz *et al.* (2002), based on principal component analysis of megaherbivore and "mesoherbivore" responses to environmental conditions across various savanna systems. They suggested that megaherbivores form a separate trophic guild from mesoherbivores, although they did not differentiate between megaherbivore feeding guilds. The current study suggests similar nutritional niches amongst megabrowsers (giraffe and black rhinoceros) and smaller eland and kudu (Figure 7.3). Even smaller browsers (group 1b) are, however, ecologically distinct from the larger species, because the former appear to eat much higher quality foods. The current analyses show no ecological differentiation between megagrazers (hippopotamus *Hippopotamus amphibius* and white rhinoceros *Ceratotherium simum*) and sympatric mesograzers, suggesting that not all megaherbivores occupy a distinct nutritional niche compared to mesoherbivores.

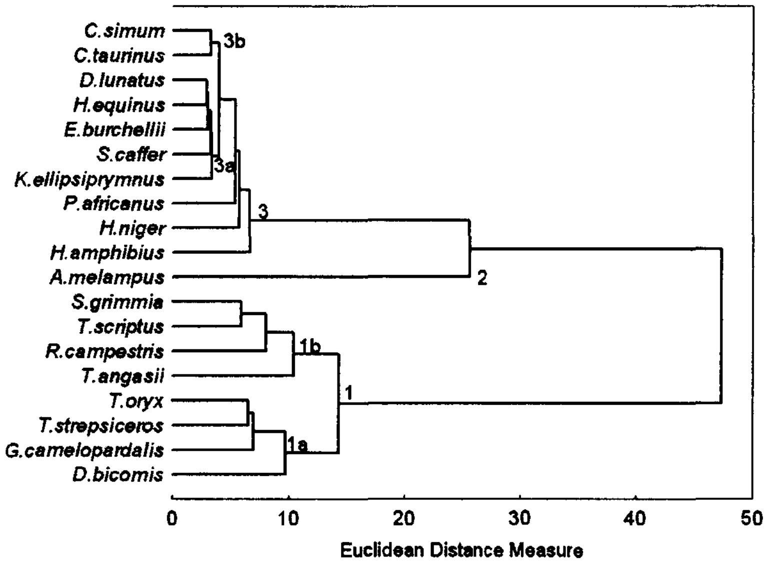

Figure 7.3. Ecological niche compartmentalization of Kruger Park ungulate species, from Euclidean distance cluster analysis based on diet type (% C_4 intake from faecal $\delta^{13}C$), and proxies for diet quality (faecal %N, NDF, ADF, and ADL).

Niche separation amongst grazers is less clear than amongst browsers. Nevertheless, two sub-groups are discernable: medium/tall-grass grazers (group 3a) and short-grass grazers (group 3b). For instance, while the white rhinoceros and blue wildebeest (group 3b) are unlikely to compete for similar ecological niches, both are selective short-grass grazers (Skinner and Smithers, 1990) and it is thus not altogether surprising that faecal proxies for diet quality reveal similar nutritional data for these two species in spite of the large size difference. Group 3a includes both large- (buffalo *Syncerus caffer*) and medium-sized grazers (Burchell's zebra, blue wildebeest, waterbuck *Kobus ellipsiprymnus*, roan antelope, and tsessebe *Damaliscus lunatus*). Waterbuck likely escape competition with sympatric species having similar nutritional requirements through their partially edaphic lifestyle, concentrating near water. That buffalo and zebra should occupy a similar nutritional niche agrees with the digesta kinetics model of Gordon and Illius (1994), which predicts that medium-sized non-ruminants such as zebra should have similar nutritional requirements to large-bodied ruminants, i.e. ~500 kg buffalo.

Of further interest is that the rare antelope on Kruger's Northern Basalt Plains (NBP), roan antelope and tsessebe, appear to occupy the same nutritional niche as the buffalo and zebra (Figure 7.3; group 3b). Recent declines in roan antelope and tsessebe numbers on the NBP have been postulated to be a result of the high density of artificial waterholes, and improved habitat conditions for zebra in this region (Harrington *et al.*, 1999; Grant *et al.*, 2002). The resultant increase in the abundance of predators, which have followed zebra onto the plains, is believed to have had a severe impact on the roan population. The current analysis implies that competition for nutritional resources with zebra may have imposed further constraints, at least for the free-ranging roan population (the majority of the remaining animals are protected in fenced enclosures). Indeed, Grant *et al.* (2002) suggested that a series of severe drought years in the early 1980's, coupled with increased grazing pressure, led to degradation of the NBP grass layer. During this period, roan and tsessebe might simply have been out-competed for resources by the more abundant zebra.

7.4. Conclusion

These results show that ecological diversity amongst ungulates extends beyond the browser/grazer dichotomy proposed by Hofmann (1989). Hofmann (1989) denied a relationship between diet and body size except for his prediction that larger-bodied species should graze whereas smaller animals should feed on concentrate browse foods. It is clear from the evidence presented here that body size is not related to feeding style, but is an important determinant of niche separation, resulting in differences in the nutritional requirements and relative quality of different species' diets, and probably also differences in digestive efficiency. Community structure, and hence evolutionary history, of ungulates may therefore in part be shaped through adaptations to different types of diets, but also by adaptations to diets of different qualities. Today, this is manifest as a range of body sizes within the browsing and grazing spectra. Amongst predominantly browser species there is a range of height classes that allow for foraging at different canopy levels, whereas grazers achieve niche separation through differential food and lifestyle preferences, including edaphic grazing, and short- or tall-grass grazing in open grasslands. Not surprisingly, in cases where ecological niches could not be partitioned based on diet quality and body size, e.g. zebra compared with roan antelope and tsessebe, the former appears to have thrived at the expense of the latter two (see Chapter 7).

Phylogenetic controls were not applied to the data presented here, since morphological and molecular evidence have provided widely different evolutionary trees for the ungulate group, especially Bovidae (see Perez-Barberia *et al.*, 2004). Nonetheless, ecological evolution usually proceeds faster than morphological change (Jernvall *et al.*, 1996), so that while phylogenetic retention of character traits may influence morphology, it is unlikely to heavily influence studies of feeding ecology.

The paucity of data for fibre digestibility of species in free-ranging conditions makes it difficult to test whether feeding style or body size is the primary determinant of niche compartmentalization. Disagreement between proponents of these two models can be traced largely to failure to integrate findings of controlled-feeding and field-based studies. In combining stable isotope and nutritional evidence for dietary ecology of free-ranging ungulates, I have made a preliminary attempt to bridge this gap. Future collaborations between nutritional physiologists and wildlife ecologists are needed in order to develop methodological and statistical approaches that can maximize the benefits of both types of research.

Table 7.1. Diets of Kruger Park ungulates from faecal stable carbon isotope data, and diet quality depicted by percent nitrogen. Landscape key: NB = northern basalts, SB = southern basalts, NG = northern granites, SG = southern granites, PM = Punda Maria.

Species	Region/ season	*n*	(mean ± 1SD)		%C_4 in Diet
			$\delta^{13}C_{VPDB}$ (‰)	%N	
Aepyceros melampus	NB Dry	88	-19.8 ± 2.6	1.9 ± 0.5	53
	NB Wet	78	-16.6 ± 1.9	1.9 ± 0.3	74
Ceratotherium simum	SB Dry	9	-14.2 ± 1.6	1.3 ± 0.3	85
Connochaetes taurinus	NB Dry	60	-14.4 ± 1.0	1.4 ± 0.4	93
	NB Wet	55	-14.7 ± 1.1	1.4 ± 0.2	87
Damaliscus lunatus	NB Dry	14	-13.2 ± 0.9	1.3 ± 0.2	98
	NB Wet	41	-14.0 ± 1.0	1.5 ± 0.4	92
Diceros bicornis	SB Dry	9	-26.1 ± 0.4	1.3 ± 0.3	9
Equus burchellii	NB Dry	59	-14.2 ± 0.8	1.2 ± 0.4	94
	NB Wet	70	-14.2 ± 1.0	1.2 ± 0.2	91
Giraffa camelopardalis	NB Dry	62	-26.4 ± 0.7	2.6 ± 0.4	4
	NB Wet	56	-26.0 ± 0.6	2.6 ± 0.3	6
Hippopotamus amphibius	SB Dry	10	-13.6 ± 1.2	1.2 ± 0.3	91
	SG Wet	5	-15.5 ± 1.1	1.3 ± 0.1	86
Hippotragus equinus	NB Dry	47	-13.6 ± 0.7	0.9 ± 0.2	98
	NB Wet	39	-13.5 ± 1.4	1.3 ± 0.3	95
Hippotragus niger	SG Dry	31	-13.1 ± 0.4	1.1 ± 0.2	97
	SG Wet	59	-13.1 ± 1.0	1.1 ± 0.2	98
Kobus ellipsiprymnus	NB Dry	20	-15.0 ± 1.4	1.9 ± 0.4	88
	NB Wet	39	-14.5 ± 1.1	1.5 ± 0.2	88
Phacochoerus africanus	NB Dry	11	-14.9 ± 1.1	2.0 ± 0.2	90
	NB Wet	29	-14.4 ± 1.1	1.6 ± 0.2	89
Raphicerus campestris	NG Dry	7	-25.3 ± 3.0	2.2 ± 0.6	12
Sylvicapra grimmia	SG Dry	7	-26.2 ± 0.6	2.2 ± 0.1	6
Syncerus caffer	NB Dry	73	-14.7 ± 0.9	1.4 ± 0.2	91
	NB Wet	73	-14.4 ± 1.1	1.5 ± 0.2	89
Taurotragus oryx	NB Dry	2	-27.4 ± 0.1	1.8 ± 0.1	0
Tragelaphus angasii	PM Dry	43	-25.4 ± 3.2	2.4 ± 0.5	14
	PM Wet	59	-23.2 ± 3.8	2.7 ± 0.7	29
Tragelaphus scriptus	SG Wet	7	-26.3 ± 1.6	2.5 ± 0.3	9
Tragelaphus strepsiceros	PM Dry	48	-26.7 ± 0.6	2.6 ± 0.4	4
	PM Wet	59	-25.9 ± 0.6	3.0 ± 0.3	11

Table 7.2. Percent fibre content of Kruger Park ungulate faeces. The number of samples pooled (middle column) to make up a single specimen is displayed in the format *x, y*, where *x* represents the number comprising the first pooled specimen, and *y* that in the second.

Species	Region/ season	*n*	Number in pool	NDF	ADF	ADL
Aepyceros melampus	NB Dry	2	1, 1	62.85	36.18	12.09
	NB Wet	2	1, 1	69.09	41.05	18.33
Ceratotherium simum	SB Dry	1	15	58.83	32.08	6.12
Connochaetes taurinus	NB Dry	2	1, 1	57.30	29.02	5.74
	NB Wet	2	1, 1	61.78	31.64	8.26
Damaliscus lunatus	NB Dry	2	2, 1	59.63	30.48	5.85
	NB Wet	2	1, 1	55.08	26.32	5.49
Diceros bicornis	SB Dry	1	6	68.42	51.24	26.78
Equus burchellii	NB Dry	2	4, 1	59.21	29.57	3.63
	NB Wet	2	1, 1	55.43	26.96	7.39
Giraffa camelopardalis	NB Dry	2	1, 1	51.80	39.06	20.79
	NB Wet	2	1, 1	70.85	56.59	40.64
Hippopotamus amphibius	SB Dry	1	8	68.86	37.14	7.04
	SG Wet	1	5	57.76	32.56	7.88
Hippotragus equinus	NB Dry	2	1	57.19	31.90	8.17
	NB Wet	2	1	57.90	29.99	5.74
Hippotragus niger	SG Dry	1	13	62.08	32.37	8.25
	SG Wet	1	20	61.08	35.51	10.86
Kobus ellipsiprymnus	NB Dry	2	3, 3	46.58	22.59	7.27
	NB Wet	2	1, 1	58.29	30.09	6.66
Phacochoerus africanus	NB Dry	2	1, 1	53.86	28.91	10.21
	NB Wet	2	1, 1	63.30	35.31	13.52
Raphicerus campestris	NG Dry	1	6	44.92	32.16	14.83
Sylvicapra grimmia	SG Dry	1	7	47.11	36.01	23.07
Syncerus caffer	NB Dry	2	1, 1	55.64	27.04	3.70
	NB Wet	2	1, 1	54.99	28.10	7.95
Taurotragus oryx	NB Dry	1	1	59.01	42.65	19.80
Tragelaphus angasii	PM Dry	2	1, 2	51.29	38.03	17.80
	PM Wet	2	4, 5	53.07	38.84	22.47
Tragelaphus scriptus	SG Wet	1	7	50.32	34.58	19.36
Tragelaphus strepsiceros	PM Dry	2	1, 1	59.40	46.28	26.54
	PM Wet	2	4, 5	59.96	43.03	23.06

CHAPTER 8

Conclusion

The organizational structure of ungulate communities is far more complex than can be explained from a dichotomous scheme differentiating browsers from grazers. Data presented in this thesis reveal different foraging strategies between browsers and grazers. Thus, at first glance, a dichotomous scheme appears sufficient for the functional diversity of modern African ungulates (du Toit 2003), but there exist several intermediate species that feed anywhere along the continuum. On a global scale, mixed-feeders are in fact equally as abundant as browsers and grazers, or even more so in many temperate regions (Hofmann 1989; Owen-Smith 1997). Hence, the proposed dietary dichotomy based on the rarity of mixed-feeders in (modern) African landscapes is incomplete, at least in evolutionary terms.

Biotic interactions within communities are characteristically a network of trophic interactions that form complex foodwebs rather than unidirectional food chains (Post *et al.*, 2002). In this thesis, isotopic evidence for monthly, seasonal and regional changes in diet divulge inconsistent patterns of variation within all three ungulate feeding guilds (browsers, grazers, and mixed-feeders). Recently, Brown *et al.* (2001) proposed, based on a decades-long study of kangaroo rats (*Dipodomys*), that while several species may indeed have similar ecologies, they are sufficiently different to respond differently to environmental perturbations. A similar lack of intra-trophic homogeneity seems to exist in ungulates in South African savannas.

Trophic positioning amongst savanna ungulates appears sufficiently variable between species components of each trophic guild so that generalized classifications can never be entirely unequivocal. In this light, it is not at all surprising that diet classification schemes for ungulates differ widely (Hofmann, 1989; Bodmer, 1990; Gagnon and Chew, 2000; Cerling *et al.*, 2003). Not only browser/grazer models suffer this drawback. For instance, body size is commonly regarded as a major driver of niche compartmentalization within ungulate communities (Demment and van Soest, 1985; Owen-Smith, 1988). Distinction is often made between the ecologies of large (megaherbivores), medium and small herbivores (Owen-Smith, 1988; Fritz *et al.*, 2002), a dubious approach given that predictions are for *allometric* scaling of body size with feeding style, diet quality, and digestive physiology (Demment and van Soest, 1985; Owen-Smith, 1988; Gordon and Illius, 1994).

Diet is continuous; in evolutionary time, the transition from browsing to grazing is likely to have been a back-and-forth process between the two extremes (Hofmann, 1989), a phenomenon existing today in the form of considerable dietary variability across space and time (Chapter 3). As shown in Chapters 5 and 6, ecological niche differentiation of ungulates most likely is (and was) driven by strategies to optimize nutritional uptake under all spatial and temporal conditions, both in terms of food quality (protein and/or fibre content) and avoiding plant secondary compounds. Given that browse and grass foods appear to offer similar metabolisable energy yields (Chapter 4), and the fact that food selection is seldom proportional to the availability of browse and grass in surrounding landscapes (Chapter 6), one must surmise that ungulate diversity is not regulated by evolutionary adaptations to maximizing the benefits of either food type.

For example, relative proportions of browse:grass consumption cannot be predicted by seasonal/rainfall (and other climatic) controls on primary productivity (Chapters 5 and 6). Further, the mechanisms underlying variations in food choice differ between trophic guilds. Browsers appear to vary their diets to avoid ingesting large doses of any one type of plant secondary compound, mixed-feeder species respond in a variety of ways to prevailing environmental conditions, and grazers eat foods that are more readily broken down by microbial fermentation, i.e. they may choose grasses having lower lignin content. Hofmann's (1989) proposal for a browser/grazer dichotomy is therefore too simplistic an explanation for the evolutionary diversification of the group.

If optimizing nutritional uptake is the primary driving mechanism for ungulate nutritional niche diversity, then differentiation is more likely achieved through adaptations to deal with different diet qualities. Chapter 7 discusses significant decreases in diet quality with increasing body mass, and those data also point to body height as functionally tied to selection of different food sources. These findings support previous predictions (Foose, 1982; Owen-Smith, 1988; Gordon and Illius, 1994, 1996) for increased digestive efficiencies amongst larger-bodied species, owing mainly to longer digesta retention times, allowing larger animals to use poorer quality foods. An exception has been noted for *Cephalophus* spp. duikers, however, because they appear to have extraordinarily long retention times for their small size (e.g. Shipley and Felicetti, 2002; Perez-Barberia *et al.*, 2004). The case of *Cephalophus* nevertheless reinforces the suggestion that diet quality is the primary mechanism for niche separation, given that fruits, which make up the bulk of the diet of these species, have high fibre content and are hence less easily digested than is commonly assumed (Chapter 4).

One major caveat to this study is the absence of data for plant secondary compounds. Hofmann's (1989) proposal for enlarged salivary glands in browsers to facilitate rapid flow-through of secondary compounds and limit their inhibitive effects of digestion by browsers was shown to be untrue for kudu (Robbins *et al.*, 1995), further supporting a lack of digestive disparity between browsers and grazers. Nevertheless, not only do secondary compounds appear to have a profound influence on variations in food selection (Chapters 5 and 6), they may have substantial effects on perceptions of plant food quality (Chapter 4). Hofmann (1989) proposed that the larger liver of browsers represents an adaptation to process high quantities of nutrients obtained from their foods. Given that browse can no longer be regarded as a concentrate food type, this function of the liver seems unlikely. It may well be, however, that the larger liver of browsers compared to grazers enables the former to effectively detoxify the bloodstream and expel harmful metabolites. Further studies will need to address both the effects of secondary compounds on food selection, and on overall digestive efficiency. The presence of nitrogen-based alkaloids and flavonoids in grasses (e.g. Ndakidemi and Dakora, 1003) also hints that secondary compounds may be as important for grass-feeders as for browsers, thus more emphasis should be placed on comparing the effects of browse- and grass-based secondary compounds, rather than only on browse-based tannins and other phenols.

This is not to suggest that nutritional factors alone underlie diversification. Undoubtedly, there are other important mechanisms that regulate community diversity (Ives, 1995; Jackson, 2001). Behavioural traits such as activity time (Mysterud, 1998), availability and distribution of water (Redfern *et al.*, 2003), climate (Vrba, 1992, 1995;

deMenocal, 2004), and predation (Duncan *et al.*, 1990) are but a few such factors. Climate may have a direct effect on animal evolution, for instance the prolonging of juvenile phases in cooler conditions (Shuter *et al.*, 1980; Wesselman, 1985). Climatic variations also regulate habitat heterogeneity at landscape- and patch-scales (Fjeldså & Lovett, 1997; du Toit, 2003; Arrington *et al.*, 2005). Biological community complexity reflects landscape complexity (Plisnier *et al.*, 2000; Jackson, 2001; La Ferla *et al.*, 2002), and it is not surprising therefore that amongst ungulates major faunal turnover pulses through the Plio-Pleistocene appear to follow major climatic shifts (Vrba, 1992, 1995; deMenocal, 2004). Patch-scale characteristics of habitats appear to influence food choice, as noted for impala *Aepyceros melampus* (Chapter 6) and kudu *Tragelaphus strepsiceros* (Own-Smith and Cooper, 1989), exemplifying that climate and landscape heterogeneity are important ultimate causes of allopatric speciation and other modes of evolutionary transition (Vrba, 1995).

Impacts of predation may filter through successive trophic levels, leading ultimately to the restructuring of communities, as is evident from studies of the effects of invasive predator species on aquatic foodwebs and communities (Cousyn *et al.*, 2001; Gorokhova *et al.*, 2005; Ouijón & Snelgrove, 2005). Amongst ungulates, predation and body size may act in tandem; larger species avoid predation simply because of sheer bulk, and smaller animals are often solitary and escape predation through flight (Skinner and Smithers, 1990). In the medium body size range, the predomination of bovid compared with equid species may also be related to nutritional ecology. Duncan *et al.* (1990) suggested that because hindgut-fermenters (e.g. Equidae) need to eat a larger quantity of food than do ruminants (e.g. Bovidae), they are more susceptible to predation because they spend longer periods of time foraging. In Kruger Park, however, carnivore activity appears to have little effect on the Burchell's zebra *Equus burchellii* population (Mills *et al.*, 1995), but in the Serengeti, survival of yearling zebra may be seriously hampered by predation (Grange *et al.*, 2005).

In this thesis, I have demonstrated the wealth of information that can be derived from stable isotope studies. I have presented data for over 2 500 faecal, and 450 plant specimens, representing a variety of high-resolution spatio-temporal scales. The ease with which samples were obtained and analyzed allowed for rapid tracking of diet shifts, and effective interpretation of dietary variations from an exceptionally robust set of data. In Chapter 5, faecal carbon isotope data are shown to trace monthly variations in plant isotope compositions, demonstrating the reliability of isotope-based records for diet.

The results of this study highlight a number of areas for future exploration. The persistence of lifetime carbon isotope compositions of animal tissues *postmortem* allows the possibility to reconstruct diet over the long-term, both historically (at decadal and centennial scales) and deep in geological time. Using suitable materials, palaeoecological diet records within different ungulate lineages can be extremely useful for informing management of changes that have occurred over the long-term in response to environmental and anthropogenic change (e.g. Tieszen *et al.*, 1989; Koch *et al.*, 1995), and for understanding the evolution of different taxa and/or guilds (e.g. MacFadden, 1998; Cerling *et al.*, 1999, 2005). One area in need of urgent attention is the discrimination of ^{15}N between diet and animal body tissues and faeces; while some progress has been made here (Chapter 3), further studies are required to refine nitrogen isotope-based models for diet and niche differentiation. The advantages of improved dual-isotope models are clearly demonstrated in Chapter 3. Added benefits also include more rigorous reconstructions of predator-prey relationships (e.g. Urton and Hobson, 2005). Higher trophic level stable isotope studies may help overcome many of the problems associated with documenting kills by large mammal carnivores in the field (see Mills *et al.*, 1995; Harrington *et al.*, 1999). Coupled with long-term evidence, these may even allow for empirical testing of hypotheses directly related to impacts of predation, for example the proposed increase in predation on roan antelope *Hippotragus equinus* populations in the northern Kruger Park during the 1980s and 1990s (Harrington *et al.*, 1999; Grant *et al.*, 2002).

8.1. Considerations for Palaeoecology

The diet-habitat tolerance ranges of extinct mammals provide an important source of information about past landscape heterogeneity, information which is useful for understanding the fundamental mechanisms that regulate dietary evolution and faunal turnover (Vrba, 1974, 1975, 1992, 1995; Kappelman *et al.*, 1997; Spencer, 1997; Bobe *et al.*, 2002). However, the vast majority of fossil faunal studies have relied on the assumption of taxonomic uniformitarianism – that extinct taxa had diets akin to that of their modern counterparts. Recent carbon isotope studies based on analysis of fossil tooth enamel have shown that this assumption is not valid for many taxa (Sponheimer *et al.*, 1999; Lee-Thorp *et al.*, submitted), *and* that most lineages included periods of mixed browsing/grazing (Cerling *et al.*, 1997a, 1999, 2005). As a result, mammalian-based reconstructions of past landscapes differ widely between studies; interpretations sometimes vary from 'open grassland' to 'riverine woodland' for a single deposit (Reed, 1997). The current study has demonstrated that, in many instances, even our knowledge of the diets of modern taxa is inadequate.

Carbon isotope-based indices of C_3- and C_4-feeder abundances have been proposed, at least in South African Plio-Pleistocene deposits, to overcome the problems associated with taxonomic uniformitarianism (Luyt, 2001; Sponheimer and Lee-Thorp, 2003a). While this approach is more empirical, the assumption that browser/grazer abundances are consistent with proportions of woody plants/grass in the landscape remains a constraint. Many extant species do not always follow habitat distributions that agree with this prediction (Hirst, 1975). One recent study of abiotic and biotic controls on ungulate community structure argues that grazer biomass is regulated by soil fertility, whereas browsers impose limitations on one another through inter-specific competition for resources between different body size guilds (Fritz *et al.*, 2005). I have shown in this study that transitions between browse- and grass-intake, not only amongst mixed-feeders but also certain recognized browsers and grazers, do not necessarily reflect changes in plant biomass. Rather, dietary variations appear to be driven by spatial and temporal changes in the relative nutritional value of plant foods.

Mixed-feeder groups may offer a means to refine interpretations of palaeolandscapes using faunal abundances/diets. Carbon isotope evidence for the diets of extinct mixed-feeders can potentially be compared with patterns observed in their modern counterparts to infer past habitat conditions, using environmental correlates for diet changes. For example, while predominantly browse-based diets in impala may not indicate a heavily wooded environment, evidence for this behaviour may reflect the presence of riparian habitats and/or edaphic grasslands (see Chapter 6). High-resolution serial profiles along teeth and tooth rows of extinct animals to examine seasonality can also be compared with data from modern descendants. Large-scale switches between browsing and grazing at this scale might be indicative of a seasonally heterogeneous landscape, whereas minimal fluctuations can be expected to be consistent with habitats such as Punda Maria in the far northern Kruger Park, which are heavily wooded but available browse is avoided throughout the seasonal cycle possibly because of high concentrations of phenolics. Nevertheless, mixed-feeder models for past habitat conditions would be limited by poor insights into environmental correlates for diet switching. For example, a recent carbon isotope study of fossil fauna from the South African Pliocene indicate a high prevalence of mixed-feeding taxa at Sterkfontein Member 4 in the Cradle of Humankind World Heritage Site, but it is unclear whether and how this phenomenon is related to past landscape conditions (Lee-Thorp *et al.*, submitted). Further studies of diet switching in modern environments, both in terms of proportions of browse/grass consumption *and* variations in diet quality, are needed to fully explore the potential for mixed feeder-based palaeohabitat reconstructions.

8.2. Considerations for Conservation Biology

Ungulate diets have direct relevance to conservation objectives and management planning. A continuing debate amongst wildlife ecologists is the impact of herbivory on vegetational landscapes. Single species are often targeted in this regard as keystone ecological units. Different species use vegetational resources in a variety of ways, such that multiple-species interactions are a more realistic approach (van de Koppel and Prins, 1998; Illius and O'Connor, 2000; Pickett *et al.* 2003; this study). Elephants, for example, have been particularly targeted for attention (e.g. Cumming *et al.*, 1997; Scholes *et al.*, 2003), because of their size and visibility, while the role of other highly abundant species such as impala and kudu in plant recruitment and habitat maintenance are largely underestimated or even ignored (Pickett *et al.*, 2003). Further, animal overstocking and resultant habitat degradation in many of southern Africa's wilderness areas can be ascribed at base to the poor understanding of dietary variations of most taxa (Bodenstein *et al.*, 2000).

Management decisions based on systemic models of reduced ecological complexity are also limited in their capacity to cater for the needs of all resident biota. For example, the high density of artificial waterholes constructed on the Northern Basalt Plains of Kruger Park may have alleviated water stress for bulk grazers such as Burchell's zebra *Equus burchellii* and buffalo *Syncerus caffer*, but appears to have been the ultimate cause of the decline of roan antelope in the region (Harrington *et al.*, 1999; Grant *et al.*, 2002). In response, a number of waterholes in the region have been closed, and it is hoped that this action will promote recovery of the landscape to its former state and facilitate recovery of the roan population. The caveat one is forced to consider is that decreasing the artificial water supply may simply undo the "solution" to an earlier problem.

Ecologists have recently begun to address themes of environmental variation and ecosystem heterogeneity as functions of natural evolutionary processes (e.g. Brown *et al.*, 2001). The result has been a paradigm shift from static to dynamic ecosystems rationale; in the past conservation was concerned with the preservation of *in situ* biodiversity, today the objective is to maintain ecosystems and evolutionary processes (e.g. Pickett &

Ostfeld, 1995; Rogers & Biggs, 1999; Weddell, 2002). Yet, wildlife management continues to manipulate various aspects of ungulate-plant systems under the guise of "sustainable utilization", which in reality is an effort to maintain the *status quo.* These include questionable conservation practices, including culling and population control, burning policies, and provision of artificial water sources, that seem often to have more negative than positive implications for wildlife refuges. The problem is that our understanding of naturally-occurring variations in ecological and evolutionary processes remains limited. Amongst ungulates, the diversity of conflicting models for niche compartmentalization, community diversity, and trophic structure that persist in the literature, and perhaps more importantly the spatio-temporal specificity of these phenomena, casts considerable doubt over our ability to predict responses to environmental change. Perhaps the best way forward is to adapt a paradigm of observing, rather than predicting, the outcomes of change. Only then can our understanding of variation equal the degree of variation that exists amongst biological processes, and only then will adaptive management become a successful reality.

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