

Diets of savanna ungulates from stable carbon isotope composition of faeces

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Abstract

Hypotheses to explain diversity among African ungulates focus largely on niche separation along a browser/grazer continuum. However, a number of studies advocate that the browser/grazer distinction insufficiently describes the full extent of dietary variation that occurs within and between taxa. Disparate classification schemes exist because of a lack of uniform and reliable data for many taxa, and failure to incorporate spatio-temporal variations into broader assessments of diet. In this study, we tested predictions for diet and dietary niche separation of African savanna ungulates using stable carbon isotope evidence from faeces for proportions of C₃ (browse) to C₄ (grass) intake among 19 species from the Kruger National Park, South Africa. Dietary predictions from the literature are confirmed in the case of browsers (black rhinoceros *Diceros bicornis*, giraffe *Giraffa camelopardalis*, bushbuck *Tragelaphus scriptus*, kudu *Tragelaphus strepsiceros*), mixed-feeders (impala *Aepyceros melampus*, nyala *Tragelaphus angasii*), and most grazers (white rhinoceros *Ceratotherium simum*, Burchell's zebra *Equus burchellii*, warthog *Phacochoerus africanus*, hippopotamus *Hippopotamus amphibius*, blue wildebeest *Connochaetes taurinus*, tsessebe *Damaliscus lunatus*, waterbuck *Kobus ellipsiprymnus*). In contrast, several species showed results differing from most expectations derived from the available literature, including eland *Taurotragus oryx*, steenbok *Raphicerus campestris*, grey duiker *Sylvicapra grimmia*, buffalo *Syncerus caffer*, roan antelope *Hippotragus equinus* and sable antelope *Hippotragus niger*. Many of these discrepancies can be accounted for by seasonal and/or regional dietary differences. Cluster analysis based on a data matrix that incorporates the extent of spatio-temporal dietary variation among Kruger Park ungulates reveals several distinct categories of feeding preferences that extend beyond a two-edged browser/grazer dichotomy, such as mixed-feeders with a preference for either forage class, and spatial/seasonal shifts between uniform and mixed-feeding styles among variable browsers (e.g. grey duiker) and variable grazers (e.g. buffalo). These results highlight the need for approaches that are sensitive to spatio-temporal variations and the continuity of diet.

Introduction

The dietary niches of ungulates are most often explained along a browser/grazer continuum, with three guilds typically recognized: browsers, grazers and mixed-feeders (Hofmann & Stewart, 1972; Jarman, 1974; Hofmann, 1989; Owen-Smith, 1997; du Toit, 2003). Consequently, models of herbivore diversity and niche compartmentalization rely largely on information about diet at this coarse scale. However, there is substantial disagreement about whether this system is sufficient to explain the full extent of dietary

diversity among 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 & Chew, 2000; Cerling, Harris & Passey, 2003).

Numerous field studies, carried out across a range of African environments, have documented proportions of browse and grass consumed by ungulates. In a major synthesis of available information, Gagnon & 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 the available literature to provide reliable data for only 42 of the 78 bovid species that they examined. Gagnon & Chew (2000) suggested that disparate dietary classification schemes are an artefact of the lack of uniformity among 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).

Stable carbon isotope ecology provides an empirical technique for diet over multiple taxonomic, habitat and time frames (Post, 2002). In African savannas, stable carbon isotope proxies for diet are especially useful because the isotopic composition of herbivore tissues and excreta reliably reflects the relative proportions of browse (C_3 -photosynthesizing trees, shrubs and forbs) to grass (C_4 -plants) consumed (Vogel, 1978; Tieszen *et al.*, 1979; Lee-Thorp & van der Merwe, 1987; Cerling & Harris, 1999). Previous carbon isotope comparisons with field studies for bovid diets in East and southern Africa revealed significant discrepancies with expectations of Gagnon & Chew (2000) for several taxa (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b). 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. Herbivore faeces, too, have been shown to reflect consistently and accurately the isotopic composition of the diet (Tieszen *et al.*, 1979; Jones *et al.*, 1981; Coates, van der Weide & Kerr, 1991; Sponheimer *et al.*, 2003c; Codron *et al.*, 2005b). Controlled-feeding studies using a variety of ungulate species fed C_3 dicot, C_3 grass and C_4 grass diets have shown that, despite representing only undigested material, faeces maintain a consistent slightly negative offset from diet ($-0.9 \pm 0.4\%$ SD; Sponheimer *et al.*, 2003c; Codron *et al.*, 2005b). Faeces are a particularly useful material for isotope studies because they provide short-term dietary information (Sponheimer *et al.*, 2003c), allowing for rapid documentation of spatial and temporal variations (e.g. Tieszen *et al.*, 1979; Codron *et al.*, 2005a,b).

Here, we use stable carbon isotope data from faeces collected over a variety of spatial and temporal scales from the semi-arid savanna of the Kruger National Park, South Africa, to test dietary predictions for African ungulates along the browser/grazer (or C_3/C_4) continuum, and whether these predictions persist when dietary variations are accounted for.

Methods

Materials and laboratory techniques

The Kruger National Park is a large ($\sim 2\,000\,000$ ha) semi-arid savanna, situated in the north-east of South Africa between the latitudes $22^\circ 20'$ and $25^\circ 32'S$, and the longitudes $30^\circ 53'$ and $32^\circ 02'E$, forming part of the 'lowveld' savanna. The mean annual rainfall varies between 500 and 700 mm in

the south to between 300 and 500 mm in the north, but is highly seasonal, falling mainly during the summer months between October and March, while April to November are dry (Venter, Scholes & Eckhardt, 2003). The area is divided longitudinally into granitic substrates forming nutrient poor soils in the west, and nutrient-rich basaltic soils in the east (Venter *et al.*, 2003; Fig. 1). The northern regions (to the north of the Olifants River) are dominated by broadleaved mopane *Colophospermum mopane* 'shrubveld' and woodland, while fine-leaved *Acacia* and *Combretum* spp. trees abound in the south.

Faeces were collected from June 2002 to May 2005, biannually during the first 2 years, and monthly from February 2004 to May 2005, from a variety of landscapes throughout Kruger Park (see Fig. 1). Only fresh faeces were collected, that is damp or wet specimens, to ensure that these represented the appropriate season. Faeces that are readily identifiable, and relatively easy to obtain, such as

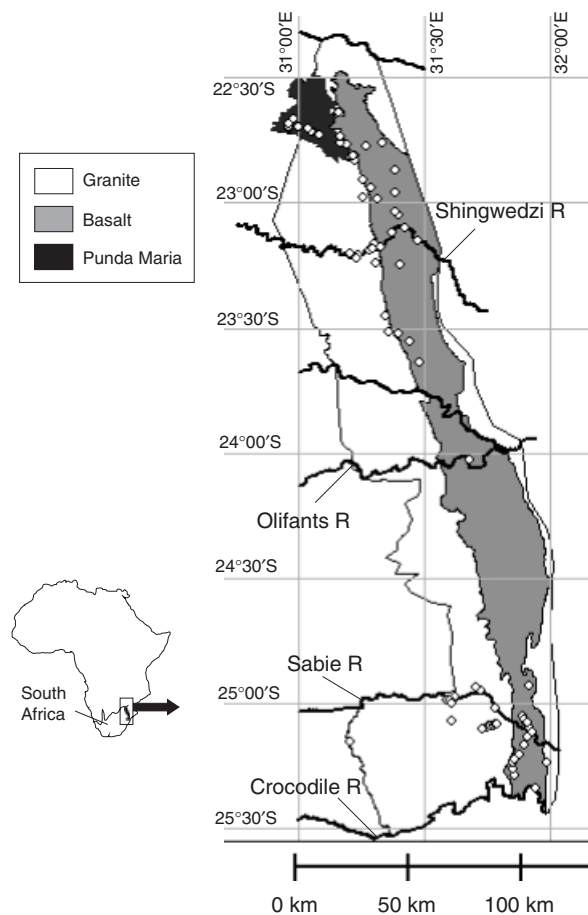


Figure 1 Map of Kruger National Park and its geographic location in Africa, showing west-east geological gradient between granites (clear region) and basalts (shaded region). The Olifants River separates northern broad-leaved *Colophospermum mopane* from southern fine-leaved *Acacia/Combretum* savannas, white circles depict faeces sampling localities.

those of buffalo *Syncerus caffer* and square-lipped (white) rhinoceros *Ceratotherium simum*, was sampled opportunistically. Collection of samples that are more difficult to identify and/or harder to locate in the field, for example kudu *Tragelaphus strepsiceros* and nyala *Tragelaphus angasii* were made by locating animals from a vehicle and then following on foot.

Faeces were oven-dried at 60 °C for 24 h, and mill-ground into a homogenous powder through a 1 mm sieve. Powdered samples were individually combusted in an automated elemental analyzer (Carlo Erba, Milan, Italy), and the resultant CO₂ gas was introduced into a Finnigan MAT 252 or a DELTA XP Mass Spectrometer (Finnigan, Bremen, Germany) via a continuous flow-through inlet system (Conflo). ¹³C/¹²C ratios are expressed in the delta (δ) notation in parts per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) standard. Standard deviations of repeated measurements of laboratory standards were less than 0.1‰.

Data analysis

To compare carbon isotope data with data from field studies, faecal δ¹³C values were converted to estimates of %C₄ grass intake using a dual-endpoint mixing model (e.g. Post, 2002; Sponheimer *et al.*, 2003b; Codron *et al.*, 2005b):

$$(\delta^{13}\text{C}_{\text{C}_3 \text{ plants}} + \Delta\delta^{13}\text{C} - \delta^{13}\text{C}_{\text{animal}}) / (\delta^{13}\text{C}_{\text{C}_3 \text{ plants}} - \delta^{13}\text{C}_{\text{C}_4 \text{ plants}})$$

where Δδ¹³C is the magnitude of discrimination between consumer tissue and source endpoints (assumed here to be -0.9‰, Sponheimer *et al.*, 2003c; Codron *et al.*, 2005b). This approach facilitates comparison of faecal δ¹³C data with data from field studies as well as with δ¹³C data from animal body tissues treated similarly (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b). Most previous studies using a similar approach have relied on global plant mean δ¹³C values (-27.0 and -12.5‰ for C₃ and C₄ plants, respectively). Here, we use regionally and seasonally specific endpoint values based on data for Kruger Park vegetation; that is northern and southern granites and basalts, respectively, Punda Maria, and dry or wet season (data from Codron *et al.*, 2005). For example, the mean δ¹³C values of vegetation for northern basalts during the dry season (April–September) were -26.0 and -12.7‰ for C₃ and C₄ plants, respectively, and these values were assigned as endpoints for faeces collected from this region during the dry season. Similarly, wet season (October–March) means for C₃ and C₄ 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 & Chew's (2000) review for bovids, and Owen-Smith (1988) and Skinner & Smithers (1990) for non-bovid taxa. Reported dietary contributions of dicots and fruit (e.g. Gagnon & Chew, 2000) were combined to represent total per cent C₃ in the diet (see Sponheimer *et al.*, 2003b). Monocots were assumed to represent C₄ intake, although some aquatic monocots (e.g. reeds and sedges) in Kruger

Park may be either C₃ or C₄ (Stock, Chuba & Verboom, 2004; Codron *et al.*, 2005). Reeds and sedges are likely to make relatively small contributions to the diets of only a few species included here, for example buffalo, blue wildebeest *Connochaetes taurinus*, waterbuck *Kobus ellipsiprymnus* and hippopotamus *Hippopotamus amphibius* (Owen-Smith, 1988; Skinner & Smithers, 1990; R. N. Owen-Smith, 2006, pers. comm.).

In a similar comparison between carbon isotope and field studies, Sponheimer *et al.* (2003b) allowed for ~15% error in isotope-based derivations of C₄ contributions to diet, in the absence of data for local vegetation, and because their data covered a broader geographical (whole of southern Africa) and temporal scale (several decades) than the current study. The use of regionally specific baseline data enhances the accuracy of isotope mixing models (Post 2002; Codron *et al.*, 2005b); hence, the 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.

Dietary niches of Kruger Park ungulates were determined using inter-group cluster analyses based on the Euclidean distance measure. To account for spatio-temporal dietary variations within Kruger Park, we used 25–75th percentile ranges of %C₄-intake ranges for each species across all regions and seasons in the analyses. Thus, the analysis is based on two variables: a minimum (25th percentile) and maximum (75th percentile) %C₄ intake for each species across space and time; we ignored values outside of this range because they are likely to be within error estimates for carbon isotope-based diet interpretations. The results of our cluster analyses are compared with dietary classification schemes derived from field study data (e.g. Gagnon & Chew, 2000) and carbon isotope data for East African bovids (Cerling *et al.*, 2003).

Results

Per cent C₄ (grass) intake of Kruger Park ungulates was similar to expectations derived from field studies for Burchell's zebra *Equus burchellii* (92% C₄; *n* = 166), the two rhinoceros species *C. simum* (90% C₄; *n* = 29) and *Diceros bicornis* (9% C₄; *n* = 9), hippopotamus (91% C₄, *n* = 26), warthog *Phacochoerus africanus* (91% C₄, *n* = 62) and giraffe *Giraffa camelopardalis* (5% C₄, *n* = 174); Table 1). However, six of the 13 bovid species analysed have faecal δ¹³C values depicting C₄ contributions to diet differing by more than 10% from those expected by Gagnon & Chew (2000). These are buffalo (88% C₄, *n* = 319), eland *Taurotragus oryx* (3% C₄, *n* = 5), roan antelope *Hippotragus equinus* (96% C₄, *n* = 86), sable antelope *Hippotragus niger* (98% C₄, *n* = 122), impala *Aepyceros melampus* (60% C₄, *n* = 606) and steenbok *Raphicerus campestris* (7% C₄, *n* = 26). By contrast, Sponheimer *et al.* (2003b) observed such differences in only three out of 27 species analysed.

In comparison with previous carbon isotope studies of bovid diets, the faecal evidence from Kruger Park differs from evidence derived from hair, bone and tooth enamel

Table 1 Mean $\delta^{13}\text{C}$ and estimated %C₄-intake from faeces of 19 ungulate species from Kruger National Park (KNP)

Taxon	Species	KNP faeces $\delta^{13}\text{C}_{\text{VPDB}}(\text{‰})$			%C ₄ in Diet				
		<i>n</i>	Mean	SD	KNP	SD	South Africa	East Africa	Field studies
Perissodactyla									
Equidae	<i>Equus burchellii</i>	166	-14.1	1.0	92	6	-	-	90
Rhinocerotidae									
	<i>Diceros bicornis</i>	9	-26.1	0.4	9	2	-	-	5
	<i>Ceratotherium simum</i>	28	-13.9	1.3	90	8	-	-	98
Artiodactyla									
Suiformes									
	<i>Hippopotamus amphibius</i>	26	-13.8	1.6	91	8	-	-	95
	<i>Phacochoerus africanus</i>	62	-14.2	1.2	91	7	-	-	95
Ruminantia									
Giraffidae									
	<i>Giraffa camelopardalis</i>	177	-26.4	0.8	5	5	-	-	0
Bovidae									
Bovini									
	<i>Syncerus caffer</i>	319	-14.7	1.1	88	7	88	100	78
Tragelaphini									
	<i>Taurotragus oryx</i>	5	-26.6	1.0	3	5	8	18	50
	<i>Tragelaphus strepsiceros</i>	145	-26.4	0.8	7	6	4	4	15
	<i>Tragelaphus angasii</i>	102	-24.2	3.7	23	26	33	-	20
	<i>Tragelaphus scriptus</i>	7	-26.3	1.6	9	12	0	0	10
Alcelaphini									
	<i>Connochaetes taurinus</i>	151	-14.5	1.1	90	7	90	100	88
	<i>Damaliscus lunatus</i>	55	-13.8	1.0	94	6	100	100	95
Reduncini									
	<i>Kobus ellipsiprymnus</i>	95	-14.4	1.2	90	7	100	92	84
Hippotragini									
	<i>Hippotragus equinus</i>	86	-13.5	1.1	96	7	91	100	85
	<i>Hippotragus niger</i>	122	-13.0	0.8	98	4	100	94	85
Aepycerotini									
	<i>Aepyceros melampus</i>	606	-18.8	2.9	60	21	51	52	45
Neotragini									
	<i>Raphicerus campestris</i>	26	-26.4	2.1	7	14	10	18	34
Cephalophini									
	<i>Sylvicapra grimmia</i>	14	-25.1	2.9	15	21	2	0	12

Comparative data for southern Africa are from Sponheimer *et al.* (2003b), and for East Africa from Cerling *et al.* (2003), based on carbon isotope composition of hair, bones and teeth. Data from field studies are from Gagnon & Chew (2000) for Bovidae, and Owen-Smith (1988) and Skinner & Smithers (1990) for other taxa.

n, number of samples; sd, ± 1 standard deviation; VPDB, Vienna PeeDee Belemnite.

from across the subcontinent (Sponheimer *et al.*, 2003b) in two species (nyala 23% C₄, *n* = 102; and grey duiker *Sylvicapra grimmia* 15% C₄, *n* = 14; Table 1). The faecal data differ from carbon isotope evidence for diets of their East African conspecifics (Cerling *et al.*, 2003) in five out of 12 cases [blue wildebeest 90% C₄, *n* = 151, as well as buffalo (88%), eland (3%), steenbok (7%) and grey duiker (15%)].

Seasonal comparisons revealed that only three out of 17 species for which dry (specimens collected from April to September) and wet season (October–March) data are available changed their diets significantly (> 10% in terms of C₃/C₄ intake; Table 2). Both mixed-feeder species increased C₄ grass consumption from dry to wet seasons (53–67% in impala, 14–29% in nyala). Grey duiker, too, appear to have switched from a pure browse diet (5% C₄) in the dry season to 30% C₄ in the wet. Thus, differences between faecal data and published data from body tissues for nyala and duiker are restricted to dry and wet seasons only, respectively.

Cluster analysis revealed the expected dichotomy between browsing (group 1; 25–75th percentiles are 0–15% C₄ in diet) and grazing (group 3; 84–100% C₄) taxa, with mixed browser/grazers (group 2; 4–76% C₄) intermediate in the tree diagram (Fig. 2). However, there are more than three discrete groups in the diagram, identifiable according to the

relative reliance on different food types, although some groups are monospecific because of the limited number of species included in this study (*n* = 19). Group 1 is represented by species with predominantly browse-based (C₃) diets, but grey duiker (group 1b; 0–15% C₄) appear to deviate from pure browsers (group 1a; 0–10% C₄), seemingly because of some (30%) C₄ grass intake inferred from wet season data for duiker. Mixed-feeders are divided into two strongly differing feeding niches, and can be defined as mixed-feeders preferring browse (group 2a, nyala; 4–33% C₄) and mixed-feeders preferring grass (group 2b, impala; 46–76% C₄). Data for grazers separate into variable (group 3a; 84–100% C₄) and obligate grazers (group 3b; 95–100% C₄).

Discussion

Comparison with previous field-based and carbon isotope studies

Stable carbon isotope evidence from faeces suggests that the diets of six bovid taxa differ from predictions of field studies. The largest discrepancies are observed for eland and steenbok, in that both consume virtually no grass despite most

Table 2 Dry-to-wet season comparison of faecal $\delta^{13}\text{C}$ and estimated % C_4 -intake among Kruger Park ungulates

Species	Dry season $\delta^{13}\text{C}$ (‰) % C_4 in diet					Wet season $\delta^{13}\text{C}$ (‰) % C_4 in diet					Significant diet change (> 10% C_4)
	<i>n</i>	Mean	SD	Mean	SD	<i>n</i>	Mean	SD	Mean	SD	
Browsers											
<i>Giraffa camelopardalis</i>	99	-26.5	0.8	5	5	78	-26.2	0.8	6	4	No
<i>Raphicerus campestris</i>	17	-26.5	2.2	6	15	9	-26.1	1.9	10	14	No
<i>Sylvicapra grimmia</i>	8	-26.5	0.9	5	5	6	-23.4	3.7	30	26	Yes
<i>Taurotragus oryx</i>	2	-27.4	0.1	0	0	3	-26.1	0.9	5	6	No
<i>Tragelaphus strepsiceros</i>	75	-26.7	0.6	4	4	70	-26.0	0.7	10	5	No
Mixed-feeders											
<i>Aepyceros melampus</i>	325	-19.6	3.0	53	21	281	-17.8	2.6	67	18	Yes
<i>Tragelaphus angasii</i>	43	-25.4	3.2	14	22	59	-23.2	3.8	29	26	Yes
Grazers											
<i>Connochaetes taurinus</i>	85	-14.2	1.1	92	6	66	-14.8	1.1	87	7	No
<i>Damaliscus lunatus</i>	14	-13.2	0.9	98	2	41	-14.0	1.0	92	6	No
<i>Equus burchellii</i>	81	-14.0	1.0	94	5	85	-14.3	1.0	91	7	No
<i>Hippopotamus amphibius</i>	17	-13.3	1.5	92	8	9	-14.8	1.1	89	7	No
<i>Hippotragus equinus</i>	47	-13.6	0.7	98	3	39	-13.5	1.4	95	9	No
<i>Hippotragus niger</i>	53	-12.9	0.5	98	3	69	-13.0	1.0	98	5	No
<i>Kobus ellipsiprymnus</i>	47	-14.2	1.2	90	6	48	-14.6	1.2	89	8	No
<i>Phacochoerus africanus</i>	32	-14.0	1.2	92	7	30	-14.5	1.1	89	7	No
<i>Syncerus caffer</i>	176	-14.7	1.0	89	6	143	-14.7	1.2	88	7	No

n, number of samples; SD, ± 1 standard deviation.

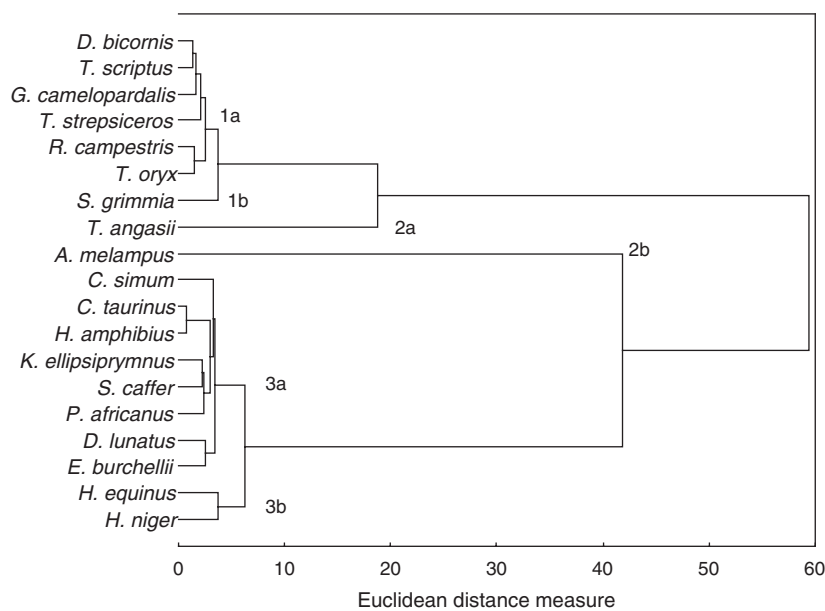


Figure 2 Diet-niche differentiation amongst Kruger Park ungulates based on stable carbon isotope data from faeces. The tree diagram shows the results of Cluster analysis Euclidean distance inter-group measures, based on 25th to 75th percentile ranges of % C_4 intake to establish degrees of dietary variation across space and time. Numbers 1 to 3 represent clusters distinguished by reliance on differential proportions of C_3 (browse) and C_4 (grass) foods.

predictions based on observational data, and stomach morphophysiology, that they are mixed feeders (e.g. Lamprey, 1963; Hofmann & Stewart, 1972; Hofmann, 1989; Gagnon & Chew, 2000). Similar results have been obtained from body tissues, although eland specimens from East Africa were found to eat more C_4 grass (~18%) than their southern African counterparts (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b). Carbon isotope evidence from faeces of eland from the Waterberg savanna, South Africa, also suggests a browse-based diet (Codron *et al.*, 2005a), as

do data from dentine collagen of animals from the treeless grasslands of the Free State Province in the central interior of South Africa (% C_4 intake ~5%, $n = 7$, D. Codron, J. S. Brink, unpubl. data). Similarly, a field study of eland in the Eastern Cape of South Africa showed that they eat very little grass (Watson & 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, although the balance may have

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. Overall, it may be that some populations of eland eat significant amounts of grass, but current evidence indicates that both eland and steenbok are fairly consistent browsers.

Three grazers also yielded results differing from literature predictions: buffalo, roan antelope and sable antelope. Grass is expected to comprise between 78 and 85% of the diet of these taxa, the balance being a mix of various C₃-based foods (Lamprey, 1963; Child & Wilson, 1964; Wilson & Hirst, 1977; Owen-Smith, 1997; Gagnon & Chew, 2000). Wilson & 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. Our data show that roan and sable antelope do not consume sufficient amounts of C₃ foods to influence the overall isotopic composition of their diets, with diets ranging between 95 and 100% C₄. Similarly, carbon isotope data from body tissues of southern and East African representatives of roan and sable agree closely with results from Kruger (Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b), suggesting that these taxa are near-exclusive grazers across many southern and East African savannas.

Some grazers, however, differ in mean %C₄ intake compared with isotope data from East Africa (Table 1). Cerling *et al.* (2003) reported diets of 100% grass in buffalo and wildebeest, while the results for Kruger Park and indeed all of southern Africa (Sponheimer *et al.*, 2003b) are consistent with >10% C₃ contributions to diet. It is possible that some of the variation here is not due to C₃-feeding in these taxa, but because of small (~1.0‰) differences in the carbon isotopic composition of grasses that follow different C₄ sub-pathways, that is nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME), nicotinamide adenine dinucleotide-malic enzyme (NAD-ME) and phosphoenolpyruvate carboxykinase (PCK) (Hattersley, 1982; Cerling & Harris, 1999). Cerling & Harris (1999) proposed that tooth enamel δ¹³C 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. This may explain the small carbon isotopic differences between Kruger Park and East African grazers, as well as differences between Kruger Park buffalo and wildebeest compared with pure grazers such as roan and sable antelope. However, the isotopic difference between these grazer groups is ~1.5‰, larger than the reported differences (~0.5‰) between grass subtypes within Kruger Park (Codron *et al.*, 2005), and indeed elsewhere (see Hattersley, 1982; Cerling & Harris, 1999). Thus, the most plausible explanation is that buffalo and wildebeest in Kruger Park do indeed utilize small, but significant, amounts of C₃ dicots, whereas their conspecifics in East Africa do not.

The other species included in this study that displayed discordant results compared with field studies is impala (60% C₄ compared with 45% predicted by Gagnon & Chew, 2000). However, impala are a notoriously enigmatic mixed

browser/grazer species (Dunham, 1980; Skinner & Smithers, 1990; Meissner, Pieterse & Potgieter, 1996; Wronski, 2002). Indeed, faecal δ¹³C shows that impala diets changed to comprise 53 to 67% C₄ grass between dry and wet seasons, and similarly large variations have been reported across habitats, both within Kruger Park (Sponheimer *et al.*, 2003a) and in bone collagen from other South African savannas (Vogel, 1978). Thus, the difference between the results of the current study and field observations can be considered an artifact of spatio-temporal dietary flux in the species. Similarly, faecal δ¹³C values for nyala reflect a diet comprising ~20% C₄ grass, similar to the expectations of Gagnon & Chew (2000), but lower than the 33% reported by Sponheimer *et al.* (2003b). However, the difference between our data and that of Sponheimer *et al.* (2003b) is limited to the dry season only; nyala in Kruger Park increased their grass intake from ~14 to 29% from the dry to the wet season, consistent with expectations (e.g. Tello & van Gelder, 1975).

For grey duiker, Kruger Park data portray an ~15% C₄ diet, as opposed to a pure C₃ diet inferred from δ¹³C values of hard tissues for both southern and East African representatives (Lee-Thorp, 1989; Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b). According to Skinner & Smithers (1990), grey duiker are almost exclusively browsers, and very seldom eat any grass. On the other hand, Gagnon & Chew (2000) expect a 12% grass diet in the species. In Kruger Park, duiker diets vary between 5 and 30% C₄ grass from the dry to the wet season, a range encompassing that of all previous estimates. However, all duikers (Cephalophini) also consume varying quantities of fruit, including, but to a lesser extent than forest-dwelling *Cephalophus* spp., the savanna species *S. grimmia* (Skinner & Smithers, 1990; Owen-Smith, 1997; Gagnon & Chew, 2000). It has been shown that fruits are 1 to 2‰ enriched in ¹³C compared with C₃ foliage in Kruger Park and elsewhere (Cerling, Hart & Hart, 2004; Codron *et al.*, 2005, 2005a); hence, the ¹³C-enriched portion of Kruger Park duiker diets may reflect some utilization of fruit, especially in the wet season. Nonetheless, wet season faecal δ¹³C for this species is sufficiently high (-23.4 ± 3.7‰, n = 6) to be consistent with at least some grass intake. It appears that grey duiker do feed to some small extent on grass, at least in certain regions/seasons.

Dietary niche separation

Our classification of ungulate dietary niches (Fig. 2) differs from previous models in several important ways. While the traditional prediction for a dichotomous arrangement between browsers and grazers does persist in these data, there are some subtle, but important, differences within these feeding classes. Moreover, mixed-feeding taxa do not form a third group, but are present in both the browser (nyala) and grazer (impala) guilds, congruent with predictions that nyala are mixed-feeders preferring browse and impala prefer grass (e.g. Hofmann & Stewart, 1972; Skinner & Smithers, 1990; Gagnon & Chew, 2000). These two

species, classed as mixed-feeders but with very different feeding habits, provide an example of misleading conclusions that can be drawn when ungulate diets are simply classified into one of three trophic guilds, that is browser, grazer or mixed-feeder. Further, our current analysis may itself be an oversimplification of niche separation, because our data include only two apparent 'mixed feeders'. While this likely reflects the African situation, mixed-feeders are in fact equally as abundant as browsers and grazers on the global scale, or even more so in temperate regions (Hofmann, 1989; Owen-Smith, 1997). Inclusion of taxa from other regions in our dataset would likely reveal an even greater variety of feeding niches.

A similar, yet less pronounced, disparity is evident among browsers; grey duiker are the only species included here that seem to eat substantial amounts of C₄ grass, particularly in the wet season. Previously, Janis (1988) showed that many small-bodied browsers, including several cephalophines, have relatively hypsodont (high-crowned) cheek teeth, an adaptation normally associated with an abrasive grazing diet. She reasoned that these animals had been mixed-feeders in the past and returned to predominantly browse-based diets only more recently in evolutionary time. Bodmer (1990), by contrast, argued that frugivory should be recognized as distinct from browsing and grazing. Our results partially support both these hypotheses – that frugivory likely represents an adaptation separate from browsing, but that frugivory is an extension of mixed-feeding (rather than browsing) character states. However, further evidence from more strongly frugivorous Cephalophini is needed.

Among grazers, we contrast between variable (consume small, but significant amounts of dicots) and obligate grazers. Testing predictions for variable grazing based on carbon isotope data alone is difficult because variations in faecal $\delta^{13}\text{C}$ are relatively small and may merely reflect the intake of NAD-ME/PCK versus NADP-ME type grasses (Cerling & Harris, 1999). Most observational studies assert that species such as white rhinoceros, hippopotamus and tsessebe are almost exclusive grazers (e.g. Child, Robbel & Hepburn, 1972; Joubert & Bronkhorst, 1977; Owen-Smith, 1988; Skinner & Smithers, 1990). Hence, several taxa included in the variable grazer group may indeed be obligate grazers. Only buffalo and wildebeest show sufficiently large variations in faecal $\delta^{13}\text{C}$ to reflect C₃ intake with certainty.

Gagnon & Chew (2000) and Cerling *et al.* (2003) also distinguish between variable and obligate grazing. However, these studies differed in terms of the species composition of these groups. For example, Gagnon & 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.*, 2003c) indicate that these species are more consistent grazers. Cerling *et al.* (2003), by contrast, classified blue wildebeest (and red hartebeest) as 'hypergrazers'. Carbon isotope data for the Kruger Park wildebeest population suggest some intake of C₃ foods (~10%), as do isotope data for specimens from across the sub-continent (Sponheimer *et al.*, 2003b). Given these disparities, which are likely related to geographical differences

in diet (e.g. between wildebeest from southern and East Africa), a distinction between variable and obligate grazers seems at best site specific, rather than a characteristic feature of certain taxa, and is possibly even redundant.

Implications for future diet studies

There is incongruence between the results of stable carbon isotope and field studies for the diets of several ungulate species. In some cases, this is likely due to variation in the quality of available observational data, which are often based on time spent feeding on a particular food item(s) and do not record actual biomass intake. A further caveat is the poorly documented phenomenon of spatio-temporal variability within taxa. Indeed, several previous studies advocate that ungulate diets should be treated as continuous, rather than using discrete categories of feeding style (Janis, 1995; Cerling *et al.*, 2003; Clauss, Lechner-Doll & Streich, 2003; Perez-Barberia *et al.*, 2004). 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 continuous. In our broad assessment of ungulate feeding habits, we were 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.

While the models presented here do control for dietary variations, we have not attempted to document variation between specific regional and seasonal scales, except for a very broad comparison across dry and wet seasons. This begs an important question for future research: 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 carbon isotope ecology is a powerful tool for documenting and expressing dietary continuity (see also Cerling *et al.*, 2003; Sponheimer *et al.*, 2003b). Further, more rigorous exploitation of this potential, especially if used in conjunction with traditional approaches, can assist with providing refined answers to questions of herbivore feeding ecology.

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