

**African wild dogs (*Lycaon pictus*) show differences in diet composition across landscape types in Kruger National Park, South Africa**

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1 Running Title: **Wild dog diet differs across the Kruger Park.**

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4 **types in Kruger National Park, South Africa**

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22  
23 The Kruger National Park (KNP) is home to the last genetically viable, minimally managed  
24 population of African wild dogs (*Lycaon pictus*, wild dogs) in South Africa. Until 2004, this  
25 population remained stable, but since has been declining. In this study, we aimed to improve our  
26 understanding of the ecology of KNP wild dogs by estimating the relative contribution of  
27 different prey types to their diet across landscape types. Based on a Bayesian mixing model, we  
28 assessed wild dog diet and foraging preferences using stable isotope analysis (SIA). We sampled  
29 73 individuals from 40 packs found in six different landscape types. In thickets, packs  
30 predominantly prey on small browsing and mixed feeding species (accounting for ~73% of their  
31 diet), but occasionally hunt large grazers (~24%), and large browsers (~3%). In open landscape  
32 types where lions (*Panthera leo*) are more or less absent, such as in the Lowveld sour bushveld,  
33 wild dogs prey on large browsers and large grazers (~67%). Our results demonstrate that KNP  
34 wild dogs occupy a broader ecological niche than previously thought, with small browsers  
35 forming an integral part of their diet. We also present the first data describing differences in wild  
36 dog diet-tissue discrimination factors for tail hair and whiskers compared to respective stable  
37 nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) values obtained from feces of captive wild dogs, as well as  
38 from those of South Africa's broader managed metapopulation. While these data should be  
39 considered preliminary, we suggest that until wild dog diet-tissue discrimination factors are  
40 calculated through a controlled feeding study, that the discrimination factors calculated for the  
41 grey wolf (*Canis lupus*) should be used for wild dog related isotope studies, rather than the often-  
42 cited values for red foxes (*Vulpes vulpes*).

43

44 **Keywords:** African wild dog; diet; feces; hair; isotopic discrimination; Kruger National Park;  
45 stable isotope analysis; South Africa; trophic ecology; whiskers.

46

**INTRODUCTION**

47 African wild dogs (*Lycaon pictus*), hereafter wild dogs, are the most endangered  
48 carnivore in South Africa, and the second-most endangered carnivore on the African continent  
49 (Davies-Mostert et al. 2016). Due to an increasing human population, wild dogs have become  
50 patchily distributed across a fragmented landscape of conservation areas, with vast terrain  
51 previously used by wild dogs being transformed into agricultural, urban, and game-breeding  
52 areas (Mills et al. 1998; Creel and Creel 2002; Davies-Mostert et al. 2015). Major anthropogenic  
53 threats to the species include high rates of snaring by poachers, introduction of diseases from  
54 domesticated animals, road accidents, and direct persecution derived from conflict with wildlife  
55 farmers (Woodroffe et al. 2007a; Gusset et al. 2008; Woodroffe and Sillero-Zubiri 2020).  
56 Compounding these issues is that wild dogs naturally live in low densities, even when prey  
57 species are abundant, and are in direct competition with other intra-guild predators such as lions  
58 (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*; Creel and Creel 2002; Woodroffe et al.  
59 2007a). This makes it difficult for packs to persist in most protected areas that also maintain high  
60 densities of other predator species. In these areas, wild dogs need to survive by resource  
61 partitioning and avoiding lions and spotted hyenas (Mills and Gorman 1997; Miller et al. 2013;  
62 Swanson et al. 2014), with the exception of Hluhluwe-iMfolozi Park (HiP) in South Africa,  
63 where both wild dogs and lions have maintained consistently high densities (Somers et al. 2017;  
64 Marneweck 2020).

65 In South Africa, efforts to minimize the extinction risk of wild dogs resulted in the wild  
66 dog managed metapopulation program being implemented in 1998. This program is aimed at  
67 creating a second, genetically-viable population of wild dogs outside of the last viable, relatively  
68 unmanaged population occurring within the Kruger National Park (KNP; Mills et al. 1998;

69 Davies-Mostert et al. 2015; Tensen et al.2019). Such management involves periodic  
70 translocation of wild dogs between game reserves that host packs to mimic natural dispersal  
71 events and gene flow as closely as possible, and avoid deleterious effects associated with a  
72 genetic bottleneck (Mills et al. 1998; Gusset et al. 2006). Overall, this approach has been  
73 successful in increasing the number of wild dogs in South Africa, with the managed  
74 metapopulation growing from 17 individuals in 1998 to 202 in 2005 (Davies-Mostert et al.  
75 2015). To date, the South African population remains small but somewhat stable at ~500  
76 individuals (Nicholson et al. 2020), while maintaining levels of genetic variability comparable  
77 with natural systems, with the current managed metapopulation maintaining ~95% of its  
78 heterozygosity (Tensen et al. 2019).

79       Of particular conservation concern is that wild dogs in the KNP, a population considered  
80 of global conservation and genetic significance (Creel et al. 2004; Tensen et al. 2016; Kuiper et  
81 al. 2018), are declining at ~3% per annum (Nicholson et al. 2020). Nicholson et al. (2020)  
82 therefore suggested that management actions to assist the KNP wild dog population be  
83 considered. Information on foraging preferences can aid in understanding species-specific animal  
84 behavior and physiological processes, as well as assist in structuring and implementing relevant  
85 conservation measures (Webster et al. 2002; West et al. 2006; Crawford et al.2008). For  
86 carnivores in particular, dietary information has traditionally been gathered by means of gut  
87 content analysis (Pezzo et al. 2003; Valdmann et al. 2005), from kill sites (Marucco et al. 2008;  
88 Webb et al. 2008), and the investigation of fecal contents for the remains of prey species  
89 (Darimont et al. 2004; Latham et al. 2013). Data derived from these methods have become the  
90 primary source of knowledge on the trophic ecology of African wild dogs (Creel and Creel 2002;  
91 van Dyk and Slotow 2003; Woodroffe et al. 2007b; Davies-Mostert et al. 2013). Such

92 approaches are not always logistically feasible, because they require long-term field excursions  
93 (Avenant and Nel 2002), which become increasingly difficult when investigating animals that  
94 occupy large home ranges (Bothma and Coertze 2004), such as wild dogs (Creel and Creel  
95 2002). The invasive nature of gut content analysis also is not appropriate when study species are  
96 of conservation concern (Darimont and Reimchen 2002), and observations of fecal contents may  
97 not always be reliable because prey items differ in their digestibility (Lockie 1959; Darimont and  
98 Reimchen 2002).

99         Stable isotope analysis (SIA) is a useful tool in trophic ecology, and has become  
100 increasingly popular as a means to complement traditional methods used to study community  
101 structure and predator-prey interactions (Roth and Hobson 2000; Codron et al. 2007; McLaren et  
102 al. 2015). Ratios of the stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$  or  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$  or  $\delta^{13}\text{C}$ )  
103 are used frequently in such studies. Stable nitrogen isotope ratios change predictably with trophic  
104 level (e.g., isotopically light  $^{14}\text{N}$  is excreted in urine; DeNiro and Epstein 1981; Peterson and Fry  
105 1987), while stable carbon isotope ratios show very little change with trophic position and reflect  
106 sources of primary productivity (DeNiro and Epstein 1978; Vogel 1978). Stable isotope analysis  
107 is advantageous when examining metabolically inert tissue types (such as whiskers, hair,  
108 feathers, and nails), because dietary information from all assimilated food sources over the  
109 period of tissue growth is integrated (Roth and Hobson 2000; Voigt et al. 2014; McLaren et al.  
110 2015). Correctly used, SIA can be used to illustrate relatively non-biased links between primary  
111 and secondary consumers that otherwise are often challenging to demonstrate (Codron et al.  
112 2007). A critical limitation to such analyses is that there currently is little information regarding  
113 species-specific isotopic discrimination factors, which account for shifts in stable isotope ratios  
114 once dietary sources are assimilated into different consumer tissue types (McLaren et al. 2015).

115           Based on data derived from direct observations of hunts, as well as scat analysis, wild  
116 dogs in the KNP have been reported to act as rate maximizing optimal foragers; specializing on  
117 impala (*Aepyceros melampus*) and kudu (*Tragelaphus strepsiceros*; Reich 1981; Mills 1992;  
118 Mills and Gorman 1997), and showing an affinity for thicket and woodland landscape types  
119 (Mills and Biggs 1993). Wild dog packs in another South African metapopulation managed  
120 reserve (HiP), use dense landscape types to ambush and trap prey, making smaller browser  
121 species amongst the most profitable to be killed (Krüger et al. 1999). Similarly, wild dogs in  
122 northern Kenya, have been shown to feed predominantly on a small browsing species such as  
123 Kirk's dik-dik (*Madoqua kirkii*), with this species making up ~70% of their diet outside of  
124 protected areas (Woodroffe et al. 2007b).

125           In this study, we aimed to improve our understanding of the trophic ecology of KNP wild  
126 dog packs by assessing the relative contribution of different prey groups to their diet across  
127 landscape types. These landscape types have been defined based on specific geomorphology, soil  
128 composition, climate, vegetation pattern and associated fauna, and form defined units for  
129 management practices in the KNP (Gertenbach 1983). This was undertaken using a Bayesian  
130 isotope mixing model based on the isotopic analysis of tail hair samples from 73 individuals  
131 across 40 packs collected between January 2009 and December 2018. To our knowledge, there  
132 currently is only one published study using SIA as it pertains to the trophic ecology of wild dogs  
133 (Crossey et al. 2020); in the current study we thus present preliminary estimates of wild dog diet-  
134 tissue isotopic discrimination factors. These estimates, based on samples collected from captive  
135 wild dogs, as well as from animals forming part of South Africa's managed metapopulation  
136 outside of the KNP, were calculated using feces as a proxy of dietary content not integrated by  
137 individuals (Crawford et al. 2008), and drawing comparisons between the fecal values obtained

138 with both tail hair and whisker  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, respectively. We predicted that wild dog  
139 diet would differ between landscape types, as the profitability of hunting prey species varies with  
140 environmental factors, and that wild dog diet-tissue discrimination factor estimates would be  
141 similar to those calculated for grey wolves (*Canis lupus*; McLaren et al. 2015), the closest  
142 phylogenetic relative of wild dogs (Gopalakrishnan et al. 2018) for which we could find  
143 published data.

144

145

## MATERIALS AND METHODS

146 *Ethical clearance and sampling permits.*— This study was undertaken with the approval  
147 of the University of Pretoria Animal Ethics Committee (Ethics clearance number: EC015-18),  
148 and followed the guidelines of the American Society of Mammalogists (ASM) pertaining to  
149 ethical research involving live animals (Sikes et al. 2016). Hair, whisker and fecal samples from  
150 wild dogs within the managed metapopulation were obtained with permission from the  
151 Endangered Wildlife Trust (EWT; data sharing agreement number: 270). All hair samples  
152 obtained from the SANParks Biobank at Skukuza in KNP were acquired through a data sharing  
153 agreement, as well as with a Threatened or Protected Species Ordinary Permit (Permit number:  
154 O 27732).

155 *Sampling sites and study animals.*—The KNP (covering ~2,000,000 ha) is situated in the  
156 Lowveld semi-arid savanna in the north-eastern corner of South Africa (Fig. 1). At ~300m above  
157 sea level, rainfall is highly seasonal and mainly occurs during the austral summer (October –  
158 March), with April – November being dry. Mean annual rainfall varies from between 500 – 700  
159 mm in the south of the park, to between 300 – 500 mm in the north (Venter et al. 2003). We



160 obtained tail hair samples from 73 wild dogs across 40 different packs (Table 1), which were  
161 stored at the SANParks Skukuza Biobank, KNP. These samples represent the period between  
162 January 2009 and December 2018, and were collected opportunistically throughout the park.  
163 Global positioning system (GPS) co-ordinates were recorded for each wild dog sampled. Wild  
164 dogs are co-operative hunters, hunting and feeding as a group (Creel and Creel 2002), and  
165 individuals within packs feed on the same prey items. We therefore considered packs, rather than  
166 individuals, as our sampling units. Packs were determined to be independently sampled based on  
167 the collection of samples from known individuals. Each wild dog pack sampled was assigned to  
168 a landscape type (Gertenbach 1983; Table 1) based on the GPS co-ordinates indicating their  
169 sampling location (Fig. 1).

170 We carried out a preliminary investigation into wild dog diet-tissue discrimination factors  
171 using opportunistically collected whisker, tail hair, and fecal samples from 13 wild dogs  
172 immobilized either for translocation or routine veterinary purposes from April to July 2018.  
173 These individuals were all sampled in South Africa, and included captive wild dogs from the  
174 Johannesburg Zoo ( $n=3$ ), Gauteng Province; temporarily captive wild dogs being readied for  
175 release into larger reserves in short-term holding facilities at Zimanga Private Game Reserve  
176 ( $n=4$ ) and Tembe Elephant Park ( $n=1$ ; both in KwaZulu Natal Province); and free ranging wild  
177 dogs from Madikwe Game Reserve ( $n=1$ ), North West Province, and HiP ( $n=4$ ) in KwaZulu  
178 Natal. To ensure a minimally invasive means of sampling, samples were collected by trimming  
179 the tail hair/whisker as close to the base of the follicle as possible. Approximately 5g of fecal  
180 material from each of these wild dogs was collected and frozen within 24 h post-defecation at -  
181 20°C until further processing.

182           *Stable isotope analysis.*—All frozen fecal samples were lyophilized, then ground and  
183 sieved using a metal strainer (mesh size: 20 $\mu$ m). This was undertaken to separate fecal powder  
184 from undigested material, such as large pieces of bone, hair, and grass (Fiess et al. 1999), which  
185 potentially could interfere with fecal stable isotope values. Whisker and tail hair samples were  
186 cleaned by suspending them in a 2:1 chloroform: ethanol solution in an ultrasonic bath for 15  
187 minutes. The solvent then was decanted, and samples dried overnight at 70°C. Fecal powder, tail  
188 hair, and whiskers (whiskers sampled from base to tip, up to a maximum of six segments) were  
189 weighed as aliquots of ~0.4 – 0.6 mg using a micro-balance (Mettler Toledo Mk5; Mettler  
190 Toledo, Columbus, Ohio), and placed in tin capsules pre-cleaned in toluene. Samples were  
191 combusted at 1020°C in an elemental analyzer (Flash EA1112 Series), coupled to a Delta V Plus  
192 stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by  
193 Thermo Fisher, Bremen, Germany). Two laboratory running standards and a blank sample were  
194 run after every 11 unknown samples (Merck Gel and DL-Valine). These running standards are  
195 calibrated against international standards (IAEA-CH-3, IAEA-CH-6, IAEA-CH-7, IAEA N-1,  
196 IAEA N-2, IAEA NO-3) produced by the International Atomic Energy Association (IAEA), and  
197 NBS22 (produced by the United States National Bureau of Standards). The precision for  $\delta^{15}\text{N}$   
198 was <0.05‰, and <0.06‰ for  $\delta^{13}\text{C}$ . All results are referenced to Vienna Pee Dee Belemnite for  
199 carbon isotope values, and to air for nitrogen isotope values (Bond and Hobson 2012). Results  
200 are expressed in delta notation using a per mille scale (‰) using the following standard equation  
201 (Coplen 2011):

$$202 \qquad \qquad \qquad \delta X = [(R_{\text{sample}}/R_{\text{standard}})-1]$$

203           (where, X=  $^{15}\text{N}$  or  $^{13}\text{C}$  and R represents  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ , respectively).

204 *Statistical analyses.*—Values for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  from tail hair and feces were obtained  
205 from samples run in duplicate. Whisker samples yielded three to six sub-sampled segments  
206 depending on the whisker length/density. We used a one-way analysis of variance (ANOVA)  
207 (Scheffe 1999), followed by Holm-Sidak's pair wise *t*-tests (Wenge and Romano 2007) to test  
208 for differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures between tail hair, whiskers, and fecal samples from  
209 the same individual. Preliminary indicators for wild dog diet-tissue discrimination factors were  
210 calculated using the mean  $\pm$  standard deviation (*SD*) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values calculated for each  
211 sample type, with mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for tail hair and whiskers subtracted from respective fecal  
212 values (as a proxy of diet; Crawford et al. 2008), to give isotopic diet-tissue discrimination  
213 estimates (Table 2). Differences in mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  tail hair values from wild dog packs  
214 sampled in different landscape types in the KNP also were tested using a one way ANOVA  
215 (Scheffe 1999), followed by Holm-Sidak's pair wise *t*-tests (Wenge and Romano 2007) to isolate  
216 which values from specific landscape types significantly differed from others. Statistical  
217 significance was set at alpha ( $\alpha$ ) = 0.05 and inferred at  $P < 0.05$ . We computed these statistical  
218 analyses using algorithms in Sigma Plot Version 14.0 (Systat Software 2017).

219 *Isotopic discrimination factor estimates.*—Our preliminary isotopic discrimination  
220 results, calculated from mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for tail hair subtracted from respective fecal  
221 values, suggest that there is an  $\sim +4.4\text{‰}$  difference in  $\delta^{13}\text{C}$ , and  $+1.6\text{‰}$  difference in  $\delta^{15}\text{N}$  values  
222 between wild dog feces and tail hair. However, these results are not fully representative of total  
223 dietary information integrated over the period of tail hair growth, with feces only exhibiting a  
224 snapshot of short-term dietary information. In addition, the growth rates for wild dog whiskers  
225 and tail hair are currently unknown, and these data should therefore be interpreted with caution.  
226 We could also not account for differences in the isotopic composition of wild dog diet across

227 geographic locations, as well as any variation in diet between individuals sampled in this portion  
228 of the study (eight of which we sampled in captivity and five of which were free-ranging). For  
229 this reason, we used isotopic discrimination factors from the closest phylogenetic relative of the  
230 wild dog (the grey wolf), for which published data were available (McLaren et al. 2015). Isotopic  
231 diet-tissue discrimination factors of +4.3‰ for  $\delta^{13}\text{C}$  and +3.1 for  $\delta^{15}\text{N}$ , as calculated for wolf hair  
232 over a four-month controlled feeding study were thus applied for respective tail hair values in  
233 our study (McLaren et al. 2015).

234 *Stable isotope analysis in R (SIAR).*—In order to assess the relative contribution of prey  
235 types to wild dog diet across landscape types in the KNP, we used the package Stable Isotope  
236 Analysis in R (SIAR) version 4.1.3 (Parnell and Jackson 2011) in the software program R (R  
237 Core Team 2016). The SIAR package fits data on animal isotopes to their dietary habits using a  
238 Bayesian isotope mixing model. This is based on a Gaussian likelihood, solving for the most  
239 likely set of dietary proportions when given the isotopic ratios in a set of possible food sources  
240 and a set of consumers (Parnell and Jackson 2011). Upper and lower estimates for the proportion  
241 of prey groups contributing to wild dog diet in the different landscape types are reported with a  
242 95% confidence interval, and illustrations were produced using Sigma Plot Version 14.0 (Systat  
243 Software, San Jose, CA, USA).

244 Published data for different KNP prey species, classified into isotopically distinct ( $\delta^{13}\text{C}$   
245 and  $\delta^{15}\text{N}$ ) prey groups, characterizing the isotopic composition of a predator's diet, were used in  
246 the development of our model (Table 2). These data were obtained from Codron et al. (2007),  
247 with prey groups from KNP categorized as follows: 1)  $\text{C}_3$  large-bodied browsers (>100 kg,  
248 comprising southern giraffe, *Giraffa camelopardalis*; and kudu, *Tragelaphus strepsiceros*); 2)  $\text{C}_3$   
249 small-bodied browsers (<100 kg, including bushbuck, *Tragelaphus scriptus*; steenbok,

250 *Raphicerus campestris*; and grey duiker, *Sylvicapra grimmia*); 3) C<sub>4</sub> large-bodied grazers  
251 (Burchell's zebra, *Equus burchellii*; African buffalo, *Syncerus caffer*; blue wildebeest,  
252 *Connochaetes taurinus*; waterbuck, *Kobus ellipsiprymnus*; reedbuck, *Redunca arundinum*; and  
253 sable antelope, *Hippotragus niger*); and 4) C<sub>3</sub>/C<sub>4</sub> mixed feeding impala, *Aepyceros melampus*, as  
254 the most abundant and predominant mixed feeding herbivore found in the KNP.

255 For the SIAR, we calculated mean and SD  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for wild dog packs  
256 sampled in different landscape types of the KNP (Gertenbach 1983). This resulted in wild dog  
257 packs being grouped into one of the following six separate categories: i) those sampled in  
258 Phalaborwa sandveld ( $n = 1$ ); ii) Mixed *Combretum/Terminalia sericea* woodland (mixed  
259 woodlands;  $n = 12$ ); iii) Lowveld sour bushveld ( $n = 5$ ); iv) Malelane mountain bushveld ( $n = 8$ );  
260 v) Thickets of the Sabie and Crocodile River (thickets;  $n = 13$ ); and vi) *Sclerocarya birrea*  
261 *caffra/Acacia nigrescens* savanna (savanna;  $n = 1$ ; Gertenbach 1983; Table 1; Fig. 1). Packs from  
262 the Phalaborwa sandveld and *Sclerocarya birrea caffra/Acacia nigrescens* savanna were  
263 excluded from analyses in SIAR as the sample size was too small ( $n = 1$  in each case) to estimate  
264 intra group variance.

265

266

## RESULTS

267 We found statistically significant differences between both the  $\delta^{15}\text{N}$  ( $F_{2,36} = 8.49$ ;  $n=13$ ;  
268  $P<0.05$ ) and  $\delta^{13}\text{C}$  ( $F_{2,36} = 50.49$ ;  $n=13$ ;  $P<0.05$ ) values obtained from the different biological  
269 sample types. Tail hair and whiskers did not significantly differ either in their  $\delta^{15}\text{N}$  values  
270 ( $t_{11}=0.33$ ;  $n=13$ ;  $P>0.05$ ) or  $\delta^{13}\text{C}$  values ( $t_{11}=0.22$ ;  $n=13$ ;  $P>0.05$ ), but had significantly different  
271  $\delta^{13}\text{C}$  (tail hair:  $t_{11}=8.81$ ;  $n=13$ ;  $P<0.05$ ; whisker:  $t_{11}=8.59$ ;  $n=13$ ;  $P<0.05$ ) and  $\delta^{15}\text{N}$  (tail hair:

272  $t_{11}=3.72$ ;  $n=13$ ;  $P<0.05$ ; whisker:  $t_{11}=3.39$ ;  $n=13$ ;  $P<0.05$ ) values compared to the respective  
273 values obtained from fecal matter. Our preliminary wild dog isotopic diet-tissue discrimination  
274 estimates were +4.4‰ and +4.3‰ for tail hair and whisker  $\delta^{13}\text{C}$  values, respectively, as well as  
275 +1.6‰ and +1.4‰ for respective  $\delta^{15}\text{N}$  values (Table 3).

276 Stable isotope results (mean  $\pm$  *SD*) obtained from wild dogs sampled in the different KNP  
277 landscape types are summarized in Table 1. Our results showed significant differences in  $\delta^{15}\text{N}$   
278 ( $F_3=11.62$ ;  $n=38$ ;  $P<0.01$ ) values between wild dog packs found in the different landscape types  
279 of KNP, but  $\delta^{13}\text{C}$  values did not differ significantly for wild dog packs across landscape types  
280 ( $F_3=0.41$ ;  $n=38$ ;  $P>0.05$ ). Differences in  $\delta^{15}\text{N}$  values were only significant for wild dogs from  
281 Lowveld sour bushveld versus those occurring in mixed woodlands ( $t_{4,11}=5.13$ ;  $n=17$ ;  $P<0.05$ ),  
282 thickets ( $t_{4,12}=5.41$ ;  $n=18$ ;  $P<0.01$ ), and Malelane mountain bushveld ( $t_{4,6}=3.03$ ;  $n=12$ ;  $P<0.05$ ),  
283 with all other pair wise comparisons yielding  $P>0.05$ .

284 Estimates for the proportion of prey groups contributing to wild dog diet in the different  
285 landscape types are reported with a 95% confidence interval (Fig. 2). Our results show distinct  
286 differences in wild dog diet across KNP landscape types. Differences in the proportion of each  
287 prey group related to the landscape type within which wild dogs were sampled were largest for:  
288 i) Large browsers in thickets (~3%) and mixed woodlands (1-3%) versus Lowveld sour bushveld  
289 (31 – 33%); ii) Small browsers, which were lowest in the Lowveld sour bushveld (21 – 23%) and  
290 highest in thickets (39 – 40%); iii) Large grazers, which varied from 7– 8% for wild dogs  
291 sampled in mixed woodland, to 23 – 24% in both thicket and Malelane mountain bushveld,  
292 respectively; and iv) Impala, which was lowest for packs sampled in Lowveld sour bushveld (15  
293 – 17%) and highest in mixed woodland (61 – 62%; Fig.2).

294

**DISCUSSION**

295 *Diet-tissue isotopic discrimination estimates.*—McLaren et al. (2015) demonstrated that  
296 grey wolf whiskers and guard hairs show stable carbon diet-to-tissue isotopic discrimination of  
297 +4.31 and + 4.25‰, with nitrogen diet-tissue isotopic discrimination factors of +3.05 and  
298 +3.09‰, respectively. Our preliminary wild dog diet-tissue discrimination estimates for carbon  
299 closely match those of grey wolves for both whiskers (+4.3‰) and hair (+4.4‰). The  
300 discrimination factors for nitrogen calculated in our study (+1.4 for whiskers and +1.6 for tail  
301 hair) are two times lower than those obtained for grey wolves. As diet composition ultimately  
302 determines the source of amino acids for tissue synthesis, and subsequent carbon and nitrogen  
303 discrimination factors (Martínez del Rio et al. 2009; Parnig et al. 2014), we speculate that  
304 variation in lipid proportion and protein quality in our wild dogs' diet may explain the  
305 differences in the respective nitrogen discrimination factors (Caut et al. 2009).

306 We also consider that there are species-specific differences in the physiological processes  
307 that affect isotopic fractionation. In particular, variability in  $\delta^{15}\text{N}$  values is known to be greater  
308 for individuals from different species raised on the same diet, than for individuals of the same  
309 species raised on a different diet (DeNiro and Epstein 1981). The selection of discrimination  
310 factors can influence estimated diet proportions when using Bayesian mixing models (Derbridge  
311 et al. 2012). As a result, taxon-, tissue-, and diet-specific discrimination factors for each study  
312 species should be determined through controlled feeding studies (Caut et al. 2009; McLaren et al.  
313 2015). Based on the close phylogenetic relationship between wild dogs and grey wolves  
314 (Gopalakrishnan et al. 2018), we are confident in our choice of isotopic discrimination factors for  
315 grey wolves, over the commonly cited values for captive red foxes (*Vulpes vulpes*; Roth and  
316 Hobson 2000). We therefore suggest that grey wolf isotopic discrimination factors (McLaren et

317 al. 2015) be used for future studies attempting to address wild dog-based SIA questions, at least  
318 until wild dog-specific diet-tissue discrimination factors can be more reliably calculated.

319 *Dietary differences.*—It has been suggested that wild dogs in KNP mainly prey upon  
320 impala and kudu (the most abundant medium-to-large sized prey in the park; Reich 1981; Mills  
321 1992; Mills and Gorman 1997). Our results suggest that prey preferences of wild dogs in KNP  
322 differ significantly across their used landscape types. Marneweck et al. (2019) calculated home  
323 range sizes for 19 of the packs included in our study over the same period as our sampling was  
324 undertaken. The data presented by these authors show that the packs we sampled maintain home  
325 ranges that include several different landscape types outlined by Gertenbach (1983). It may  
326 therefore be the case that the landscape type within which we sampled wild dog packs is not the  
327 only landscape type used by these packs while hunting. Despite the possibility for this overlap,  
328 and the high likelihood that packs spend an unequal amount of time either moving through or  
329 hunting in different landscape types, our results indicate significant variation in the diet of wild  
330 dogs depending on the landscape type where they were sampled.

331 Growth and isotopic turnover rates for wild dog hair have not been investigated.  
332 However, McLaren et al. (2015) demonstrated that grey wolf hair does not grow continuously,  
333 growing more slowly in the period from 60 – 120 days than from days 0 – 60. In addition, these  
334 authors demonstrated that grey wolf hair grown over a 60-day period exhibited significantly  
335 different  $\delta^{13}\text{C}$  values compared to day zero, but a significant difference could not be detected  
336 between days 60 – 120. Assuming that wild dog hair grows in a similar fashion to that of grey  
337 wolves, the stable isotope values of wild dog hair represent a long-term dietary signal, and this is  
338 representative of the average cumulative diet of packs (thus elucidating their most commonly  
339 consumed prey species). The model we generated predicted that large browsers (predominantly



340 kudu in the case of wild dogs (Mills 1992; Mills and Gorman 1997; Creel and Creel 2002), and  
341 impala combined contribute 50% or more to wild dog diet in both mixed woodland and Lowveld  
342 sour bushveld. Whereas a combination of small browsers and large grazers comprise up to 55%  
343 and 64% of wild dog diet in Malelane mountain bushveld and thicket, respectively.

344         In our study, the highest number of packs sampled in a single landscape type were found  
345 in thicket (13), with mixed woodland a close second (12). In thicket, our model predicts that  
346 small browsers (~40%) contribute almost as much as impala (~33%) to wild dog diet. This is  
347 congruent with the results of Krüger et al. (1999), who found that in HiP, nyala (*Tragelaphus*  
348 *angasii*, a mixed-feeder similar to impala), as well as red duiker (*Cephalophus natalensis*), and  
349 bushbuck (both browsers), are the most profitable species to be targeted in dense habitat types.  
350 This comes as the profitability of prey types differ with various factors (such as the size and  
351 vulnerability of prey across different landscape types), with wild dogs adjusting their prey  
352 selection based on ease of prey-capture (Reich 1981). In dense habitats in HiP, wild dogs use  
353 ambush techniques to flush prey, and chases seldom exceed 1 km (Krüger et al. 1999; similar  
354 behavior has also been recorded for packs in the Selous Game Reserve in Tanzania—Creel and  
355 Creel 1995). The higher proportion of small browsers consumed by packs sampled in KNP  
356 thickets may also be a result of impala behavior, because they are known to avoid dense habitat  
357 types due to an increased risk of predation (Ford et al. 2004). Such a reliance of wild dogs on  
358 small browsers as a major part of their diet has not been reported previously for packs in the  
359 KNP. These results are rather similar to those for wild dogs in northern Kenya, where Woodroffe  
360 et al. (2007b) showed that packs living outside of protected areas feed predominantly (~70%) on  
361 Kirk's dik-dik (a small browsing antelope ~15% that of a wild dog's body weight), with impala  
362 estimated to be the second most consumed species in these areas. The hunting of small browsing

363 prey species in thicket and dense vegetation types could also prove beneficial if KNP packs are  
364 adopting similar hunting strategies to those recorded by Hubel et al. (2016) for wild dog packs in  
365 dense habitat types in Botswana. These authors suggested that a hunting strategy that makes use  
366 of several simultaneous, opportunistic chases, where packs pursue multiple smaller prey items,  
367 may be the key to their hunting success in closed habitat types.

368         We found that large grazers make up ~24% of wild dog diet for packs sampled in the  
369 dense thicket landscape type. Creel and Creel (2002) demonstrated that wild dogs in Selous  
370 Game Reserve exhibit prey preferences based on pack size, with smaller packs disinclined to  
371 hunt larger prey such as Burchell's zebra and blue wildebeest, but larger packs hunting blue  
372 wildebeest proportionately to the rate at which they encountered them. These authors reported  
373 that in 85% of the hunting incidences they observed there were successful kills when wild dogs  
374 chased their prey into dense vegetation. This most likely is because fleeing prey must make  
375 decisions as to the best route around an obstacle, in some cases freezing altogether, whereas wild  
376 dogs, which often are more agile, are able to follow the exact line taken by their target without  
377 needing to make independent decisions (Estes and Goddard 1967; Creel and Creel 2002). The  
378 use of dense vegetation as a potential obstacle for prey is likely a key factor assisting some KNP  
379 wild dog packs to hunt large grazers in these areas. This is not surprising because wild dogs have  
380 been known to kill prey species as large as eland (*Taurotragus oryx*; an animal weighing on  
381 average >400 kg) by using man-made fences and structures as a means of trapping prey (van  
382 Dyk and Slotow 2003; Davies-Mostert et al. 2013).

383         Wild dogs hunt for ~3.5 hours per day, but would need to increase their hunting activity to  
384 12 hours per day to meet energy requirements should they lose a quarter of their food to  
385 kleptoparasitism (Gorman et al. 1998). This poses a serious risk to wild dogs, which naturally

386 live in low densities, and show a negative relationship in density as hyena and lion numbers  
387 increase (Fanshawe and Fitzgibbon 1993; Creel and Creel 1996; Mills et al. 1998). Wild dogs in  
388 KNP lose a large number of kills to kleptoparasitism by spotted hyenas and lions, particularly in  
389 open habitats where visibility is good (Kruuk 1972; Fanshawe and Fitzgibbon 1993). In densely  
390 wooded areas both in Selous Game Reserve and KNP, however, spotted hyenas are rarely able to  
391 take food from wild dog packs (Mills and Biggs 1993; Creel and Creel 1995). Hunting in  
392 thickets in the KNP likely plays the dual role of allowing wild dogs to manage their energy  
393 requirements through opportunistic and ambush hunting strategies, where chase durations are  
394 reduced, while simultaneously avoiding kleptoparasitism by spotted hyenas and lions (Davies et  
395 al. 2021). In mixed woodland, where visibility is greater than in thickets, but still poor enough to  
396 confer an advantage in kleptoparasitism avoidance, our model shows that wild dogs are acting as  
397 rate maximizers. Here, it appears that packs are trading-off the benefits of opportunistic hunting,  
398 and instead acting as foraging specialists, hunting the most abundant medium-to-large-sized prey  
399 species available (Ginsberg and Macdonald 1990), with impala comprising ~62% of their diet.  
400 As seen for the packs sampled in thicket, small browsers still make up a sizable portion of wild  
401 dog diet for packs sampled in the mixed woodland landscape type (26-27%).

402       Approximately 39% of natural pup mortality, and 43% of natural adult wild dog mortality  
403 in KNP is caused by lions (van Heerden et al. 1995). In addition, Mills and Gorman (1997)  
404 demonstrated that KNP wild dogs avoid habitats chosen by lions, and Swanson et al. (2014)  
405 showed declines in the wild dog population of the Serengeti National Park, Tanzania, as lion  
406 numbers tripled between 1966 and 1998. Lions tend to select their habitat based on the density of  
407 their favoured prey (blue wildebeest, buffalo, and Burchell's zebra), which has resulted in KNP  
408 lion prides showing a strong affinity for savanna, where these prey species are abundant

409 (Gertenbach 1983; Mills and Gorman 1997; Marneweck et al. 2019). It therefore is not surprising  
410 that we only were able to collect a single sample from a wild dog in open savanna, with  
411 Marneweck et al. (2019) finding no wild dog home ranges along the eastern boundary of the  
412 KNP, and these areas being almost completely avoided by wild dog packs.

413         As the name suggests, the Lowveld sourveld is characterized by the presence of sour  
414 grass species, such as *Hyperthelia dissolute*, and generally is avoided by buffalo (Gertenbach  
415 1983). In this landscape type, where lion density is comparatively lower than in savanna (Mills  
416 and Gorman 1997), packs that we sampled seem to predominantly feed on large browsers (31 –  
417 33%; Mills 1992; Mills and Gorman 1997; Creel and Creel 2002), and large grazers (~34%).  
418 Small browsers still make up a substantial proportion of their diet (up to 23%), but it is likely  
419 that in the relative absence of lions, wild dogs find it more profitable to hunt larger prey species  
420 more regularly than smaller species, particularly if their pack size is large enough (Creel and  
421 Creel 1995; Courchamp and Macdonald 2001). Mills and Gorman (1997) demonstrated that  
422 Lowveld sour bushveld and Malelane mountain bushveld both are preferred by KNP wild dogs,  
423 despite impala showing no preference for these landscape types. They suggested that the absence  
424 of impala is countered by the presence of kudu (the second-most conspicuous prey species) in  
425 both of these landscape types. Our model supports these findings for the Lowveld sour bushveld,  
426 indicating that kudu make up a sizeable portion of wild dog diet in this landscape type (31 –  
427 33%), but also suggests that the presence of small browsers (29 – 31%) may make up for the  
428 fewer impala being present in the Malelane mountain bushveld.

429         Rogers et al. (2020) showed that SIA modelling approaches that analyze whole hair  
430 samples, and not serially sectioned tissue segments, are unlikely to overestimate dietary niche  
431 breadth, but have the potential to underestimate niche breadth estimates for species occupying

432 broad isotopic niches, and which exhibit temporal variations in diet. Wild dogs exhibit temporal  
433 variation in diet, both seasonally, and particularly during droughts (Creel and Creel 2002;  
434 Skinner and Chimimba 2005). We, therefore, are confident in the accuracy of our model's  
435 prediction that KNP wild dogs occupy a broader dietary niche than previously reported.  
436 However, our results may represent an under-estimate of the breadth of wild dog foraging  
437 preferences in the park. We suggest that such preferences may be better elucidated using wild  
438 dog-specific diet-tissue discrimination factors (as well as hair and whisker growth rates),  
439 whereafter SIA can be more readily integrated with other traditional sampling methodologies for  
440 monitoring the species.

441 The results of our study show a higher level of adaptability in the foraging behavior of  
442 wild dogs in KNP than previously reported, with small browsers comprising a greater percentage  
443 of wild dog diet than was originally thought. These results may be considered preliminary,  
444 because investigations into specific wild dog diet-tissue discrimination factors could improve the  
445 robustness of the assumptions upon which our model were based.

446

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452

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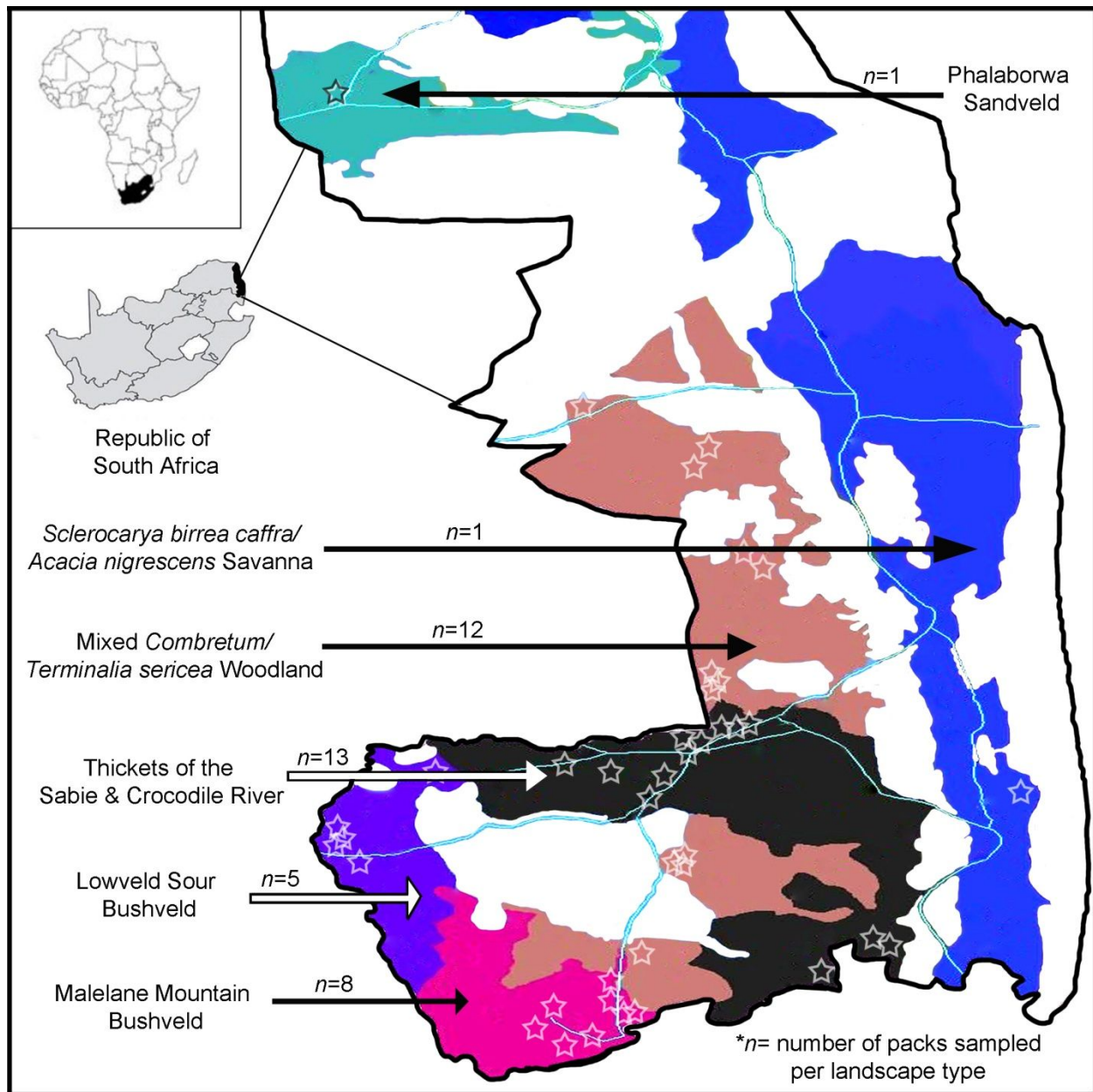
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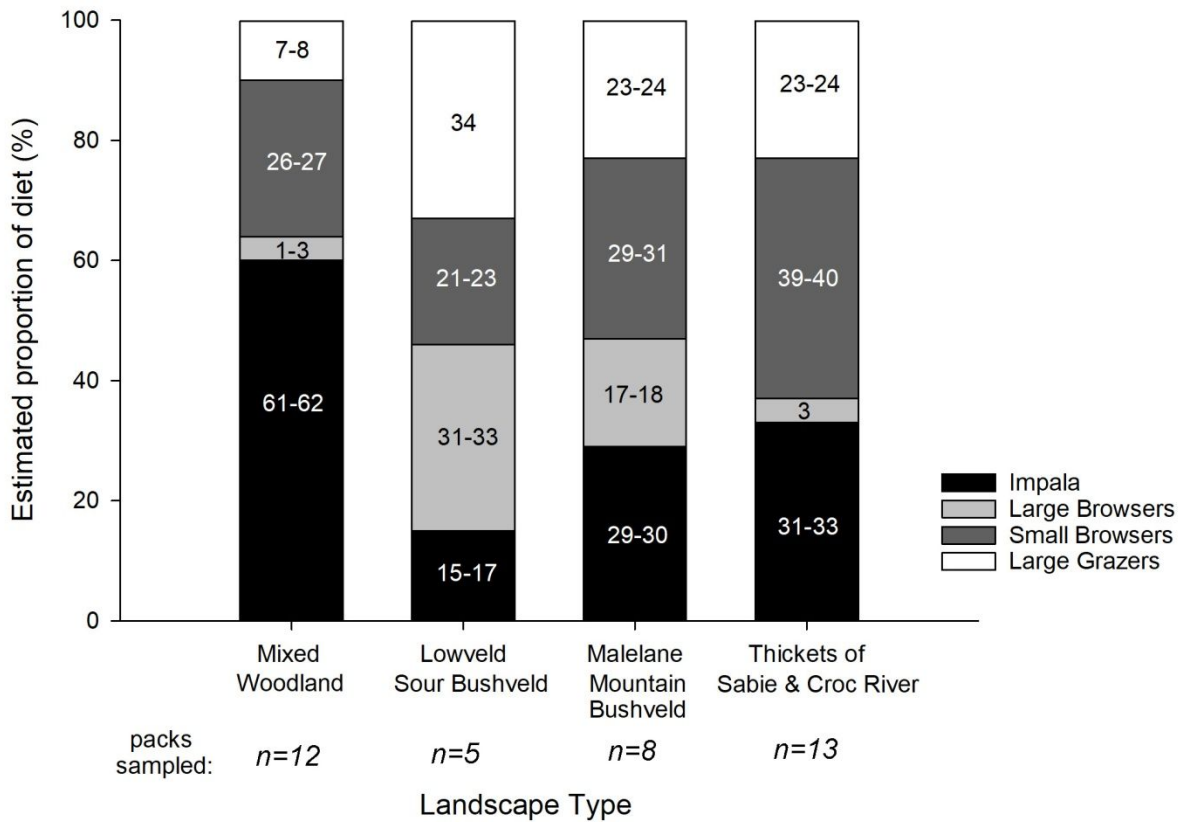


651 **Fig. 1.**—Landscape types of the Kruger National Park, South Africa (Gertenbach 1983) in which

652 African wild dog (*Lycaon pictus*) packs were sampled. Star symbols indicate the location where

653 each pack was sampled and lines indicate the presence of tarred roads in the Park.





656 **Fig. 2.**—Stacked bar plots reporting the upper and lower 95% confidence intervals of the  
 657 estimated proportion of prey species consumed by African wild dog (*Lycaon pictus*) packs  
 658 sampled in different landscape types in the Kruger National Park, South Africa (Gertenbach  
 659 1983).

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665 **Table 1.**—African wild dog (*Lycaon pictus*) tail hair carbon ( $\delta^{13}\text{C}_{\text{VPDB}}$ ) and nitrogen ( $\delta^{15}\text{N}_{\text{Air}}$ )  
 666 stable isotope values (mean  $\pm$  SD) calculated for 40 packs sampled in different landscape types  
 667 (Gertenbach 1983) in the Kruger National Park, South Africa.

Landscape type	Wild dogs sampled ( <i>n</i> )	Packs sampled ( <i>n</i> )	Pack tissue data			
			$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)		$\delta^{15}\text{N}_{\text{Air}}$ (‰)	
			Mean	SD	Mean	SD
Mixed <i>Combretum</i> / <i>Terminalia sericea</i> woodland	25	12	-14.7	2.0	+11.4	0.6
Lowveld sour bushveld	7	5	-15.1	0.7	+9.2	0.9
Malelane mountain bushveld	10	8	-15.2	1.2	+10.6	1.2
Thickets of the Sabie & Crocodile River	27	13	-15.2	1.0	+11.5	0.7
<i>Sclerocarya birrea</i> <i>caffra</i> / <i>Acacia nigrescens</i> savanna	1	1	-16.5	-	+11.7	-
Phalaborwa sandveld	3	1	-16.3	0.2	+12.3	0.2

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669 **Table 2.**—Converted muscle estimate carbon ( $\delta^{13}\text{C}_{\text{VPDB}}$ ) and nitrogen ( $\delta^{15}\text{N}_{\text{Air}}$ ) stable isotope  
 670 values (mean  $\pm$  SD) calculated for presumptive prey species of African wild dog (*Lycaon pictus*)  
 671 packs in the Kruger National Park, South Africa. \*Data for prey species were obtained from hair  
 672 samples collected and analyzed by Codron et al. (2007), and categorized into: Large browsers  
 673 (southern giraffe, *Giraffa camelopardalis*, and kudu, *Tragelaphus strepsiceros*); Small browsers  
 674 (bushbuck, *Tragelaphus scriptus*; steenbok, *Raphicerus campestris*; and grey duiker, *Sylvicapra*  
 675 *grimmia*; and Large grazers (Burchell's zebra, *Equus burchellii*; African buffalo, *Syncerus*  
 676 *caffer*; blue wildebeest, *Connochaetes taurinus*; waterbuck, *Kobus ellipsiprymnus*; reedbuck,  
 677 *Redunca arundinum*; and sable antelope, *Hippotragus niger*).

Prey species	Hair samples ( <i>n</i> )	Converted muscle estimates			
		$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)		$\delta^{15}\text{N}_{\text{Air}}$ (‰)	
		Mean	SD	Mean	SD
Large browsers	26	-24.6	1.2	+5.8	2.0
Small browsers	8	-24.8	0.9	+7.2	2.4
Large grazers	100	-12.2	0.8	+6.7	1.1
<i>Aepyceros melampus</i>	42	-17.1	3.3	+8.0	2.1

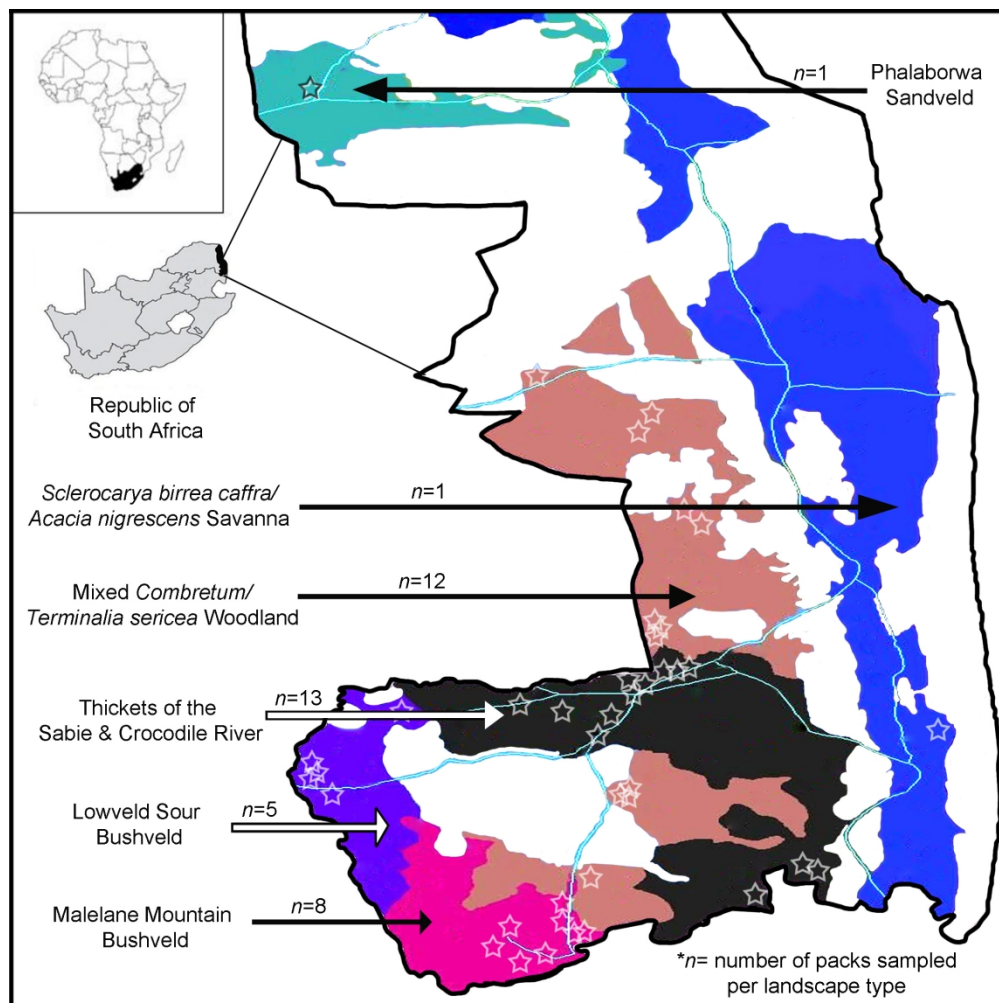
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679 **Table 3.**—Calculated carbon ( $\delta^{13}\text{C}_{\text{VPDB}}$ ) and nitrogen ( $\delta^{15}\text{N}_{\text{Air}}$ ) stable isotope values (mean  $\pm$   
 680 SD) for different biological tissue types (feces, tail hair, and whiskers) and accompanying diet-  
 681 tissue discrimination factor estimates for 13 African wild dogs (*Lycaon pictus*) from South  
 682 Africa.

Biological tissue	<i>n</i>	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)		$\delta^{15}\text{N}_{\text{Air}}$ (‰)		$\delta^{13}\text{C}$ diet-tissue discrimination factor estimate	$\delta^{15}\text{N}$ diet-tissue discrimination factor estimate
		Mean	SD	Mean	SD		
Feces	13	-17.6	1.6	+11.0	1.2	-	-
Tail hair	13	-13.2	1.3	+12.6	1.1	+4.4	+1.6
Whiskers	13	-13.3	0.8	+12.4	0.9	+4.3	+1.4

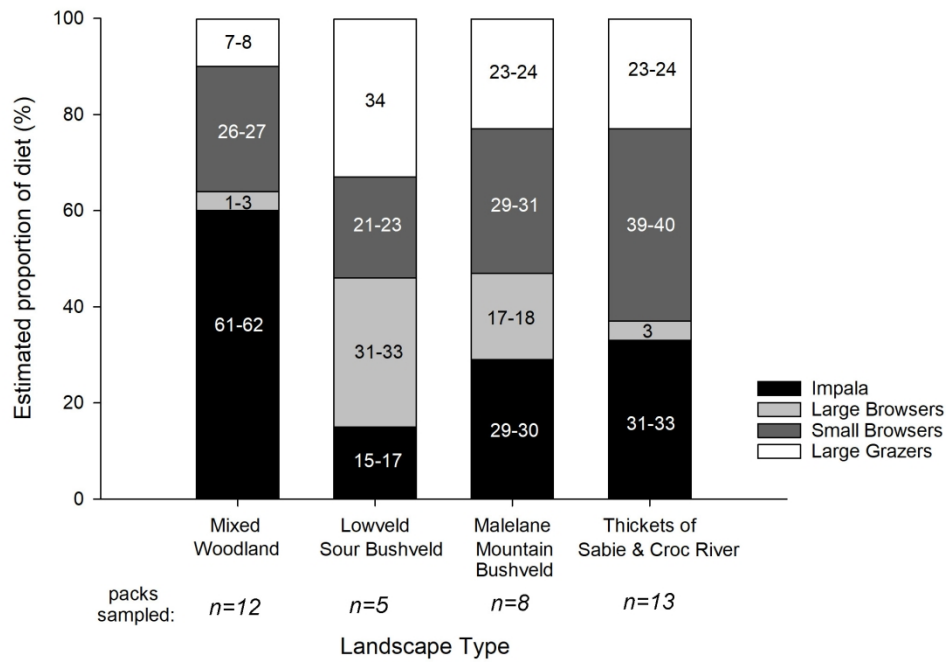
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Landscape types of the Kruger National Park, South Africa (Gertenbach 1983) in which African wild dog (*Lycaon pictus*) packs were sampled. Star symbols indicate the location where each pack was sampled and lines indicate the presence of tarred roads in the Park.

199x199mm (300 x 300 DPI)



Stacked bar plots reporting the upper and lower 95% confidence intervals of the estimated proportion of prey species consumed by African wild dog (*Lycaon pictus*) packs sampled in different landscape types in the Kruger National Park, South Africa (Gertenbach 1983).

184x128mm (300 x 300 DPI)