What's in the diet? DNA-based analysis for qualitative and quantitative assessment of animal diet

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Exploring the effect of fencing on the diet composition and prey preference of lions using DNA-based diet analysis: a case-study in four Kenyan National Parks

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5.1 | Abstract

Fences, omnipresent and often man-made structures worldwide, play a crucial role in biodiversity conservation. While their short-term effects, such as protecting large carnivores from poaching and livestock predation, are well-known, the long-term consequences, including behavioral shifts in lions and their prey, remain incompletely understood. Notably, there has never been a direct, simultaneous comparison of lion diet and preferences in both fenced and unfenced natural areas using identical methodologies and timeframes. To address this gap, we harnessed the power of being able to analyze large sample sizes using DNA metabarcoding to enable a direct comparison of lion diet composition and prey preference between four different National Parks (NPs) as a means to study the effect of fencing on lion diet. Our objective was to investigate whether differences existed in the diet preferences and compositions of lions between fenced and unfenced NPs. The findings revealed distinct changes in lion diet composition and prey preferences along a gradient from fully fenced to completely unfenced NPs. Remarkably, the fenced park exhibited an almost opposing pattern in terms of prey preferences, particularly concerning specific prey body weights, compared to the unfenced NP. Lions in the small, fenced reserve displayed a pronounced preference for smaller prey, contrasting with the preferences observed in other NPs. Partly fenced NPs yielded varied diet results, generally aligning within the preference boundaries of both fenced and unfenced NPs and lacking outliers for specific prey species. Despite the limited statistical power arising from the study of only four NPs, our research provides evidence that fencing influences lion diets. Exploratory studies like these contribute to unraveling the impacts of fences on ecosystems across trophic levels, ultimately benefiting the conservation of large carnivores.

5.2 | Introduction

Fences are ubiquitous, widespread and often manmade infrastructures existing all over the globe and frequently used to conserve and protect biodiversity (Woodroffe et al., 2014; Løvschal et al., 2017; Jakes et al., 2018). The intended aims of fencing include preventing the introduction of invasive species and reducing human-wildlife conflicts and thus persecution, if a fence is managed and maintained correctly (Ewen et al., 2011; Kesch et al., 2015). Additionally, fences are a powerful tool for conserving and restoring wildlife in landscapes that are highly modified by human activity and these areas are increasingly viewed as nuclei for more extensive restoration efforts entailing progressive removal of fencing (Woodroffe et al., 2014). On the other hand, fences can obstruct large-scale animal movements and constrain herbivores' abilities to escape predators. Additionally, it can cause ecosystem fragmentation, genetic isolation, reduced conservation value of buffer zones, and fence materials can be utilized for wire snare poaching (Creel et al., 2013; Davies-Mostert et al., 2013; Woodroffe et al., 2014). In addition, fences can cause an altered landscape of fear (i.e. predation pressures), by which prey species may alter their feeding behavior, and create a negative ecological cascade causing destabilization of the ecosystem inside the fenced area (Cassidy et al., 2013). This dichotomy and plethora of potential effects on the ecosystem of using fences as management practice evidently illustrates the currently limited comprehension regarding fencing impacts on ecosystem functioning (Trinkel et al., 2016).

Predators, especially apex carnivores, form a key component of an ecosystem, with their top-down effects, and are suited to study ecosystem functioning alterations due to fencing (Ford & Goheen, 2015; Ford et al., 2014; Feit et al., 2019). A high-profile debate concerning the use of fences to protect African lions highlights a more general need to evaluate the role of fencing in predator conservation (Packer et al., 2013a; Creel et al., 2013; Packer et al., 2013b). Packer et al. (2013a) state that lion populations in fenced reserves are significantly closer to their estimated carrying capacities and have reduced costs of management than unfenced populations. Whereas Creel et al., (2013) prioritize and argue for large and intact ecosystems to conserve lions, instead of fencing. In that context, the high lion densities found by Packer et al. (2013a) might actually indicate that food webs are profoundly altered by fencing (Woodroffe et al., 2014). For example, Louw et al. (2012) demonstrate that lions seem capable of locating the preferred prey species with more ease in reserves where the movement of the prey species is constrained by fences and also Davies-Mostert et al. (2013) shows that boundary subjected (fenced) areas are likely to affect carnivores' hunting strategies and thereby their diet composition. Moreover, both Power (2002) and Lehmann et al. (2008) conducted a study on prey selection in a single small, fenced reserve and also showed deviations from the general findings for lion diet composition. Thus, predator-prey dynamics and diet composition within fenced reserves can change, and might even lead to dietary range shifts, in response to being held in a constrained habitat (Dupuis-Desormeaux et al., 2016). Furthermore, fencing confines predator populations into smaller areas, and might thereby induce density-dependent effects, especially when the carrying capacity of a fenced area is reaching its maximum for a predator species (Lindsey et al., 2017). In summary, short term effects of fencing are generally known (Packer et al., 2013a; Woodroffe et al., 2014; Kesch et al., 2015) and aid in the protection of large carnivores (e.g. reduced poaching and livestock predation), however long term effects (e.g. behavioral changes in lions and their prey) are not fully understood (Jakes et al., 2018; McInturff et al., 2020). Moreover, while these studies do indicate or propose that fencing influences a carnivores diet, a direct comparison of lion diet and preferences in fenced and unfenced nature areas using the exact same methodology and timeframe has never been made.

Therefore, we set up to study the diet of lions in four differently fenced or unfenced Kenyan National Parks (NPs), as an explorative case study. Specifically, we aim to address whether differences are found in diet preferences and diet composition of lions between fenced and non-fenced NPs. To accomplish this we established a method where we could analyze feeding behavior simultaneously in these NPs to prevent confounding effects of temporal dynamics and observer bias (e.g. biased census data, see Louw et al., 2012). Therefore, we adopted the method of Chapter 4. They performed DNA-based diet analysis as it favors lower costs, reduced effort and time and increased dietary resolutions, hence a better assessment of the large carnivore dietary ranges and components as compared to traditional scat analysis and carcass counts, which is needed to accurately compare spatial differences in lion diets. Ultimately, this understanding on the effect of fencing on lion feeding behavior could benefit in improving large predator conservation practices.

5.3 | Materials and Methods

5.3.1 | *Study sites*

To study the effect of fencing on the diet of lions, four NPs in Kenya were selected for their varying degree of fencing and their presence of lions (Figure 5.1). Northwest of Nairobi and south of the city of Nakuru lies Lake Nakuru National Park (LNNP, 188 km^2, 0°18'- 0°30' S; 36°2'- 36°9' E) (Kassilly et al., 2008). The park was established in 1961 and is completely enclosed with an electric fence since 1986. The landscape, enclosing a saline lake, consists of grasslands, swamps and marsh, with rocky cliffs and outcrops. There are areas of woodland and rocky hillsides covered with bushland and forest (Kassily et al., 2008). Meru National Park (MNP, 884 km^2), a partly fenced (70km, 24% of the total perimeter) savannah bushland, was established as a National Park in 1967 and is located in the Eastern Province of Kenya (0°20'- 0°10' S; 38°0'- 38°25' E) (Sitienei et al., 2014). Vegetation and ecosystem types within MNP predominantly consist of thorny Acacia and Commiphora bushland and Acacia wooded grassland (Bundotich et al., 2016). Approximately 7 km south of the city Nairobi, situated in the Nairobi Province, lies Nairobi National Park (NNP, 117 km^2, 01°20'- 01°26' S; 36°50'- 36°58' E). This park, established in 1946, consists predominantly of savannah grassland and was semi-fenced in 1955, with about 56% (65 km) of the park's border being fenced to create a separation between the city and the National Park (Lesilau, 2019). Established in 1974, located Southeast of NNP, close to the Tanzanian, border lies Amboseli National Park (ANP, 392 km^2, 02°37 S; 37°15 E), a non-fenced savannah grassland with seasonal flooding (Okello et al., 2008). Lions here have the opportunity to disperse to other reserves and to the surrounding environment (Huqa, 2019).

We have chosen to use the number of kilometers fence to order the parks from fully fenced to not fenced (LNNP; fully fenced → MNP; partly fenced for 70 km → NNP; partly fenced for 65 km → ANP; not fenced). In general, road accessibility within the NPs was satisfactory. However, in the case of MNP, the southern part of the park posed challenges due to poor or non-existent roads and dense vegetation. This resulted in disproportionate sampling across the surface of the National Park. Nevertheless, prey transects and scat sampling sites did overlap, and availability and diet occurrences could thus be related. Moreover, as we ordered the NPs by the degree of fencing, our transect and sampling sites in MNP did concur with the location of the fenced park boundaries and the potential effect of fencing is thus most likely to occur in the part surveyed. So due to the partial sampling, the apparent fencing proportion is likely substantially higher than 24%. All NPs have two rainy seasons: long rains from March to May and short rains from October to December with dry spells in between.

5.3.2 | Lion surveys

Lion population numbers and social structure in the NPs can influence feeding ecology (Hayward & Kerley, 2005). Therefore, we performed lion surveys to estimate population numbers and social structure in the four different NPs. Fieldwork took place from 4th February, 2019 until 17th April, 2019. During this period, we performed direct counts from opportunistic observations and call-up surveys where different vocalizations were broadcasted to attract lions to a survey point to obtain data for population and social structure. Lions were distinguished from each other by their unique whisker spot pattern (using photos of vibrissae patterns) and by investigating typical characteristics such as unique marks, scars, ear notches and presence of scrotum (Pennycuick & Rudnai, 1970). We kept track of the land surface area surveyed (in km^2) for the presence of lions per NP using a GPS unit (Garmin eTrex). Furthermore, lions were grouped into two age categories namely cubs (0-1 year) and adults (> 1 year) as it was not possible to assign gender to cubs by sighting. Additional notes were made on date, time, sex, and if the lion was in a group or pride and the composition of that group or pride.

5.3.3 | Prey transects

Prey availability was measured for calculating prey preferences. Therefore, we conducted prey transects to count available prey species for lions in each NP simultaneously during the study period. All vertebrate prey species (above 5 kg body weight) within 500 meters (measured using a Mileseey Golflaser rangefinder) on each side of the transect (Figure 5.1) were counted and identified. For birds, this includes ostrich and kori bustard, and for reptiles, amongst other the Nile crocodile, since they all have a bodyweight > 5kg. Species were identified using the field guide from Stuart & Stuart (2013) and prey weights were taken from the same guide. Prey transects were conducted two days a week. Every transect day, transect counts were conducted while driving around the park in clockwise direction (at 06:30 in the morning) and in the opposite direction (at 15:00 in the afternoon). This order was alternated every week. Transects were 2 km long following a park track and chosen to cover the majority of habitats in the park.

5.3.4 | DNA-based diet analysis

A DNA-based diet analysis was carried out according to the method described in Chapter 4. In short, lion scat samples were collected between the 4th of February and the 16th of April 2019 (Figure 5.1). Scat samples were collected opportunistically along transects during patrols thrice a week. Stuart & Stuart (2013) pocket guide was used to identify the scats and additional information was reported (date and time of collection, GPS coordinates, habitat type, weather conditions, and scat freshness). The categories for freshness of scats were specified from 1 to 4 (1 = inside and outside of the scat were soft; 2 = inside soft, outside hard; 3 = inside and outside hard; 4 = only hairs left) with 1 being the freshest. Five picks were taken from inside the scat with sterilized forceps, preserved in vials with 1 mL 99% ethanol and stored in a fridge at the end of each fieldwork day. After all fieldwork was completed, the samples were transferred to a $-20^{o}C$ freezer. To extract DNA from the scat samples, the protocol for 'Isolation of DNA from Stool for Pathogen Detection' of the QIAamp DNA Stool Mini Kit handbook was followed. After extraction, samples were tested for lion DNA to ensure correct scat collection.

Next, samples were selected for freshness (≤ 3) to prevent degradation bias as much as possible and location to make sure that multiple prides were taken into account (when present). Subsequently, a PCR with vertebrate-specific primers, Mod_RepCOI_F and VertCOI_7216_R, was performed to amplify a 244-bp fragment of the cytochrome c oxidase subunit I (COI) gene which has been demonstrated to have high-resolution power for identifying the genera across most vertebrate taxa (Reeves et al., 2018). Amplification was carried out in a final volume of 20 µL, using 3 µL DNA extract, 15 µL TaqMan™ Environmental Master Mix 2.0 (Applied Biosystems, Waltham, United States) and 0.1 µM of forward and reverse primer. A single cycle of 10 min at 95 °C, followed by 45 cycles of

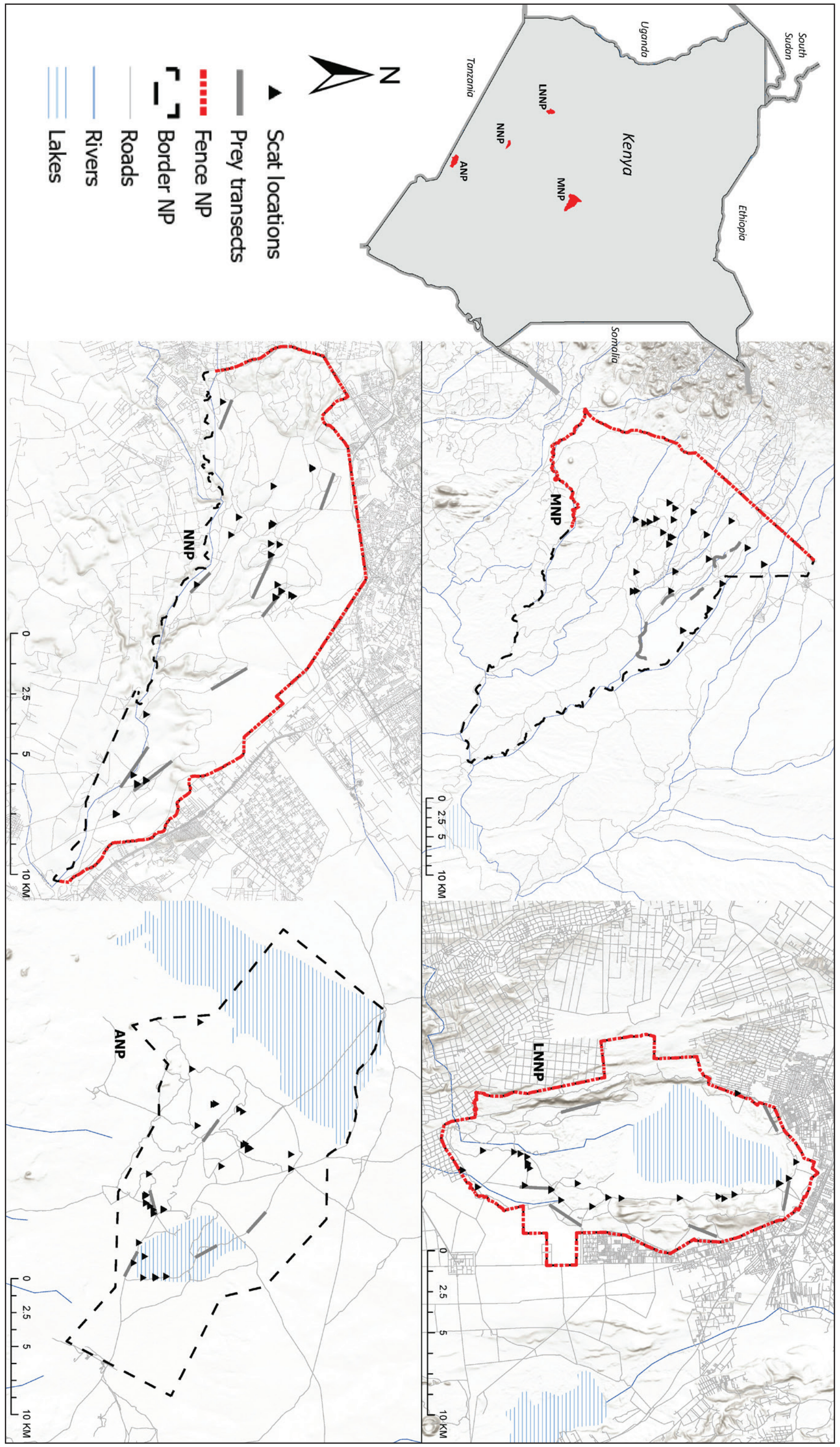

Figure 5.1 | Map of the study sites with fences depicted (red borders). Grey lines represent prey transects conducted and black triangles show where scat samples were collected in (following anticlockwise direction) fully fenced Lake Nakuru National Park (LNNP), partly fenced Meru National Park (MNP) and Nairobi National Park (NNP) and non-fenced Amboseli National Park (ANP). The map on the top left corner shows Kenya with the four study sites depicted in red. The southern part of MNP was inaccessible due to bad or non-existing roads and dense vegetation.

15 s at 95 °C, 30 s at 48.5 °C and 40 s at 72°C with a final extension step of 5 min at 72°C was used as PCR programme. Finally, Nextera i7/i5 index adapters were added to the amplicons for the purpose of pooling at sample level. Positive (POS) controls; DNA extracts of Chilean flamingo (***Phoenicopterus chilensis***, geographically restricted to South America; BirdLife International, 2018) and wood mouse (***Apodemus sylvaticus***), and no-template controls (NTC) were included in the metabarcoding amplifications to check for contamination. Additionally, for every park, negative field control(s) (NEG), by dipping sterilized forceps used for sampling in 99% ethanol, were included in the diet analysis. Final PCR products were pooled in equimolar concentrations and sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Data output of the NGS run was prepared by merging the demultiplexed reads, primer trimming and sequence trimming as detailed in Chapter 4. Next, OTUs were created and the presence of chimera sequences was checked. The created OTUs were BLASTed to the custom reference database used in Chapter 4. Subsequently, a least common ancestor analysis was performed to account for missing identifications and a table including all OTUs with their number of reads per sample was created. The reads in the control samples (POS, NTC, NEG) were used to filter out any contamination by subtracting the number of sequence reads found in the controls per taxon from the number of reads found in the samples. Lastly, a taxonomy worksheet was created, displaying all occurrences for every taxonomic unit for every sample. If samples had missing identifications at species-level and composed ≥1% of the total number of reads in a sample a higher taxonomic level was used. For details on sequencing results, e.g. generated reads, quality scores and mean number of reads, see Chapter 4 and NGS raw data output can be found at: doi:10.5061/dryad.zs7h44jfm.

5.3.5 | *Data analysis*

We calculated the number of sequence hits for each species in each sample. Next, we omitted all hits from species below 5 kg as these were not counted during the prey transects and no abundance data was thus available (see Chapter 4 for results on prey < 5kg in the lion diet). Furthermore, we removed reads from mesopredators (***Canis sp.***, ***Leptailurus sp.***, ***Crocuta sp.*** and ***Ichneumia sp.***), humans (***Homo sapiens***) and host DNA (***Panthera sp.***) following the reasoning and analysis performed in Chapter 4.

The presence of a prey taxa (regardless of the number of sequences of that prey taxon) within a fecal sample was counted as an occurrence, where presence of multiple taxa means multiple prey within one fecal sample. The diet of lions was quantified as a proportion of occurrence of an individual prey taxon over all occurrences found in all fecal samples (i.e. frequency of occurrence per food item, expressed as a percentage of the number of occurrences of one food item of the total number of occurrences of all food items). To obtain proportions of occurrences per park, only the respective fecal samples of that particular park were used. Lions, as carnivores, feed sporadically and in discrete foraging events therefore prey occurrences may provide a meaningful indication of how often each taxon is predated thereby justifying the use of occurrence data to interpret the lion diet (Deagle et al., 2018). However, as the latter study recommends, we also calculated the relative read abundance per prey species and NP, using the formula in Deagle et al. (2018), for completeness.

The resulting diet information based on occurrences does not take into account the difference in prey availability per NP. Therefore, to compare the diet between parks and account for differences in prey abundance, we used the Jacobs' index (D) which calculates a prey preference using the formula: $D = (r - p) / (r + p - 2rp)$. Where r represents the number of times a species is killed as a proportion of the total number of kills and p represents the proportional availability of that species (Jacobs, 1974). In this study, the proportion of occurrence (%TX) (as described above) of a species in the diet is interpreted as r and the relative species abundance of that species is used as availability (p). The relative species abundance was calculated by adding the number of individuals counted for a species and dividing that number by the total number of animals counted per NP. The Jacobs' index produces values from -1 to 1. A positive index ($D > 0.1$) indicates a preference while a negative index ($D < -0.1$) means a species is avoided and consumed less frequently than expected based on the abundance of that species (Hayward & Kerley, 2005). All figures were made using the ***ggplot2*** package (Wickham, 2009) in R (R Core Team, 2022). We added trend lines to scatter plots using locally weighted smoothing (LOESS, formula = y ~ x). Species accumulation curves were produced using the ***specaccum*** function (default settings) in the ***vegan*** package (Oksanen et al., 2022).

5.4 | Results

5.4.1 | *Lion surveys*

Totaled for all NPs, we observed 122 lions during the lion surveys (Table 5.1). With highest number of individuals (45), of which 15 cubs, was found in Amboseli NP, but highest density (31.6 ind./100 km^2) was found in Nairobi NP. Lowest abundance (9 ind.) and density (6.7 ind./100 km^2) were found in Lake Nakuru NP. Number of lion prides were highest in Amboseli with 5 prides found, and an average group size of (45 ind. / 5 prides) 9 individuals. Group sizes in Nairobi , Meru and Lake Nakuru NP were 12, 8 and 5 lions per pride, respectively.

Table 5.1 | Lion population numbers and social structure, including land surface area surveyed, for each National Park (NP) based on our lion surveys.

National Park (NP)	**Land surface area surveyed (km^2)**	**Abundance (ind.)***	**Density (ind./100 km^2)**	**Cubs (lions < 1-year-old)**	**Sex ratio (♀:♂)****	**Number of prides**
Amboseli NP	392	45	11.5	15	1.7 : 1.0	5
Nairobi NP	117	37	31.6	12	1.6 : 1.0	3
Meru NP	381	31	8.1	10	1.1 : 1.0	4
Lake Nakuru NP	135	9	6.7	3	1.0 : 1.0	2

* All individuals (adults + cubs)
** Lions >1-year-old only

5.4.2 | *Prey transects*

We recorded a total of 94 490 individuals (ANP: 33 505, NNP: 24 939, MNP: 26 374, LNNP: 9 672) spread over 30 prey species in 24 transects (ANP: 5, NNP: 8, MNP: 5, LNNP: 5) for all NPs combined. The total transect length was 1 920 km, combined with a coverage of 500 meter on each side of the transect, we thus surveyed an area of 1 920 km^2. Highest number of individuals within prey transects were observed for plains zebra (***Equus burchellii***) (18 792 total individuals; highest number in NNP with 7 382 individuals), shoat (***Ovis aries***/***Capra aegagrus hircus***) (15 931 total individuals; highest number in MNP with 13 400 individuals) and blue wildebeest (***Connochaetes taurinus***) (118 470 total individuals; highest number in ANP with 10 350 individuals). Highest number of individuals within prey transects in fully fenced Lake Nakuru NP are found for African buffalo (*Syncerus caffer*) with 5 114 individuals and second highest for plains zebra with 1 728 individuals. Prey availability (proportions of counted individuals per species versus total individuals of all species per NP) for all prey species observed are given in Table S5.1. Sixteen out of the 30 (53%) prey species counted were not found in the diet of lions. All species found in the diet of lions were observed in the prey transects (14), except for two, more cryptic, species which were not observed inside the prey transects, although observed outside transects, namely crested porcupine (*Hystrix cristata*) and the Nile crocodile (*Crocodylus niloticus*).

5.4.3 | DNA-based diet analysis

5.4.3.1 | Comparison of lion diet composition

The DNA-based diet analysis resulted in 16 assigned prey species, with a body weight greater than 5 kg, in 171 scat samples (Figure 5.2). The number of species found in the diet of lions in ANP is 10, in NNP 7, in MNP 9 and in LNNP 7. Sampling effort per NP was compared using species accumulation curves (Figure S5.1). Relative read abundance per prey item and per NP can be found in Table S5.1. Proportions of occurrences did not significantly differ from percentages of relative read abundances (Wilcoxon signed-rank test; V = 348 (N =33), p = 0.23).

Lion diet composition based on proportions of occurrences of prey items in, non-fenced, Amboseli NP is similar to the diet composition found in, partly fenced, Nairobi NP, while diet composition of lions in Meru NP (partly fenced) and Lake Nakuru NP (fully fenced), strongly diverge (Figure 5.2A). In both, the two least fenced, NPs (ANP and NNP) the highest proportions of occurrences were found for plains zebra (ANP 46.88 %, NNP 38.20 %) and blue wildebeest (ANP 31.25 %, NNP 22.47 %). Plains zebra is, however, less observed in the diet of lions in the most fenced NPs (MNP and LNNP, 8.47 % and 4.76 %, respectively). Blue wildebeest does not occur in MNP and LNNP and is thus not present in the diet. In contrast, the proportion of occurrences for the diet of lions in most fenced NPs (MNP and LNNP) was strongly dominated by African buffalo (54.24 % and 47.62 %) but was lacking for least fenced NPs (ANP, 3.13 % and NNP, 13.48 %). Although livestock was regularly observed during prey transects in each NP (average availability in transects 9.4 %), except LNNP, occurrences of livestock in the diet of lions were few and found only in MNP where 5.08 % of the lion diet was contributed to cow (*Bos taurus*). Proportion of occurrences plotted against minimum prey weight demonstrate that lions in unfenced ANP and partly fenced NNP, generally prey more frequently on prey species lighter than 500 kg whereas in the most fenced NPs, MNP and LNNP (Figure 5.2B) most occurring prey weight lies within 500-600 kg body weight.

5.4.3.2 | Comparison of lion prey preference

To fully assess diet differences between NPs, we took prey availability within NPs into account. Lion prey preference in each NP, as calculated by the Jacobs' index (JI), is shown for different functional groups (see Table S5.1 for group assignment) (Fig. 5.3A) and prey body weight (Fig. 3B). Figure 5.3 represents prey species found in the DNA-based diet analysis only. Index values for prey species found during prey transects but not in the diet are treated as avoided (D = -1) and are added to Table S5.1 (but are thus not included in Figure 5.3).

Rodents and reptiles are only found in the diet of lions in Meru NP, both were not frequently counted during transects and therefore receive a high index value (D = 1) and are therefore considered preferred. Similarly, Dik-dik's in NNP and LNNP also got a D = 1 value for preference, as they were not observed but found in the diet using DNA analysis. As these results might have an (substantial) impact on the trendline of MNP, NNP and LNNP in Fig 3B, and to evaluate robustness, we added the same figure without the results of rodents and reptiles in MNP and Dik-dik's in NNP and LNNP to the supplementary material (Figure S5.2). Removing these datapoints did not alter overall trends which illustrates the robustness of the results.

The sole livestock species found in the diet of lions is cow and is marginally favored (D = 0.18). Suids are equally preferred in all NPs (D = 0.3 – 0.5), except MNP, where no suids were found in the lion diet. Primates are highly preferred in all NPs (D > 0.8), except NNP where no primates were found in the diet of lions. Most apparent differences in prey preference between NPs were found within the equids, large ungulates and antelopes. In fully fenced LNNP, lions tend to avoid equids (D = -0.6), where these are generally preferred in the other NPs (D > 0.2). Additionally, lions in LNNP show less preference, although still preferred (D < 0.5), for large ungulates (African buffalo and giraffe) compared to the partly and non-fenced NPs which show high preference (D > 0.5). Antelopes are generally favored by lions among all NPs, with highest mean preference for antelopes in LNNP (D > 0.5) and lowest mean preference in partly fenced NNP (D =0.2) due to highly species-specific prey preferences for this functional group.

Differences in prey preferences in terms of prey body weight were especially found in the only enclosed NP, Lake Nakuru, where lions clearly prefer lower prey weights (~100 – 200 kg) and avoid prey of higher body weight (200 to 500 kg). Prey preference for prey heavier than 700 kg is high in all NPs (D > 0.5). Meru NP follows the same strong preference as LNNP for lighter prey. However, lions here do not at all avoid heavier prey and actually favor them. Contrastingly, lions in Nairobi NP generally avoid light prey and prefers medium sized prey (~300 kg) and prey above 500 kg. Lions in non-enclosed Amboseli NP do not avoid any prey but generally tend to have a larger preference for heavier prey (> 400 kg) compared to lighter body weights (around 250 kg).

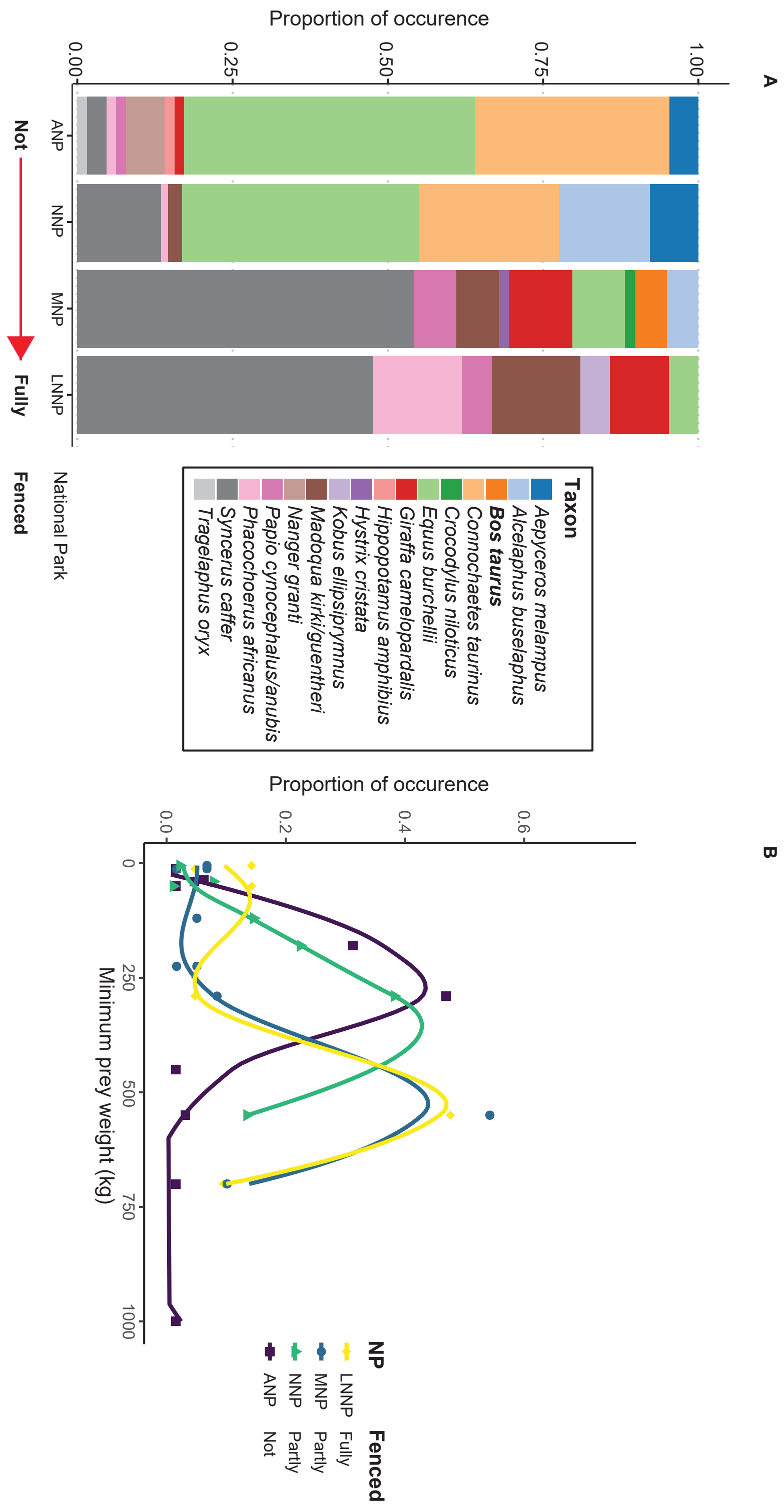

Figure 5.2 | Lion diet composition based on prey occurrences (prey body weight > 5 kg) in the diet of lions in four different fenced National Parks (NPs) in Kenia. ANP = Amboseli NP where N = 44 scat samples, NNP = Nairobi NP where N =46, MNP = Meru NP where N = 50, and LNNP = Lake Nakuru NP where N = 31 scat samples. The extent of fencing is added to the legend of the figure, ordered by km fence. **(A)** Proportions of occurrence found in the diet of lions ordered from non-fenced ANP to fully fenced LNNP. Livestock species are given in bold. **(B)** Proportions of occurrence plotted against minimum prey body weight. A LOESS trendline is added for each NP with smoothing parameter α = 0.8 where each point represents a prey species.

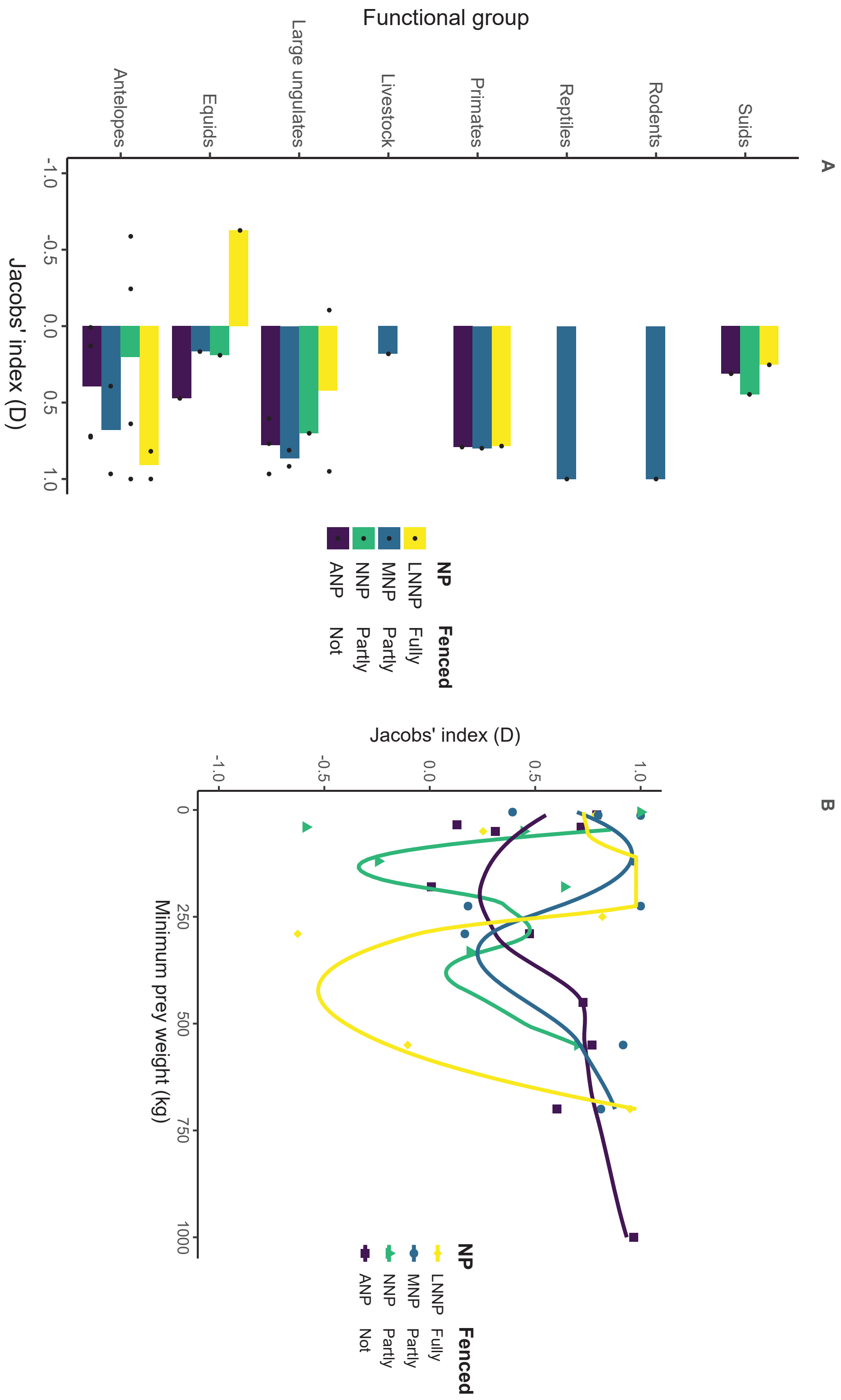

Figure 5.3 | Lion prey preference (prey body weight > 5 kg) based on the Jacobs' index, in which prey availability is included, in four different fenced National Parks (NPs) in Kenia. ANP (N = 44 scat samples) = Amboseli NP, NNP (N = 46) = Nairobi NP, MNP (N = 50) = Meru NP, and LNNP (N = 31) = Lake Nakuru NP. Prey preference is given per functional group **(A)** and per species plotted against prey body weight **(B)** for each NP. Black dots represent individual species belonging to that functional group in figure A. Functional groups are assigned according to the information in Table S5.1. Please note that for this figure, only species found in the DNA-based diet analysis are used. Preferences of species not found in the diet but observed during prey transects are given in Table S5.1. For Figure B, a LOESS trendline was added for each NP with smoothing parameter α =0.98.

5.5 | Discussion

We found a contrasting lion diet composition and prey preference between the fully enclosed NP (LNNP) and the completely unfenced NP (ANP). Strikingly, LNNP also shows almost an opposite pattern in terms of prey preferences for specific prey body weights compared to ANP. The lions in this small and fenced reserve show high preference for smaller prey (< 250 kg), especially antelopes (highest mean preference of all NPs), and avoidance for prey weights that are generally preferred in other NPs and according to the scientific literature (most preferred prey weight of lions is considered to be 350 kg according to Hayward & Kerley, 2005). Partly fenced NPs (NNP and MNP) generally show varying diet results, but are nearly always situated within the preference boundaries set by the prey preferences of LNNP and ANP and show no particular outliers for certain prey species.

The lions in the least fenced NPs preyed most dominantly upon plains zebra, followed by blue wildebeest, whereas African buffalo prevailed as dominant prey in the diet of lions in the most fenced NPs. Both plain zebra and African buffalo do occur in all NPs, while prey availability of buffalo is extremely high in LNNP (52.9 %) compared to the other NPs (< 5.0%). Although highest proportions of occurrences were found in LNNP, lions in LNNP showed no preference for African buffalo. In all other NPs lions showed high preferences for buffalo. Additionally, plains zebras were also non-preferred by lions in the enclosed LNNP, whereas the lions in other NPs preferably hunt on this prey species. Availability of plain zebra was generally similar for each NP (~20 %), with proportionally slightly less zebras in MNP (6.2%).

Overall, lions in LNNP demonstrate remarkable differences in diet composition and preference in comparison to the other NPs, which clearly shows that feeding behavior in the only fully fenced NP in this study is substantially different to the unfenced and partly fenced NPs. Completely attributing this difference to fencing is difficult and caution should be taken, as diet can be influenced, aside from prey density, by other factors (e.g. climatic events such as droughts and lion demography, see Hayward & Kerley (2005) and Huqa (2019)). Although, by sampling all NPs during the same period we have tried to minimize the effect of seasonal climatic events. Furthermore, large regional differences in weather between the NPs were not observed. However, in regards to lion demography within NPs, we need to stress that lion population numbers in LNNP are the lowest among all four NPs and the obtained diet might thus be the result of a smaller lion population, including lowest number of lion individuals, lowest density, fewest prides and lowest groups size. However, this argument of lion demography does not fully explain the resemblance of Meru NPs' trend to that found in LNNP (trend of MNP is similar to LNNPs' trend except with less extreme preferences) as lion numbers in MNP are higher, with larger group size. So, we suggest that also fencing - in addition to or in combination with population structure – is an important explanation for the observed patterns.

Our results corroborate that lions tend to hunt smaller prey when lion numbers are low (as collective hunting by lions allows lions to take larger-bodied prey) and avoid larger prey such as buffalo which agrees with our results (Hayward & Kerley, 2005). Furthermore, we noted high lion offspring mortality due to conflicts with African buffalo in LNNP due to the extremely high presence of these prey species in the park. Notwithstanding, a small lion population might also be an indirect effect or characteristic of an enclosed, unbalanced system with less genetic diversity and no (im)migration possibilities (Creel et al., 2013; Creel et al., 2019; Naha et al., 2023). As Mesa-Cruz et al. (2016) also demonstrated that outside of protected areas (i.e. unnatural systems), large cats shifted their diet to smaller prey which concurs with our results.

Lions in ANP, the only unfenced NP in this study, showed a preference for all prey species considered in this study, except 2 antelopes, which were not preferred nor avoided. Highest preferences were seen for species > 250 kg, although proportions of occurrences also showed high proportions of smaller prey in their diet. Combined, these results suggest that in open unfenced areas lions can be regarded as opportunistic hunters, that hunt everything they can get, but with preference for heavier prey. This concurs with other lion prey preference studies in natural systems (i.e. unfenced) where prey might be more difficult to get, since it is not confined to a closed area, and prey selection is not always possible when hungry (Creel et al., 2013; Davies-Mostert et al., 2013; Woodroffe et al., 2014).

As discussed before prey body weight preferences of the lions in partly fenced MNP follow the same trend as the lions in LNNP however with less extremes. When we consider that the area surveyed within MNP is mostly within bounds of the fenced part of the NP, and is thus likely most affected by it, we could infer that the prey preference trend that is observed (MNP behaving like LNNP), might thus likely be directly attributed to the effect of fencing. Combined with the fact that NNP, also partly fenced but surveyed across the whole park area, does not follow the same trend as MNP or LNNP (but rather shows more agreement with the trend of ANP) supports our argument that fencing was a driver of the patterns found. We do note that both ANP and LNNP could benefit from increased sampling effort as accumulation curves show that the asymptote has not been reached, which is reached for NNP and MNP, and the curve is still climbing. Although total number of species found in the diet did not differ much between NPs (7 species for LNNP, 7 for NNP, 9 for MNP and 10 species for ANP). Additionally, Trites and Joy (2005) calculated that for 8 or 11 species in a diet, between 46 and 31 scats need to be collected to infer differences between diets among locations with enough statistical power (based on a diet with linearly declining proportions of occurrences for prey species). Scat samples collected within NPs differed between 31 and 50, we therefore, although barely, abide by these set numbers and therefore we do not expect a difference in observed patterns with increased sampling effort.

Multiple studies show deviating lion ecology in small enclosed nature areas (Lehmann et al., 2008; Packer et al., 2013a; Creel et al., 2013), which forms an increasing body of evidence of fence-induced influences on ecosystem functioning. This study adds to this evidence by directly comparing diet composition and preferences between NPs with varying degree of fencing. However, as only 4 NPs have been studied here and only two of four NPs were either completely fenced or unfenced stating statistically based differences with regards to the effect of fencing was not possible. Nonetheless, despite statistical power issues and alternative explanations, we provide strong evidence that fencing influences the diets of lions. We therefore do stress the need for further research into the impact of fences on wildlife ecology. Diet studies would for instance benefit from scenarios where fences are being erected, and a before and after situation can both be studied, combined with for example remote sensing techniques to evaluate changes in vegetation over time or monitor herbivore activity (Kröschel et al., 2017). Such studies could help disentangling the impact of fences on ecosystems along trophic levels and ultimately benefit the conservation of large carnivores.

5.6 | Supporting information and data accessibility

No non-public available data is used for this publication.

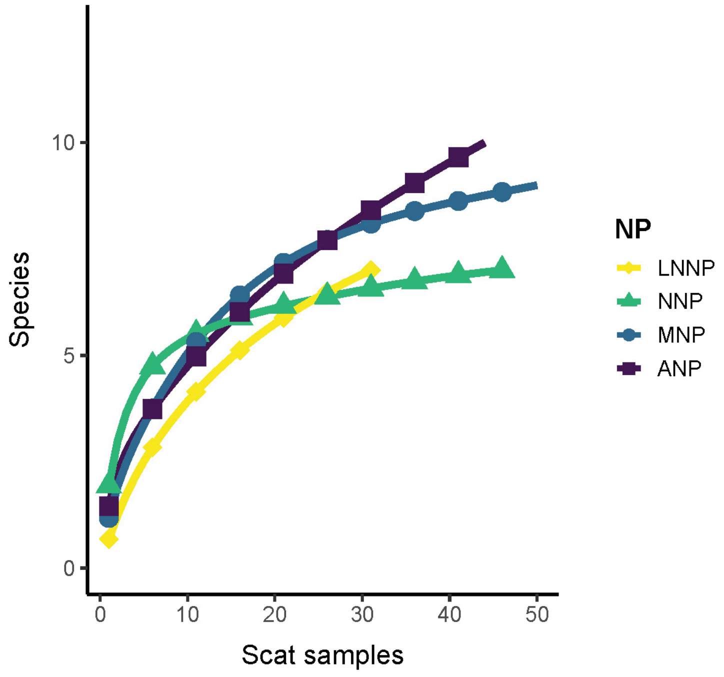

Figure S5.1 | Species accumulation curves for scat samples collected in four National Parks in Kenia. ANP (N = 44) = Amboseli NP, NNP (N = 46) = Nairobi NP, MNP (N = 50) = Meru NP, and LNNP (N = 31) = Lake Nakuru NP.

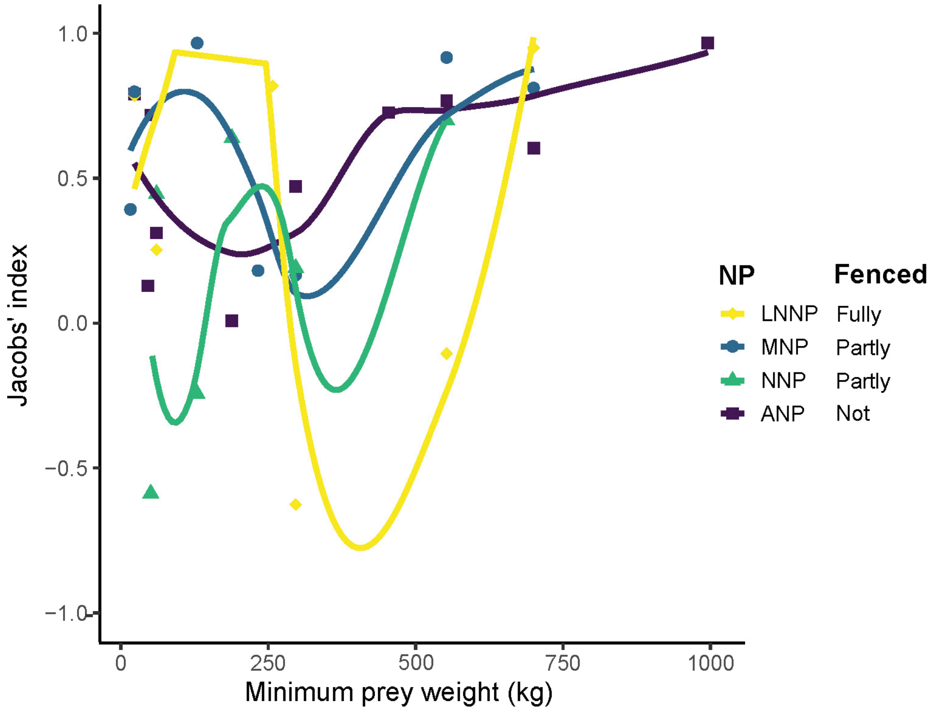

Figure S5.2 | Lion prey preference (prey body weight > 5 kg) based on the Jacobs' index, in which prey availability is included, in four different fenced National Parks (NPs) in Kenia. ANP (N = 44 scat samples) = Amboseli NP, NNP (N = 46) = Nairobi NP, MNP (N = 50) = Meru NP, and LNNP (N = 31) = Lake Nakuru NP. Prey preference is given per species plotted against prey body weight for each NP. Please note that for this figure, only species found in the DNA-based diet analysis are used. However, data points with a Jacobs' index value of 1 (i.e. species found in diet but not observed during transects) are excluded (but shown in Table S5.1). Preferences of species not found in the diet but observed during prey transects are also given in Table S5.1. A LOESS trendline was added for each NP with smoothing parameter α =0.98.

Table S5.1 | Overview of prey species analyzed during this study with corresponding prey availability based on results of prey transects and proportions of occurrences in lion diets found based on the results from the DNA-based diet analysis. Prey preferences are calculated using the Jacobs' index (D). Species that receive index values of D > 0.5 are considered highly preferred prey (++) and values of D < -0.5 are highly avoided prey (--). Species with D > 0.1 and ≤ 0.5 are preferred prey (+) and D < -0.1 and ≤ -0.5 are avoided prey (-). Index values for prey species found during prey transects but not in the diet are treated as avoided (D = -1.0). A blank space means that a particular prey species does not occur in that particular NP. An x denotes that the calculation of the Jacobs' index was not possible due to no availability and no occurrence in the diet of that prey species, but the prey species is known to occur in the area. Species' taxonomic families are given as well as assigned functional groups (l. u. = large ungulate). Functional groups 'elephant' and 'bird' are not presented in Figure 5.3A, as no occurrences were found in the diet of lions for these groups in any NP, but are included here for completeness.

Species[a]	Scientific name	Family (functional group)	Body mass (min - max, kg)	Preference [D] (Jacobs' index)				Availabilty [p] (%)				Proportion of occurrence [r] (%)				Relative read abundance (%)[b]			
				ANP	*NNP*	*MNP*	*LNNP*	*ANP*	*NNP*	*MNP*	*LNNP*	*ANP*	*NNP*	*MNP*	*LNNP*	*ANP*	*NNP*	*MNP*	*LNNP*
African buffalo	*Syncerus caffer*	Bovidae (l. u.)	550 - 700	++ 0.77	++ 0.70	++ 0.92	~ -0.1	0.42	2.67	4.89	52.87	3.13	13.48	54.24	47.62	2.44	21.18	73.06	49.97
Baboon	*Papio cynocephalus/ anubis*	Cercopithecidae (primate)	12 - 45	++ 0.80	-- -1.0	++ 0.80	++ 0.78	0.19	0.45	0.81	0.60	1.56	0	6.78	4.76	1.22	0	4.73	5.56
Black rhinoceros	*Diceros bicornis*	Rhinocerotidae (l. u.)	800 - 1100		-- -1.0	x	-- -1.0		0.10	0	0.01		0	0	0		0	0	0
Blue wildebeest	*Connochaetes taurinus*	Bovidae (antelope)	180 - 250	~ 0.008	++ 0.64			30.89	6.00			31.25	22.47			39.37	20.29		
Bohor reedbuck	*Redunca redunca*	Bovidae (antelope)	35 - 65	-- -1.0	-- -1.0		x	0.12	0.17		0	0	0		0	0	0		0
Bushbuck	*Tragelaphus sylvaticus*	Bovidae (antelope)	45 - 80	x	-- -1.0	-- -1.0		0	0.03	0.004		0	0	0		0	0	0	
Common duiker	*Sylvicapra grimmia*	Bovidae (antelope)	18 - 21	x	-- -1.0	x	x	0	0.01	0	0	0	0	0	0	0	0	0	0
Common eland	*Tragelaphus oryx*	Bovidae (antelope)	450 - 900	++ 0.73	-- -1.0	-- -1.0	-- -1.0	0.25	1.83	0.20	1.12	1.56	0	0	0	0.01	0	0	0
Common ostrich	*Struthio camelus*	Struthionidae (bird)	63 - 150	-- -1.0	-- -1.0	-- -1.0	-- -1.0	0.79	2.23	0.43	0.08	0	0	0	0	0	0	0	0
Common warthog	*Phacochoerus africanus*	Suidae (suid)	50 - 105	+ 0.31	+ 0.45	-- -1.0	+ 0.25	0.83	0.43	0.61	9.04	1.56	1.12	0	14.29	0.47	1.43	0	16.67
Cow	***Bos taurus***	**Bovidae (livestock)**	**225 - 395**	**-- -1.0**	**-- -1.0**	**+ 0.18**	**x**	**11.31**	**0.12**	**3.58**	**0**	**0**	**0**	**5.08**	**0**	**0**	**0**	**1.18**	**0**
*Crested porcupine**	*Hystrix cristata*	*Hystricidae (rodent)*	*13 - 27*	*x*	*x*	*++ 1.0*	*x*	*0*	*0*	*0*	*0*	*0*	*0*	*1.69*	*0*	*0*	*0*	*0.02*	*0*
Dik-dik's	*Madoqua kirki/ guentheri*	Bovidae (antelope)	5 - 6	x	++ 1.0	+ 0.39	++ 1.0	0	0	3.07	0	0	2.25	6.78	14.29	0	0.24	4.88	5.59
Donkey	***Equus africanus asinus***	**Equidae (livestock)**	**80 - 480**	**-- -1.0**	**x**	**-- -1.0**	**x**	**0.05**	**0**	**4.03**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Dromedary	***Camelus dromedarius***	**Camelidae (livestock)**	**300 - 600**	**-- -1.0**	**x**	**-- -1.0**		**0.16**	**0**	**16.23**		**0**	**0**	**0**		**0**	**0**	**0**	
East African oryx	*Oryx beisa*	Bovidae (antelope)	175 - 175	x		-- -1.0		0		0.06		0		0		0		0	
Elephant	*Loxodonta africana*	Elephantidae (elephant)	2800 - 6500	-- -1.0		-- -1.0		7.51		0.17		0		0		0		0	
Gerenuk	*Litocranius walleri*	Bovidae (antelope)	30 - 50	x		-- -1.0		0		0.25		0		0		0		0	
Giraffe	*Giraffa camelopardalis*	Giraffidae (l. u.)	700 - 1400	++ 0.60	-- -1.0	++ 0.81	++ 0.95	0.39	1.50	1.16	0.27	1.56	0	10.17	9.52	0.02	0	3.64	11.11
Grant's gazelle	*Nanger granti*	Bovidae (antelope)	35 - 80	+ 0.13	-- -1.0	-- -1.0	-- -1.0	4.89	2.84	4.15	3.49	6.25	0	0	0	2.89	0	0	0
Hartebeest	*Alcelaphus buselaphus*	Bovidae (antelope)	120 - 150	x	- -0.24	++ 0.97		0	21.96	0.09		0	14.61	5.08		0	11.48	2.60	
Hippopotamus	*Hippopotamus amphibius*	Hippopotamidae (l. u.)	1000 - 2000	++ 0.97	-- -1.0	x	x	0.03	0.43	0	0	1.56	0	0	0	0.37	0	0	0
Impala	*Aepyceros melampus*	Bovidae (antelope)	40 - 50	++ 0.72	-- -0.59	-- -1.0	-- -1.0	0.80	24.72	0.94	11.94	4.69	7.87	0	0	2.45	8.46	0	0
Kori bustard	*Ardeotis kori*	Otididae (bird)	5.5 - 19	-- -1.0	x	-- -1.0	x	0.23	0	0.05	0	0	0	0	0	0	0	0	0
Lesser kudu	*Tragelaphus imberbis*	Bovidae (antelope)	62 - 100	x	x	-- -1.0		0	0	0.55		0	0	0		0	0	0	
Mountain reedbuck	*Redunca fulvorufula*	Bovidae (antelope)	30 - 30		-- -1.0		x		0.08		0		0		0		0		0
Nile crocodile**	*Crocodylus niloticus*	Crocodylidae (reptile)	*225 - 750*	x	x	++ 1.0	x	0	0	0	0	0	0	1.69	0	0	0	0.04	0
Plains zebra	*Equus burchellii*	Equidae (equid)	290 - 340	+ 0.47	+ 0.19	+ 0.17	-- -0.63	24.01	29.60	6.21	17.87	46.88	38.20	8.47	4.76	50.75	36.91	9.84	5.56
Shoat	***Ovis aries/ Capra hircus***	**Bovidae (livestock)**	**45 - 100**	**-- -1.0**	**-- -1.0**	**-1.0**	**x**	**7.02**	**0.72**	**50.81**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Thomson gazelle	*Gazella thomsoni*	Bovidae (antelope)	15 - 25	-- -1.0	-- -1.0		-- -1.0	10.11	4.01		2.14	0	0		0	0	0		0
Waterbuck	*Kobus ellipsiprymnus*	Bovidae (antelope)	250 - 270	-- -1.0	-- -1.0	-1.0	++ 0.82	0.003	0.02	1.71	0.50	0	0	0	4.76	0	0	0	5.56
White rhinoceros	*Ceratotherium simum*	Rhinocerotidae (l. u.)	1400 - 2300		-- -1.0	x	-- -1.0		0.07	0	0.07		0	0	0		0	0	0

* *Not a representative result as transects likely miss this nocturnal animal and results in no availability,* ** Common but not in transects

[a] **Livestock species in bold**, [b] Differences between prop. of occurrence vs. rel. read abundance >5% are underlined