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Research Article

Minimal impact of spotted hyenas on livestock and endangered species in a prey-rich ecosystem

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The diet of large carnivores is of great interest to conservation managers, as it can reveal the extent of human–carnivore conflict and the impact of carnivores on species of high conservation priority. Metabarcoding of environmental DNA can identify species and is often more reliable than observational or morphological methods, particularly when it comes to detecting rare and elusive species. Here, we used DNA metabarcoding of feces to determine the diet of spotted hyenas *Crocuta crocuta* in the Ngorongoro Crater, a protected area in northern Tanzania with high densities of wild ungulates surrounded by areas co-inhabited by pastoralists. We assessed which species hyenas preferably consumed over a 24-year period and how frequently they consumed livestock and black rhinoceros, a species of high economic value and conservation priority. We further estimated the effects of three key socio-demographic variables – age, social rank, and sex – on the propensity of hyenas to consume livestock. We detected DNA from 20 species in 371 hyena feces. Livestock was rarely consumed (4.1% of detections) and mostly by old hyenas that are less capable of hunting fleet-footed and powerful wild prey. No DNA of black rhinoceros was detected in any of the samples. Our findings suggest that the impact of Crater hyenas on livestock and wildlife of high conservation priority is minimal. Our study highlights the potential of DNA metabarcoding to assess the extent of human–carnivore conflict and to guide evidence-based conservation efforts to promote coexistence of carnivores, humans, and species of high conservation priority.

Keywords: black rhinoceros, diet, DNA metabarcoding, domestic animals, human–carnivore conflict, multiple-use protected areas, spotted hyenas, threatened prey species

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Introduction

The effective management of multi-use protected areas (MUPA), where humans and wildlife cohabit, depends on gathering rigorous evidence to answer pressing conservation questions such as what ecological factors drive human–wildlife interactions (Watson et al. 2016). Finding answers to these questions is especially urgent for the conservation of threatened wildlife species (Western et al. 2009). Using such an evidence-based approach helps to balance the needs of priority wildlife species with those of local stakeholders, and the promotion of human–wildlife coexistence (Wevers et al. 2020).

There is currently a lack of evidence and conservation research conducted in protected areas, and particularly MUPA (Coetzee 2017). This has hamstrung successful conservation and led to harmful management decisions, such as the broadly applied indiscriminate culling of animals. While these actions may help reduce the perceived conflict in the short term, they can have major negative effects on populations and ecological communities, exacerbating the initial problem (Swan et al. 2017). For example, poorly justified lethal control of rodents *Rodentia* spp. may have weakened multiple ecosystem services, reduced the food base for predatory birds and mammals, and caused habitat loss for other burrowing animals (Singleton et al. 2007). Management decisions may also overlook individual-level tendencies in a population. For example, 'problem animals' may develop a predilection for consuming domestic animals, such as livestock. This behavior may be influenced by individual socio-demographic traits such as age, social rank, or sex (e.g. in brown bears *Ursus arctos*: Morehouse et al. 2016). For instance, older age has been suggested to be positively associated with the killing of livestock or even humans by large carnivores (Reza et al. 2002). This is because old individuals may be less capable of successfully hunting fleet-footed, powerful wild prey due to age-related disease and injury, and loss of dexterity, endurance, speed, and strength (Peterhans and Gnoske 2001).

DNA metabarcoding is a recently developed, non-invasive technique to identify species in multi-species samples, including from environmental DNA (eDNA) samples. It has increasingly been used to assess biodiversity and determine the diet of wild carnivores (Morin et al. 2016, Berry et al. 2017, Ruppert et al. 2019, Havmøller et al. 2021). In carnivore research, scientists collect fecal samples from the carnivore, extract eDNA from the sample, and identify the consumed species based on the DNA using high-throughput sequencing of taxonomically informative markers (Alberdi et al. 2019, Buzan et al. 2024). DNA metabarcoding poses several advantages over traditional diet assessments based on direct observation of consumed prey or morphological analyses of prey hair in feces. It often detects consumed taxa otherwise missed (Berry et al. 2017). It reduces representation bias associated with major differences in surface-area-to-volume ratios and hair densities across different species (Wachter et al. 2012). It can verify the identity of the putative consumer (Shehzad et al. 2015). It is also less likely to lead to misidentification between closely related domestic and wild taxa than morphological approaches are (Monterroso et al. 2019). Although DNA metabarcoding cannot distinguish between hunting and scavenging (Toju and Baba 2018), distinguishing the two behaviors was not the focus of our study.

In this study, we used DNA metabarcoding of fecal samples collected over a period of 24 years to assess the diet of spotted hyenas *Crocuta crocuta* (henceforth 'hyena') in the Ngorongoro Crater (henceforth 'Crater'), a caldera within a large MUPA and UNESCO World Heritage Site. Hyenas are apex predators and scavengers ranging across much of sub-Saharan Africa (Bohm and Höner 2015). The Crater hyena population has been the subject of a long-term study since 1996 (Bailey et al. 2024). Past research has suggested that hyenas exhibit considerable age-, rank-, and sex-related behavioral differences (Serengeti National Park, Tanzania: Hofer and East 1993, Benhaiem et al. 2012; Ngorongoro Crater, Tanzania: Höner et al. 2007, 2010, Davidian et al. 2021; Masai Mara National Reserve, Kenya: Boydston et al. 2003, Yoshida et al. 2016), although this has not yet been assessed in terms of diet. To reduce this gap, we addressed three conservation questions of high importance to the local authorities and of broad interest to applied ecologists and wildlife managers. First, we determined which animal species hyenas preferably consumed and how frequently they left the Crater floor to consume pastoralist livestock in the areas surrounding the Crater. Second, we assessed how often they consumed black rhinoceros *Diceros bicornis* (henceforth 'rhino'), a critically endangered species and a species of great economic value for tourism (Emslie 2020). Third, we investigated how hyena socio-demographic variables affect the propensity of hyenas to consume domestic animals.

Material and methods

Study area

The Ngorongoro Crater (Fig. 1) is located in the Ngorongoro Conservation Area (NCA), Tanzania (03°12′36″S, 35°27′36″E), and is part of the Greater Serengeti ecosystem. The NCA is a MUPA established in 1959 and a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site with a double mandate to protect the interests of wildlife and local human communities (Charnley 2005). The NCA is inhabited by Maasai, a semi-nomadic, pastoralist ethnic group ranging from central Kenya to southern Tanzania (Fratkin 2001). The Maasai resided in the Crater until 1974, when they were evicted and required to live in other parts of the NCA (Moehlman et al. 2020). They were allowed to enter the Crater with livestock to conduct pastoralist activities during daytime until the end of 2016 (Melubo and Lovelock 2019).

Throughout the 24-year study period, the Crater was exceptionally dense in wild ungulates (Moehlman et al. 2020). In the areas surrounding the Crater, the populations of humans and their livestock greatly increased during this

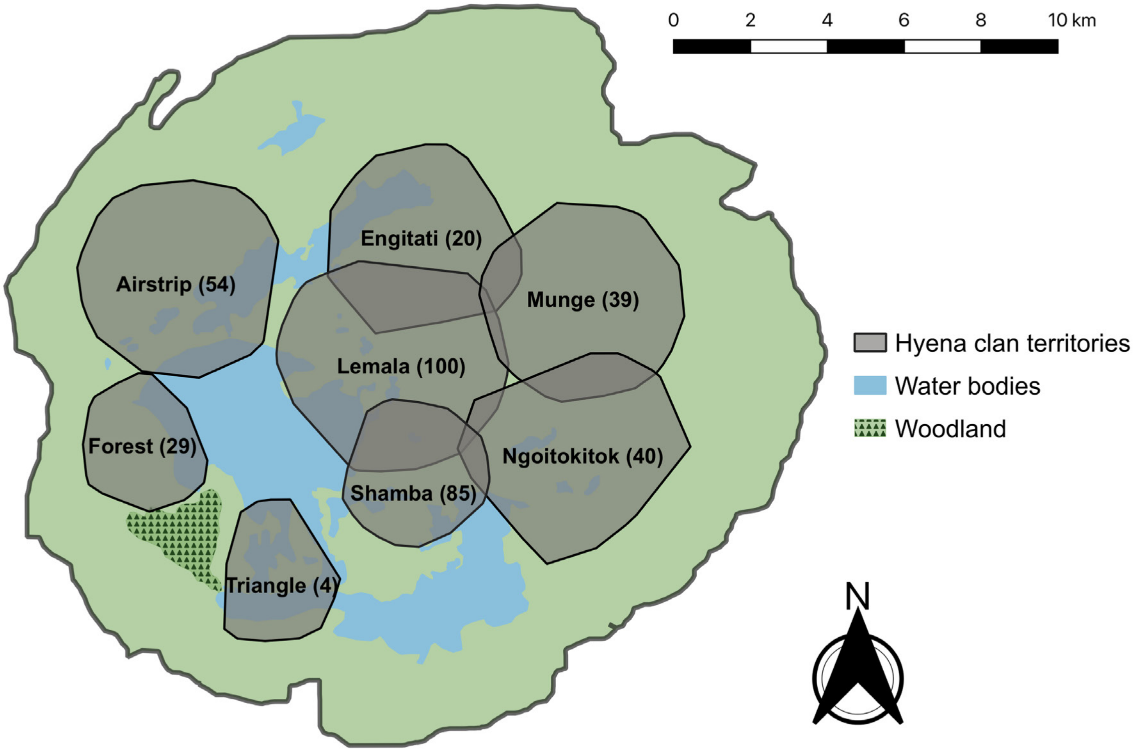

Figure 1. Location of the territories of the eight spotted hyena study clans in the Ngorongoro Crater. Territory boundaries are based on 85% minimum convex polygons (MCP) of adult female hyena sightings from 1996 to 2019 for each clan. MCP of 85% were chosen to accurately represent the locations and sizes of clan territories across the study period. Territories are labeled with corresponding clan names. Numbers in parentheses indicate the number of samples we analyzed from the corresponding clan.

period. The human population increased from under 43 000 to over 100 000 (Manzano and Yamat 2018), the cattle *Bos taurus* population more than doubled from under 120 000 to over 240 000, while the donkey *Equus asinus*, goat *Capra hircus*, and sheep *Ovis aries* populations increased to over 20 000, 220 000, and 340 000, respectively (National Bureau of Statistics Tanzania 2017). Other domestic animals in the NCA included cats *Felis catus*, chickens *Gallus gallus*, dogs *Canis familiaris*, dromedary camels *Camelus dromedarius*, and pigs *Sus domesticus*.

Data collection

Collection of field data occurred between April 1996 and December 2019, on a near-daily basis, between 06:00 and 19:00 h. During the study period, the population of spotted hyenas consisted of eight clans of average size ranging between 26 and 73 members; total population size ranged between 165 and 609 individuals. We recognized individual hyenas based on their pelage patterns, ear notches, scars, and other traits. We estimated the age of cubs based on pelage, body size, behavior, and locomotion, with an accuracy of ± 7 days (Pournelle 1965) and identified the sex of individuals through observation of external genitalia (Frank et al. 1990). We collected fecal samples opportunistically, immediately after defecation by identified individuals. Following collection, we mechanically mixed and subsampled the feces, stored them in liquid nitrogen, and transported them to the laboratory in Berlin, Germany, on dry ice, where we stored them at −80°C until processing them for extraction and analyses.

DNA extraction

We extracted DNA from 505 fecal samples using a Stool DNA Kit (Roboklon GmBH,, Germany), following manufacturer's instructions, with the following adjustments. We placed at least 200 mg of feces into individual bead tubes from the kit and then homogenized the sample (Tissue Lyser II, Qiagen, Germany) twice for 20 s. We used the entire lysate for the remaining extraction procedure and eluted the samples in a final volume of 70 µl. The concentration of extracted DNA was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Germany). We then stored samples at −20°C until sequencing.

Sequencing summary

Out of the 505 fecal samples, we retained results from 371 (73.5%) for our analyses (Supporting information). Of these, 64 samples had read counts between 1000 and 2000, 95 samples between 2000 and 3000, and 212 samples > 3000. Of the 134 samples we removed, 41 had low total read counts (< 1000 counts; Johnson et al. 2021). Another 70 samples had low read counts and a DNA concentration that was too low ($n = 63$; < 50 ng μl^{-1}) or too high ($n = 7$; > 600 ng μl^{-1}) to be reliably sequenced (Koetsier and Cantor 2019), possibly due to inhibitors preventing amplification of the target fragment (De Barba et al. 2014). The remaining 23 samples removed were either duplicates derived from the same stool (i.e. samples collected from the same 'defecation event'), or definite cases of contamination. For example, we had one sample with a total read count of ≥ 1000, and most of its reads were of red fox *Vulpes vulpes*, which are not extant in the NCA (Hoffmann and Sillero-Zubiri 2016), but samples of which were analysed in the same lab during the same period.

PCR amplification and sequencing

Because DNA in fecal samples can be heavily degraded, we used a 108-base pair (bp) fragment within the 12S rRNA gene (Riaz et al. 2011) with a two-step metabarcoding protocol. Briefly, vertebrate DNA was first amplified with primers containing Illumina adapters and a blocking primer specifically designed to inhibit the amplification of host DNA (i.e. hyena DNA; 5′-AGATACCCCACTATGCC TAGCCCTAAACTCAGATAGATAATT-Spacer_C3- 3′). Depending on the DNA concentration, we used either 5 µl or 200 ng of DNA in a 20 µl PCR mix containing 1× Herculase buffer, 0.2 nM of both dNTPs and forward and reverse primer, 10 nM of blocking primer, 4 nM of Mg^{2+}, and 1.25U of Herculase II polymerase. Thermocycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and extension at 72°C for 1 min, and ended with a 10-min final elongation step at 72°C. We cleaned the PCR products using CLEAN NGS magnetic beads (GCBiotech, the Netherlands) in a 0.8:1 ratio of beads to PCR product and washed them twice, using 80% EOTH. We re-suspended products in 25 µl of 0.1× T.E. buffer, and then recovered 24 µl for the second PCR step. In order to de-replicate the individual samples post-sequencing, we PCR-ligated each sample with a unique combination of P7 and P5 indices, containing the sequencing primer and part of the flow cell adapter. As such, we added 10 µl of cleaned PCR product to a 15 µl mixture containing 1× Herculase buffer, 0.1nM dNTPs, 4% DMSO, 0.21U of Herculase II Polymerase and 0.25 nM of both P7 and P5 indices. Thermocycling conditions included an initial denaturation at 95°C for 5 min, followed by 12 cycles of 94°C for 30 s, 52°C for 30 s, extension at 72°C for 1 min, and ended with a 10-min final elongation step at 72°C. We cleaned the now-indexed samples using the method described after the first PCR. Quantification of cleaned libraries were measured using a Quant-iT™ Picogreen™ dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Germany). Based on the concentration, we pooled samples equi-mass with 15 ng of DNA. We cleaned the pool twice to remove any remaining primer dimers or sequencing products that were less than 100 bp, using the steps outlined above. We recovered a total of 20 µl following the final elution. We checked libraries for a single fragment size of 320 bp using an Agilent Tapestation (Applied Biosystems, Germany) and quantified in replicate using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Germany). We carried out sequencing on a MiSeq, v3 600 Cycles Reagent Kit (Illumina, USA).

Quality control and analysis of sequences

We assessed all reads for quality using 'FASTQC' (Babraham Bioinformatics, UK) and 'MultiQC' software (Ewels et al. 2016). We determined the number of reads generated per sample from the R1.fastq.gz and retained samples with a total read count of ≥ 1000 (Clarke et al. 2020). We assembled read 1 and read 2 and removed poor-quality sequences and sequencing adapters using Paired End reAd mergeR (PEAR, HITS, Heidelberg, Germany), keeping only reads with quality ≥ 18, based on the MultiQC output, by defining it with parameter: *-q 18*. We then removed primers using cutadapt: *-g forwardprimer…reverseprimer*, specifying that adapters are linked and requiring both to be removed (Martin 2011). We removed chimeras using abundance rather than a reference with *Uchime_denovo* with the 'Vsearch' programme (Rognes et al. 2016) after de-replication. Following the removal of chimeras, we re-replicated samples and appended sample names into the header of the sequence using *Obiannotate –sample S:sample_name*, within the 'OBItools' programme (Boyer et al. 2016). We de-replicated sequences again using *Obiuniq – m sample*, as the output contained the number of reads for each unique sequence cluster. Based on the read counts in the *Obiuniq* output, we only retained sequences with read counts of $\geq 5\%$ of the total count within the sample ('discardPercentageofmaxderep_Fasta.py', Supporting information). Finally, we allocated species assignments using a BLASTn search on Genbank, which contains a reference library of over 450 000 formally described species (Sayers et al. 2020), including all terrestrial mammals known to be extant in the NCA (Moehlman et al. 2020). We assumed each individual detection of a given species' DNA in a sample represented one 'meal', i.e. one case of the hyena eating an animal of that species (Davidson et al. 2019, McLennan et al. 2022).

Verification of species identification

To validate our method to distinguish between the DNA of cattle, the most important livestock species to the Maasai community (Goldman 2011), and the closely related African buffalo *Syncerus caffer* (henceforth 'buffalo'), we conducted a feeding experiment with a hyena housed at Tierpark Berlin, a zoo in Berlin, Germany, in 2019. On a designated morning, the hyena keeper fed the hyena exclusively cattle meat. The next morning, based on the speed of the hyena digestive process (4–48 h; authors' observation; Goymann et al. 1999), the keeper collected a fecal sample from the hyena. In this sample, the extracted DNA was clearly identified as cattle DNA. In addition, we were able to verify the species identification three times based on direct field observations. First, $\geq 5\%$ of reads of a fecal sample collected on 7 July 1998 from a hyena that ate blue wildebeest *Connochaetes taurinus* (henceforth 'wildebeest') on 6 July 1998, were from wildebeest. Second, a sample from a hyena that we saw eating from a wildebeest carcass 6 h prior to sample collection contained only wildebeest DNA. Third, the feces collected on 29 November 2012 from a hyena that we observed eating from a buffalo on 28 November 2012 contained buffalo and wildebeest DNA ($\geq 5\%$ of the reads for both species). We did not see this hyena eating wildebeest on 28 November 2012, but it is feasible that it did so at a time when we were not monitoring it (e.g. at night, given that we were restricted to daytime observations).

Age, social rank, sex, and consumed species category

The 371 analyzed samples were from 255 individual hyenas that were ≥ 1 year old on the date of collection. Of these, 234 samples (63.1%) were from males and 137 from females. The hyenas represented all eight Crater clans. For more details, see the Supporting information. We attributed to each sample a unique ID as well as the sex, age, and social rank of the corresponding defecating hyena on the day of sample collection. We determined ordinal ranks based on the history of recorded agonistic interactions and our knowledge of rank inheritance and social queuing (for details, see Davidian et al. 2021). We converted the ordinal rank *OrdRanki* of an individual (*i*) into a proportional rank *PropRanki* bounded between −1 (bottom rank) and 1 (top rank), accounting for clan size *N*, using the following formula:

$$\text{PropRank}_i = \frac{N - \text{OrdRank}_i}{\frac{N-1}{2}} - 1$$

We estimated the effects of hyena age (in years: numeric and continuous), social rank (proportional: between −1 and 1), and sex (categorical: female or male) on the propensity of hyenas to consume domestic animals using a generalized linear mixed-effects model (GLMM) with a binomial distribution using package 'lme4' (Bates et al. 2015). We included a random effect for the ID of the fecal sample, given that some samples had more than one species detected. We used the binomial distribution because the response variable was categorical, with two levels: 'domestic' or 'wild'.

We computed predictions and associated 95% confidence intervals from the GLMM for any significant independent variable(s) using the function *ggemmeans* from package 'ggeffects' (Lüdecke 2018). For ease of interpretation, we calculated the percent change in the absolute value of the probability of domestic animal consumption resulting from a one-unit change in the value of the focal independent variable compared to its reference, calculated as $[\exp(\text{coefficient})-1] \times 100$.

Feeding preferences

We used data from biannual, transect-based Crater censuses (n = 43; Moehlman et al. 2020) to estimate the composition and size of the typically available food base for the hyena population over the course of our study period. We determined feeding preferences for the different species by calculating Manly's standardized selection ratio *B* (Manly et al. 1993) using package 'adehabitatHS' (Calenge 2006). To ensure our estimates of *B* were rigorous (Manly et al. 1993), we restricted analyses to the five species for which there were reliable census data and at least 10 detections: wildebeest, buffalo, zebra *Equus quagga* (henceforth 'zebra'), Grant's gazelle *Nanger granti*, and Thomson's gazelle *Eudorcas thomsonii*, the five most commonly hunted herbivores by Crater hyenas (Höner et al. 2002). The ratio *B* provides an estimate of selection for a given resource (i.e. the consumed species) relative to selection for all other resources, based on the proportional availability of each resource within the entire community. The value of *B* is the probability that a randomly chosen detection of any species will belong to a given species, if all species are equally frequent in the original population (Manly et al. 1993). Thus, the ratio can range from 0 (minimum selection) to 1 (maximum selection). To determine if the proportional consumption of each species was significantly different from its corresponding proportional availability, we used a χ^2 test with Bonferroni-corrected p-values.

Statistical analyses and graphical representation

We conducted all statistical analyses in R software 4.2.0 (www.r-project.org). Means are presented with standard deviation (± SD), unless stated otherwise. The threshold for statistical significance was set to $\alpha = 0.05$. Figure 1 was created using QGIS ver. 3.34.7.

Results

Animal species detected in spotted hyena feces

We detected 20 animal species in 371 feces from 255 hyenas (Fig. 2). The total number of detections – inclusive of all species – was 434. The majority of feces (n = 312; 84.1%) only contained one species, the others either contained two (n = 55) or three species (n = 4).

Wildebeest was by far the most commonly detected species, followed by zebra and buffalo. There were three detections of Maasai giraffe *Giraffa camelopardalis tippelskirchi* (henceforth 'giraffe'), a species only extant outside the Crater. Domestic animals (cattle, dog, donkey, goat, and sheep) were detected 18 times, which corresponds to 4.1% of all detections. There were no detections of rhino.

Detections according to socio-demographic variables and species categories

The hyenas in whose feces domestic animal DNA was detected were older (mean age = 9.08 ± 4.16 years, n = 18 detections) than the hyenas that consumed wildlife species (6.19 ± 3.04 years, n = 416; two-sample permutation test; Z = 3.82, $p < 0.001$; Fig. 3A). The proportional ranks of hyenas consuming domestic (−0.39 ± 0.62) and wild (−0.23 ± 0.63) animals were similar (Z = −1.06, p = 0.29; Fig. 3B).

The majority of detections (n = 273; 62.9% of all detections) were from males, and the majority of both domestic (n = 13; 72.2%) and wild (n = 260; 62.5%) animal detections also came from males.

Model results

There was a significant positive effect of age, but not of social rank or sex, on the predicted probability of domestic animal

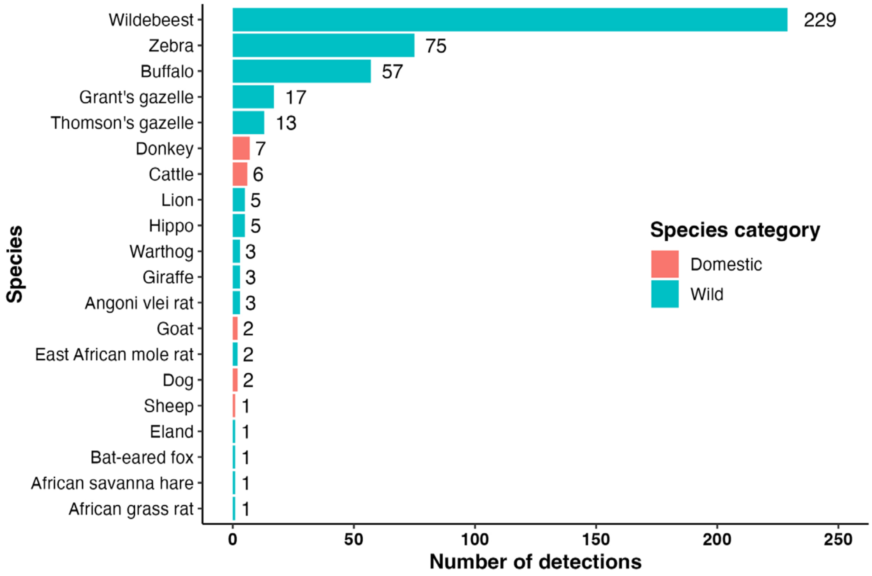

Figure 2. Total number of detections of DNA of different animal species in spotted hyena feces from the Ngorongoro Crater. There were 434 detections across 371 feces from 255 hyenas. Each detection represents one case of DNA of the given species being detected. Detections were classified as 'domestic' (domestic animal species) or 'wild' (wild animal species).

consumption (Table 1). A one-year increase in a hyena's age led to a predicted 26.8% increase in the probability of domestic animal consumption.

Dietary preferences

Wildebeest was selected for the most strongly, followed by Grant's gazelle, buffalo, zebra, and then Thomson's gazelle (Table 2).

Discussion

DNA metabarcoding of 371 feces of spotted hyenas collected over a 24-year period in the Ngorongoro Crater revealed that the diet of hyenas consisted mainly of wild animals, with only few detections of domestic animal DNA in hyena feces. The likelihood of hyenas to consume domestic animals increased with increasing age. No DNA of black rhinoceros was detected in any of the hyena feces, suggesting that Crater hyenas do not frequently consume this critically endangered species. Below, we provide possible explanations for and interpretations of our results in the context of evidence-based large carnivore conservation.

The diet of Crater hyenas predominantly consisted of 15 species of wild animals, with wildebeest being the most strongly preferred species, followed by Grant's gazelle. These results are similar to those of Höner et al. (2002), who analyzed direct observations of hunts and found that Crater hyenas preferably hunted wildebeest and juvenile gazelles (Grant's and Thomson's combined). One of the species detected in the current study was giraffe, a species of wild herbivore that lives in the areas surrounding the Crater but not on the Crater floor. This shows that resident Crater hyenas occasionally undertake foraging trips to areas outside the Crater, corroborating indirect evidence from an earlier study (Höner et al. 2005).

The domestic animals whose DNA was detected in Crater hyena feces also most likely originated from outside the Crater because Maasai pastoralists and their livestock do not reside in the Crater. Livestock were permitted to enter the Crater during daytime until 2017, but they were very well protected by the pastoralists (Dheer et al. 2022). The small number of detections of livestock suggests that Crater hyenas rarely consume livestock, despite the high abundance of livestock in the areas surrounding the Crater. Furthermore, the fact that the detections include both depredation and scavenging of dead livestock and that there were frequent outbreaks of fatal diseases among livestock during the study period (Dheer et al. 2021) suggests that livestock depredation by Crater hyenas overall is very rare. It seems likely that the high abundance of wild herbivores in the Crater makes hunting and scavenging from wild animals relatively easy. This would be consistent with past research, which suggested that large carnivores only regularly consume domestic animals when wild prey is very scarce (Yirga et al. 2012, Khorozyan et al. 2015, Parsons et al. 2022). It also corroborates the finding of a recent review that wild prey abundance is the primary predictor of livestock depredation by wild carnivores, surpassing factors like livestock biomass, carnivore weight, and livestock protection measures (Alquinta et al. 2025). Our results also have implications for conservation management in the Crater and surrounding areas as they suggest that most depredation leading to human–hyena conflict in the NCA (Dheer et al. 2021) is by hyenas from outside the Crater and not by hyenas resident in the Crater.

We found a positive effect of age on the propensity of Crater hyenas to consume domestic animals. This may be because older hyenas are less likely to successfully hunt wild

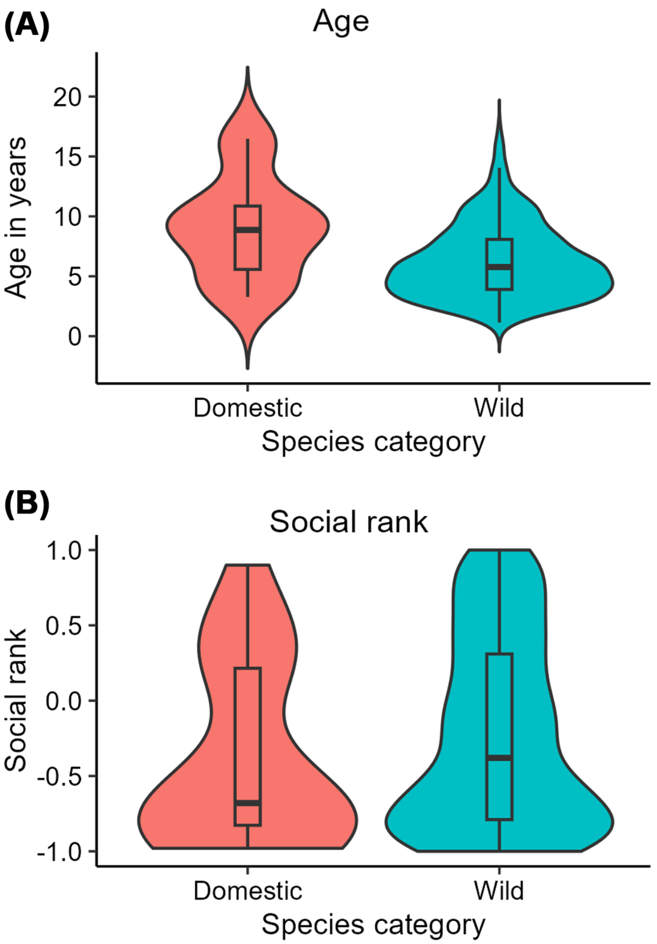

Figure 3. Consumption of domestic and wild animals by spotted hyenas from the Ngorongoro Crater as a function of hyena age (A) and social rank (B). Boxes indicate the interquartile range around the median (horizontal bar), vertical bars represent data that lie within 1.5 times the interquartile range.

Table 1. Effects of socio-demographic variables on the propensity of spotted hyenas from Ngorongoro Crater to consume domestic animals. Covariates consist of age, social rank, and sex. The intercept for the model corresponds to the domestic species category and the female sex, with age and rank held at their means. The column 'SE' provides standard errors on parameter estimates. The columns 'Z' and 'p' give, respectively, the Z- and p-values associated with the likelihood ratio test. Data in bold were deemed significant. Results are based on a binomial generalized linear mixed-effects model (GLMM). Random variance was estimated for sample ID (n=371 samples) at 0.74.

	Estimate	SE	% change	Z	p
Intercept	−5.35	0.87			
Age	**0.24**	**0.07**	**26.8**	**3.29**	**0.001**
Social rank	−0.07	0.63	−6.6	−0.11	0.91
Sex male	0.55	0.83	73.2	0.66	0.51

prey and more likely to know where to find and scavenge or hunt livestock. Past reports of culled 'man-eaters' or 'problem animals' suggested that old and injured large carnivores may be forced to rely on scavenging and feeding on domestic animals and humans due to age- and injury-related physical impairments (Rabinowitz 1986). Other studies suggested that conflict-prone individuals (at least in large felids; *Felidae* spp.) tend to be young transients who have not yet established territories with sufficient wild prey (Patterson et al. 2003, Woodroffe and Frank 2005). This is less likely in spotted hyenas, as females are usually resident in their natal clans for life (Höner et al. 2007), most males disperse only as adults (at around 3.5 years of age; Höner et al. 2010, Davidian et al. 2016), and transience generally is rare. In the Crater, only 15 of 470 males who joined a Crater clan were transient at some point in their life, and transient individuals were on average 7.9 ± 3.9 years old at the beginning of the transience period. These findings warrant further research into the effects of individual socio-demographic traits on the consumption of domestic animals by large carnivores. This can inform evidence-based large carnivore conservation actions tailored towards preventing specific individuals or classes of individuals from entering areas where they are likely to come into conflict with humans (Snijders et al. 2019).

The absence of detections of rhino DNA in hyena feces is consistent with behavioral observations that the Crater hyenas rarely consume rhinos. The potential effect of hyena predation on rhinos has been raised as a major conservation issue in the NCA and more globally in East Africa, but such concerns have lacked robust evidence (Sillero-Zubiri and Gottelli 1991, Davidson et al. 2019). Our results may further assuage such concerns. While hyenas are known to scavenge and occasionally even attack and kill rhinos (Kruuk 1972, Owen-Smith and Mills 2008), the lack of rhino DNA

Table 2. Dietary preferences by spotted hyenas in the Ngorongoro Crater. Abundances were estimated based on transect surveys conducted within designated census blocks from 1996 to 2019 by the Ngorongoro Conservation Area Authority, usually bi-annually (Moehlman et al. 2020). Presented are results for the five species for which abundance estimates existed and that were detected ≥ 10 times. Column '*B*' provides Manly's standardized selection ratio for the given species, with higher values indicating greater selection for the given species. Columns 'χ^2' and 'p' provide the χ^2 test statistic and Bonferroni-corrected p-value, respectively, for the proportional consumption and availability of each species. Column 'Sign' shows whether the proportional consumption was significantly greater than expected (+), less than expected (–), or as expected (0). Data in bold were deemed statistically significant.

Species	Abundance ± SD	*B*	χ^2	p	Sign
Wildebeest	**8218.8 ± 2924.4**	**0.30**	**25.75**	**< 0.001**	**+**
Grant's gazelle	**833.7 ± 396.4**	**0.22**	**5.58**	**0.018**	**+**
Buffalo	3176.8 ± 1540.1	0.19	3.16	0.075	0
Zebra	4274.1 ± 1331.1	0.19	0.09	0.76	0
Thomson's gazelle	**1392.4 ± 632.0**	**0.10**	**24.15**	**< 0.001**	**–**

in 371 hyena feces indicates that it is very unlikely to occur regularly. Our results therefore suggest that hyenas do not pose a credible threat to the persistence of the black rhino population in the Ngorongoro Crater. This is supported by a recent study that showed that the Crater rhino population increased substantially and more strongly during our study period than predicted under favorable conditions (Moehlman et al. 2020). We consider this a positive sign of the rhino population's continued persistence, and the result of ongoing conservation efforts geared towards the protection of rhinos by the NCAA, including intensive surveillance and anti-poaching operations, and an evident lack of significant predation pressure by hyenas and lions *Panthera leo*, the other main carnivore in the Crater.

Our study highlights the potential of DNA metabarcoding to assess the extent of human–carnivore conflict and to guide evidence-based conservation efforts to promote coexistence of carnivores, humans, and species of high conservation priority.

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Data availability statement

Data are available in the Supporting information.

Supporting information

The Supporting information associated with this article is available with the online version.

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