RESEARCH PAPER

Diet of common leopard and leopard cat in Murree, Kotli Sattian and Kahuta National Park, Pakistan: contrasting patterns of domestic animal and wild carnivore consumption

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Abstract

Predator communities are structured based on how sympatric species differentially use shared resources. To better understand the carnivore community in Murree, Kotli Sattian and Kahuta National Park in Pakistan, we determined the dietary breadth and niche overlap of sympatric carnivore Common leopards (*Panthera pardus*) and Leopard cats (*Prionailurus bengalensis*). We identified prey species through scat analysis after molecular confirmation of the source predator species for all Leopard cat scats (n = 42) and a subset of Common leopard scats (n = 38). Common leopard diet was relatively diverse (dietary breadth = 0.8) and comprised 19 mammalian prey species, including large mammal species (ungulates and domestic dogs; Frequency of Occurrence, FO = 34.6%), small (FO = 32.3%) and mid-sized mammals (FO = 27.7%). Even excluding domestic dogs, the frequency of domestic ungulate prey was slightly higher (15.4%) than the frequency of wild ungulates (14.6%). There was a notably high consumption of other carnivore species; five carnivore taxa were consumed by Common leopard. Leopard cat diet was similarly diverse (dietary breadth = 0.6), and included 9 mammalian prey species, as well as wild birds, reptiles and insects. Leopard cats relied heavily on rodents with the FO higher in winter (89.5%) than summer (62.6%). Although there was moderate dietary overlap between the two species (O_{jk}= 0.53), the observation that Common leopard focuses more on large prey and Leopard cat hunts primarily small mammals suggests competition for dietary resources is relatively low.

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Introduction

Sympatric species have the potential to compete for shared resources. For mammalian carnivores this competition may be especially strong for taxa that are closely related, are similar in size, or have a similar foraging ecology (Palomares *et al.*, 1996; Sunsquist *et al.*, 1989; Rosenzweig, 1966). To reduce competition, these taxa may show niche segregation of dietary resources where sympatric (Chamberlain and Leopold, 2005; Jedrezejewski *et al.,* 1989). Given that prey availability is a critical feature underpinning carnivore distributions, densities and behavior, studies of prey use by sympatric carnivores are essential to determine not only the extent of dietary overlap, but also for understanding how co-occurring carnivores are able to persist (Mills, 1992). Although numerous studies have examined mechanisms that support the coexistance of sympatric carnivores, including patterns of resource partitioning, habitat or activity segregation, and reduced niche overlap (e.g., Vanak *et al.,* 2013; Vanak and Gompper, 2009; Janssen *et al.,* 2007; Glen and Dickman, 2005; Fedriani *et al.,* 2000; Jones and Barmuta, 2000), there remain communities for which the extent of resource overlap and prey partitioning is unknown. An example is the carnivore community of eastern Pakistan, which as a whole is poorly studied, but known to contain species such as the Leopard cat (*Prionailurus bengalensis*), for which even simple natural history data is limited. Further, even species such as Common leopard (*Panthera pardus*) that are well studied across their range have been subject to limited work in Pakistan despite being critical agents of human-wildlife conflict in the country (Khatoon *et al.,* 2019; Khatoon *et al.*, in review). Therefore there is an important need to better understand the dietary ecology of these species in the region In Pakistan, the Common leopard is found in the highlands of Baluchistan and Sindh, and mountain forests of Punjab, Khyber-Pakhtoonkhwa and Azad Jammu and Kashmir. It inhabits hilly or mountainous country throughout Waziristan, Baluchistan and Sindh Kohistan in association with Acacia scrub forest. In the northern mountainous region, it is found in the Murree Hills, Swat, Kohistan, Dir, Chitral, Abbottabad, Kaghan Valley, Gilgit,Margalla Hills National Park and Neelum Valley in Azad Jammu and Kashmir. Conflicts with expanding human populations have made the Common leopard extremely rare in Pakistan including in remote mountain regions (Roberts, 1997), where the species is considered Critically Endangered (Sheikh and Molur, 2004). In contrast, the Leopard cat is a small (<8 kg; Sunquist and Sunquist, 2009) felid that is found in diverse environments, from semi deserts to tropical forests, woodlands to pine forests, and scrub land to agricultural land and can be found in close proximity to human populations (Sunquist and Sunquist, 2009; Scott *et al.,* 2004). In Pakistan, this species is categorized as "data deficient" as little information exists about the extent of its occurrence and its habitat requirements (Sheikh and Molur, 2004).

Here we contrast the feeding ecology of the Common leopard and the Leopard cat in the newly established Murree, Kotli Sattian and Kahuta National Park in eastern Pakistan. We focus on the seasonal variation in prey selection and dietary overlap among the two sympatric carnivores, combining scat analysis to determine prey content with molecular identification of the scats to avoid misidentification of the predator species. Given the potential role of both species in human-wildlife conflict in the region, we also focused on determining the extent to which these wild carnivores are consuming domestic animals.

Materials and methods

Study area

Murree, Kotli Sattian and Kahuta National Park (121,117 ha) is located in district Rawalpindi. It includes portions of three zones situated on the southern slopes of the northwestern extremities of the Himalayas, and features large mountain tracts with rich valleys traversed by mountain rivers. A description of the park environment can be found in Khatoon *et al.* (2019). In brief, park elevation varies from approximately 300 m to >2100 m with a steady increase from the south to north. Climate is temperate, with average June and January

temperatures of 32°C and10°C, respectively, and an average annual rainfall of 1.25 m, most of which falls in the monsoon season, and which peaks in July (GOP, 2006). The park has three principal forest types: Sub-tropical broad-leaf forest occurs at lower elevations, sub-tropical chirpine forest covers steep mountain slopes occurring between approximately 900 and 1,400 - 1,800 m, and a moist temperate coniferous forest zone with some admixture of oak and deciduous broad-leaved trees occurring above 1,400-1,800 m (Siddiqui *et al.,* 2010; Khan, 1994).

The landscapes of eastern Pakistan contain a diverse array of small and mid-sized mammals (Roberts, 1997), although the make-up of the community within the park remains uncertain. Larger non-carnivore wild mammals includes Barking deer (*Muntiacus muntjac*), Goral (*Naemorhedus goral*), Wild boar (*Sus scrofa*), and Rhesus macaque (*Maccaca mulatta*) (Habiba *et al.,* 2019; Roberts, 1997). Known or suspected mammalian carnivores of the park include Common leopard, Jungle cat (*Felis chaus*), Leopard cat, Golden jackal (*Canis aureus*), Red fox (*Vulpes vulpes*), Small Indian civet *(Viverricula indica),* Yellow throated marten *(Martes flavigula*), Indian grey mongoose (*Herpestes edwardsi*) and Small Indian mongoose (*H. auropunctatus*) (Khatoon *et al.*, 2019; Roberts, 1997). The presence of weasel and otter species (*Mustela*, *Lutra* and *Lutrogale* spp.), Palm civets (*Paradoxurus* and *Paguma* spp.), Gray wolf (*C. lupus*) and Asian black bear (*Ursus thibetanus*) is uncertain. Free-ranging domestic dogs (*C. familiaris*) and cats (*F. catus*) are common in and near villages in the park.

Collection and identification of scats
Scats were collected monthly for 24 months between 2016 and 2018 from 23 sites in the park (Khatoon *et al.,* 2019). At each site a 1-4 km transect along human or animal trails was identified and walked during each site visit. All scats were collected and identified to a source species following criteria of Jackson and Hunter (1996). On return to the laboratory, samples were placed in 98% ethanol for subsequent genetic analyses (Khatoon *et al.,* 2019).

A subset of scats, ultimately including all Leopard cat scats and five of 38 Common leopard scats, were subjected to genetic analyses to confirm the predator species (for details, see Khatoon *et al.,* 2019). In brief, approximately 200 mg of dried scat sample was scraped from the surface of each scat with sterile razors and tweezers and subjected to DNA extraction. DNA amplification was conducted using a polymerase chain reaction (PCR) approach and primers were designed to amplify a fragment of the carnivore mitochondrial control (d-loop) region. All PCRs included a positive control to confirm the success of the reaction and a negative (no DNA) control to detect reagent contamination. For samples that failed to amplify using the d-loop primers, we attempted to amplify and sequence approximately 350 bp of the mitochondrial cytochrome b gene using universal primers CB1/CB2 (Khatoon *et al.,* 2019; Kessing *et al.,* 1989; Kocher *et al.,* 1989). Gel electrophoresis was performed to confirm the success of PCR. The gel was visualized using an UV transilluminator to identify PCR products, which were then purified and sequenced. The resulting sequences were aligned, edited and compared to sequences in the NCBI Gen Bank database to identify the source species (Khatoon *et al.,* 2019).

Prey identification
Diet composition of each carnivore were determined by macro and microanalysis of scats, with prey species identified from tooth and bone fragments and by comparison of medullary and scale patterns on the hair remains with reference slides of the wild and domestic prey species potentially occurring in the park. Hairs were provided by the Pakistan Museum of Natural History, Islamabad, and were collected in complete tufts from different body parts to include a representative sample of hair types for the potential prey species. Microphotographs of all reference hairs were taken using a Leica DM1000 LED microscope.

This allowed for the development of a photographic reference key for 24 known species to use to identify the prey species recovered from Common leopard and Leopard cat scats.

Scat samples were washed in warm water to dissolve mucus and then dried on blotting paper. Thereafter, remains such as hairs, bones, teeth, nails and feathers were segregated using forceps.

Individual hairs were cleaned in carbon tetrachloride. Casts of cuticular scale patterns of hairs were made with clear nail enamel polish (Vanak and Gompper, 2009). Two to three drops of medium were placed on a slide, and then a clean hair was placed vertical to the long axis of the slide, with one end of the hair projecting over the edge of the slide so it could be easily grasped for removal.

The hair was removed using forceps, leaving the cast. Cuticular scale patterns of hairs and medullary structure were compared to reference hairs from known species.

Quantitative analyses
Scat samples of each carnivore species were categorized by season (Summer: April to September; Winter: October to March). Representation of each prey type in the diet was expressed as frequency of occurrence (FO), defined as
FO = s/N

where s is the number of occurrences of each prey type and N is the total occurrences of all prey types in all samples (Khatoon *et al.,* 2017; Khan *et al.,* 2017; Nadeem *et al.,* 2012). For each carnivore species and season we calculated prey species diversity (H'), prey richness (S, the total number of animal prey species consumed by each carnivore in a specific season) and prey evenness (E, calculated as E = H'/lnS) (Akrim *et al.,* 2018; Mahmood *et al.,* 2013; Nadeem *et al.,* 2012). Prey species diversity was calculated as
$H' = -\Sigma [p_i \times \ln p_i]$

where p_i represents the relative percentage of each prey item i. We measured dietary niche breadth (BA) using Levins' (1968) index:

$$BA = \left(\frac{1}{\Sigma p_i^2}\right) - 1 \big/ n - 1$$

Where n is the number of food items. Dietary niche overlap between the carnivore species was calculated by using Pianka's index (1973):

$$O_{jk} = \Sigma P_{ij} P_{ik} \big/ \sqrt{\left(\Sigma P_{ij}^2 \Sigma P_{ik}^2\right)}$$

Where p_{ij} and p_{ik} are the FO of prey item i in the diet of species j and k, respectively (Pianka, 1973). Pianka's index varies between 0 (total separation) and 1 (total overlap).

Results
A total of 80 scats were collected, 38 from Common leopard (5 genetically confirmed) and 42 from Leopard cat (all genetically confirmed). From these scats, 129 dietary items from 19 mammal species were identified for Common leopard, and 88 dietary items were isolated and attributed to 9 mammalian prey species for Leopard cat (Table 1).

Both species also consumed wild bird species, and reptiles and insects occurred in the scats of Leopard cat. Plant materials occurred in 6 Common leopard scats and in 9 Leopard cat scats. Excluding the plant materials, which were assumed to be accidentally consumed or consumed for non-dietary purposes, and excluding non-mammals which were not identified to species, the diet of Common leopard was somewhat broader (BA = 0.8) than that of Leopard cat (0.6). We recorded moderate niche overlap between Leopard cat and Common leopard (O*jk* = 0.53), with this overlap principally due to the consumption of the same rodent species.

Diet of Common leopard
Common leopards relied on both domestic and wild animals. Collectively the FO of large mammal species (ungulates and domestic dogs) was of 34.6%, followed by small (FO = 32.3%) and mid-sized mammals (FO = 27.7%). Across seasons, birds occurred in 3.8% of fecal samples. Domestic prey, including both livestock and dogs, constituted 20% FO of all food items. Even excluding domestic dogs (FO = 4.6%), the frequency of domestic ungulate prey was slightly higher (15.4%) than the frequency of wild ungulates (14.6%). Among the smaller and mid-sized mammals killed by

leopards, the most common taxa included mongooses (most likely *H. auropunctatus*, but possibly also including *H. edwardsii*), House rat and Rhesus macaques, which collectively had a FO of 24 % (Table 1).

Prey species richness for Common leopard was similar in summer (S = 15) and winter (S = 16), and there was a slightly higher dietary diversity and evenness during the summer (H' = 2.3; E = 0.88) compared to winter (H' = 2.1; E = 0.78). We identified strong seasonality in domestic animal use but not in in the consumption of wild ungulates. Overall, the FO of larger mammals, including both wild and domestic prey, varied significantly across seasons (x^2=12.2, P < 0.05, df = 5), with that

difference being a function of shifts in patterns of consumption of domestic prey.

The FO of domestic prey was higher in summer (31.4% including dogs; 22.4% excluding dogs) than in winter (8.1%), and the summer domestic animal consumption was entirely goat and dog. In contrast, the winter consumption of domestic animals lacked evidence of killing dogs, and included both goats and sheep, each at relatively low frequencies.

For wild ungulates, the FO were similar in winter (14.5%) and summer (15%). Among wild ungulates Goral, Wild boar and Barking deer all occurred in feces at similar frequencies across seasons (Table 1).

Table 1. Percent frequency of occurrence of prey items identified in the diet of Common leopard and Leopard cat in Murree, Kotli Sattian and Kahtua National Park. Samples are divided into those collected during the winter and summer (n = number of scat samples).

Prey items	Common leopard					Leopard cat				
	Winter (n=18)		Summer (n=20)		Total (n=38)	Winter (n=18)		Summer (n=24)		Total (n=42)
	FO	% FO	FO	% FO	% FO	FO	% FO	FO	% FO	% FO
Large Mammals										
Domestic goat (*Capra hircus*)	3	4.8	15	22.4	13.9					
Barking deer (*Muntiacus muntjac*)	2	3.2	3	4.5	3.8					
Wild boar (*Sus scrofa*)	3	4.8	3	4.5	4.6					
Domestic dog (*Canis familaris*)	0	0	6	9	4.6					
Domestic sheep (*Ovis aries*)	2	3.2	0	0	1.5					
Grey goral (*Naemorhedus goral*)	4	6.5	4	6	6.2					
Mesomammals										
Small Indian civet (*vivirricula indica*)	5	8.1	0	0	3.8					
Flying squirrel (*Petaurista petaurista*)	0	0	4	6	3.1	0	0	7	12.5	7.9
Common red fox (*Vulpes vulpes*)	3	4.8	3	4.5	4.6					
Rhesus macaque(*Maccaca mulatta*)	0	0	8	11.9	6.2					
Mongoose spp. (*Herpestes spp.*)	6	9.7	5	7.5	8.5	1	3.2	1	1.8	2.3
Jungle cat (*Felis chaus*)	0	0	2	3	1.5					
Small Mammals										
Indian gerbil (*Tatera indica*)	0	0	5	7.5	3.8	2	6.3	6	10.7	9.1
Murree vole (*Hyperacrius wynnei*)	4	6.5	2	3	4.6	3	9.4	7	12.5	11.4
Bandicoot rat (*Bandicota bengalensis*)						6	18.7	2	3.6	9.1
House shrew (*Suncus murinus*)	4	6.5	2	3	4.6	2	6.3	2	3.6	4.5
Turkistan rat (*Rattus turkistanicus*)	3	4.8	0	0	2.3	4	12.5	4	7.2	9.1
House mouse (*Mus musculus*)	6	9.7	0	0	4.6	6	18.7	4	7.2	11.4
House rat (*Rattus rattus*)	9	14.5	3	4.5	9.3	6	18.7	10	17.8	18.2
Indian parcopine (*Hystrix indica*)	2	3.2	2	3	3.1					
Birds	5	8.1	0	0	3.8	1	3.2	6	10.7	7.9
Insects	1	1.6	0	0	0.78	1	3.2	4	7.2	5.7
Reptiles	0	0	0	0	0	0	0	3	5.4	3.4

There were also significant seasonal differences in the FO of small mammals (x^2=12.60, P <0.05, df = 6). House rat, House mouse, and House shrew were more prevalent during the winter, with House rats (FO = 14.5%) and birds (FO = 8.1%) being particularly important in winter. Among the mid- sized mammals, species such as Civets were solely consumed in winter, while Rhesus macaques and Flying squirrels were only observed in the diet in summer. (Table 1).

There was a high rate of use of consumption of other carnivore species. Remains of 5 carnivore species were consumed (treating *Herpestes* as a single species) by Common leopard. While there were strong seasonal differences in the FO of domestic dogs (summer only) and Small Indian civets (winter only), the FO of Red fox and of mongooses were similar across seasons (Table 1).

Diet of Leopard cat
Combining seasons, rodents were the dominant prey group of Leopard cat, collectively comprising FO =72.8% of the diet, with six species each having a FO of >9%. Mid-sized mammals (Flying squirrel and mongoose spp.) collectively totaled just FO = 10.2%. Additional important components included birds (7.9%), insects (5.7%) and reptiles (3.4%) (Table 1).

The Leopard cat diet showed strong seasonality in prey species consumption. Prey species richness and diversity were slightly higher in the summer (S = 12; H' = 2.1) than the winter (S = 10; H' = 1.98), with patterns of evenness that were identical (winter E = 0.86; summer E = 0.86)). Small mammals varied seasonally and significantly in the Leopard cat diet (ied14.7, P < 0.05, df = 6) and the FO was higher in winter (89.5%) compared to summer (62.6). In contrast, while the occurrence of mid-sized mammals, birds, reptiles and insects were all higher in the winter than the summer (Table 1), these differences were not statistically significant (p>0.05 for each).

Discussion
In cases where sympatric carnivores compete for resources, one potential mechanism for reducing competition is partitioning of the prey community (Linnell and Strand, 2000; Jedrzejewski *et al.,*1989).

Common leopards and Leopard cats inhabiting Murree, Kotli Sattian and Kahtua National Park have diverse diets with a moderate level of overlap, but differ greatly in the extent to which larger prey are used. Common leopard makes use of larger and mid-sized mammalian prey, and the smaller Leopard cat consumes principally small mammals and non-mammalian prey. While such differences may suggest strategies for reducing competition, given the size difference in the two predator species, extensive competition might not be expected *a priori*. Further, recent work suggests that while competitive dynamics are often considered fundamentally important in structuring carnivore communities, factors such as habitat availability may also underpin the ability of carnivores to co-occur (Gompper *et al.,* 2016; Lesmeister *et al.,* 2015). As such, if small mammals are not a limiting resource in the park, the extent of exploitation competition between Common leopard and Leopard cat inhabiting the park is relatively low.

In addition to gaining a better understanding of the natural history of these two species, our results provide insights into three phenomena: the use and seasonal variability in consumption of domestic animals by Common leopards in the region, the extent to which Common leopards in the region consume smaller carnivore species, and the reliance of Leopard cats on rodents. Common leopards of Murree, Kotli Sattian and Kahtua National Park rely heavily on domestic animals. Such findings are not rare. For instance, Kshettry *et al.* (2018) found 80% of the contributed dietary biomass in a leopard population in India was derived from domestic prey, principally cattle, goats and pigs. We found strong seasonality in consumption of domestic prey compared to wild ungulates in the Common leopard's diet. The FO of domestic prey was higher in summer (31.8% including dogs; 22.7% excluding dogs) than in winter (8.1%), and the summer domestic animal consumption was entirely goat and dog. For wild ungulates, the FO were similar in winter (14.5%) and summer (13.6%). The heavy predation on domestic animals in the summer is likely a function of grazing practices used by local people. During the summer, livestock owners often move their animals further

from villages and deeper into the park to access better forage habitat, and such animal husbandry practices may underpin the severe local human-carnivore conflict (Khatoon *et al.,* in review).

A striking finding was the extent to which Common leopards consumed other carnivore species. Leopards consumed domestic dogs, as well as at least (given that mongooses may comprise more than one species) four other carnivores including civet, fox and Jungle cat. These carnivores together comprised 23 % of the diet of Common leopard. While larger carnivores often kill smaller carnivore species, such killing is typically viewed in an intraguild competition context underpinned by selection to reduce the number of co-occurring resource competitors (Donadio and Buskirk, 2006; Palomares and Caro, 1999). Typically, the killing species does not consume the killed species. In contrast, our findings suggest a system wherein Common leopards actively hunt not only domestic dogs (which has been repeatedly reported (e.g. Kshettry *et al.,* 2018; Athreya *et al.,* 2016; Butler *et al.,* 2014), but also diverse small carnivores.

The Leopard cat is among the least studied felid species. At <8 kg (Sunquist and Sunquist, 2009), Leopard cats are approximately the same size as large house cats (Fernandez and De Guia, 2011). Our results suggest the species is principally a predator of rodents, with wild birds and insects also contributing to their diet. Such findings are similar those reported by the few other studies that have examined the species (Rajaratnam *et al.,* 2007; Grassman *et al.,* 2005; Grassman, 2000; Rabinowitz, 1989).

In Pakistan, Shezad *et al.* (2012) used molecular approaches to assess the diet of populations from Ayubia National Park and Chitral Gol National Park in Khyber Pakhtunkhwa province. These populations also showed a focus on rodents, and as in our study, the House rat was the most abundantly identified prey item in feces. In contrast to the findings for Common leopard, there was no use of domestic animals, although several of the rodent species consumed can be human commensals.

Forest carnivore communities, and the interactions of the species that comprise these communities, are poorly studied throughout much of Asia. Yet these predator communities are diverse and interact strongly with local human and domestic animal communities (Khatoon *et al.* 2019; Khatoon *et al.* in review). The findings from this dietary study also suggest that while the food competition dynamics between the focal species may be low, there is extensive intra guild killing occurring within the broader carnivore community. As such, studies of the carnivore community of Murree, Kotli Sattian and Kahtua National Park, and similar poorly studied communities elsewhere in Asia, may provide insights into circumstances underpinning why some carnivore communities appear to be strongly interactive.

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Conflict of interest
None.

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