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Winter diet analysis of leopard (*Panthera pardus*) in the Nagarjun Forest of Shivapuri Nagarjun National Park

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The leopard (*Panthera pardus*) is a common and wide-ranged wild cat species listed in the vulnerable category of the IUCN Red List of Threatened Species. It is distributed widely in different protected areas and the human-dominated landscape of Nepal. The current study explored the winter diet of leopard in the Nagarjun Forest, Shivapuri Nagarjun National Park. A total of 16 scat samples of leopards from Nagarjun Forest were opportunistically collected between November and January 2022 and were analyzed for their diet composition based on microhistological analysis. Altogether 13 prey species were identified in the scats, compromising 11 wild and 2 domestic species. The primary wild prey was the wild boar with a percent occurrence of 81.25% and percentage biomass consumption of 30.76%. Among domestic prey was the dog with a percent occurrence of 43.75% and percentage biomass consumption equal to 12.25%. The measurements for length, overall hair diameter, medullar diameter, and medullary index were determined. The findings suggests that the winter diet of leopards of Nagarjun Forest is primarily sustained by wild prey, with smaller contributions from livestock. We suggest further studies on the seasonal dietary composition of this apex predator.

Key words: Biomass; Diet composition; Prey species; Scat analysis.

The leopard *(Panthera pardus)* is a solitary wild cat of the bush and forest and is mostly nocturnal. Leopards are the most prevalent wild cats around Asia and Africa (Nowell & Jackson, 1996) inhabited by mountains, rain forests, suburban areas, and semiarid environments, throughout the Middle East, South Asia, the Russian Far East, and sub-Saharan Africa (Stein & Hayssen, 2013). The leopard's home range is influenced by prey availability and the natural environment. They exhibit remarkable tolerance to the fluctuation of altitude, temperature, and rainfall (Sunquist, 1999). Leopards inhabit in the mountainous areas to an elevation of 4,600 m on Mount Kenya and 5,200 m in the Himalayan region (Stein et al., 2016).

Despite their broad range of distribution from arid to mountain, leopards are increasingly endangered due to habitat loss and degradation, prey depletion, poaching, and retaliatory killings by local communities in response to livestock depredation and human injuries (Kandel, 2019). Currently, leopards occupy only 25 to 37% of their historical range (Jacobson et al., 2016). According to the IUCN Red List, the species is categorized as Vulnerable (VU) due to an estimated global population decline of over 30% over the past three generations (Stein et al., 2016). It is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

In Nepal, leopards are generally distributed from the lowland Terai region (<100 meters above sea level) to the mountainous areas exceeding 4,000 meters. They primarily inhabit areas outside protected zones particularly forests and forest corridors, and seldom across the agricultural regions (Dhungana et al., 2019). Leopard's habitat coincide with the Royal Bengal tiger (*Panthera tigris*) within national parks and buffer zones in both the lowland Terai

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and mountainous regions (DNPWC, 2018; Subedi et al., 2021).

Leopards possess the most varied diets among greater obligate carnivores (Hussain et al., 2019). Their widest distribution among wild felid species is because of their diverse feeding habits, extremely flexible hunting style, and solitary nature (Chattha et al., 2015). A leopard's food preference is largely determined by its ability to seize and hold onto its prey (de Jesus, 2021). They consume a variety of prey, including various-sized ungulates, tiny rodents, other small mammals, birds, as well as livestock (Nowell & Jackson, 1996). Although leopards favor prey between the sizes of 10 to 40 kg, they may also eat bulkier prey if there are no competing predators (Hayward et al., 2006; Stein, 2008).

Leopards can live in close vicinity to humans and are primarily nocturnal. In a large portion of their territory, habitats have been converted to agricultural land to meet the demands of a growing human population. This has led to habitat degradation and fragmentation, posing significant threats to local leopard populations. Knowledge about prey preference and diet is essential to understanding the structural and physiological behavior of carnivores and to the conservation and management of top predators (Miquelle et al., 1996). Both the number and availability of the wild as well as domestic prey species are the main aspect that considers the potential carrying capacity of bigger carnivores in the human-inhabited environment (Boitani & Powell, 2012). Winter is a challenging season, with harsh climatic conditions and scarcity of food for large predators. Thus, it is important to study the winter diet composition of leopards. Such studies will be beneficial for determining whether a location has enough wild food species available for leopards as well as to plan efficient conservation and management strategies for this apex predator species (Hussain et al., 2019). Therefore, this study aims to analyze the winter diet composition of leopards in the Nagarjun Forest of Shivapuri Nagarjun National Park, Nepal, through scat analysis to identify prey species composition and biomass consumption. We assume that leopards primarily rely on wild prey during winter, with domestic animals constituting a smaller portion of their diet.

Materials and methods

Study Area

Shivapuri Nagarjun National Park (SNNP) is situated within the boundaries of Nuwakot, Kathmandu, Dhading, and Sindhupalchwok districts covering an area of 159 square kilometers. The park encompasses two distinct forest patches: Shivapuri and Nagarjun Forests. Nagarjun Forest, located between latitudes 27°43' to 27°46' North and longitudes 85°13' to 85°18' East, spans 16 square kilometers at the border of Nuwakot and Kathmandu districts (Figure 1). The forest ranges in elevation from approximately 1,350 meters above sea level (masl) to 2,100 masl. Nagarjun Forest comprises largely of quartzite rock along with siliceous limestone, limestone, and calcisilicate rocks for certain areas (Hagen, 1959). The forest's soill composition varies from dry, light brown to black soil and has varying levels of humus (Kanai et al., 1970). The forest experiences its highest humidity during July, August, and September, with peak rainfall occurring in July and August. The average monthly temperature ranges from 3.5°C to 30.2°C, humidity levels fluctuate between 78.73% and 87.73%, and rainfall ranges from 5.5 mm to 552.8 mm (Rijal, 2015). Poudyal et al. (2023) documented a total of 65 mammalian species within SNNP that belong to eight different orders. The fauna of Nagarjun Forest includes diverse species of birds, herpetofauna, and mammals. Among the fauna in the forest, the leopard and sambar deer (*Rusa unicolor)* are listed as Vulnerable (VU) on the IUCN Red List, while other species such as the wild boar (*Sus* sp.), leopard cat *(Prionailurus bengalensis*), large Indian civet *(Viverra zibetha)*, barking deer (*Muntiacus vaginalis*), jungle cat (*Felis chaus*), masked palm civet (*Paguma larvata*), Himalayan porcupine (*Hystrix brachyuran*), yellow-throated marten (*Martes flavigula*) are categorized as Least Concern (LC). The Chinese pangolin (*Manis pentadactyla*) is classified as Critically Endangered (CR) and the Assam macaque *(Macaca assamensis)* is listed as Near Threatened (NT) on the IUCN Red List (Dhital et al., 2020). Koju et al. (2022) documented the presence of the Burmese ferret badger (*Melogale personata*) through camera trapping inside Nagarjun Forest.

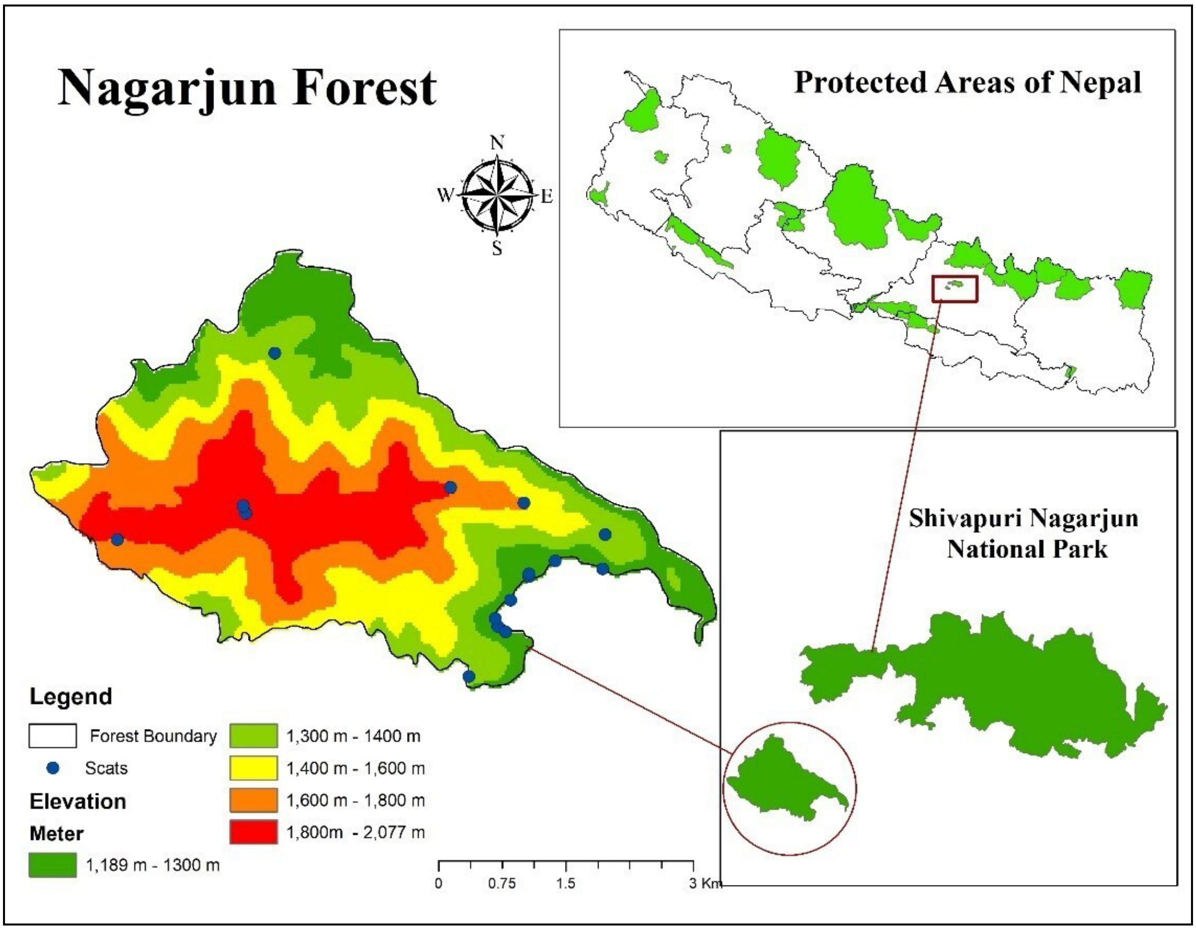

Figure 1: Map of the study area with the location leopard scats

Methods

Collection of scats

Scats of leopards were collected opportunistically from November to January 2022 by walking all available human trails, roads, and animal trails covering a distance of more than 19 kilometers. A portion of each scat was intentionally left to prevent alterations to the natural mark of the leopard (Schwarz & Fischer, 2006). Zip-lock plastic bags were used to collect the scat samples, with the date and GPS coordinates labeled on each bag (Kandel, 2019). To differentiate leopard scats from those of other species, signs of leopard such as pugmark and scrape were observed along with shape, which is characterized by pointed ends and several lobes concerning the diameter (Edgaonkar & Chellam, 2002).

Scat analysis

All the collected leopard scat samples were first washed with tap water using a fine-mesh sieve, to separate the undigested remains such as bones and teeth, to help identify the types of prey that the leopard had eaten. The washed samples were furthermore cleaned in a 1:1 ether-alcohol solution and further dried using blotting paper. Subsequently the samples were oven-dried at approximately 60 °C (Oli, 1993; Koju et al., 2023). Following the method described by Mukherjee et al. (1994), 20 random prey hair samples were taken from each scat for histological study. To ensure impartiality and randomization, an A_4 sheet paper was drawn with 2.5×2.5 cm^2 and 20 colored boxes were randomly chosen. After that dried hairs were spread randomly in paper and one hair sample was chosen from each selected box for further examination. The variation in prey consumption obtained from the number of hairs in the scats of leopards was statistically analyzed using the chi-square (χ^2) test. The prey species were categorized into two major categories: wild and domestic.

Mounting of hair and prey species identification

Each hair sample was mounted on a glass slide using DPX (Dibutylphthalate Polystyrene Xylene) as the mounting medium and covered with a cover slip. Thus prepared slides ware then examined under the microscope at 400× magnification and photographs were taken. Prey species were identified by examining the structure of the medulla and comparing it with reference slides of hair samples (Bahuguna, 2010; Shrestha, 2015; Schacker et al., 2018). Reference slides for domesticated and pet animals were prepared using hair samples collected from the study area.

Biomass calculation

The relative composition of prey species was determined using Ackerman's equation (Ackerman et al., 1984), presuming that the leopard has similar digestive physiology to that of the Mountain Lion (*Puma concolor*) (Karanth & Sunquist, 1995), to prevent bias resulting from varying prey body size, as follows:

$Y = 1.98 + 0.035X$

Where, Y= Weight of prey devoured per scat
X = Assumed prey species weight (kg)

Several studies have employed this method for leopards (Karanth & Sunquist, 1995; Andheria et al., 2007; Mondal et al., 2012; Athreya et al., 2014). We used Athreya et al. (2014), Bhandari et al. (2017), Hussain et al. (2019), Desai et al. (2021), and Rasphone et al. (2022) to get the prey species' assumed weight (X).

Microscopic analysis

The mounted hair samples were analyzed for micrometry and the following distances and medullary index were calculated (Kshirsagar et al., 2009). 1) Shaft's overall diameter, 2) Medulla diameter, and 3) Medullary Index: Medulla diameter/ Shaft's overall diameter. Pearson Correlation test was conducted between biomassof the prey species with recorded medullary index to assess whether hair thickness is related to biomass of animal.

Results

Diet composition

A total of 13 different pre species were identified from the analysis of 16 leopard scat samples (Table 1). Of these samples, 10 contained remains of three prey species, 4 containeed four species, and 2 contained five species. Among the wild prey, the wild boar was the most frequently occurring species (81.25%), followed by yellow-throated marten at 68.75%. Two domestic animal species dog and goat were also identified. Among them, dogs (43.75%) were the most favored species during the winter season. Among small mammals, rodents (43.75%), crab-eating mongoose (43.75%), Himalayan striped squirrels (18.75%), and Indian hares (18.75%) were also commonly detected in the leopard scats during winter.

The chi square test ($\chi2$) on variations of preys consumption obtained from number of hairs in the scats of leopards suggested that wild and domestic prey species in the leopard's diet is significantly different from an equal distribution, indicating a preference for wild prey ($\chi2=6.23$, df=1, p-value is 0.0126)

Biomass consumption

In terms of biomass consumed, wild prey constituted a major fraction (80.09%) of the leopard diet where

Table 1: Percent occurrence of prey species of scat sample of leopard

Name of species	Scientific Name	Number of samples with presence	Percent occurrence %
Wild boar	*Sus scrofa*	13	81.25
Yellow throated marten	*Martes flavigula*	11	68.75
Assamese monkey	*Macaca assamensis*	8	50
Rodent	*Rodentia* spp.	7	43.75
Crab eating mongoose	*Herpestes urva*	7	43.75
Himalayan striped squirrel	*Tamiops macclellandii*	3	18.75
Indian hare	*Lepus nigricollis*	3	18.75
Rhesus monkey	*Macaca mulata*	2	12.5
Himalayan langur	*Semnipithecus entellus*	2	12.5
Barking deer	*Muntiacus vaginalis*	1	6.25
Large Indian civet	*Viverra zibetha*	1	6.25
Dog	*Canis lupus*	7	43.75
Goat	- *Capra* spp	5	31.25
Unknown	-Not applicable	8	50

wild boar contributed the highest (25.08%) followed by Assamese monkey (13.03%). Domestic animals constituted 19.05% of prey biomass consumed by the leopard. Among domestic animals, the dog was the important constituent in the diet of leopards accounting for 10.22% of the consumed biomass (Table 2).

Medullary Index

The medullary index of hair samples from prey species varied by species (Table 3). Among wild prey, barking deer exhibited the highest hair medullary index (0.84), followed by Himalayan striped squirrel (0.83), crab eating mongoose (0.83), and Assamese monkey (0.82). Among domestic animals, the goat showed the highest medullary index (0.74). In contrast, the rhesus monkey had the lowest medullary index (0.34).

The Pearson correlation test yielded a Pearson correlation coefficient (r) of approximately 0.104, indicating a very weak positive correlation between

Table 2: Calculation for biomass consumption by leopard

Name of species	Assumed weight (a)	Biomass per scat (b)	No. of scats (c)	Biomass consumed (d)	Percentage (%) biomass consumption (e)
Wild boar	38	3.31	13	43.03	25.08
Yellow throated marten	1.5	2.0325	11	22.3575	13.03
Assamese monkey	10	2.33	8	18.64	10.86
Rodents	0.5	1.9975	7	13.9825	8.15
Crab eating mongoose	2	2.05	7	14.35	8.36
Himalayan striped squirrel	0.8	2.008	3	6.024	3.51
Indian hare	2.5	2.0675	3	6.2025	3.61
Rhesus monkey	10	2.33	2	4.66	2.71
Himalayan langur	10	2.33	2	4.66	2.71
Barking deer	20	2.68	1	2.68	1.56
Large Indian civet	8.5	2.2775	1	2.2775	1.32
Dog	15	2.505	7	17.535	10.22
Goat	30	3.03	5	15.15	8.83

a = Assumed weight (kg) of the prey species
b = Estimated weight of prey consumed per scat ($b = 1.98 + 0.035 \times a$) (Ackerman et al., 1984)
c = Number of scats in which prey species were identified
d = Biomass consumed ($b \times c$)
e = Percentage consumption ($b \times cC/\sum [b \times c] \times 100$) (Khatoon et al., 2017)

Table 3: Medullary index of prey species of leopards

Prey Species	No. of prey species	Overall diameter mean (µm)	± SD	Medullar diameter mean (µm)	± SD	Medullary index
Wild boar	64	96.34	36.20	68.08	42.93	0.71
Assamese monkey	44	115.91	43.08	95.13	43.27	**0.82**
Yellow throated marten	36	58.57	16.99	29.44	8.30	0.50
Rodents	35	97.22	44.02	73.14	41.77	0.75
Himalayanlangur	19	73.83	17.46	53.23	17.97	0.72
Barking deer	18	147.46	24.29	123.17	22.09	**0.84**
Crab eating mongoose	14	123.27	21.29	101.84	24.72	**0.83**
Himalayan striped squirrel	8	117.50	17.84	97.14	18.39	**0.83**
Large Indian civet	7	90.20	19.58	54.29	17.22	0.60
Rhesus monkey	6	44.29	12.36	15.24	2.33	0.34
Indian hare	3	36.19	16.25	14.29	2.86	0.39
Dog	26	56.37	17.15	36.81	13.70	0.65
Goat	10	76.00	22.14	56.29	24.39	**0.74**

the assumed body weight and the medullary index of the prey species.

Discussion

The dietary analysis revealed that the most preferred wild prey of the leopard was the wild boar, followed by the yellow-throated marten, as determined by the percentage occurrence of prey species in the leopard scat samples. The high consumption rate of wild boar in the current study may be attributed to its abundance and widespread distribution across the study area. This finding is consistent with the study by Ghoddousi et al. (2017) in Golestan National Park, Iran, where wild boar was alos the predominant prey species in the leopard's diet due to its high population density. The preference for wild boar can be explained by its larger body mass, which provides substantial nourishment, especially in the absence of other prey species.

Despite being a preferred prey item, the wild boar is considered a highly aggressive and dangerous species for leopards (Ramakrishnan et al., 1999), which may explain why it is often classified as a less favorable prey choice in other studies (Hayward et al., 2006). Leopards may avoid predation on mature wild boar and instead target juveniles to minimize the risk associated with hunting large and aggressive individuals (Sugimoto et al., 2016). This preference for wild boar could result from a prey switching strategy (Ghoddousi et al., 2017), likely driven by the lower densities of other ungulates in the study area (Upadhyay et al., 2019).

In the present study, a low representation of barking deer was observed, with a percentage occurrence of 6.25% and biomass consumption of 1.87% in the diet of leopards. It may be due to the seasonal fluctuations and the number of scat samples analyzed. Although crab-eating mongoose, Himalayan langur, and Indian hare are absent from the study area, they were still found in the scat samples. This may be due to the Nagarjun Forest being nearer to the Shivapuri Forest, which provides a corridor for leopards to migrate between forest patches to fulfill their dietary needs. Leopards have a large home range and may migrate to nearby forests, suggesting that the available prey species innone forest may appear in the scat sample of another forest patch.

Rodents contributed approximately 10% of the prey biomass consumed by the leopard. This highlights the importance of rodents in the leopard's diet, specially during the scarcity of native prey in the forest, which may force leopards to rely on rodents for nutrition (Kandel, 2019). In our study, domestic animals accounted for 22.83% of the leopard's diet in terms of biomass consumption. Among domestic animals, the dog was the important prey both in percentage occurrence and biomass consumption. No consumption of other domesticated animals such as goats, was found in the study. This might be due to the implementation of predator-proof corrals, which help reduce human-wildlife conflict (Athreya et al., 2014).

This study revealed that wild prey were the major food items of the leopard's winter diet, although domestic livestock were consumed. Our findings are somehow similar to the study of Chattha et al. (2015) and Dar and Bhat (2022), who reported that leopards consumed more wild prey species than domestic livestock during the winter season. This may be due to the reduced availability of livestock for predation as the livestock remain caged in sheds. While domestic prey species do contribute to the diet of large felids, they are rarely the sole food source (Athreya et al., 2014). However in human settlement, domestic prey populations can exceed those of wild prey (Kshettry et al., 2018). Leopard depredation on livestock may primarily result from a depletion of wild prey.

Similalry, the study also found that the medullary index value obtained for goats aligns with the findings of Shrestha (2008), and the value for dogs aligns with with the results reported by Negi et al. (2017). The width of hairs in the medulla and cortex varies by species, forming distinctive scale patterns that can be used for accurate species identification (Wiley, 2004). These findings supports the conclusion that using hair parameters and the medullary index together ensures more reliable method for prey species identification in diet analysis (Mihaylov & Kirilov, 2022).

Conclusion

This study shows that leopards (*Panthera pardus*) in Nagarjun Forest consume a variety of prey species. During the winter season, leopards prefer wild prey as the food source over domestic animals. The findings of this study provides baseline informatiom on the winter diet of leopards, which will be helpful in the development of effective, evidence-based long-term strategies for the conservation and management of this apex predator and its wild prey base. Furthermore, the findings can also contribute to mitigating human-leopard conflict in areas of shared habitat.

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Author's contribution statement

S. Shrestha: Conceptualization, methodology, field work, validation, formal analysis, writing original draft, writing review & editing; **N. P. Koju:** Conceptualization, methodology, field work, validation, formal analysis, writing original draft, writing review editing; **A. Thapa Magar:** Methodology, field work, formal analysis, writing review & editing; **S. B. Shrestha:** Methodology, field work, formal analysis, writing review & editing; **S. Ghimire:** Methodology, field work, formal analysis, writing review & editing.

Declaration of competing interest

The authors declare there is no conflict of interest.

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