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#### RECOMMENDATION

This is to recommend that the thesis entitled "DIET ANALYSIS OF BARKING DEER (*Muntiacus vaginalis*, Boddaert 1785) IN NAGARJUN FOREST OF SHIVAPURI NAGARJUN NATIONAL PARK, NEPAL" has been carried out by Kasturi Gurung for the partial fulfillment of Master's Degree of Science in Zoology with special paper Ecology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Supervisor Prof. Dr. Tej Bahadur Thapa, Head of Department, Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal Date: 9th ] qo 1202 2.

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#### LETTER OF APPROVAL

On the recommendation of supervisor "Dr. Tej Bahadur Thapa" this thesis submitted by Miss. Kasturi Gurung entitled "DIET ANALYSIS OF BARKING DEER (*Muntiacus vaginalis*, Boddaert 1785) IN NAGARJUN FOREST OF SHIVAPURI NAGARJUN NATIONAL PARK, NEPAL" is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper ecology.

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#### CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Kasturi Gurung entitled "DIET ANALYSIS OF BARKING DEER (*Muntiacus vaginalis*, Boddaert 1785) IN NAGARJUN FOREST OF SHIVAPURI NAGARJUN NATIONAL PARK, NEPAL" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper ecology.

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#### DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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#### ABSTRACT

The composition and selection of food by ungulates is a fundamental element to understand their dietary habits. This study aimed to assess the winter diet composition, niche breadth, and preference of forage plants for Barking Deer in the Nagarjun Forest of Shivapuri Nagarjun National Park, Nepal in 2019. The line transect method along with quadrat sampling was used for the collection of Barking Deer pellets and reference plant species. Micro-histological technique was used to prepare micro-photographs of reference food plants and deer pellets in the laboratory. A total of 1500 fragments of 50 fecal samples were analyzed to identify undigested plant parts in the pellet of Barking Deer. Diet composition was expressed in terms of percentage of occurrence, Levin's niche breadth was calculated for the evaluation of the degree of food selectivity and browse to grass ratio to determine whether the Barking Deer adopts browsing or grazing strategy. Food preference of Barking Deer was evaluated by using Ivlev's Electivity Index and statistical tool, Chi-square test was used for data analysis.

Barking Deer consumed 26 plant species. Of these, 18 species belonged to browse, four species to grass, two species to fruits, and two other species to fern and herb. The contribution of browse species was higher in the overall diet (61.13%), followed by fruits (16.66%), grasses (10.87%), and others (1.87%). Smilaxaspera (10.33%), Litsea chartacea (7.87%), Schima wallichii (5.4%), Prunus cerasoides (5.13%), etc. were the other major browse plants in the diet. Choerospondiasaxillaris (15.86%) formed the largest proportion of all the food species and was the important fruit for Barking Deer. The important grass species present in the diet were Imperata cylindrica (4.13%) and *Carex* sp. (3.2%). The Browse to grass ratio in the Barking Deer diet was 5.62 showing a strong preference towards the browse plants. The niche breadth of the food plants consumed by Barking Deer was found to be 0.512 indicating its generalist nature of feeding, consuming a variety of species. Choerospondias axillaris (IEI = 0.5), Litsea chartacea (IEI = 0.51), Prunus cerasoides (IEI =0.5) and Smilax aspera (IEI = 0.51) were four plant species found to be strongly preferred. Castanopsis indica, (IEI = 0.07), Myrica esculenta (IEI = 0.05) and Berberis asiatica (IEI = 0.04) were found to be used in proportion to availability whereas Rhododendron arboreum (IEI = -0.58), Pinus roxburghii (IEI = -0.7), Lithocarpus elegans (IEI = -0.6), etc. were some other avoided food plants. Researches on nutritive value of important dietary species and their niche overlap with other herbivore competitors are recommended.

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# LIST OF ABBREVIATIONS

- CDZ: Central Department of Zoology
- CMOS: Complimentary Metaloxide Semiconductor
- DCM: Digital Camera Module
- DNPWC: Department of National Park and Wildlife Conservation
- GoVN: Government of Nepal
- GPS: Global Positioning System
- IUCN: International Union for Conservation of Nature
- IEI: Ivlev's Electivity Index
- MS: Microsoft
- NHPL: National Herbarium and Plant Laboratories
- PSM: Plant Secondary Metabolites
- **RIV: Relative Importance Value**
- SNNP: Shivapuri Nagarjun National Park
- USB: Universal Serial Bus

#### **1. INTRODUCTION**

#### **1.1 Species Introduction**

The Species of the genus "*Muntiacus*" (Rafinesque, 1815) are considered to be the most primitive species in their order, Artiodactyla, and are also regarded to be the ancestor of all living Cervidae (Oli and Jacobson, 1995).Groves and Grubb (2011) suggested that the species in the Northeast India, Nepal and Parts of Myanmar was *Muntiacus vaginalis* (Boddaert, 1785) which were distinct from red muntjacs in the north-west and central India "*Muntiacus aureus*" and the red muntjacs in the Western Ghats and Sri Lanka "*Muntiacus malabaricus*". At present, IUCN recognizes 13 species of Barking Deer or muntjac deer: Reeves' Muntjac (*M. reevesi*), Puhoat Muntajc (*M. puhoatensis*), Large Antlered Muntjac (*M. vuquangensis*), Sumatran Muntjac (*M. montanus*), Bornean Yellow Muntjac (*M. atherodes*), Roosevelts' Muntjac (*M. rooseveltorum*), Black Muntjac (*M. crinifrons*), Northern Red Muntjac (*M. vaginalis*), Leaf Muntjac (*M. muntjak*), Fea's Muntjac (*M. feae*) andAnnamite Muntjac (*M. truongsonensis*) (Amato *et al.*, 1991; Nowak, 1991; Giao *et al.*, 1998; Shi and Ma, 1998; Timmins *et al.*, 1998; Wang and Lan, 2000; Groves and Grubb, 2011).

# **1.1.1 Geographical Distribution**

Barking Deer have a broad geographic range distributed throughout Asia from sea level to 3000 meters in the Himalayas (Mishra, 1982) and are found in Indo- Malayan countries, China, Formosa, Japan, Sri Lanka, North India and Nepal (Prater and Barruel, 1971). They have also been introduced to Britain and the United States wherein some areas they are well established (Chapman *et al.*, 1993). In Nepal, the species is commonly distributed in Terai and mountains and are common in all national parks of Terai foothills (Shrestha, 1997). It is reported from Chitwan National Park, Bardia National Park, Sagarmatha National Park, Langtang National Park, Makalu Barun National Park and Conservation Area, Shivapuri Nagarjun National Park, Parsa Wildlife Reserve, Annapurna Conservation Area (Chaudhary, 1998), Rara National Park, Dhorpatan Hunting Reserve, Shey Phoksundo National Park (Shrestha, 1997).



Figure 1: Global distribution of Barking Deer (*Muntiacus vaginalis*) (Source: IUCN, 2008)

# 1.1.2 Morphology

The animal takes its name from its call which is dog-like bark (Shrestha, 1997). Barking Deer is distinguishable by its deep red or bright chestnut coat on the back, paler on the flanks and almost whitish or buff underside of the body and tail (Nowak, 1991). The colour of the coat varies from dark brown to chestnut brown, hence the local name "Raate" or "Ratuwa" (Yonzon, 1978). Males are distinguishable by their outwardly curvy tusks and long and hairy pedicels from which antlers grow whereas tufts of bristly hair replace the horns in does (Shrestha, 1997). The height at the shoulder of an adult is from 50 to 75 cm and it weighs 22 to 23kg (Shrestha, 1997). The males tend to be larger than the females.

# 1.1.3 Behaviour

Barking Deer is primarily a solitary species (Dubost, 1971; Barrette, 1977; Yonzon, 1978; Mishra, 1882) but can be seen occasionally in a group of four or five animals (Krump, 1971; Mishra, 1982). The animal browses at early dawns and in the evening; mid-day is spent resting under the cover of a bush or a rock. It emits barking calls when bound from

danger with the white underside of its tail exposed and as it makes audible thumps on the ground with the hind hooves (Barrette, 1977). It exhibits two patterns of defecation in captivity and even in the wild. It defecates through its enclosure without regard to existing pellet groups, and it repeatedly uses specific areas, which are called latrines (Dubost, 1970). Young muntjacs are usually born in dense jungle growth, where they remain hidden until they can move about with the mother (Rafinesque, 1815).

# 1.1.4 Habitat

The smaller, forest-dwelling and sexually size-monomorphic muntjacs were observed inhabiting a more uniform and stable habitat with less strict territoriality but their welldefined home ranges and high fidelity indicated some form of site-specific dominance (Odden and Wegge, 2007). The muntjac inhabits rain forests, areas of dense vegetation, hilly country and monsoon forests (Rafinesque, 1815) close to water resources because they usually drink water at least once a day, mainly in the morning or at noon hours (Yonzon, 1978). Small forest ungulates like muntjac choose to inhabit and hide in thick cover to avoid predation (McCullough et al., 2001). Seeking dense canopy cover is an important thermal strategy in winter (Mysterud and Ostbye, 1996) and provides a means to avoid heat stress during summer (Sargent et al., 1994). Both the forage availability and vegetation cover were very important factors for Barking Deer habitat selection as per a study in Hainan Island of China, (Teng et al., 2004). Also, the choice of ungulate habitat is strongly influenced by nutrients and energy demands for the growth of bone and body mass in males (Bronson, 1989) as well as gestation and lactation of calves in females (Belovsky, 1986). The muntjacs were found to show preference towards Sal and riverine forests and were often seen on meadows (Tamang, 1982) because riverine forest and Sal forest are provided with optimal food conditions, habitat requirements and escape cover. In Nagarjun forest Barking Deer was found preferred mixed broadleaved forest in the spring season and pine and mixed broadleaved forest in the rainy season (Nagarkoti and Thapa, 2007).

#### 1.1.5 Food Habit

Several studies on food habits in a wide range of habitats have shown that the Barking Deer is a selective feeder, subsisting on easily digestible food offering few fibres, but rich in energy and proteins (Barrette, 1977; Maloiy *et al.*, 1988; Kranz, 1991; Estes, 1993; Brotherton and Manser, 1997). It is a selective browser, taking food items such as

flowers, twigs, fruits, and seed pods (Hofmann and Stewart, 1972; Jarman, 1974; Hofmann, 1989). When they do browse, they take only tender leaves, buds and flowers. Whenever the leaves are large, only the leaf tends to be cropped, and the relatively fibrous stem is left on the twig (Barrette, 1977). Muntjacs are virtually omnivorous and feed on bamboo shoots, foliage, bark, fruits, carrion (Kurt, 1981), herbs, sprouts, seeds, grasses, bird's eggs and small mammals and warm-blooded animals using their canines to bite and their forelegs to deliver strong blows to catch (Humas, 2004). Their preference for food choices includes abundant fruits, seeds and all young, easy to digest leaves with soft palatable trunks mostly from fast growing pioneer colonists rather than closed forest species that contained a low levels of tannin and lignin (Farida et al., 2006). The main food plant species of Barking Deer on the lowlands of Nepal are Imperata spp, Shorea robusta, Ziziphus spp. (Yonzon, 1978; Shrestha, 1984; Gaire, 2016), Terminalia Bellerica, Bombax ceiba, Mimosa spp. (Yonzon, 1978; Shrestha 1984), Syzygium cumini (Shrestha, 1984; Gaire, 2016). Whereas Imperata cylindrica, Arundinaria falcata, Moghania strobilifera, Smilax aspera and fruits of Rubus ellipticus, Prunus cerasoides and Berberis asiatica are amongst the preferred food species on mid-hills of Nepal (Nagarkoti and Thapa, 2007). Similarly, Indigofera heterantha, Calamintha umbrosum, Bergenia ligulata, Flemingia strobilifera, Ranunculus lactus are some major noticeable diet species recorded from the Himalayan region of Kumaon (Ilyas and Khan, 2003). In Thailand, it was found particularly fond of fallen fruits and browsed more than it grazed (Chaplin, 1977).

#### **1.1.6 Conservation Status**

*Muntiacus vaginalis* is enlisted as the least concern in the IUCN list of endangered species, they are facing several threats in different areas. Among these threats, habitat destruction, encroachment, habitat disruption, hunting and poaching are very common throughout its distribution range (IUCN, 2021; Bennett and Gumal, 2001). Nationally it is a protected and vulnerable species due to an observed decline of populations in the wild of more than 30% over the past 15 years and globally Least Concern species in the IUCN category (DNPWC, 2011). Like so many of the species in the Terai region, especially herbivores, this species is threatened by habitat loss and degradation and due to human encroachment, conversion of land into agriculture since the eradication of malaria in the 1950s and poaching throughout the country except in protected areas. Local people's increasing need for forest resources such as feed, grass, timber, and firewood puts more

strain on supplies (Thapa, 2003). If the herbivore species like Barking Deer and other prey species continue to dwindle as a result of these threats, Large carnivores like leopards and tigers will be affected (Barrette, 1977; Mishra, 1982a; Chapman, 1993; Oli and Jacobson, 1995; Pei *et al.*, 1995; Suwal and Verheught, 1995; Chapman *et al.*, 1997; Chen *et al.*, 2001; Ernest, 2003; Baral and Shah, 2008).

## **1.2 Objectives of the Study**

The general objective of the study was to gather ecological information on the food and feeding habits of Barking Deer in Nagarjun Forest, Kathmandu, Nepal. The specific objectives were to;

- Examine the diet composition of Barking Deer.
- Determine the dietary niche breadth and
- Assess the diet preference of Barking Deer.

#### **1.3 Rationale**

Food habit analysis is a crucial process in understanding an animal's ecology because it reflects resource use and can provide insights into habitat utilization and competitive interaction. (Litvaitis, 2000).In Nepal, most of the studies have been carried out focusing on the population status, distribution, and habitat pattern of Barking Deer with limited information available on feeding behaviour (Yonzon, 1978; Dinerstein, 1979b; Shrestha 1984; Nagarkoti and Thapa, 2007; Pokharel *et al.*, 2015). In addition, studies on Barking Deer have been concentrated in the lowlands of Nepal. Barking Deer is considered to be the major prey species for a long-range of carnivores, thus acting as an important component of the food chain of the forest ecosystem (Prater, 1971). So it is hoped that the knowledge generated about diet compositions, food resource utilization, and factors affecting animal's food choices from this study will play important role in developing effective conservation strategies and sound management programmes thereby enhancing the long term survival of this nationally vulnerable species (Jnawali *et al.*, 2001) and other key predatory species relying on Barking Deer in the Nagarjun forest as well as other parts of central hills of the country.

# **1.4 Limitations of the Study**

- Due to the rugged topography of the study area, the field survey was limited to a small study area.
- This study only represents the data from the winter season diet.
- Considering the accessibility of Barking Deer, reference plant species up-to a certain heights were collected.

#### **2. LITERATURE REVIEW**

#### 2.1 Study of Barking Deer Diet in Nepal

Yonzon (1978) studied general behavior and feeding habits (through direct observation methods) of Barking Deer in Chitwan National Park, where he found Barking Deer grazing in the forest fringes and sometimes feeding on more palatable up-shoots of Imperata cylindrica spp. in the morning and afternoon hours in the grassland areas that had appeared after burning. Major food plants consumed were Imperata spp., Mimosa spp., Ziziphus spp., Bombax ceiba, Shorea robusta, Bauhinia racemosa, Anthocephlous cadamba and Terminalia bellerica. From a limited number of sightings, Dinerstein (1979b) observed some important plants that the Barking Deer fed on. The plants consumed by deer were Imperata cylindrica, Cynodon dactylon and fruits of trees including Ficusglomerata, Schleicheratrijuga and Eugenia jambolana. Similarly, Shrestha (1984) reported that the Barking Deer consumed 13 tree species, one grass and one herb in Chitwan National Park throughout the study. The plants consumed were Adina cordifolia, Anthocephalus cadamba, Artocarus integra, Bombax ceiba, Bauhinia malabaricum, Ficus glomerata, Ficus religiosa, Imperata cylindrica, Mimosa pudica, Shorea robusta, Syzigium cumini, Tamarindus indica, Terminalia belirica, Trewia nudiflora, Ziziphus mauritiana.

Barking Deer was found to be mixed feeders, consuming both the grasses and browse species according to availability in the Nagarjun forest of SNNP. Grass, browse and soft mass made up 55.36%, 38.5%, 2.49% and 42.62%, 48.44%, 2.08% of the Barking Deer's diet during the spring and rainy seasons, respectively. Whereas a higher proportion of grasses were recorded in the diet in the rainy season with few species of lichens. Regardless of individual plant species, grass *Imperata cylindrica*, contributed significantly to the spring season diet. *Imperata cylindrica*, *Smilax aspera*, *Moghania strobilifera* and *Arundinaria falcata* were the most preferred food items in both seasons including fruits of *Prunus cerasoides*, *Berberis asiatica* and *Rubus ellipticus* (Nagarkoti and Thapa, 2007). A study of niche partitioning in Bardia National Park between Four Horned Antelope and Barking Deer revealed that both the species diet was largely composed of  $C_3$  plants hence they were categorized as browsers. Overlapping of diet was recorded during the rainy season when resources were prevalent whereas the differentiation of diets was recorded during the resource-limited dry season (Pokharel *et* 

*al.*, 2015). Based on food habit studies, a total of 44 plant species were recorded in the Barking Deer diet, with 32 species of browse (56.84%) occupying the largest proportion, followed by 6 species of forbs (13.34%) and 6 species of grass (11.16%). With niche breadth value 0.67, Barking Deer was found to be a generalist feeder also the browse to grass ratio was found to be 3.37, with a strong preference towards the browse plant species. The important food species that contributed to the diet were *Justicia spp.* (8.83%), *Shorea robusta* (5.88%), *Syzygium cumini* (5.44%), *Thysanolaena maxima* (4.67%), *Pogostemon benghalensis* (4.22%), *Aegle marmelon* (4.06%), *Mitragyna parvifora* (3.33%) (Gaire, 2016).

#### 2.2 Study of Barking Deer Diet outside Nepal

Muntjacs were observed as browsers feeding primarily on tender leaves, buds, flowers, and fallen fruits in Wilpattu island, Srilanka (Barrette, 1977). Barking Deer's preference towards thick forest was noticed where they utilized forest species and understory plants for browsing more than grassland species. They avoided open and clear areas although the area contained many species of food plants. The highest diversity of diet was observed in June and July when most plant species are at their peak fruiting season. The fruit species belonging to the family Moraceae was found most favoured by Barking Deer. Similarly, *Ficus* spp., *Irvingia malayana*, *Horsfieldia* spp., were amongst the other highly consumed fruit species (Kassim, 1987).

Ilyas and Khan (2003) studied the seasonal diet of Barking Deer in India and found that *Indigofera heterantha, Lannea secunda, Galium aperina, Desmodium triguatrum, Plectranthus striatus, Geranium collinum, Bergenia ligulata*, and *Parthinocissus himalayana* were significant diet plants recorded during the pre-monsoon season with a browse to grass ratio of 6.7. Similarly, *Myrica esculenta, Indigofera heterantha, Thalictrum foliolossum, Launea secunda, Bergenia ligulata*, and *Flemingia strobilifera* were among the most important diet plants recorded during the post-monsoon season with a browse to grass ratio of 3.54 in the overall diet of the Barking Deer. Results obtained from this study revealed that Barking Deer was predominantly a browser in both seasons.

A total of 215 plants species consumed by 364 muntjac groups were recorded at 817 feeding sites in Hainan Daitan National Nature Reserve of China within one year including 114 shrubs (53.02%), 95 herbs (44.19%) and 6 ferns (2.79%) respectively. Although no significant differences were found in seasonal food usage by muntjacs,

seasonal differences in food selection were detected. According to feeding parts, Indian muntjacs belonged to concentrate selectors that preferred tender leaves and twigs of fruits. Among six habitat types, Indian muntjac preferred thorn shrub and grassland throughout the year (Teng et al., 2004). Barking Deer were observed mostly inhabiting forest edges covered with dense bush and their diet was found composed of 40 plant species with 25 browse, five climbers, 15 grass and one herb species. Selection of foraging plants by Barking Deer in Indonesia was determined by the utilization techniques. The result revealed that deer used a type of savanna and monsoon forest for feeding activity. The botanical composition of the forage was found dominated by herbaceous dicot (Forbes), next is the woody plants and grasses. Through the study, Barking Deer was classified as browser type or concentrate selectors and found consumed 33 plant species during the rainy season and 25 in the dry season. Seventeen species of plants were recorded eaten throughout the season. Species of forbs plants showed a high selection index (preference) in both seasons. Some of them were Commelina benghalensis, Boerhavia diffusa, Desmodium triflorum, and Synedrella nodiflora, Tribulus terrestris, Streblus asper, justicia sp. (Giantra and Wahyuni, 2014). Barking Deer were seen feeding on elephant dung infested with dung beetles, which contained partially digested fruits of *Dilleniaindica* Linnaeus. The coprophagy record of Barking Deer was the first of its kind. It didn't appear to be a case of 'Pika,' a disorder characterized by a voracious appetite for non-nutritive substances, but the partially digested fruit might have attracted frugivorous deer, and the deer took advantage of feeding on partially digested fruit that would otherwise be unavailable to small grounddwelling mammals like Barking Deer. (Ranade and Prakash, 2015).

Analysis of fecal samples of Barking Deer concluded high preference of animal for trees in both summer and winter seasons. The diet was found to be 37.03% tree species followed by 22.22% of grasses and shrubs in summer and 42.10% of trees, 36.84% of shrubs, and 15.78% of grasses in winter. The Barking Deer were browsers during the winter and browser and grazer (mixed feeders) in the summer. Dominant tree species were *Phyllanthus emblica*, *Acaciamodesta*, *Grewia optiva*, *Bauhinia variegata*, *Ziziphus nummularia*, *Justicia adhatoda*, *Carissa opaca*, *Woodfordia fruticosa*, *Maytenus royleanus* and *Myrsine africana* were shrub species with a large proportion in the diet. Preferred herbal species were *Oxalis corniculata*, *Adiantum incisum*, *Micromeria biflora*and *Saussurea heteromella*. Only two grass species *Apluda mutica* and *Bothriochloa ischaemum* were recorded in large quantities from fecal samples (Habiba *et al.*, 2021).

#### 2.3 Micro-histological Analysis

This technique of analyzing diet composition from the faeces of herbivores has been a widely used method for the study of ungulate diet (Baumgartner and Martin, 1939; Kiley, 1966; Anthony and Smith, 197; Holechcek and Gross, 1982). This technique allows for practically unlimited sampling (Anthony and Smith, 1974). The basic principle of this method is that the epidermal cuticle of the plant is a non-digestive part and can be identified by comparable plant reference materials (Sparks and Malechek, 1968, Dawson and Ellis, 1979; Jnawali, 1995; Steinheim et al., 2005). This method required the collection, preservation and preparation of fecal samples and reference slides. This method is efficient for studying the diet of secretive and/or endangered species (Anthony and Smith, 1974) due to its simplicity and effectiveness (Holechek et al., 1982). So, the method adopted was followed by Sparks and Malechek (1968) and Anthony and Smith (1974), as modified by Vavra and Holechek (1980) and Jnawali (1995). The enigmatic and sensitive nature of ungulates makes it difficult to obtain direct information on their feeding ecology. As a result, pellet group analysis utilizing the micro-histological methodology has emerged as the most acceptable indirect way for investigating their food components and feeding ecology (Ilyas, 2001). In Nepal, this method has been applied in diet analysis of Rhino (Jnawali, 1995; Pradhan et al., 2008), Swamp Deer (Pokharel, 1996), Red Panda; (Panthi et al., 2012; Thapa and Basnet, 2015), Barking Deer (Nagarkoti and Thapa, 2007; Pokharel et al., 2015; Gaire, 2016), etc.

# **3. MATERIALS AND METHODS**

## 3.1 Study Area

The study area, Nagarjun forest (27° 43' 37.13" to 27° 46'22.84" N and 85° 13' 52.97" to 85° 18' 14.38" E) is inside the Shivapuri Nagarjun National Park in the northernmost border of Kathmandu valley (Figure 2). The forest is located on the western part of the national park which covers an area of 16.45 km2 at the border of the Kathmandu and Nuwakot districts. The study area extends from the base of Nagarjun forest (around 1300m a. s. l.) to its highest peak at Jamacho (2188m a. s. l.). Many spurs of the hill run in different directions forming gullies and narrow valleys. Since 1972, infrastructure development works have been carried out with the main objective of achieving effective protection against deforestation and further loss of wild fauna. A 29 km fence wall and 31 km of the motorable road up to Jamacho had been constructed. This forest is one of the important natural areas along the Kathmandu valley rim and comes under Royal protection.



Fig 2: Map of the study area (Nagarjun Forest, SNNP, Nepal) showing GPS co- ordinates of pellet groups collected.

## 3.1.1 Climate

Nagarjun forest is a typical Mahabharata hill 1392m above sea level and enjoys mostly subtropical type of climate and partly temperate climate (Chaudhary, 1998), with rainy summer and dry winter. The southern side is sunlit and appears to be far dryer than the forested northern side. As the climatic data of the Nagarjun area is not available, the other relevant climatic record from nearest meteorological stations i.e. Dhunibesi (270 43'' N and 850 11'' E, and elevation at 1085m) and Panipokhari, Maharajgunj (270 44'' N and 850 20'' E, and elevation at 1335m) were used for analysis.



Fig 3: Monthly average (5 yearly, 2015-2019) rainfall recorded at Panipokhari and Dhunibesi stations, Nepal. (Data source: DHM/GoVN)



Fig 4: Monthly average minimum and maximum temperature (5 yearly, 2015-1019) recorded at Panipokhari and Dhunibesi stations, Nepal. (Data source: DHM/GoVN).



Fig 5: Monthly average relative humidity (5 yearly, 2015-2019) recorded at Panipokhari and Dhunibesi stations, Nepal. (Data source: DHM/GoVN).

The average monthly temperatures of the area ranged from 4.52°C (January) recorded at Panipokhari to 32.62°C (July) at Dhunibesi (Fig 4). Similarly, average relative humidity in the September was maximum (86.52%) at Panipokhari and in April it was minimum (59.64%) at Dhunibesi (Fig 4). Likewise rainfall in July was maximum (439.16 mm) and minimum (0.32mm) at Dhunibesi station (Fig 3). November, December and January were the coldest months while June, July and August were the hottest months. August and September were the most humid months, and July and August were the most precipitous months.

#### **3.1.2 Biodiversity**

Biodiversity abounds in the study area. This could be due to the forest receiving the most secure protection from security forces of any protected area in Kathmandu.

## 3.1.2.1 Vegetation

Forests in Nagarjun can be categorized into four types along with a few small patches of grassy meadows: *Schima wallichii* Forest, Pine Forest, Mixed Broadleaved and Dry Oak Forest (Kanai and Shakya, 1970). Among the four types of forests recognized in Nagarjun hill, the *Schima wallichii* forest represented nearly 2/3rd of the overall forest cover. Out of the four types of forests identified, *Schima wallichii*, mixed broadleaved forest, pine forest, and dry oak forest coverage on Nagarjun hill was found to be 61.29%, 27.91%, 9.08%, and 1.72%, respectively, according to GIS analysis (Nagarkoti and Thapa, 2007). *Schima wallichii* forestis mostly associated with other tree species like *Castanopsis* 

*indica, C. tribuloides, Juglans regia, Quercus spicata, Pyrus pashia*,etc and undergrown shrub species like *Sarcococca coriacea*, *Smilax aspera*, *Arundinaria falcata*, *Myrsine semiserrata*,etc at different altitudinal regions of the hill. *Pinus roxburghii* forest dominates the southern slopes of the hill, mostly at lower elevations with small trees of *Myrica esculenta* and *Schima wallichii* forming the second stratum along with most prevalent shrub species like *Sarcococcacoriacea*, *Berberisasiatica*, *Myrsinesemiserrata*, *Colebrookeaoppositifolia*, *Glochidionvelutinum*,*Rubus ellipticus* etc. *Phoebe lanceolata*, *Machilus duthiei*,*Acer oblongum*, *Quercus glauca*, *Camellia kissi*, *Lindera pulcherrima*, *Sarcococca hookeriana etc* and other species make up the mixed broadleaved forest that grows on the ridge's concave northern slopes. The dry oak forest dominated by *Quercus lanuginosa* has very few *Lyonia ovalifolia* and *Rhododendron arboreum* plants. *Berberis asiatica*, *Caryopteris grata*, *Desmodium floribundum*, *Gaultheria fragrantissima*, *Rubus ellipticus* etc. are the common shrubs in this forest type usually grow at edges and in open places.

## 3.1.2.2 Fauna

The fauna of Nagarjun Forest has been described by HMG/ADB/FINNIDA (1988), Shrestha (1997), Malla (2000) and others. The major mammalian fauna present in Nagarjun Forest of SNNP includes Bat species Hipposideros armiger, Megaderma lyra, Miniopteros schreibersii, Rhinolophus affinis, Rhinolophus pusillus) (Malla, 2000), Hoary bellied squirrel (Calloscirus pygerythrush lokroides), Wild Boar (Sus scrofa), Rhesus Monkey (Macaca mulatta), Spotted leopard (Panthera pardus), Clouded leopard (Neofelis nebulosa), leopard cat (Prionailurus bengalensis), pangolin (Manis sp.), Barking Deer (Muntiacus vaginalis), , Sloth Bear (Melursus ursinus) Himalayan yellow throated Marten (Martesflavigula), etc. (HMG/ADB/FINNIDA, 1988). The important birds of forest are Bonelli's Eagle (Hieraaetus fasciatus), Red-Billed Blue Magpie (Urocissa erythrorhyncha), Spotted Babbler (Spelaeornis caudatus), Large Yellow-naped Woodpecker(Picus flavinucha), Eurasian Kingfisher(Alcedo atthis), Golden-throated Barbet(Megalaima franklinii) etc. (Shrestha, 1997). Importantly, the Spiny Babbler (Turdoides nipalensis), Wren Babbler Pnoepygapusilla are the endemic birds found in Shivapuri Nagarjun National Park. Ophiophagus Hannah, Naja kaouthia, Bufo melanostictus, Amphiesma syhadrensis, Asymble pharus platyceps, Limnonectes sikimmensis etc are some noticeable herpetofaunal species of the area (Pokharel et al., 2011).

# 3.1.3 Boundary and Access

The Nagarjun Forest is bordered by concrete walls and barbed wire fences in almost all directions. The forest has entry points at 7 different places- Raniban, AinDanda, Mudkhu, Baikhu, Ichangu, Batase and Badri danda. The forest is accessible by road. Even inside the forest, there is a gravel road to go to different places within the forest.

# **3.2 Materials**

The scientific instruments and chemicals used during the field survey and laboratory work are as follows

- GPS(Garmin eTrex 10)
- Topographic map of the area
- Measuring tape
- Compound Microscope (Proway)
- Digital camera for microscope (DCM510; USB2.0; 5M pixel, CMOS chip)
- Sodium Hypochlorite (4%)
- Staining Chemical: Methylene blue (liquid)

# 3.3 Methods

# **3.3.1 Preliminary Survey**

A preliminary field survey was conducted during September 2019 in Nagarjun Forest by general observations using pre-existing trails to gather some information about the study area and potential habitats of Barking Deer to choose the most suitable sampling technique according to topographical conditions of the area. Discussions were made with the forest ranger, security forces, and local villagers to identify the most probable sights with a high possibility of Barking Deer to be seen so that samples could be collected in enough amounts for further studies.

# 3.3.2 Field Survey

The field survey was carried out in November 2019 by the following methods to collect data on individuals for further laboratory procedure and analysis.

### **3.3.2.1 Sampling Design**

Five random sites located at different altitudes of Nagarjun Forest were selected as study sites. In selecting sampling sites, those areas that were most representative of the vegetation and were known as potential Barking Deer home ranges as in the previous study (Nagarkoti and Thapa, 2007) were chosen. In each site, 1<sup>st</sup> sampling point was chosen randomly and line transects each of different lengths was laid maintaining an interval of 100m with the consecutive transect in North-South and East-West direction depending upon the topography and site accessibility. Also for the ease of walking, pre-existing trails were used as transects in areas where straight-line transects were not feasible to conduct. Pellets were collected at the interval of 100m distance along transects by plotting  $10m \times 10m$  quadrats.

The availability of forage plants in the sampling site was assessed within the plots along the line transects established for collecting pellet groups.Quadrats of  $10m \times 10m$  were assigned to tree saplings and seedlings. Within a quadrat,  $4m \times 4m$  quadrats were allocated randomly in the corner for shrub species. Likewise, grasses, herbs, and plants including ferns and forbs were recorded from nested sampling of  $1m \times 1m$  quadrat within the  $4m \times 4m$  quadrat (Schemnitz, 1980). Considering the average shoulder height of adult Barking Deer (50-75 cm), (Shrestha, 1997), plant species up to 1.2m height were collected and profile recorded with different parameters. However, fruiting species were recorded without any height limitations. In each quadrat total number of individual tree and shrub species were counted and the frequency of each plant species within  $1m \times 1m$ quadrat was recorded. Altogether 50 quadrats were plotted within all sampling locations and 50 samples of pellet groups were collected for further laboratory analysis.

## **3.3.2.2 Pellet Identification and Collection**

Fecal pellets of Barking Deer were identified as typical comma shaped (Dinerstein, 1980; Pokharel *et al.*, 2015) which are much smaller than that of spotted and Sambar Deer. Fresh and old (without fungus) both the pellet samples were collected, packed individually in the zip-lock plastic bags. Each plastic bag was labelled with a numbering and GPS point. A total of 50 pellet samples were collected in November. The samples were then sun dried to remove moisture content and fungal infections and brought to the laboratory of the Central Department of Zoology, Tribhuvan University for further analysis.

#### **3.3.2.3 Reference Plant Collection**

Sixty-two different plant species including their different parts (leaves, twigs, fruits, flowers, and bark) found within the Barking Deer habitat were collected during the field survey for the preparation of reference slides. All the collected plant materials were preserved in the herbarium and brought to the Central Department of Zoology. Reference plant materials were identified in the Central Department of Botany, TU Kirtipur and National Herbarium and Plant Laboratories, Lalitpur. Additionally, other plant micro-histological photographs were studied as reference keys available on M.sc thesis (Nagarkoti and Thapa, 2007) and some provided by the seniors Raju Gaire and Kiran Thapa Magar of CDZ and Ume Habiba of PMAS Arid Agriculture University, Rawalpindi.

#### 3.3.3. Micro-histological Analysis

Micro-histological analysis has become the most widely used method to evaluate the botanical diet composition of fecal matter of wild herbivores. Micro-histological techniques of identifying diet constituents have been first described by Baumgartner and Martin (1939). The technique was later verified by Sparks and Malechek (1968). A review done by Holechek *et al.*, (1982) on botanical composition determination of range herbivore diets found this method to be widely applicable because fecal analysis gives greater sampling precision and does not require animal sacrifice. Micro-histological techniques can be aided by the use of software developed to help the management of reference collections to determine herbivore diet (Degano *et al.*, 1998). This software is useful in the redetermination of the epidermis and comparisons of the similar epidermis.

#### **3.3.3.1 Slide Preparation**

The samples of reference plants identified were air dried in the oven at 60 °C in the laboratory of CDZ, Tribhuvan University. Both the dried samples (plants and fecal pellets) were separately ground in the electric blender into tiny fragments. The fragments were sieved through sieve mesh size 1mm to 0.3 mm to ensure homogeneity in the size of the fragments. The fragments remaining on the 0.3 mm sieve was chosen as slide preparation of both samples.

The method introduced by Norbury (1988) was adopted for the preparation of clearer slides because of its effectiveness and being less time consuming (Singh 2015; Kunwar *et al.*, 2016). In this method, 0.5 gm from each sample was placed in Petri dishes and bleached with 50 ml of 4% Sodium hypochlorite for 6-12 hours at room temperature to remove mesophyll tissue and to render the epidermis identifiable. The bleached fragments were then rinsed under running tap water thoroughly in a sieve and treated with a few drops of staining substance, methylene blue solution for 5 sec and again rinsed with water. The stained fragments were mounted on standard microscope slides in a glycerin medium with cover slip of 22 X 50 mm. Both reference slides and sample slides were observed in a compound microscope at magnifications 4X, 10X and 100X and each fragments were photographed using a digital camera for microscope (DCM510; USB2.0; 5M pixel, CMOSDCM chip) in a laptop using software- Scope Tek Scope Photo; Version: x84, 3.1.615 (http://www.scopetek.com)

#### 3.3.3.2 Slide Interpretation

The key features of the epidermis such as shape, size and arrangement of epidermal cells, vascular vessels, hair and trichome, crystal, shape and size of stomata and inter-stomatal cells, fibre structure and arrangement of veins, etc. of the fragments of the reference plants were first photographed through a 10x 40x and 100x microscope. Then for each fecal sample, non-overlapped and distinguishable 30 random fragments were stained and observed while moving the slides from one end to another end under the microscope and identified by comparing the key features of reference plants.

## 3.4 Data Analysis

All the collected information was categorized and tabulated according to the objective of the study to examine the diet composition, determine dietary niche breadth and assess the diet preference of barking deer. Data were manually processed and analyzed in a descriptive way as well as by statistical measure.

## 3.4.1 Diet Composition

The plant fragments identified from the micro-histological analysis of the pellet samples were assigned into one of the following four levels of classification with different categories under each classification: (1) Functional Category: (i) Browse, (ii) Grasses, (iii) Fruits and (iv) Others; (2) Broad Category: (i) Monocots and (ii) Dicots; (3) Family;

and (4) Species. The purpose of this categorization was to determine the relative contribution of different plant taxa to the diet of Barking Deer in each classification. Diet composition was expressed as the percentage occurrence (O %) of species (Cavallini and Lovari, 1991).

Percentage of Occurrence (O %) =  $\frac{\text{Total number of fragments identified for each species}}{\text{Grand total of fragments count made from all samples}} \times 100\%$ 

A chi-square goodness-of-fit test was carried out to determine whether Barking Deer consumes all potential forage plants in uniform proportion. The test was performed by setting the hypothesis that the Barking Deer would feed on all plant species, family and functional categories (browse, grass, fruits, others) uniformly. The hypothesis was tested at a 5% level of significance. All tests were performed using MS excel 2016 and Graph Pad software Quickcalcs online calculator (https://www.graphpad.com/quickcalcs/).

Under H<sub>0</sub>, the test statistic is given by:

$$\mathbf{x}^2 = \sum \frac{(\mathbf{0} - \mathbf{E})^2}{\mathbf{E}} (\mathbf{n} - \mathbf{1}) \, \mathrm{d}\mathbf{f}$$

Where, O = Observed frequency

E = Expected frequency

#### **3.4.2** Niche Breadth

To determine the degree of selectivity of the plant species consumed in the diet, Levin's measure of Niche Breadth (Levins, 1968) described by Krebs (1999) was used which measures how uniformly resources are being utilized. The equation is,

$$\mathbf{B} = \frac{1}{\Sigma P_i^2}$$

Where B = Levin's Measure of Niche Breadth

 $P_i$  = proportion of diet contributed by plant species i (i =1,2,3,...,n)

Hulbert (1978) suggests the following measure for standardized niche breadth:

$$\mathbf{B}_{s} = \frac{\mathbf{B}-1}{\mathbf{n}-1}$$

Where,

 $B_s$  = Levin's standardized niche breadth

n = number of possible resource states (Total no. of plant species in all fecal samples) A high value of  $B_s$  indicates that the animal is a generalized feeder and a low value indicates that the animal is a selective or specialized feeder.

#### 3.4.3 Browse to Grass Ratio

To evaluate whether the deer is a browser or grazer browse to grass ratio was calculated.

Browse to grass ratio  $(\mathbf{B/G}) = = \frac{\Sigma(\mathbf{B}_{\mathbf{p}})}{\Sigma(\mathbf{G}_{\mathbf{p}})}$  Where,

Bp =Percent occurrence of all browse plant species in the diet Gp =Percent occurrence of all grass plant species in the diet.

#### 3.4.4 Availability, Use and Preference of Food Plants

For the determination of the food preference of Barking Deer, The availability of each plant species recorded within the field was converted in terms of percentage. Then was compared with its percentage of use in diet. The average availability percentage of each tree and shrub species throughout the field was calculated by dividing the total number of each species from that of the total number of all individuals sampled for all species multiplied by 100 whereas, for plants recorded within 1m×1m quadrat (grass, herb, forb and ferns), percent availability was calculated based on their Relative Frequency. The forage species recorded from sampling plots but found absent in pellet samples were excluded from availability calculations. The percentage of used forage plants in the diet was calculated by dividing the number of plant fragments identified for each species by the total number of identified plant fragments of all species excluding unidentified plant fragments. The average percentage availability of forage plants in deer habitat was calculated separately for Tree, shrub, grasses and plants in category others according to their respective quadrat size used during sampling.

Frequency = Number of quadrats of species occurence Total number of quadrats studied

Relative Frequency =  $\frac{\text{Frequency of individual species}}{\text{Sum of frequency of all the species}} \times 100$
Food preference of Barking Deer was determined by calculating Ivlev's Electivity Index (IEI) (Ivlev, 1961) described by (Krebs, 1989) using the following equation:

$$IEI = \frac{U_{i\%-A_i}\%}{U_i\%+A_{i\%}}$$

Where, A<sub>i</sub>% = percentage of forage species i along with all the sampled quadrats (i.e., its availability in the habitat)
U<sub>i</sub>% = percentage of forage species in the Barking Deer diet.

The IEI values range from -1 up to 1, with preference level categories are as follows: Strongly preferred (0.5 to 1), preferred (0.1 to 0.49), proportional (0.09 to -0.09), least preferred (-0.1 to -0.49) and avoided (-0.5 to -1).

# 3.4.5 Relative Importance Value

The Relative Importance Value (RIV) of each plant species observed in the fecal sample was calculated using the formula described by Jnawali (1995) and Thapa and Basnet (2015).

# $RIV_x = D_x \sqrt{F_x}$

Where, RIV = Relative Importance Value for species X

 $D_x$  = Mean Percentage of species X in fecal sample  $F_x$  = Frequency of species in fecal sample

# **4. RESULTS**

A total of 1500 plant fragments (30 non-overlapping fragments from each pellet group sample) prepared from 50 groups of pellet samples were evaluated for the dietary analysis of Barking Deer.

# 4.1 Diet Composition

A total of 1358 fragments of 26 plant species (browse = 18; 11 trees and 7 shrubs, grass = 4, fruits = 2 and others = 2) belonging to 20 different families were identified in the diet of Barking Deer (Table 1). Between the category level (including unidentified) the browse species dominated the fecal material with >50%, followed by fruits and grasses; the proportion of species belonging to category others were very low, While <10% (142 fragments) plants present in the diet was found unknown to be categorized as unidentified (Fig 6).



Figure 6: Percentage occurrence of functional plant categories identified in the pellets of Barking Deer in Nagarjun Forest, SNNP, Nepal

Regarding individual diet species, Fruits of *choerospondias axillaris* alone constituted by far the largest proportion of the overall diet. Similarly, Presence of the other key dietary items in fecal samples were recorded in the following order: *Smilax aspera* > *Litsea chartacea* > *Schima wallichii*>*Prunus cerasoides*>*Castanopsis indica*> *Rubus ellipticus* > *Imperata cylindrica* >*Smilax lanceifolia* > *Carex* sp. >*Myrica esculenta*. Whereas, Contribution of *Fraxinusfloribunda*, *Ziziphusincurva*, *Pogonatherumpaniceum* and *Berberisasiatica* were recorded between 2-3%. The rest of the plant species were consumed in trace amounts (Table 1).

Table 1: Percentage composition of various plant categories (F.C = Functional category; B.C = Broad category, family and species) identified in pellets of Barking Deer in Nagarjun Forest, SNNP, Nepal. F = fragments of plants in pellet group, O% = Percentage of occurrence, T = Trees, S = Shrubs, \* = herb, # = fern.

FC	BC	Family	Species	Parts used	F	0%
Browse	Dicot	Betulaceae	Betula utilis (T)	Leaf	35	2.33
		Ericaceae	Rhododendron arboretum (T)	Leaf	28	1.87
		Fagaceae	Castanopsis indica (T)	Bark	71	4.73
		Lauraceae	Litsea chartacea (T)	Leaf	118	7.87
		Oleaceae	Fraxinus floribunda (T)	Leaf	34	2.26
		Rhamnaceae	Ziziphus incurve (T)	Leaf	32	2.13
		Rosaceae	Prunus cerasoides (T)	Leaf	77	5.13
			Rubus ellipticus (S)	Leaf	62	4.13
		Myricaceae	Myrica esculenta (T)	Leaf	45	3.0
		Theaceae	Schima wallichii (T)	Leaf, bark	81	5.4
		Acanthaceae	Justicia adhatoda (S)	Leaf	14	0.93
		Berberidaceae	Berberis asiatica (S)	Leaf	34	2.27
		Clusiaceae	Hypericum cordifolium (S)	Leaf	12	0.8
		Lamiaceae	Colebrookea oppositifolia (S)	Leaf	25	1.67
	Monocot	Smilacaceae	Smilax aspera (S)	Leaf, stem	155	10.33
			Smilax lanceifolia (S)	Leaf, stem	54	3.6
		Gramineae	Dendrocalamus sp. (T)	Leaf	12	0.8
	Gymnosperm	Pinaceae	Pinus roxburghii (T)	Leaf, bark	28	1.87
Total					917	61.13
Grass	Monocot	Gramineae	Imperata cylindrica	Leaf	62	4.13
			Pogonatherum paniceum	Leaf	38	2.53
			Saccharum spontaneum	Leaf	15	1.0
		Cyperaceae	Carex sp.	Leaf	48	3.2
		Т	otal		163	10.87
Fruits	Dicot	Anacardiaceae	Choerospondias axillaris (T)	Exocarp	238	15.86
		Fagaceae	Lithocarpus elegans (T)	pericarp	12	0.8
Others	Dicot	Asteraceae	*Ageratum conyzoides	Leaf	21	1.4
		Dennstaedtiaceae	#Pteridium aquilinum	Leaf	7	0.47
Uniden tified					142	9.47
Total					1500	100

Barking Deer appeared not to feed all plant species uniformly at the species level ( $\chi^2 = 1257.26$ , df = 25, p<.0001), family level ( $\chi^2 = 1220.23$ , df = 19, p<.0001) and functional category level ( $\chi^2 = 598.21$ , df = 4, p<.0001).

The Barking Deer consumed 20 plant families in total, with Anacardiacea (238 fragments) having the highest proportion, followed by a few other notable families; Smilacaceae (209 fragments), Rosaceae (139 fragments), Gramineae (127 fragments), Lauraceae (118 fragments), Fagaceae (83 fragments), Theaceae (81 fragments), Cyperaceae (48 fragments), and Myricacea (45 fragments).Families such as Acanthaceae, Asteraceae, Pinaceae, Ericaceae, Clusiaceae, Lamiaceae, and Dennstaedtiaceae were present in small numbers. (Fig 7)



Fig 7: Percentage occurrence of different plant families in the diet of Barking Deer in Nagarjun Forest, SNNP, Nepal.

# 4.2 Niche Breadth

Standardized Levin's Measure of Niche Breadth ( $B_s$ ) of the food plants consumed by Barking Deer in Nagarjun Forest of SNNP, Nepal was found to be 0.512 (Table 2).

S.N	Diet plant species	Pi	S.N	Diet plant species	Pi
1.	Betula utilis	0.0233	15.	Colebrookea oppositifolia	0.0167
2.	Rhododendron arboreum	0.0187	16.	Rubus ellipticus	0.0413
3.	Castanopsis indica	0.0473	17.	Smilax aspera	0.1033
4.	Litsea chartacea	0.0787	18.	Smilax lanceifolia	0.036
5.	Fraxinus floribunda	0.026	19.	Imperata cylindrica	0.0413
6.	Ziziphus incurva	0.0213	20.	Pogonatherum paniceum	0.0253
7.	Prunus cerasoides	0.0513	21	Saccharum spontaneum	0.01
8.	Myrica esculenta	0.03	22.	Carex sp.	0.032
9.	Schima wallichii	0.054	23.	Choerospondias axillaris	0.1586
10.	Dendrocalamus sp.	0.008	24.	Lithocarpus elegans	0.008
11.	Pinus roxburghii	0.0187	25.	Ageratum conyzoides	0.014
12.	Justicia adhatoda	0.0093	26.	Pteridium aquilinum	0.0047
13.	Berberis asiatica	0.0227	27.	Unidentified	0.0947
14.	Hypericum cordifolium	0.008			
n = 27				$B_s=0.512$	

Table 2: Proportion of diet contributed by plant species  $(P_i)$  and Niche Breadth  $(B_S)$  of plant species identified in fecal sample of diet of Barking Deer in Nagarjun Forest, SNNP, Nepal.

# 4.3 Browse to Grass Ratio

The study identified 917 fragments of browse (trees and shrubs) and 163 fragments of grass. The browse to graze ratio was found to be 5.62 in the present study.

# 4.4 Diet Preference

Barking Deer fecal samples revealed a strong preference for browse species, with most parts of trees and shrubs being consumed. Of the 26 plant species consumed, four were strongly preferred (*Choerospondias axillaris*, *Litsea chartacea*, *Prunus cerasoides*, and *Smilax aspera*), six were preferred (*Rubus ellipticus*, *Schima wallichii*, *Smilax lanceifolia*, *Carex* sp., and *Pogonatherum paniceum*), three were used in proportion to availability (*Castanopsis indica*, *Myrica esculenta* and *Berberis asiatica*), five were least preferred (*Fraxinus floribunda*, *colebrookia oppositifolia*, *ziziphus incurva*, *Saccharum spontaneum* and *Ageratum conyzoides*) and seven were avoided (*Rhododendron arboretum*, *Pinus roxburghii*, *Lithocarpus elegans*, *Dendrocalamus* sp., *Hypericum cordifolium*, *Justicia adhatoda* and *Pteridium aquilinum*) based on their preferences indices values (IEI value). However browse species *Betula utilis*did not occur in vegetation sampling but was recorded in the diet (Fig 8).

Table 3: Tree species preference by Barking Deer in Nagarjun Forest, SNNP, Nepal (A% = percentage availability, U% = percentage of used plant species, IEI= Ivlev's Electivity Index, PC = preference category; SP=strongly Preferred, P = Preferred, Pr = Preferred in proportional to availability, LP = least preferred, Av = avoided)

S.N	Species	A%	U%	IEI value	PC
1.	Betula utilis	_	4.32	_	_
2.	Rhododendron arboreum	13.23	3.45	-0.58	Av
3.	Castanopsis indica	7.6	8.75	0.07	Pr
4.	Litsea chartacea	4.72	14.55	0.51	SP
5.	Fraxinus floribunda	9.84	4.19	-0.4	LP
6.	Ziziphus incurva	6.56	3.95	-0.24	LP
7.	Prunus cerasoides	2.87	9.49	0.5	SP
8.	Myrica esculenta	4.92	5.54	0.05	Pr
9.	Schima wallichii	7.6	9.9	0.1	Р
10.	Dendrocalamus sp.	5.74	1.47	-0.5	Av
11.	Pinus roxburghii	20.31	3.45	-0.7	Av
12.	Choerospondias axillaris	9.54	29.35	0.5	SP
13.	Lithocarpus elegans	7.07	1.47,	-0.6	Av
		100	100		

Table 4: Shrub species preference by Barking Deer in Nagarjun Forest, SNNP, Nepal ( A% = percentage availability, U% = percentage of used plant species, IEI = Ivlev's Electivity Index, PC = preference category; SP = strongly Preferred, P = Preferred, Pr = Preferred in proportional to availability, LP = least preferred, Av = avoided).

S.N	Species	A%	U%	IEI value	РС
1.	Justicia adhatoda	15.77	3.93	-0.6	Av
2.	Berberis asiatica	8.72	9.55	0.04	Pr
3.	Hypericum cordifolium	16.28	3.37	-0.65	Av
4.	Colebrookea oppositifolia	17.28	7.02	-0.42	LP
5.	Rubus ellipticus	13.92	17.42	0.11	Р
6.	Smilax aspera	14.09	43.53	0.51	SP
7.	Smilax lanceifolia	12.24	15.17	0.1	Р
		100	100		

Table 5:  $1m \times 1m$  quadrat species (Grasses and others) preference by Barking Deer in Nagarjun Forest, SNNP, Kathmandu (A% = percentage availability, F = number of plant fragments in pellet, U% = percentage of used plant species, IEI = Ivlev's Electivity Index, .PC = preference category; SP = strongly Preferred, P = Preferred, Pr = Preferred in proportional to availability, LP = least preferred, Av = avoided, \*= herb, #= fern)

S.N	Species	Frequency (%)	Relative frequency% (A %)	F	U%	IEI value	PC
1	Imperata cylindrica	32	15.23	62	32.46	0.36	Р
2.	Pogonatherum paniceum	28	13.33	38	19.8	0.19	Р
3.	Saccharum spontaneum	36	17.14	15	7.85	-0.37	LP
4.	Carex sp.	40	19.05	48	25.13	0.13	Р
5.	*Ageratum conyzoides	30	14.28	21	10.9	-0.13	LP
6.	#Pteridium aquilinum	44	20.95	07	3.66	-0.7	Av
		TF=210	100	191	100		



Fig 8: Ivlev's Electivity Index (IEI) values for various dietary items of Barking Deer in Nagarjun Forest, SNNP, Nepal

# 4.5 Relative Importance Value of Species

Among the different consumed food plants, *Choerospondias axillaris* was found to have highest RIV (Relative Importance Value) (244.68), followed by *smilax aspera* (128.61), *Litsea chartacea* (85.49), *Schima wallichii* (48.6), *Prunus cerasoides* (45.02), *Castanopsis indica* (39.86), *Imperata cylindrica* (32.52), *Rubus ellipticus* (32.52), *smilax lanceifolia* (26.45) etc. while *pteridium aquilinum*, *Lithocarpus elegans*, *Hypericum cordifolium*, *Dendrocalamus sp.*, *Saccharum spontaneum* etc were the species which had comparatively low RIV value in the list of consumed food plants (Table 6). These RIV of various plant species may show the relative preference of the animal for its diet supplement.

S.N	Plant species in diet	Fx	<b>D</b> <sub>x</sub> (%)	RIV
1.	Betula utilis	35	2.33	13.78
2.	Rhododendron arboretum	28	1.87	9.9
3.	Castanopsis indica	71	4.73	39.86
4.	Litsea chartacea	118	7.87	85.49
5.	Fraxinus floribunda	34	2.26	13.18
6.	Ziziphus incurva	32	2.13	12.05
7.	Prunus cerasoides	77	5.13	45.02
8.	Myrica esculenta	45	3.0	20.12
9.	Schima wallichii	81	5.4	48.6
10.	Dendrocalamus sp.	12	0.8	2.77
11.	Pinus roxburghii	28	1.87	9.9
12.	Justicia adhatoda	14	0.93	3.48
13.	Berberis asiatica	34	2.27	13.24
14.	Hypericum cordifolium	12	0.8	2.77
15.	Colebrookea oppositifolia	25	1.67	8.35
16.	Rubus ellipticus	62	4.13	32.52
17.	Smilax aspera	155	10.33	128.61
18.	Smilax lanceifolia	54	3.6	26.45
19.	Imperata cylindrical	62	4.13	32.52
20.	Pogonatherum paniceum	38	2.53	15.59
21.	Saccharum spontaneum	15	1.0	3.87
22.	Carex sp.	48	3.2	22.17
23.	Choerospondias axillaris	238	15.86	244.68
24.	Lithocarpus elegans	12	0.8	2.77
25.	Ageratum conyzoides	21	1.4	6.42
26.	Pteridium aquilinum	7	0.47	1.24
27.	Unidentified	142	9.47	

Table 6: Relative Importance Value (RIV) of different plant species identified in fecal samples of barking deer in Nagarjun Forest, Kathmandu. D = Mean percent of species in sample, F= Frequency of fragments of species in sample

#### **5. DISCUSSION**

#### **5.1 Diet Composition**

Data from the present study reveals that during winter, the Barking Deer in the Nagarjun forest of SNNP adopt a browsing strategy to feed extensively on tree and shrub layers, utilizing a diverse range of plant species available in the habitat rather than grazing.

Smilax aspera, Litsea chartacea, Schima wallichii, Castanopsis indica, Prunus cerasoides, Smilxa lanceifolia, Rubus elipticus etc were the most frequently consumed browse plants which constituted around two-third of the overall Barking Deer diet. Imperata cylindrica (Yonzon, 1978; Shrestha 1984; Farida et al., 2006; Nagarkoti and Thapa, 2007), Rubus ellipticus, Prunus sp., Myrica esculenta (Ilyas and khan, 2004; Nagarkoti and Thapa, 2007) and, Justicia adhatoda (Habiba et al., 2021) were among the common food plants the animals were found to consume in the various ecological regions. One species, Betula utilis, present in the Barking Deer diet was found absent throughout the field sampling. Its absence from sampling could be due to its low abundance or is confined to specialized areas that were perhaps under-sampled. Schima wallichii. Castanopsisindica, Berberisasiatica, Imperatacylindrica, Rhododendronarboreum, Pogonetherumpaniceum, and Colebrookiaoppositifolia were common food plants recorded in a previous study (Nagarkoti and Thapa, 2007) in the rainy and spring seasons in the same study area. However, the proportion of most browse plants was significantly higher in the current study. Since the pellet samples analyzed were from the winter season, browse species are likely to have contributed in higher quantity because browse provides a major proportion of nutrients, especially proteins, during critical times of the season when grasses become low in nutritional value & digestibility and with ample fiber content (Schaller, 1998; Wagner and Peek, 2006).

The results of this study, which show a higher contribution of browse in the diet, are consistent with those of Barrette (1977) in Srilanka, Lekagul and McNeely (1977) in Thailand, Khan and Ilyas (2003) in India, Teng *et al.*, (2004) in Hainan Island, China and Pokharel *et al.*, (2015) and Gaire (2016) in Nepal. However, it shows contradiction with the results concluded by Yonzon (1978), Nagarkoti and Thapa (2007), I Giantra and Walyuni (2014) in Indonesia and Habiba *et al.*, (2021) in Pakistan. Yonzon (1978) in Chitwan National Park observed Barking Deer mostly grazing over palatable shoots of *Imperata cylindrica* grown after burning. Similarly, Nagarkoti and Thapa (2007) in Nepal

and Habiba *et al.*, (2021) in Pakistan categorized Barking Deer as a mixed feeder (browser and grazer both) based on their studies in two different seasons. Giantra and Walyuni (2014) in Indonesia reported forb domination in the Barking Deer forage. These studies clearly show that Barking Deer has great flexibility in feeding habits. Such a distinct difference in feeding nature and forage choices could be due to the wide distribution of Barking Deer in different ecological regions with significant variation in floral compositions which is linked with associated ecological conditions such as elevation, drainage, precipitation, sunlight and other factors and partly due to lack of data on seasonal diet composition of Barking Deer in the present study.

In the present study, the consumption of grasses were found to be comparatively much less than that of browse species. However, the previous study by Nagarkoti and Thapa (2007) in Nagarjun Forest, SNNP, had shown that the Barking Deer had the maximum proportion of grass in the spring season (55.36% of grass and 38.5% of browse) and a more or less equal proportion of grass and browse in the rainy season (42.62% of grass and 48.44% of browse). Because during the monsoon season, grass availability is high, the ungulates behave more like pure grazers because they can find palatable grass everywhere, but they behave more like browsers in winter, a season of resource scarcity (Pradhan et al., 2008). This discrepancy in findings may be attributed to the low availability and quality declination of graze species during winters. Soil moisture plays an important role in the abundance of new growth of grasses and sedges which starts to reduce as the cool dry season progresses (Dinerstein, 1979b). Barking Deer differs from other ruminants in that it doesn't like grasses in the vegetative phase but prefers more nutritious shoots and young buds of tall, coarse grasses that emerge during rain or after the forest fire (Yonzon, 1978; Dinerstein, 1979; Semiadi, 1996). Also, the willingness to consume young buds in burnt fields is linked with the effort of the Barking Deer and other Cervidae species to meet their mineral requirements and is especially so for male animals growing velvet (Semiadi, 1996). Hence conclusions can be made that food choices in Barking Deer might be affected by the nutritional requirements, the need to decrease fiber intake, and maximization of protein intake to increase digestibility.

The fruits of *Choerospondias axillaris* were found to be consumed in significantly greater amounts (15.85%) than any other plant species in the overall diet. Being protein and calcium-rich, the fruit of *C. axillaris* offers a good nutritional reward to the frugivorous

animals (Chen *et al.*, 2001). This nutrition enrichment property triggers the deer to feed on a large amount of easy to reach fallen fruits which can be understood as the smartest attempt of the ungulate to fulfill energy requirements for their small body weight with less effort within a short period (Barrette, 1978). Dinerstein (1979b) hypothesized that the small rumen to body size ratio of the Barking Deer and higher nutritional requirement restricts the animal to the forested habitat where fruits, leaves, flowers and buds are more abundant. Herb representation in the Barking Deer diet was very less (1.4%) while flower fragments were found completely absent in fecal samples. Herbs being fugacious and appear for a shorter duration, have limited availability and hence low in consumption. Another reason for the low presence of herb and no sign of flowers consumed in deer diet is perhaps due to their high digestibility. They have softer tissues, hence expected to face higher digestion and lower representation as identifiable fragments in fecal samples. In this study, no evidence of animal remains recorded in the diet (Kurt, 1981; Humas, 2004).

The proportion of plant fragments that remained unidentified was found to be 9.47% in this study. These were perhaps the plant species recorded as available that couldn't be identified due to poor fragment structure recognition during sample preparation. Thus the large percentage of unidentified food parts in the pellets could be because of the degradation of plant particles due to the high degree of mastication and efficient digestive system of the deer (Korschgen, 1971). Jnawali (1995) also reported that fecal analysis does not incorporate all species in herbivore's diet. Fecal analysis methodology assumes that fragments of nearly every ingested plant species and plant parts within species are recoverable and identifiable in fecal samples (Storr, 1961) and the recovery or identification rates of plant fragments are consistently proportional to ingestion rates of plant parts or that digestion correlation factors can be developed to account for the differential digestion biases (Dearden et al., 1975, Fitzgerald and Waddington 1979). Herbaceous species, such as forbs are likely to be digested more than woody species (Mangold, 1997) resulting in biases towards the browse (tree, shrub) category (Anthony and Smith, 1974). While in some cases grasses are overestimated because of the greater resistance of grasses to digestion than Forbes (Havstad and Donart, 1978; Vavra et al., 1978; Vavra and Holechek, 1980; Bartolome et al., 1995). Other factors that may cause biases include the presence of woody materials (Holechek and Valdez, 1985a, 1985b), observer error and training (Holechek and Gross, 1982a; Alipayo et al., 1992), calculation procedure for analysis (Holechek and Gross, 1982b) and sample size (Anthony and Smith, 1974).

#### 5.2 Niche Breadth

In the present study, the niche breadth of Barking Deer was calculated 0.512 which indicates the generalist feeder strategy of the animal consuming a wide variety of plant species as niche breadth ranges between 0-1 according to Krebs (1999). Also, the specialists have traditionally been defined as animals consuming at least 60% of their diet from one plant genus (Dearing et al., 2000). In Nagarjun Forest, SNNP, Barking deer consumed a more diverse diet in which no single genus comprises >60% of its diet. Such a greater diversity of diet species may be according to the foraging abundance hypothesis (Weckerly and Kennedy, 1992). When the supply of nutritious food reduces, foraging theory suggests broadening one's diet breadth (Pianka, 2000). Such strategies enable herbivores to utilize a variety of dietary food plants to get the optimum supply of various key nutrients and detoxify enormous amounts of chemically similar PSMs with this method. The Nutrients Constraint Hypothesis asserts that no single plant species can meet all of the herbivores' nutrient needs (Westoby, 1978) and the Detoxification limitation hypothesis contends that mammalian herbivores are unable to reduce equivalent PSMs detoxification burdens (Freeland & Janzen, 1974). Adopting a generalist feeding strategy is an alternative to circumvent these restrictions (Wiggins et al., 2006).

#### **5.3 Diet Preference**

In the current study, a total of 13 plant species (nine browse, one fruit and three types of grasses) were recorded as preferred by the animal out of identified 26 plant species from the diet. Fruits of *choerospondias axillaris*, browse species *Litsea chartacea*, *Prunuscerasoides*, *Smilax aspera*, *Smilaxlanceifolia*, *Schimawallichi*, *Rubus elipticus* and grass *Imperata cylindrica*, *Pogonetherum paniceum*, *Carex sp.* were the preferred forage plants followed by *Castanopsis indica*, *Myrica esculanta* and *Berberis asiatica* that were consumed in proportion to availability. *Smilax aspera*, *Prunus Cerasoides*, *Imperata cylindrica* and *Berberis asiatica* were also recorded as the preferred diet in earlier studies (Nagarkoti and Thapa, 2007). As the study was conducted in November, the food preference of Barking Deer determined through comparison of the percentage of availability and use indicates only the winter season's food preference.

The current study concludes that Barking Deer strongly preferred browse species during winter. Similar trends of browse preference in winter by Barking Deer were observed by Habiba *et al.*, (2021) in Pakistan. Hobbs *et al.*, (1983) and Schaller (1998) upon studying the diet of three mountain ungulates (Elk, Mountain sheep and Mule Deer) and *Axisaxis* on nutrient level respectively documented the similar browse shifting strategy of the animal in winter where they observed reduced proportion of grass and increased proportion of dicots in their diet. Their study showed quality degradation of grasses throughout the winter due to crude protein level declination and fiber content increment.

Although Choerospondias axillaris trees were very located in the study area, it was the most preferred fruit species accounted for in the overall diet. Perhaps ripened fruits simply contain more fructose and are preferred because they contain more energy (Schall and Ressel, 1991). However the oak fruit Lithocarpus elegans was found to be avoided. Such high preference towards the fruits was also seen in the study conducted by Selwyn (2020) and Chen et al., (2001). The conifer species, Pinus roxburghii was estimated as lean winter food of Barking Deer despite higher availability. Chemical constituents in plants might be the reason behind such a low level of utilization. Conifers contain essential oils that inhibit rumen functions to some degree (Oh et al., 1970). Consumption of such low quality conifer needles (Prieditis, 1984; Sauve and cote, 2007) must be an alternative diet adjustment for herbivores when forage choices are limited (Ahmad et al., 2016). However, some most abundant plant species were moderately used while some were nearly avoided. Pinus roxburghii, Rhododendron arboreum, Fraxinus floribunda, Colebrookea oppositifolia, Justicia adhatoda, Hypericum Dendrocalamus sp. cordifolium, Lithocarpus elegans, Ageratum conyzoides, Saccharum spontaneum and *Pteridiumaquilinum* were used less than their availability.

From the overall study and results obtained, a Conclusion can be made over the feeding choices of animals that selection and preference of plants as forage cannot be explained by food availability alone rather depends most importantly on herbivore's body size, anatomy and digestive physiology (Schwartz and Ellis, 1981), seasonal quality of available forage, the extent to which the animals can exploit it for their growth (Milton, 1979), and possibly other factors such as nutrient level, secondary chemical compounds, etc.

## 6. CONCLUSION AND RECOMMENDATIONS

# 6.1 Conclusion

The study conducted in Nagarjun Forest of Shivapuri Nagarjun National Park of Kathmandu district, Nepal in November, 2019, confirmed that Barking Deer shows strong affinities towards browsing during the winter season. Altogether, 18 browse (61.13%), four types of grasses (10.87%), two fruits (16.66%) and one species of herb and fern each were recorded in the diet. Litsea chartacea, Smilax aspera, Smilax lanceifolia, Prunus cerasoides, Rubus elipticus, Schima wallichii, Castanopsis indica, Berberis asiatica, Imperta cylindrica, Pogonetherum paniceum and Carex sp. were the preferred dietary plants according to estimated preference Index values. Anacardiaceae, Smilacaceae, Gramineae, Rosaceae, Lauraceae and Theaceae were the significant families consumed in frequent amounts. Species belonging to gymnosperm, bamboo, Herb and Fern were readily consumed by deer as an alternative food rather than selection and browse species including both trees and shrubs were critical to the dietary composition which was consumed at relatively high rates followed by choerospondias axillaris (15.86 %) fruits alone in the whole diet. Niche breadth (0.512) of the food items justifies that the species is generalist feeder relying on a wide range of forage species. There is a persistent need to evaluate the quality of winter food items for ascertaining the nutritional importance of each item concerning the survival of the Barking Deer in the area.

# **6.2 Recommendations**

Based on the study, following recommendations are put forward.

- Future research on seasonal nutrient analysis of important food plants of Barking Deer should be carried out.
- Study on feeding niche overlap of other herbivores in the study area should be done to assess the degree of competition for food and space with Barking Deer.
- Effective programmes should be brought into action for the suppression and eradication of invasive plant species that is covering the potential growth area of deer's preferred plant species.

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# 8. PHOTOGRAPHS

# I. Photographs from study area



1. Different sampling sites at Nagarjun Forest



3. Barking Deer encountered



4. Barking Deer pellet collection

5. Quadrat sampling



6. Separating sample fragments after washing

7. Taking micro-photographs of slides



8. Reference plant samples and fecal samples respectively in NaOCl solution under bleaching process.

**II.** Micro-histological photographs of some of the collected reference plants from the sampling area in Nagarjun Forest, SNNP, Nepal.



4. Pinus roxburghii (leaf- 40X and 100 X)



8.Colebrookia oppositifolia (leaf- 40X and 100 X)



12.Litsea chartacea (leaf- 40X and 100 X)



16.Pogonatherum paniceum (leaf- 40X and 100 X)



17.Lithocarpus elegans (pericarp) (leaf- 40X and 100 X)



18.Myrisine semiserrata (leaf- 40X and 100 X) (Source :Raju Gaire, M.Sc ,CDZ, Nepal)



19.Betula utilis (leaf- 40X and 100 X) (source: Kiran Thapa Magar, M.Sc CDZ Nepal)



20.Berberis asiatica (lef 100x and 40 X) (source: Kiran Thapa Magar, M.Sc, CDZ, Nepal)



24.Rhododendron arboreum (leaf- 40X and 100 X)


28. Thysanolaena maxima (leaf-100X and 40 X) (Source : Raju Gaire, M.Sc , CDZ, Nepal)

**III.** Selected micro-histological Photographs of plant species identified in the pellets of Barking Deer



7.Imperata cylindrica (leaf-40X)

8. *Pogonatherum paniceum* (leaf- 40X)



10.Smilax aspera (Leaf- 40X)

11. Pinus roxburghii (Leaf-40X)