Predator-prey interactions in subtropical forest and ecology and conservation of swamp deer or barasingha (*Rucervus duvaucelii duvaucelii*)

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Frontispiece

Swamp deer in Shuklaphanta National Park (Photo: Yadav H.)

In the western lowland of Nepal, Shuklaphanta National Park was established to protect the habitat of the last remaining population of the swamp deer (*Rucervus duvaucelii duvaucelii*). Endemic to Nepal and India, Swamp deer in Shuklaphanta represents the largest population and serves as an essential prey of tiger (*Panthera tigris tigris*).

Abstract

The tiger, an apex predator, is an indicator of a healthy ecosystem. Therefore, the conservation of this immensely important umbrella species necessitates ecological knowledge on its interaction with co-predators and status of significant prey species sharing the habitat. Royal Bengal tiger (*Panthera tigris tigris*) and common leopard (*Panthera pardus fusca*) are two sympatric carnivores in suitable habitats of the Indian subcontinent where sympatric ungulates cervids are prevailing as major prey species. This thesis investigated the diet overlap of these sympatric apex predators and the population status of prey species in the subtropical lowland landscape popularly known as Terai Arc Landscape (TAL), Nepal. This thesis further explored ranging behaviour, habitat preference, movements, conservation genetics and food habits of the swamp deer or barasingha (*Rucervus duvaucelii duvaucelii*), the largest cervid prey species of tiger in the western portion of TAL, Nepal.

The line transect survey revealed high density and biomass of major cervid prey species compared to other tiger bearing protected areas, with large-sized swamp deer, medium-sized spotted deer (*Axis axis*) and hog deer (*Axis porcinus*) being main prey species. Scat analysis revealed that wild ungulates were the main food of both predators. Large-sized wild preys (mainly swamp deer) were found more frequently in tiger scats, and domestic cattle were found more frequently in leopard scats. Both predators consumed the medium-sized spotted deer in high proportions resulting in a high diet overlap. Swamp deer played a critical role in diet partition between the two cats. Dominant tigers displaced leopards to the degraded fringe habitat where the latter subsisted on the domestic livestock, a major cause of human-wildlife conflict.

The monitoring of nine radio-collared swamp deer showed a high 95% Minimum Convex Polygon (MCP) and 95% Fixed Kernel (FK) home ranges compared to other sympatric ungulates such as spotted deer, hog deer, and barking deer (*Muntiacus muntjac*). There was no significant variation in 95% FK annual home ranges between sexes and across three seasons. Overall, the grassland was the most preferred habitat of swamp deer in all the seasons, and the dense Sal (*Shorea robusta*) forest was the least preferred. In the monsoon season, the moderately dense Sal forest followed the grassland. Within grassland, swamp deer preferred *Imperata cylindrica* assemblage followed by *Imperata cylindrica* – *Narenga porphyrocoma* and *Narenga porphyrocoma* assemblage. A range shift from the grassland to the Sal (*Shorea robusta*) forest, only during the monsoon season, indicated that the grassland had reached the carrying capacity during this season. The radio-collared swamp deer crossed the international border. During the rutting season, they consistently used contiguous habitat patches of Lagga-Bagga area of Pilibhit Tiger Reserve, India, thus emphasising the need for transboundary cooperation to conserve this species.

The genetic variability, population structure and effective population size of Shuklaphanta National Park (SNP) and Bardia National Park (BNP) populations of swamp deer were examined using the mitochondrial (mt) DNA and microsatellite markers. The analysis revealed moderate to high genetic diversity compared to other swamp deer populations in India. Neutrality tests, which are used to evaluate demographic effects, did not support population expansion. The multimodal pattern of mismatch distribution indicated that both swamp deer populations are under demographic equilibrium. Furthermore, population bottleneck analysis indicated no signature of a bottleneck for both populations. Bayesian cluster analysis and population differentiation test revealed

two population clusters with low population differentiation. The effective population size in BNP was below 50, which is often regarded as a threshold below which inbreeding depression is likely to occur. It is recommended to design and implement an effective conservation strategy to enhance the genetic diversity and increase the population size of swamp deer in BNP through an in-situ conservation program and translocation of some breeding individuals from SNP to BNP.

Faecal microhistological analysis of swamp deer, sympatric hog deer, and spotted deer from both grassland and the Sal forest habitats revealed that graminoids constituted the majority of the diet of these species in both habitats. However, the proportion of woody plants in diets of spotted deer was significantly higher than the other two. Apart from the graminoids, woody plant Shorea robusta and herb Phoenix humilis were major plant species consumed in the Sal forest. Among graminoid species, early successional tall grasses, especially *Saccharum spontaneum*, were the dominant food of all three deer species in both habitats. The importance of early successional tall grasses in their diet emphasised the key role of the threatened alluvial floodplains in conserving threatened mammal species in South Asia. Swamp deer foraged more in late succession tall grasses (*Saccharum narenga* and *Themeda* spp.) and short grasses (*Imperata cylindrica*, *Cyperus* spp., *Chrysopogon zizanioides*, *Cymbopogon* spp.) than hog deer and spotted deer. Despite the similarity of their diet, the three ungulates coexisted through differential consumption of plants species and seasonal habitat partitioning.

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Chapter 1 General Introduction

Successful conservation of threatened species requires a detailed understanding of their population status, interaction with habitat, and other co-occurring species in a community. Therefore, a fundamental aspect is quantifying information about species abundance, impacts of predator carnivores on prey species, habitat use, genetic diversity, diet competition, and coexistence of sympatric species. Such ecological information is crucial for science-based conservation of species, community and ecosystem as these aid in the formulation of management strategies and action plans to be implemented by conservation managers of protected areas.

Large carnivores, because they are at the top of the trophic level in an ecosystem, often require expansive habitats (Ripple, Estes, Beschta, et al., 2014). As a keystone species, they play an essential role in maintaining natural communities structures and biodiversity by regulating ungulate populations and suppressing meso-predators through predation and intraguild competition (Carter, Jasny, Gurung, et al., 2015; Ripple et al., 2014). Additionally, large carnivores, because of their position at the top of the food web, naturally have low population densities (Ripple et al., 2014). Globally, they are vulnerable to extinction due to habitat loss and degradation/fragmentation, persecution, utilisation (food, curatives, or trophies), prey reduction, and conflicts with human and livestock (Karanth & Chellam, 2009; Ripple et al., 2014). Due to vulnerability to extinction, ability to structure ecosystems and role as keystone species, the conservation of large carnivores is vital.

The tiger *Panthera tigris* and the common leopard *Panthera pardus* (henceforth leopard) are the two large sympatric carnivores in Asian forests (Carter et al., 2015; Goodrich, Lyam, Miquelle, et al., 2015; Jacobson, Gerngross, Lemeris Jr., et al., 2016; Lamichhane, Leirs, Persoon, et al., 2019; Lovari, Pokheral, Jnawali, et al., 2015; Pokheral & Wegge, 2019; Ramakrishnan, Coss, & Pelkey, 1999; Simcharoen, Savini, Gale, et al., 2014; Simcharoen, Simcharoen, Duangchantrasiri, et al., 2018; Stein, Athreya, Gerngross, et al., 2016). Palaeontological and molecular studies suggest that the leopard after its evolution in Africa ca. 3.5 million years ago (Turner, 1990) dispersed to Asia ca. 2 million years ago. However, the tiger endemic to Asia appeared ca. 1.5 million years ago (Lovari et al., 2015; Turner & Anton, 1997).

Out of nine subspecies of tiger, only five subspecies are known to exist in the wild. They are distributed over a wide range of geographical regions, from tropical forests of southern Asia to the temperate and boreal forests of the Russian Far East. The subspecies, Royal Bengal Tiger (*Panthera tigris tigris*), exists only in the Indian subcontinent, including Nepal (Weber & Rabinowitz, 1996; Goodrich et al., 2015). The leopard distribution is more widespread throughout Asia, Africa, the Middle East and South-Eastern Europe (Uphyrkina, Johnson, Quigley, et al., 2001). There are nine subspecies of leopard, with the common leopard (*Panthera pardus fusca*) existing in the Indian subcontinent, including Nepal (Stein et al., 2016). Regardless of conservation efforts, both carnivores, due to poaching, prey depletion, habitat destruction and conflict with humans are now mostly restricted to protected areas. Due to the continuously declining population and range shrinkage, tiger and leopard are listed as 'endangered' and 'vulnerable' respectively in IUCN red list (Goodrich et al., 2015; Stein et al., 2016).

In Nepal, tigers and leopards exist sympatrically in lowland Terai protected areas such as Parsa National Park (Thapa, Shrestha, Karki, et al., 2014), Chitwan National Park (Lamichhane et al., 2019; Seidensticker, 1976), Bardia National Park (BNP) (Odden, Wegge, & Fredriksen, 2010) and Shuklaphanta National Park (SNP) (Lovari et al., 2015). The conservation of these carnivores is guided by the landscape-level conservation approach aiming to increase the habitat for tigers (Smith, Ahearn, & McDougal, 1998) and restore the connectivity between protected areas (Wikramanayake, Mcknight, Dinerstein, et al., 2004). The Terai Arc Landscape (TAL) is one such landscape, situated in the foothills of the Himalayas and proximate plains, for the conservation of large mammals, including tigers and leopards (Chanchani et al., 2014). TAL connects 15 protected areas of Nepal and India and is recognised as a conservation landscape of global importance (Chanchani et al., 2014; Wikramanayake et al., 2004).

The global wild population of the tiger is continuously declining from an estimated 100,000 individuals at the turn of the 19th century to 5,000 to 7,000 individuals in 1998 (Seidensticker et al., 1999), and further to as low as 3,200 in 2010 (GTRP, 2010). During a summit in 2010, participating countries representing all 13 tiger habitats committed to double the wild tiger populations by 2022 by endorsing the Global Tiger Recovery Program (GTRP, 2010). Nepal committed to double the country's tiger population count from 121 to 250 individuals by 2022 by implementing the National Tiger Recovery Program (NTRP) (Dhakal et al., 2014). For doubling tiger populations, the availability of the prey base is one of the most determining factors (Aryal, Lamsal, Ji, et al., 2016). Among available prey species, tiger prefers to kill large-sized prey because they are the most profitable prey in terms of the ratio of energy gain to handing time (Karanth &

Sunquist, 1995). The swamp deer *Rucervus duvaucelii* is one of the largest cervid prey species inhabiting the western landscape of TAL.

The swamp deer or barasingha (*Rucervus duvaucelii*) is an obligate swampy grassland-dwelling large cervid endemic to India and Nepal (Tewari & Rawat, 2013; Qureshi et al., 2004) (Figure 1.1). Historically swamp deer were widely distributed throughout the Indo-Gangetic plains and the lowlands areas across the southern Himalayas, covering Bangladesh, Nepal, Pakistan, and India (Groves, 1982; Sankaran, 1989; Schaller, 1967). However, the population is in decline across its range, with a current global population size of <5,000 individuals (Duckworth et al., 2015). It is extinct from Bangladesh and Pakistan and currently restricted only to some isolated habitats in north, north-east and central India and south-west Nepal (Qureshi et al., 2004). Swamp deer is categorised as "Vulnerable" on the IUCN Red List (Duckworth et al., 2015) and is listed in Appendix I of CITES. There are three subspecies of swamp deer, as reported by Groves (1982). The northern subspecies *Rucervus duvaucelii duvaucelii* (G. Cuvier, 1823) (also called wetland barasingha) occurs in the north Indian states of Uttar Pradesh and Uttarakhand and southern Nepal. The central subspecies *Rucervus duvaucelii branderi* (Pocock, 1943) (also called hard ground barasingha) occurs as a single population in central India, and the eastern subspecies *Rucervus duvaucelii ranjitsinhi* (Groves, 1982) occurs in the Indian state of Assam.

The northern subspecies *Rucervus duvaucelii duvaucelii* is the most abundant, comprising ca. 80 % of the global population (Qureshi et al., 1995, 2004). This subspecies occurs as small, fragmented populations across the states of Uttar Pradesh (Hastinapur Wildlife Sanctuary, Bijnor Forest Division, Pilibhit Tiger Reserve, Kishanpur Wildlife

Sanctuary, Dudhwa National Park and Katerniaghat Wildlife Sanctuary) and Uttarakhand (Jhilmil Jheel Conservation Reserve) states of India (Qureshi et al., 2004). A recent study further confirms the presence of this subspecies in areas between Jhilmil Jheel Conservation Reserve and Hastinapur Wildlife Sanctuary and the adjoining regions of northern India (Paul, Pandav, Mohan, et al., 2018; Paul, Sarkar, Patil, et al., 2020).

In Nepal, swamp deer inhabit the terai region (an extension of the north Indian Gangetic plain, which fringes the southern edge of Nepal) and is protected by the National Parks and Wildlife Conservation Act (1972). As late as the 1950s, swamp deer were still widely distributed in Banke, Bardia, Kailali, and Kanchanpur districts of western Nepal. In 1957, many swamp deer were also reported from then extensive marshy grasslands of the Chitwan valley of central Nepal located in the north of the Rapti river, just outside the area which is now the Chitwan National Park. A few animals, in this valley survived as late as 1963. However, none occurs here today (Schaaf, 1978).

At present, two isolated swamp deer populations exist in Nepal. A small population of ca. 100 individuals inhabit BNP in mid-western and ca. 2000 individuals in SNP in far-western Nepal (Figure 1.1). In addition, Schaaf (1978) reported a population of unknown size and status in the Dhaka area, which now falls on the eastern side within the boundary SNP.

Schaller (1967) was the first to provide data on the biology of the central subspecies of swamp deer from his work in the Kanha National Park, India. Martin (1976), succeeding Schaller, assessed the cause of the sharp decline of the swamp deer population in Kanha, studying ecology during 1971 -1973. He concluded that the leading causes of this decline were loss of habitat and poaching, along with deer’s need for seasonal migration to satisfy requirements for food, water, and suitable fawning ground. In Kanha, swamp deer exhibited spatially distinct dry season and monsoon season home ranges and showed a preference for grasslands inside the Sal forest. These seasonal home range extensions took the swamp deer outside the park, causing conflict with the humans. Like central subspecies, in Dudhwa, northern subspecies too exhibited seasonal ranges. Swamp deer utilised the grasslands within the park between late winter and summer (January to June); however, they moved about 2 km and lived predominantly in agricultural areas between monsoon and early winter (July to December) (Sankaran, 1989).

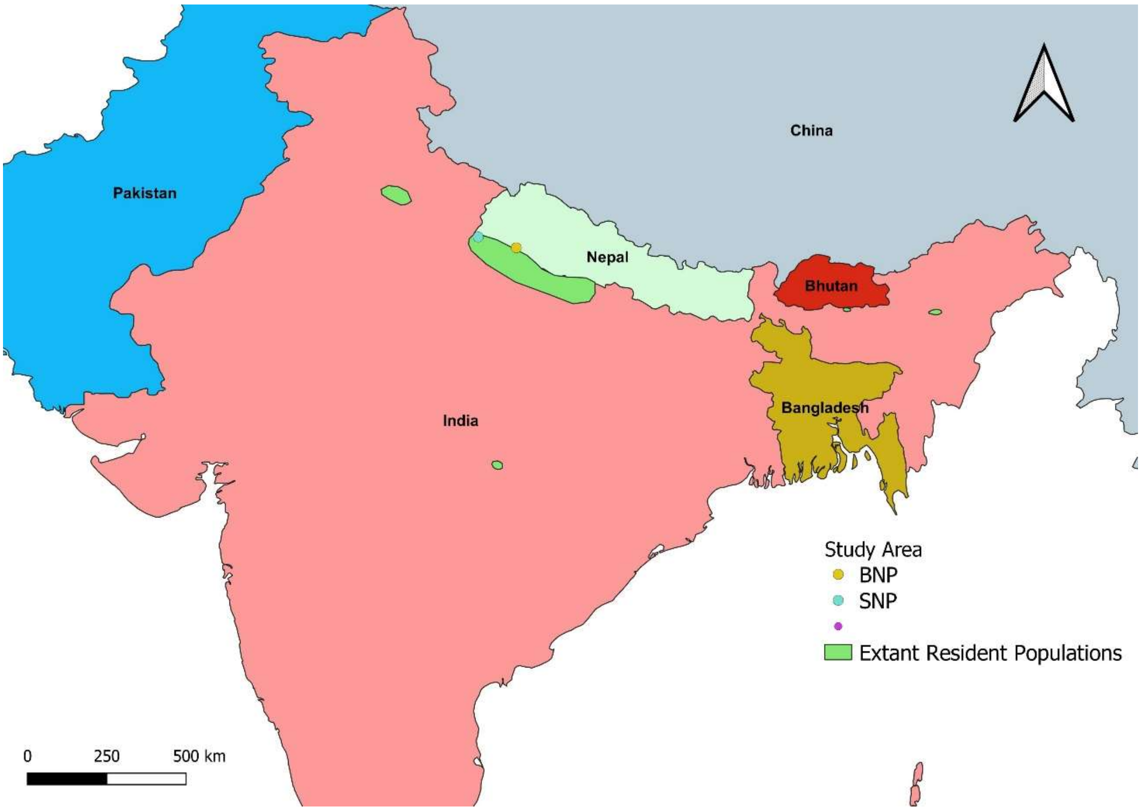

Figure 1. 1 Map showing extant resident swamp deer populations and study areas in Shuklaphanta National Park (SNP) and Bardia National Park (BNP) of Nepal. The polygons of extant resident populations in the map is taken from IUCN, 2008. (https://www.iucnredlist.org/ species/4257/22167675).

Swamp deer in Jhilmil Jheel preferred mostly hydrophytic habitat and showed different preferences in monsoon, summer and winter seasons (Tiwari, 2009).

Schaaf (1978) studied northern subspecies of swamp deer in SNP between 1974 and 1976, a pioneering study of this subspecies in Nepal. During the study periods, swamp deer remained mostly in grasslands and avoided forest habitats in all seasons. However, from my personal experience, while working for SNP, I reckoned that much might have changed compared to the 1970s, particularly behaviours related to populations status, habitat use and food habit of swamp deer. As a part of regular wildlife monitoring activities, I encountered swamp deer frequently in the Sal forest during the monsoon season but not in the dry seasons, which I found to be a striking difference compared to the findings of Schaaf (1978). However, such observed behavioural changes have not been tested. The crucial components of conservation genetics (genetic diversity, population genetic structure and effective populations size) are instrumental in managing swamp deer populations. Though the genetic diversity and populations structure of extant major Indian populations are recently studied (Kumar, Ghazi, Hussain, et al., 2017), none are explored for Nepalese populations. Similarly, data on effective population size is not available for any populations of its distribution range. Regarding the diet of swamp deer, previous studies (Pokharel, 1996; Tewari & Rawat, 2013; Wegge, Shrestha, & Moe, 2006) are restricted to the dry seasons only, and only one study (Wegge et al., 2006) covered diet comparison and competition among sympatric ungulates. However, nothing is known on the monsoon season diet and the coexistence of swamp deer with hog deer and spotted deer, specifically when swamp deer might have changed the habitat use pattern compared to the 1970s.

1.1 Thesis outline

In this study, I explored the diets of the two large predators, tiger (*Panthera tigris tigris*) and leopard (*Panthera pardus fusca*), their prey population and biomass density, and population status and habitat requirements of the large prey species, i.e., swamp deer (*Rucervus duvaucelii duvaucelii*). These pieces of knowledge will enable researchers and conservationists to enhance an understanding of the degree of diet overlap and prey availability for these two sympatric carnivores in the western lowland protected area of TAL, Nepal, i.e., SNP. This will, in turn, help with formulating the optimal conservation strategies to protect these top predators, their principal prey species and the ecosystem integrity. This information can further be utilised for reducing possible conflicts of people with wildlife, especially with carnivores. The scientific information generated on the ranging behaviour, habitat use, and food habit of swamp deer will be instrumental in the habitat management and mobilisation of logistic resources focussed on swamp deer conservation. The genetic information will help project the future survival of swamp deer and devise conservation programs to reduce the chances of extinction of this species.

1.2 Thesis structure

The thesis encompasses four research/data chapters (Chapters 2 to 5), with chapters 1 and 6 being introductory and concluding discussion chapters, respectively. Each research/data chapter has been written in the format of journal articles.

Chapter 1 introduces the studied species with an overview of current knowledge and gaps in understanding related to aspects of predators and prey species and their coexistence in Nepal.

Chapter 2 investigates the prey abundance and diet of sympatric tiger and leopard in SNP. The specific questions this chapter seeks to answer are:

- What is the current population status of major prey species in terms of abundance and population density?
- What is the current diet composition of tiger and leopard? Is there diet partitioning between these predators? Does large-sized swamp deer play a role in the diet partitioning of these predators?

Chapter 3 Assesses habitat requirements of swamp deer in the SNP, aiming to answer the following questions:

- What are the seasonal home range size and the habitat preferences of swamp deer?
- Are there trans-border movements of swamp deer between SNP, Nepal and bordering Lagga-Bagga sector of Pilibhit Tiger Reserve, India?

Chapter 4 investigates conservation genetics of swamp deer residing in two protected areas, SNP and BNP of Nepal. This chapter aims to answer the following questions:

- What is the genetic diversity of swamp deer populations in Nepal?
- Is there gene flow between the two populations of swamp deer in Nepal?
- What is the effective population size of two populations?

Chapter 5 explores the dietary requirements of swamp deer and the other two co-occurring major ungulate prey species, i.e., hog deer and spotted deer, in SNP. This chapter answers the following questions:

- What are the seasonal diet compositions and diet overlap among three ungulates?
- Is there potential competition among prey species that potentially affect the swamp deer population?

Chapter 6 summarises the key findings of this study concerning the conservation of tiger and swamp deer. The significance of this research and future research direction are further outlined. Lastly, management considerations are also proposed.

Chapter 2 Prey abundance and diets of sympatric carnivores: tiger (*Panthera tigris tigris*) and leopard (*Panthera pardus fusca*) in Shuklaphanta National Park, Nepal

2.1 Abstract

Understanding diet composition and niche partition of large predators like tigers (*Panthera tigris tigris*) and leopards (*Panthera pardus fusca)* are essential for understanding their ecological needs and potential competitions. Such knowledge is crucial for the conservation of these top predators and the management of human-wildlife conflicts. It has been reported that leopard avoids dominant tiger, and the two cats might co-exist through diet, spatial and/or temporal partitioning. Between 2015 and 2016, the predators' prey density and diet composition were explored in the sub-tropical habitat of western lowland, Nepal. Line transect of distance sampling estimated high density and biomass of wild prey (105.9 individuals per km ^{2}, 9224.9 kg per km ^{2}) and domestic prey (30.3 individuals per km ^{2}, 5621.4 kg per km ^{2}). Scat analysis revealed that wild preys (mainly ungulates) were the main food of both predators (tiger 92%; leopard 60%). Tiger consumed large-sized wild prey (tiger 36%, leopard 8%) more often and domestic cattle (tiger 8%, leopard 40%) less often than the leopard. Both predators concentrated on the medium-sized wild prey in high proportions (tiger 59%; leopard 83%), resulting in a considerably high diet overlap (Pianka index 0.90). Although extensive diet overlap existed, diet partitioning was observed between the two cats. Also, the tiger displaced the leopard to the fringe habitat where the latter consumed available livestock, causing conflict with people. In conclusion, the study area had high prey availability, and the two carnivores might have co-existed through diet and spatial partitioning.

2.2 Introduction

Globally vulnerable to extinction due to habitat loss and degradation/fragmentation, persecution, utilisation (food, curatives, or trophies), prey reduction, and conflicts with human and livestock (Karanth et al., 2009; Ripple et al., 2014), large carnivores play a vital role in the structuring of ecosystems by playing a dual role of regulation of ungulate populations and suppression of mesopredators through predation and intraguild competition respectively (Carter et al., 2015; Ripple et al., 2014). Due to their keystone species status and their vulnerability to extinction, the conservation of large carnivores is extremely important.

Theoretically, closely related species, including carnivores, may coexist without competition if the shared resources are not limited. However, if resources are a limiting factor, competition is anticipated through the mechanism of resource exploitation or interference (Putman, 1996). Carnivores tend to avoid interspecific interference through segregation of diet, space and temporal activity patterns (Harmsen, Foster, Silver, et al., 2009; Hayward & Slotow, 2009; Kronfeld-Schor & Dayan, 2003; Lamichhane et al., 2019; Lovari et al., 2015; Mills & Gorman, 1997; Palomares, Gaona, Ferreras, et al., 1995; Pereira, Alves da Silva, Alves, et al., 2012; Pokheral et al., 2019; Romero-Muñoz, Maffei, Cuéllar, et al., 2010; Vanak, Fortin, Thaker, et al., 2013). In addition, top-down cascade effects of dominant predators on subordinate predators force the latter to explore the prey in a way to avoid the risk of an encounter with the former, leading them to adapt accordingly in terms of diet, movements, habitat use and activity rhythms (Lovari et al., 2015; Vanak et al., 2013).

The tiger *Panthera tigris* and the leopard *Panthera pardus* are the two large sympatric carnivores in Asian forests (Carter et al., 2015; Goodrich et al., 2015; Jacobson et al., 2016; Lamichhane et al., 2019; Lovari et al., 2015; Pokheral et al., 2019; Ramakrishnan et al., 1999; Simcharoen et al., 2014, 2018; Stein et al., 2016), with former dominant over the latter (Karanth & Sunquist, 2000; Odden et al., 2010; Palomares, Caro, Byers, et al., 1999; Seidensticker, 1976). Tiger is larger than leopard (65 – 306 and 28 – 90 kg respectively) and is known to kill the latter (Pokheral et al., 2019; Seidensticker, 1976). They are both cryptically coloured, have a similar social structure, and apply the same approaches for hunting prey (Pokheral et al., 2019). Studies in Asia shows that leopard avoids tiger (Harihar, Pandav, & Goyal, 2011; Lamichhane et al., 2019; Odden et al., 2010; Pokheral et al., 2019). Similarly, high diet overlap (Karanth et al., 2000; Lovari et al., 2015; Selvan, Veeraswami, Lyngdoh, et al., 2013; Wegge, Odden, Pokharel, et al., 2009) and spatial overlap (Simcharoen et al., 2018) between these two carnivores have been reported.

Based on a study in Nagarhole, India, Karanth and Sunquist (1995) suggested that prey selection by the tiger and co-occurring predators is primarily governed by the structure of the prey community, mainly in terms of the abundance of different sized prey. Where tiger and leopard coexist, if both large (> 100 kg) and medium (> 25 to 100 kg) sized prey are abundant, the tiger would select large prey, enabling optimum conditions for the coexistence with leopard. Where large preys are scarce, the tiger would switch to medium-sized prey, causing competition with the leopard. However, if both large and medium-sized prey is scarce, the leopard would benefit more because of their ability to survive on smaller prey (5 to 25 kg).

The diet of tiger and leopard predominantly includes deer species (Sunquist, 1981; Wegge & Storaas, 2009). The livestock also contributes to a significant proportion of large predators' diet. When wild prey becomes scarce, predators increase predation on livestock to survive (Baker, Boitani, Harris, et al., 2008; Khorozyan, Ghoddousi, Soofi, et al., 2015; Zhang, Zhang, & Stott, 2013), causing human-wildlife conflict (Bhandari, Chalise, & Pokheral, 2017; Inskip & Zimmermann, 2009; Nowell and Jackson, 1996).

Despite conservation efforts for both tiger and leopard, due to poaching, prey depletion, habitat destruction, and conflict with humans, they are now mostly restricted to protected areas. Due to the continuously declining population and range shrinkage, tiger and leopard are categorised as 'endangered' and 'vulnerable' species respectively in IUCN red list (Goodrich et al., 2015; Stein et al., 2016) and are protected species by National Parks and Wildlife Conservation Act 1973 of Nepal.

This chapter estimates the density and biomass of prey species and analyses the food habits, prey selection, and diet overlap of tiger and leopard in Shuklaphanta National Park (SNP). I also explored seasonal diet patterns and the effects of habitat management on the diet of the tiger. SNP is famous for grassland habitats covering 27% of the park's total area. Furthermore, the grassland named Shuklaphanta, covering an area of 34 km^2 and located on the southern side of the largest continuous Sal forest of SNP, is the most extensive intact grassland in Nepal (SNP, 2017). The southwestern side of the park is mostly grassland, contiguous with the Lagga-Bagga part of Pilibhit Tiger Reserve in India, providing a favourable habitat for the trans-border movement of the threatened tiger (Chanchani et al., 2014) and swamp deer (Chapter 3).

SNP has a rich community of wild prey species of different sizes, ranging from large-sized prey like swamp deer and nilgai to medium-sized prey like spotted deer, hog deer and wild boar and small-sized prey, mainly primates like monkey and langur. In addition, domestic livestock such as cattle, buffaloes and goats have also been observed grazing inside the park. Due to the availability of different sized prey, I expect low exploitative competition between two carnivores, with tiger consuming large to medium prey more often than leopard and leopard consuming medium to small prey more often than the tiger. Additionally, I hypothesise that the swamp deer, a large prey, is important in tiger diet, primarily contributing to the diet partition between the two cats.

2.3 Methods

Study area

The study was conducted in the south-westernmost (about 100 km^2) of 305 km^2 SNP (N: 28.7193 to 29.0515; E: 80.0609 to 80.4120) in the far western lowland Terai region of Nepal (Figure 2.1). The altitude ranges from 90 -270 m above sea level. The climate is mainly subtropical and monsoonal, with more than 90% of the annual precipitation (1,000-2,000 mm) falling between July and September. The temperature ranges from 10^0-12^{0}C during winter (February/March) to 40^0-42^{0}C during summer (May/June) (Henshaw, 1994). There are three distinct seasons: cold dry (November–February), hot dry (March–June) and monsoon (July–October). The habitat in the study area ranges from early successional floodplain tallgrass to climax stage Sal (*Shorea robusta*) forest. The different types of habitats in the study area consisted of Sal forest *Shorea robusta* (30%), Mixed deciduous forest (30%), early successional Khair-Sissoo forest (5%) and grassland (35%). The ground vegetations of the forest, mainly Sal forest, is covered with grass species most similar to grassland habitat. On the south-central part of the park, primarily large patches of tall grassland occur, providing prime habitat for swamp deer. Other ungulates in the study area are spotted deer *Axis axis*, hog deer *Axis porcinus*, Indian muntjac *Muntiacus muntjak* and nilgai antelope *Boselaphus tragocamelus*. Other prey species in the area are wild boar *Sus scrofa*, common langur *Presbytis entellus* and rhesus macaque *Macaca mulatta*. Farmland and settlements surround the park except for the southern side, which borders the forest of India. So apart from the wild ungulates, SNP is grazed by domestic ungulates entering from the human settlement sides.

The habitat structure of the forest and grassland of SNP is altered seasonally through already existing cutting and burning practices. The purposes of such habitat management are providing thatching materials for local people, preventing succession from grassland to forest, improve forage quality for wild ungulates as the grasslands regenerate and avoiding a wildfire later in the dry season (Schaaf, 1978; Peet et al., 1999). The burning generally initiates in the mid of cold dry season in December. Additionally, the burning generally starts from grassland patches, and it spreads to peripheral areas in Sal forest by pre-monsoon hot dry season in March and April. Habitat management affects the ecological factors such as cover and prey community structure, hence may affect predation success. I defined July to December as pre-management and January to June as the post-management period. The grasses in both grassland and forest grow tall in the pre-management situation, and the forest canopy is more closed. In contrast, in the post-management condition, the grass is low, and the forest canopy is open, thus reduces the cover for animals.

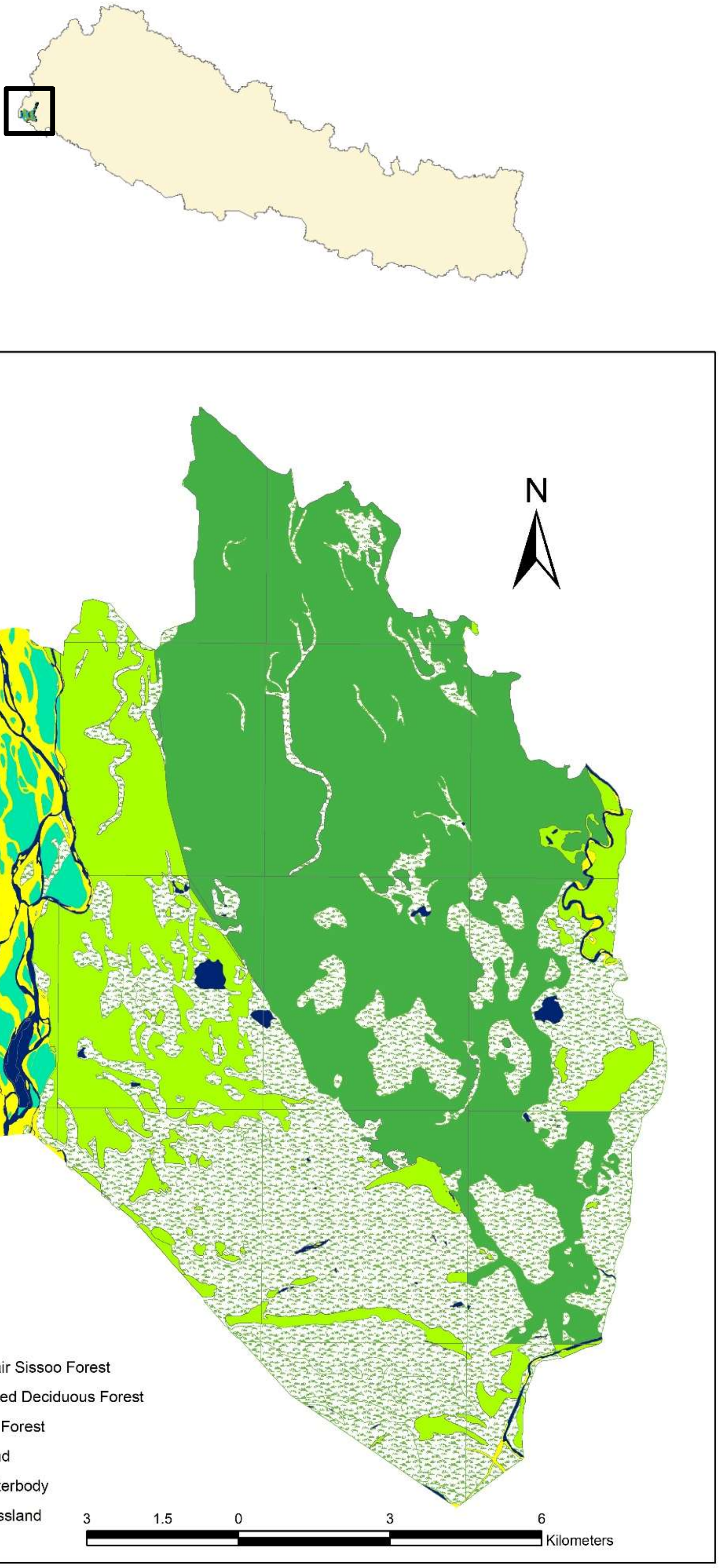

Figure 2. 1 Map showing study area of Shuklaphanta National Park.

Density and biomass of prey species

The line transect method (Buckland, Anderson, Burnham, et al., 1993; Burnham, Anderson, & Laake, 1980) was used to estimate the densities of prey species as it has now become one of the standard methodologies for monitoring prey species. This method has been widely used to determine animal densities in tropical conditions (Bagchi, Goyal, & Sankar, 2003; Biswas & Sankar, 2002; Jathanna, Karanth, & Johnsingh, 2006; Yadav, 2006; Karanth & Sunquist, 1992, 1995; Khan, Chellam, Rodgers, & Johnsingh, 1996; Varman & Sukumar, 1995; Wegge, Odden, Pokharel, & Storaas, 2009; Wegge & Storaas, 2009). This method gives relatively unbiased results if certain assumptions are fulfilled (Buckland et al., 1993). Ten systematic parallel line transects varying in length between 2.71 and 6.1 km and totalling 45.17 km were run nine times (three times in each cold dry, hot dry and monsoon season), resulting in a total effort of 135.51 km per season. An experienced, well-trained wildlife technician assisted the monitoring along transects during the morning and late afternoon when animals are most active. We carried out the survey on the elephant back (Wegge & Storaas, 2009). For each sighting of prey species along the transect, the parameters: (1) the angle between the observer and the prey (with a compass); (2) distance between the individuals and the prey (with range finder); (3) group size of prey, were noted. The density of prey species was estimated using Distance software version 7.3 (Thomas, Buckland, Rexstad, et al., 2010). The farthest sightings of the prey species on the transects (10% of all observations) were truncated to achieve a reliable density estimate (Buckland et al., 1993). The density estimates obtained from transects were used to calculate the biomass density of prey species in the study area by multiplying the individual density estimates with the average live weight of the prey species available from literature (Biswas et al., 2002).

Diet composition of tiger and leopard

Scat analysis was used to estimate the proportion of different prey species consumed by tiger and leopard since this method is non-destructive, non-invasive, and cost and time effective (Biswas et al., 2002; Mumma, Adams, Zieminski, et al., 2015). This method has been widely used to study the food habit of carnivores (Aryal & Kreigenhofer, 2009; Bagchi, Goyal, & Sankar, 2003a; Bhandari et al., 2017; Biswas et al., 2002; Karanth et al., 1995; Lamichhane et al., 2019; K Mondal, Gupta, Bhattacharjee, et al., 2012; Krishnendu Mondal, Gupta, Qureshi, et al., 2011; Sankar & Johnsingh, 2002; Stoen & Wegge, 1996; Sunquist, 1981; Yang, Dou, Baniya, et al., 2018). Scats of both predators were collected whenever encountered while carrying out line transect sampling for prey density estimation and monitoring of radio-collared swamp deer in 2015 and 2016 (Chapter 3). However, mostly scats were encountered on forest roads, trails and elephant paths, which were known to be used for scat deposition by tiger and leopard (Karanth et al., 1995; Odden et al., 2010). The scats of two predators were identified based on size and morphology and secondary signs such as scrape marks and pugmarks (Bhattarai & Kindlmann, 2012; Biswas et al., 2002; Simcharoen et al., 2018). Scats of the tiger are much larger and have a lower degree of coiling and relatively larger distance between two successive constrictions within a single piece of scat. Tiger pugmarks (> 8 cm pad width) and scrape marks (> 35 cm long and > 19 cm wide) are larger than leopard pugmarks (< 6.5 cm pad width) and scrape marks (< 25 cm long and < 15 cm wide). A recent study based on molecular identification of carnivore scats reported high accuracy of field identification technique (Upadhyaya, Musters, Lamichhane, et al., 2018). Ambiguous and unidentified scats were excluded from the analysis. I used morphological analysis of predator scats as this method has several advantages over the molecular method (Mumma et al., 2015). Unlike the molecular method, morphological analysis is straightforward and cost-effective. It allows quantification of different prey items and biomass consumption by predators and evaluating their prey selection when prey availability is known (Mumma et al., 2015). I followed Mukherjee et al. (1994) to identify prey species in the scat through microscopic analysis of medullary and cuticular structures of hairs found in the scat of predators. Microscopic analysis of hair was carried out at the laboratory maintained at the Shuklaphanta Conservation Program (SCP) of the National Trust for Nature Conservation (NTNC). Prey species present in the scat were identified by comparisons of hair structure with reference samples maintained at NTNC and the Wildlife Institute of India (Bahuguna, 2010).

Occurrence of prey, relative biomass and number of preys consumed

The diet of tiger and leopard was quantified using the percentage of occurrence of prey items (number of times a specific item was found as a percentage of all items found) (Ackerman, Lindzey, & Hemker, 1984). Wild prey species consumed by predators were classified into three different classes based on their mean body weight. Species with a mean body weight between 5 and 25 kg were classified as 'small-sized prey' (langur and monkey), >25 – 100 kg as 'medium-sized prey' (spotted deer, hog deer and wild boar) and >100 kg as 'large-sized prey' (swamp deer and nilgai) (Lovari et al., 2015). However, high variability in body sizes of prey species causes an overestimation of smaller prey in the diet (smaller prey species have more hair per unit body weight than larger prey and thus produce relatively more scats per unit prey weight consumed). Therefore, the frequency of occurrence did not adequately represent the proportion of different prey species consumed (Karanth et al., 1995). To overcome this problem, I used the following regression equation developed by Ackerman et al. (1984). This equation relates the average live weight of a prey animal consumed (X) by tiger and leopard to the weight of that prey represented in one field collectable scat (Y):

$$Y = 1.980 + 0.035X$$

From the above equation, scat production ($\lambda_i = X_i/Y_i$, the average number of collectable scats produced by a given predator from an individual animal of each prey species), relative biomass and numbers of each prey killed were calculated as follows:

If

X = average body weight;

Y = estimated weight of prey consumed per collectable scat produced;

A = percentage of occurrence of prey items;

D = relative biomass consumed by tiger or leopard;

E = relative number of prey animals consumed.

Then, $D = (A \times Y)/\sum (A \times Y)$; $E = (D \div X)/ \sum (D \div X)$

Estimation of prey selectivity

The selectivity of prey by predators was estimated by comparing observed counts of each prey item in the scat with the estimated prey availability of each prey item in the environment using multinomial likelihood ratio tests (Karanth et al., 1995; Link & Karanth, 1994). The expected number of scats having a particular prey species based on the null hypothesis of random, non-selective predation was calculated following Karanth & Sunquist (1995) as $\pi i = \ di\lambda i/(\sum idi\lambda i)$, where prey species i has population density di, and λi (λi = X_i/Y_i derived from Ackerman's equation) is the number of scats produced from a single kill of species i. The online version of computer program SCATMAN (https://www.mbr-pwrc.usgs.gov/software/scatman.html) (developed by J.E. Hines and W. A. Link; Link & Karanth, 1994) was used to calculate the expected proportions of prey species in scats. The variability in density estimates of prey species and the number of scats generated from a particular kill of any prey species might increase the likelihood of the Type 1 error (Link et al., 1994). As suggested, I implemented 1000 times parametric bootstrapping functionality of the SCATMAN program to alleviate the above problem (Link et al., 1994). Each prey item was given equal weightage for scat frequency when more than one species were detected in a scat (Bagchi et al., 2003; Biswas & Sankar, 2002; Grey, 2009; Karanth & Sunquist, 1995).

The relationship between prey species found in the scats and prey available in nature was further depicted using Jacobs index (Jacobs, 1974): $D_i \ = (r_i \ - a_i)/(r_i + \ a_i - 2r_i a_i)$ where r_i = % occurrence of prey items in the scats; a_i = % availability of prey species in the environment. D_i values range from +1 (maximum preference) to −1 (maximum avoidance) (Jacobs, 1974). I presented Jacob’s index value graphically at three levels: annual selection by predators for major prey items and different sized prey and selection by tiger for prey among seasons and between habitat management scenarios.

The diet overlap between tiger and leopard was measured by using Pianka's niche overlap index (Pianka, 1973) as follows:$O = \sum_{i=1}^{M} p_{it}\, p_{il} \Big/ \sqrt{\sum_{i=1}^{M} p_{it}^{2} \sum_{i=1}^{M} p_{il}^{2}}$, where p_i is the proportion of prey species i (with i = 1,………, M) found in the diet of the tiger (t) or common leopard (l). The index value ranges from 0 (no overlap) to 1 (complete overlap).

2.4 Results

Density and biomass of prey species

There was a high wild ungulate density in the study area. The density of wild ungulates was 101.7 animals km^{-2}, constituting 96% of the total wild prey species density (Table 2.1). Of the total wild prey density, spotted deer, swamp deer and hog deer contributed 41.9%, 36.8% and 18.8%, respectively. Primates contributed 4% to the overall wild prey density in the study area. The density of domestic preys (cattle and buffalo) were 30.3 animals km^{-2} (Table 2.1). The cattle density alone was 28.5 animals/ km^{2}, 21.6 % of total ungulate density (wild and domestic). Overall, the prey density in the area was 136.2 animals/km ^{2} (Table 2.1). Among wild preys, 36% were large-sized prey, 60% were medium-sized prey, and only 4% were small-sized prey (Table 2.1). Similarly, of the total animal density, 78 % were wild prey, and the remaining 22% were domestic prey (Table 2.1). The total biomass density estimate in the study was 14,846.3 kg km $^{-2}$ (Table 2.1), of which 62 % were wild prey, and 38% were domestic prey (Table 2.1). Similarly, in terms of the size of animals, of the total wild prey biomass density, 65% was contributed by large-sized prey, 35% by medium-sized prey, and only 0.2% by small-sized prey (Table 2.1). Among wild prey, the biomass density of swamp deer was the highest (64.6%), followed by spotted deer (25.5%), hog deer (8.3%), and others (wild boar, nilgai, langur and monkey) 1.6%. Cattle alone contributed 34.5% of the total biomass density in the study area.

Table 2. 1 Annual numerical and biomass density estimates of principal prey species in Shuklaphanta National Park between July 2015 and June 2016.

[n= total number of groups detected; D_G = density of groups; D_I = density of individuals; GS= mean group size; C_VD_G = coefficient of variation of density of groups; C_VD_I = coefficient of variation of density of individuals; C_ID_I=95 % confidence intervals of density estimates of individuals.; b (Lovari et al., 2015)]

Species	**n**	**D_G (km^2)**	**C_VD_G (%)**	**GS**	**D_I (km^2)**	**C_VD_I (%)**	**C_ID_I (km^2)**	**Biomass density (kg/km^2)**
Wild prey								
Spotted deer	195	5.2	9.4	8.2	42.7	16.2	31.2 – 58.7	2348.5
Hog deer	98	4.4	13.4	4.3	19.2	20.8	12.8 – 28.8	768
Swamp deer	160	2.7	9.8	14.0	37.5	16.9	26.9 – 52.3	5962.5
Wild boar	21	0.7	26.8	2.8	2.1	35.4	1.1 – 4.1	79.7
Nilgai	4	0.1	61.2	2.5	0.2	72.2	0.06 – 0.9	36.8
Total (ungulate)					**101.7**			**9195.5**
Langur	5	0.2	77.2	9.4	2.1	82.6	0.4 – 10.1	16.8
Monkey	7	0.2	74.6	9.7	2.1	82.5	0.5 – 9.7	12.6
Total (wild)		**13.5**			**105.9**			**9224.9**
Domestic prey								
Cattle	11	0.3	38.3	89.5	28.5	44.9	11.7 – 69.7	5130
Buffalo^b	73	0.2	45.2		1.8	51.0	0.7 – 4.8	491.4
Total (domestic)		**0.5**			**30.3**			**5621.4**
Grand total		**14.0**			**136.2**			**14846.3**
Wild Prey Category					**%**			**%**
Small prey (5 – 25 kg)					4			0.4
Medium prey (>25 – 100 kg)					60			34.6
Large prey (>100 kg)					36			65
Total					**100**			**100**
Wild Vs Domestic Prey					**%**			**%**
Wild prey					78			62
Domestic prey					22			38
Total					**100**			**100**

Diet composition of tiger and leopard

I collected 247 and 86 tiger's and leopard's scats, respectively. However, only 210 tiger and 59 leopard scats were used for final analysis because microbes degraded the remaining scats. The plot of the number of scats analysed and the accumulated number of prey species revealed that the asymptote reached 75 scats for the tiger (Figure 2.2). Although the number of prey species appears to have reached a plateau at 50 scats for leopard (Figure 2.3), whether the asymptote reached is unknown. All scat samples included in the analysis revealed twelve and eleven prey species in tiger and leopard, respectively. I recorded 293 and 66 prey items in tiger and leopard scats, respectively. For tiger, 64.7%, 31.9%, 2.4%, and 1% of the scats consisted of one, two, three and four prey species, respectively. For leopard, 78% and 22% of the scats contained one and two prey species, respectively. The spotted deer, a medium-sized prey, was the dominant species for both tiger (38.9%) and common leopard (40.9%) (Table 2.2). For tiger, the frequency of occurrence of spotted deer items was followed by hog deer (16.7%), swamp deer (13.3%), wild boar (8.2%) and nilgai (5.1%). The presence of mongoose, langur, monkey, porcupine and civet represented less than 3%. For leopard, spotted deer was followed by cattle (16.7%), hog deer (10.6%), wild boar, and domestic dog (each 7.6%). Other prey items in leopard scat represented less than 3%. The wild prey constituted 96% and 73%, respectively, in tiger's and leopard's scats (Table 2.3). Domestic animals (cattle, buffalo and dog) contributed 4% and 27% respectively in tiger's and leopard's scats (Table 2.3). Wild ungulates species constituted 82% and 61% of identifiable items in tiger's and leopard's scats, respectively. In terms of prey size, large, medium and small prey contributed 19%, 69% and 12% respectively in tiger's scat (Table 2.3). In leopard, large, medium and small prey constituted 4%, 83% and 13%, respectively (Table 2.3). No remains of nilgai and porcupine were found in leopard scats, and domestic dog remains was not found in tiger scats. With 12 and 11 prey species respectively killed by tiger and leopard, the diet overlap measured using the Pianka overlap index (Pianka, 1973) was 0.90.

The estimated relative biomass contributed by different prey species to predators' diet (Table 2.3), calculated using the equation developed by Ackerman et al. (1984), revealed that spotted deer was the primary prey species for both tiger and leopard with a contribution of about 35% for each predator. Swamp deer contributed 23% to the tiger's diet, whereas only 5% to the leopard's diet. The proportion of cattle in leopard scats was 32%, whereas only 6% in tiger scats. Wild prey made up 92% and 60% of the total biomass of tiger's and leopard's diet, respectively (Table 2.3). Similarly, domestic prey's proportion in tiger's and leopard's scats was 8% and 40%, respectively (Table 2.3). In tiger, the biomass of large, medium and small-sized prey constituted 36%, 59% and 5%, respectively (Table 2.3). Large, medium, and small prey contributed 8%, 83%, and 9% in leopard, respectively (Table 2.3). This study found that tiger and leopard consumed smaller (mongoose; 1kg) to large-sized (buffalo; 375 kg) prey species.

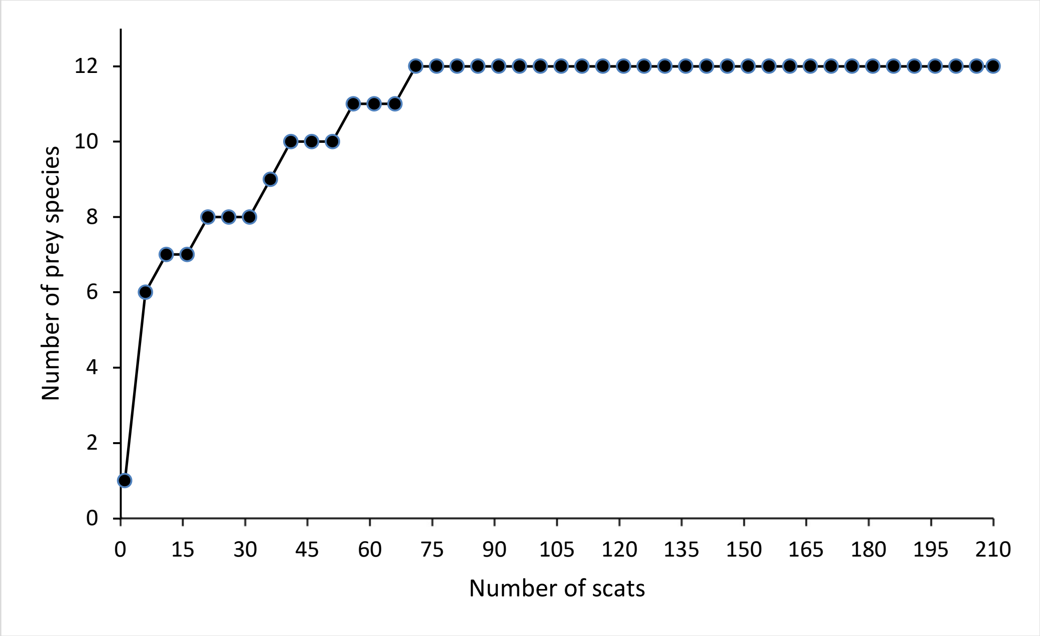

Figure 2. 3 Scat sample stabilisation curve of tiger in Shuklaphanta National Park.

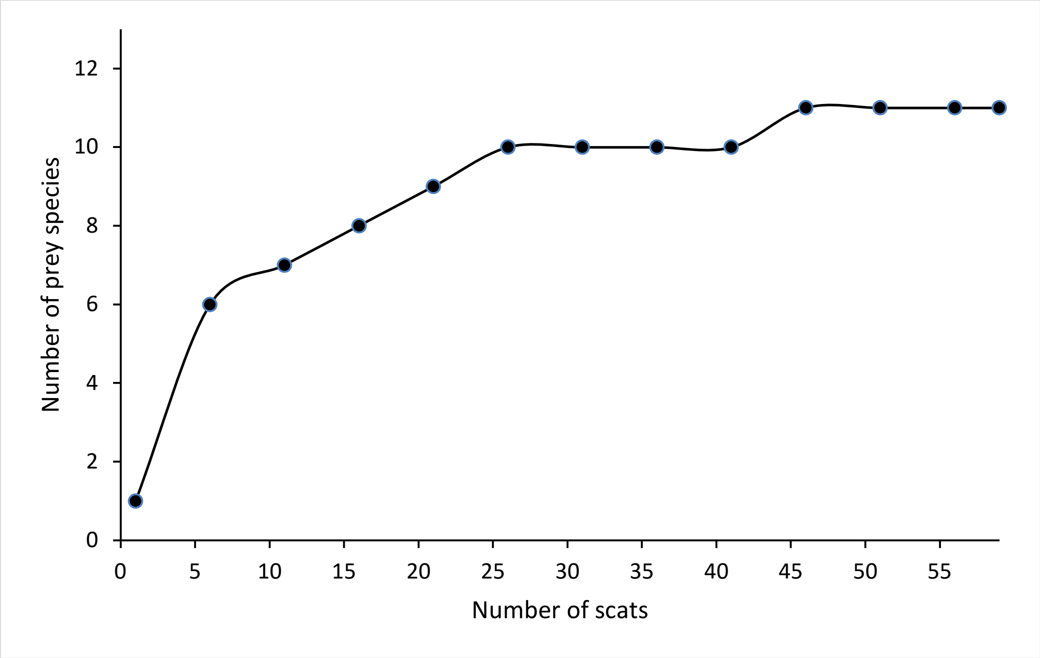

Figure 2. 2 Scat stabilisation curve of leopard in Shuklaphanta National Park.

Table 2. 2 Composition of tiger and leopard diet in the study area of Shuklaphanta National Park.

		Tiger (210)		Leopard (N = 59)	
	Prey Species	FOI	% FOI N = 293	FOI	% FOI N = 66
Wild	Spotted deer	114	38.9	27	40.9
	Hog deer	49	16.7	7	10.6
	Swamp deer	39	13.3	2	3
	Wild boar	24	8.2	5	7.6
	Nilgai	15	5.1	0	0
	Mongoose	9	3.1	2	3
	Langur	7	2.4	1	1.5
	Monkey	6	2	1	1.5
	Porcupine	6	2	0	0
	Civet	3	1	2	3
	Sub-total	**272**	**92.8**	**47**	**71.2**
Domestic	Cattle	9	3.1	11	16.7
	Buffalo	3	1	1	1.5
	Dog	0	0	5	7.6
	Sub-total	**12**	**4.1**	**17**	**25.8**
Unknown		9	3.1	2	3.0
Total		**293**	**100**	**66**	**100**

% FOI = frequency of occurrence of items (number of times a specific item was found as a percentage of all items found)

Table 2. 3 The relative biomass and the relative number of prey individuals consumed by tiger and leopard in the study area of Shuklaphanta National Park.

				Tiger (N = 210)			Leopard (N = 59)		
	Prey Species	X (kg)	Y (kg/scat)	A (%)	D (%)	E (%)	A (%)	D (%)	E (%)
Wild	Spotted deer	55	3.9	38.9	34.6	16.9	40.9	36.4	18.6
	Hog deer	40	3.4	16.7	12.9	8.6	10.6	8.2	5.7
	Swamp deer	159	7.5	13.3	22.9	3.9	3.0	5.2	0.9
	Wild boar	38	3.3	8.2	6.2	4.4	7.6	5.7	4.2
	Nilgai	184	8.4	5.1	9.8	1.4	0.0	0	0
	Mongoose	1	2	3.1	1.4	37.8	3.0	1.4	39.0
	Langur	8	2.3	2.4	1.2	4.1	1.5	0.8	2.7
	Monkey	6	2.2	2.0	1	4.6	1.5	0.8	3.5
	Porcupine	2	2.1	2.0	1	12.8	0.0	0	0
	Civet	3	2.1	1.0	0.5	4.3	3.0	1.4	13.5
	Sub-total				**91.5**	**98.8**		**59.9**	**88.1**
Domestic	Cattle	180	8.3	3.1	5.8	0.9	16.7	31.5	5.0
	Buffalo	273	11.5	1.0	2.7	0.3	1.5	4.0	0.4
	Dog	20	2.7	0.0	0	0	7.6	4.6	6.5
	Sub-total				**8.5**	**1.2**		**40.1**	**11.9**
Total					**100**	**100**		**100**	**100**
	Wild Prey Category			**%**	**%**		**%**	**%**	
	Small prey			12	**5**		13	**9**	
	Medium prey			69	**59**		83	**83**	
	Large prey			19	**36**		4	**8**	
Total				**100**	**100**		**100**	**100**	
	Wild Vs Domestic			**%**	**%**		**%**	**%**	
	Wild prey			**96**	**92**		**73**	**60**	
	Domestic prey			**4**	**8**		**27**	**40**	
Total				**100**	**100**		**100**	**100**	

X = average body weight; Y = estimated weight of prey consumed per collectable scat produced; A = percentage of occurrence of prey items; D = relative biomass consumed by tiger or leopard; E = relative number of prey animals consumed. D = (A × Y)/∑ (A × Y); E = (D ÷ X)/ ∑(D ÷ X) (Ackerman et al., 1984).

Diet composition of the tiger (seasonally and habitat management situations)

The frequency of occurrence of spotted deer and hog deer in tiger scats was high in cold dry season compared to hot dry and monsoon season (Table 2.4); however, they were not significantly different (spotted deer: F = 3.22, d. f. = 2, P = 0.060; hog deer: F = 1.97, d. f. = 1, P = 0.150). Similarly, the frequency of occurrence of swamp deer in tiger scats was not different across seasons (F = 0.65, d. f. = 2, P = 0.525). However, the occurrence of wild boar was significantly different across seasons (F = 8.09, d. f. = 2, P = 0.002), being higher in the monsoon season than cold dry and hot dry seasons. Considering the frequency of occurrence of prey items before and after habitat management, that of spotted deer, hog deer and swamp deer were not different between two habitat management situations (spotted deer: F = 0.006, P = 0.934; hog deer: F = 0.0005, P = 0.981, swamp deer: F = 0.775, P = 0.384; d. f. = 1 for all three species), while, that of wild boar was significantly high before habitat management period from July to December (F= 5.61, d. f. = 1; P = 0.026) (Table 2.4). The frequency of occurrence of other prey species was similar under both habitat management situations.

Table 2. 4 Number and proportion (%) of prey species in the tiger diet in Shuklaphanta National Park (annual, seasonal, and during management situations).

Species	**Annual (N = 210)**		**Monsoon (N = 63)**		**Hot dry (N = 78)**		**Cold dry (N = 69)**		**Pre mgmt. (N = 95)**		**Post mgmt. (N = 115)**	
	n	%	n	%	n	%	n	%	n	%	n	%
Spotted deer	114	38.9	27	29	46	41.4	41	46.1	47	35.6	67	41.6
Hog deer	49	16.7	12	12.9	18	16.2	19	21.3	21	15.9	28	17.4
Swamp deer	39	13.3	13	14.0	13	11.7	13	14.6	18	13.6	21	13.0
Wild boar	24	8.2	17	18.3	2	1.8	5	5.6	20	15.2	4	2.5
Nilgai	15	5.1	8	8.6	6	5.4	1	1.1	8	6.1	7	4.3
Mongoose	9	3.1	2	2.2	6	5.4	1	1.1	2	1.5	7	4.3
Langur	7	2.4	4	4.3	1	0.9	2	2.2	4	3.0	3	1.9
Monkey	6	2	0	0.0	6	5.4	0	0.0	0	0.0	6	3.7
Porcupine	6	2	2	2.2	3	2.7	1	1.1	2	1.5	4	2.5
Civet	3	1	1	1.1	2	1.8	0	0.0	1	0.8	2	1.2
Cattle	9	3.1	2	2.2	4	3.6	3	3.4	4	3.0	5	3.1
Buffalo	3	1	2	2.2	0	0.0	1	1.1	2	1.5	1	0.6
Unknown	9	3.1	3	3.2	4	3.6	2	2.2	3	2.3	6	3.7
Total	293	100	93	100	111	100	89	100	132	100	161	100

(n = number prey items in the diet, N = number of scats analysed)

Prey selection by tiger and leopard (Annual)

Though two predators killed diverse prey, six species (spotted deer, hog deer, swamp deer, wild boar, nilgai and cattle) provided 92.2% of the biomass killed by a tiger and 87% by a leopard, so the diet selectivity investigation was restricted only to these principal prey components.

The multinomial likelihood ratio tests showed that both predators overall non-randomly selected prey species when prey availability was estimated based on individual density (tiger, $\chi2 = 518.03$; df = 5, P = 0.0001; leopard, $\chi2 = 61.55$; df = 5, P = 0.0018) as well as group density (tiger, $\chi2 = 80.99$; df = 5, P = 0.0647; leopard, $\chi2 = 76.04$; df = 5, P = 0.0011). Since there was evidence of selective predation among all prey species, selectivity for each prey species was then individually examined.

Tiger significantly preferred wild boar ($P < 0.01$) and nilgai ($P < 0.01$). Spotted deer, hog deer, swamp deer and cattle were utilized in proportion to their availability suggesting no selection ($P > 0.05$). Leopard demonstrated positive selection for spotted deer (P <

0.05) and wild boar (P <0.01); negative selection for swamp deer (P< 0.05) and no selection for hog deer, nilgai and cow (P > 0.05) (Table 2.5).

Overall, annual prey base selection by tiger and leopard based on Jacob’s index is graphically presented in Figure 2.4. The findings are generally similar to the multinomial likelihood ratio test results except for negative selection by the leopard for nilgai and cattle obtained using Jacob’s index.

Table 2. 5 Observed and expected selection among major prey by tiger and leopard in Shuklaphanta National Park based on likelihood ratio test using the SCATMAN program.

		Spotted deer	Hog deer	Swamp deer	Wild boar	Nilgai	Cattle
Tiger	Observed frequency	80.15	32.91	26.16	20.5	10.01	8.5
	Expected frequency	47.35	17.84	62.06	1.89	0.38	48.71
	$\chi2$	30.93	14.14	31.85	184.81	246.29	45.67
	P value	0.105	0.121	0.113	**0.000**	**0.000**	0.098
	Inference	+	+	-	**+ ***	**+ ***	-
Leopard	Observed frequency	22.50	4.5	1	4.5	0.0	10.5
	Expected frequency	11.42	4.3	14.97	0.46	0.09	11.7
	$\chi2$	14.62	0.009	20.00	36.17	0.091	0.183
	P value	**0.045**	0.946	**0.028**	**0.000**	0.772	0.850
	Inference	**+ ***	+	**- ***	**+ ***	-	-

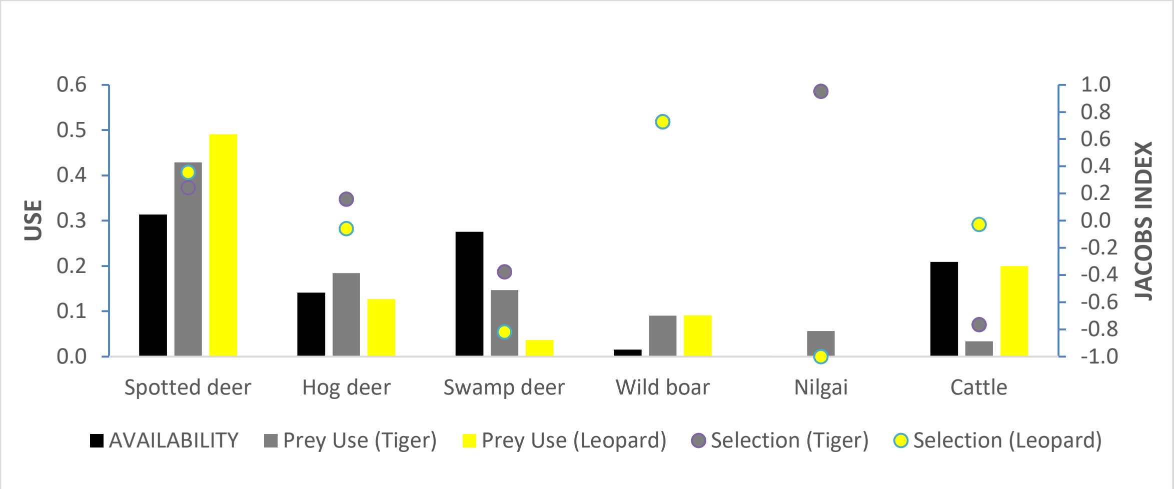

Figure 2. 4 Relative prey availability, prey use and prey selection (Jacobs index) of tiger and leopard in Shuklaphanta National Park.

Prey selection by tiger (seasonal, pre-and post-management)

SCATMAN analysis results depicting prey selection across seasons and management durations are shown in Table 2.6 and 2.7 respectively. Tiger preferred spotted deer in all seasons and management situation. Hog deer selection was also positive, but was not statistically significant across seasons (monsoon, P = 0.705; hot dry, P = 0.108, cold dry, P = 0.061) and between management durations (pre-management, P = 0.267; post-management, P = 0.086). Statistically non-significant avoidance (negative selection) was seen for swamp deer (monsoon, P = 0.178; hot dry, P = 0.139; cold dry, P = 0.158; pre management, P = 0.118; post management, P = 0.185) and cattle (monsoon, P = 0.088; hot dry, P = 0.132; cold dry, P = 0.107; pre management, P = 0.110; post management, P = 0.113) in all seasons and management situations. Tiger preferred wild boar and nilgai in which selection of wild boar was highly significant in monsoon (P = <0.001) and pre management situation (P = <0.001). Similarly, selection of nilgai was highly significant in all seasons (monsoon, P = <0.001; hot dry, P = <0.001) and management situations (pre management, P = <0.001; post management, P = <0.001) except cold dry season (P = 0.345). Prey base selection by tiger based on Jacob's index among seasons and between management situations is graphically presented in Figure 2.5 and 2.6 respectively. The findings are similar to the multinomial likelihood ratio test results.

Table 2. 6 Observed and expected selection among major prey by the tiger in three seasons of Shuklaphanta National Park, based on likelihood ratio test using the SCATMAN program.

Seasons		**Spotted deer**	**Hog deer**	**Swamp deer**	**Wild boar**	**Nilgai**	**Cattle**
Monsoon	Observed frequency	16.66	6.58	8.33	15	5.18	1
	Expected frequency	14.01	5.28	18.37	0.56	0.11	14.42
	χ2	0.68	0.35	8.41	376.07	230.38	17.18
	P value	0.678	0.705	0.178	**0.000**	**0.000**	0.088
	Inference	+	+	-	**+ ***	**+ ***	-
Hot dry	Observed frequency	31.66	12.5	8.33	1	4.33	3.5
	Expected frequency	16.29	6.14	21.35	0.65	0.13	16.76
	χ2	19.74	7.32	12.18	0.188	136.10	14.43
	P value	**0.048**	0.108	0.139	0.700	**0.000**	0.132
	Inference	**+ ***	+	-	+	**+ ***	-
Cold dry	Observed frequency	31.83	13.83	9.5	4.5	0.5	3
	Expected frequency	16.78	6.32	21.99	0.67	0.13	17.26
	χ2	18.38	9.91	10.88	22.08	1.00	16.21
	P value	0.057	0.061	0.158	**0.0005**	0.345	0.107
	Inference	+	+	-	**+***	+	-

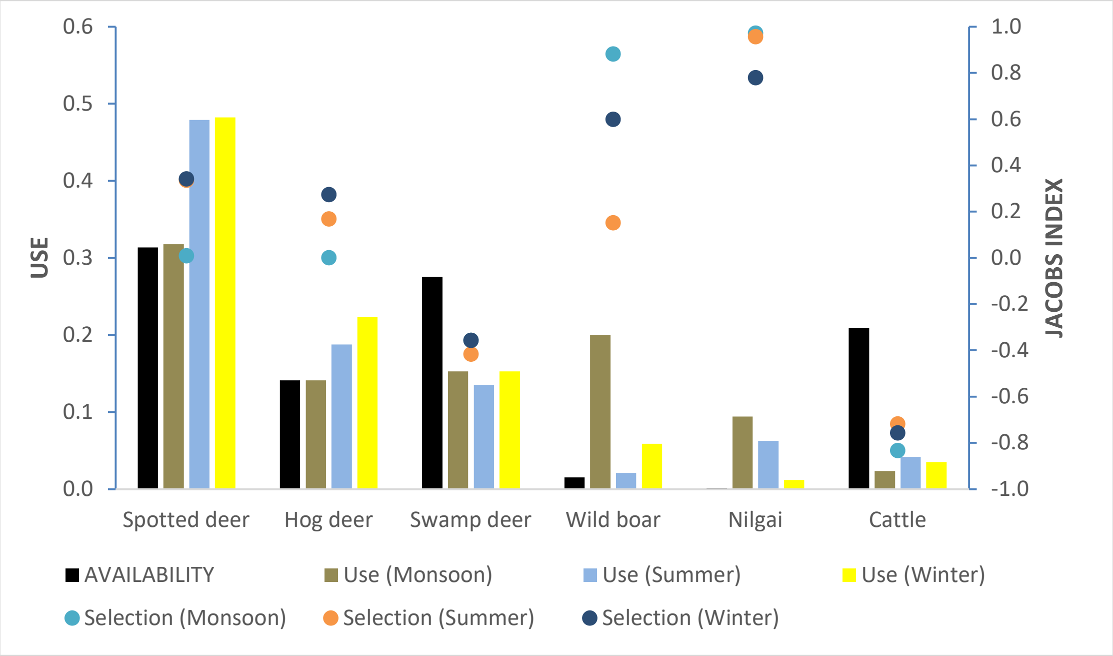

Figure 2. 5 Relative prey availability, prey use and prey selection (Jacobs index) of the tiger in Shuklaphanta National Park (seasonally).

Table 2. 7 Observed and expected selection among major prey by the tiger during pre- and post-management periods of Shuklaphanta National Park, based on likelihood ratio test using the SCATMAN program.

		Spotted deer	**Hog deer**	**Swamp deer**	**Wild boar**	**Nilgai**	**Cattle**
Pre-Management	Observed frequency	33.16	14.08	11.33	18	5.18	4
	Expected frequency	22.78	8.58	29.86	0.91	0.18	23.44
	$\chi 2$	6.43	3.91	17.63	324.03	137.84	22.18
	P value	0.305	0.267	0.118	**0.000**	**0.000**	0.110
	Inference	+	+	-	**+ ***	**+ ***	-
Post Management	Observed frequency	46.99	18.83	14.83	2.50	4.83	4.50
	Expected frequency	24.57	9.26	32.20	0.98	0.20	25.28
	$\chi 2$	27.86	11.00	14.37	2.36	109.85	23.49
	P value	**0.046**	0.086	0.185	0.194	**0.000**	0.113
	Inference	**+ ***	+	-	+	**+ ***	-

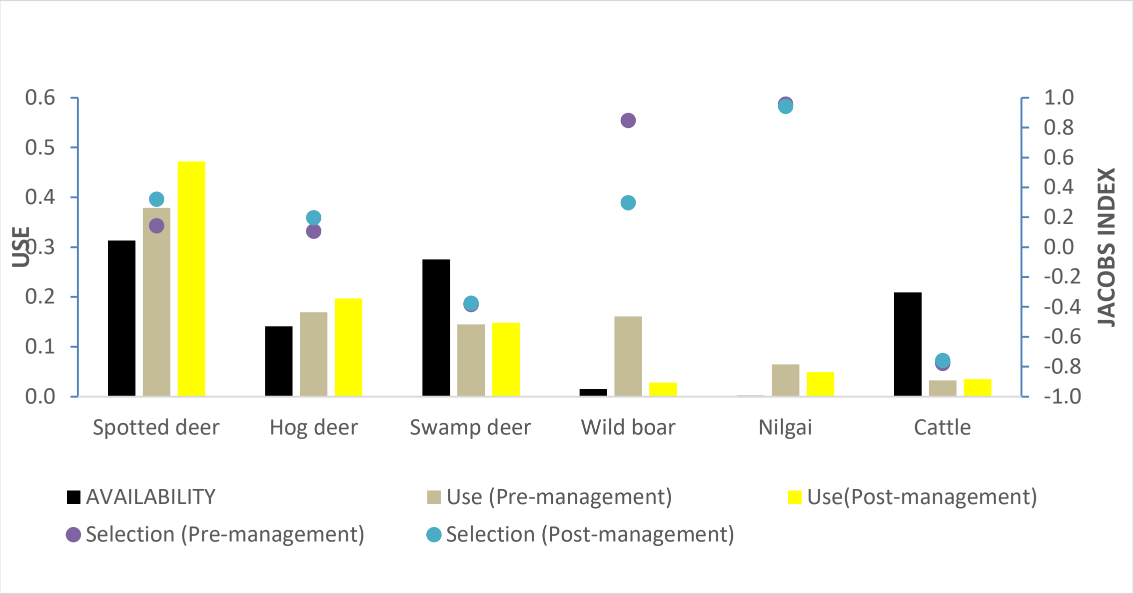

Figure 2. 6 Relative prey availability, prey use and prey selection (Jacobs index) of the tiger in Shuklaphanta National Park (habitat management situations).

2.5 Discussion

Density and biomass of prey species

Comparison of prey densities estimated in the present study with that of other areas in South Asia (Table 2.8 & 2.9) revealed that SNP (Shuklaphanta) holds a high density of ungulate prey, mainly swamp deer, spotted deer and hog deer. Especially, swamp deer density in SNP is the highest among all areas harbouring this species. The study area has large extensive grasslands and high habitat heterogeneity created due to the assemblage of grasslands patches interspersed within open canopied forests favouring a high density of ungulate deer species. The density of nilgai, langur and monkey is probably underestimated because these animals were poorly represented on transects.

Table 2. 8 Densities of wild prey species from tiger bearing Protected Areas (PA) in South Asia

Areas	Spotted deer	Hog deer	Swamp deer	Wild boar	Nilgai	Langur	Monkey	Cattle
Shuklaphanta - NP (Present study)	42.7	19.2	37.5	2.1	0.2	2.1	2.1	28.5
Shuklaphanta - NP (Lovari et al., 2015)	41.0	11.6	32.3	1.8	0.1	6.0	9.5	48.5
Bardia – NP (DNPWC & DFSC, 2018)	56.4	13.2[a]	0.4[a]	2.04	0.3[a]	15.2	5.47[b]	NA
Chitwan – NP (DNPWC & DFSC, 2018)	43.8	13.4	NA	3.8	NA	12.78[c]	15.14[C]	NA
Parsa - NP (DNPWC & DFSC, 2018)	8.8	NA	NA	4.9	NA	NA	NA	NA
Pench - IN (Biswas & Sankar, 2002)	80.8	NA	NA	2.6	0.4	77.2	NA	NA
Ranthambhore - IN (Bagchi et al., 2004)	31	NA	NA	9.7	11.4	21.7	NA	NA
Kaziranga - IN (Karanth & Nichols, 1998)	NA	38.6	14.2	2.6	NA	NA	NA	NA
Anamalai - IN (Kumaraguru et al, 2011)	20.5	NA	NA	20.6	NA	NA	NA	NA
Sariska - IN (Mondal et al., 2011)	33.8	NA	NA	54.1	42.7	50.6	NA	28.03
Nagarhole - IN (Karanth & Sunquist, 1992)	50.6	NA	NA	4.2	NA	23.8	NA	NA
Kanha - IN (Karanth & Nichols, 1998)	49.7	NA	3.0	2.5	NA	NA	NA	NA
Mudumalai - IN (Ramesh et al., 2012)	43.8	NA	NA	NA	NA	31.0	NA	NA
Rajaji - IN (Harihar et al., 2011)	51.0	NA	NA	2.9	1.7	15.4	NA	16.67

NP = Nepal; IN = India; a (Wegge et al., 2009); b (Dhakal et al., 2014); c (Bhattarai & Kindlmann, 2012); NA = either absent or not found during the survey.

Domestic ungulate buffalo was not encountered in the present study; also, the density estimate of cattle in the present study was smaller than the previous estimate in 2010 and 2011 (Lovari et al., 2015). This might be because the current research focused on the western portion of SNP, where grazing by domestic ungulates is less than that of the eastern portion of the park. Like SNP, domestic animals graze other protected areas of South Asia, and the estimate of SNP is similar to Sariska while more than Rajaji (Table 2.8). Considering biomass density, comparisons with other areas revealed that SNP has the third-highest prey biomass density after Sariska and Anamalai (Table 2.9). The high biomass density in these areas is due to the high density of large-size ungulate prey like swamp deer in SNP; nilgai and sambar in Sariska; Nilgiri tahr, gaur and sambar in Anamalai. The present estimate of prey in terms of total individual and biomass density in SNP is similar to the findings of previous studies (Lovari et al., 2015; Yadav, 2006).

Table 2. 9 Density and biomass of major wild prey species of large predators in the Indian Subcontinent.

Areas	**Density**	
	Wild prey Individual (animals/km^2)	Biomass (kg /km^2)
Shuklaphanta – NP (Present study)	105.9 (101.7)	9224.9
Shuklaphanta – NP (Lovari et al., 2015)	109.8 (85.8)	8073.5
Shuklaphanta – NP (Yadav, 2006)	(108.32)	9520
Chitwan – NP (DNPWC and DFSC, 2018)	(74.85)	5280.47
Bardia – NP (DNPWC and DFSC, 2018)	74.98 (59.96)	3615.64
Parsa – NP (DNPWC and DFSC, 2018)	(15.91)	1137.32
Banke – NP (DNPWC and DFSC, 2018)	(8.1)	NA
Ranthambhore – IN (Bagchi et al., 2003,2004)	96.65 (74.9)	6263
Pench – IN (Biswas & Sankar, 2002)	167.65 (90.49)	6013.25
Kaziranga – IN (Karanth & Nichols, 1998; Biswas & Sankar, 2002)	(58.1)	4252
Anamalai – IN (Kumaraguru et al., 2011)	(74.16)	14204
Sariska – IN (Mondal et al., 2011)	207.77 (157.1)	15458.6
Nagarhole – IN (Bagchi et al., 2004; Karanth & Sunquist (1992)	103.4 (74.1)	7638
Kanha – IN (Karanth & Nichols (1998)	(57.3)	3635.5
Mudumalai – IN (Ramesh et al. (2012)	86.4 (55.4)	6491.8
Rajaji – IN (Harihar et al., 2011)	81.83 (66.43)	5357

NP = Nepal; IN = India; NA = not calculated; values in the bracket indicate the individual density of wild ungulate species only.

Diet and Prey Selection

Information concerning the food habits of large predators is central to understanding the ecological niche they occupy (Kumaraguru, Saravanamuthu, Brinda, et al., 2011). Food habits vary depending on habitat conditions and the availability of prey species. The present study in SNP showed that the tiger diet includes an assemblage of medium and large-sized wild prey. However, the diet of leopard mainly consists of medium-sized wild prey as well as domestic prey. The tiger generally consumed spotted deer, hog deer, swamp deer, wild boar and nilgai. The leopard mostly consumed spotted deer, cattle, hog deer, and wild boar and domestic dog. In terms of relative biomass contribution, spotted deer was the dominant prey species for both tiger and leopard, followed by swamp deer for tiger but cow for leopard. For both predators, spotted deer was the dominant prey species consumed, which may be due to its abundance and wide distribution (Karki 2011; Lovari et al., 2015). Spotted deer is ubiquitous in distribution and uses varied habitat ranging from grassland to forest.

In general, spotted deer, wild boar and hog deer are the most abundant prey species for tiger and leopard in Nepal (Bhandari et al., 2017; Bhattarai et al., 2012; Karki 2011; Stoen & Wegge 1996). However, in the SNP, swamp deer additionally contributed 23% and 5% of total biomass for tiger and leopard diet, respectively. The cattle were found surprisingly high in leopard scat and stand as a second position in terms of frequency of occurrence of hair remains, and biomass consumed. Grey (2009) reported that domestic animals contributed 3.56% of the total diet of the tiger in BNP. However, in this study, domestic animals contributed 8.5% to the total biomass of the tiger. High livestock densities within protected areas are also believed to increase the chances of domestic animals being preyed upon by predators (Sekhar, 2003). Sunquist (1981) reported that when livestock is available, the tiger will readily prey on it. Domestic animals (cow, buffalo, and dog) in large predator scat indicate possible conflict between people and wildlife and might be challenging for wildlife conservation in human-dominated areas (Bhandari & Chalise, 2016; Bhandari et al., 2017; Lovari et al., 2015).

Pianka's diet overlap index between tiger and leopard in SNP is similar with the findings in other areas such as Chitwan: 0.90 (Lamichhane et al., 2019), Shuklaphanta: 0.85 (Lovari et al., 2015), Sariska: 0.94 (Mondal et al., 2012), Mudumalai: 0.72 – 0.82 (Ramesh, Snehalatha, Sankar, et al., 2009), Rajaji: 0.77 – 0.89 (Harihar et al., 2011), Bhutan: 0.92 (Wang & Macdonald, 2009), Bandipur: 0.84 (Andheria, Karanth, & Kumar, 2007), Nilkeri, Nagarhole: 0.94 (Karanth et al., 1995).

The interference among large sympatric carnivores tends to increase with taxonomic relatedness, diet overlap and decreasing body size differences between competitors (Donadio & Buskirk, 2006; Palomares et al., 1999). However, they coexist by avoiding competitive interactions through the mechanism of diet, space and/or temporal partitioning. As the tiger is larger than the leopard and the former is dominant over the latter, they will coexist through the partitioning process.

Tiger preys on medium to large-sized prey, whereas leopard kills small to medium-sized prey (Andheria et al., 2007; Jarman, 1974; Karanth et al., 1995, 2000; Kumaraguru et al., 2011). These differences in prey selection help the two predators to coexist (Karanth et al., 1995, 2000). In the present study, the diet overlap was quite high (90%); however, the tiger, more often, killed larger preys than the leopard did (Figure 6a & 6b, 7a & 7b). Though there existed competition for food exploitation, diet partitioning was still prevalent. Large-sized prey (e.g., swamp deer) were used far more often by the tiger than by the leopard. Though small-sized preys were not much represented in leopard scats, medium-sized prey, mainly spotted deer, was maximally consumed by both predators indicating competitive exploitation. However, it looks like high density and evenly distribution of spotted deer throughout all habitat types might also help the coexistence of two predators.

In this study, a large diet overlap between tiger and leopard suggests temporal and/or spatial partitioning might be occurring apart from food partitioning alone, allowing the coexistence of two predators. Smaller predators can alter their activity and movements to minimize the risk of encounters with dominant predators (Durant, 1998; Harrington, Harrington, Yamaguchi, et al., 2009; Vanak et al., 2013). Karanth & Sunquist (2000), in a study in the tropical forests of Nagarahole, southern India, found an extensive temporal overlap suggesting the absence of temporal separation of predatory activities between tiger and leopard. In a recent study in CNP, Nepal, Lamichhane et al. (2019) documented leopard temporally avoiding tiger with leopard being more active during the day in the presence of a tiger. However, Pokheral and Wegge (2019) found no significant temporal separation between tiger and leopard in the present study area. They suggested that this might be due to the low density (≤ 3 /100 km^2) of both predators in SNP. There are reports on spatial partitioning between these two cats in which tiger displaced leopard to areas underutilized by tiger (Harihar et al., 2011; Odden et al., 2010; Pokheral et al., 2019). Tiger was more concentrated in the core and relatively undisturbed habitat with a high density of large-sized ungulates. However, the leopard was displaced towards marginal degraded habitat where local communities graze their cattle (Lamichhane et al., 2019). My study confirmed this spatial partitioning of two predators through habitat segregation. The tiger diet constituted more large-sized wild prey while the leopard had more domestic cattle in their diet. Also, leopard scats were more prevalent in the fringe habitat and tiger scats in core habitat.

This study suggests that in SNP, two large predators may coexist due to high prey density and food and spatial partitioning. More precisely, the high density of large-sized prey, mainly swamp deer, and medium-sized prey, mostly spotted deer, is the key to the coexistence of two cats in the SNP.

Chapter 3 Home range and habitat use of swamp deer or barasingha (*Rucervus duvaucelii duvaucelii*) in Shuklaphanta National Park, Nepal

3.1 Abstract

Shuklaphanta National Park (SNP), one of the tigers bearing protected area of Terai Arc Landscape (TAL), represents about one-third of the world's population of swamp deer or barasingha. I used VHF radio-collars on 9 swamp deer individuals (3 male and 6 female) to study their home range, habitat preference and trans-border movement. The average annual 95% Minimum Convex Polygon (MCP) and 95% Fixed Kernel (FK) home ranges were 22.90 (SE 3.64) km^2 (range 4.00 - 31.96) and 15.88 (SE 2.62) km^2 (range 4.46 - 27.87) respectively. There was no significant variation between 95% FK annual home range for male (20.86 km^2, SE 4.98) and female (13.39 km^2, SE 2.79). The 95% MCP home range in the hot dry season was significantly larger than in the cold dry and monsoon seasons. However, there was no variation in 95% FK home ranges across the seasons. Overall, the grassland was the most preferred habitat in all the seasons and the dense Sal forest, the least preferred. The Sal forest of moderate density was the second most preferred habitat after the grassland in the monsoon season. The swamp deer presence was highly associated with grassland that contained *Imperata cylindrica* assemblage followed by *Imperata cylindrica – Narenga porphyrocoma* and *Narenga porphyrocoma* assemblage. The radio-collared barasingha crossed the border and used contiguous habitat patches of the Lagga-Bagga area of Pilibhit Tiger Reserve (PTR), India, mainly in the rutting season. This cross-border movement emphasises the need for transboundary cooperation between Nepal and India to protect threatened wild animals better across TAL.

3.2 Introduction

Understanding the relationship between animals and their habitats is crucial for conserving animals and managing their habitats (Morrison, Marcot, & Mannan, 2006). The pattern of habitat use by animals is determined by the spatial arrangement of habitat patches that vary with habitat quality, including food abundance (Deacon & Smit, 2017; Lawson & Rodgers, 1997). Other factors that affect habitat use and home range size are body mass (Odden & Wegge, 2007), seasonal changes in the environment, and breeding status (Aung, McShea, Htung, et al., 2001). This study focusses on habitat selection of swamp deer, a main tiger prey species, playing a vital role in the coexistence of tiger and leopard through diet partitioning (Chapter 2). The knowledge of habitat selection by animals is essential as it aids in concentrated conservation efforts on protection or restoration of habitats that are important to the target animal species (Hull, Zhang, Huang, et al., 2016).

Earlier studies reported different findings regarding swamp deer habitat use. Schaaf (1978) documented that swamp deer of Shuklaphanta confined mostly to grasslands and avoided forest habitats consistently throughout all seasons. In Kanha, swamp deer had spatially distinct dry and monsoon season home range and exhibited a preference for grasslands associated inside the Sal forest (Martin, 1977). In Dudhwa, too, swamp deer showed seasonal ranges. Between late winter and summer, swamp deer utilized the grasslands within the park, however between monsoon and early winter, the deer moved about 2km and lived predominantly in agricultural areas (Sankaran, 1989). Swamp deer in Jhilmil Jheel preferred mostly hydrophytic habitat and showed different preferences in monsoon, summer and winter seasons (Tiwari, 2009).

This study investigated the home range, habitat preference, and trans-border movement of swamp deer in SNP, Nepal, based on VHF telemetry tracking of nine swamp deer. The value of the SNP for swamp deer conservation is immense due to its largest population size and contiguous habitat with the Lagga-Bagga area of Pilibhit Tiger Reserve (PTR), India. Swamp deer population of SNP represents one-third of the world population (Schaaf, 1978). At a landscape level, SNP is one of the protected area members of 49,500 km^2 Terai Arc Landscape (TAL), consisting of six protected areas of Nepal and nine of India. TAL is recognised as a conservation landscape of global importance. It is envisioned to restore connectivity of isolated habitats in Nepal and India for providing dispersal corridors and migration routes for tiger, rhino, elephant and many other species, including swamp deer, which are crucial for the functioning of an ecosystem (Chanchani et al., 2014). Therefore, understanding home range, habitat preference, and trans-border movement of swamp deer are important for formulating conservation-oriented management strategies to restore an ecosystem of higher prey biodiversity and abundance in which big predators and human can better coexist.

3.3 Methods

Ethics statement

I obtained permission to capture and collar swamp deer from Ministry of Forest and Soil Conservation, Nepal, under the National Parks and Wildlife Conservation Act 1972, after the recommendation from the technical committee established at Department of National Parks and Wildlife Conservation (DNPWC). The capture and collaring of swamp deer were performed under the supervision of veterinary officer mobilized by the Chief Conservation Officer of SNP.

Study site

For details of the study site, see methods section of Chapter 2.

Field Methods

Collaring of swamp deer

I used cotton nets (Dhungel & O'Gara, 1991; Moe & Wegge, 1994; Odden et al., 2007; Odden, Wegge, & Storaas, 2005a) to capture the swamp deer. Fifteen nets, each 10 m long and 3 m high, were erected with a flexible wooden stick on swamp deer movement routes within the study area. They were camouflaged under the tall grass cover dominated by *Narenga porphyrocoma*. The captures were conducted in the morning before 7 AM from 20 May 2015 to 17 June 2015. Groups of swamp deer were herded towards the net with the help of elephants positioned at strategic locations. Once the deer was caught, the erected wooden sticks would fell, and animals would be wrapped in the net without causing any injuries. For reducing stress to the captured animal, the eye and the ear were covered with cloth. Immediately, a blood sample was collected from each captured deer and VHF radio collars fitted (Telonics, MOD-500-2, and weight - 265 g) before it was released (Table 3.1). To reduce the stress, morphological measurements were not taken, and the handling process of each deer did not exceed five minutes. During the first months after the collaring, the data collected were not included in analyses to minimise the possible effect of capture on their behaviour. The transmitters had a detect range of about 2 km from the ground (3-4 km from an elevated position) and an average battery life of >2 years. Radio-collared deer were located at least four days/week from dawn to dusk using a 4-element Yagi antenna and a 12-

channel radio receiver. Tracking was done on foot, motorbike and vehicle during dry seasons and the backs of elephants during monsoon season. As far as possible, the deer was located by direct sighting and locations was taken once the deer had moved. When deer fled before the visual sighting, locations were only recorded if deer were estimated to have been less than 50m of the observer before it ran. For each located deer, date, time, habitat type, dominant plant species and if seen, behaviours, group size composition, plant species consumed were recorded.

Table 3. 1 Details of radio-collared swamp deer in Shuklaphanta National Park.

Collaring date	**Sex**	**Animal ID**	**Frequency (MHz)**	**Monitoring period**	**Remarks**
25th May 2015	Female	1 F	150.37000	26 July 2015 to 15 December 2016	
25th May 2015	Female	2 F	150.38000	26 July 2015 to 15 December 2016	
25th May 2015	Male	3 M	150.39000	23 July 2015 to 15 December 2016	
25th May 2015	Female	4 F	150.41000	24 July 2015 to 15 December 2016	
15th June 2015	Female	5 F	150.42000	24 July 2015 to 24 September 2015	Killed by a tiger in the third week of September 2015
16th June 2015	Female	6 F	150.54000	23 July 2015 to 15 December 2016	
16th June 2015	Female	7 F	150.55000	23 July 2015 to 15 December 2016	
17th June 2015	Male	8 M	150.57000	28 July 2015 to 15 December 2016	
17th June 2015	Male	9 M	150.58000	24 July 2015 to 15 December 2016	
17th June 2015	Female	10 F	150.59000	23 July 2015 to 15 December 2016	
17th June 2015	Female	11 F	160.100	23 July 2015 to 29 December 2015	Did not transmit signal from 29th December 2015
17th June 2015	Female	12 F	160.500		Did not work

Habitat mapping of the study area

I used 30M resolution cloud-free Landsat 8 satellite imagery from November 2015 downloaded from the USGS Earth Explorer website (https://earthexplorer.usgs.gov/) for the habitat map preparation of the study area. After the ground validation, I first did the classification unsupervised, followed by supervised classification. Then, I used semi-automatic classification plugin (https://plugins.qgis.org/plugins/SemiAutomaticClassificationPlugin/) for the preprocessing and post-processing of Landsat image. Finally, I followed the TAL forest classification report for the criteria of classification of the habitat of the study area (Joshi et al., 2003).

Home range

I used two non-parametric methods: the Minimum Convex Polygon (MCP) (Mohr & Stumpf, 1966) and the Fixed Kernel (FK) (Worton, 1989) for the estimation of annual and seasonal home ranges of collared swamp deer. I calculated 95% MCP and 95% FK home ranges to minimise the effect of animals' outlier and exploratory movement. I determined the adequacy of the number of fixes for home range estimates (Harris, Cresswell, Forde, et al., 1990) by plotting home range sizes against the number of fixes incremented (Kernohan et al., 2001; Worton, 1989). I used GME (version 0.7.4.0) software for the estimation of 95% FK home range (Beyer, 2015) and QGIS (http://qgis.osgeo.org) for 95% MCP home range. One-way ANOVA was used to test whether the home range size varies with sex and season.

Habitat use and preference

I used compositional analysis (Aebischer, Robertson, & Kenward, 1993) to compute the swamp deer's annual and seasonal habitat preference. This analysis considers habitat use as the percentage of locations in each habitat type (Aebischer et al., 1993; White & Garrott, 1990). The 95% FK home range was considered habitat available from which swamp deer has to choose different habitat types. I used ArcGIS 10.3.1 version (http://www.esri.com) to compute the available habitat and the percentage of locations in each habitat types within 95% FK home range. Each swamp deer was considered as a sample for statistical analysis (Garton et al., 2001). Similarly, I used Compos Analysis, version 6.3, plus software for compositional analysis (Smith, 2010). Ivlev's Index (Ivlev, 1961) was used for graphical presentation of habitat preference of individual swamp deer within its 95% FK home range.

To further assess the association of swamp deer with different grassland assemblages, I used 8.5 X 8.5 m sampling plot to survey percentage cover of plant species (Lehmkuhl, 1994; Peet et al., 1999). First, I collected information on grass species composition from 621 plots (cold dry season = 178; hot dry season = 266; monsoon season = 177) where the collared swamp deer were sighted. Then, I calculated the annual and seasonal relative frequency of grassland assemblages identified based on key recommendations for Nepalese subtropical grassland (Lehmkuhl, 1994; Peet et al., 1999).

Movement and Trans-border movement

Annual movements of swamp deer were calculated from 95% MCP home range by measuring straight line distance between two most distant fixes on the polygon. One-way ANOVA was used to check whether there were differences in movement between sexes.

The trans-border movement was analyzed from the fixes of the radio-collared swamp deer. First, I obtained the location of collared animals across the Nepal-India border by triangulation method (Moe et al., 1994). These locations were then plotted on the map, and coordinates were obtained for further analysis. Next, I used ArcGIS 10.3.1 version (http://www.esri.com) to calculate the percentage of the home range of individual animals falling towards the Indian side. Finally, I counted the groups which were nearby the border with the help of binocular. From the mid cold dry season (January) to the hot dry season (till June), when the visibility in the forest is suitable as a result of fire, enabled to count the animals accurately.

3.4 Results

Habitat Map

The following habitat classes were identified (Table 3.2, Figure 3.1)

Table 3. 2 Habitat classes in the study area of Shuklaphanta National Park.

S.N.	Habitat	Area (km^2)	%	Description of habitat
1	Agriculture	9.7	5.01	These are mainly towards the western side of the SNP, mainly in the fringe area and Mahakali river. Mainly composed of invasive vegetation, e.g., Ipomoea species
2	Degraded Forest (DF)	2.3	1.19	These are the scrub type of vegetation with scattered tree species.
3	Dense Mixed Forest (DMF)	9.4	4.85	This forest type has 86% mean canopy closure, 16.1 % mean basal area, and 9.5% mean ground coverage. The primary forest species consists of deciduous trees like *Terminalia tomentosa*, *Adina cordifolia*, *Schleicheria oleosa*
4	Dense Sal Forest (DSF)	12.1	6.25	This forest type has 83.6 % mean canopy closure, 25.4 % mean basal area and 34.9% ground coverage. Sal (*Shorea robusta*) is the dominant tree species. Other associated species are *Terminalia tomentosa*, *Terminalia chebula*, *Terminalia belerica*, *Lagerstroemia parviflora, Garuga pinnata, Cassia fistula, Adina cordifolia, Phyllanthus emblica* etc.
5	Grassland (GL)	57.8	29.85	It consists of **short grassland (or phanta)** (less than 2m tall) dominated by *Imperata cylindrica*, **tall grassland** with species such as *Saccharum spontaneum*, *Saccharum bengalense*, *Phragmites karka*, *Typha elephantine*, *Narenga porphyrocoma*, *Themeda* spp. and **wooded savannas** like grassland having sparsely distributed tree species of *Bombax ceiba, Butea monsoperma* and *Dalbergia sissoo* and the grass species such as *Imperata cylindrica, Saccharum spontaneum, Saccharum bengalense, Cymbopogan* spp., *Narenga porphyrocoma, Desmostachya bipinnata, Cyperus* spp. and *Cynodon dactylon.*
6	Moderately Dense Mixed Forest (MDMF)	19.5	10.07	Tree species composition same as dense mixed forest, but mean canopy closure is 57.8 %, basal area 16.5 % and ground cover 29.4%.
7	Moderately Dense Sal Forest (MDSF)	27.6	14.25	Tree species composition same as dense Sal forest, but mean canopy closure is 72.2 %, basal area 22.1 % and ground cover 36.1%.
8	Riverine Forest (RF)	27.00	13.94	Mostly evergreen trees: *Mallotus philippensis Syzygium cumini*, *Ficus* spp.
9	Sand Exposed Surface (SES)	19.23	9.93	Includes the exposed, sandy and marshy area
10	Waterbody (WB)	9.0	4.65	Includes all areas with flowing or standing water like rivers, ponds, lakes and streams.
Total		193.63	100.00	

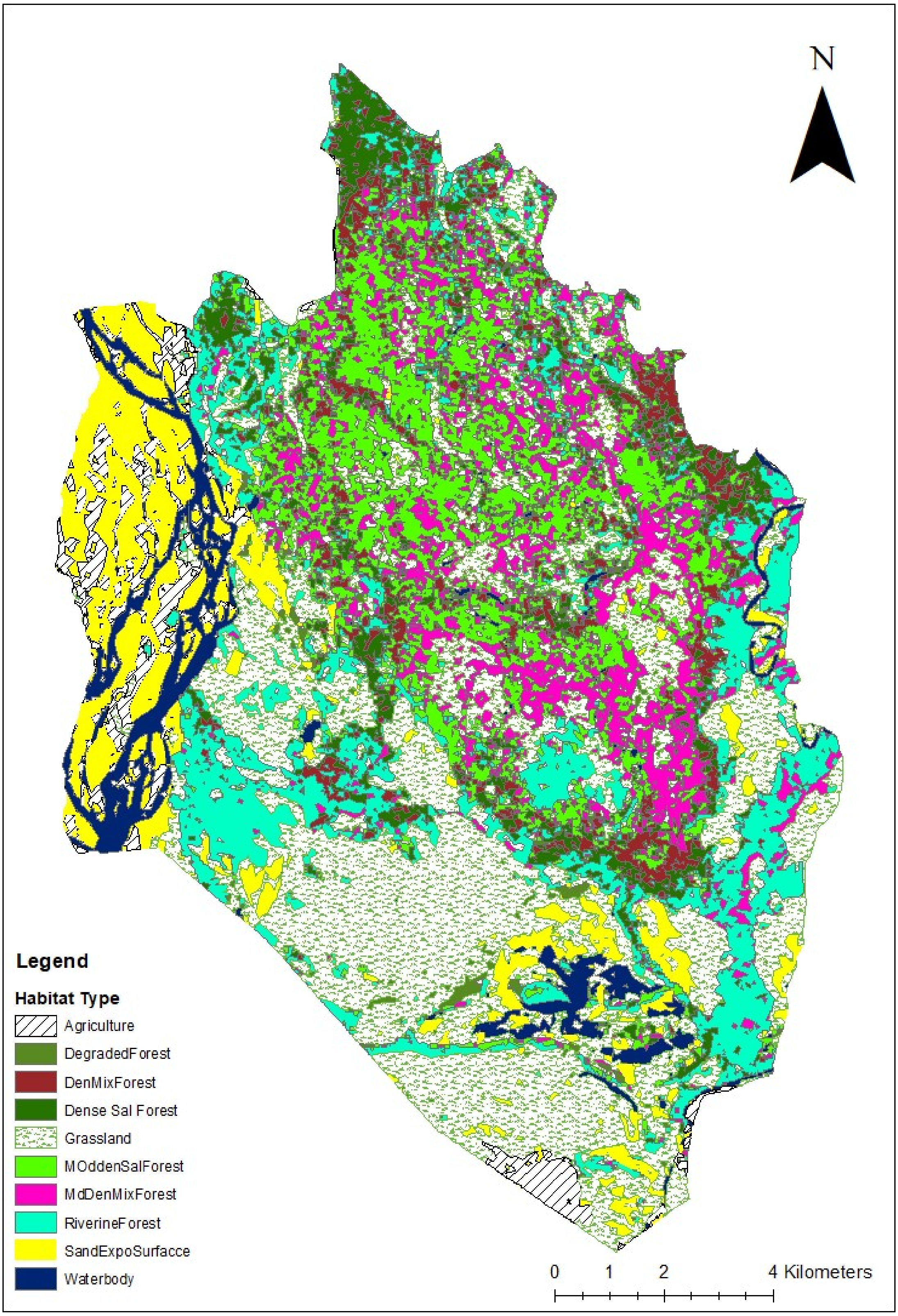

Figure 3. 1 Habitat classification of the study area in Shuklaphanta National Park.

Home ranges of swamp deer

Among 12 radio-collared swamp deer, the ID 5F was killed by the tiger. The ID 11F did not transmit signals after some weeks and, ID 12F did not transmit immediately after the collaring (Table 3.1). As fixes from above three deer were relatively few and were not included in further analyses. The number of tracking days for rest of nine swamp deer individuals (six females and three males) varied from 150 to 200.

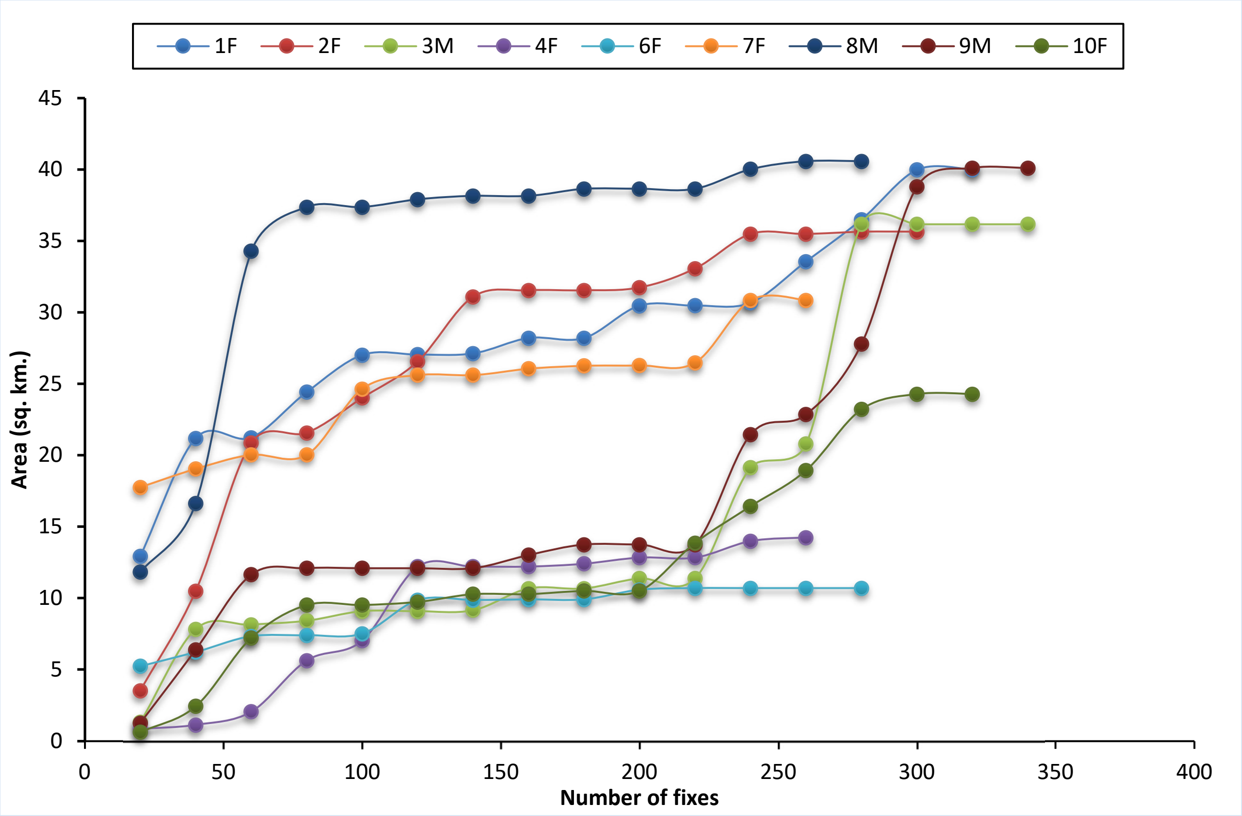

Figure 3. 2 Areas (95% MCP) of home ranges of swamp deer achieved with the number of fixes in Shuklaphanta National Park.

On average, home ranges reached an asymptote at 260 fixes (Figure 3.2), and the number of fixes used for home range estimations was 313 ± 26 for males and 290 ± 11 for females. Average sizes of the annual home ranges based on MCP were 28.22 ± 6.07 km^2 for males and 20.24 ± 4.49 km^2 for females (Table 3.3) and varied greatly among individuals of the same sex (males, 19.67 – 39.96 km^2; females, 4.00 – 32.12 km^2) (Figure 3.3). There were no differences between sexes in annual home ranges ($F = 1.07$, d. f. = 1, $P = 0.333$), but home ranges differed significantly between seasons ($F = 7.02$, d. f. = 2, $P = 0.003$), larger in the hot dry season (Table 3.3). There were no differences in home ranges of male seasonally ($F = 2.61$, d. f. = 2, $P = 0.152$); however, home ranges differed significantly for females across seasons, being larger in the hot dry season ($F= 5.45$, d. f. = 2, $P = 0.016$) (Table 3.3).

Based on FK method, average annual home range sizes of males and females were 20.87 ± 4.98 km^2 and 13.39 ± 2.80 km^2 respectively and did not differ between sexes ($F = 2.03$, d. f. = 1, $P = 0.196$) and among seasons ($F= 0.08$, d. f. = 2, $P = 0.915$) (Table 3.3). However, annual home ranges were highly varied among individuals of males (11.23 – 27.87 km^2) and females (4.46 – 22.77 km^2) (Figure 3.4). There were no differences in seasonal home ranges of males ($F= 0.31$, d. f. = 2, $P = 0.740$) and females ($F= 0.06$, d. f. = 2, $P = 0.939$) (Table 3.3).

The average annual and seasonal home ranges of all individuals ($n = 9$) based on 95% MCP and 95% FK method varied, however, they were not significantly different (annual, $F = 2.44$, d. f. = 1, $P = 0.137$; cold dry, $F = 3.18$, d. f. = 1, $P = 0.093$; hot dry, $F = 2.92$, d. f. = 1, $P = 0.106$; monsoon, $F = 2.80$, d. f. = 1, $P = 0.113$) (Figure 3.5).

Table 3. 3 Annual and seasonal home range size (km^2) of males (n = 3) and females (n = 6) swamp deer based on 95% MCP and 95% FK methods in Shuklaphanta National Park.

Season	**No. of fixes**	**95% MCP**				**95% FK**			
		Males (n =3)		Females (n = 6)		Males (n = 3)		Females (n = 6)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cold-dry	526	8.54	1.48	3.59	1.17	15.71	3.45	6.59	1.78
Hot-dry	1774	19.51	4.96	11.36	3.33	10.80	1.90	7.4	1.80
Monsoon	380	7.03	5.15	2.38	0.77	12.34	6.66	6.92	1.23
Annual	2680	28.22	6.07	20.24	4.49	20.87	4.98	13.39	2.80

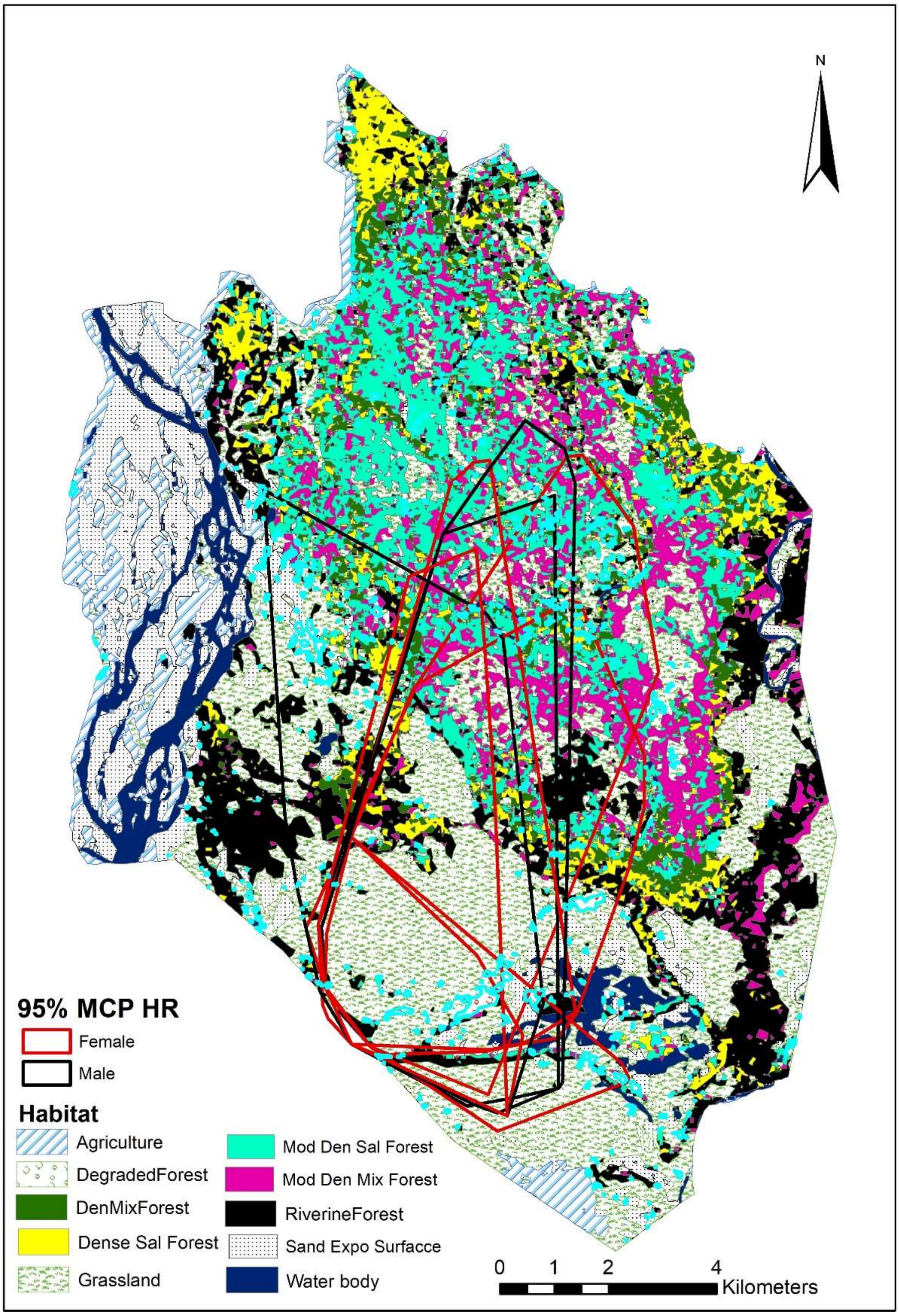

Figure 3. 3 95% MCP home ranges (km^2) of radio-collared swamp deer (n = 9) based on sex (male = 3, female = 6) in Shuklaphanta National Park.

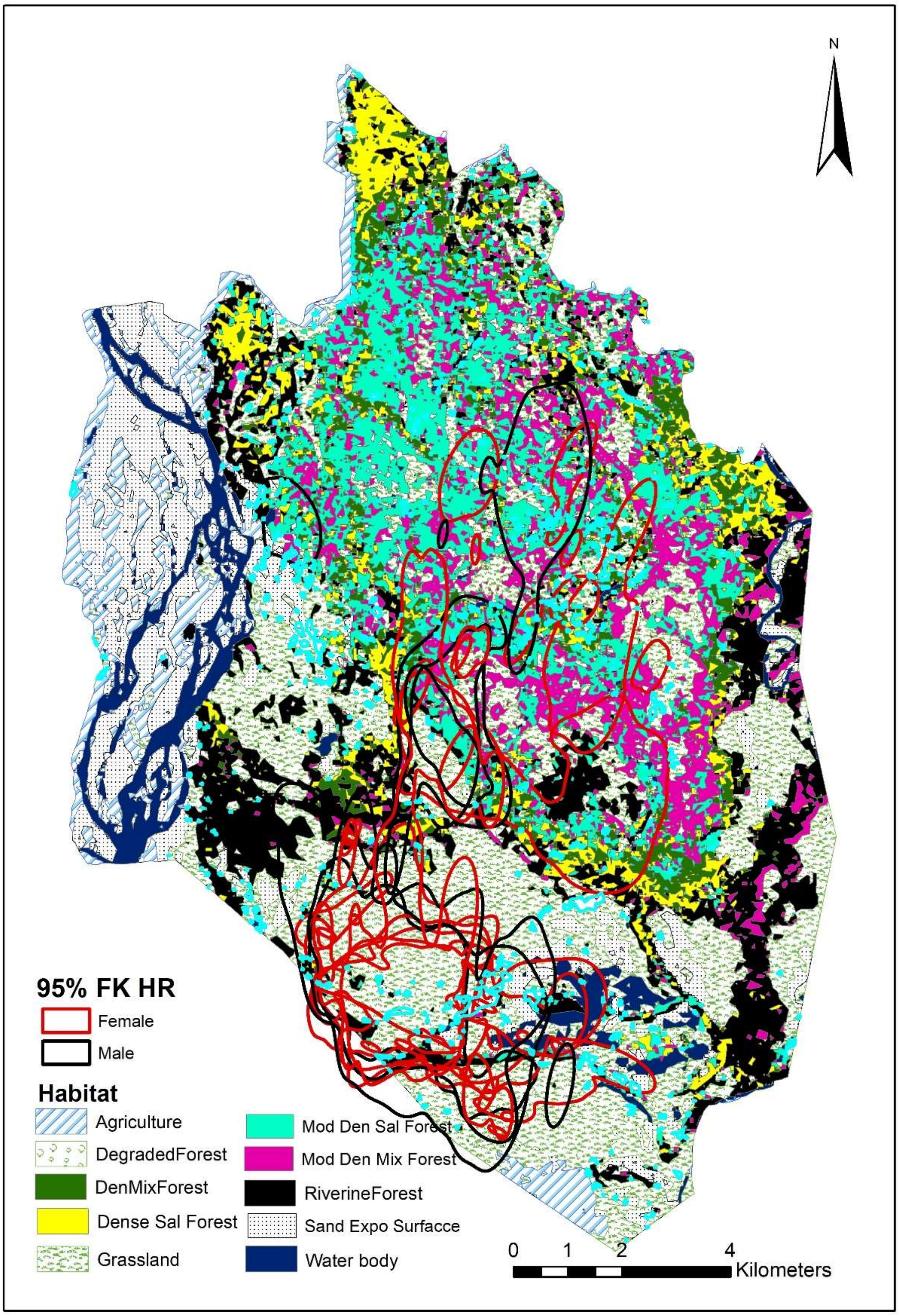

Figure 3. 4 95% FK home ranges (km^2) of radio-collared swamp deer (n = 9) based on sex (male = 3, female = 6) in Shuklaphanta National Park.

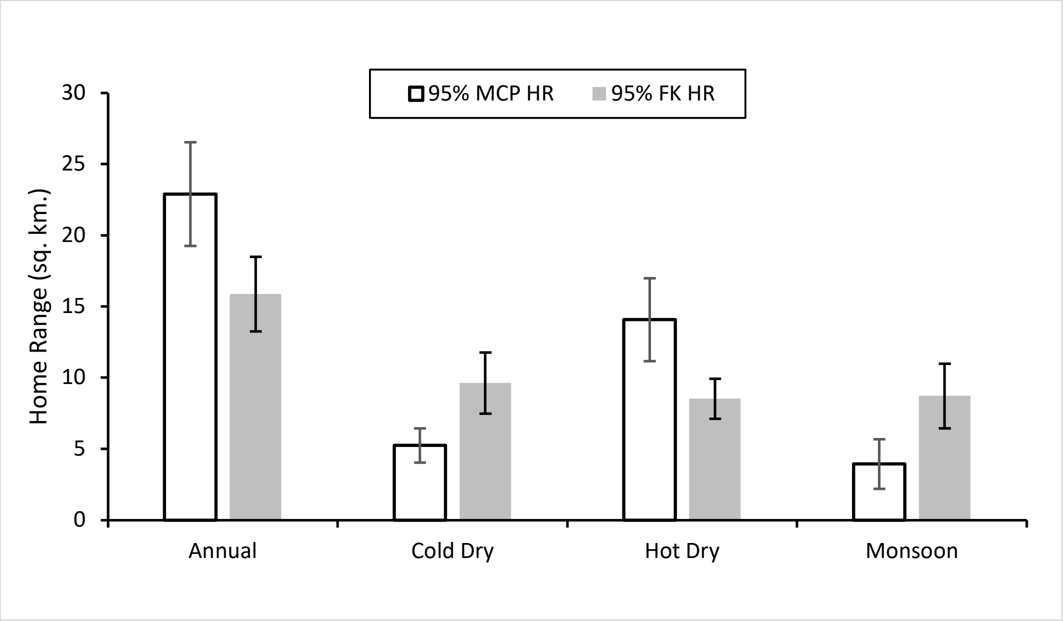

Figure 3. 5 Average annual and seasonal 95% MCP and 95% FK home range (km^2) of swamp deer (n = 9) in Shuklaphanta National Park.

Habitat use and preference of swamp deer

Compositional analysis revealed that swamp deer exhibited annual and seasonal habitat preferences. Therefore, the orders of habitat preference are given in Table 3.4.

Table 3. 4 Annual and seasonal habitat preferences of swamp deer (n = 9) in Shuklaphanta National Park.

Seasons	Order of preference (from most to least preferred)
Annual (χ^2 =37.72, df = 8, $p < 0.05$)	Grassland>Waterbody>Degraded forest>Sand exposed surface>>>Riverine forest>Moderate dense Sal forest>Dense mixed forest>Moderate dense mixed forest>Dense Sal Forest
Cold Dry Season (χ^2 =49.71, df = 8, $p < 0.05$)	Grassland > Sand exposed surface > Waterbody= Dense mixed forest> Degraded forest= Riverine forest > Dense Sal Forest > Moderate dense mixed forest > Moderate dense Sal forest
Hot Dry Season (χ^2 =38.06, df = 8, $p < 0.05$)	Grassland > Degraded forest >>> Riverine forest > Sand exposed surface > Waterbody >>> Moderate dense Sal forest > Dense mixed forest > Dense Sal Forest > Moderate dense mixed forest
Monsoon Season (χ^2 =38.12, df = 8, $p < 0.05$)	Grassland > Moderate dense Sal forest > Sand exposed surface > Moderate dense mixed forest > Dense mixed forest > Riverine forest > Dense Sal Forest > Waterbody > Degraded forest

As predicted, annually and seasonally, the highest preference was for grassland and the least for the dense Sal forest. In the cold dry season, the lowest preference was for the moderate dense Sal forest. In contrast, in the hot dry season and monsoon season, the moderate dense mixed forest and degraded forest were the least preferred habitat, respectively. Ivlev's index for the individual swamp deer also showed a preference for grassland and similar patterns of habitat selection as the results of compositional analysis (Appendix 1.1).

Imperata cylindrica (IMPC) assemblage was the most used grassland type among seven grass assemblages identified in the study area (Figure 3.6). The moderate use was seen for the *Imperata cylindrica – Narenga porphyrocoma* (ICNP) assemblage and the least for the *Narenga porphyrocoma* (NARP) assemblage. The habitat uses for *Phragmites karka* (PK), *Phragmites karka – Saccharum spontaneum – Saccharum arundinaceum* (PKSSSA), *Saccharum spontaneum* (SS) and *Themeda arundinacea* (TA) assemblages were marginal (RF % < 3). However, there was no seasonal variation in the use of different grassland assemblages (Wilcoxon Singed-Rank Test: n = 6, α = 5%, signed-rank test statistic > critical value among seasons).

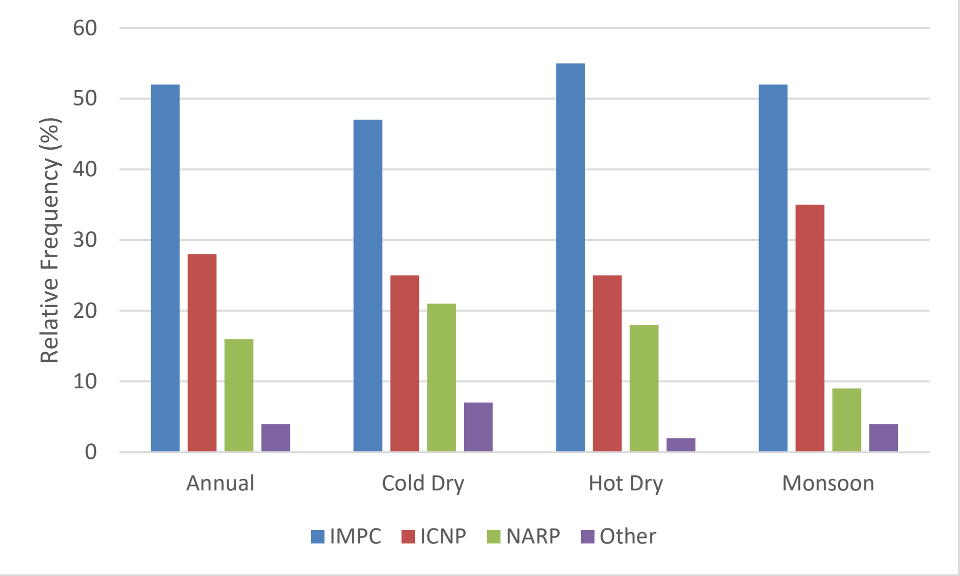

Figure 3. 6 Annual and seasonal percentage relative frequency of grassland assemblages used by swamp deer in Shuklaphanta National Park. (IMPC = *Imperata cylindrica*, ICNP = *Imperata cylindrica – Narenga porphyrocoma*, NARP = *Narenga porphyrocoma*, Other = *Phragmites karka*, *Phragmites karka – Saccharum spontaneum – Saccharum arundinaceum*, *Saccharum spontaneum* and *Themeda arundinacea*).

Movement and trans-border movement

Movement

Annually, radio-collared swamp deer moved a straight-line distance of 10.14 ± 0.85 km ranging from 5.90 to 12.78 km. Male swamp deer (12.18 ± 0.41 km) travelled longer distance than the female (9.11 ± 1.03), however they did not differ significantly (F= 3.95, d. f. = 1, P = 0.087) (Figure 3.7). Among nine deer, two females (4F & 6F) stayed in the southern grassland throughout the year (straight line distance moved = 6 – 7 km) and did not emigrate to the northern forest habitat (Figure 3.8). All other seven deer (3 males & 4 females) showed a clear range shift from southern grassland to northern forest habitat (straight line distance moved = 9 – 13 km) (Figure 3.8). The timing of the movement appeared to be consistent. The departure from the grassland to forest occurred in the late hot dry season and before the onset of the monsoon season (second week of May) through a fixed route (Figure 3.8). The departure from the forest to grassland occurred at the end of the monsoon season, just before the onset of the cold dry season (last week of October) through two routes (Figure 3.8).

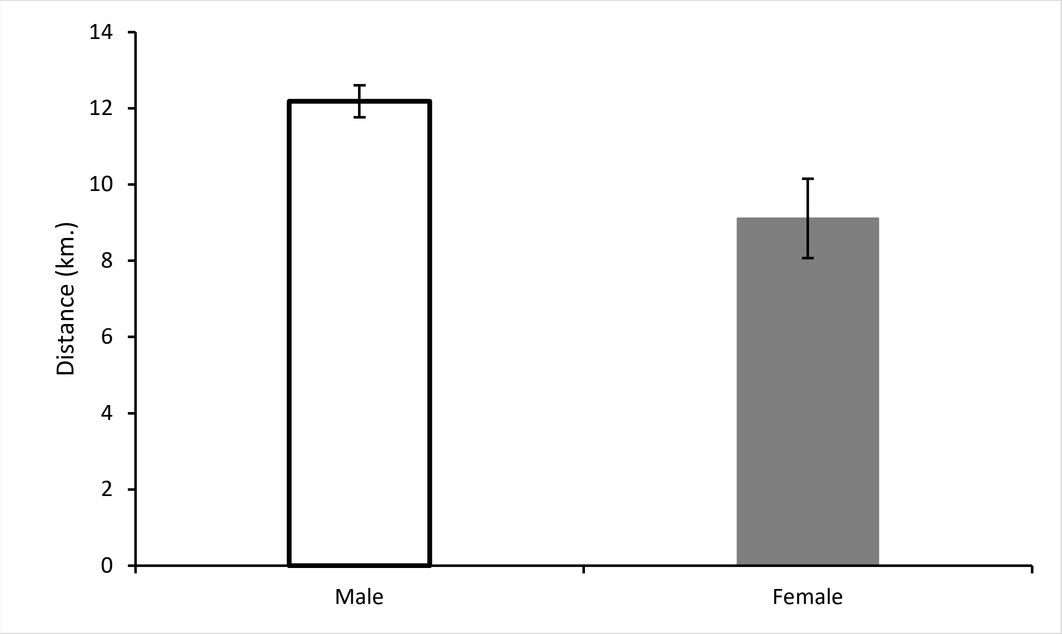

Figure 3. 7 Annual straight-line distance (km) moved by swamp deer (n = 9) based on sex (male = 3, female = 6) in Shuklaphanta National Park.

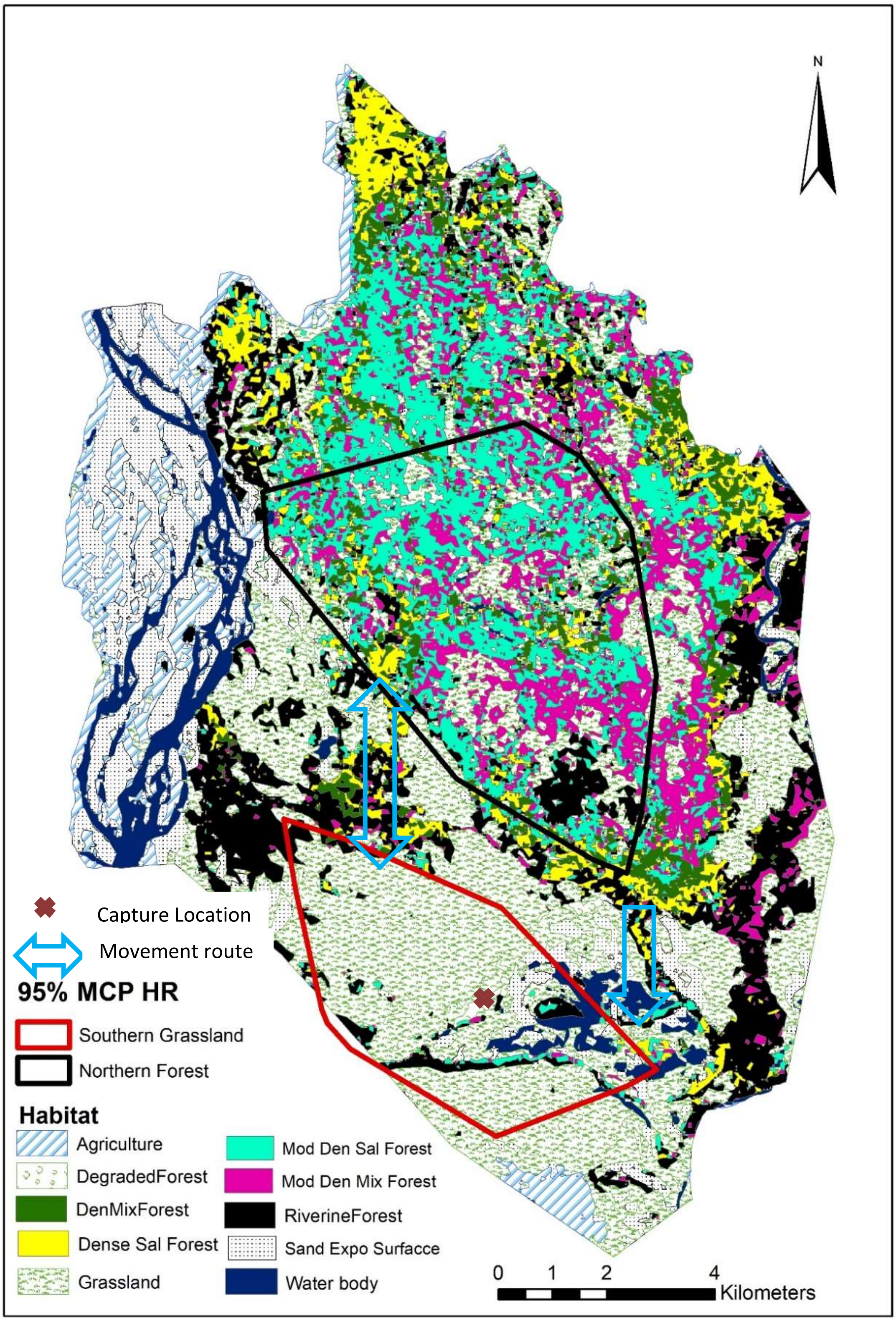

Figure 3. 8 95% MCP home range of Swamp deer (n = 9) showing ranges in grassland and forest habitat of Shuklaphanta National Park. Among nine individuals, two stayed in the southern grassland, and seven moved from the southern grassland to the northern forest in the monsoon season.

Trans-border movement

I found the movement of swamp deer across the international border in all seasons except the peak hot, dry (May, June) and early monsoon (July, August) season (Table 3.5). The collared animals moved an average 400 m (range 10 – 675m) further south from the southern border of SNP, an international border between Nepal and India. Overall, 4.33 % (0.59 km^2) of total 95% FK home range of swamp deer was in India. Similarly, the average percentage of the total 95% FK home range of males and females was 3.47% (0.82 km^2) and 4.77% (0.47 km^2), respectively, in India. During monitoring, on an average, 426 ± 96 swamp deer individuals were counted in the hot dry season during 11 occasions crossing the border and utilizing the continuous habitat of Lagga-Bagga area of PTR, India. However, the duration of stay in India was very short.

Table 3. 5 Percentage of 95% FK Home Range (HR) of swamp deer falling across the Indo-Nepal border of Shuklaphanta National Park.

Swamp deer	Total fixes	Fixes (India)	Total HR (95% FK)	HR India (%)	Month in India (number of fixes)
3M	340	1	11.23	0.21 (1.90)	April (1)
8M	262	2	27.87	1.51 (5.40)	April (2)
9M	338	2	23.5	0.74 (3.10)	April (2)
Mean Male	313	1.67	20.86	0.82 (3.47)	
Standard error	26	0.33	4.98	0.38 (1.03)	
1F	320	0	18.66	0.99 (11.40)	-
2F	300	1	22.77	0.56 (2.50)	April (1)
4F	260	0	8.06	0.01 (0.1)	-
6F	280	11	4.46	0.35 (7.8)	September (3), October (1), November (4), January (1), February (1), April (1)
7F	260	0	10.83	0.36 (3.3)	-
10F	320	2	15.55	0.54 (3.5)	February (1), April (1)
Mean Female	290	2.33	13.39	0.47 (4.77)	
Standard error	11.25	1.76	2.79	0.13 (1.67)	
Mean overall	298	2.11	15.88	0.59 (4.33)	
Standard error	11	1.15	2.62	0.15 (1.14)	

3.5 Discussion and Management Recommendation

This study contributes to understanding home range, habitat use, selection and trans-border movement of the threatened swamp deer. In the Shuklaphanta, the swamp deer had home range size of an average of ≈23 km^2 (MCP) and ≈16 km^2(FK). Overall, grassland and moderately dense Sal forest mingled with patches of swampy areas and riverine forest were the preferred habitats for the swamp deer. The swamp deer was found to be highly associated with *Imperata cylindrica* grass assemblage. The movement of swamp deer was extensive between the grassland and the forest habitat, with a distinct range shift between two habitats during the part of a year. Swamp deer utilized the contiguous habitat, including Lagga-Bagga area of PTR, India.

Home range

FK home range is the most accurate estimator of the area used by an animal during normal activities (Worton, 1989). However, the MCP home range more reflects the habitat available for individuals. It covers the area where the animal has reached but do not indicate range use intensity (Harris et al., 1990).

Table 3. 6 A comparison of home range size (km^2) for selected species of South Asian cervids.

Attributes	Barking deer	Hog deer	Spotted Deer	Thamin deer	Swamp deer
Body weight (kg)	18-21[b]	27- 43 [b]	70-90[d]	70-130[d]	170-180[d]
Home Range (MCP) (km^2)	0.12-0.2 (CNP)[a]	0.6-0.8 (CNP, N)[a] 0.12 – 1.37 (BNP, N)[b]	0.15 – 0.2 (CNP, N)[a] 1.34 – 1.82 (BNP, N)[c]	12.47-16.76 (CWS, M)[d]	10 (India) [e] 3.81-4.73 (BNP, N, HDY)[f] 19.26 – 26.54 (Shuklaphanta)[g]
Home Range (95% FK) (km^2)	0.53 –0.76 (BNP, N)[b]	0.37 – 0.54 (BNP, N)[b]	1.48 – 2.03 (BNP, N)[c]	7.25 – 9.04 CWS, M[d]	13.26 – 18.50 (Shuklaphanta)[g]

a(Dhungel et al., 1991); b(Odden et al., 2007); c(Moe et al., 1994), d(Aung et al., 2001); e(Schaller, 1967); f(Moe, 1994); g this study; CNP, N = Chitwan National Park, Nepal; BNP, N = Bardia National Park, Nepal; CWS, M = Chatthin Wildlife Sanctuary, Myanmar, HDY = Hot Dry Season

The estimated home range size of swamp deer from the present study was significantly larger than other South Asian cervids (Table 3.6). The average annual MCP home range of swamp deer (≈23 km^2) is about 143, 31 and 14 times larger than barking deer, hog deer and spotted deer, respectively. Similarly, average annual 95% FK estimation (≈16 km^2) of SNP swamp deer is by far larger than these three ungulates. The smaller size of home ranges of barking deer, hog deer and, spotted deer compared to swamp deer are probably due to a difference in body size (Geist, 1998; Aung et al., 2001). There are positive relationships between body mass and home range sizes, and mammals adjust their home ranges to include enough resources to fulfil their metabolic needs (Odden et al., 2007; Ottaviani, Cairns, Oliverio, et al., 2006). The bodyweight of thamin deer and swamp deer are closer. However, remarkable large home range size of swamp deer than that of thamin deer is probably due to the large group size of swamp deer (maximum group size = 1175 in April, present study) than thamin deer (maximum group size = 70+ in April, (Aung et al., 2001)). Many individuals exploiting the same resource area increase the home range sizes possibly (Damuth, 2008). The maximum group size of swamp deer in Bardia was 33 individuals (Ghimire, 1996). Smaller group size may also explain their smaller hot dry season home range size (3.81 – 4.73 km^2) (Moe, 1994) compared to Shuklaphanta (14.08 km^2). Absence of sex-related differences in home range size of swamp deer is similar to the findings on thamin deer (Aung et al., 2001), hog deer (Dhungel et al., 1991; Odden et al., 2007) and barking deer (Odden et al., 2007). Individuals of both sexes exhibited site fidelity and demonstrated the same pattern of ranging behaviour. Hence, the home range estimated from the present study represents the maximum attainable range by swamp deer in this site. Such site fidelity is quite common in deer species (Cederlund, Sandegren, & Larsson, 1987; Craighead, Craighead, Ruff, et al., 1973; Martin, 1977; Verme, 1973).

MCP estimation of home range size in the hot dry season is significantly larger than the cold dry and monsoon season. This difference is attributed to the movement of the animals from the main southern grassland to the northern Sal forest at the end of the hot dry season, thus increasing the home range size. The pre rutting activity of swamp deer occurs during the monsoon season (July to October). The peak rut is from the end of the monsoon season to the beginning of the cold dry season (October to mid-November). Increased movements during the rut are common for ungulates (Aung et al., 2001; Relyea & Demarais, 1994), leading to a larger home range. Swamp deer start to move for pre rutting activity from the end of the hot dry season, so the home range is more extensive in the hot dry season. Sal forest in this area consists of forest, grassland, water bodies and associated riverine forest patches, which reduces the animals’ need for long-distance movements to obtain food. This heterogeneous habitat structure is likely the reason for the reduced home range size in the monsoon season (Clutton-Brock, Guinness, & Albon, 1983; Moe et al., 1994). Small home range size in the cold dry season coincided with grassland management intervention. The cutting and burning of grassland improve grassland's nutrient quality due to growth of new shoots (Moe & Wegge, 1997), leading to enhanced habitat productivity, resulting in the smaller home range of swamp deer (Odden et al., 2007).

Habitat preference

Degraded forests (or scrubland) are tiny patches scattered within the extensive grassland. However, patches of riverine forest are embedded in both grassland and other forests, mainly on wet sites along and around water bodies. Swamp deer frequently use these forest patches during dry seasons (both cold and hot dry) for high-quality forages, mineral-rich flowers, and fruits and proteins found in leaves of *Mallotus philippinensis*, *Ficus racemosa* and *Syzygium cumini* (Dinerstein, 1979b). Apart from high-quality forage, the riverine forest also provides shade and cover during the dry season (Moe et al., 1994). This configuration possibly explains the high preference for degraded forest and riverine forest in dry seasons when most deer have already arrived in the main grassland habitat from the forest habitat.

Similarly, the waterbody or aquatic habitat preference, especially in the dry season, might be due to the importance of aquatic plants for swamp deer, similar to the swamp deer of Bardia (Moe, 1994). The Calcium (Ca) and Sodium (Na) content of the terrestrial grasses are far less than aquatic plants, and swamp deer by eating aquatic plants may compensate for the low Na and Ca content in the terrestrial grasses. In addition, a high concentration of Ca in aquatic plants might be an important source for lactating females and growing fawns (Moe, 1994). Therefore, after the onset of cold dry season, all collared deer hit the aquatic habitat straight and continued to live nearby wet areas until the hot dry season.

In the monsoon season, the lowest preference for degraded forest and the riverine forest is because animals in this season are mostly in the forest area, where the highest preference is for the moderate dense Sal forest (open Sal forest) after grassland. A similar preference for the Sal forest was observed for the spotted deer in Bardia, where Sal forest was heavily utilised in the monsoon and the early part of the cold dry season (Moe et al., 1994). After the arrival of monsoonal rain, animals are attracted to grass patches existing within the Sal forest (Dinerstein, 1980) and utilizes grasses and sedge (Dinerstein, 1979b) and possibly protein and mineral-rich mushrooms (Moe, 1993). Furthermore, the leaves of Sal (*Shorea robusta*) and palm tree (*Phoenix humilis*) are essential forage species of deer species in the Sal forest (Chapter 5).

Among grassland assemblages, swamp deer exhibited more association with IMPC, ICNP and NARP assemblages. The highest association with IMPC assemblages throughout all seasons is like previous findings (Schaaf, 1978; Dinerstein, 1979b; Peet et al.,1997). The association with ICNP assemblage is also similar to the previous finding (Peet et al., 1997). The association of swamp deer with NARP assemblages increased when new nutritious shoots were available after cutting and burning of grassland (Peet et al., 1997). In the hot, dry season, when the grass started to mature and became less palatable, the association with NARP decreased and became the least associated in the monsoon season (Figure 3.15). The less association with assemblages PK, PKSSSA, SS and TA is similar to previous findings (Peet et al., 1997).

Movement and trans-border movement

The habitat of swamp deer in SNP can be classified into two broad categories: grassland dotted with forest patches (mainly degraded forest or scrubs and riverine forest) and forest (mostly Sal forest) encompassing small patches of grasslands, water bodies and associated riverine forests. In the monsoon season, due to the movement of some collared swamp deer (n = 6) from the main grassland to Sal forest, range shift was observed (Figure 3.7). However, the remaining deer (n = 3) did not move across broad habitats and continued to use the main grassland (Figure 3.7). This movement pattern implies that the swamp deer population in SNP is divided between habitats during monsoon season. In 1975, Schaaf (1978) did not observe more than two swamp deer both in pre-monsoon (May-June) and monsoon season (August-September) in the Sal forest. In the present study, the density of swamp deer in the northern Sal forest and the southern grasslands was 17 individuals/km^2 and 13.3 individuals/km^2, respectively.

Similarly, the census population size of swamp deer increased from 805 in 1975 (Schaaf, 1978) to 1898 in 2016 (present study). Therefore, it looks like the carrying capacity of the main grassland of SNP has crossed its maximum to hold the increased population in the monsoon season. Furthermore, swamp deer may need larger area during the rutting, and the available habitat in grassland is congested. Therefore, part of the swamp deer population from the grassland starts to shift to the Sal forest after the arrival of the pre-monsoon (May). And again, start their return to grassland after the onset of the cold dry season (last week of October).

For the first time, I documented the movement of swamp deer across the border from SNP in Nepal to Lagga-Bagga area of PTR, India. The movement of deer in late cold dry (December, January) and the hot dry (April) season is mainly for the exploration of water and palatable forage emerging due to early burning of grassland towards India side. This movement is generally nearby the border on either side. However, in peak hot dry period, groups of swamp deer were observed drinking water in Mahakali river towards India. In addition, I observed strict site fidelity during rutting season for ID6F. This deer utilised the same area towards the Indian side with the same seasonal movement pattern in 2015 and 2016. Similar trans-border movements were observed for other animals such as tigers between Shuklaphanta and Lagga-Bagga area of PTR (Chanchani et al., 2014).

Management recommendations

This study emphasises the importance of management of grassland patches, especially dominated by IMPC and ICNP assemblages, for swamp deer's sustainable future. The associated wetland/water bodies and forest patches that form habitat mosaics must be identified and appropriately managed. The moderately dense Sal forest having grass understory is the prime habitat of swamp deer after the grassland during the monsoon season, which coincides with rutting of this species. Owing to the site fidelity during rutting, the movement routes from the main grassland to Sal forest and towards India after crossing Indo-Nepal border needs to be identified and conserved. This study further established the trans-border movement of swamp deer between SNP and Lagga-Bagga forest of PTR; the transboundary cooperation is essential for conserving threatened swamp deer under the umbrella of the major apex predator tiger inhabiting TAL.

Chapter 4 Conservation genetics of swamp deer or barasingha (*Rucervus duvaucelii duvaucelii*) in Nepal

4.1 Abstract

Endemic to Indian sub-continent, the swamp deer (*Rucervus duvaucelii*) are threatened due to anthropogenic activities, leading to a drastic decline of wild populations. Even though the species has recently shown signs of recovery, it is still vulnerable to extinction due to the small population size and its role as a major prey species of the endangered tiger (*Panthera tigris tigris*). Among three subspecies of swamp deer, the subspecies *Rucervus duvaucelii duvaucelii* exist in northern India, and two isolated populations inhabit Bardia National Park (BNP) and Shuklaphanta National Park (SNP) of southern Nepal. Whereas the genetic variations of Indian populations are studied recently, such information lacks for Nepalese populations hindering effective conservation planning. In this study, both populations' genetic variability, population structure, and effective population size were examined using the mtDNA and microsatellite markers. The analysis revealed moderate to high genetic diversity compared to other swamp deer populations in India. Neutrality tests, which are used to evaluate demographic effects, did not support population expansion. The multimodal pattern of mismatch distribution indicated that both populations are under demographic equilibrium. Furthermore, population bottleneck analysis indicated no signature of a bottleneck for both populations. Bayesian cluster analysis revealed two population clusters. However, fixation index values were low, indicating low population differentiation between populations. The effective population size in BNP was below 50, which is often regarded as a threshold below which inbreeding depression is likely to occur. Due to relatively low genetic diversity and effective population size below 50, it

is recommended to increase the population size of swamp deer in BNP through an in situ conservation program coupled with the translocation of few swamp deer individuals from SNP for upgrading the genetic diversity of BNP population.

4.2 Introduction

Genetic diversity, population structure and effective population size are essential components of conservation genetics (Mukesh et al., 2015). Such genetic information is crucial for managing species, especially those threatened to extinction due to various anthropogenic factors (e.g., habitat loss, introduced species, overexploitation and pollution). Threatened species have small and declining population size susceptible to multiple stochastic effects, including genetic stochasticity in the form of inbreeding depression and loss of genetic diversity (Frankham, 2003). It is now established that both inbreeding and loss of genetic diversity would pose extinction risk in threatened species since former reduces reproduction and survival rates, and the latter reduces the ability of populations to evolve to cope with environmental changes (Frankham, 2003). Effective population size will play a crucial role in determining the degree to which populations can avoid extinction from stochastic events (Boyce, 1992; Frankham, 2003), as it helps predict the rate of inbreeding and loss of genetic variation in wildlife (Frankham, 1995). Therefore, monitoring of populations genetic parameters of threatened wildlife species in terms of genetic diversity, effective population size and populations structure is extremely important to devise adequate conservation and management strategies (Huang, Wang, Li, et al., 2014; Mukesh, Kumar, Sharma, et al., 2015; Schwartz, Luikart, & Waples, 2007).

Endemic to Indian sub-continent, swamp deer (*Rucervus duvaucelii*) or swamp deer is a threatened cervid species which were once widely distributed over the Ganga-Brahmaputra and Indus basins (Martin, 1977). Swamp deer constitutes one of the main diets of the critically endangered top predator, tiger (*Panthera tigris*) (Chapter 2). A large-sized ungulate species, swamp deer, plays a crucial role in the coexistence of sympatric tiger and leopard that prey primarily on medium to smaller prey species (Chapter 2) and plays an essential role in the lowland forest ecosystem of Indian sub-continent. However, this species has undergone significant population reduction due to anthropogenic activities and is now restricted to small isolated, fragmented habitats in India and Nepal (Duckworth et al., 2015; Qureshi et al., 1995, 2004). Although this species is downlisted from endangered to the vulnerable category in IUCN Red List in 1996 (Duckworth et al., 2015), there are still threats prevailing to the extant populations from population isolation, poaching, habitat degradation, diseases and small populations. Three subspecies of swamp deer have been reported (Groves, 1982). *R. d. duvaucelii* (G. Cuvier, 1823), the wetland swamp deer is restricted to Indo-Gangetic plain in north India and south-west Nepal. *R. d. branderi* (Pocock, 1943), the hard-ground swamp deer is confined between Ganges and Godavari river in Madhya Pradesh, central India. *R. d. ranjitsinhi* (Groves, 1982), the eastern swamp deer is found in the Brahmaputra floodplains of Assam, India. Swamp deer is extinct from Bangladesh and Pakistan (Qureshi et al., 2004). Swamp deer are listed as protected species by the National Parks and Wildlife Conservation Act 1973 of the government of Nepal. Up to 1950s, the wetland swamp deer were widely spread in terai (flat land which fringes southern edge of Nepal), mainly in central (Chitwan valley) and western Nepal. Due to the conversion of grasslands to cultivated land, the Chitwan Valley (present-day Chitwan National Park) population of this subspecies in central Nepal is extinct. In western Nepal, swamp deer is now restricted to two isolated populations in protected areas, Shuklaphanta National Park (SNP) and Bardia National Park (BNP) (Schaaf, 1978). Population size in SNP is larger (1883 individuals, present study) than BNP (105 individuals, BNP, 2012). Due to effective conservation efforts like the operation of antipoaching campaign, habitat management, the population size of each isolated population is in the increasing trend. However, the population increase is slow, and both populations are still small, especially in BNP. There is a growing concern for the future survival of the swamp deer population in BNP. In order to increase tiger populations in both protected areas, it is important to maintain healthy prey populations. The genetic information of these two swamp deer populations is crucial for evaluating the populations status of this important prey species.

The population genetics of swamp deer have been studied less compared to other deer species of the world. Recent genetic study of Indian populations of swamp deer using the mitochondrial DNA (mtDNA) control region and microsatellite markers revealed a geographic pattern in the population structure, with moderate levels of genetic diversity (Kumar et al., 2017). This study encompassed all the extant captive as well as wild populations of swamp deer in India, including geographically isolated populations of Dudhwa National Park (DNP), Jheelmil Jheel Conservation Reserve (JJCR), Kanha Tiger Reserve (KTR) and Kaziranga National Park (KZNP). However, there is a lack of such studies for Nepalese populations. Studies showed that species occurring in genetically isolated or small populations are at higher risk of extinction due to anthropogenic factors, loss of genetic diversity, inbreeding and stochastic processes (Mukesh et al., 2015). So, it is essential to know genetic diversity and demographic history of such fragmented populations of swamp deer to formulate conservation strategy to manage this species. Both nuclear microsatellite loci and mitochondrial DNA sequence data are instrumental in revealing aspects of genetic diversity and predicting genetic fitness for the survival and monitoring of wildlife populations (Brown, Ramey, Tamburini, et al., 2004; Davis & Shaw, 2001) such as Bavarian red deer (Kuehn, Schroeder, Pirchner, et al., 2003), Scottish highland red deer (Pérez-Espona, Pérez-Barbería, Goodall-Copestake, et al., 2009), Siberian roe deer (Lee, Markov, Voloshina, et al., 2015), Kashmir red deer (Mukesh et al., 2015), swamp deer (Kumar et al., 2017) and hog deer (Gupta, Kumar, Angom, et al., 2018).

In this study, I investigated the genetic diversity, population structure and effective population size of existing two populations of swamp deer in Nepal (Figure 4.1) using the mitochondrial DNA (mtDNA) control region and ten microsatellite markers. I aim to answer the following questions:

1. What is the status of genetic diversity of swamp deer populations in Nepal? Is there any genetic bottleneck in these populations?

2. Are there genetic population structures of populations due to spatial isolations?

3. What is the effective population size of SNP and BNP swamp deer populations, and the future trend of these two populations?

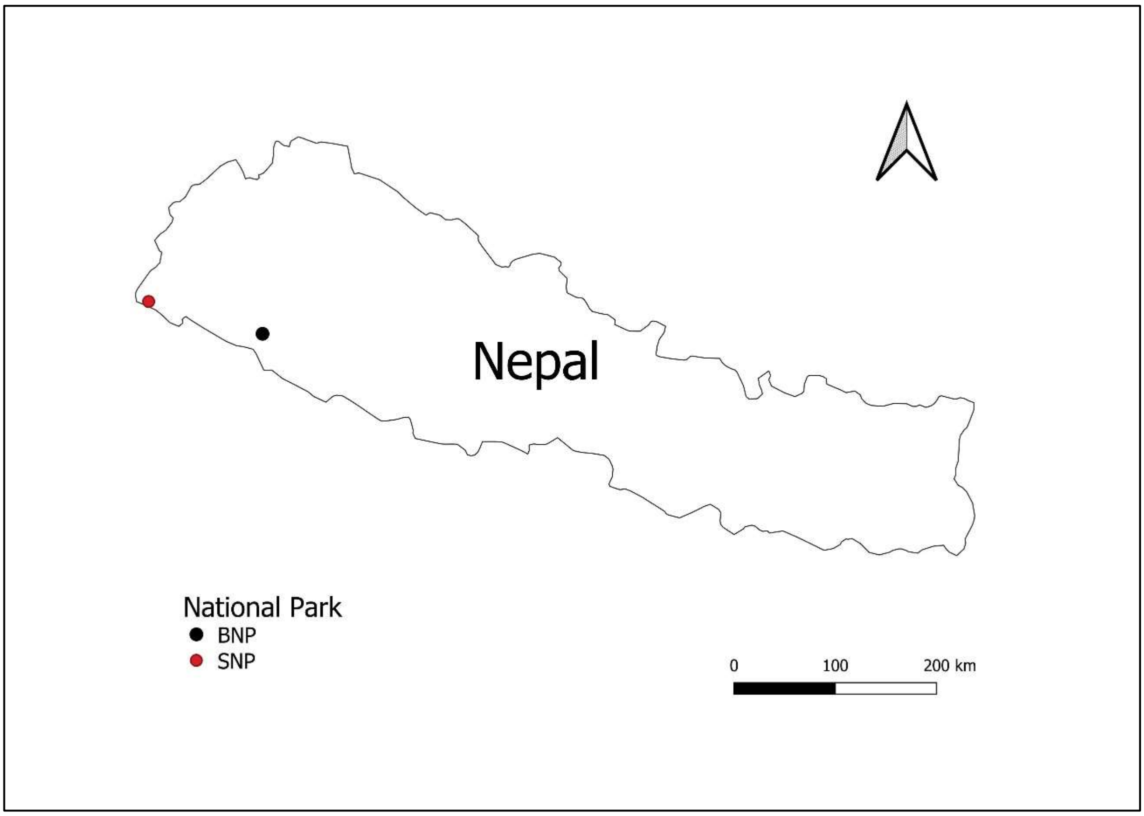

Figure 4. 1 Map showing two swamp deer populations in Bardia National Park (BNP) and Shuklaphanta National Park (SNP) of Nepal.

4.3 Methods

Sample collection and DNA extraction

Three types of swamp deer samples were used for NDA extraction: antlers, faecal pellets and muscle tissue (Table 4.1). Shed antlers were cut into pieces by hand saw and stored at room temperature. The tissues samples were collected from the animals that died naturally or killed by predators and were stored at room temperature in absolute ethanol. I collected fresh faecal samples from different herds of swamp deer. In order to avoid picking a sample from the same individual swamp deer, the herds were monitored with the help of binoculars and samples were collected from different locations of a large herd. The faecal samples were stored in silica beads at room temperature. DNA from the tissue was extracted using DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. The antlers were first pulverised before treated with 0.5 M EDTA for 48 hours which allowed the decalcification of antlers, and then, DNeasy Blood & Tissue Kit (Qiagen, Germany) was used for DNA extraction (Gupta, Kumar, & Hussain, 2013). The DNA from faecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) by following the manufacturer's instructions.

Table 4. 1 Details of samples used for the genetic analysis of swamp deer populations.

Sites	Number of samples used for DNA extraction				Number of samples used for mtDNA analysis				Number of samples used for microsatellite analysis			
	faecal	Tissue	Antler	Total	Faecal	Tissue	Antler	Total	faecal	Tissue	Antler	Total
SNP	42	17	30	89	31	16	18	65	21	15	14	50
BNP	65	0	0	65	35	0	0	35	32	0	0	32
Total	107	17	30	154	66	16	18	100	53	15	14	82

PCR amplification and DNA sequencing

I used deer specific primers, Cerv.tPro (5'-CCACYATCAACACCCAAAGC-3') and Cerv.CRH (5'-GCCCTGAARAAAGAACCAGATG-3') (Balakrishnan, Monfort, Gaur, et al., 2003) to amplify the control region of mitochondrial DNA. PCRs were conducted in 20 µl volume with 1-2 µl genomic DNA, 1 µl each of primers, 1 µl Bovine Serum Albumin (BSA) and remaining nuclease-free water. I used BSA only for faecal and antler samples. The PCR reaction conditions were as follows: an initial denaturation of 10 min at 95^0C, followed by 35 cycles at 95^0C for 45 s, 55^0C for 1 min and 72^0C for 1 min, with a final extension of 72^0C for 15 min. The PCR products were electrophoresed on 1.2 % agarose gel and visualized under UV light in the presence of ethidium bromide. The unidirectional sequencing of the selected PCR products was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

PCR amplification and microsatellite genotyping

I used a total of 10 microsatellite loci (Table 4.2) that were previously used for the population genetic analysis of swamp deer (Kumar et al., 2017) and spotted deer (Gaur, Singh, Arunabala, et al., 2003). Multiplex PCR was conducted in 20 µl volume consisting 1-2 µl genomic DNA, 1 µl labelled forward primers, 1 µl unlabelled reverse primer, 1 µl Bovine Serum Albumin (BSA) and remaining nuclease-free water. The PCR reaction was carried out under the conditions: preheating at 95^{0}C for 10 min, followed by 35 cycles at 95^{0}C for 45 s, 55^{0}C for 1 min (for ABS12 50^{0}C for 1 min) and 72^{0}C for 1 min, with a final extension of 60^{0}C for 30 min. The quality of PCR products was assessed by gel electrophoresis using 1.2% agarose gel and visualized under UV light in the presence of ethidium bromide. Good quality PCR products of each sample amplified for all the loci in a group was mixed and then subjected to genotyping using ABI 3170 Genetic Analyzer (Applied Biosystems) and analysed using GeneMapper version 3.7 (Applied Biosystems).

Table 4. 2 List of 10 microsatellite loci used for genetic analysis of swamp deer populations.

Loci	Allele size	Forward Sequences	Dye	References
T156	143-189	TCT TCC TGA CCT GTG TCT TG	TMR	(Jones, Levine, & Banks, 2002)
T108	158-190	CAT GTG GAG ATA GGT AGA CAG A	FAM	(Jones et al., 2002)
T507	140-167	AGG CAG ATG CTT CAC CAT C	FAM	(Jones et al., 2002)
BM1225	220-260	TTT CTC AAC AGA GGT GTC CAC	FAM	(Bishop, Kappes, Keele, et al., 1994)
BM848	360-400	TGG TTG GAA GGA AAA CTT GG	FAM	(Bishop et al., 1994)
BM203	210-240	GGG TGT GAC ATT TTG TTC CC	HEX	(Bishop et al., 1994)
ABS12	120-160	CTT GGG GGT CTC AAG GAA TT	HEX	(Slate, Coltman, Goodman, et al., 1998)
TGLA226	110-130	AGT GGA ATC CAG ATA AGA TGT ATC A	FAM	(Slate et al., 1998)
IDVGA55	190-249	GTG ACT GTA TTT GTG AAC ACC TA	HEX	(Slate et al., 1998)
Ca67	181-195	TAA TCC TAA CTC CTG GAC CC	TMR	(Gaur et al., 2003)

Data analysis

(1). Mitochondrial control region

a. Genetic variability and demographic history

Good quality raw DNA sequences were edited by visual inspection using the BioEdit version 7.2.6 software (Hall, 1999). Then, a similar length of the sequences was generated after proper trimming for further analysis. Three sequences (NC020743, JN632696 and EU921907) were obtained from the gene bank and included in the analysis. Multiple sequence alignment was performed using Clustral X (Thompson, Gibson, Plewniak, et al., 1997). The program DnaSP version 5.10 (Librado & Rozas, 2009) was used to calculate the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), the average number of nucleotide differences (K) and mismatch distribution test for demographic expansion, equilibrium or bottleneck (Rogers & Harpending, 1992). The population that has experienced a recent demographic expansion exhibits unimodal mismatch distribution, whereas ragged and multimodal distribution represent populations at demographic equilibrium (Balakrishnan et al., 2003; Mukesh et al., 2015).

I performed two statistical tests, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997), to evaluate the past population expansion (demographic effects) using Arlequin version 3.5.2.2 (EXCOFFIER & LISCHER, 2010).

(2). Nuclear microsatellites

a. Genetic variability

The computer program CERVUS version 3.0.7 (KALINOWSKI, TAPER, & MARSHALL, 2007) was used to quantify the Polymorphic Information Content (PIC), the number of alleles per locus, observed heterozygosity (H_o) and expected heterozygosity (H_e). Furthermore, the probability test approach (Guo & Thompson, 1992) was employed for the estimation of the Hardy-Weinberg Equilibrium (HWE) using the program GENEPOP 4.6 version (Raymond & Rousset, 1995). Furthermore, Wright's inbreeding coefficient (F_{IS}) (Weir & Cockerham, 1984) was estimated and tested the linkage disequilibrium using GENEPOP 4.6 version (Raymond et al., 1995). Finally, the allelic richness and pairwise Fst values between populations were estimated using FSTAT, version 2.9.3 (Goudet, 1995).

I employed two different approaches to detect molecular evidence of bottleneck events in the populations. Firstly, I tested for deviations of the expected heterozygosity (He) from the heterozygosity expected at drift-mutation equilibrium (H_{eq}) by Wilcoxon sign-rank test (Luikart, Allendorf, Cornuet, et al., 1998) using the programme BOTTLENECK version 1.2.02 (Cornuet & Luikart, 1996; Piry, Luikart, & Cornuet, 1999). I followed a two-phase mutation model (TPM) (Di Rienzo, Peterson, Garza, et al., 1994) using a setting of 10% multiple-step mutations and 90% single-step mutations with 1000 simulations in the program. Secondly, I used a mode-shift test, which checks a mode-shift in distributions of allele frequencies from the L-shaped distribution under mutation-drift equilibrium. In the population with recent bottleneck, distorted distribution is expected compared to a normal L-shaped distribution (G Luikart et al., 1998). The program CONVERT, version 1.31, was used to convert the input file into the required formats for different software (Glaubitz, 2004).

b. Population genetic structure

The genetic structure in the data was estimated by the Bayesian assignment method (EVANNO, REGNAUT, & GOUDET, 2005), implemented in Structure 2.3.4 version (Pritchard, Stephens, & Donnelly, 2000). The admixture model was run with correlated allele frequencies with burn-in periods of 50,000 and 5, 00,000 MCMC iterations. Fifteen independent replicates were run with the number of genetic clusters (K) between 1 and 10 populations. The most probable value of K was established by comparing the log-likelihood estimates at different K values and by the rate of change in the log probability of the data between successive K values (Delta K / ΔK) using the web-based program Structure Harvester (Earl & vonHoldt, 2012).

c. Effective population size

The effective population sizes (N_e) and their confidence intervals (95%) were estimated using a bias-corrected version of the Linkage Disequilibrium method (LD) (Hill, 1981; WAPLES & DO, 2008) based on point-in-time sample (single sample method) as implemented in program N_eEstimator 2.01 version (Do, Waples, Peel, et al., 2014). This method assumes that LD signature arises only from genetic drift (i.e. genetic drift will create non-random combinations of alleles of different loci in small populations with few parent individuals) (Gordon Luikart, Ryman, Tallmon, et al., 2010; Zachos, Frantz, Kuehn, et al., 2016). This method also assumes that markers are neutral, unlinked and the population was closed (Mukesh et al., 2015). This approach, in general, is reliable if the analysis is based on ten or more loci with population sample sizes are more than 25 individuals (Zachos et al., 2016). Rare alleles with very low frequencies may significantly impact the LD value and eventually can bias the analytical results. Therefore, N_e value was estimated by fixing the critical threshold value (P_{crit}) at 0.01, 0.02 and 0.05, which allows the software to remove allele frequencies below these thresholds during analysis. The estimation of N_e at different P_{crit} allowed us to compare the estimates calculated. This software implements an upgraded method to account for missing data by calculating a unique fixed-inverse variance-weighted harmonic mean (Peel, Waples, Macbeth, et al., 2013). Also, it allows the users to choose between the confidence intervals generated by the standard parametric chi-squared method and non-parametric jack-knife method of Waples and Do (2008) relevant for their analysis.

4.4 Results

Out of 154 samples collected, 100 samples (SNP = 65, BNP = 35) yield high-quality DNA, from which mtDNA loci was amplified and produced clean sequences for further analysis (Table 4.1). For microsatellite analysis, 82 (SNP = 50, BNP = 32) samples were used.

mtDNA Genetic variability and demographic history

Fragments of 420 to 450 bp of the mitochondrial DNA control region was obtained. However, trimming yielded a final equal length of 420 bp for further analysis. In total, 100 sequences had 26 polymorphic sites, and of these, 3 were singletons, and 23 were parsimony informative sites (Table 4.3). Eleven unique haplotypes were obtained from two geographic locations of swamp deer populations (Table 4.4). In SNP, ten haplotypes (Hap - 1, Hap 3 - 11) were observed from 65 samples. Of these, 25 samples (38%) shared the same haplotypes (Hap - 2). Three haplotypes (Hap - 2, 3 & 9) were obtained from 35 samples in BNP. Of these, 25 samples (71%) shared the same haplotypes (Hap - 2). SNP and BNP shared two haplotypes (Hap - 3 & 9). All the existing swamp deer mtDNA control region sequences deposited in the GeneBank shared haplotypes with Nepalese swamp deer population (Table 4.3).

Both haplotype and nucleotide diversity indices varied between SNP and BNP. In SNP, haplotype and nucleotide diversity values were 0.799 ± 0.036 and 0.018 ± 0.001 respectively whereas in BNP, it was 0.455 ± 0.086 and 0.009 ± 0.002 respectively. The overall haplotype diversity of all the samples comprising two populations was 0.843 ± 0.018.

The Tajima’s D and Fu’s Fs neutrality tests (Table 4.4), which was performed to detect the past population growth rate and infer the demographic history of two spatially located populations of swamp deer, gave positive value for the populations. The observed estimates of the neutrality tests were not statistically significant ($P > 0.10$), which suggest that the populations have not passed through a bottleneck or population expansion. Furthermore, both populations showed a multimodal ragged pattern of mismatch distribution, which further validated the neutrality tests, indicating that the swamp deer populations to be under demographic equilibrium (Figure 4.2). In order to test my findings, I estimated the raggedness index statistic (Rg) under the demographic expansion model for each population. The non-significant value of Rg for both the population again rejected the recent population expansion hypothesis suggesting that swamp deer populations in Nepal have been under demographic equilibrium and thus a stable population.

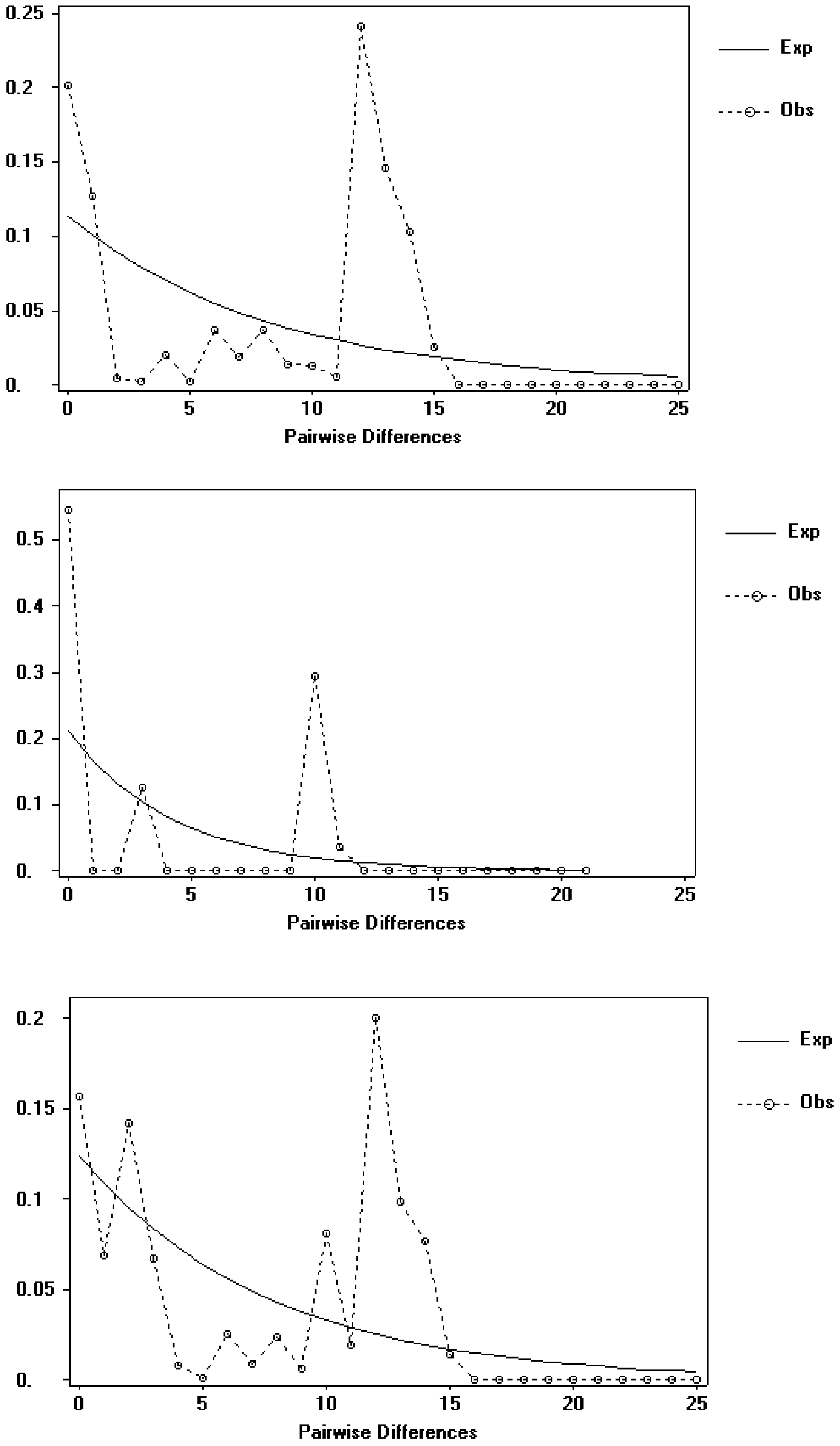

Figure 4. 2 Mismatch pairwise distribution graphs for swamp deer populations. The X-axis shows the number of pairwise differences, and the Y-axis shows the frequency of pairwise comparisons. The expected and observed frequencies of mismatch distribution were represented in the solid and dotted line, respectively. The graph at the top is for SNP populations; the middle for BNP population and the bottom for both populations.

Table 4. 3 Polymorphic positions among 11 haplotypes of the mitochondrial DNA control region in the swamp deer populations.

Haplotypes	n	Variable sites																								
		90	140	141	164	167	171	204	214	228	268	269	294	295	301	314	315	320	321	336	337	359	374	375	377	397	407	Populations
Hap-1	25	T	C	C	C	C	C	T	C	A	T	T	T	C	T	C	A	A	A	T	G	C	A	G	C	A	A	All SNP
Hap-2	25	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	All BNP
Hap-3	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	SNP-9, BNP-3
Hap-4	6	.	T	A	T	.	T	.	T	G	C	.	C	T	.	T	G	.	.	.	.	.	.	A	T	G	.	All SNP
Hap-5	6	.	T	A	T	.	T	.	.	.	C	.	C	T	.	T	G	.	.	C	.	.	.	A	.	G	.	SNP, NC020743, JN632696
Hap-6	1	.	T	A	T	.	T	.	.	.	C	.	C	T	.	.	.	.	.	C	A	.	.	A	.	G	G	All SNP
Hap-7	1	.	T	A	T	T	T	.	.	.	C	.	C	T	.	.	.	.	.	C	.	T	.	A	.	G	G	All SNP
Hap-8	2	C	T	A	T	.	T	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	A	.	G	G	All SNP
Hap-9	8	.	T	.	.	.	.	C	.	G	C	.	C	T	A	.	.	G	.	.	.	.	G	A	.	G	G	SNP-1, BNP-7, EU921907
Hap-10	4	C	T	A	T	.	T	.	T	.	C	.	.	.	A	.	G	.	.	C	.	.	.	A	.	G	G	All SNP
Hap-11	10	C	T	A	T	.	T	.	T	.	C	.	.	.	.	.	G	.	.	C	.	.	.	A	.	G	G	All SNP

Table 4. 4 Genetic diversity of the swamp deer populations.

Parameters	SNP	BNP	Total
mtDNA control region			
n	65	35	100
s	25	12	26
h	10	3	11
hd	0.799 ± 0.036	0.455 ± 0.086	0.843 ± 0.018
Pi	0.018 ± 0.001	0.009 ± 0.002	0.017 ± 0.001
k	7.830	3.707	7.058
Neutrality Tests			
Tajima's D	1.529 (0.945)	0.860 (0.839)	1.214 (0.902)
Fu's Fs statistic	5.904 (0.953)	7.784 (0.993)	5.888 (0.946)
Rg	0.096 (0.518)	0.483 (0.696)	0.080 (0.376)
Microsatellite analysis			
n	42	26	68
Na	9.40	5.90	9.80
Ar	8.43	5.84	8.14
Ho	0.764	0.727	0.749
He	0.756	0.706	0.746
Fis	-0.011	-0.034	-0.005
Bottleneck Tests			
Wilcoxon sign-rank tests* (TPM)	0.883	0.161	
Mode shift	None (normal L shaped distribution)	None (normal L shaped distribution)	
n number of samples, S number of polymorphic sites, h number of haplotypes, Hd haplotype diversity, Pi nucleotide diversity, k average number of nucleotide differences, r raggedness statistic. * One tail probability for observed heterozygosity excess compared to the heterozygosity expected at drift mutation equilibrium (Heq)			

Nuclear microsatellite Genetic Variability and Population Genetic Structure

The linkage disequilibrium test for ten microsatellite loci used in this study showed that the loci used were independent of each other. Nine out of ten loci were polymorphic for each of the population. Loci (BM1225, BM848, BM203, and T108) in SNP and loci (T156, BM203 and T108) deviated from HWE (Table 4.5). Table 4.4 presents the estimate of genetic diversity indices. The set of loci used were highly polymorphic, and the Polymorphic Information Content (PIC) ranged from a low value of 0.401 (TGLA226) to a high value of 0.880 (T156) with an average value of 0.705. However, all loci except TGLA226 and T507 exhibited PIC value higher than 0.5, indicating that 8/10 loci are informative (Table 4.5). In SNP, mean allele number (Na) was 9.40, ranging from 5 to 13 per locus, while in BNP, it was 5.90, ranging from 2 to 11 per locus. The mean allelic richness (Ar) in SNP was 8.43, while in BNP, it was 5.84, with an overall average of 8.14. The mean observed heterozygosity (H_o) in SNP was 0.764 (range 0.547 – 1), and mean expected heterozygosity (H_e) was 0.756 (range 0.481 - 0.898). Similarly, in BNP, H_o was 0.727 (range 0.480 – 1) and H_e was 0.706 (range 0.456 – 0.866). The overall H_o value was 0.749 (range 0.588 – 1) and H_e value was 0.746 (range 0.468 – 0.896). The inbreeding coefficient (F_{is}) estimates of SNP ranged from - 0.241 to 0.208 with a mean value of - 0.023 ± 0.053. The F_{is} estimates of BNP ranged between - 0.331 and 0.368 with a mean value of - 0.052 ± 0.070. The negative inbreeding coefficient indicated outbreeding in both populations.

Both tests to investigate population genetic bottlenecks revealed no signature of a bottleneck for the two populations. Both populations showed high expected heterozygosity under equilibrium compared with the observed heterozygosity (Table 4.4). The mode shift test also yielded a normal L- shaped allele distribution curve (Table 4.4), indicating a large proportion of allele classes with low frequency, indicating the lack of a genetic bottleneck in the swamp deer populations. So, both populations lack genetic bottleneck and are regarded as stable populations.

The Bayesian clustering analysis of dataset gave the maximum value for ΔK (mean likelihood of K (mean LnP[X/K] = -2679.91) when K = 2 (Figure 4.3). This is the value of ΔK where the best convergence of log-likelihoods was obtained, and the majority of the individuals were assigned in clusters indicating two structures (clusters) of the swamp deer population in Nepal. However, I presented structure output plots for K = 3 and 4 also (Figure 4.3).

Table 4. 5 Summary of multilocus genotype data for swamp deer populations in Nepal. Key: Na = number of alleles; Ho = observed heterozygosity; He = expected heterozygosity; PIC = Polymorphic information content; * = deviation from HWE (P<0.05)

Loci	All populations (n = 82)					SNP (n=42)				BNP (n=26)			
	Size range	Na	Ho	He	PIC	Na	Ho	He	FIS	Na	Ho	He	FIS
BM1225*	242-280	13	0.761	0.839	0.814	11	0.714	0.808	0.118	11	0.840	0.866	0.031
BM848*	348-372	12	0.671	0.856	0.834	11	0.707	0.890	0.208	7	0.615	0.759	0.193
T156*	148-196	13	0.820	0.896	0.880	13	0.857	0.898	0.047	8	0.760	0.848	0.106
ABS12	124-162	8	0.681	0.633	0.581	8	0.682	0.653	-0.046	4	0.680	0.609	-0.118
BM203*	206-238	14	0.634	0.825	0.797	14	0.736	0.849	0.135	7	0.480	0.754	0.368
TGLA226	124-132	5	0.588	0.468	0.401	5	0.595	0.481	-0.241	4	0.576	0.456	-0.271
T108*	153-193	10	1.000	0.801	0.770	10	1.000	0.809	-0.239	6	1.000	0.756	-0.331
T507	143-156	5	0.575	0.582	0.491	5	0.547	0.615	0.112	2	0.625	0.509	-0.232
IDVGA55	191-213	10	0.926	0.829	0.800	9	0.928	0.816	-0.139	6	0.923	0.835	-0.107
Ca67	177-201	8	0.833	0.725	0.680	8	0.875	0.739	-0.185	4	0.769	0.667	-0.156
Mean		9.80	0.749	0.745	0.705	9.40	0.764	0.756	-0.023	5.90	0.727	0.706	-0.052
S.E.		1.03	0.045	0.044		0.956	0.046	0.042	0.053	0.808	0.051	0.045	0.070

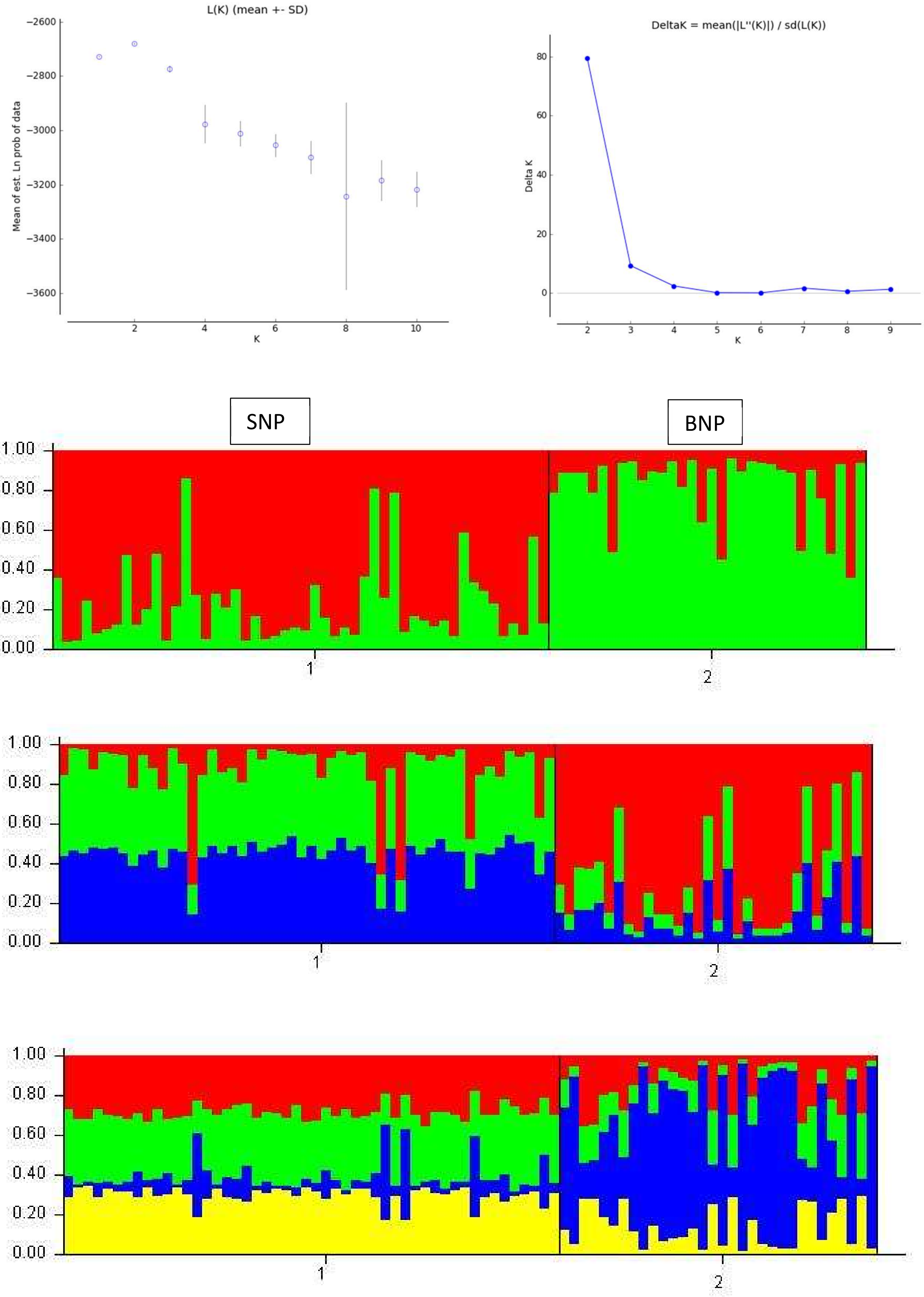

Figure 4. 3 Bayesian clustering patterns of swamp deer population.; (A) L (K) (mean ± SD) over 15 runs for each value of K = 1 – 10; (B) ΔK; (C) bar plots of individual assignments (K = 2 to 4).

Genetic Differentiation

The AMOVA revealed low molecular variances between populations based on mtDNA (34.34%) and microsatellite analysis (3.04%), compared to high molecular variances within populations. Accordingly, the fixation indexes F_{st} were also low, being 0.343 at mtDNA level and 0.030 at microsatellite level, indicating low genetic differentiation between SNP and BNP (Table 4.6).

Table 4. 6 Analysis of molecular variance of the swamp deer populations.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation
Based on haplotype frequencies (mtDNA analysis)				
Among populations	1	8.428	0.17776 Va	34.34
Within populations	98	33.312	0.33992 Vb	65.66
Total	99	41.740	0.51768	F_{ST} : 0.343, $P < 0.001$
Based on the number of different alleles (microsatellite analysis)				
Among populations	1	6.211	0.05649 Va	3.04
Within populations	162	291.917	1.80196 Vb	96.96
Total	163	298.128	1.85845	F_{ST} : 0.030, $P < 0.001$

Effective Population Size (N_e)

The estimates of Ne values as calculated by the LD method are given in Table 4.7. SNP shows high N_e value compared with BNP. The value of N_e in BNP is below 50, which is often regarded as a threshold below which inbreeding depression is likely to occur (Zachos et al., 2016).

Table 4. 7 Effective population size (Ne) estimates calculated from NeEstimator based on the LD approach. For each population, Ne estimates are given for three different frequency thresholds (Pcrit = 0.01, 0.02 and 0.05) with a 95% confidence interval based on parametric and jack-knife on loci. n: number of samples used for analysis. The interpretation of "infinite" values refers to cases where there is no evidence for variation in genetic characteristic caused by genetic drift due to a finite number of parental individuals, i.e. all can be described by sampling error (Do et al., 2014; Waples & Do, 2010).

			Frequency threshold (P_{crit})		
Population	n	Effective population size (N_e)	0.05	0.02	0.01

SNP	50	Estimated N_e	136.5	176.0	549.8
		95% CI (parametric)	70.3 - 733.3	99.8 - 573.8	180.3 - Infinite
		95% CI (Jackknife on Loci)	62.9 - Infinite	87.3 - 1674.7	138.0 - Infinite
		N_e/N*	0.084	0.109	0.342
BNP	32	Estimated N_e	26.6	28.3	44.9
		95% CI (parametric)	17.1 - 48.1	18.8 - 48.4	27.1 - 100.3
		95% CI (Jackknife on Loci)	15.5 - 58.3	17.2 - 56.9	24.8 - 131.2
		N_e/N*	0.350	0.372	0.590

*N_e/N is the ratio of effective population size to actual counted adult population size.

4.5 Discussion

Genetic diversity

At mtDNA level, the haplotype diversity (Hd) of swamp deer from SNP (0.799±0.036) was higher than that of BNP (0.455±0.086). The high Hd could be due to the large effective number of individuals found in SNP. The low Hd in BNP implies that the current population in Bardia probably originated from relatively fewer individuals than SNP. The overall Hd of swamp deer populations in Nepal (0.843±0.018) was comparable with that of Indian populations (0.813±0.029; Kumar et al., 2017). The Hd of SNP from Nepal is comparable with Dudhwa (0.722±0.159) and Kanha (0.720±0.044) from India (Kumar et al., 2017). Similarly, relatively low Hd estimate of BNP was comparable with Jheelmil Jheel (0.525±0.137) in India (Kumar et al., 2017). At cross-species level, the mitochondrial DNA variability of Kashmir red deer (*Cervus elaphus hanglu*) (Hd 0.589±0.091; Mukesh et al., 2015) is similar to estimates from BNP in Nepal and Jheelmil Jheel in India. The Hd of swamp deer from SNP is comparable with eld's deer (*Cervus eldi thamin*) (Hd = 0.77; Balakrishnan et al., 2003) from Myanmar. At microsatellite level, both populations of swamp deer had comparable average heterozygosity (H_e) values ranging from 0.705 (BNP) to 0.765 (SNP). These estimates were slightly higher than swamp deer populations from India, where H_e ranged from 0.542 in Kaziranga to 0.601 in Dudhwa (Kumar et al., 2017).

Genetic differentiation and population structure

AMOVA results based on both mtDNA and microsatellite marker showed a low population differentiation between SNP and BNP swamp deer. The low F_{st} value possibly indicates that the BNP population may be derived from the SNP population. A similar low genetic differentiation was found between two spatially isolated Dudhwa and Jhilmil Jheel populations of swamp deer in India, where F_{st} value was 0.218 at mtDNA level and 0.059 at nuclear level (Kumar et al., 2017). The Bayesian cluster analysis indicated a substructure in the population because the majority of the individuals (more than 80%) were strongly assigned to one of the two clusters. This result strongly revealed the presence of geospatial population structure in swamp deer populations in Nepal. The genetic structure analysis among spatially isolated wild swamp deer populations indicated the existence of four clusters (Dudhwa, Jhilmil Jheel, Kaziranga and Kanha) from India (Kumar et al., 2017) and two clusters (Shuklaphanta & Bardia) from Nepal (present study). However, the relationship between Nepalese and Indian subpopulations are the subject of further study. Here, Dudhwa, Jhilmil Jheel, Shuklaphanta and Bardia represent the northern populations of *R. d. duvaucelii*; Kaziranga and Kanha represent the eastern and central populations of *R. d. ranjitsinhi* of *R. d. branderi,* respectively. A similar genetic population structure was found in Kashmir red deer (Mukesh et al., 2015) and hog deer (Gupta et al., 2018).

Effective population size

The N_e of BNP is less than SNP, which is expected because of the large census population size (N) of SNP compared to BNP. The N_e/N value in both populations is comparable, though in SNP is comparatively less than in the BNP (Table 4.7). Studies (Ficetola, Padoa-Schioppa, Wang, et al., 2010; Palstra & Ruzzante, 2008; Pray, Goodnight, Stevens, et al., 1996) show a negative relationship between N_e/N and N within species. Pray et al. (1996) found that the N_e/N ratio decreased as census size increased, and large populations had a proportionately smaller N_e/N ratio than small populations. The N_e/N value in a population or species differs greatly depending upon demography and life history. A recent review found a median N_e/N value of approximately 0.15 with many low fecundity vertebrate species having this value of more than 0.15 and in high fecundity groups, such as marine fishes, having extremely low N_e/N (<0.001) (Palstra et al., 2008). The N_e/N value of swamp deer is comparable with elk deer (0.23 and 0.41), white-tailed deer (0.52- 0.65) (Frankham, 1995). SNP, which possibly holds the largest population of swamp deer in the distribution range, has N_e estimate similar with Bavarian red deer calculated for 11 different subpopulations in Germany (N_e = 80.6 to 301 & N = 90 - 5000; Kuehn et al., 2003). Similar comparable N_e estimates are available for European red deer at continental scale consisting of twenty populations from different European countries (Zachos et al., 2016). The Linkage disequilibrium approach for estimating N_e is viewed as a reliable method overall. However, the estimate should be interpreted with caution because there are many unknowns in any calculations of effective population size (Gordon Luikart et al., 2010), and the results can be biased with the sample size and markers used (Mukesh et al., 2015; Pudovkin, Zaykin, & Hedgecock, 1996).

For mtDNA, both populations of swamp deer exhibited multimodal mismatch distribution curve, indicating that these are under demographic equilibrium and did not undergo a bottleneck in the past. Neutrality tests further supported, indicating no demographic sign of population expansion. Additionally, no population bottleneck case was identified at microsatellite level, which was indicated by both the presence of a normal L-shaped distribution curve (mode shift test) and obtaining a statistically nonsignificant higher value of expected heterozygosity at drift mutation equilibrium compared to observed heterozygosity under gene diversity excess test. So, regardless of the BNP population's low effective population size, there are moderate to high genetic diversity indices at mtDNA and microsatellite level, indicating that both isolated populations of swamp deer in Nepal are genetically stable.

Management Recommendations

Overall, this study suggested that the SNP population of swamp deer has more genetic variation and effective population size than BNP; however, both are stable populations with two genetic clusters and low population differentiation.

Swamp deer population in BNP has relatively low genetic diversity, and it needs specific attention due to its low census and effective population size. It is known that populations which are entirely isolated or exist in small numbers are more prone to genetic erosion than populations that are contiguous or exist in larger numbers (Frankham, 2010). The possible cause for the sluggish increase in population size of swamp deer in BNP might be due to heavy predation by tiger or habitat quality deterioration or competition with sympatric ungulates. Therefore, it is recommended to design and implement an effective conservation strategy in order to maintain the genetic diversity and increase the population size of swamp deer in BNP through in situ conservation program as was done for the recovery of the endangered Asiatic lions (Banerjee & Jhala, 2012) in Gir Forests, Gujarat and the hard-ground swamp deer (*R. d.*

branderi) (Kumar et al., 2017) in India. Such efforts increased Asiatic Lion populations from 177 in 1968 to about 411 by 2010 (Banerjee et al., 2012). Similarly, in central India, the population of *R. d. branderi* increased from 66 individuals in 1970 to 335 individuals in 2004 (Qureshi et al. 2004).

As the genetic diversity of the BNP swamp deer population is relatively less and the effective population size is below the threshold of 50, It is recommended to translocate swamp deer individuals from the SNP to BNP to improve the genetic diversity.

As there are only two extant populations of swamp deer in Nepal, it is reasonable to recommend re-establishing a viable population in its previous distribution range (Banke and Chitwan) where habitats are still available to thrive. Similarly, it is suggested to initiate the captive breeding program for Shuklaphanta and Bardia populations to maintain viable populations in a zoo for future safe breeding stock as done in India for hard-ground swamp deer *R. d. duvaucelii* (Sankarnarayanan et al., 1995).

Chapter 5 Seasonal diets of sympatric ungulates in Shuklaphanta National Park, Nepal

5.1 Abstract

In the subtropical climate with monsoonal rain, nutrient status of plant species varies with seasons. Seasonal diets of three sympatric ungulate prey species of the tiger, such as swamp deer *Rucervus duvaucelii*, hog deer *Axis porcinus* and spotted deer *Axis axis*, were compared based on micro-histological analyses of faecal material collected from alluvial grassland and associated climax Sal (*Shorea robusta*) forest in Shuklaphanta National Park, Nepal. All three deer species had similar diets in grassland habitat, with graminoids contributing more than 95% of the swamp deer and hog deer diet and 83% of spotted deer. The proportions of woody plants were higher in spotted deer (17%) than swamp deer (1.3%) and hog deer (2.4%). The proportions of herbs in the diet of all three species were negligible (<2%). In Sal forest, apart from graminoids (50-68%), woody plants and herbs composed significant proportions (32% – 50%) of diets of all ungulates. Woody plant *Shorea robusta* and herb *Phoenix humilis* were the major plant species consumed in the Sal forest. Among graminoid species, early successional tall grasses, especially *Saccharum spontaneum*, were the dominant food of all the three deer species in both habitats. The importance of early successional tall grasses in their diet emphasised the key role of the threatened alluvial floodplains in the conservation of threatened mammal species in South Asia. Swamp deer foraged more in late succession tall grasses (*Saccharum narenga* and *Themeda* spp.) and short grasses (*Imperata cylindrica*, *Cyperus* spp., *Chrysopogon zizanioides*, *Cymbopogon* spp.) than hog deer and spotted deer. Despite the similarity of their diet, the three ungulates coexisted through differential consumption of plants species and seasonal habitat partitioning.

5.2 Introduction

Diet and foraging behaviour are essential aspects of animal ecology. Knowing the diet composition of herbivores is crucial for understanding their foraging ecology and their role in regulating plant community structures in the ecosystem (Barcia et al., 2007). The quality of food animals consumes significantly affects their reproduction, growth, survival, and population dynamics (Pekins, Smith, & Mautz, 1998). Furthermore, for the management of threatened species, it is important to understand interspecific interactions in terms of diet and habitat partitioning (Jones & Barmuta, 1998; Mysterud, 2000; Schoener, 1974); and dietary patterns can provide insight into potential competitions (Putman, 1996).

Generally, high diet similarity between species indicates competitive interaction when density is high and food resources are limited (Wegge et al., 2006). Among sympatric herbivores, niche differentiation is primarily interrelated to body size differences (Bell, 1971; Demment & Van Soest, 1985; Jarman, 1974) and morpho-physiological characteristics (Gordon & Illius, 1988; Hofmann, 1989). Smaller ungulates, because they have relatively higher metabolic requirements per unit body weight, may feed on higher-quality diets (Bell, 1971; Jarman, 1974) and therefore, they are predicted to be more selective in their choice of food uptake (Demment et al., 1985; Jarman, 1974). On the other hand, Larger species require a higher amount of food and less selective (Owen-Smith, 1988).

I used Shuklaphanta National Park (SNP) as a study site to explore the competitive interaction and coexistence of three main prey species: swamp deer (*Rucervus duvaucelii)* (c. 160 kg body weight), hog deer (*Axis porcinus)* (c. 32 kg) and spotted deer (*Axis axis)* (c. 60 kg), of the critically endangered tiger (*Panthera tigris*) (Chapter 2; Lovari, Pokheral, Jnawali, Fusani, & Ferretti, 2015). Swamp deer is a principal prey of the tiger and plays an essential role in diet partition between the tiger and common leopard (Chapter 2). Understanding the dietary interaction and potential competition between swamp deer and other ungulates is essential for conserving this important prey species. These ungulates are known to prefer tall grasslands and alluvial floodplains interspersed with forests (Wegge et al., 2006). Whereas many studies have been conducted on food interactions among sympatric ungulates in North America and Africa, only a few have compared diets of coexisting ungulates in the subtropical region of Asia (Johnsingh, 1991; Bagchi, Goyal, & Sankar, 2003; Dinerstein, 1980; Khan, 1994; Martin, 1982; Steinheim, Wegge, Fjellstad, Jnawali, & Weladji, 2005; Wegge et al., 2006). Those studies are restricted to the dry season, and little is known about their diets in the monsoon season. In this study, I examined the seasonal diets of sympatric swamp deer, hog deer and spotted deer and explored the extent of interspecific forage competition.

Both swamp deer and hog deer prefer grasslands (Mishra, 1982; Schaaf, 1978). Furthermore, hog deer shows preference exclusively for tall grassland (Odden, Wegge, & Storaas, 2005b). Therefore, I predict high diet similarities between these two species. On the other hand, spotted deer is mainly a forest species and uses grasslands mostly after burning grasslands (Mishra, 1982; Schaaf, 1978). Therefore, I predict less overlap of spotted deer with swamp deer and hog deer. During monsoon season, both swamp deer and hog deer were also found to utilise Sal forest habitat, so these two deer species may compete with the spotted deer. As swamp deer and hog deer use only grassland habitat during dry seasons, spotted deer may compete with swamp deer and hog deer.

5.3 Methods

Study area

Shuklaphanta National Park (SNP), (N: 28.7193 to 29.0515; E: 80.0609 to 80.4120) covers 305 km^2 in the far western lowland Terai region of Nepal. This study was carried out in westernmost (about 60 km^2) area of SNP (Figure 5.1). The southern part of the study area is contiguous with Lagga-Bagga sector of Pilibhit Tiger Reserve of India, providing favourable habitat for the trans-border movement of the threatened tiger (Chanchani et al., 2014) and swamp deer (Chapter 3). The altitude ranges from 90 -270 m above sea level. The climate is mainly subtropical and monsoonal, with more than 90% of the annual precipitation (1,000-2,000 mm) falling between June and September. The temperatures range from 10^0-12^{0}C during winter (February/ March) to 40^0-42^{0}C during summer (May/June) (Henshaw, 1994). There are three distinct seasons: the cold dry season (November–February), hot dry season (March–June) and monsoon (July–October). The vegetation ranges from early successional tallgrass floodplain to climax stage Sal (*Shorea robusta*) forest, and according to Pokheral & Wegge (2019), it can be classified into five types (Table 5.1).

Table 5. 1 Vegetation types in the study area of Shuklaphanta National Park (after Pokheral and Wegge, 2019).

Broad habitat (approx. area %)	Specific habitat	Approx. area (%)	Major species
Forest (43)	Sal forest	33	Sal forest is dominated by Sal (*Shorea robusta*). Other associated species are *Terminalia tomentosa*, *Terminalia chebula*, *Terminalia belerica*, *Lagerstroemia parviflora, Garuga pinnata*, *Cassia fistula, Adina cordifolia*, *Phyllanthus emblica*. Understorey species are *Colebrookea oppositifolia*, *Grewia tiliifolia*, *Asparagus racemosus*, and *Phoenix humilis*. Sal forest with open canopies (open Sal forest) usually have a ground cover of grasses, including *Eulaliopsis binata, Narenga porphyrocoma, Themeda arundinacea, Saccharum bengalensis, Saccharum spontaneum, Desmostachya bipinnata.*
	Mixed deciduous forest	10	This forest occurs along river channels. Common tree species are *Syzygium cumini*, *Trewia nudiflora*, *Mallotus philippensis*, *Ehretia laevis*, *Butea monosperma*, *Bombax ceiba*, *Ficus glomerata*, *Albizia procera*, *Holarrhena antidysenterica*, *Careya arborea*, *Cedrela toona* and *Lagerstroemia parviflora.* The understorey species comprises *Murraya koenigii*, *Cannabis sativa*, *Clerodendron viscosum*, *fern species*, *Calamus tenuis*, *Colebrookea oppositifolia*

			and *Callicarpa macrophylla*. The ground cover is dominated by grasses like *Imperata cylindrica, Saccharum spontaneum, Narenga porphyrocoma, Vetiveria zizanoides.*
	Khair-Sissoo forest	0	Sissoo *Dalbergia sissoo* and Khair *Acacia catechu* are pioneer species on unstable riverine sites only occurring on the banks of the Mahakali river in the southwest corner of the park.
Grassland (57)	Short grassland or phanta	50	These are short perennial grasses (less than 2 m tall) dominated by *Imperata cylindrica*, *Vetiveria zizanoides*, *Cymbopogan* spp., *Cyperus* spp. and *Cynodon dactylon*.
	Tall grassland	7	These area tall perennial grasses such as *Saccharum spontaneum*, *Saccharum bengalensis*, *Phragmites karka, Typha elephantine, Narenga porphyrocoma and Themeda spp*.

Sal forest represents the climax vegetation (Dinerstein, 1979a). In open Sal forest, the ground cover grass species are more or less continuous; however, they occur more patchily in the mixed deciduous forest (Schaaf, 1978; Henshaw, 1994). Short grasslands (or phanta), dominated by *Imperata cylindrica,* result from anthropogenic interventions such as forest clearing, burning, livestock grazing, cultivation and abandoned villages (Lehmkuhl, 1989; Pokharel, 1993). On previously cultivated sites, short *Imperata* dominated swards usually succeed to tall *Narenga porphyrocoma* dominated swards, forming a tall and short grassland mosaic. Tall grasslands represent an early successional stage (Peet et al., 1999). On permanently wet or seasonally inundated sites, tall grassland is dominated by *Saccharum spontaneum, Saccharum bengalensis, Phragmites karka and Typha elephantine.* However, on better developed non-inundated soil, the dominant species are *Narenga porphyrocoma*, *Themeda spp*. Except for one large patch of tall grassland in the extreme southwest, other grasslands were interspersed with forested habitats across the study area (Figure 5.1).

Annual cutting and burning have been practised for the management of Nepalese grasslands, including SNP. Such management aims to prevent succession from grassland to forest, provide good quality forage for ungulates as the grasslands regenerate, and prevent destructive hot burns late in the dry season (Peet et al., 1999; Schaaf, 1978). In Sal forest, too, for avoiding damaging wildfire in the late dry season, deliberate early burning of ground vegetation is common. However, the timing of fire in grassland and forest is different, being early (December/January) in southern grassland and later (March/April) in northern Sal forest.

In addition to the three focal deer species, other coexisting mammals include barking deer *Muntiacus muntjak*, nilgai antelope *Boselaphus tragocamelus*, wild boar *Sus scrofa*, Asian elephants *Elephas maximus* and One-horned rhinoceros *Rhinoceros unicornis*. According to population survey in April and May of 2016, the population estimate of swamp deer in the study area was 1883 ± 22 (SD) individuals compared to 805 individuals in 1975 (Schaaf, 1978). The estimated density of swamp deer, hog deer and spotted deer was 37.5 (26.9 – 52.3), 19.2 (12.8 – 28.8) and 42.7 (31.2 – 58.7) animals/km^{2,} respectively (Chapter 2).

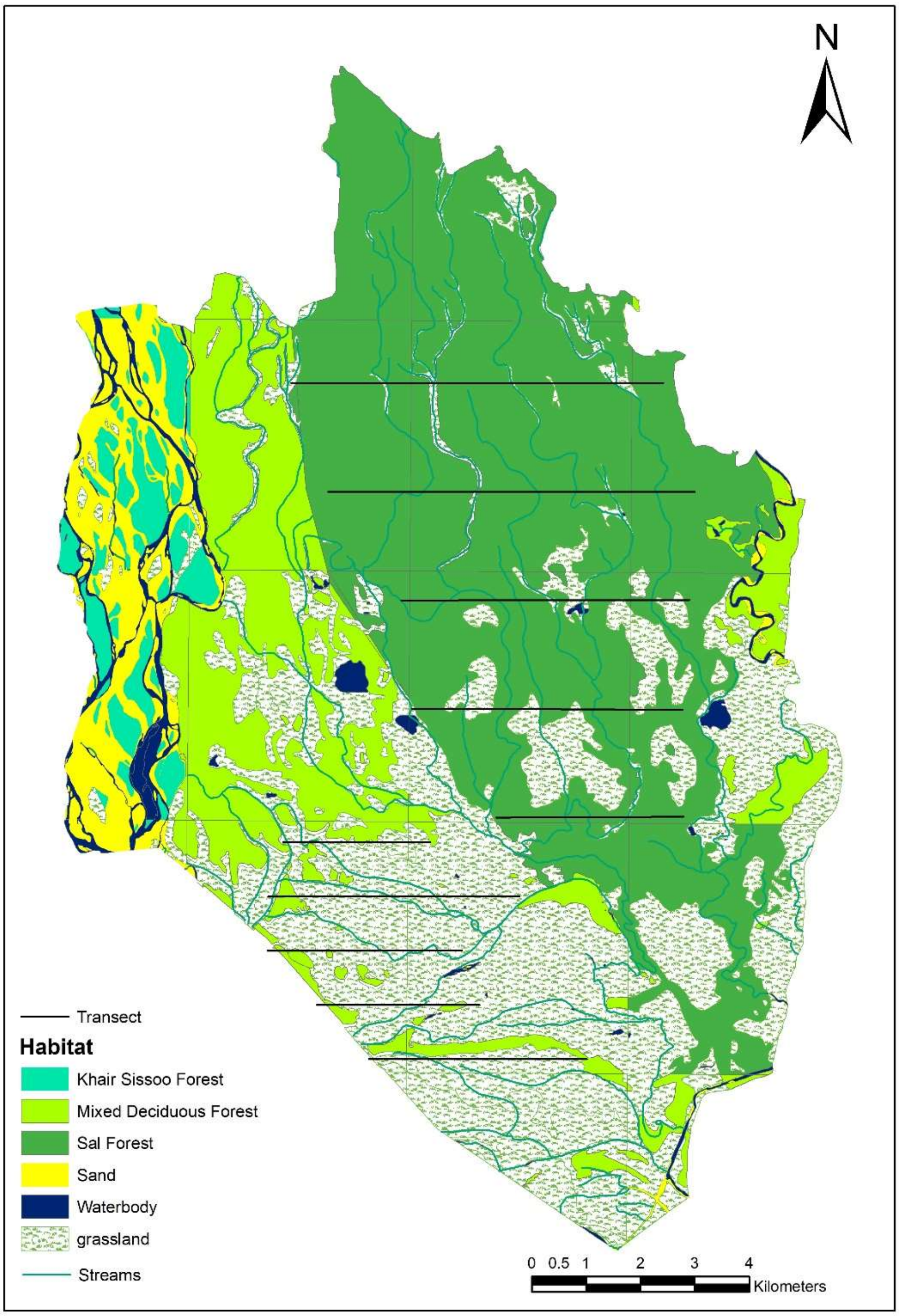

Figure 5. 1 Line transects showing the study area in Shuklaphanta National Park.

Micro-histological analysis of faeces

The diets of ungulates were determined using faecal microhistological analysis (Norbury, 1988). The waxy cuticle of plants carries a permanent imprint of underlying epidermal characteristics, which survives ruminant digestion (Stevens, Stevens, Gates, et al., 1987). Therefore, the undigested epidermal parts of the plant in the faeces are identified by comparing them with known plant reference material (Norbury, 1988; Sparks & Malechek, 1968). This technique is regarded as reliable, particularly for comparative studies of diets (Butet, 1985; Garnick, Barboza, & Walker, 2018; Wegge et al., 2006). Furthermore, the faecal analysis gives greater sampling precision and does not require the sacrifice of animals (Holechek, Vavra, & Pieper, 1982). This method has successfully been used to diet studies of free-ranging animals such as rhino (Jnawali, 1995; Steinheim et al., 2005), swamp deer (Pokharel, 1996; Tewari & Rawat, 2013), hog deer (Wegge et al., 2006), Elk deer (Sandoval, Holechek, Biggs, et al., 2005), mule deer (Sandoval et al., 2005), takin (Wangchuk, Wegge, & Sangay, 2016), Asian elephant (Koirala, Raubenheimer, Aryal, et al., 2016; Steinheim et al., 2005), hispid hare (Achyut Aryal, Brunton, Ji, et al., 2012), four-horned antelope (Kunwar, Gaire, Pokharel, et al., 2016) and mountain ungulates (Harris & Miller, 1995; Khadka, Singh, Magar, et al., 2017; Shrestha, Wegge, & Koirala, 2005).

Faecal sample collection

Fresh faecal samples (less than two days old) of swamp deer, hog deer and spotted deer were collected in cold dry (December, January 2015/2016), hot dry (April, May, June 2016), and monsoon (August, September 2016) seasons. The feeding herds of studied species were mostly located from a distance, and then 10 – 15 fresh pellets from each pellet group were collected after the animals had moved away (Wegge et al., 2006). In some cases, when animals were not seen defecating, pellets of deer species were differentiated based on shape and size (Dinerstein, 1980) by comparing with confirmed pellets of each species. In grassland habitat, faecal samples were collected for all three deer species and all three seasons. However, in Sal forest, the sample size was smaller and restricted to monsoon season when swamp deer and hog deer emigrated to this habitat (Chapter 3). In total, 115 pellet groups were sampled of each deer species (Table 5.2).

Table 5. 2 Details on the number of pellet groups used to analyse diets of ungulates in Shuklaphanta National Park.

Deer species	**Number of pellet groups**				**Total**
	Grassland			Sal forest	
	Cold dry	Hot dry	Monsoon	Monsoon	
Swamp deer	40	30	35	10	115
Hog deer	40	30	35	10	115
Spotted deer	40	30	35	10	115
Total	120	90	105	30	345

Preparation of composite samples and slides

From different pellet groups, I prepared composite samples for each species (Table 5.3). First, five pellet groups were randomly selected from each species. Five pellets were then picked from each group and pooled to make a composite sample (Wegge et al., 2006). Next, the composite samples were ground with an electric blender and sieved through Endecott sieves of mesh size 1 – 0.3 mm. The fragments that remained on the 0.3 mm sieve was used as final samples for slide preparation.

Table 5. 3 Details on the number of composite samples (number of fragments) used to analyse diets of ungulates in Shuklaphanta National Park.

Species	**Number of composite samples (fragments)**				**Total**
	Grassland			Sal forest	
	Cold dry	Hot dry	Monsoon	Monsoon	
Swamp deer	8 (800)	6 (600)	7 (700)	2 (200)	23 (2300)
Hog deer	8 (800)	6 (600)	7 (700)	2 (200)	23 (2300)
Spotted deer	8 (800)	6 (600)	7 (700)	2 (200)	23 (2300)
Total	24 (2400)	18 (1800)	21 (2100)	6 (600)	69 (6900)

Reference plant material, slide preparation and plant species identification

A total of 71 potential forage species were collected based on previous dietary studies of swamp deer (Pokharel, 1996; Schaaf, 1978; Tewari & Rawat, 2013; Wegge et al., 2006), hog deer (Dhungel et al., 1991; Wegge et al., 2006) and spotted deer (Dinerstein, 1980; Johnsingh and Sankar, 1991; Khan, 1994), and from my observation on plants grazed by these species. I followed the methods suggested by Norbury (1988) to prepare the slides of faecal and reference plants. Both reference slides and faecal slides were observed at 100x and 400x magnifications with a compound microscope, and each fragment were photographed using a digital camera for microscope (DCM510; USB2.0; 5M pixel, CMOS chip) in a laptop using software - ScopeTek Scope Photo; Version: x64, 3.1.615 (http://www.scopetek.com).

I used five slides for each composite sample. Two transects were randomly chosen on each slide. On each transect, I identified the first encountered ten non-overlapping fragments by matching the fragments with the reference photographs based on special histological features of the epidermis such as shape, size and arrangement of the epidermal cell, cell wall structure, hairs and trichomes, shape, size and orientation of stomata etc. A total of 2300 fragments (cold dry = 800, hot dry = 600, monsoon = 700 and Sal forest (monsoon) = 200) were identified for analysis of diet of each species (Table 5.3). The fragments that could be identified to a forage category but could not be identified to species or genera were classified into ‘‘unidentified graminoids’’, ‘‘unidentified woody plants’’ or ‘‘unidentified others’’. Two grass species, *Phragmites karka* and *Arundo donax,* were grouped due to difficulty in separating them. Fragments that could not be identified even to the forage category were classified as ‘‘unknown’’.

Data analysis

All monocot species in the diet were included in ‘‘graminoids’’ category. All woody dicot species (trees, shrubs and climbers) were included in the ‘‘woody plants’’ category. Herbs were included in the ‘‘others’’ category. The relative frequency of each category and each plant species was expressed as a percentage to determine the diet composition (Wegge et al., 2006). Chi-square test statistic was used to test whether the proportions of forage categories were similar across species and seasons. One-way ANOVA was used to determine if the proportions of graminoids, woody plants and others varied in the diet of the three focal species. In the case of non-normality, a Kruskal-Wallis test was used. When there were significant differences, pairwise multiple comparisons using Bonferroni simultaneous confidence intervals with adjusted critical P-value (Byers, Steinhorst, & Krausman, 1984), or Dunn’s method in the case of Kruskal–Wallis ANOVA, were carried out.

Based on successional affinity, dominance and height (Peet et al., 1999), graminoid food plants were further categorised as follows to analyse the dietary pattern at a finer scale (Wegge et al., 2006).

(a) ‘’Early succession tall grasses’’ included *Saccharum spontaneum*, *Arundo donax* /*Phragmites karka*, *Saccharum bengalensis* and *Saccharum ravennae*

(b) ‘’Late succession tall grasses’’ included *Themeda* spp. and *Saccharum narenga*, and

(c) ‘’Short grasses’’ consisted of *Imperata cylindrica*, *Cynodon dactylon*, *Chrysopogon zizanioides*, *Cymbopogon* spp., *Desmostachya bipinnata*, *Apluda mutica* and *Cyperus* spp.

Niche breadth: Niche breadth was measured using the Shannon–Wiener diversity index (Krebs, 1989):

$$\boldsymbol{H' = \sum Pjlog\ Pj}$$

Where H' is niche breadth measured with the Shannon–Wiener index of plant species, Pj is the proportion of j^{th} plant species (*j* = 1, 2, 3,..., n), and n is the total number of species included. Since this equation gives results ranging from 0 to ∞, the evenness measure, J’, was calculated to standardize the scale ranging from 0 to 1 (Krebs, 1989) as follows:

$$\boldsymbol{J' = \frac{H'}{\log n}}$$

Where J' is the evenness measure of the Shannon–Wiener function and n is the total number of plant species included.

Diet similarity: Diet similarity at forage categories (at broader scale) and within forage categories (at finer scale) level between ungulate pairs was calculated using the percentage similarity index (Gauch Jr., 1973). This index ranges from 0 (no similarity) to 100% (complete similarity).

$$\boldsymbol{PSI = \frac{2 \times \sum \min(x, y)}{\sum(x + y)} \times 100}$$

Where x and y are the frequencies of each plant species recorded in the diet of ungulate pair 1 and 2, respectively, and min (x, y) is the minimum frequency of each plant species recorded between ungulate pair 1 and 2.

Multivariate analysis: The similarity and dissimilarity in diets among ungulate species were also explored using non-metric multidimensional scaling (NMDS) analysis of multivariate analysis technique. In NMDS, the Bray- Curtis distance was used to construct a map in which species having more similarity in diets were placed near and having lower similarity away. I used PERMANOVA+ package in PRIMER v6 software (Anderson, Gorley & Clarke, 2008) to perform PERMANOVA (Anderson, 2017) to test diet differences among species and seasons and NMDS mapping.

5.4 Results

Annual diet composition

Swamp deer

Swamp deer were found to feed on 37 species of plants (15 graminoids, 18 woody plants, and 4 herbs) belonging to 19 families (Appendix 1.2). More than 75% of the food species were from the family Poaceae, followed by Cyperaceae (7%) in grassland. In Sal forest, Poaceae also dominated with 43%, followed by Arecaceae (38%) and Dipterocarpaceae (6.5%) (Appendix 1.3).

In both habitats, graminoids dominated (grassland, 98%; Sal forest, 50%) in diet (Table 5.4 & 5.5, appendix 1.2 & 1.3). The tall grass *Saccharum spontaneum* was the most common species in the diets (grassland, 39%; Sal forest, 35%) (Table 5.4 & 5.5). In grassland, woody plants and others each contributed minimally (<2%); however, these categories made up 13% and 38% in Sal forest, respectively.

Tall grass *Saccharum spontaneum* was the main forage plant in all seasons. In grassland, the proportions of forage categories were not significantly different among the three seasons (χ^2=2.8, *df* = 4, *p*>0.05). In Sal forest, among woody plants, *Shorea robusta* (6.5%) was the main food plant, and in others category, *Phoenix humilis* (38%), the only species recorded was an essential food plant (Table 5.5).

Hog deer

Hog deer were found to feed on 30 plant species (13 graminoids, 12 woody plants, and 5 herbs) belonging to 17 families (Appendix 1.2). In grassland, 85% of the food species were from the family Poaceae, followed by Moraceae (1.3%). In Sal forest, also Poaceae dominated the diet with 66% contribution, followed by Arecaceae (17%), Dipterocarpaceae (8%) (Appendix 1.3).

In both habitats, graminoids constituted major diets (grassland, 97%; Sal forest: 68%) (Table 5.4 & 5.5, Appendix 1.2 & 1.3) and the tall grass *Saccharum spontaneum* was the most dominant species grazed (grassland, 56%; Sal forest: 57%) (Table 5.4 & 5.5).

Tall grass *Saccharum spontaneum* was the main forage plant in all seasons. In grassland, the proportions of forage categories were not significantly different among the three seasons (χ^2=5.2, df = 4, p>0.05). In Sal forest, *Shorea robusta* in woody plants and *Phoenix humilis* in others category were dominant, contributing 8% and 17%, respectively (Table 5.5).

Spotted deer

Spotted deer was found feeding on 39 species of plants (13 graminoids, 23 woody plants, and 3 herbs) representing 20 families (Appendix 1.2). More than 70% of the food species were from the family Poaceae, followed by Moraceae (2.5%) in grassland. In Sal forest, also Poaceae dominated the diet with 45% contribution, followed by Dipterocarpaceae (32%) and Arecaceae (12%) (Appendix 1.3, Table 5.4 & 5.5).

In both habitats, the percentage of graminoids were highest in the faecal samples (grassland, 83%; Sal forest, 55%) (Table 5.4 & 5.5, Appendix 1.2 & 1.3). Like the other two cervids, tall grass *Saccharum spontaneum* was the most important food plant, contributing 38%.

In grassland, the proportions of forage categories were not significantly different among the three seasons (χ^2=2.2, *df* = 4, *p>0.05*). In Sal forest, the percentage of tall grass *Saccharum spontaneum* in the faecal samples (30%) was like that of woody species *Shorea robusta* (31.5%). Woody species contributed 20% and 34% in grassland and Sal forest, respectively. *Phoenix humilis*, the only plant species identified in others category in Sal forest, contributed around 12% (Table 5.5).

Table 5. 4 Proportion (%) of plant species in the faecal samples of ungulates in the grassland habitat of Shuklaphanta National Park.

	Cold dry			Hot dry			Monsoon		
Plant species	Swamp	Hog	Spotted	Swamp	Hog	Spotted	Swamp	Hog	Spotted
Graminoids									
Tall grasses									
Early successional tall grasses									
Saccharum spontaneum	40.3	65.4	53.3	35.8	47.7	30.5	39.5	54.6	29.4
Saccharum bengalense	6.5	4.0	10.0	4.7	2.7	1.7	4.0	3.3	4.5
Saccharum ravennae	5.5	4.5	3.3	1.7	3.5	2.0	1.7	2.6	4.3
Phragmites karka	0.8	0.0	0.3	1.5	6.8	2.0	0.6	0.2	2.0
Total early successional tall grasses	**53.1**	**73.9**	**66.9**	**43.7**	**60.7**	**36.2**	**45.8**	**60.7**	**40.2**
Late successional tall grasses									
Saccharum narenga	5.1	2.1	2.0	9.8	1.3	4.7	5.6	1.6	3.5
Themeda spp.	3.1	0.1	0.8	3.2	3.5	0.7	6.5	0.1	2.5
Total late successional tall grasses	**8.2**	**2.2**	**2.8**	**13.0**	**4.8**	**5.4**	**12.1**	**1.7**	**6.0**
Short grasses									
Imperata cylindrica	2.1	3.3	5.5	4.5	3.5	4.5	5.5	1.8	9.0
Chrysopogon zizanioides	0.9	7.1	0.0	11.0	1.5	16.0	8.7	1.1	5.0
Cymbopogon spp.	4.0	0.8	0.5	3.5	2.5	0.3	3.6	0.0	3.0
Cyperus spp.	10.0	0.0	0.5	5.2	3.3	1.0	5.6	16.6	2.5
Apluda mutica	1.1	0.0	0.0	1.5	0.0	0.0	1.3	0.0	0.0
Eulaliopsis binata	0.0	0.0	0.8	0.0	0.3	0.0	0.3	0.0	1.5
Chrysopogon aciculatus	0.0	0.0	1.0	0.2	0.2	0.0	0.0	0.3	1.5
Banso	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dubo	2.1	2.4	0.5	2.3	3.5	2.3	1.7	4.6	3.5
Typha spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Total short grasses	**20.5**	**13.6**	**8.8**	**28.2**	**14.8**	**24.1**	**26.7**	**24.5**	**26.0**
Unidentified graminoids	14.4	4.1	6.4	13.9	18.4	18.4	14.2	11.4	7.3
Total graminoids	**96.2**	**93.8**	**84.9**	**98.8**	**98.7**	**84.1**	**98.8**	**98.3**	**79.5**
Woody plants									
Identified	1.4	3.8	10.9	0.6	0.6	5.6	0.8	0.6	8.4
Unidentified	0.8	0.8	3.1	0.2	0.5	10.3	0.3	0.9	11.2
Total woody plants	**2.2**	**4.6**	**14.0**	**0.8**	**1.1**	**15.9**	**1.1**	**1.5**	**19.6**
Total others	**1.7**	**1.8**	**1.1**	**0.4**	**0.2**	**0.0**	**0.1**	**0.2**	**0.9**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 5. 5 Proportion (%) of plant species in the monsoon season diet of ungulates in the Sal forest of Shuklaphanta National Park.

Plant species	Swamp deer	Hog deer	Spotted deer
Graminoids			
Tall grasses			
Early successional tall grasses			
Saccharum spontaneum	35.0	57.0	30.0
Saccharum bengalense	1.0	4.0	5.5
Saccharum ravennae	1.5	3.0	1.0
Total early successional tall grasses	**37.5**	**64.0**	**36.5**
Late successional tall grasses			
Saccharum narenga	1.5	0.0	1.5
Total late successional tall grasses	**1.5**	**0.0**	**1.5**
Short grasses			
Imperata cylindrica	1.5	1.0	2.0
Chrysopogon zizanioides	0.5	0.0	3.0
Cyperus spp.	0.0	1.0	0.0
Eulaliopsis binata	0.0	0.0	1.5
Dubo	2.0	1.0	0.0
Total short grasses	**4.0**	**3.0**	**6.5**
Unidentified graminoids	6.5	1.0	10.0
Total graminoids	**49.5**	**68.0**	**54.5**
Woody plants			
Shorea robusta	6.5	8.0	31.5
Helicteres isora	3.5	0.0	0.0
Others identified woody plants	2.5	4.0	1.0
Unidentified woody plants	0.0	3.0	1.5
Total woody plants	**12.5**	**15.0**	**34.0**
Others			
Phoenix humilis	38.0	17.0	11.5
Total others	**38.0**	**17.0**	**11.5**
Total	**100**	**100**	**100**

Seasonal diet

Multivariate analysis

The NMDS plot (Figure 5.2) shows a clear diet partition between swamp deer, hog deer and spotted deer regarding the intake of different forage species. However, the diet of hog deer overlapped both with that of swamp deer and spotted deer but more similar to that of the former. The PERMANOVA test further shows variation in differential use of diet among species (F= 43.7, d. f. = 2, P = 0.001), seasons (F = 39.6, d. f. = 2, P = 0.001) and interaction of both species and seasons (F = 17.2, d. f. = 4, P = 0.001).

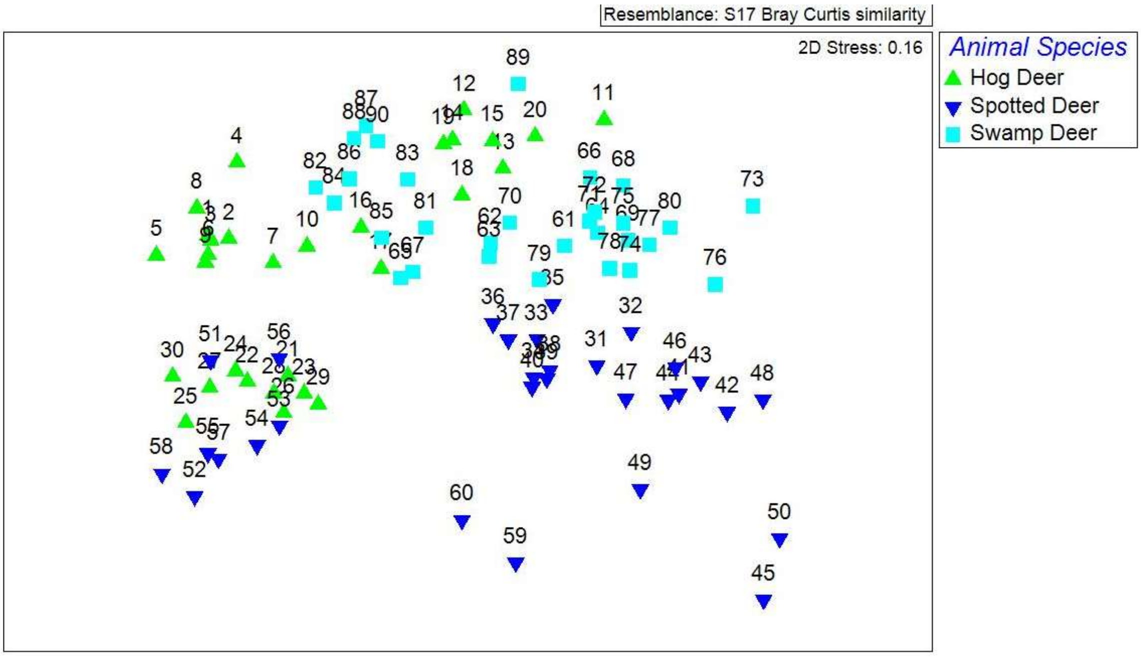

Figure 5. 2 NMDS plot showing partitioning in the diet of ungulates in Shuklaphanta National Park.

Grasslands

Cold dry season

For all food categories, spotted deer and hog deer had the highest overlap (≈75%), followed by nearly similar overlaps between spotted deer and swamp deer (≈65%) and swamp deer and hog deer (≈63%). Similarly, the diet of spotted and hog deer had the highest overlap (≈ 80%) for the graminoids category, and the least overlap was between that of swamp deer and hog deer (≈64%). Pairwise, spotted deer and hog deer and swamp deer and hog deer consumed similar proportions of woody plants (≈30%) and spotted deer and swamp deer had the least overlap of this food category (≈19%) (Table 5.6).

The proportions of forage categories were significantly different among the three species ($\chi 2 = 12.4$, df = 2, $p < 0.001$) (Figure 5.3). However, they did not differ in consumption of graminoids ($H = 1.7$. df = 2, $P > 0.05$) but consumed significantly different proportions of woody plants ($H = 7.1$, df = 2, $P < 0.05$). Pairwise Bonferroni comparisons revealed significant differences in woody plants consumption between swamp deer and spotted deer ($H = 4.5$, df = 1, $P < 0.05$) and between spotted deer and hog deer ($H = 4.8$, df = 1, $P < 0.05$), with spotted swamp deer consuming more than other two. Swamp deer and hog deer did not differ in their proportions of woody plants ($H = 0.02$, df = 1, $P > 0.05$).

The proportions of different subcategories of graminoids were significantly different among three deer species ($\chi 2 = 12.6$, df = 4, $p < 0.05$) (Figure 5.4, Table 5.4). “Early succession tall grasses” were the most important food plants for all three cervids (>80%), but they did not differ in their relative consumption of this subcategory ($H = 0.154$, $P = 0.926$). Hog deer consumed the highest proportion of the early succession tall grasses, especially *Saccharum* *spontaneum*, followed by swamp deer and spotted deer. Swamp deer consumed relatively more “late succession tall grasses” followed by spotted deer and hog deer; however, their consumptions were not significantly different (F = 3.47, P = 0.165). The consumption of “short grasses” was also similar (H = 3.08, P = 0.213).

Hot dry season

Swamp deer and hog deer had the highest overlap in all (≈86%), graminoids (≈86%), and woody plants (≈21%) categories indicating these two cervids were consuming similar plant species. Conversely, spotted deer and hog deer had the least overlap in all (≈66%) and graminoids (≈71%) categories; however, for woody plants, the least overlap (≈2%) was between spotted deer and swamp deer (Table 5.6).

The proportions of forage categories were significantly different among the three deer species ($\chi2 = 25.9$, df = 2, $p < 0.001$) (Figure 5.3). They did not differ in graminoids consumption (H = 0.5, df = 2, $P > 0.05$), however, consumed significantly different proportions of woody plants (H = 9.7, df = 2, $P < 0.05$). Pairwise Bonferroni comparisons revealed significant differences in woody plants consumption between swamp deer and spotted deer (H = 7.4, df = 1, $P < 0.05$) and spotted deer and hog deer (H = 4.4, df = 1, $P < 0.05$) with spotted swamp deer consuming more than other two. Swamp deer and hog deer did not differ in their proportions of woody plants (H = 1, df = 1, $P < 0.05$).

The proportions of different subcategories of graminoids were significantly different among three deer species ($\chi2=13.2$, df = 4, $P < 0.05$) (Figure 5.4, Table 5.4). “Early succession tall grasses” were the most important food plants for all three cervids, but they did not differ in their relative consumption of this subcategory (H = 1.8, df = 2, P = 0.392). Hog deer consumed the highest proportion of the early succession tall grasses, especially *Saccharum spontaneum*, followed by swamp deer and spotted deer. Swamp deer consumed more "late succession tall grasses" than other two deer; however, their consumptions were not significantly different ($F = 0.97$, $df = 2$, $P = 0.472$). Swamp deer and hog deer consumed more short grasses than spotted deer, but their consumptions were not significantly different ($H = 1.07$, $df = 2$, $P = 0.585$).

Monsoon season

Pairwise, swamp deer and hog deer and swamp deer and spotted deer had similar overlaps (≈68%) in all categories; however, spotted deer and hog deer had the least similarity (≈55%). For graminoids, the highest overlap (≈75%) was between swamp deer and spotted deer, followed by swamp deer and hog deer (69%) and the least between spotted deer and hog deer (≈61%). In consumption of woody plants, swamp deer and hog deer had the highest overlap (≈54%), followed by spotted deer and hog deer (≈13%) and spotted deer and swamp deer (≈10%) (Table 5.6).

The proportions of forage categories were significantly different among the three deer species ($\chi2 = 32.8$, $df = 2$, $p < 0.001$) (Figure 5.3). They did not differ in graminoids consumption ($H = 1.8$, $df = 2$, $P > 0.05$) but consumed different proportions of woody plants ($H = 9.3$, $df = 2$, $P < 0.05$). Pairwise Bonferroni comparisons revealed significant differences in woody plants consumption between swamp deer and spotted deer ($H = 5.6$, $df = 1$, $P < 0.05$) and spotted deer and hog deer ($H = 6.4$, $df = 1$. $P < 0.05$) with spotted deer consuming more than other two. Swamp deer and hog deer did not differ in their proportions of woody plants ($H = 0.3$, $df = 1$, $P > 0.05$).

The proportions of different subcategories of graminoids were significantly different among three deer species ($\chi2 = 10.9$, df = 4, $p < 0.05$) (Figure 5.4, Table 5.4). “Early succession tall grasses” were the most important food plants for all three cervids, but they did not differ in their relative consumption of this subcategory (H = 0.7, df = 2, P = 0.693). Hog deer consumed the highest proportion of the early succession tall grasses, especially *Saccharum spontaneum*, followed by swamp deer and spotted deer. They consumed significantly different proportion of “late succession tall grasses” (F = 20.1, df = 2, $P <0.05$). Pairwise Bonferroni comparisons revealed significant differences in consumption between swamp deer and hog deer (F= 35.3, df= 1, $P< 0.05$) and swamp deer and spotted deer (F = 20.5, df = 1, $P < 0.05$) with swamp deer consuming more than other two. Spotted deer and hog deer did not differ in their proportions of late succession tall grasses consumption (F = 5.6, df = 1, $P > 0.05$). The consumption of “short grasses” among three species was similar (H = 1.5, df= 2, P = 0.455).

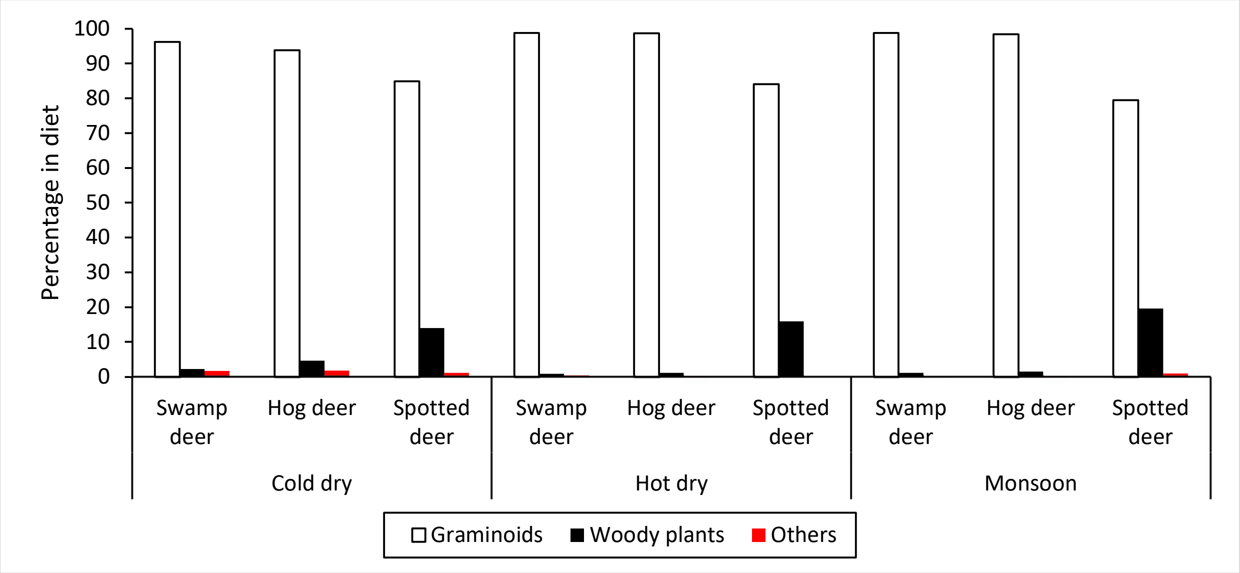

Figure 5. 3 Proportions of forage plant categories in the seasonal diets of ungulates in the grassland of Shuklaphanta National Park.

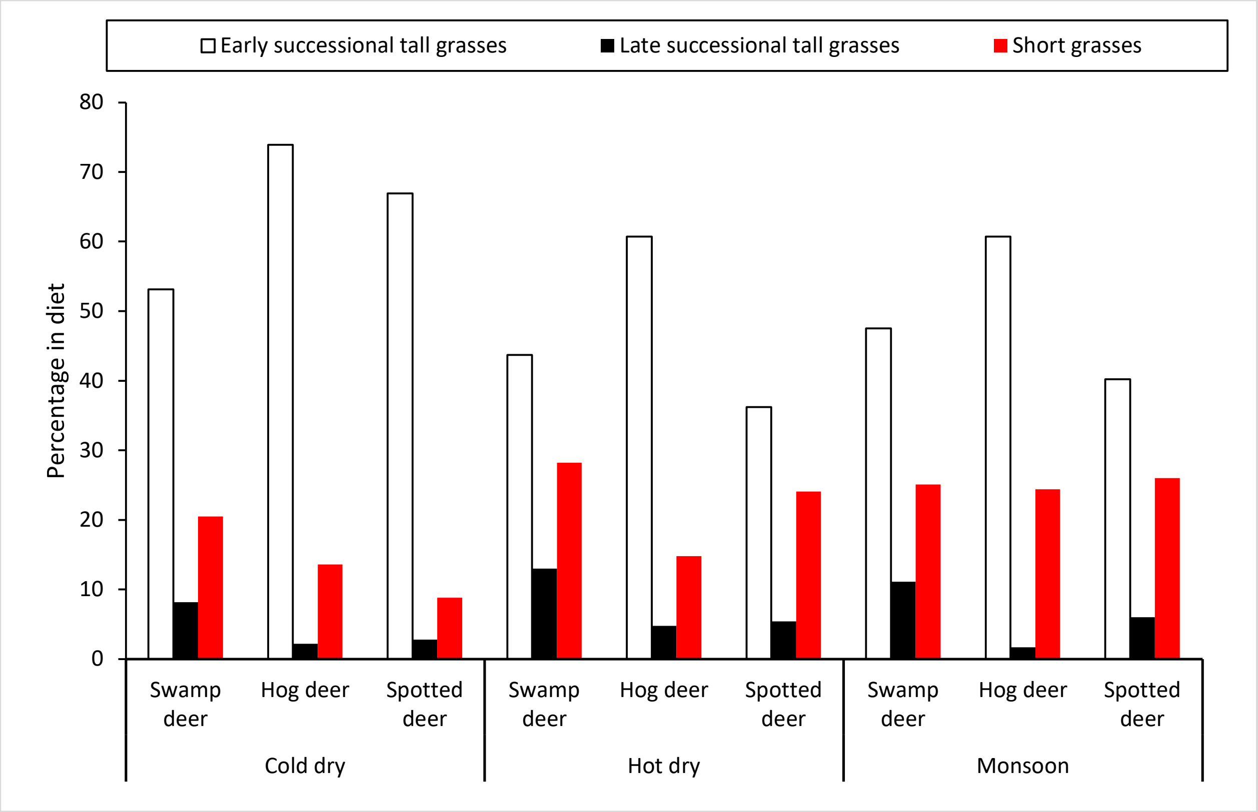

Figure 5. 4 Proportions of subcategories of graminoids in the seasonal diets of ungulates in the grassland of Shuklaphanta National Park.

Table 5. 6 Seasonal percentage similarity indices (adapted by Gauch Jr., 1973) of diets among ungulates at forage category levels in the grassland of Shuklaphanta National Park.

Species	**Spotted deer**				**Swamp deer**			
Seasons	Cold dry	Hot dry	Monsoon	Overall	Cold dry	Hot dry	Monsoon	Overall
Swamp deer								
All categories	65.4	74.0	68.0	79.2				
Graminoids	69.8	80.7	75.2	86.2				
Woody plants	18.5	2.4	9.7	11.6				
Others – percentage contribution was negligible so not calculated								
Hog deer								
All categories	74.6	66.1	55.1	73.0	62.7	85.5	68.8	77.0
Graminoids	79.6	71.3	60.5	78.1	64.2	86.4	69.0	78.0
Woody plants	30.1	10.6	13.3	23.6	29.4	21.1	53.8	35.4
Others – percentage contribution was negligible so not calculated								

Sal forest

The annual diet overlap was similar for all categories, ranging from 59% to ≈65% (Table 5.7). For graminoids, the highest overlap was between swamp deer and spotted deer (≈81%), followed by swamp deer and hog deer (≈69%) and hog deer and spotted deer (≈60%). For woody plants, the highest overlap found between swamp deer and hog deer (≈51%), followed by spotted deer and hog deer (≈43%), and swamp deer and spotted deer (≈30%). For "others" category, the highest overlap existed between spotted deer and hog deer (≈81%), followed by swamp deer and hog deer (≈62%) and swamp deer and spotted deer (≈47%).

The proportions of forage categories were significantly different among the three species (χ^2= 34.3, df = 4, $p < 0.001$) (Figure 5.5). The intake of graminoids was not different (H = 1.2, df = 2, P > 0.05). However, they ate significantly different proportions of woody plants (H = 7.6, df = 2, P < 0.05). Pairwise Bonferroni comparisons revealed spotted deer consuming more than swamp deer (H = 4.8, df = 1, P < 0.05) and hog deer (H = 5.3, df = 1, P < 0.05). However, Swamp deer and hog deer did not differ in their proportions of woody plants (H = 1.7, df = 1, P > 0.05). The "others" category dominated exclusively by *Phoenix humilis* occurred in different proportions (F = 12.55, df = 2, P <0.001) with the highest in swamp deer. Pairwise Bonferroni comparisons revealed that swamp deer consumed more than hog deer (F = 13.14, df = 1, P <0.005) and spotted deer (F= 15.7, df = 1, P < 0.001). Pairwise hog deer and spotted deer ate in similar proportions (F = 1.01, df = 1, P >0.05).

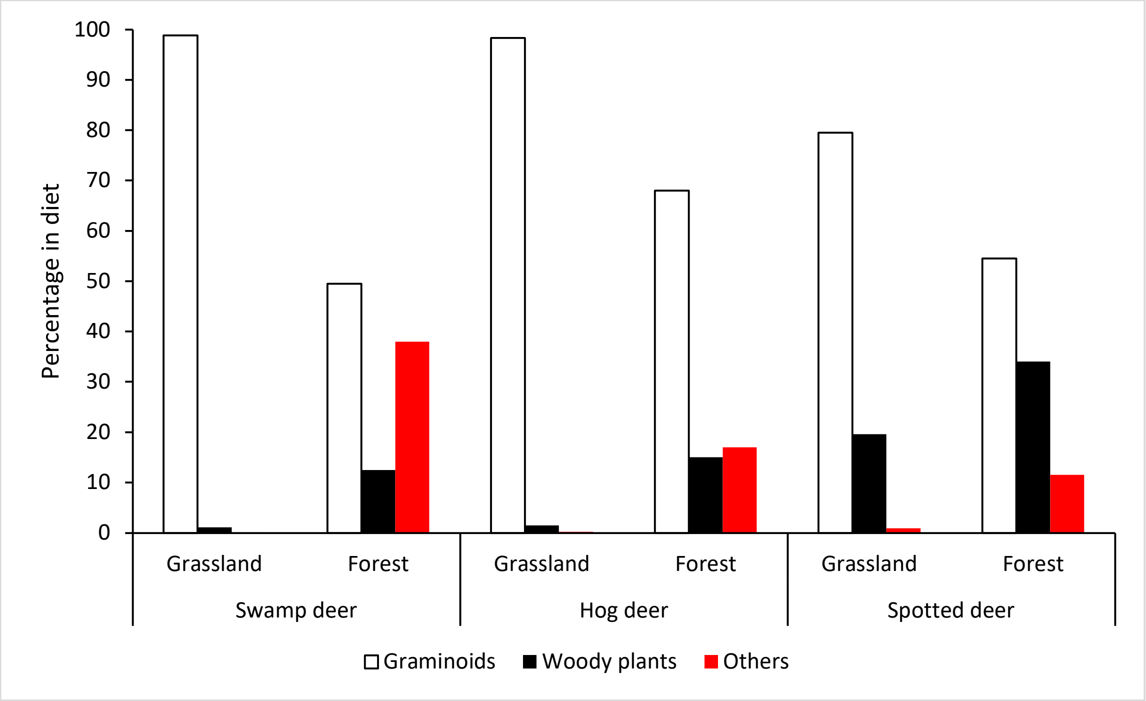

Figure 5. 5 Proportions of forage categories in the diets of ungulates in the grassland and Sal forest of Shuklaphanta National Park (monsoon season).

Table 5. 7 Percentage similarity indices (adapted by Gauch Jr., 1973) of diets among ungulates at forage category levels in the Sal forest of Shuklaphanta National Park (monsoon season).

Species	**Spotted deer**	**Swamp deer**
Swamp deer		
All categories	60.5	
Graminoids	80.8	
Woody plants	30.1	
Others	46.5	
Hog deer		
All categories	59.0	64.5
Graminoids	60.4	68.9
Woody plants	42.9	50.9
Others	80.7	61.8

The proportions of different subcategories of graminoids were not different among three deer species ($\chi 2 = 6.0$, df = 4, P > 0.05). “Early succession tall grasses” were the most dominant food plants for all three deer species, but they did not differ (H = 0.694, df = 2, P = 0.706). Hog deer consumed the highest proportion of the early succession tall grasses, especially *Saccharum spontaneum,* than other two deer. *Saccharum narenga*, the only late succession tall grass, was not found in the diet of hog deer; however, its presence in swamp deer and spotted deer was same. Spotted deer consumed more short grasses than other two deer, but were not different (H = 0.927, df = 1, P = 0.628).

Comparison between grassland and Sal forest

For both all and graminoid categories, ungulate pairs' diet was similar in grassland and Sal forest. However, in terms of woody plants consumption, diet similarities between swamp deer and spotted deer and hog deer and spotted deer were nearly three times higher in Sal forest than in grassland. On the other hand, between swamp deer and hog deer, woody plants' diet was similar in both habitats (Table 5.6 & 5.7).

All deer consumed more graminoids in the grassland than in the Sal forest and they were significantly different (swamp deer, H = 7.0; hog deer H = 3.1; spotted deer, H = 5.3; P < 0.05 for all) (Figure 5.5). In Sal forest, proportions of "woody plants" were 12.5%, 15% and 34% compared to 1%, 1.5% and 19.6% in grassland for swamp deer, hog deer and spotted deer respectively. These proportions were different for swamp deer (H = 10.7, df = 1, P < 0.05) and hog deer (H = 8.0, df = 1, P < 0.05), however not different for spotted deer (H = 3.6, df = 1, P > 0.05). Similarly, proportions of category "others" in Sal forest was 38%, 17% and 11.5% in swamp deer, hog deer and spotted deer respectively compared to less than 1% in all deer in grassland and these were significantly different (swamp deer, H = 16.3, df = 1, P < 0.05; hog deer, H = 14.9, df = 1, P < 0.05; spotted deer, H = 9.3, df =1, P < 0.05).

‘‘Early succession tall grasses’’ were the dominant food plants consumed by three cervids, and they were similar in both habitats (swamp deer, $H = 0.7$; hog deer, $H = 1.1$; spotted deer, $H = 0.1$; $P > 0.05$ for all). ‘‘Late succession tall grasses’’ comprised relatively low proportions of the total diets of all three species in both habitats. Similarly, “short grasses” consumption was similar in both habitats (swamp deer, $H = 1.0$; hog deer, $H = 1.8$; spotted deer, $H = 3.2$, $P > 0.05$ for all).

Out of 54 identified forage species representing 30 families (Appendix 1.2 and 1.3), 8 plant species namely *Saccharum spontaneum*, *Imperata cylindrica*, *Chrysopogon zizanioides*, *Saccharum narenga*, *Cyperus* spp., *Themeda* spp., *Saccharum bengalense*, *Phragmites karka*, *Shorea robusta* and *Phoenix humilis* were important food plants of three herbivores (Table 5.4 & 5.5, Appendix 1.4). *Saccharum spontaneum* was by far the most important food plant, as it comprised on an average more than 30% of diets each ungulate in every season. There were significant differences among the three species in their consumption of *Saccharum spontaneum* in each of cold dry, hot dry and monsoon season (Appendix 1.4). Pairwise Bonferroni comparisons showed that hog deer ate more than swamp deer and spotted deer ($P < 0.05$), whereas proportions among swamp deer and spotted deer were not different ($P > 0.05$). In hot dry season, consumption of *Imperata cylindrica* was similar ($F = 0.63$, $P > 0.05$) among deer, however they were different in cold dry ($F = 7.40$, $P <0.05$) and monsoon season ($F = 22.58$, $P < 0.001$). Similarly, there were significant differences in consumption of *Chrysopogon zizanioides* (cold dry: $F = 53.31$, hot dry: $F = 17.02$, monsoon: $F = 30.10$; $P < 0.001$ for all seasons), *Saccharum narenga* (cold dry: $F = 14.52$, $P < 0.05$; hot dry: $F = 35.58$, $P < 0.001$; monsoon: $F = 5.62$, $P < 0.05$), *Cyperus* spp. (cold dry: $F = 71.81$, $P < 0.001$; hot dry: $F = 3.81$, $P < 0.05$; monsoon: $F = 75.74$, $P < 0.001$), *Themeda* spp. ($F = 39.97$, $P < 0.001$), *Saccharum* *bengalense* (F = 16.48, P < 0.001), *Phragmites karka* (F = 15.79, P < 0.001), *Shorea robusta* (F = 28.45, P < 0.001) and *Phoenix humilis* (F = 12.55, P < 0.001) (Appendix 1.4).

During the year, swamp deer consumed the highest number of grass species (N = 15), while spotted deer consumed the highest number of woody plants (N = 23). The overall annual niche breadth, expressed by the evenness measure, was the highest in spotted deer (0.66), followed by swamp deer (0.63) and hog deer (0.52) (Table 5.8). In all seasons, niche breadth was high for the spotted deer, followed by the swamp deer and the least for the hog deer (Table 5.8).

Table 5. 8 Standardised niche breadth of ungulates in the Shuklaphanta National Park (number in brackets indicates niche breadth values in the Sal forest).

Species	Cold dry	Hot dry	Monsoon	Overall
Swamp deer	0.67	0.72	0.67 (0.61)	0.63
Hog deer	0.49	0.63	0.52 (0.58)	0.52
Spotted deer	0.70	0.72	0.75 (0.72)	0.66

5.5 Discussion

Swamp deer

Swamp deer were found to feed on 37 species of plants in this study, compared to 42 species recorded in Jhilmil Jheel (Tewari et al., 2013) and 32 species in Bardia (Pokharel, 1995). The majority of the plant species were from the family Poaceae that is in line with previous findings in Nepal (Bhatta, 2004; Pokharel, 1995; Schaaf, 1978; Wegge et al., 2006) and India (Khan et al., 2004; Martin, 1977; Qureshi et al.; 1995; Singh, 1984; Tewari & Rawat, 2013). Apart from the graminoids, swamp deer consumed woody plants in similar proportions as in a previous study (Pokharel, 1995; Wegge et al., 2006); however, *Shorea robusta*, the major woody species discovered in SNP, was not detected in the previous study. Herb species *Phoenix humilis*, the vital diet of swamp deer in SNP, was also found in a much lower percentage in swamp deer diet in Bardia (Pokharel, 1995; Wegge et al., 2006). Compared to the small population of swamp deer in the Bardia, a large population of SNP used significantly more Sal forest.

Hog deer

A higher number of plant species (30) were recorded in faecal samples of hog deer in this study compared to previous studies [(15 species in Bardia (Wegge et al., 2006) and 17 species in Chitwan (Dhungel et al., 1991)]. Like my findings, graminoid species make up most of the diets (Dhungel et al., 1991; Wegge et al., 2006). Hog deer was found to forage in Sal forest in SNP, and Sal (*Shorea robusta*) was an essential component of their diet in monsoon season. However, hog deer were not found to feed Sal forest in Chitwan (Dhungel et al., 1991) and Bardia (Odden et al., 2005a). I presume that the high density of hog deer coupled with high swamp deer density forced these ungulates to adjoin open Sal forest with ground cover vegetation similar to the grassland. On the other hand, hog deer with less density in Chitwan and Bardia than SNP (Chapter 2) found adequate grassland habitat for fulfilling the dietary requirements.

Spotted deer

Thirty-nine plant species comprising 13 graminoids, 23 woody and 3 herbs were recorded in the diet of spotted deer. However, Khan (1994) documented 50 species foraged by this species encompassing 10 graminoids and 40 woody species in Gir Lion Sanctuary, Gujarat, India. The high number of graminoid species in SNP may be due to a large grassland area compared to Gir. In Gir, the proportion of grass in spotted deer diet was lowest in winter (55%) and highest in monsoon (92%) despite much smaller seasonal variation in the proportion of grass available, ranging from 79.5% in monsoon to 85% in winter. Unlike Gir, where the proportion of grass in the diet was 92% in monsoon season, in SNP, the grass proportion was far less (54.5%) in the forest habitat. The diet contribution by graminoid and non-graminoid (woody plants and herbs) species were nearly the same in monsoon season in the forest habitat of SNP. This is because the ground storey of forest (mainly open Sal forest) habitat is covered with layers of grasses, herbs, shrubs, and different aged tree species, thus providing optimum conditions for ungulates to exploit ranges of food species to their nutritional requirements.

Competition and coexistence

The diet of deer is influenced by dominance and spatial distribution pattern of plant species (Martin, 1977; Tewari et al., 2013). In SNP, habitat mosaics formed due to forest and grassland habitats resulted in the polyphagous feeding habit of swamp deer and hog deer, more a grazer in grassland and more a mixed feeder in the forest. However, spotted deer is a mixed feeder in both habitats. The sympatric swamp deer, hog deer and spotted deer overlapped widely in their seasonal food habits. All three species consumed a high proportion of grass; the most noticeable interspecific difference was that spotted deer was found to eat woody browse more than the other two species. This study documents the importance of tall floodplain grasslands for the conservation of endangered herbivores in south Asia. The early successional tall grass *Saccharum spontaneum* was the most important food plant species for all three deer species in all seasons. *Saccharum spontaneum* has unique basal sprouting ability during year-round (Lehmkuhl, 1989), making it an important food resource for the deer species even during the resource-limited dry season. The results agree with previous diet studies on swamp deer (Pokharel, 1996; Wegge et al., 2006) and hog deer (Dhungel et al., 1991).

To explore the coexistence of ungulates with high diet similarity, it is important to understand seasonal habitat use patterns by these species. Both line transect survey (Chapter 2) and telemetry study (Chapter 3) revealed that swamp deer and hog deer used upland Sal forest from the end of hot dry to the entire monsoon (July – October) and early cold dry season (November). After the grassland management through cutting and burning starts in December, both deer gradually congregate in grassland along with spotted deer. When the extensive southern grassland is thoroughly managed, and new sprouts are enormous, swamp deer and hog deer did not use the Sal forest in the dry season (mid-January to mid-May). However, spotted deer were found to use both habitats in all seasons.

In all three seasons, high diet similarity implies potentially high competition among three ungulates. However, a visible niche separation is noticed in the consumption of significantly more woody plant species by spotted deer than swamp deer and hog deer. Additionally, the statistical tests showed niche separation in diets through differential use of major individual food plants species (Appendix 1.4). This food consumption pattern is further supported by NMDS plot (Figure 5.2) and PERMANOVA test.

In the cold dry season, apart from high use of woody plants, spotted deer depended on *Saccharum bengalense* and *Imperata cylindrica*. Hog deer, however, consumed more *Saccharum spontaneum* and *Chrysopogon zizanioides*. Swamp deer ate more *Cyperus* spp., a short succulent grass found on moist soil, similar to swamp deer in northern India (Tewari et al., 2013). Hog deer ate more woody plants, especially *Ficus benghalensis,* than swamp deer and spotted deer.

In the hot dry season, despite diet partition through more consumption of woody food plants, spotted deer ate more *Chrysopogon zizanioides*. On the other hand, Hog deer depended more on tall grass species such as *Saccharum spontaneum* and *Phragmites karka.* Swamp deer demonstrated attraction for *Saccharum narenga* and *Cyperus spp*.

In the monsoon season, spotted deer consumed more *Imperata cylindrica*; however, hog deer favoured *Cyperus* spp. and swamp deer ate more *Chrysopogon zizanioides, Saccharum narenga, Themeda spp.* Aquatic plants like *Pani leu* are less represented in the diet of swamp deer. However, during monitoring of radio-collared swamp deer, they have frequently encountered grazing inside wetlands in dry seasons (cold dry and hot dry), similar to those swamp deer of Bardia and Jhilmil Jheel (Moe, 1994; Tewari et al., 2013). Hog deer and spotted deer were never found to feed inside the wetlands during the study period. Like grassland, in Sal forest, differential use of three major plant species viz, *Saccharum spontaneum*, *Shorea robusta* and *Phoenix humilis* by three deer illustrate niche separation for their coexistence.

After the grassland management, a high diet similarity indicates high competition in dry seasons (Table 5.6). However, the coexistence of deer species is facilitated by the availability of highly productive newly sprouted grasses available throughout a large patch of grassland and later in the Sal forest. On the other hand, in the monsoon season, grasses start to mature, and nutritional quality deteriorates. In general, graminoids, especially when matured and senescent, have lower nutritive quality than browse (Gwynne & Bell, 1968; Robbins, 1983). The effective grazing ground available for deer species severely reduces as most of the southern, eastern, and western sides of grassland are partially submerged. The increasing population of swamp deer and possibly high hog deer population further requires more spaces and food. The pre rutting season of swamp deer and hog deer coincides with the monsoon season, further requiring more space. The overall picture is that in monsoon season, the grassland alone is not adequate to accommodate deer species, and thus there is competition for food and space. To avoid competition, part of swamp deer and hog deer population shifts to upland Sal forest to exploit different proportions of nutritious herbs (*Phoenix humilis*) and browse species (*Shorea robusta* and others) apart from the graminoid species (Table 5.5 & Appendix 1.4) for their coexistence with spotted deer. Such seasonal segregation indicates competition (Rosenzweig, 1981).

In this study, diets of three ungulates were quite similar. However, high diet similarity does not necessarily signify competition between these species. Competition occurs only when the shared resources are short supply, limiting survival, growth, or reproduction of competing species (Kirchhoff & Larsen, 1998). These species may coexist through differential consumption of forage species and seasonal habitat partitioning. Further studies on population densities and habitat carrying capacity considering species interactions are required to better understand the interspecific competition in SNP.

In summary, the diet overlaps between swamp deer, hog deer, and spotted deer is relatively low during the monsoon season in both habitats. This season, swamp deer shift ranges from the grassland to the Sal forest (Chapter 3). This shift indicates a favourable condition for the future of swamp deer to flourish through switching habitat use between habitats. Habitat partitioning between swamp deer and other species during monsoon season reduces the possibility of competition over food recourses. In the last three decades, the population size of swamp deer increased from 805 in 1975 (Schaaf, 1978) to 1883 (present study) in 2016. Such an increase may be due to the habitat management in the dry seasons and range shift of swamp deer from the grassland to the Sal forest in the monsoon season. Additionally, Sal forest and grassland areas in the eastern part of SNP that historically occupied by swamp deer (Schaaf, 1978) provide habitat for further increase in the swamp deer population. Active habitat management through cutting and burning in dry seasons improves resource availability and facilitates the coexistence of swamp deer with hog deer and spotted deer, and is a necessary intervention with positive conservation outcomes.

Chapter 6 General discussion

6.1 Summary of research findings

The conservation of large predators requires information on the abundance of prey species, particularly those which constitute their major food items. This thesis provided findings on the prey abundance and seasonal food habit of the tiger along with possible alteration on a diet due to habitat management regimes in SNP. The coexistence of carnivores, i.e., tiger, and leopard, are facilitated by diet partitioning. For the first time, this thesis provides understandings of the home range, habitat preference, transborder movement, conservation genetics, food habit of swamp deer, the largest cervid and one of the major preys of the tiger, and that of other sympatric ungulates. Such knowledge will be instrumental in enhancing the knowledge base in formulating management strategies for the conservation of predators and focal preys in the western landscape of TAL, Nepal.

To investigate the possibility of competition between two large apex predators, tiger and common leopard, I studied the density and biomass of major prey species and compared the diet of tiger and leopard. The density and biomass of prey species were high in SNP compared to other tiger bearing protected areas. The notable finding was that the large-sized swamp deer and medium-sized spotted deer had a high but comparable density in the study area, indicating the most favourable condition for the coexistence of both predators. The spotted deer, the most abundant prey species with uniform distribution in all habitat types ranging from grasslands to forests, were the most dominant prey species in the diet of both predators. The diet overlap was high (90%), suggesting the existence of competition for food. However, a diet partitioning occurred through the consumption of the large-sized swamp deer far more often by tigers than leopards. A spatial partitioning through habitat segregation between two carnivores was also seen in which tigers were more concentrated in the core and relatively undisturbed habitat with a high density of large-sized ungulates such as swamp deer. Leopards were displaced towards degraded marginal habitat where local communities graze their cattle, forcing them to consume domestic livestock, a major cause of human-wildlife conflicts in the study area.

Knowing that the threatened swamp deer is one of the important diets of endangered tiger and playing a crucial role in diet partitioning between the tiger and leopard (Chapter 2), in chapter 3, I explored the home range, habitat preference and movement of this species. Analysis of nine VHF collared swamp deer tracking data showed that both 95% MCP and FK average annual home ranges (22.90 ± 3.64 km^2 and 15.88 ± 2.62 km^{2,} respectively) were by far larger than other Asian cervids like barking deer, hog deer and spotted deer. For example, the average annual MCP home range of swamp deer was 143, 31, 14 times larger than that of barking deer, hog deer and spotted deer, respectively. There were no differences in home range sizes between male and female swamp deer. The home range size of swamp deer was larger in the hot dry season (March to June) than cold dry (November to February) and monsoon (July to October) season. Overall, grassland was the most preferred habitat in all seasons. In dry seasons, in addition to grassland, riverine forest and water bodies, other important habitats were used by the swamp deer. In the monsoon season, moderately dense Sal forest followed the grassland. Among grassland assemblages, swamp deer showed the highest association with IMPC assemblage in all seasons, followed by ICNP and NARP. Unlike the previous study in SNP by Schaaf (1978), I found a remarkable movement of swamp deer from the southern lowland grassland to the northern adjoining upland Sal forest in the monsoon season. This range shift from the grassland to the Sal forest during the monsoon season implied that the grassland alone was not sufficient to hold the population of swamp deer during rutting. The swamp deer population increased from 805 in 1975 (Schaaf, 1978) to 1898 (present study) in 2016. Furthermore, the swamp deer was found to move across the international border to Lagga-Bagga forest of Pilibhit Tiger Reserve in India during dry and monsoon seasons.

In chapter 4, I investigated the conservation genetics of two remaining populations of swamp deer in Nepal. Ten haplotypes were recorded in SNP and only three haplotypes in BNP. Both populations shared two haplotypes. Both haplotype and nucleotide diversity of SNP population was higher than that in BNP. At microsatellite level, both populations of swamp deer showed similar average heterozygosity, which was slightly higher than that of swamp deer populations of India. At the mtDNA level, neutrality test and multimodal pattern of mismatch distribution indicated both swamp deer populations are under demographic equilibrium. Furthermore, the population bottleneck analysis indicated no signature of a bottleneck for both populations. The Bayesian cluster analysis indicated two populations clusters. However, low fixation indexes indicated low population differentiation. Although both populations of swamp deer seem to be genetically stable at present, the low effective population size of BNP, which is below a threshold level of 50, indicates that inbreeding is likely to occur if appropriate management intervention is not taken into consideration.

The prey population, especially the ungulate cervid populations in SNP, has been in increasing trend. The population of sympatric swamp deer, hog deer and spotted deer, which are the major food of tiger, had increased reasonably due to effective protection measures in place coupled with habitat management interventions. Chapter 5 covered diets, competition and coexistence of swamp deer with hog deer and spotted deer sharing the same common habitat in SNP. With a diversified habitat formed due to mosaics of forest and grassland, swamp deer and hog deer exhibited polyphagous feeding habit, being more a grazer in grassland and more a mixed feeder in the forest. Unlike these two deer, spotted deer, on the other hand, consistently showed mixed feeding behaviour in both habitats. The early successional tall grass *Saccharum spontaneum*, having unique ability of basal sprouting all the year-round, was the most important food plant species for the three deer species in all three seasons. There was a high similarity in diets of ungulates indicating high competition; however, they showed niche separation through differential use of individual plant species and seasonal habitat segregation. The most significant finding was that in the monsoon season, the diet overlap among deer was relatively low in both habitats. In the monsoon season, swamp deer range shifted from the grassland to the Sal forest (Chapter 3), signifying a favourable condition to flourish swamp deer in both habitats. The availability in both habitats will help in the even (uniform) distribution of swamp deer (rather than previously mostly localised in southern grassland Schaaf (1978)) in SNP, thus enabling tiger to focus more on this large-sized prey and leaving other medium and small-sized prey for the leopard.

My study highlights the importance of community structure of prey species in supporting the apex predator populations. Thus, these findings are important not only for the conservation of endangered species but also for mitigating human-wildlife conflict in the human-dominated landscape of the western TAL.

6.2 Future directions and management recommendations

This study and the previous study documents domestic animals inside SNP (Lovari et al., 2015; Pokheral, 2011; Yadav, 2006). Further, these domestic animals were a substantial proportion of the diet of both predators. From a conservation perspective, this might create multiple problems: transmission of disease, human predator conflict and potential competition of wild and domestic ungulates.

Livestock grazing inside national parks is regarded as an illegal activity by the National Parks and Wildlife Conservation Act 1973 (NPWCA 1973) promulgated by the Government of Nepal. However, massive illegal grazing inside SNP could be a serious conservation threat because of the possibility of disease spreading between wildlife and livestock. Disease outbreaks can diminish population sizes of carnivores by more than 35% (Pokheral, 2013). The incidence of Foot and Mouth Disease (FMD) in SNP in 2010 resulted in the death of many wild ungulates, specifically swamp deer, hog deer and spotted deer (personal observation). Therefore, there is a need for wildlife diseases (for carnivores and wild ungulates) study in protected areas and adoption of the management strategy to eliminate the contact between cattle and wildlife for the long-term survival of these species.

Although the study area is rich in wild prey species, the diet study of carnivores showed a substantial killing of domestic animals. The killing of domestic animals by large carnivores has escalated conflicts with local communities throughout their range. The population decline and extinction of many carnivore species are linked to retaliative killing due to livestock depredation (Kala, 2013; Karanth & Sunquist, 1995; Mishra, Van Wieren, Heitkönig, et al., 2002). There is a possibility of escalation of conflicts unless depredation and illegal grazing inside the national park are addressed. In SNP, major prey species are concentrated in the western area (ca. 155 km^2). Due to massive human pressure, poaching, illegal livestock grazing and encroachment, the density of wild prey species in the eastern section is lower. However, in the eastern area, livestock density is very high (186 animals/km^2; Yadav, 2006). Since large carnivores require a large undisturbed area for survival, the pressure on the western part can be decreased by creating extra suitable habitat for wild prey species and hence for carnivores by effective protection of western sector from illegal grazing, encroachment and poaching. This will ultimately help to reduce human-wildlife conflict. Further research is recommended on the impact of domestic livestock, human disturbance on predator and prey in SNP.

Schaaf (1978) emphasised that if swamp deer are observed in large numbers in the upland Sal forest of SNP, it could indicate either that disturbances in the grassland or that the carrying capacity of the grassland had been exceeded, causing animals to move into the Sal forest. During this study, I did not observe any disturbances in the southern grassland; rather, I frequently encountered swamp deer in the Sal forest during monsoon season with an estimated density of 17 individuals/ km^2 (Chapter 3). However, in the dry season, after the management of Shuklaphanta grassland (through cutting and burning), swamp deer congregate here almost completely without any individuals remaining in the northern Sal forest. In the dry season, the census population size of swamp deer in Shuklaphanta grassland enlarged from 805 in 1975 to 1898 in 2016 (Chapter 3). This situation indicates that the carrying capacity of the grassland has reached during the monsoon season. Therefore, I recommend scientific research on the habitat carrying capacity for swamp deer, hog deer, and spotted deer, considering all possible grassland patches, particularly those on the eastern Dhakka area, which already contains a few remaining populations of this species. I also recommend habitat suitability study of other possible grasslands of SNP taking Shuklaphanta grassland and adjoining Sal forest as reference. Furthermore, the small population of swamp deer in the Dhakka area must be conserved. If needed, Dhakka population should be supplemented by the swamp deer of the western side (Shuklaphanta grassland) by employing local translocation to establish a second viable population of this species in SNP.

The BNP population of swamp deer has relatively low genetic diversity, and effective population size is below 50 (Chapter 4). So, this population is susceptible to experience inbreeding depression. Therefore, I recommend an in-situ conservation program and the translocation of some individuals from SNP to BNP for the population recovery and upgrading genetic diversity of swamp deer in BNP.

Due to trans-border movements of swamp deer and tiger between SNP and adjoining Indian protected area (Chapter 3), I emphasise enhancing and strengthening transboundary cooperation to conserve endangered species, including curbing any form of wildlife crime.

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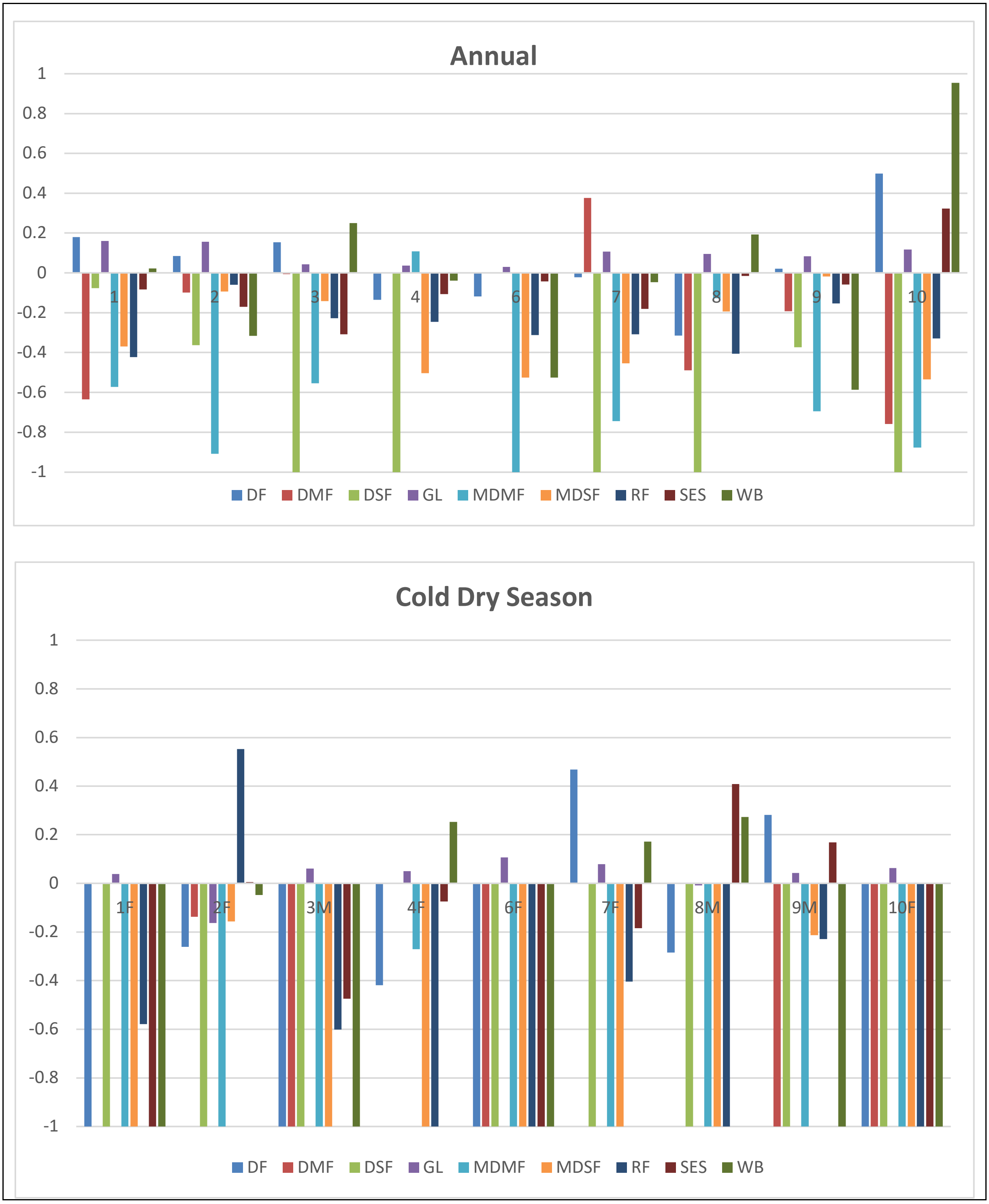
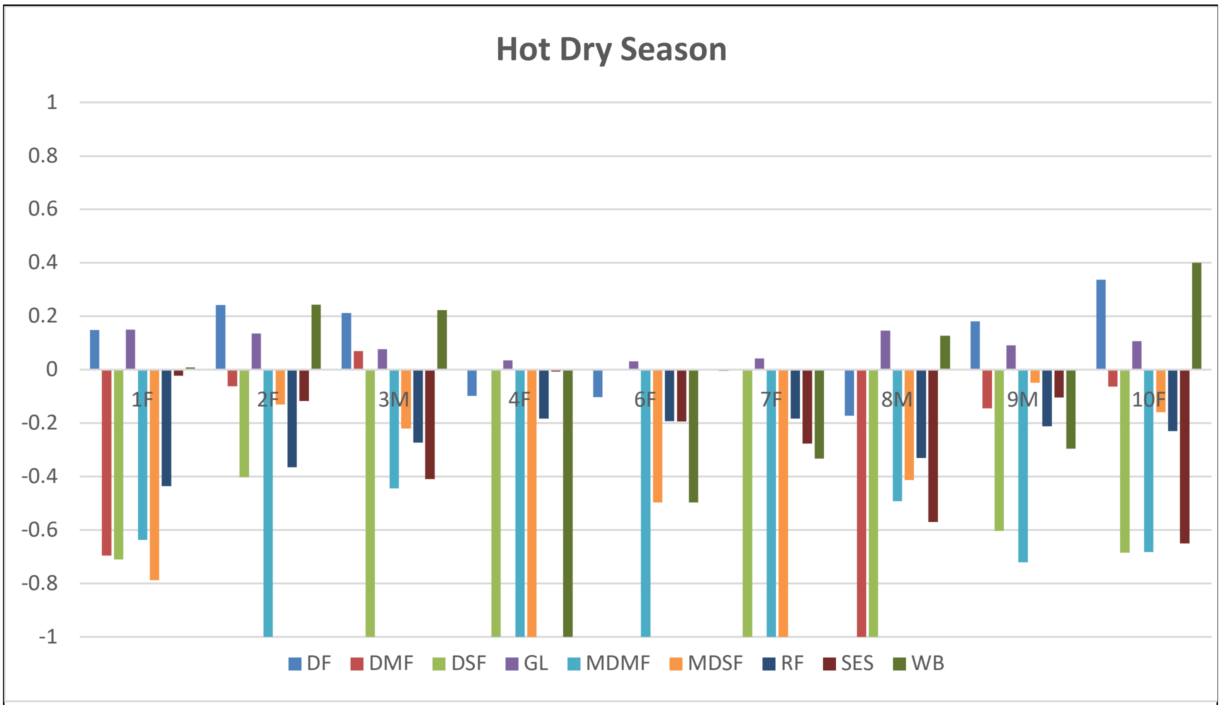
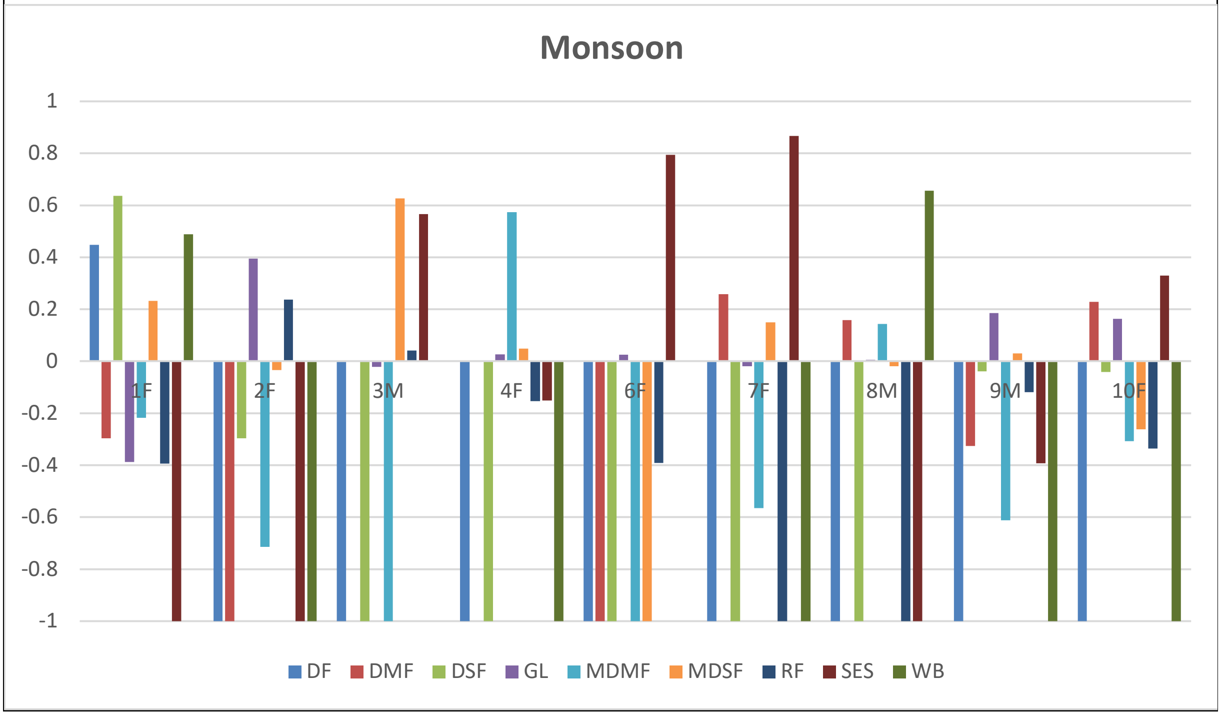
Appendices

Appendix 1. 1 Annual and seasonal habitat preferences of swamp deer individuals (n = 9) in Shuklaphanta National Park.




Hot Dry Season
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
1F
2F
3M
4F
6F
7F
8M
9M
10F
DF
DMF
DSF
GL
MDMF
MDSF
RF
SES
WB


Monsoon
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
1F
2F
3M
4F
6F
7F
8M
9M
10F
DF
DMF
DSF
GL
MDMF
MDSF
RF
SES
WB

Appendix 1. 2 Proportion (%) of plant species in the diet of swamp deer, hog deer, and spotted deer in cold dry, hot dry and monsoon seasons in the grassland of the Shuklaphanta National Park.

Plant species		**Swamp deer**				**Hog deer**				**Spotted deer**			
	Family	Cold dry %	Hot dry %	Monsoon %	Mean (SD)	Cold dry %	Hot dry %	Monsoon %	Mean (SD)	Cold dry %	Hot dry %	Monsoon %	Mean (SD)
Graminoids													
Saccharum spontaneum	Poaceae	40.3	35.8	39.5	38.5 (2.4)	65.4	47.7	54.6	55.9 (8.9)	53.3	30.5	29.4	37.7 (13.5)
Saccharum bengalense	Poaceae	6.5	4.7	4.0	5.1 (1.3)	4.0	2.7	3.3	3.3 (0.7)	10.0	1.7	4.5	5.4 (4.2)
Saccharum ravennae	Poaceae	5.5	1.7	1.7	3.0 (2.2)	4.5	3.5	2.6	3.5 (0.9)	3.3	2.0	4.3	3.2 (1.2)
Imperata cylindrica	Poaceae	2.1	4.5	5.5	4.0 (1.7)	3.3	3.5	1.8	2.9 (0.9)	5.5	4.5	9.0	6.3 (2.4)
Chrysopogon zizanioides	Poaceae	0.9	11	8.7	6.9 (5.3)	7.1	1.5	1.1	3.2 (3.4)	0.0	16	5.0	7.0 (8.2)
Saccharum narenga	Poaceae	5.1	9.8	5.6	6.8 (2.6)	2.1	1.3	1.6	1.7 (0.4)	2.0	4.7	3.5	3.4 (1.4)
Themeda spp.	Poaceae	3.1	3.2	6.5	4.3 (1.9)	0.1	3.5	0.1	1.2 (2.0)	0.8	0.7	2.5	1.3 (1.0)
Cymbopogon spp.	Poaceae	4.0	3.5	3.6	3.7 (0.3)	0.8	2.5	0.0	1.1 (1.3)	0.5	0.3	3.0	1.3 (1.5)
Cyperus spp.	Cyperaceae	10.0	5.2	5.6	6.9 (2.7)	0.0	3.3	16.6	6.6 (8.8)	0.5	1	2.5	1.3 (1.0)
Phragmites karka	Poaceae	0.8	1.5	0.6	1.0 (0.5)	0.0	6.8	0.2	2.3 (3.9)	0.3	2	2.0	1.4 (1.0)
Apluda mutica	Poaceae	1.1	1.5	1.3	1.3 (0.2)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Eulaliopsis binata	Poaceae	0.0	0.0	0.3	0.1 (0.2)	0.0	0.3	0.0	0.1 (0.2)	0.8	0.0	1.5	0.8 (0.8)
Chrysopogon aciculatus	Poaceae	0.0	0.2	0.0	0.07 (0.1)	0.0	0.2	0.3	0.2 (0.2)	1.0	0.0	1.5	0.8 (0.8)
Banso	Poaceae	0.3	0.0	0.0	0.1 (0.2)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Dubo	Poaceae	2.1	2.3	1.7	2.0 (0.3)	2.4	3.5	4.6	3.5 (1.1)	0.5	2.3	3.5	2.1 (1.5)
Typha spp.	Typhaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Unidentified graminoids		14.4	13.9	14.2	14.2 (0.3)	4.1	18.4	11.4	11.3 (7.2)	6.4	18.4	7.3	10.7 (6.7)
Total graminoids		96.2	98.8	98.8	97.9 (1.5)	93.8	98.7	98.4	96.9 (2.7)	84.9	84.1	79.5	82.8 (2.9)
Woody plants													
Ficus religiosa	Moraceae	0.3	0.0	0.3	0.2 (0.2)	0.1	0.0	0.3	0.1 (0.2)	0.3	0.3	1.1	0.6 (0.5)
Grewia sapida	Malvaceae	0.1	0.2	0.0	0.1 (0.1)	0.1	0.0	0.0	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Ficus racemosa	Moraceae	0.0	0.0	0.1	0.03 (0.1)	0.4	0.2	0.0	0.2 (0.2)	0.0	3	0.1	1.0 (1.7)
Spatholobus parviflorus	Leguminosae	0.5	0.0	0.1	0.2 (0.3)	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.5	0.2 (0.3)
Trewia nudiflora	Euphorbiaceae	0.1	0.0	0.0	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)	3.3	0.0	0.9	1.4 (1.7)
Calamus tenuis	Arecaceae	0.1	0.0	0.0	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Casearia tomentosa	Salicaceae	0.3	0.0	0.1	0.1 (0.2)	0.0	0.0	0.0	0.0 (0.0)	0.5	0.0	0.5	0.3 (0.3)
Colebrookea oppositifolia	Lamiaceae	0.0	0.2	0.0	0.07 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Careya arborea	Lecythidaceae	0.0	0.2	0.0	0.07 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Dalbergia sissoo	Leguminosae	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Stereospermum chelenoides	Bignoniaceae	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.1	0.03 (0.1)
Helicteres isora	Sterculiaceae	0.0	0.0	0.0	0.0 (0.0)	0.3	0.0	0.0	0.1 (0.2)	1.3	1.0	0.1	0.8 (0.6)
Ficus benghalensis	Moraceae	0.0	0.0	0.0	0.0 (0.0)	2.6	0.2	0.0	0.9 (1.4)	1.5	0.7	0.5	0.9 (0.5)
Dillenia pentagyna	Dilleniaceae	0.0	0.0	0.0	0.0 (0.0)	0.1	0.0	0.0	0.0 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Acacia catechu	Leguminosae	0.0	0.0	0.0	0.0 (0.0)	0.1	0.0	0.0	0.0 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Syzygium nervosum	Myrtaceae	0.0	0.0	0.0	0.0 (0.0)	0.1	0.0	0.1	0.1 (0.1)	0.5	0.0	0.3	0.3 (0.3)
Syzygium cumini	Myrtaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.5	0.0	0.5	0.3 (0.3)
Holarrhena pubescens	Apocynaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.2	0.0	0.1 (0.1)	0.3	0.0	0.0	0.1 (0.2)

Lagerstroemia parviflora	Lythraceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.1	0.0 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Shorea robusta	Dipterocarpaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.3	0.0	0.0	0.1 (0.2)
Garuga pinnata	Burseraceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.3	0.0	0.1	0.1 (0.2)
Butea monosperma	Leguminosae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.3	0.0	0.0	0.1 (0.2)
Bombax ceiba	Malvaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.8	0.3	1.3	0.8 (0.5)
Ehretia laevis	Boraginaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.5	0.0	0.0	0.2 (0.3)
Litsea monopetala	Lauraceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.5	0.0	0.1	0.2 (0.3)
Zizyphus nummularia	Rhamnaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.3	1	0.4 (0.5)
Terminalia bellirica	Combretaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.8	0.3 (0.5)
Schleichera oleosa	Sapindaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.1	0.0 (0.1)
Mallotus philippensis	Euphorbiaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.3	0.1 (0.2)
Bauhinia purpurea	Leguminosae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.1	0.0 (0.1)
Unidentified woody plants		0.8	0.2	0.3	0.4 (0.3)	0.8	0.5	0.9	0.7 (0.2)	3.1	10.3	11.2	8.2 (4.4)
Total woody plants		2.2	0.8	1.1	1.3 (0.7)	4.6	1.1	1.5	2.4 (1.9)	14.0	15.9	19.6	16.5 (2.8)
Others													
Diplazium esculentum	Dryoptericaceae	0.8	0.2	0.0	0.3 (0.4)	1.8	0.0	0.0	0.6 (1.0)	0.8	0.0	0.9	0.6 (0.5)
Ludwigia perennis	Onagraceae	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.1	0.03 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Pani Leu		0.9	0.0	0.0	0.3 (0.5)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Phoenix humilis	Arecaceae	0.0	0.2	0.0	0.07 (0.1)	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)
Cirsium wallichii	Compositae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.2	0.0	0.1 (0.1)	0.0	0.0	0.0	0.0 (0.0)
Daucus carota	Apiaceae	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0 (0.0)	0.3	0.0	0.0	0.1 (0.2)
Total others		1.7	0.4	0.1	0.7 (0.9)	1.8	0.2	0.2	0.7 (0.9)	1.1	0.0	0.9	0.7 (0.6)
Total		100	100	100	100	100	100	100	100	100	100	100	100

Appendix 1. 3 Proportion (%) of plant species in the diet of swamp deer, hog deer, and spotted deer in the Sal forest of Shuklaphanta National Park.

Plant species	Swamp deer		Hog deer		Spotted deer	
	%	SD	%	SD	%	SD
Graminoids						
Saccharum spontaneum	35.0	1.57	57.0	1.5	30.0	2.7
Saccharum bengalense	1.0	0.0	4.0	0.5	5.5	0.8
Saccharum ravennae	1.5	0.71	3.0	0.5	1.0	0.0
Imperata cylindrica	1.5	0.6	1.0	0.5	2.0	0.6
Chrysopogon zizanioides	0.5	0.0	0.0	0.0	3.0	1.0
Saccharum narenga	1.5	0.0	0.0	0.0	1.5	0.7
Cyperus spp.	0.0	0.0	1.0	0.3	0.0	0.0
Eulaliopsis binata	0.0	0.0	0.0	0.0	1.5	0.7
Dubo	2.0	0.0	1.0	0.3	0.0	0.0
Unidentified graminoids	6.5	0.95	1.0	0.5	10	4.5
Total graminoids	49.5	2.02	68.0	1.7	54.5	2.5
Woody plants						
Ficus religiosa	0.0	0.0	1.0	0.3	0.5	0.0
Ficus benghalensis	0.0	0.0	1.0	0.3	0.0	0.0
Syzygium nervosum	0.0	0.0	1.0	0.3	0.0	0.0
Holarrhena pubescens	0.5	0.0	0.0	0.0	0.0	0.0
Helicteres isora	3.5	0.96	0.0	0.0	0.0	0.0
Shorea robusta	6.5	0.92	8.0	0.8	31.5	2.45
Acacia catechu	0.5	0.0	1	0.3	0.5	0.0
Zizyphus nummularia	0.5	0.0	0.0	0.0	0.0	0.0
Bauhinia vahlii	0.5	0.0	0.0	0.0	0.0	0.0
Phyllanthus emblica	0.5	0.0	0.0	0.0	0.0	0.0
Unidentified woody plants	0.0	0.0	3.0	0.4	1.5	0.7
Total woody plants	12.5	1.43	15.0	2.3	34.0	2.6
Others						
Phoenix humilis	38.0	3.17	17.0	1.3	11.5	1.8
Total others						
Total	100		100		100	

Appendix 1. 4 The frequency distribution of main plant species in the seasonal diets of ungulates in grassland and Sal forest of Shuklaphanta National Park.

Grassland/ cold dry season

Species	Swamp deer	Hog deer	Spotted deer	P-value	Swamp Vs hog deer	Hog Vs spotted deer	Swamp Vs spotted deer
Saccharum spontaneum	40.3	65.4	53.3	<0.001 (S)	S	S	NS
Imperata cylindrica	2.1	3.3	5.5	<0.05 (S)	NS	NS	S
Chrysopogon zizanioides	0.9	7.1	0.0	<0.001(S)	S	S	S
Saccharum narenga	5.1	2.1	2.0	<0.05 (S)	S	NS	S
Cyperus spp.	10	0.0	0.5	<0.001 (S)	S	NS	S
Saccharum bengalense	6.5	4.0	10.0	<0.001 (S)	NS	S	S
All	70.4	86.4	74.6				

Grassland/hot dry season

Species	Swamp deer	Hog deer	Spotted deer	P-value	Swamp Vs hog deer	Hog Vs spotted deer	Swamp Vs spotted deer
Saccharum spontaneum	35.8	47.7	30.5	<0.001 (S)	S	S	NS
Imperata cylindrica	4.5	3.5	4.5	>0.05 (NS)	NS	NS	NS
Chrysopogon zizanioides	11.0	1.5	16.0	<0.001 (S)	S	S	NS
Saccharum narenga	9.8	1.3	4.7	<0.001 (S)	S	S	S
Cyperus spp.	5.2	3.3	1.0	<0.05 (S)	NS	S	S
Phragmites karka	1.5	6.8	2.0	<0.001 (S)	S	S	NS
All	74.2	70.3	63.9				

Grassland/monsoon season

Species	Swamp deer	Hog deer	Spotted deer	P-value	Swamp Vs hog	Hog Vs spotted	Swamp Vs spotted
Saccharum spontaneum	39.5	54.6	29.4	<0.001 (S)	S	S	NS
Imperata cylindrica	5.5	1.8	9.0	<0.001 (S)	S	S	NS
Chrysopogon zizanioides	8.7	1.1	5.0	<0.001 (S)	S	S	S
Saccharum narenga	5.6	1.6	3.5	<0.05 (S)	S	NS	NS
Themeda spp.	6.5	0.1	2.5	<0.001 (S)	S	S	S
Cyperus spp.	5.6	16.6	2.5	<0.001 (S)	S	S	S
All	71.1	81.7	60.7				

Sal forest/monsoon season

Species	Swamp deer	Hog deer	Spotted deer	P-value	Swamp Vs hog	Hog Vs spotted	Swamp Vs spotted
Saccharum spontaneum	35.0	57.0	30.0	<0.001 (S)	S	S	NS
Shorea robusta	6.5	8.0	31.5	<0.001 (S)	NS	S	S
Phoenix humilis	38.0	17.0	11.5	<0.001 (S)	S	NS	S
All	79.5	82.0	73.0				