Landscape Ecology of Grey Wolf (*Canis lupus*) in Northern Pakistan

BY

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DEPARTMENT OF ANIMAL SCIENCES
FACULTY OF BIOLOGICAL SCIENCES
QUAID-I-AZAM UNIVERSITY, ISLAMABAD, PAKISTAN
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بسم الله الرحمن الرحيم

DEDICATION

I am proud to dedicate this research to my family who are heaven for me and whose super moral training allowed me to build my personality. I dedicate this work to my supervisor and friends whose moral encouragement and support during tense and stressful moments allowed me to complete my dissertation. These feelings of closeness will always be with me like the banks of streams - which always go together.

I ALSO DEDICATE THIS WORK TO ALL THOSE WHO ARE BUSY IN SERVING HUMANITY WITH FAITH AND WITHOUT FEAR

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Muhammad Kabir

Date:

CERTIFICATE OF APPROVAL

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Islamabad, Pakistan (2019)

LIST OF ABBREVIATIONS

%	Percentage
+VE	Positive
AIC	Akaike Information Criteria
AJ&K	Azad Jammu and Kashmir
AM	Ante Meridiem
ASCII	American Standard Code for Information Interchange
ASL	Above Sea Level
AUC	Area Under Curve
CKNP	Central Karakoram National Park
CR	Capture-recapture
CSV	Comma Separated Values
CV	Cross Validation
DEM	Digital Elevation Model
DNA	Deoxyribonucleic Acid
eDNA	Environmental DNA
GIS	Geographic Information Systems
GPS	Global Positing System
HKKH	Hindu Kush-Karakorum-Himalaya
IUCN	International Union for the Conservation of Nature
KG	Kilogram
Km^2	Square Kilometer
M	Meter
MAXENT	Maximum Entropy
MODIS	Moderate Resolution Imaging Spectroradiometer
NAs	Northern Areas
NASA	National Aeronautics and Space Administration
NDVI	Normalized Difference Vegetation Index
NGO	Non-government Organization
NGS	Next-generation Sequencing
OA	Occupancy and Abundance
^oC	Degrees Centigrade
P	Detection Probability
PAs	Protected Areas
PCA	Principle Component Analysis
PCR	Principle Component Regression
PKR	Pakistani Rupees
PM	Post Meridiem

PMNH	Pakistan Museum of Natural History
PSCL	Political Science Computational Laboratory
Psi “ψ”	Occupancy
RHC	Reference Hair Collection
SDMs	Species Distribution Models
SE	Standard Error
SECR	Spatially Explicit Capture-recapture
TSS	True Skill Statistic
US$	United States Dollar
USGS	United States Geological Survey
UTM	Universal Transverse Mercator
-VE	Negative
VRMINT	Vector Ruggedness Measure
ZSD	Zoological Science Division

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GENERAL ABSTRACT

Background: The grey wolf *(Canis lupus)* has been extirpated from most of its historic range in Pakistan primarily due to its effect on livestock and livelihoods. Lack of scientific knowledge is likely to be an important issue in Asian countries. The scientific information is necessary to inform practical conservation strategies, which are unfortunately lacking across the distribution range of the grey wolf in Pakistan. Field research on the grey wolf is challenging because it lives in remote areas, and its low-density population makes it difficult to monitor. Such scarcity of scientific information hinders effective conservation planning of the grey wolf. Current management efforts are ad-hoc, which may or may not deliver conservation targets. Effective conservation of the grey wolf, therefore, requires a detailed understanding of factors that govern the specie's spatial distribution and habitat use. Information about resource availability and selection, conflict with humans, and feeding ecology are important requisites to devise a conservation strategy for the species. The grey wolf has been extirpated from most of its historic range in Pakistan primarily due to its effect on livestock and livelihood of rural people. It is often considered a problem animal in mountain ecosystems in northern Pakistan.

Methodology: The first objective of the study was to understand the habitat requirements of the wolf and update its geographical distribution in Pakistan. Habitat suitability models are useful to understand species distribution. The study used non-invasive survey data from camera traps and genetic sampling to develop a habitat suitability model for the grey wolf in northern Pakistan and explored the extent of connectivity among populations. The suitable habitat was investigated through Maximum Entropy (Maxent) approach (Maxent ver. 3.4.0) and movement corridors were identified using the Circuitscape 4.0 tool (McRae and Shah, 2011). After the identification of a suitable habitat, the study investigated local sites used by wolves through occupancy modelling using camera trap data. The probability of wolves encountering camera traps is a function of the micro-habitat where the camera traps were installed. Different combinations of survey and site covariates were tested using the Presence software (version 12.7_170921, Hines 2006) to describe variation in the probability of detection and markability and occupancy of wolves at the site level.

The third aspect of the study related to understanding the socio-ecological factors that contribute to human-wolf conflicts. Interviews with 2,317 local people were held and a mixed-effect nested negative binomial regression model was constructed using the "lme4" package and the "pscl" package in R (software) (https://cran.r-project.org/) to determine the influence of socio-ecological factors on livestock losses due to grey wolf predation. Once the average rate of livestock predation was estimated for different valleys, it was extrapolated to entire study area using geostatistical analysis in ArcGIS (10.2).

Determining the diet profile of a predator is essential to understand its role in regulating given ecosystem and rationalizing public claims of livestock depredations. I analyzed wolves' scats to document diet composition and prey selection of the species. We searched and collected 1,186 unidentified scats samples belonging to different carnivore species found in the suspected grey wolf range, in both protected and non-protected areas, from 2009 to 2017. Upon genetic validation of the samples through mitochondrial DNA analyses, confirmed wolf samples were further processed for physical analysis. We estimated biomass consumption rate by following the linear relationship developed by Ackerman et al. (1984).

Results: The habit suitability model predicted ca. 23,129 km^2 (15% of study area) of area to be potentially suitable for the grey wolf, though most of the suitable habitat was in remote and inaccessible areas that appeared to be connected through movement corridors. The Maxent model demonstrated high levels of predictive performances (area under curve: 0.971±0.002, and true skill statistics: 0.886±0.021). The main predictors for habitat suitability included the distance to road and rivers and the mean temperature of the wettest quarter. The corridor modeling generated estimates of habitat connectivity among scattered wolf populations in northern Pakistan. Four patches of suitable habitats were identified within the Himalayas, Pamirs, Hindukush, and Karakoram mountains ranges of northern Pakistan. These habitat patches have strong but unprotected connectivity and corridors of movement exist between all major habitats. The model identified weak linkages between populations found at lower altitudes with high disturbance rates. The mean occupancy of wolves across the sampling sites was 0.43 (SE = ±0.09). Detection probability for wolves varied across different types of terrain and the effect of efforts and plateau had a positive influence on it, whereas the cliff base had a negative effect on probability of detecting wolves on cameras. In the top three models, three site covariates including average distance to road, average distance to settlements, and available habitat were found to collectively affect the probability of wolf occupancy.

The human-wolf interaction analysis revealed that 7,583 livestock were killed from 2011 to 2015. Small ruminants were more likely to be killed by wolves as compared to large livestock. Wolves preferred to attack small ruminants in open pastures and large ruminants in corrals. Several factors were positively correlated with livestock predation, including the size of livestock holding, mortality due to diseases, and occupation of the owner (livestock rearing). The negative binomial model suggests that predation incidences tend to decline as the education level of respondents increases. In contrast, predation counts doubled when livestock holding increased. Livestock disease was a stronger cause of loss per household with an average of 3.5 animals dying annually.

Diet analysis, confirmed that wolves consumed 12 different prey species, including wild and domestic ungulates. Domestic ungulates were a substantial part of the wolf diet (49%) and goats were the most common domestic prey (19%), followed by sheep and yak (12% each). Wild prey constituted 51% of the wolf diet with a major contribution of Himalayan ibex (18%), followed by blue sheep (7%) and Himalayan marmots (7%). A minor fraction of small mammals and birds were also consumed. In terms of biomass consumption, livestock dominated (62.2%), followed by wild ungulates (28.7%).

Conservation of the research species: The study demonstrates that non-invasive study techniques such as camera trapping, genetics sampling, and interviews in combination with statistical and analytical tools is a promising approach to understand landscape ecology of threatened carnivore species. The study represents the first large-scale assessment of wolf distribution, habitat suitability, and movement in Pakistan. The identified movement corridors suggest that wolves can potentially expand their range in northern Pakistan in the presence of a conducive environment. Management of protected areas with rigid restrictions is not a practical solution in northern Pakistan, partly due to heavy dependence of people on natural resources. Habitat suitability map generated in the study can help authorities in identifying key conservation areas where management efforts should be focused. The occupancy estimates, being the first effort in Pakistan, set a credible baseline against which change in wolf population and distribution could be gauged in the future in response to conservation efforts.

The outcome of this study has policy implications for managing human-wolf conflicts. Increasing population of livestock is fostering conflicts and undermining support for wolf conservation in Pakistan. The study highlights livestock loss by the wolf as a real and serious problem and this issue needs to be addressed to promote wolf acceptance in the rural communities of Pakistan. Better guarding could prevent wolf attacks, particularly in a high-depredation season. I recommend establishing veterinary centers with the allocation of substantial budgets to reduce livestock mortality due to diseases. A major obstacle for wolf conservation and extensive killing is livestock depredation in the area. The study suggests multi-pronged conservation management programs that include sustained compensation schemes, livestock vaccination, and awareness campaigns to sustain the co-existence of wolf populations with rural communities.

LANDSCAPE ECOLOGY OF GREY WOLF *(CANIS LUPUS)* IN NORTHERN PAKISTAN

CHAPTER 1

General Introduction

1 GENERAL INTRODUCTION

Large carnivores form a naturally rare, elusive, ecologically significant and globally threatened group of wild mammals (Ripple et al. 2014). They occupy top ranks in the ecosystems, exercise key roles and are used as flagship species in conservation programs in different parts of the world (Poyarkov and Subbotin 2002). They function as keystone species in various ecological processes of ecosystems. Their protection is strongly linked with the biodiversity of mountain ecosystems in Central Asia (Estes et al. 2011; Ripple et al. 2014). In particular, large carnivores play key ecological functions, such as controlling prey abundance, regulating macropredator populations through resource competition, and sustaining the stability of biological diversity in wildlife communities (Beschta and Ripple 2009; Hawlena and Schmitz 2010; Ripple et al. 2013). In Pakistan, mammalian carnivores are represented by at least 36 species of Palearctic and Oriental origin, and most of them are listed as threatened in the country (Roberts 1997; Sheikh and Molar 2004). In northern parts of the country, 18 carnivore species are known to occur (Roberts 1997), including the grey wolf (*Canis lupus*).

1.1 Conservation Status of the Grey wolf in Pakistan

Grey wolf is widely distributed in the Palearctic and the Neoarctic biogeographic regions (Vila et al. 2002), distributed in all the continents except Antarctica (Sillero-Zubiri et al. 2004). It is unevenly distributed throughout Europe from transcontinental Turkey up to central Switzerland. The distribution range stretches from the French Alps to southern Norway and the south-central region of Scandinavia (Skogen et al. 2008; Wabakken et al. 2001). It spread from Russia into Central Asia and China, up to Mongolia and northern Afghanistan (Hinrichsen 2000; Stevens et al. 2011). In the Middle East, its range extends from the deserts of Saudi Arabia into Iran (Cunningham and Wronski 2010). In the sub-continent, India got the vast distribution range of grey wolf. These are randomly dispersed across India and have a presence in many states, including Andhra Pradesh, Rajasthan, Maharashtra, Madhya Pradesh, Gujrat, Haryana, Karnataka, etc. (Shahi 1982; Jhala 2003).

The grey wolf is a least concern species (IUCN 2018) is not particularly habitat-specific but can adapt to a variety of habitat types (Mech and Boitani 2003). In Pakistan, two subspecies of grey wolf are present, *i.e.* the Indian wolf (*C. l. pallipes*) and Tibetan wolf (*C. l. chanco*), inhabiting the southern region of Kashmir and the Himalayas, respectively (Lydekker and Blaine 1907). Both subspecies of grey wolf has been declared an endangered species in Pakistan and is smaller in size and weight than the wolf of the subarctic region in the northern hemisphere (Sheikh and Molur 2004). It has a wide range of distribution and has adapted to live in varied habitats like subtropical scrubland, tropical dry scrubland, hot deserts, up to open areas and tropical thorn forests in Pakistan (Sheikh and Molur 2004; Kabir et al. 2017). Its habitat extends from the mountainous areas of Balochistan in the south up to the high elevated areas of Chitral and Gilgit-Baltistan (Roberts 1997; Kabir et al. 2017). A small population occurs in the deserts of Tharparkar (Sindh) and Cholistan (Punjab) stretching to the coastal belt of Makran with sporadic sightings in the Indus River plains (Sheikh and Molur 2004). Abbas et al. (2013) assessed grey wolf numbers using a questionnaire survey to be 350–400 individuals dispersed over an area of 35,000 km^2 in Gilgit-Baltistan as of 2006.

Although there are sanctions on killing, wolves are being persecuted by shooting, blocking or smoking active den-sites with pups or adults inside and by poisoning following depredation on domestic goats and sheep. Wolf population decline continues in Pakistan (Roberts 1997; Kumar and Rahmani 2000; Jhala 2003). Wolves may occasionally attack humans causing injuries and deaths and may spread rabies (Shahi 1982). These incidences are rare and often as a result of human interference like destruction of dens, setting traps and persecuting pups (Linnell et al. 2002; McNay 2002). This cause high rate of wolf mortality, for example, 66–87 wolves were killed between 2005 and 2006, mainly in the winter, and in retaliation to livestock attacks in the Gilgit-Baltistan region of northern Pakistan (Abbas et al. 2013).

In northern Pakistan, the trade in wolf body parts, primarily skin, has been rampant. Pelts were widely available and sold for up to Rs. 10,000 (~US$ 50–70), possibly for export (Abbas et al. 2013). Other body parts of wolves are commonly sold in markets, including fat which is perceived to have aphrodisiac qualities, paws for supposed medicinal value and bones are thought to have supernatural value (Abbas et al. 2013). Due to massive killing of wolves in the past, the species now prefers remote, isolated patches of fragmented habitat (Promberger et al. 2000; Kabir et al. 2017). Other threats, besides retaliatory killing, include disease transmission from domestic ungulates, poaching, prey depletion, shrinking of habitat, competition with other sympatric carnivore species (Jhala 2003; Irshad 2010).

1.2 Conservation Challenges

Although large carnivores are important species, they are suffering from a decline in populations worldwide and are classified as the most threatened types of species (Ceballos et al. 2005). The key ecological and biological factors involved in global population declines, which ultimately result in extinction, are large home range, body size, high energy demands and slow rates of population growth (Karanth et al. 2011; Marshall 2015). Among environmental factors, habitat degradation and fragmentation, prey depletion, competition for resources, poaching, persecution by human and land conversion practices caused population declines in recent decades (Gese 2001; Cardillo et al. 2004). Large carnivore species are experiencing a sustained global recession which is almost entirely caused by human activities. Growing human population and consequent competition for resources have led to changes in landscapes and habitats of many species are being destroyed and fragmented. The loss of natural prey and persecution over livestock depredation are the most prevailing threats for the survival of large carnivores, and developmental activities are posing a persistent threat to their continued existence in natural habitats (Burkey and Reed 2006). These threats vary in intensity across the landscape, and species living in more severely affected areas are more vulnerable to extinction (Ceballos and Ehrlich 2002; Parks and Harcourt 2002). The IUCN Red List data indicate that 59% of all large carnivore species and 60% of species of wild ungulate are on the verge of extinction (Ripple et al. 2016), with more declines in past few decades (Naves et al. 2003; Rodríguez and Delibes 2003).

The removal of top predators from natural habitats adversely affects biodiversity, which often leads to destabilizing the structure of ecosystems and communities by changing the composition of the food chain (Terborgh et al. 2002), affecting interacting species ecological and communities, changing basic ecosystem processes (Duffy et al. 2007; Delibes-Mateos et al. 2008). This undermines human and ecological stability by disturbing the services of the ecosystem, reducing social integration (Ripple et al. 2014; Brashares et al. 2014). Currently, large carnivores have received serious conservation concern because of their charismatic and conflicting image, ecological significance and lack of information on their ecology (Gittleman et al. 2001; Kruuk, 2003; Ray et al. 2005). Luckily, the growing recognition of carnivores' conservation needs (Miller and Cohen 2001; Sergio et al. 2008) mobilize financial, political and social commitment from all over the world for successful conservation and long-term survival of carnivores (Ripple et al. 2016).

1.3 Scarcity of Scientific Knowledge

The lack of scientific knowledge is the most pivotal issue for wolf conservation in Asian countries. Scientists are increasingly convinced and endorse the fact that the extinction of many carnivore species is mostly unpredicted and occurring in a short period of time (Pimm and Raven 2000; Thomas et al. 2004). Scientific information is necessary to formulate practical conservation strategies, which are unfortunately lacking across the distribution range of the grey wolf in Pakistan. Current management efforts are *ad hoc*, which may or may not deliver conservation targets. Indeed, a profound knowledge of previous ecological literature can be utilized as an apparent and essential part of devising excellent hypotheses, framing alternative views of ecosystems and, in turn, developing ecological research (Lindenmayer and Likens 2010). Inadequate scientific data and expertise in ecological studies has been a major roadblock in formulating strong conservation initiatives and effective resolution of heated conflicts. Therefore, information that describes resource competition and patterns of conflict is an important requisite to devise a conservation strategy for the species.

The grey wolf has been nearly extirpated from most of its historic range in Pakistan primarily because of its effect on livestock and livelihoods. Scientific research on wolf ecology from Pakistan is rare (Din et al. 2013; Bischof et al. 2014; Ahmad et al. 2016; Kabir et al. 2017) and only brief descriptions and basic information on wolves could be found. There are limited studies describing human-carnivore conflict in Pakistan (Dar et al. 2009; Din and Nawaz 2011; Perveen and Abid 2013; Kabir et al. 2013), despite wide prevalence of the issue, particularly in northern Pakistan, where various large carnivores (snow leopard *Panthera uncia*, Asiatic black bear *Ursus thibetanus*, brown bear *Ursus arctos*, wolf and lynx L*ynx lynx)* often come into contact with humans and contribute to appreciable economic losses. There has been no study carried out to understand or estimate the occupancy and variables that affect the occupancy estimates of wolves in northern Pakistan. Management across regions and disjunct areas of suitable habitat is often necessary because these large carnivores have large spatial habitat requirements, but prey selection and its importance in diet is still not examined in Pakistan.

Different national and international researchers have collected data on Pakistan's carnivore fauna and the problems associated with their conservation; notably Schaller (1976), Roberts (1997), Hussain (2003), and Abbas et al. (2013). Until the current decade, wildlife research relied largely on sign surveys and sighting reports. Moreover, past studies covered a limited geographic area of northern Pakistan, thus leaving this carnivore species in the country least known, and their geographic ranges unexplored (Sheikh and Molur 2004), which underscores the need for research on carnivores with modern technology. The use of standardized research techniques in the study of landscape ecology remains an important issue in Pakistan.

A variety of the latest and non-invasive ecological research techniques including genetic sampling, camera trapping, track and scat surveys, radio telemetry and aerial counts, have been used for the study of large carnivores (Gompper et al. 2006; Bischof et al. 2014; Ahmad et al. 2016; Kabir et al. 2017). Yet many study techniques have limitations for use in the field due to time constraints, feasibility, financial constraints and applicability in the field (Potvin and Breton 2005). In all aspects of ecological management, there is an essential requirement for standards and an organized assessment of interferences to inform strategic outcomes (Pullin and Knight 2001; Sutherland et al. 2004). The unsustainable devotion to such standards hinders the protection of large carnivores (Treves et al. 2016). Modern techniques have recently been introduced to Pakistan such as molecular analysis (Shehzad et al. 2015; Kabir et al. 2017) and camera traps (Din et al. 2013; 2015; Bischof et al. 2014; Hameed et al. 2014; Ahmad et al. 2016; Kabir et al. 2017), which are effective for studying rare and elusive species.

1.4 Habitat Requirements for Carnivore Conservation

Understanding on how species are distributed spatio-temporally is an important question in landscape ecology of large carnivore species including wolves (Schadt et al. 2002; Fernandez and Picard 2003). The habitat influences animal's activities such as resource selection, interspecific interactions, movement and dispersal, territory marking and reproduction, at various level of time and space scales (Gaillard 2010). The aforementioned requisites regulate species distribution (Hirzel and Le Lay 2008).

The habitat categories such as “structural” and “functional” can be observed as external powers which influence demographics and are known as significant in elaborating changes in an individual’s performance, like survival rate (Van Moorter et al. 2009; Basille et al. 2013; DeCesare et al. 2014) and breeding success (Mcloughlin 2007; Fisher 2014). The survival requirements of wild predators are linked with the availability of suitable habitat (Melville et al. 2004).

Increasing human population growth exerts pressure on the remaining natural habitats and their resources through habitat conversion and commercial exploitation (Kolowski and Holekamp 2006), with more conflicts between humans and wildlife as a consequence (Bulte and Rondeau 2005; McCleery 2009). Large carnivores, due to their large home ranges and habitat requirements, need more space and healthy habitat, are frequently vulnerable to human changes in their habitat, likely to be trapped, poached and killed (Enserink and Vogel 2006; Gordon and Loison 2009). This may lead to a genetic disorder, which may have a strong negative effect on the viability of populations (Keller and Waller 2002; Liberg et al. 2005). Habitat links and connectivity not only depend on spatio-temporal patterns of landscape characteristics, but are also linked with the patterns of biological and ecological characteristics of species, including habitat selection and use, safe movement along various landscape features and avoidance of vulnerability to death risk over several landscape types (Uezu et al. 2005; Fitzgibbon et al. 2007). Today, the major concern in conservation efforts is the identification of healthy and suitable habitat that is considered to support the survival of threatened species (Mesler 2015). In fact, identifying environmental conditions linked to habitat selection and use is a key task in any conservation plan (Guisan and Zimmermann 2000). Moreover, the investigation of ecological movement corridors can support the linking of local populations so that a fragmented population can move freely and easily (Beier and Noss 1998; Kabir et al. 2017).

At the landscape level, finding data on the spatial distribution of large threatened carnivore species is a challenging task because of their elusiveness and rarity (Karanth et al. 2011). Developing practical and efficient study techniques for carnivores is of great concern and a priority across the wolf’s distribution range due to global declines in population under prevailing threats (Inskip and Zimmermann 2009; Treves and Karanth 2003). Landscape ecology highlights how changing landscape patterns affect species presence, distribution, abundance and persistence (Singh et al. 2010) and brings a fast and exciting advancement in both application and theory. The main focus of ecologists is measuring the occurrence, range and distribution of species (Engler 2004) and modeling habitat selection (Gibson 2004).

Camera trapping is increasingly used technology that provides knowledge on species presence, including nocturnal wildlife (Jackson et al. 2006; McCarthy et al. 2008; Bischof et al. 2014; Ahmad et al. 2016; Kabir et al. 2017). Species distribution modeling (SDM) is a known and accepted approach for habitat suitability mapping of species, assessing interactions between species, and effects of spatial/environmental features (Franklin and Lindenmayer 2009). Maxent (Maximum Entropy Modelling) is one of such tools widely used (1,000 applications of Maxent are published) in environmental niche and species distribution modeling (Phillips and Dudík 2008). SDMs, being highly useful in exploring requirements for the survival of endangered species (Hirzel et al. 2001), play a key role in devising conservation strategies by identifying priority areas (Rhodes et al. 2006). Increasing studies on landscape ecology, such as mapping and evaluation of suitable habitat, by applying latest technologies and new analytical approaches have permitted the advancement in spatial explicit modelling that can help conservationist to understand species interaction with different landscape features (Jepsen and Fischer 2005; Drielsma et al. 2007; Larue and Nielsen 2008; Kabir et al. 2017).

1.5 Occupancy Studies and Elusive Carnivore Species

The speedy prevalence of camera trapping studies has resulted in its wide range of applications, but the technique is not yet sufficiently standardized. It has a drawback when individual identification is difficult, or in some cases, impossible (Oliveira-Santos et al. 2009) as in the wolf (Genovesi 2002). Occupancy-based abundance is a useful technique for monitoring unmarked and elusive animals like the wolf, as it permits trend monitoring through the percentage of occupied parts (Stanley and Royle 2005; Steenweg et al. 2016). To avoid bias in population sampling of rare, elusive and free-ranging species, recent advancements in ecological studies have improved the statistical and biological complexities of modern study techniques of population analysis (Williams et al. 2002; Thompson 2004). The statistical design and components of a survey are important factors in demographic studies of animals (Williams et al. 2002).

Photographs captured by remote cameras are used to build presence/absence data for species, and this data are entered in various software programs to model site occupancy and habitat suitability (MacKenzie et al. 2002; MacKenzie and Royle 2005; Sharma et al. 2014). The site occupancy study technique has been developed on the principle that the variation in the amount of available habitat occupied by a species is linked with the fluctuation in population size of that species (Royle and Nichols 2003). Occupancy gives a realistic measure for status estimation and changes, and also provides a cost-effective, credible alternative to assessment over large scale, multi-species monitoring programs (Mackenzie et al. 2002; Pollock et al. 2002). Data on species occupancy can be used to develop vigorous inferences about many variables like resources selection, distribution, population size, species interaction and metapopulation dynamics (MacKenzie and DeLuca 2006). The principle or criteria of occupancy-based abundance revealed that the variation in quantity of area occupied by a species is correlated with the variation in its population abundance in that area (Royle and Nichols 2003).

1.6 Human-Carnivore Interactions

Human-carnivore interaction over livestock predation has existed for millennia (Kruuk 2002), but both the problem and magnitude of the possible response have increased greatly over recent generations (Gittleman et al. 2001; Treves and Karanth 2003; Western and Waithaka 2005). Human-carnivore conflict is a common income and conservation subject in mountain communities living near wildlife habitats (Khan et al. 2014). Combined with habitat loss and fragmentation, human-induced mortality has contributed to widespread carnivore population declines, along with declines of their important ecosystem functions (Ripple et al. 2014). Still, in pastoral landscapes, the presence of large carnivores is controversial because they kill livestock and are exposed to retaliatory killings (Mishra et al. 2003; Suryawanshi et al. 2014; Jackson 2015; Mishra and Novakowski 2016). Large home ranges and overlapping diet requirements of large carnivores make them more vulnerable to retaliatory killing (Linnell et al. 2001; Macdonald and Sillero-Zubiri 2002). Reducing conflict between humans and carnivores is fundamental for the conservation of large carnivores and is frequently of financial significance where communities exist together with carnivores (Li et al. 2013).

Human-carnivore conflicts are complex and shaped by a suite of cultural, economic, historical, and ecological factors that may affect the use and effectiveness of techniques for mitigating livestock depredations (Messmer 2000; Dickman 2010). Multiple varying factors are contributing to conflict situations across the world, e.g., livestock grazing in habitats with carnivore abundance or in areas of low natural prey, lack of predator avoidance behavior in domesticated animals (Namgail et al. 2007; Suryawanshi et al. 2013; Aryal et al. 2014). Carnivores switch to preying on livestock when the availability of their natural prey is reduced (Treves and Karanath 2003; Dar et al. 2009; Sharma et al. 2015; Shehzad et al. 2015; Babrgir et al. 2017). Similarly, low levels of herd protection include the lack of guarding and no or poorly walled and/or roofed enclosures for animals at night (Dar et al. 2009; Suryawanshi et al. 2013). In all cases, actual or perceived conflict with livestock was the driving factor behind wolf extirpation from most of their historic range (Fritts et al. 2003).

Large carnivore livestock depredations can result in substantial economic hardships for livestock producers and ultimately weaken local support for conservation (Wang and Macdonald 2006; Karlsson et al. 2010; Lindsey et al. 2013). Carnivore-caused property losses are making the achievement of conservation goals difficult as they result in low tolerance and negative attitudes of affected agro-pastoral communities, particularly those belonging to low-income groups that rely largely on livestock rearing as a livelihood means and live close to wild habitats (Mishra et al. 2003; Linkie et al. 2007; Dar et al. 2009; Redpath et al. 2015). Negative attitudes toward wildlife often encourage people to kill wild animals (Williams et al. 2002; Bagchi and Mishra 2006), which takes a toll on conservation efforts. Consequently, it triggers antagonism towards predators and wolves suffer intense retaliatory killing where they have been known to be poisoned and hunted by people who are also rewarded for these actions (Namgail et al. 2007).

Livestock depredation by large carnivores and their retaliatory persecution by pastoralists are worldwide conservation concerns (Bagchi and Mishra 2006; Graham et al. 2005). Knowledge about the ecological and human dimensions of conflict is important for effective resolution of the problem, especially for understanding how livestock losses caused by wild animals affect local people’s responses to them (Mir et al. 2015). Understanding the factors that contribute to the complexity of conflict issues is crucial if conservationists are to facilitate the development of appropriate mitigation strategies (Dickman et al. 2013). Most often, there is an existing conflict between competing human priorities and conservation policies (Redpath et al. 2015). Affiliating socioeconomic interests with conservation efforts is the only feasible way to reduce conflict effectively (Mishra et al. 2003). There is an urgent need for interdisciplinary applied research (Nyhus et al. 2003; Ogada et al. 2003) that can assist in developing appropriate conflict management strategies (Treves and Karanth 2003). Addressing these human-carnivore interactions is, thus, essential for the effective conservation of these species (Kolowski and Holekamp 2006).

1.7 Foraging Ecology and Conflict Resolution

Another issue in determining the livestock depredation is the wrong identification of the perpetrator and overstating the number of livestock loss intentionally or erroneously (Suryawanshi et al. 2013). Diet composition depends mainly on the food availability of the area where wolves live, and this is the reason it is important to study their diet at a local scale. Wolves occur across a gradient of human landscape-transformation, from human-dominated regions to relatively undisturbed areas (Peterson and Ciucci 2003; Chapron et al. 2014). In places where the availability of prey populations fluctuates throughout the year, wolf depredation patterns can be difficult to understand (Mech and Boitani 2003; Jhala 2003). Furthermore, the lack of detailed information on the feeding ecology of large carnivores in dense human-use landscapes also hinders science-based conservation and management of such populations (Ghosal et al. 2013; Odden et al. 2014).

The ecology of the wolf regarding its selection of prey and its importance in diet is still not examined in Pakistan. Furthermore, persecution of the wolf has been a major issue in the Himalayan region without any detailed assessment of its prey selection and the reason behind its mass depredation on livestock. The damage caused by wolves, namely the loss of livestock, remains a point of contention (Ogada et al. 2003; Spinkyte-Backaitiene and Petelis 2012). Knowledge of species' dietary habits is crucial for the study of complex ecosystem processes (Treves and Karanth 2003), predator-prey dynamics and trophic interactions such as interspecific resource partitioning (Symondson et al. 2002; Sheppard and Harwood 2005; Klare et al. 2011), resource selection, population change, and physiological health (Deagle et al. 2010). As animals at or near the apex of food webs, predators can exert a disproportionate effect on ecosystem functioning relative to their biomass through predation (Estes et al. 2011). To facilitate a informed discussion on grey wolf conservation and management, it is important to develop a clear understanding of dietary ecology in landscapes with varying levels of human influence.

Wolf scat analysis forms an integral part of wolf ecology studies and may provide important or complementary information on wolf food habits which are otherwise difficult to obtain (Peterson and Ciucci 2003). Scat analysis is a widely used technique for determining the diet composition of various carnivores (Oli 1993; Litvaitis 2000; Kaunda and Skinner 2003; Ott et al. 2007; Aryal and Kreigenhofer 2009) and samples can be collected over large spatial scales (Spaulding et al. 2000; Wasser et al. 2004). Scat analysis is the basic method of studying the feeding ecology of elusive carnivores (Li and Ruhe 2008), but where several similar-size carnivores co-occur, reliable identification of the correct donor species has been a substantial impediment to dietary analysis (Oli 1993; Farrell et al. 2000). Non-invasive genetic sampling (NGS) techniques, which use DNA extracted from animal signs such as hair, scat, saliva, urine, or regurgitates (Waits and Paetkau 2005), have become an effective method for studying wildlife populations and are the preferred monitoring method for some species and populations (De Barba et al. 2010; Borthakur et al. 2011). Molecular methods have been successfully used in developing diet profiles of wild animals, including the snow leopard (Shehzad et al. 2012a), leopard cat (Shehzad et al. 2012b), and leopard (*Panthera pardus*) (Shehzad et al. 2015). In fact, despite their costs, molecular techniques can provide more exhaustive information than any other method (Lukacs and Burnham 2005; Waits and Paetkau 2005; Lukacs et al. 2007).

1.8 Aims and Objectives of this PhD Study

The grey wolf is generally ignored in ecological investigations in Pakistan. In particular, its distribution, habitat selection and behavior are poorly understood. Increasing human population and dependence on livestock rearing generate human-wolf conflicts, retaliatory killing and depletion of natural prey, which are major threats to the wolf's survival in Pakistan. The lack of knowledge of the aforementioned ecological aspects of wolves constitute an obstacle in devising effective conservation strategies to cope with threats that wolf populations are facing in Pakistan. By applying the latest field and analytical techniques, i aimed to fill important information gaps in the landscape ecology of the grey wolf in northern Pakistan. To accomplish this, I used non-invasive study techniques, including camera trapping, genetic sampling, site occupancy survey and scats analysis. I used Maxent, Presence and R package for statistical analysis and developed spatially explicit models for habitat suitability and movement corridors. This study attempts to address the lack of data and supports wildlife managers, policymakers and conservation biologists in developing conservations plans based on ground realities. The following aspects were studied in detail:

i. Distribution and occupancy of the grey wolf in Pakistan, and factors that influence these parameters.

ii. Habitat suitability and movement corridors of the grey wolf in Northern Pakistan.

iii. An understanding of human-wolf interactions and avenues of co-existence.

iv. Feeding ecology and prey selection of the grey wolf in northern Pakistan.

1.9 Structure of the Thesis

This doctoral thesis comprises five chapters arranged in four independent papers (chapters 2–5). Chapter 1 describes the study background and general introduction. In chapters 2–5, detailed studies on the proposed core objectives of the thesis are explained, together with the materials and methods used to attain these objectives. Details of the statistical computations of the attained data are presented in each chapter along with recommendations for the conservation of the species and its habitat.

CHAPTER 1 narrates the general introduction, study area description, background research and study research question, terminating on the problem statement and objectives of the thesis. The problem statement and objectives of the thesis are provided at the end of chapter 1.

In **CHAPTER 2,** the availability of suitable habitat and the effect of environmental variables on species distribution modeling is quantified. I used non-invasive study techniques to develop a habitat suitability model and explore the extent of connectivity among populations. This chapter entailed the sampling strategy, sampling sites, methodology and model construction and software used in habitat suitability modeling. I also identified possible and suitable movement corridors of wolves influenced by various landscape elements. This chapter also identifies the movement corridors of wolves across their distribution range in northern Pakistan. The findings presented in this chapter have been published (Kabir et al. 2017).

CHAPTER 3 provides information on the occupancy of grey wolves in northern Pakistan. I quantified the influence of site and survey covariates on wolf occupancy to assist in population monitoring and to better understand the factors that influence the distribution of wolves. I used a single-season, single-species occupancy model, and the AIC value was considered to select the best model. I used a logistic regression equation using the coefficients generated for the top model to predict occupancy estimates in the grid cells that were not sampled. The model results illustrated detailed information on wolf occupancy which are assumed as surrogates of abundance and are particularly important for the conservation management of wolf populations in Pakistan.

CHAPTER 4 comprises information on patterns of livestock losses. I collected information through questionnaire and group discussion with key stakeholders in the area which explains the trend of livestock rearing and dependency of local livelihood on farming. I explored how wolf predation is influenced by socio-ecological factors, including education level, income sources and relative abundance of livestock and herd size. I developed maps and highlighted the areas with more predation risk of wolves thus resulting in negative interaction with local communities. The outcomes of the study highlighted and suggested proactive approaches to minimize the losses of local communities in the form of livestock depredation. The outcome of the study could be useful for defining mechanisms of human-wolf coexistence.

CHAPTER 5: For this chapter, I studied the diet composition of the wolf, and provided information about key prey species, frequency of occurrence and contribution of domestic versus wild ungulates in diet. Wolf scats samples were confirmed through genetic analysis, and prey species were identified by comparing the cuticular and medullary hair patterns obtained from scats samples.

1.9.1 References of the Study Published Under Chapter 3

Kabir, M., Hameed, S., Ali, H., Bosso, L., Din, J.U., Bischof, R., Redpath, S., Nawaz MA. 2017. Habitat suitability and movement corridors of grey wolf (*Canis

lupus) in Northern Pakistan. PLoS One 12:e0187027. https://doi.org/10.1371/journal.pone. 0187027.

1.10 Study Area

Pakistan has a variety of ecosystems existing within a wide range of ecological zones starting from the Indus delta and coastal wetlands in the south, covering a vast area of dry tropical deserts and thorn forests in the central plain areas of the river Indus and the semi-arid scrub lands of Pabbi hills of Pothohar. It further extends to subtropical broad-leaved evergreen scrub forests, dry and moist temperate coniferous forests and cold deserts occurring in the confluence of world-famous Karakorum, Himalayas and Hindu Kush mountains in the northern parts of the country (Pak-NBSAP 2015). This study was conducted in northern Pakistan, which lies between 35-37° N and 72-75° E. Surveys were conducted during 2009–2016 in the following national parks: Broghil National Park, Qurumber National Park, Shandoor-Handrab National Park, Deosai National Park, Machiara National Park and Musk Deer National Park, and study sites outside the national parks and protected areas, including Phandar Valley, Terich Valley, Khanbari, Hisper-Hoper, Shimshal, Chapursan and Misgar, covering the distribution range of the grey wolf in northern Pakistan (Figure 1.2.1).

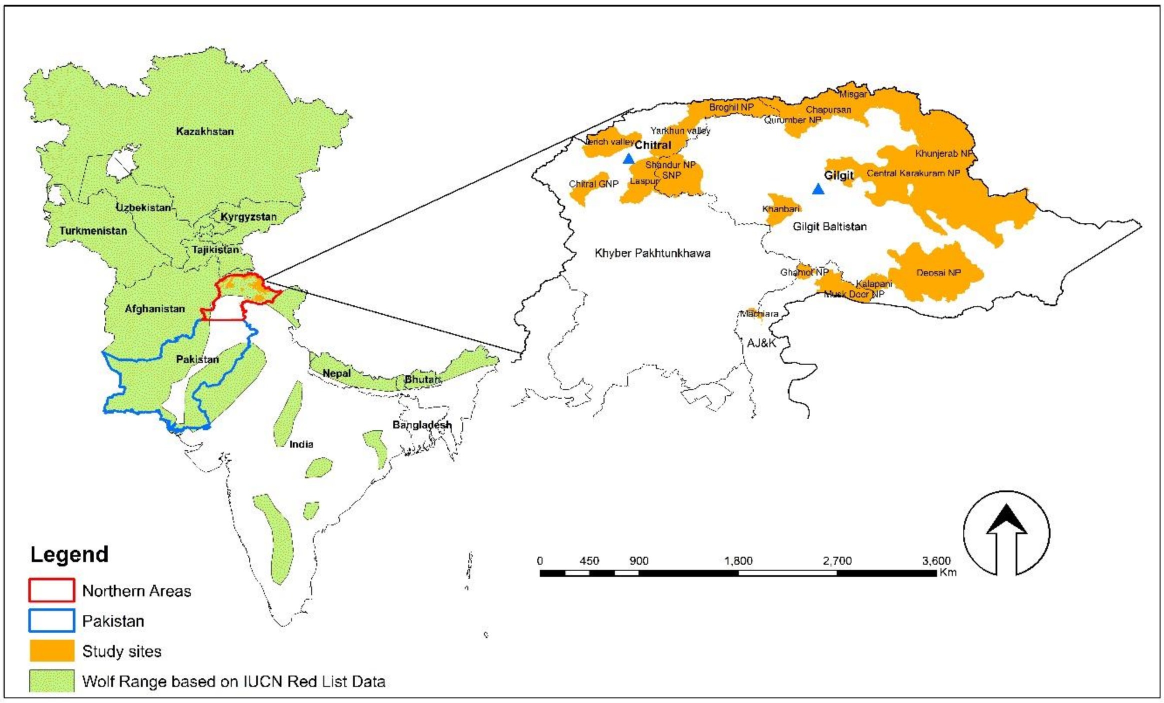


Figure 1.10.1: Study sites in Northern Pakistan

1.11 Topography

Northern Pakistan encompasses mighty mountain ranges including the chains of Hindukush, Himalayas, Karakorum and Pamir. Geographically, the landscape is surrounded by the Hindukush in the west, the Karakoram in the east, the Pamirs in the north, and the lesser Himalayas in the south (Khan et al. 2014). These mountain ranges converge near Jaglot in Gilgit, creating distinctive geographic features. Geologically, both the Himalaya and Karakorum ranges are active areas at the junction where the two continents collide with each other, making them interesting phenomena for earth scientists. These wide-ranging mountains were formed about 40 million years ago and have a significant effect on climate change, directly or indirectly (EOS 2018).

These copious amounts of large and bare rocks are vulnerable to atmosphere degradation and withered by carbon dioxide. This phenomenon plays a significant role in making global climate cool through the removal of green gases in the atmosphere, and also play a vital role in activating current ice age series. Narrow valleys, cliffs, rough ridges and glaciers dominate the area (Bischof et al. 2014). These mountain ranges have a collection of the highest and most vertiginous peaks of the world, formed a watershed of big rivers and streams. These are considered an important lifeline for the rest of country as the watersheds of these mountains provide water for agriculture, and industrial and domestic use, and they also serve as a chief source of freshwater for drinking. From the total 72% annual influx in the Indus comes from the Hunza, Astore, Shyok, Ghizer, and Shigar rivers. The fascinating landscape of these mountains attracts a large number of tourists, climbers, trackers and nature loving communities to explore the unique topographic features and biodiversity of this hidden paradise on earth.

1.11.1 Karakorum Mountain Range

The main Karakoram mountains represent the continental divides of South Asia and connect borders between China, India and Pakistan at different locations, including Xinjiang (China), Ladakh (India) and northern Pakistan. This range extends up to 500 km and is surrounded by the Tibetan Plateau from the northeast and the Pamir mountains from the north edge. The range also has more than 60 peaks which are above 7,000 m (22,960 ft). Siachen (70 km) and Biafo (63 km) are the world’s second and third largest glaciers located outside the polar regions. This mountainous area comprises various types of rocks (sedimentary, igneous and metamorphic) and a high elevation range and rugged mountains with steep slopes. At some places, very deep gorges have formed while at other locations sharp cliffs and peaks stand out. Because of their sloping surface, the mountain soils are very unstable and subject to active water erosion. Heavy glaciations are exhibited by the Karakoram (especially on the southern slopes) due to their immense height. The glaciers lie at 2,900 m asl and the snow line extends up to an elevation of 4,700 m asl along the southern Karakorum slopes. The complex networks of glacial systems are not only occupying the valleys, but in some locations the whole watershed. The seasonal melting of these glaciers results in heavy floods in the southern slopes. Along the Indus river valley, signs of prehistoric glaciation have been observed at altitudes as low as 2,600 m asl and 850 m asl.

1.11.2 Himalayas (Western) Mountain Range

The Himalayas are spread across Asian countries, including India, China, Nepal, Bhutan and Pakistan (Ives 2006). The Western Himalayas are located in northern Pakistan and the Kashmir valley and extend to the south and east of the Indus River, and are dominated by the Nanga Parbat block. The world’s ninth highest and dubbed the ‘killer’ mountain, Nanga Parbat is the backbone of the western Himalayas. The Himalayas are affected by various climatic conditions. From June to December, the monsoon rain prevails. The summer of the Himalayas (April–June) is hot, while in winter (November–March) it is extremely cold. In winter, temperatures are below freezing, and snowfall exceeds 2,000 m asl (Negi 1998). The Himalayas have tropical forests below 1,500 m asl, but small patches of oak trees can be seen even above 1,500 meters. Coniferous forests are found at more than 2,500 m asl, but some alpine meadows occur around 3,500 m asl.

1.11.3 Hindu Kush Range

The Hindu Kush is among the magnificent mountain system of Central Asia. Broadly demarcated, it is 800 km long and 240 km wide and the southwest of the Pamirs rise from the Hindu Kush mountains. It extends from the Wakhjir Pass near the junction of Pamirs and Karakorum to the Khawak Pass north of Kabul. The 966-km Hindu Kush mountain range is located in northwestern Pakistan, across Afghanistan and Pakistan.

1.11.4 Climatic Conditions

Mountain ranges characterize the climate of the area by advancing and regulating the long-term disparity in precipitation and temperature. From a global climate point of view, these mountains belong to the Central Asian mountainous belt and have their most northern ranges in the Tien-Shan to the south, followed by Pamir, up to the most southern: the Hindukush and Karakorum (Pak-NBSAP 2015). Strong winds, concentrated sun rays, wide ranges of temperatures and rarefied air are distinctive climatic attributes of the region. The climate of the Northern Areas is extremely cold in winter and relatively hot in summer. Temperatures range from -2.4ºC in winter to 48ºC in summer depending elevation. According to a study by Hussain et al. (2005), the combined effect of this increase/decrease of temperature has led to Karakoram glaciers' expansion. Precipitation and snow are less on the middle and lower slopes where annual precipitation is not greater than 100 mm. Above 4,900 m asl, elevation precipitation is in solid form which has led to the formation of Karakoram glaciers (Winiger et al. 2005).

The difference in the precipitation times in the Himalayas, Karakorum and Hindukush (HKKH) region is a consequence of different circulation patterns prevailing above described parts (Winiger et al. 2005). Rainfall is not enough to meet the water requirements for streamflow and crops. Ice-melt and snow are chief sources of precipitation which contribute to high elevation water resources in the upper Indus. Northern slopes are very dry whereas southern slopes get moist monsoon breezes from the Indian ocean. In the Himalayan region, monsoon winds from the south west of India bring with it rains that usually start in June and last till September. While western winds affect HKKH climate, the Himalayan region is under the influence of the winds from the Indian Ocean, and the westerly winds come from the Caspian and Mediterranean Sea (CKNP 2014).

Table 1.11.1: Meteorological Data (2018) of Northern Pakistan

	Himalaya (Muzaffarabad)				Karakorum (Gilgit)				Hindukush (Chitral)			
Month	Maxi. temp. (ºc)	Mini. temp. (ºc)	Mean temp. (ºc)	Mean rainfall (mm)	Maxi. temp. (ºc)	Mini. temp. (ºc)	Mean temp. (ºc)	Mean rainfall (mm)	Maxi. temp. (ºc)	Mini. temp. (ºc)	Mean temp. (ºc)	Mean rainfall (mm)
January	12.32	2.84	7.58	7.25	10.23	-1.41	4.41	0.36	8.6	-0.68	3.96	3.38
February	20.16	5.92	13.04	2.02	13.61	2.37	7.99	0.47	10.08	-0.16	4.96	5.77
March	21.35	7.59	14.47	2.23	18.19	5.48	11.83	0.16	17.30	4.51	10.90	1.39
April	28.67	12.06	20.36	5.24	23.06	8.38	15.72	1.52	25.02	10.14	35.16	0.42
May	32.73	16.04	24.38	1.38	31.17	13.79	22.48	1.40	30.69	14.31	22.5	0.35
June	34.22	19.01	26.61	3.18	33.9	16.18	25.04	0.33	35.82	18.16	26.99	0.01
July	34.83	21.82	28.32	10.16	34.32	19.16	26.74	0.33	36.26	21.92	29.09	0.0
August	34.63	21.93	28.28	4.74	33.66	17.59	25.62	0.33	35.08	18.66	26.87	0.06
September	33.19	18.82	26.00	1.25	31.03	12.86	21.94	0.10	32.98	14.15	23.56	0.15
October	30.95	13.57	22.26	0.0	26.53	6.66	16.59	0.0	27.31	7.76	17.53	0.0
November	22.47	7.75	15.11	1.15	19.78	-0.53	9.62	0.0	19.9	1.48	10.69	0.08
December	18.36	4.26	11.31	2.11	13.58	-1.80	5.89	0.15	13.3	0.14	6.72	0.60

Source: Meteorological Department, Islamabad

1.12 Ecological Zones and Corresponding Flora and Fauna

Wide-ranging altitude and varied climatic conditions have led to the formation of distinct ecological zones characterized mainly by vegetative communities and fauna associated with them (Roberts 1997). Climatic variations (-20^{o}C in winters to 45^{o}C in summers) and steep gradients allow assemblage of globally important animals and economically significant plants (IUCN 2003). Northern Pakistan is well known for its rich biodiversity and its distinctive geographical position is home to many threatened wildlife species, including birds, mammals and reptiles. The remoteness of these ranges favors the survival of many plant and animal species in the area. The rich biodiversity is supported by diverse ecological zones and vegetation types (Table 1.2.2.).

Table 1.12.1: Species Diversity in Northern Pakistan (as reported in the Biodiversity Action Plan for Pakistan, Roberts 1977).

Taxon	Reported for Pakistan	Estimated for northern Pakistan	Endemic to the northern Pakistan
Mammals	174	54	2
Birds	668	230	-
Reptiles/amphibians	177/22	23/6	4/2
Freshwater fish	198	20	4
Insects	> 5000	?	?
Plants	> 5700	?	?

1.12.1 Fauna

The diverse ecosystem and climatic conditions support many endangered and rare species of birds and mammals. The study area embraces rich faunal diversity owing to its vegetative communities and ecological niches. The iconic wildlife species and charismatic large carnivore species of the region have adapted to survive at such high altitudes described below (Table 1.12.2).

Table 1.12.2: Key Wildlife Species in Northern Pakistan (Roberts 1977; Sheikh and Molur 2004; Kabir et al. 2017).

	Species name	Scientific name	IUCN status
1	Snow leopard	*Panthera uncia*	Vulnerable
2	Brown bear	*Ursus arctos*	Vulnerable
3	Grey wolf	*Canis lupus*	Least concern
4	Himalayan lynx	*Lynx lynx*	Least concern
5	Himalayan ibex	*Capra sibrica*	Least concern
6	Blue sheep	*Pseudois nayaur*	Least concern
7	Flare-horned markhor	*Capra falconeri cashmirensis*	Near threatened
8	Ladakh urial	*Ovis orientalis vignei*	Vulnerable
9	Musk deer	*Moschus chrysogaster*	Endangered
10	Marco Polo sheep	*Ovis ammon polii*	Near threatened
11	Mountain weasel	*Mustela altaica*	Near threatened
12	Woolly flying squirrel	*Eupetaurus cinereus*	Endangered
13	Pallas cat	*Otocolobus manul*	Near threatened
14	Leopard cat	*Prionailurus bengalensis*	Least concern
15	Grey langur	*Semnopithecus ajax*	Endangered

The diverse habitat also provides shelter for a variety of birds, including rock pigeon (*Columba livia domestica*), chukar partridge (*Alectoris chukar*), booted eagle (*Hieraaetus pennatus*), snow pigeon (*Columba leuconota*), oriental turtle dove (*Streptopelia orientalis*), common snow cock (*Tetragallus himalayensis*) and common kestrel (*Falco tinnunculus*), which are some famous residents. In summer, the area serves as a breeding ground to various bird species like common hoopoe (*Upupa epops*), common swift (*Apus apus*), common cuckoo (*Cuculus canora*) and Eurasian nightjar (*Caprimulgus europaeus*). In winter, it receives guests (migratory birds) from many parts of the world including Eurasian goldfinch (*Carduelis carduelis*), skylark (*Alauda arvensis*), Spanish sparrow (*Passer hispaniolensis*), hen harrier (*Circus cyaneus*), pine bunting (*Emberiza leucocephalos*) and Himalayan accentor (*Prunella himalayana*). Rare birds residing here include Himalayan monal (*Lophophorus impejanus*), snow partridge (*Lerwa lerwa*), golden eagle (*Aquila chrysaetos*), Hume's wheateater (*Oenanthe albonigra*) and a variety of finches. The area is also known for high-altitude wetlands and lakes and hosts a number of water fowl and migratory birds during the spring and winter migration seasons.

1.12.2 Flora

Flora varies with the increase in elevation from moist temperate to alpine zone, with variations of animals and plants among different zones. The region lies in the rain shadow of the Himalayas and, except for some areas in the Himalayan region which get better summer precipitation, most is characterized as a cold desert with scattered vegetation cover, except some isolated patches of forest cover that cover four percent of the area. Dense forests are uncommon, and trees mostly grow in patches and are scattered. The dry, temperate mountain forests are generally confined to the south-western parts of northern Pakistan. These forests are generally found at elevations of 1829–3048 m asl. Upper and lower tree lines are present in the area; cold delimits the upper area whereas aridity limits lower areas. Between both are only sparse, degraded tree cover. Dominant vegetation in this alpine dry steppe zone is *Artemisia* and *Juniperus spp*.

In the northern-most regions and highest altitudes as typified by the Karakoram Mountains and Hunza and northern Chitral, the vegetation is often more xerophytic than in those alpine zones associated with smaller mountain masses. Major plant species here are *Juniperus communis, Potentilla desertorum, Salix denticulate* and *Mertensiatibetica*. The sub-alpine scrub zone is represented by narrow tree belts aligned with streams, often neighboring ravines. Main plant species here include *Butela, Lonicera, Juniperus, Berberis, Cotoneaster, Rhododendron, Anemone, Primula* and *Ranunculaceae*. Above the tree line, breathtakingly beautiful lush green pastures in moist regimes are featured. Vegetative communities here are composed of grasses, like *Poa spp.* and *Carex spp.*

1.13 Socioeconomic Conditions

Except for a few large towns, most human population is scattered in small settlements in isolated valleys. Most of the population is present along the valley floors and exist on alluvial fans and terraces on either side of the existing rivers and streams in deep gorges and valleys based on water availability for human consumption and agriculture. The region is extremely prone to land sliding and other natural disasters as compared to the rest of the country.

Northern Pakistan hosts over 2.5 million people and livestock holdings representing significant sources of livelihood and assets on which farmers can rely on (AKRSP 2000; Hussain 2003; Afridi et al. 2009). In the Karakoram-Hindu Kush-Himalayan ranges, 80% of the local community depends on livestock rearing for their livelihood (Khan 2014). Cattle are used for ploughing fields and providing milk and animal manure is used as fertilizer. As soon as crops are sown after winter, villagers begin moving all livestock except lactating animals to pastures, following the snowmelt, and ultimately spending the summer months in alpine pastures. At the end of summer, they gradually start moving their livestock back towards the village and arriving there after crops have been harvested. The animals are then free to roam around and feed on stubble in agricultural fields. The winter months are extremely deficient in feed and most animals lose a lot of body weight.

Goat and sheep populations have risen apparently from 1.2 million in 2000 to 1.71 million in 2010, with a 3.3% annual growth rate. Therefore, there is very heavy grazing pressure on the ecosystems which are already degraded (Source: Livestock Department GB, and P&DD 2013). The amount of grazing in most grasslands exceeds its carrying capacity (Beg 2010). Due to climate change and under increasing grazing pressure the pasture grazing resource base is slowly shrinking. Due to overgrazing, drought and climate change, pastures are losing their productivity and biodiversity. With the collaboration of the local community, the government developed a community-controlled hunting area which contributes to local income and conservation of wildlife species. In urban areas, people are engaged in trade, hotel businesses and tourism.

1.14 Biodiversity Conservation

The major threats to biodiversity are habitat degradation and fragmentation, indiscriminate hunting, grazing competition between domestic and wild ungulates and killing predators to control livestock losses. The fragile ecosystems of the region are degrading at a very fast rate due to growing populations, unplanned, haphazard development and the construction of mini and mega energy projects. Climate change represents a major threat to mountainous biodiversity and ecosystem integrity. To representative samples of ecosystems and protect threatened species, a number of protected areas, national parks, game reserves, wildlife sanctuaries and community-controlled hunting areas have been established across the region.

There are nine national parks in northern Pakistan covering the iconic, unique and threatened biodiversity of the Himalayas, Karakorum and Hindu Kush mountain ranges (Table 1.2.1). In addition, to recover populations of rapidly declining game animals, the government has introduced a trophy hunting program where local communities are encouraged to protect populations of big game and keep 80% of permit fees. The major species being managed for trophy hunting are the Kashmir markhor, Himalayan ibex, and blue sheep.

Table 1.14.1: Protected Areas and their Key Wildlife Species

National Park	**Ecological significance**
Chitral Gol National Park (CGNP)	CGNP is located at 35^o 56'N latitude to 71'40^{o}E longitude in the northwestern part of Khyber Pakhtunkhwa Province, Pakistan. The fauna of CGNP has affinities with the Palearctic Faunal Region with only a slight southern oriental mixture. Dry and arid temperate climates have been compensated for their species richness by large altitudinal variations. Major wildlife includes the snow leopard, Himalayan lynx, grey wolf, Himalayan ibex and Markhor (Din and Nawaz 2010).
Shundur-Hundrub National Park (SHNP)	The SHNP is situated along the boundaries of district Ghizer and its borders along the Wakhan strip on its north-west. The Langer wetland complex in Shundur supports one of the largest populations of trout and is an ideal breeding ground for the fish. Key wildlife species include the snow leopard, brown bear and wolf.
Broghil National Park (BNP)	BNP is situated in the extreme north of district Chitral at an altitude ranging from 3048 m to 4267 m with an elevation of around 3109 m. It is the habitat of other globally significant wildlife species, including the snow leopard, brown bear and wolf. The complex network of wetlands in BNP collectively

	provide breeding grounds for water birds and staging grounds for waterfowl mostly in autumn and spring.
Qurumber National Park (QNP)	QNP is one of the highest altitude parks in Pakistan at an elevation of 2286 m up to 5182 m and falls in the Western Tibetan Plateau Alpine Steppe. A beautiful perennial water body situated at an elevation of 4304 m is Qurumber Lake which is considered a unique high-altitude wetland spot in Pakistan. Its water clarity level is the highest ever recorded in the lakes of Pakistan, *i.e.* 13.75 (Secchi Disc reading). Wildlife species include the snow leopard, wolf, Pallas's cat, blue sheep and ibex.
Khunjerab National Park (KNP)	KNP and the adjacent Taxkorgan (Tash Kurghan) Nature Reserve in China are refuge for high-altitude animals. The fauna in KNP is composed of a mixture of Palearctic and Indo-Malay elements containing taxa from the Ethiopian region, making biodiversity very interesting and diverse. The highest photo-captured record of the snow leopard obtained from this park and other large mammalian species include the Marco Polo sheep, blue sheep, ibex, bear and wolf.
Central Karakorum Park (CKNP)	CKNP stretches over an area of 10,000 km^2. The characteristic features of CKNP are its large glaciers, diverse topography, wide-ranging altitude (*i.e.* 1,500–8,000 m above sea level) and distinct ecological zones. It has four peaks over 8,000 meters, including K-2 (8,611 m). The Trango Towers are a family of the world's tallest rock towers located in Central Karakoram National Park. The presence of the Baltoro, Biafo, Hisper and Siachen glaciers make CKNP the largest glacial complex in the world after Antarctica. Mammalian species worth mentioning include the brown bear, snow leopard, grey wolf, Himalayan lynx, blue sheep, markhor, Ladakh urial, musk deer and Marco Polo sheep.

Musk deer National Park (MDNP)	MDNP provides maximum protection to the musk deer and its habitat, ensuring the maintenance of viable populations of this globally endangered species. MDNP is rich with respect to faunal diversity. Reported mammal species include the brown bear, wolf, common leopard, giant red flying squirrel, leopard cat, and musk deer.
Deosai National Park (DNP)	DNP is located where two biogeographical provinces merge in the Himalayan and Karakorum-Pamir highlands. Due to this, it has rich biodiversity as species are channeled in the main crest of the Himalayas, the Karakorum range, the Indus valley, Ladakh range and Zanskar range. This area was designated with the chief objective of the conservation and protection of the Himalayan brown bear which is native to this part of world. The park is part of Conservation International Himalayan Biodiversity Hotspots and contains a rich variety of species, including a population of Tibetan wolf (*Canis lupus chanco*).
Ghamot National Park (GNP)	GNP is located in the upper Neelum valley which is part of the inner Himalayas. The area comprises dry temperate conifer forest, sub- and high-alpine pastures, and cold desert (Roberts 1977). Rich habitats support the survival of carnivores, including the leopard cat, black bear, brown bear, common leopard, snow leopard, wolf, ibex and musk deer.

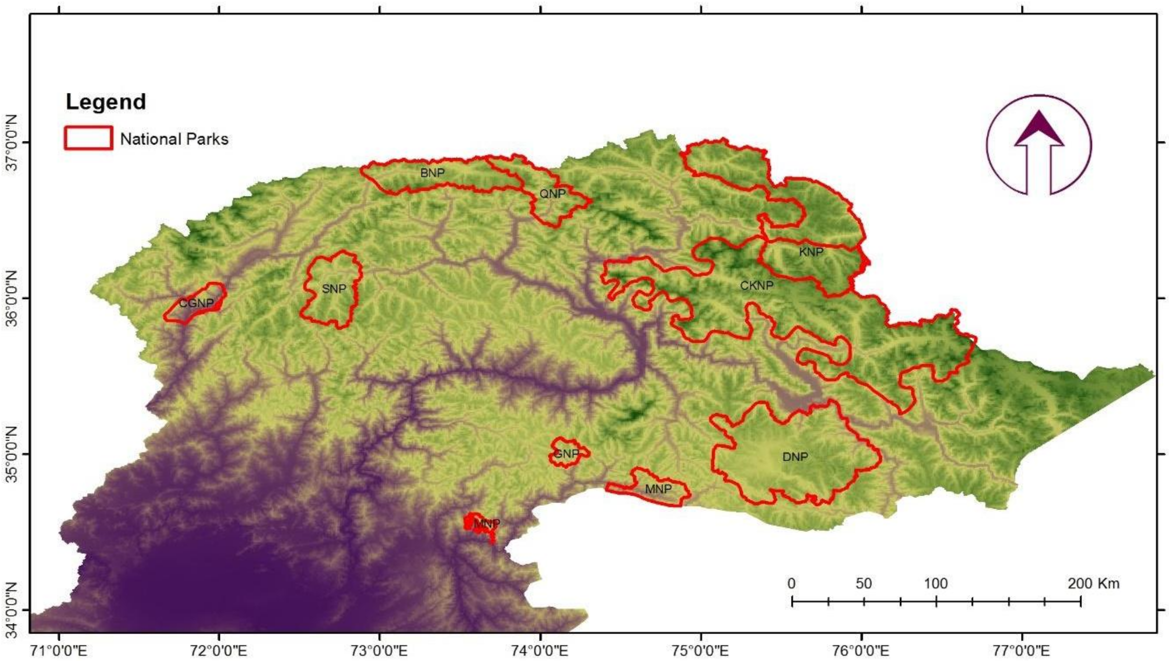


Figure 1.14.1: Locations of National Parks (red polygons) in Northern Pakistan

CHAPTER 2

Habitat Suitability and Movement Corridors of the Grey Wolf *(Canis lupus)* in Northern Pakistan

2 Habitat Suitability and Movement Corridors of the Grey wolf *(Canis lupus)* in Northern Pakistan

ABSTRACT

Habitat suitability models are useful to understand species distribution and to guide management and conservation strategies. The grey wolf has been extirpated from most of its historic range in Pakistan, primarily due to its effect on livestock and livelihoods. I used non-invasive survey data from camera traps and genetic sampling to develop a habitat suitability model for *C. lupus* in northern Pakistan and to explore the extent of connectivity among populations. I detected suitable habitat for the grey wolf using a maximum entropy approach (Maxent ver. 3.4.0) and identified suitable movement corridors using the Circuitscape 4.0 tool. Our model showed high levels of predictive performances as seen from the values of the area under curve (0.971±0.002) and true skill statistics (0.886±0.021). The main predictors for habitat suitability for *C. lupus* were distance to road, mean temperature of the wettest quarter, and distance to river. The model predicted ca. 23,129 km^2 of suitable areas for the wolf in Pakistan, with the majority of suitable habitat in remote and inaccessible areas that appeared to be well-connected through vulnerable movement corridors. These movement corridors suggest that potentially the wolf range can expand in Pakistan's northern areas. Managing protected areas with stringent restrictions is challenging in northern Pakistan, in part due to people's heavy dependence on natural resources. The habitat suitability map provided by this study can inform future management strategies by helping authorities identify key conservation areas.

Keywords: Habitat suitability, Maxent, *Canis lupus,* Pakistan, Karakoram, Himalaya, Hindukush

2.1 INTRODUCTION

The distribution of species in space and time is a central topic in ecology. Species distribution models (SDMs) are increasingly important for investigating the requirements of species and for conservation planning (Jarvis and Robertson 1999; Hirzel et al. 2001; Bosso et al. 2016a; Sheehan et al. 2017; Smeraldo et al. 2017). Such models provide valuable quantitative information on threats, such as areas where there is a high risk from humans, or where there are the required resources (Guisan and Thuiller 2005). They also help identify conservation priorities (Rhodes et al. 2006; Roscioni et al. 2013; Roscioni et al. 2014; Russo et al. 2015; Oberhauser et al. 2017).

The conservation of large carnivores remains challenging, in part due to a poor understanding of the complex spatial dynamics that facilitate population persistence (Hinton et al. 2016). The habitat requirements of such species deserve particularly close attention because they generally require large home ranges, are negatively wedged by changes in land use and are killed because of the threats they pose to livelihoods (Enserink and Vogel 2006; Gordon 2009; Doherty et al. 2017; Newsome et al. 2016). The grey wolf is a prime example of the drastic reduction in former ranges as a result of intense persecution. Wolves were once widely distributed throughout the Palearctic and Nearctic biogeographic regions (Vila et al. 1999; Mech and Boitani 2003). The global wolf range has shrunk by 33% over the last century (Mech and Boitani 2003). In many areas, agricultural expansion into marginal areas of wolf habitat has increased depredation of livestock and subsequently increased poaching, resulting in a numerical and spatial contraction of grey wolf populations (Mech 1981).

A major concern of modern conservation efforts is identifying remaining habitat that is suitable for a species to occupy (Mech 1981; Hilty et al. 2012; Lindenmayer and Fischer 2013; Saura et al. 2014; Mesler 2015). SDMs have proven to be effective at predicting habitat suitability for large carnivores, including wolves (Hendricks et al. 2016; Otis et al. 2017; Subba et al. 2017). Habitat and conflict management can be implemented using the results from monitored wolf populations once potential areas are identified. In addition, knowledge of potentially suitable wolf habitat can be integrated into landscape planning (Rhodes et al. 2006). Indeed, evidence from elsewhere suggests that map-based conservation planning can help facilitate human-wolf coexistence by identifying areas where the potential conflict, caused by livestock depredation, is high (Carroll et al. 1999; Mech et al. 2000).

Several studies have shown that the long-term survival of large vertebrates is achieved by protecting source populations and providing dispersal opportunities between suitable patches (Margules and Pressey 2000; Mech and Hallett 2001). Ecological corridors can help to connect populations, allowing individuals free dispersal between populations (Beier and Noss 1998). Wolf dispersal patterns across the landscape can better predict where new wolf populations may appear (Mesler 2015). Animals use a wide variety of mechanisms to select suitable habitat and being aware of habitat use details is important for corridor design (Danchin et al. 2001). Connectivity analysis is particularly important for wolves because it allows them to know this animal can move through the existing habitat (Ciucci et al. 2009).

Wolf populations in Pakistan have suffered population declines and range contraction (Mech and Boitani 2003; Sheikh and Molur 2004). They are now confined to remote, barren, mountainous regions and extensive deserts (Roberts 1997). Numerous factors are thought to be responsible for this decline, encroachment caused habitat loss. The movement of herders up the altitudinal gradient because of climate change has further reduced available habitat and increased the effect of retaliatory killings of wolves. These predators move to lower altitudes during heavy snowfall, further increasing the chances of being killed due to predator control by livestock herders (Lovari et al. 2007).

Although several studies have addressed GIS (Global Information System) and modeling analyses of *Canis lupus* in different areas of the world (Hendricks et al. 2016; Otis et al. 2017; Subba et al. 2017), yet no research has been conducted on the grey wolf in Pakistan. Our goal was to model, through the use of non-invasive survey data from camera traps and genetic sampling, a habitat suitability model for grey wolf in northern Pakistan and to explore the extent of connectivity between populations. I identified: a) the geographic distribution of *C. lupus* in northern Pakistan and ecological factors that may be limiting species distribution; and b) existing corridors through northern Pakistan that could facilitate dispersal of *C. lupus.* I did so using non-invasive survey data obtained through camera trapping and genetic sampling. Our results improve understanding of landscape permeability for large carnivores in a mostly unsuitable matrix and present conservation agencies with useful information should grey wolves continue to disperse into the region.

2.2 MATERIALS AND METHODS

The field surveys were conducted during the period of 2009–2017 within the following protected areas (PAs): Machiara National Park, Musk Deer National Park, Khunjerab National Park, Broghil Valley National Park, Qurumber National Park, Shandoor-Handrab National Park, and Deosai National Park, and outside the PAs in Phandar Valley, Kotli, Misgar, and Chapursan, covering suspected wolf range (Figure 2.2.1).

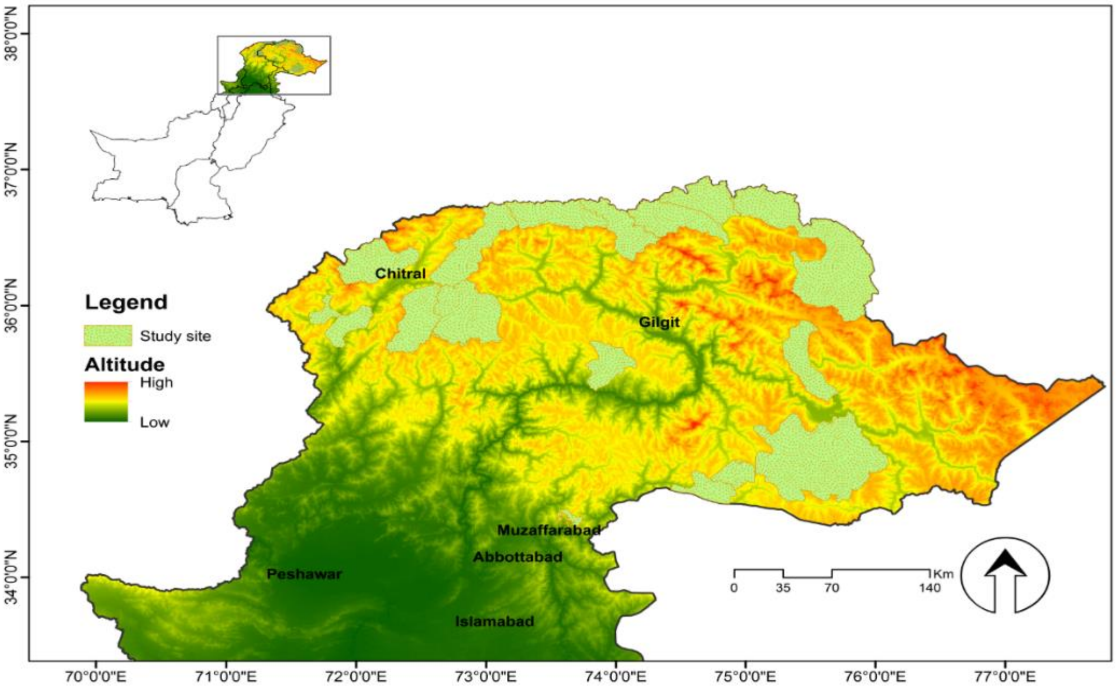


Figure 2.2.1: Study area (northern Pakistan). Scales show the altitude ranging from low (dark green) to high (red)

2.2.1 Genetic Sample Collection

Survey areas were divided into grid cells of 5×5 km^2 (except in Khunjerab National Park and Shimshal where the grid size was taken as 10 × 10 km^2) on GIS maps. Survey points were randomly selected within each grid cell and a 50 m radius around each point was searched for wolf scats. We searched 1,736 points within the study area. We also searched for scats at camera trap locations and while hiking along livestock trails and humanmade tracks (which were also used by wildlife). We searched and collected 1,186 scat samples of carnivores and preserved in 20 ml bottles filled with 95% alcohol.

I confirmed that: 1) the institution leader of this research (Carnivore Conservation Lab, Department of Animal Sciences, Quaid-i-Azam University, Islamabad) has all field permits to work in all protected areas; 2) the land owners of non-protected study sites authorized data collection; and 3) no wolves were harmed or sacrificed during this research. I only collected wolf scats and images through camera traps.

2.2.2 DNA Analysis and Species Identification

Deoxyribonucleic acid (DNA) was extracted from 15 mg of feces using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) recovered in a total volume of 200 µL. Blank extractions were systematically performed to monitor possible contaminations. Species identification was performed through next-generation sequencings (NGS) by amplifying DNA extract using primer pair 12SV5F (5’-TAGAACAGGCTCCTCTAG-3’) and 12SV5R (5’- TTAGATACCCCACTATGC-3’) (Riaz et al. 2011) targeting about 100-bp of the V5 loop of the mitochondrial 12S gene (Shehzad et al. 2012). The sequencing was carried out on the Illumina Genome Analyzer IIx (Illumina Inc.), using the Paired-End Cluster Generation Kit V4 and the Sequencing Kit V4 (Illumina Inc.), following the manufacturer’s instructions. The sequence reads were analyzed using OBI tools (http://www.prabi.grenoble.fr/trac/OBITools). Taxon assignation was achieved using the ecoTag program (Pegard et al. 2009) in comparison with a reference database for vertebrates. This reference database was built by extracting the relevant part of the mitochondrial 12S gene from the European Molecular Biology Laboratory’s (EMBL) nucleotide library using the ecoPCR program (Ficetola et al. 2010). Genetic results revealed identification of 80 samples belonging to wolves.

2.2.3 Camera Traps

Camera traps were installed in 800 locations during the period of 2009–2017 and were separated by a horizontal buffer of at least 1 km (Table 2.21). Camera trap locations were identified based on landscape characteristics—ridges, cliff bases, draw preferred by carnivores and the presence of carnivore signs (Bischof et al. 2013). A single motion-triggered digital camera with infrared flash (HC500/PC900, Reconyx, Holmen, WI, USA) was deployed at each location on a steel pole (50–60 cm) driven into the ground. Camera traps were set to take consecutive images (1-s picture interval) when triggered and were typically kept active at a given location for 10–40 days (Bischof et al. 2013). Camera trap sites were baited with fish oil. Commercial trapping scent lures were deployed in some randomly selected sites, as well (Bischof et al. 2014).

Table 2.2.1: Details of camera trapping studies and photo-captured record of the wolf in northern Pakistan

No.	Site	Year	Camera Station No.	Camera station with wolf presence
1	Chitral	2006	19	1
2	Chitral	2007	18	5
3	Chitral Gol National Park	2008	21	3
4	Chitral Gol National Park	2009	20	0
5	Tooshi game reserve	2009	30	0
6	Khunjerab National Park	2010	10	0
7	Laspur valley	2010	20	0
8	Khunjerab National Park	2011	86	1
9	Shimshal	2011	36	0
10	CGNP, TGR and Buffer	2011	22	11
11	Broghil and Qurumber	2012	80	6
12	Deosai National Park	2013	116	9
13	Yarkhun valley, Chitral	2013	58	4
14	Misgar and Chapursan	2013	59	0
15	Astore valley	2013	25	0
16	Musk deer National Park	2014	36	1
17	Khanbari, Diamer	2014	48	11
18	Tirch valley, Chitral	2015	28	0
19	Hisper valley, Nagar	2016	38	0
20	Bhasha valley, CKNP	2017	30	3

2.2.4 Model Preparation: Selection of Presence Data and Environmental Variables

Records obtained by scats collection and camera trapping of *C. lupus* were screened in ArcGIS (version 9.2) for spatial autocorrelation using average nearest neighbor analyses to remove spatially correlated data points and guarantee independence (Bosso et al. 2016a; Kwon et al. 2016; Bosso et al. 2017a). After this selection, from an initial dataset of ca. 134 presence records, only 25 unrelated locations were used to generate current SDMs of *C. lupus*.

I initially considered a set of 28 environmental variables to produce SDMs for *C. lupus* in northern Pakistan (Table 2.2.2). I included altitude, 19 bioclimatic variables, land cover, slope, soil type, distance to roads, distance to rivers, distance to settlements, vector ruggedness measures, and normalized difference vegetation index (NDVI). Bioclimatic variables and altitude were obtained from the WorldClim database (www.worldclim.org/current) (Hijmans et al. 2005). Land cover was obtained from the Global Land Cover 2000 database (available from https://lta.cr.usgs.gov/glcc/globdoc2_0). Distance to roads, distance to rivers, distance to settlements were calculated using the Euclidean distance tool in Arc GIS 10.0. Soil (FAO 2003, digital soil map of the world) and vector ruggedness measures (SRTM 90m DEM by the Center for Nature and Society, Peking University), and a normalized difference vegetation index were obtained from NASA's website (http://modis-land.gsfc.nasa.gov/vi.html). The MODIS normalized difference vegetation index product is computed from atmospherically corrected, bi-directional surface reflectances that have been masked for water, clouds, heavy aerosols, and cloud shadows. Global MOD13A2 data are provided every 16 days at 1-kilometer spatial resolution as a gridded level-3 product in the Sinusoidal projection.

To remove any variables that were highly correlated before generating the models, i calculated a correlation matrix using Pearson's technique and selected only the variables for which $r < 0.70$ (Booth et al. 1994). From this first set of predictors, i selected only those variables that were most representative of the species' ecological requirements (Vila et al. 1999; Mech and Boitani 2003; Ciucci et al. 2009; Mesler 2015; Hendricks et al. 2016; Hinton et al. 2016; Newsome et al. 2016; Otis et al. 2017; Subba et al. 2017).

After this analysis, eight environmental variables were selected considering their applicability to the scale of our study area, relevant predictive power, and their suspected biological importance (Guisan and Zimmermann 2000; Post and Forchhammer 2002). All the variables were prepared—conforming cell size [30-arc second resolution (0.93 × 0.93 km = 0.86 km^2 at the equator)], geographic extent, projection, and ASCII—using the 'resample', 'clip', 'mask', and 'conversion' tools in ArcGIS 10.0. Finally, the following eight environmental variables were used for model training: distance to roads (m), distance to rivers (m), mean temperature of wettest quarter (°C), mean diurnal range (°C), soil, annual precipitation (mm), altitude (m), and global land cover.

Table 2.2.2: The environmental variables used for habitat modeling of the grey wolf in northern Pakistan

Environmental variable	Acronym	Data source
Mean diurnal range	bio_2	www.worldclim.org/current
Isothermality (Bio2/Bio7) (* 100)	bio_3	
Temperature seasonality (standard deviation *100)	bio_4	
Max temperature of warmest month	bio_5	
Min temperature of coldest month	bio _6	
Temperature annual range (Bio5-Bio6)	bio_7	
Mean temperature of wettest quarter	bio_8	
Mean temperature of driest quarter	bio_9	
Mean temperature of warmest quarter	bio_10	
Mean temperature of coldest quarter	bio_11	

Annual precipitation	bio_12	
Precipitation of wettest month	bio_13	
Precipitation of driest month	bio_14	
Precipitation seasonality mean temperature of driest quarter	bio_15 bio_9	
Precipitation of wettest quarter	bio_16	
Precipitation of driest quarter	bio_17	
Precipitation of warmest quarter	bio_18	
Precipitation of coldest quarter	bio_19	
Elevation above sea level (m)	Alt	SRTM/www.worldclim.org/current
Slope of the area	Slope	derived from alt in Arc GIS 10.0
Euclidean distance to rivers (m)	river_distt	Calculated by using Euclidean distance tool in ArcGIS 10.0.
Euclidean distance to rivers (m)	road_distt	
Euclidean distance to rivers (m)	sett_distt	
Normalized difference vegetation index	Ndvi (MODIS)	(http://modis-land.gsfc.nasa.gov/vi.html)
Digital soil map of the world	Soil	FAO, 2003, digital soil map of the world
Vector ruggedness measure	vrmint	SRTM 90m DEM by Center for Nature and Society, Peking University
Global land cover 2000	glc2000	https://lta.cr.usgs.gov/glcc/globdoc2_0

2.2.5 Maxent Model

SDMs rely on presence-absence data or presence-only data (Ficetola et al. 2007; Rebelo and Jones 2010; Roscioni et al. 2014; Bosso et al. 2017a; Oberhauser et al. 2017). The use of presence-only is recommended when absence data has a high degree of uncertainty relative to presence data, which is especially true when detection rates are poor (Otis et al. 2017; Subba et al. 2017). I modeled wolf distribution using Maxent (ver. 3.4.0) as it is recognized as a better performer with presence-only data, especially with small numbers of occurrence points (Engler et al. 2004; Elith et al. 2006; Wisz et al. 2008). To build the models, i used the presence records (defined “sample” in Maxent) of *C. lupus* selected as described above and the environmental variables (defined “environmental layers” in Maxent). In the setting panel, i selected the following options: auto features; random seed; write plot data; remove duplicate presence records; give visual warming; show tooltips; regularisation multiplier (fixed at 1); 10,000 maximum number of background points; 1,000 maximum iterations; and, finally, i achieved a 20 replicates effect with cross-validation run type as suggested by Pearson et al. (2007) for testing small samples. This run type makes it possible to replicate n sample sets removing a location at each step (Bosso et al. 2017b; Guo et al. 2017; Tang et al. 2017). All other parameters were left by default. These settings are conservative enough to allow the algorithm to get close to convergence and optimize performance (Phillips et al. 2017).

The final logistic output gave suitability values from 0 (unsuitable habitat) to 1 (suitable habitat). The 10th percentile (*i.e.* the value above which the model correctly classifies 90% of the training locations) was selected as the threshold value for defining the species’ presence. This is a conservative value commonly adopted in species distribution modeling studies, particularly those relying on datasets collected over a long time by different observers and methods (Russo et al. 2015; Bosso et al. 2017a). This threshold was used to reclassify our model into a binary presence/absence map.

I used Jackknife sensitivity analysis to estimate the actual contribution that each variable provided to the geographic distribution models. Maxent generated three models during this process. First, each environmental variable was excluded in turn, and a model was created with the remaining variables to check which one was the most informative. Second, a model was created individually adding each environmental variable to detect which variable had the most information not featuring in the other variables. Third, a final model was generated based on all variables. Response curves derived from univariate models were plotted to examine how each environmental variable influenced presence probability.

2.2.6 Model Validation

I tested the model with different validation methods: receiver operated characteristics, analyzing the area under curve (AUC) (Fielding and Bell 1997), and the true skill statistic (TSS) (Allouche et al. 2006). AUC assesses the discrimination ability of the models and its value ranges from 0 (equaling random distribution) to 1 (perfect prediction). AUC values > 0.75 correspond to high discrimination performances (Fielding and Bell 1997). TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. It considers both omission and commission errors, and success as a result of random guessing. Its values range from -1 to +1, where +1 corresponds to perfect agreement and 0 or less to a performance no better than random (Allouche et al. 2006).

2.2.7 Modeling Potential Movement Corridors

A spatial corridor model was developed using the distribution map of wolves in Circuitscape 4.0 software (http://www.circuitscape.org/downloads) (McRae and Shah 2009). I used Circuitscape 4.0 to model the connectivity and movement corridors of the grey wolf in Pakistan across the landscape. Circuitscape treats the landscape as a conductance surface where each pixel represents a resistor with an assigned resistance value. Pairwise electrical resistances between locations are calculated by running a theoretical electrical current between each population pair, with one population being set as the current source and the other as the ground (McRae and Shah 2009). Contrary to least-cost resistance methods, Circuitscape does not assume that animals disperse according to previous knowledge of the surroundings, but is based on random walks (McRae and Shah 2009). It thus links populations through multiple pathways (McRae and Shah 2009) such that connectivity between habitat patches increases according to the number of connected pathways, and the effective resistance between two populations is derived from the overall resistance across all pathways. I used SDM output as a conductance layer and 24 nodes to run movement corridors of the grey wolf in Circuitscape 4.0. The nodes were used to represent different areas where i have confirmed wolf presence in northern Pakistan. Clearly, i have not used all of the nodes to run Circuitscape because otherwise, this procedure would become too complex. I have used a very low number of nodes and have chosen them as the most important areas of wolf movement in northern Pakistan. I converted the nodes into a grid file in ArcGIS 10.0. Both the habitat suitability map (created by Maxent) and the nodes file were converted into ASCII format for a Circuitscape model run. I used the option of conductance instead of resistance because the landscape is represented as a conductive surface with low resistances assigned to landscape feature types (Saura et al. 2011; Cushman et al. 2013; Roscioni et al. 2014). The final movement corridors map was reclassified into three categories - low, moderate, and high to better represent the most important areas for *C. lupus* movements.

2.3 RESULTS

2.3.1 Camera Traps and Genetic Analysis

We obtained 51 wolf presence records from camera traps and 80 from the genetic analysis of scat samples collected from the distribution range of wolves (Figure 2.3.1). Most presence records were obtained from national parks. Presence records along the altitudinal gradients ranged from 3,000 m (Musk Deer National Park) to 4,700 m (Khunjerab National Park). PAs with a higher frequency of presence records were Deosai National Park (Himalayan range), Chitral Gol National Park (Hindu Kush range), Khunjerab (Karakorum range) and Broghil National Park (Pamir range). Outside PAs, the highest wolf encounter was recorded from the Khanbari Valley in Gilgit-Baltistan. There was no presence record from Terich, Astor, Misgar, Chipurson, Shimshal, and Hisper Hooper Valley. Overall, wolf detection was low, suggesting thin and patchy populations.

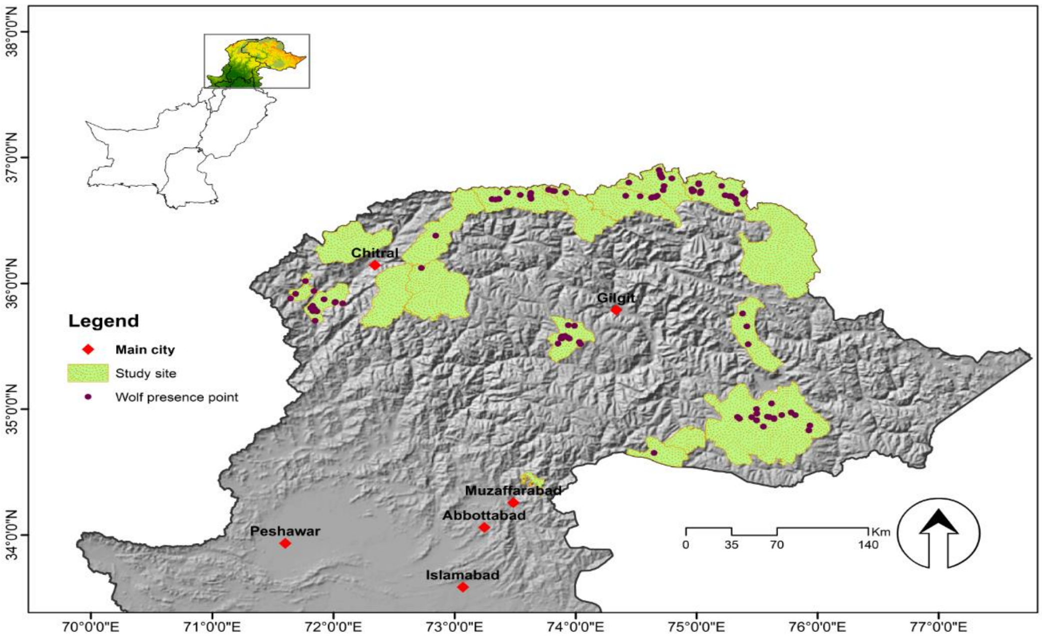


Figure 2.3.1: Genetic or photographic detections of wolves in the study area. The presence points were obtained from camera trap results and DNA analysis of scat samples collected from the Northern Areas of Pakistan (2009–2015). Altitude map in grey

2.3.2 Habitat Suitability and Model Validation

The Maxent model suggested that there was suitable wolf habitat within the areas chosen as suspected wolf habitat range (Figure 2.3.2). The binary map discriminated between areas typically used by wolves and those considered unsuitable (Figure 2.3.3). The most suitable areas identified from the models were located predominantly within PAs and most inaccessible areas with minimum human disturbance, and overall, mainly along the narrow valley and around summer livestock pastures. The model suggested that there was less suitable habitat in lower altitude areas with more human access.

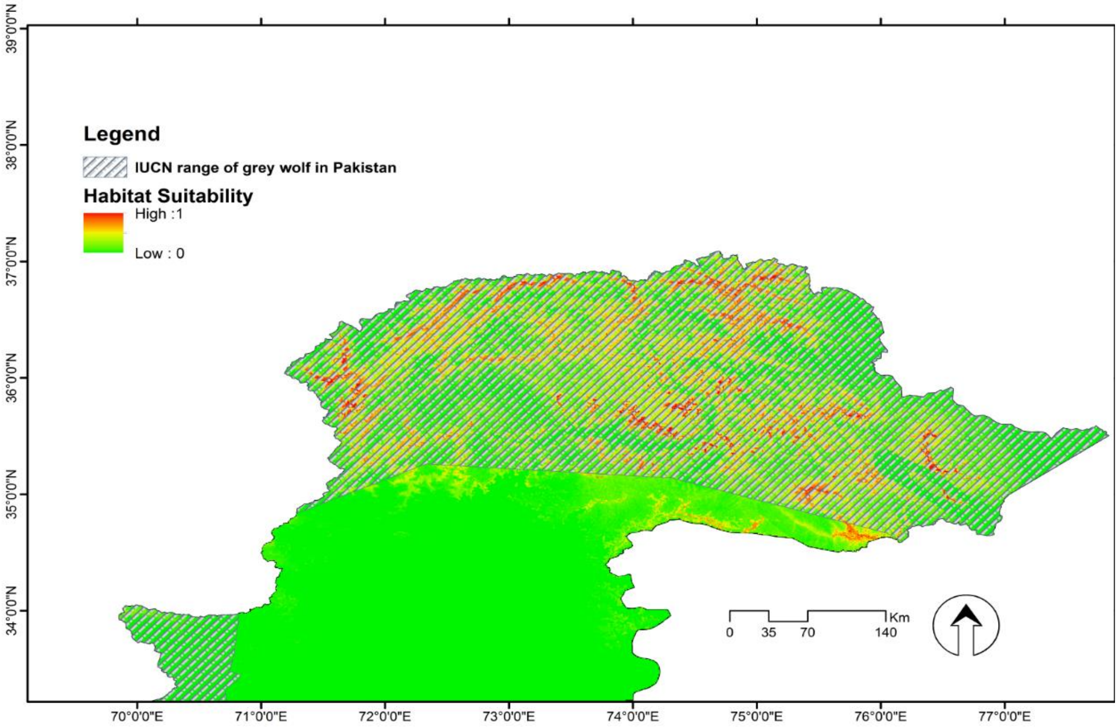


Figure 2.3.2: Wolf habitat suitability in northern Pakistan generated through Maxent. Scales show the probability of presence ranging from 0 to 1

In relation to the distribution range, suitable areas were quantified based on habitat suitability modelling (Figure 2.3.3). The model predicted ca. 23,129 km^2 of potential wolf distribution in northern Pakistan. The jackknife-cross-evaluation test yielded the relative contribution and permutation of each environmental variable using Maxent. Distances to roads, mean temperature of the wettest quarter and distance to rivers contributed most to the model. Soil, altitude, annual precipitation and land cover contributed relatively little.

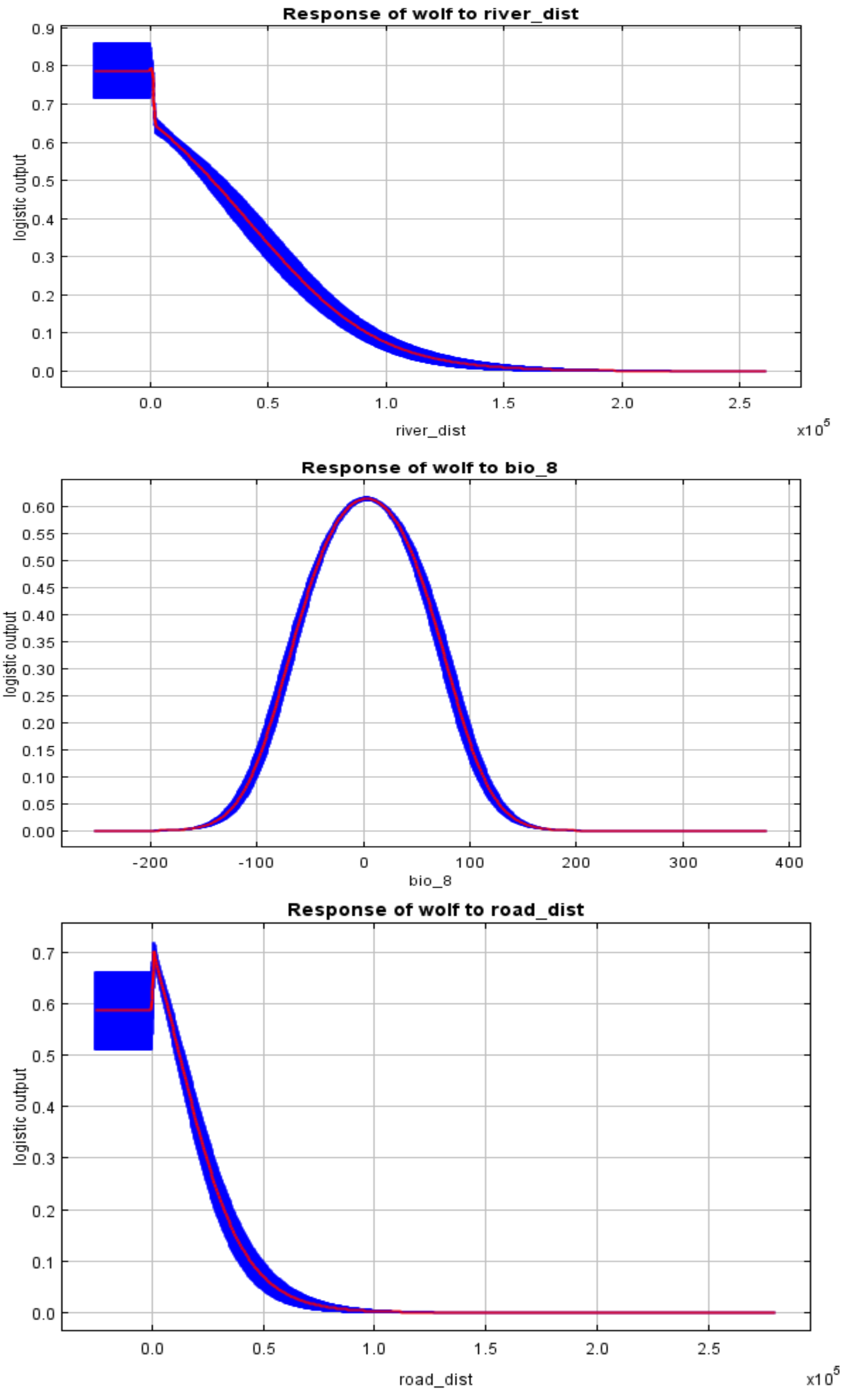


Figure 2.3.3: Response curves of probability of presence of grey wolf to (A): distance to river (B): mean temperature of wettest quarter (C): distance to road. The red curves represent the mean trends while the blue shades show the mean +/- the standard deviation. In each graph, the x-axis shows the change in each environmental variable, while the y-axis shows the species' probability of presence.

Response curves showed how the logistic prediction changed as environmental variables varied while keeping all other environmental variables at their average sample values. The probability of wolf presence was 0.7 at 0 km^2 of distance from the road and stream, but it increased suddenly to 0.05 at a distance of 0.5 km^2. Mean temperature of the wettest quarter ranged from -10°C to 10°C with a maximum value (0.6) of probability of presence around 0 °C.

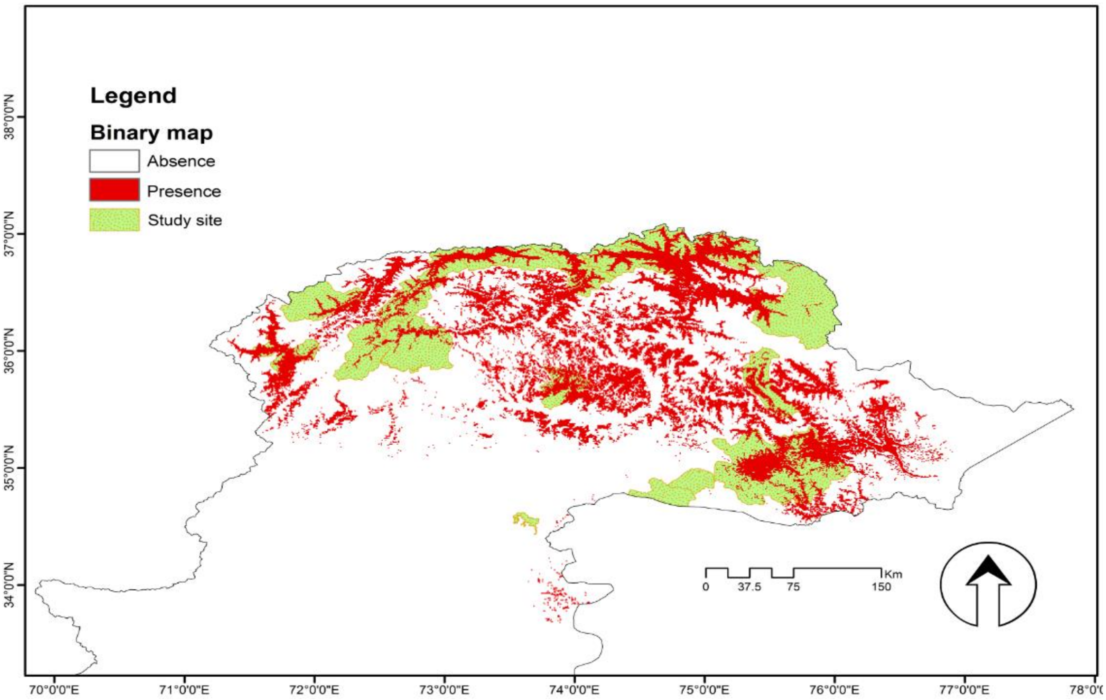


Figure 2.3.4: Binary map of *C. lupus* in Pakistan. White = absence; red = presence.

The jackknife test also revealed the importance of different variables and their effect on model efficacy. Distance to river was the most important variable in determining model prediction in training, testing, and AUC evaluation. Distance to river increased the gain more than any other variables when added in isolation. On the other hand, mean temperature of the wettest quarter variable decreased the gain most when omitted, *i.e.* it contains the most information, not present in others (Figure 2.3.3). Finally, our model showed high levels of predictive performances as can be seen from the values of area under curve (0.971–0.002) and true skill statistics (0.886–0.021).

Table 2.3.1: Estimates of relative contributions of the environmental variables to the species distribution model (SDMs)

	Environmental variables	**Acronym**	**Percent contribution**	**Permutation importance**
1	Euclidean distance to rivers (m)	river_dist	37.3	16.7
2	Mean temperature of wettest quarter	bio_8	25.5	43.1
3	Euclidean distance to rivers (m)	road_dist	19.9	29.1
4	Mean diurnal range	bio_2	5.4	3.5
5	Digital soil map of the world	soil	4.4	2.3
6	Elevation above sea level (m)	alt	3.5	2.2
7	Annual precipitation	bio_12	2.2	1.7
8	Global land cover 2000	glc2000	1.8	1.4

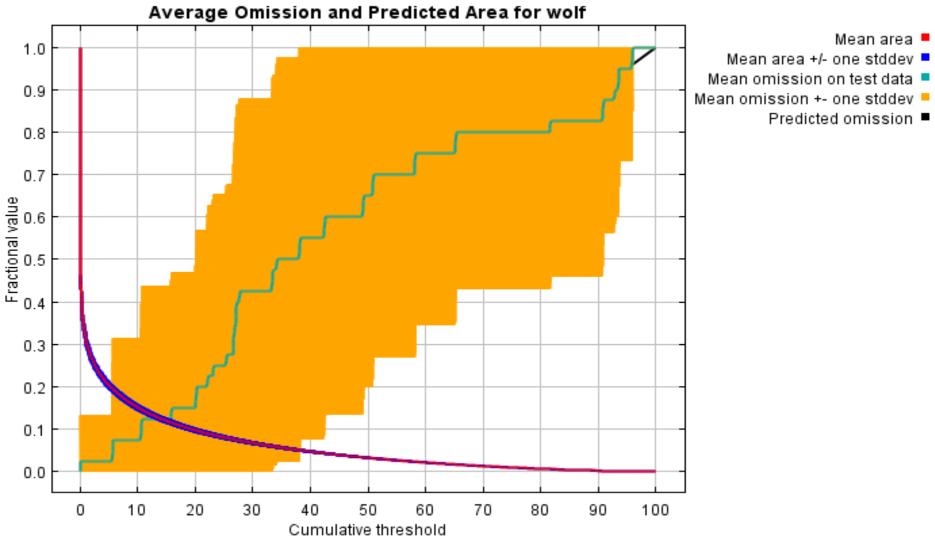


Figure 2.3.5: Averaged omission and predicted area for wolf presence depicted the test omission rate and predicted area as a function of the cumulative threshold, averaged over the replicate runs.

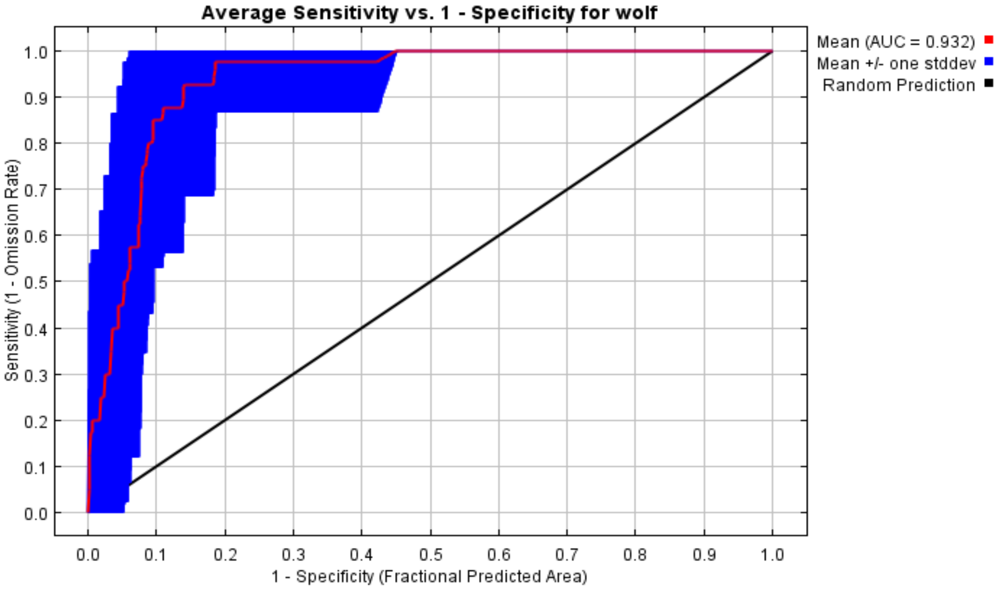


Figure 2.3.6: Maxent output: Analysis of omission/commission showing how well the model accurately predicted wolf presence; the AUC of 0.970 means that the model is an excellent predictor of wolf presence.

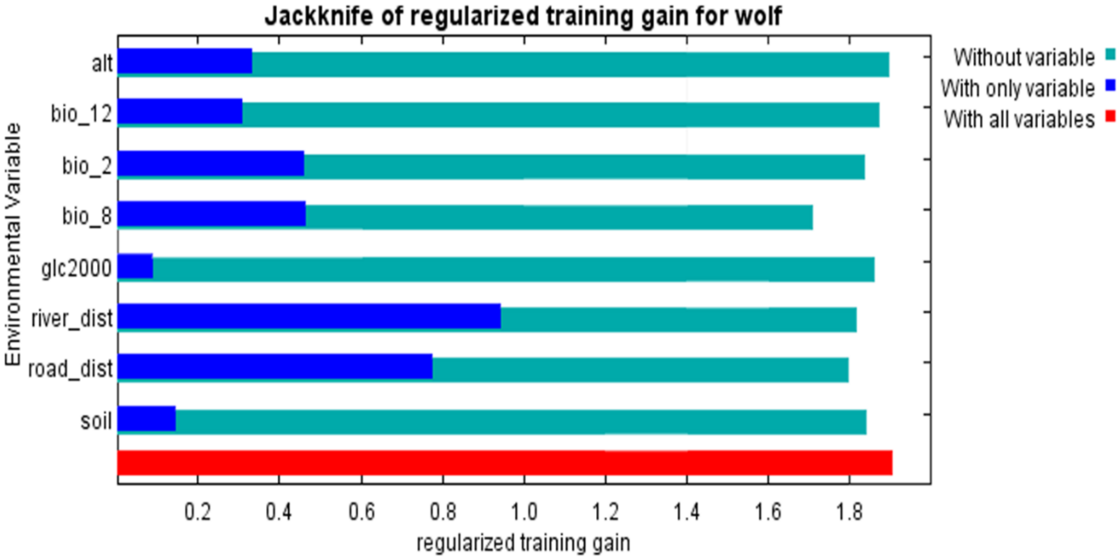


Figure 2.3.7: Jackknife analysis of variables. It shows how important each variable is in explaining wolf presence when used separately (cobalt blue), and how the model is affected when each variable is left out (aqua). Dark blue bars = importance of single variable, light blue bars = loss in model gain when variable is omitted. Red bar = total model gain. Alt = Altitude; bio_12 = annual precipitation; bio_2 = mean diurnal range (mean of monthly); bio_8 = mean temperature of wettest quarter; glc2000 = global land cover 2000; road_dist = distance to roads; river_dist = distance to rivers; soil = soil.

2.3.3 Potential Movement Corridors

The corridor modeling generated estimates of habitat connectivity among scattered wolf populations in northern Pakistan (Figure 2.3.8). Four patches of suitable habitat were identified within the Himalayas, Pamirs, Hindukush, and Karakorum mountain ranges of northern Pakistan. Sub-populations have strong, but unprotected connections, and corridor movement existed between all major areas of wolf habitat. The model identified weak linkages between populations found at lower altitudes with high disturbance rates. Among the PAs, Chitral Gol, Broghil, and Qurumber National Park had wide potential corridors comprising suitable habitat connecting core areas. Furthermore, the analysis revealed appropriate dispersal habitat between Musk Deer and Deosai National Park, and similarly between Qurumber, Broghil, and Khunjerab National Park to allow for wolf dispersal.

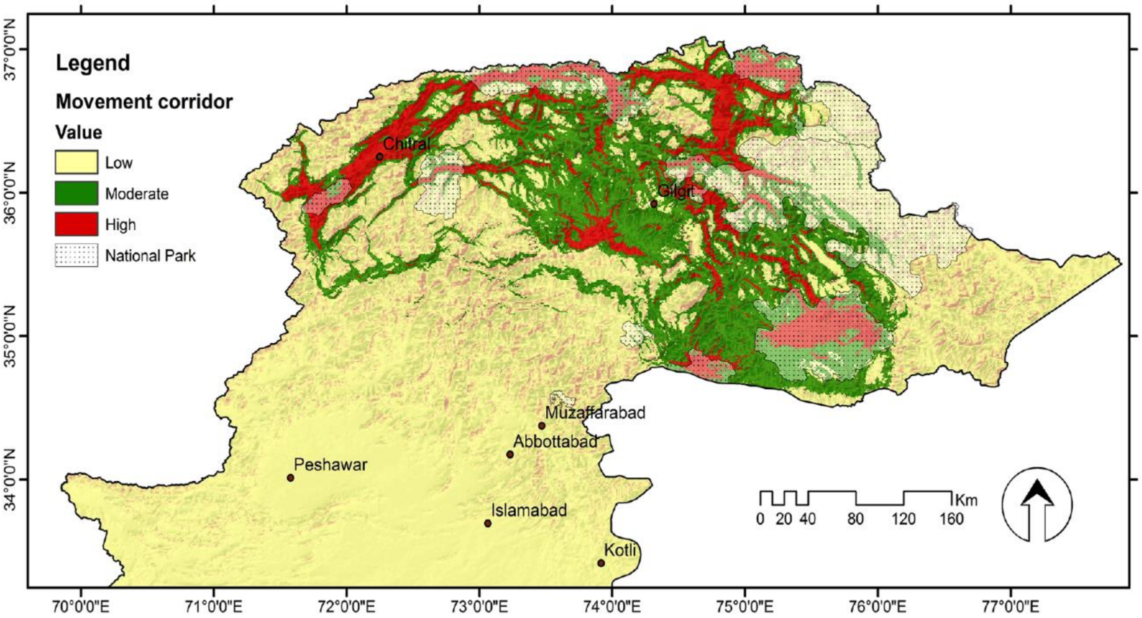


Figure 2.3.8: Potential movement corridors of grey wolf in northern Pakistan. Red areas are strong links while yellowish areas are the weakest. The map also illustrates population connections found in National Parks.

2.4 DISCUSSION

2.4.1 Maxent Model and Movement Corridors of the Grey wolf in Pakistan

This study represents the first large-scale assessment of wolf distribution, habitat suitability, and movement corridors in Pakistan. Our model identified areas with suitable habitat and corridors through which wolves may travel to reach new territories across northern Pakistan. The model showed considerable predictive performance, showing AUC value > 0.9 that may be placed among the highest in published models (Rebelo and Jones 2010; Domíguez-Vega et al. 2012; Smeraldo et al. 2017) and excellent values of the True Skill Statistic, corresponding to a very high predictive capacity (Hanspach et al. 2010; Wogan 2016; Taylor et al. 2017).

Overall, our model found that the most suitable areas for wolves are located in mountainous regions where human disturbance is limited (Ansorge et al. 2003). But it was also clear that wolves are relatively flexible in their use of habitat at the landscape scale. Along altitudinal gradients, wolf presence was recorded ranging from a moist temperate zone in Musk Deer National Park up to the alpine zone in Khunjerab National Park. In general, wolves can potentially live in any area where human tolerance and prey populations are adequate to support viable numbers (Fritts et al. 2003). Wolves show different patterns of habitat selection based on time (year, season, time of day) and areas in which they are observed (Ciucci et al. 2003; Milakovic et al. 2011). Our data supported previous observations that wolves occur in various types of habitat and shows low habitat specificity and high levels of ecological resilience compared with other large carnivores (Carroll et al. 2001; Fritts et al. 2003; Mech and Boitani 2003).

Wolf habitat selection patterns at a fine scale appear to be influenced by complex interactions between habitat attributes and human disturbances (Ciucci et al. 2003; Houle et al. 2010). I found that wolf presence depends on the type of anthropogenic disturbance in the area. Most roads in the study area are unpaved with minimum disturbance and traffic pressure. I observed that wolves avoid main roads and tracks, but follow livestock tracks and small, unpaved roads with low disturbance. We suspect that wolves use roads for traveling, scent-marking, and territorial patrolling, but have also developed cryptic behavioral responses to roads, likely driven by the increased risks associated with human presence (Barja et al. 2004; Zimmermann et al. 2014). Wolves are likely to select secondary gravel or unpaved roads for hunting due to the greater visibility and mobility (Mesler 2015). The presence of road networks may drive wolves toward suitable habitat types. Wolves tolerance to human disturbances increased in suitable habitat types (Lesmerises and Dussault et al. 2013).

The results of our model suggest that distance to road was an important predictor of wolf presence (Whittington et al. 2005; Houle et al. 2010; Gurarie et al. 2011). This finding suggest that wolves may seek to minimize the probability of encountering humans (Whittington et al. 2005; Gurarie et al. 2011). The proximity to rivers was the second most important predictor. Riparian habitats provide wolves with increased opportunities to hunt wild prey and are also important in den selection (Packard 2003; Latham 2009). In our landscape, shepherds prefer moving along streams and established temporary stay areas which may also attract wolves to feed on livestock. Wolves were concentrated in lower areas due to snow-caused aggregation of prey during the winter season. Frozen rivers and lakes are often used by wolves to travel faster (Musiani et al. 1998; Ansorge et al. 2003). Others environmental variables such as altitude, annual precipitation, and land cover were among the variables that contributed least to the SDMs for *C. lupus* in northern Pakistan.

We found that summer huts, temporary settlements, and grazing pastures limited wolf distribution. Wolves exhibited some tolerance towards humans, enabling them to persist within a mosaic of human-altered and naturally occurring habitat. Wherever primary habitat is rare, wolves tend to be dispersed in meadows and rangelands, or in less-natural landscapes such as mixed-use agro-ecosystems (Jędrzejewski et al. 2008; Treves et al. 2011). A similar relationship between the number of inhabitants of settlements and avoidance of close surroundings by wolves was observed in Poland (Theuerkauf et al. 2003). Wolf populations in closer contact with human-active areas indicate tolerance to human activities (Ahmadi et al. 2013).

Resistance modeling indicated the presence of habitat corridors for wolves in northern Pakistan. These corridors could link potential habitats and movement corridors between PAs. Habitat connectivity is not uniform in the Himalayas and population connectivity between the Pamirs and Himalayas range is very weak, based on our corridor modeling analysis. Wolf populations in the Hindu Kush appear to be well connected with the population of the Pamirs and Karakoram, which is also enhanced by the establishment of PAs, including the Broghil, Qurumber, and Khunjerab National

Parks. PAs have become islands of habitat within a mosaic of agriculture and development, and although at a slower rate than non-PAs, anthropogenic activities persist even within the boundaries of PAs (Gaveau et al. 2009). Chitral, Broghil, and Qurumber were identified as areas of likely wolf activity based on habitat quality and connectivity to other patches of high-quality habitat. The wolf population in Deosai appears to be connected with the population of Central Karakorum National Park, but only weakly connected with another potential habitat. The wolf populations in Musk Deer and Khanbari study sites appear to be isolated.

Highly suitable habitat was also detected outside PAs with minimum levels of anthropogenic activities. For large carnivores, sub-optimal habitats might serve as corridors linking habitats necessary for survival and reproduction, and also prevent inbreeding depression (Mech 2006). Grey wolves are able to travel through habitats considered poor in the search for an area to form a new pack (Merrill and Mech 2000). “Pioneering” wolves have been known to disperse over large distances and settle in new habitats far from the nearest source population (Wabakken et al. 2001; Mech and Boitani 2003). The populations of the Hindu Kush, Pamirs, and Karakorums appear to be connected through movement corridors, but these need to be protected to facilitate safe use by dispersing wolves.

2.4.2 Model Constraints

There are two main limitations to our model. First, I did not have prey availability estimates. Second, data was only collected during winter and wolf habitat selection patterns may vary between seasons. The dataset was influenced by the species’ patchy distribution, its rarity throughout the landscape, its seasonal surface occurrence, and its location (when active) on steep and rocky (often impassable) terrain (Mooney 2010). Previous studies showed that wolf distribution at the landscape scale was influenced primarily by prey availability and human infrastructure (Potvin et al. 2005). Assuming prey biomass varies with habitat type, studies on carnivores demonstrate the potential for deriving accurate habitat and connectivity models (Wikramanayake et al. 2004; Linkie et al. 2006).

2.4.3 Application of Habitat Modeling

The model predicted habitat suitability reasonably well with respect to species presence. Among the four major patches of high-quality habitat identified, one is protected, one is partially protected, and the rest are weakly protected. The most suitable habitat in the Hindu Kush range (Khanbari) lacks PAs and has poor connections with other populations. The eastern part of the Pamir range and southern Himalayas are partially connected with the western Karakorum and northern Himalayan populations, respectively. Our study provides a better idea of where wolves may disperse to in case numbers increase in the future, and helps identify priority areas for community engagement, management zones, and proactive planning (Houts 2003).

Habitat models developed in the current study will support wolf conservation in three ways. First, habitat maps provide a tool to identify suitable habitat and movement corridors and provide a guide map for investing limited conservation resources. Second, wildlife managers prioritize the establishment of more PAs covering suitable habitat and movement corridors to extend the PA network for the long-term survival of wolf populations in Pakistan. Third, it is a challenging issue in northern Pakistan to manage protected areas in stringent categories due to people's heavy dependence on natural resources.

APPENDIX 2.1: CAMERA TRAP STATION SHEET

		CAMERA TRAP STATION SHEET	Set by:					
STATION ID		LURE TYPE	☐skunk + fish oil	☐castor + fish oil	☐fish oil	☐none		
WATERSHED		HABITAT	☐scrub	☐forest	☐pasture	☐barren	☐agric.	
e.g. MISGAR-WS1		(in immediate surroundings)						
N	☐☐.☐☐☐☐☐☐	TERRAIN	☐ridge	☐cliff base	☐draw	☐valley	☐saddle	☐plateau
E	☐☐.☐☐☐☐☐☐	SUBSTRATE	☐sand	☐soil	☐rock/gravel	☐snow	☐vegetation	
ELEVATION	meters	Station potential	☐good	☐medium	☐poor			
CAMERA ID								

STATION VISIT⇩	**Sign in buffer area ⇨**					
	DATE	**TIME**	**SIGN AT STATION**	**SD CARD**	**Camera Operational**	**NR NEW PHOTOS**
SETUP					☐ YES ☐ NO	
RE-BAITING					☐ YES ☐ NO	

LANDSCAPE ECOLOGY OF GREY WOLF *(CANIS LUPUS)* IN NORTHERN PAKISTAN

CHAPTER 3

Distribution Status and Occupancy of the Grey wolf *(Canis lupus)* in Northern Pakistan

3 Distribution Status and Occupancy of the Grey wolf *(Canis lupus)* in Northern Pakistan

ABSTRACT

Grey wolf (*Canis lupus*) is an elusive carnivore and its population faces survival threats caused by many socio-ecological factors. Its conservation is a challenging issue because of large home range and territorial behavior and low population density. Occupancy based modeling provides a framework for monitoring of population structure and territorial range. I used camera trap data of 800 locations installed during the period 2009–2017, following a 5x5 grid, and each station separated by a minimum distance of 1 km. I tested two survey covariates on detection, and eight site covariates on occupancy ‘Presence’ software (version 12.7_170921, Hines 2006). Presence uses likelihood-based methods on detection non-detection data to estimate probability of sites being occupied or used by a species of interest. Single-season single-species occupancy model yielded mean occupancy of wolf across the sampled sites to be 0.43 (SE = ±0.09). The effect of efforts and plateau was positive whereas cliff base had negative effect on probability of detecting wolf on cameras. In the top three models, three site covariates (distance to road, distance to settlements and available habitat) were retained. An understanding of wolf behavior indicates that the probability of encountering wolves on camera traps, given presence could be a function of the micro-habitat where the camera trap was installed.. The outcomes of this study in the form of occupancy estimates, provide a baseline to assist in population monitoring and to prioritize and gauge conservation efforts.

Keywords: *Canis lupus, occupancy, variables, Karakoram, Himalaya, Hindukush*

3.1 INTRODUCTION

Enjoying the highest trophic level, large carnivores regulate the biological composition and structure of ecosystem. Large carnivore conservation has become a global priority because of their functionally important role as an umbrella species (Wang and Macdonald 2009). Abundance and distribution monitoring of wild animals is fundamental in conservation management of carnivore populations (Wilson and Delahay 2001). The study of pattern and rate of population dispersal is challenging to develop sustainable conservation strategies and to significantly handle the effect that large carnivores exert on their key prey, including domestic and wild ungulates (Luikart et al. 2010).

Across the geographic range, studies are focused on determining carnivore occupancy or distribution abundance and relative abundance (Patterson et al. 2004; Gompper et al. 2006; Long et al. 2007; Johnson et al. 2009). The significance of studying the abundance of animals over a wide spatial range to suggest a better knowledge of interaction between human disturbance, natural habitat and animal species is largely recognized (Baum et al. 2003; Karanth et al. 2004). The essential of monitoring population of large carnivores and to for monitoring carnivore and to know the population status is highly acknowledged due to increasing concerns on their distribution and status across the range (Woodroffe 2001). Population estimation is a key component in wildlife ecology, conservation and management (Alibhai et al. 2008). Prioritization and allocation of resources for evaluation of the success of conservation programs can best be done by monitoring populations (Sharma et al. 2005). Estimation of wild carnivore abundance is important for conservation of wildlife habitats (Latham 2014). Knowledge of animal abundance, relative abundance and variables influencing abundance are important in many ecological research studies (e.g., habitat, demography), management (harvest quotas, pest management), and conservation planning (Stanley et al. 2005).

The grey wolf has adapted and flourished in diverse habitat ranging from arctic tundra to Arabian deserts (Mech and Boitani 2003). Wolf have been classified as least concern globally and is classified as endangered in Pakistan and known to occupy the southern region of Kashmir and Himalayas respectively (Lydekker 1907; Sheikh and Molur 2004). By investigating the category of threats by IUCN, Ripple et al. (2016) reported that 59% largest carnivores and 60% of the largest ungulates were categorized as facing threats of extinction worldwide. Promoting co-existence and tolerance to large carnivores is a vital societal issue, as they are among the most vulnerable to extinct and become locally extinct first (Ripple et al. 2014). Carnivore species are particularly sensitive to disturbance, habitat exploitation and fragmentation by human (Ginsberg 2001; Woodroffe 2001).

Globally, over the last century, wolf range has shrunk by 33% and Pakistan wolf populations have experienced both numerical declines and about 80–90% range contraction (Mech and Boitani 2003; Sheikh and Malur 2004). The wolves are persecuted by shooting, poisoning and smoking den sites in retaliation of depredation on livestock (Roberts 1997; Jhala 2003) while the habitat destruction occurs due to increasing human population, land conversion, urbanization, overgrazing, prey depletion, poaching and competition on limited resources (Jhala 2003; Irshad 2010). During the winter season, because of snowfall, wolves prefer to move downward along the altitude (Mech 1970), leading to interactions with humans that in turn increase their chances of being killed (Lovari et al. 2007). After centuries of persecution, wolves have survived mainly in wild, remote, and often isolated areas (Promberger et al. 2000).

The wolf symbolizes a keystone species which can regulate the ecosystem; Mech and Boitani (2003) reported that “wolves are probably the most important wild predator”. “wolves are perhaps the single most important predators of large mammals”. Limited research (Dar et al. 2009; Anwar and Nadeem 2011; Abbas et al. 2013; Saad et al. 2015; Ahmad et al. 2016; Ali et al. 2016; Din et al. 2016; Kabir et al. 2017) has been conducted so far on the ecology of wolves in Pakistan. Inadequate scientific data has been a major concern to formulate strong conservation initiatives to save the survival of the sparsely distributed population of wolves in Pakistan. Estimation of wolf occupancy and space use is important for species conservation specially to ensure its long-term survival. This study aimed to provide information about the status of wolf populations that could be used for their conservation.

Conservation of large carnivores is worldwide priority, but their population monitoring remains challenging task because of their large home range, low density and elusive in nature (Gittleman et al. 2001; Thompson 2004). Evaluating the successful conservation and management program conducted on focal species depends on reliable estimation of population size (Cubaynes et al. 2009). Carnivores of terrestrial landscape have low densities and large home range, therefore reflecting in estimating their status and abundance reliably (Long et al. 2008). Population monitoring of elusive and wide-ranging carnivore species like wolves, which are widely distributed in forest areas with low density is hard and often expensive (Galaverni et al. 2012). Irrespective of the used monitoring and study techniques, financial constraints often hinder reliable estimates of population trends, particularly at wide spatial level (Andelman and Fagan 2000). Commonly used study techniques for carnivore species includes model of demographic population or mark recapture-based estimates of abundance by tagging the animals, but in absence of identifiable markings signs on their body, can only be achieved through invasive methods or genetics that are challenging and expensive (Gompper et al. 2006).

Developing effectual and practical study techniques for carnivores across their distribution range is growing urgency with respect to declining population and growing threats (Inskip and Zimmermann 2009; Treves and Karanth 2003). Although carnivore species are innately nocturnal and elusive, an inability to locate them is also among the major issues of survey biasness (Karanth and Nichols 2002; Linkie et al. 2007). On such elusive species, recently non-invasive survey techniques developed to strengthen the advancement in ecological studies (Mackay 2008; Long et al. 2008). Large carnivores such as wolves are usually wide-ranging habitat generalists, scarce, nocturnal and elusive, it is often to make their abundance by using traditional survey methods using direct sighting record information (Sharp et al. 2001; Marucco et al. 2011).

Presence/absence data is usually low-cost, and their use are prompting with latest emerging and efficient technologies including camera trapping and eDNA (Schmidt et al. 2013; Burton et al. 2015). The non-invasive survey methods like camera trapping and genetic have recently been deployed to monitor the population of large carnivore species with different success rates (Marucco et al. 2009; Waits and Paetkau 2005). Camera trapping is a latest efficient tool for studying wildlife species, with rapid increase in research studies using this technique in past decade (Kays and Slauson 2008; O’Connell et al. 2011). Camera traps have been effectively used in many research studies on large carnivores worldwide, on various topics including distribution, occurrence, population structure, abundance, behavior and habitat use (Wilson and Delahay 2001; Jackson et al. 2006; Karanth et al. 2006). Camera trapping is considered to estimate the relative abundance of study species, making assumption that high detection rate of photo capture is linked with more abundance of animals (Carbone et al. 2001; Moruzzi et al. 2002) are subjected to unknown factors that limit our ability to use these methods in non-homogenous habitats. Camera trapping studies has effectual potential which may use to collect information on nocturnal species (Kelly and Holub 2008). Wildlife authorities are using camera trapping as a monitoring tool, and to determine the presence record of medium and large sized mammals and to assess the pattern of species, abundance across different management zones, which are measurable by using camera trapping (Jenks et al. 2011).

Remote camera trapping tools provide reliable occupancy estimates and count statistics (number of areas surveyed/sampled in relation to the area occupied) with respect to size of population where the identification of individuals animals is not feasible (Thompson 2004). Remote camera trapping is also an inexpensive tool of studying species abundance and richness (Manley and Van Horne 2004; Larrucea et al. 2007).

The camera trapping technique has been employed to detect absence/presence (Gompper et al. 2006; Moruzzi et al. 2002; Rosellini et al. 2008) and population status of large carnivores (Karanth et al. 2006), but this technique has rarely been used to study wolf species (Berzi and Groff 2002; Palumbo et al. 2010). Camera trapping based obtained small datasets can provide an important and reliable species occurrence relative abundance, information of species richness and also provide information about species activity pattern (O’ Brien et al. 2003; Kelly and Holub 2008; O’ Brien et al. 2010; Suntaro et al. 2013). For unmarked species lacking distinctive stripes, spots and other markings camera trapping provide reliable data on species presence/absence, that can be used in monitoring of occupancy trends, species distribution and relative abundance (Steenweg et al. 2016).

Recently, the methods relevant to population analysis (Burnham and Anderson 2002; Williams et al. 2002) have great advancement and permitting wildlife biologist to tackle with both statistical and biological complexities and challenging involved in population sampling of free-ranging, low density, and elusive carnivore species (Thompson 2004). The indices are often used for unmarked species that cannot be distinguished separately from photographs, and it varies over species, time and space (O’ Brien et al. 2003; O’ Brien 2011). Moreover, ecological research studies relating to population dynamics, prey-predator interaction, mortality to disease, contributed to developing reliable population estimates of animals (Wilson and Delahay 2001). Camera trapping studies are imperfect due to less species specificity, where individual identification is often tough or impossible (Oliveira-Santos et al. 2009), particularly in wolves (Genovesi 2002). In case of unmarked species like grizzly bear (*Ursus arctos horribilis*) and other species such as the wolf are not identifiable individually, so occupancy is permitting the trends monitoring through the percentage of occupied parts (Stanley and Royle 2005). In 2 and 5 IUCN standards for evaluating the status of threatened species use occupancy metrics such as “area of occupancy “and “extent of occurrence” (Mace et al. 2008).

Occupancy based modeling gives an outline for the monitoring of population and distribution of territorial, low-density and elusive carnivore species. Occupancy modeling can also be applied in making robust inferences for many variables including geographic distribution, population estimates, natural resources selection, species interactions and meta-population dynamics (MacKenzie et al. 2006). Occupancy, is often used by means of substitute of abundance, is calculated through the data collection on detection/non-detection of species and permitting the collection of data over wide spatial scale but relatively with low cost (Blanc et al. 2014). Site occupancy-based surveys have been conducted worldwide, over large areas extending from small habitat patches to the entire area (Karanth et al. 2011). Occupancy modelling requires uniformed sample sizes to achieve estimation of area occupied by a study species (MacKenzie et al. 2002; 2006). To guesstimate the likelihood of finding, at site level, some form of duplication is also required (MacKenzie et al. 2002; 2006). The optimal numeral of replicates and site depend on approach of sampling and detectability and occupancy of the focal species (MacKenzie and Royle 2005). It requires surveying sites multiples either temporally or spatially within in a very short period adequate to reduce the chances of variation in occupancy throughout the sampling duration (Hines et al. 2010; MacKenzie et al. 2002, 2006).

Occupancy is considered as useful index to population status (Royle et al. 2012). Using data from various latest study techniques and incorporating their probability of detections occupancy-based modeling predict an important framework for abundance estimation and species distribution (MacKenzie et al. 2006). Generally, definition of occupancy is the percentage of areas occupied by the species (Gaston et al. 2000; MacKenzie et al. 2002), but the criteria differs along spatial and temporal sampling units (Royle and Dorazio 2008; Noon et al. 2012). Miller et al. (2011, 2013) described multi-state framework of occupancy in the form extension (Royle and Link 2006; Nichols et al. 2008), permits for combination of the data obtained from multiples sources, which may have some observation like false positive detection. Interaction between occupancy and abundance (OA) is a basic and preliminary ecological procedure and area of research, site occupancy-based model is progressively used in understanding of ecological interactions and to monitor population trends (Steenweg et al. 2018).

Occupancy modelling incorporate the presence or detection process, improving for this possible biasness (MacKenzie et al. 2006). They entail data collection on detection and non-detection status, often obtained through less effort and cost than demographic data (Thorn et al. 2010). To improve the imperfect detection (false absence) and spatial and temporal changes in probability of detection information theoretic modelling is specifically used (MacKenzie et al. 2002, 2006). This generate perfect maximum probability of estimates from many variables linked with large variety of study/research, conservation and management applications (MacKenzie et al. 2006). The categorical covariates can also be modeled to infer interaction among observation pattern and fundamental procedures and thus extrapolate the patterns in areas which have not been surveyed (MacKenzie et al. 2006). Occupancy model provide data on abundance estimates (Royle and Nichols 2003) when an area is confirmed, occupied by carnivore species, then it is predicted that one individual of species is present on that site at least (MacKenzie et al. 2005). This has led to the use of occupancy models as a source of abundance (MacKenzie and Nichols 2004; Sólymos et al. 2012).

Due to the inaccessibility or impossibility of conducting exhaustive population censuses of free-ranging wildlife species, surveying techniques aim at yielding a count of a representation of the total population (Blanc et al. 2014). The landscape/occupancy models for such species are usually derive from a small dataset of some individuals, or larger categorical data (absence/presence), instead of using continues (population status) data (Fuller et al. 2001; Zielinski et al. 2005). The utilization of presence/absence data for long term monitoring of species and habitat selection studies has amplified manifolds recently (Fleishman et al. 2001; MacKenzie et al. 2002). As a consequence, experts in some ways have to guesstimate the likelihood that an individual presence and is actually detected during sampling and, consequently included in a count (Williams et al. 2002). Recent advancements in non-invasive survey techniques (Long et al. 2008) and occupancy modeling, which is likelihood-based (MacKenzie et al. 2002; MacKenzie and Baily 2004; MacKenzie et al. 2006), now allow both concurrent estimation of detectability and site occupancy and detection/non-detection collection of data from large, free-ranging carnivore species (Royle and Nichols 2003). Abundance is usually estimated by developing framework of assumption then building statistical models that links parameters and datasets, so that an estimate can be made (Stanley et al. 2005).

Detectability of the animals is a significant variable in species monitoring studies. Some species could be detected only at those sites where they exist (Mackenzie et al. 2002). The detection/non-detection information are also useful in distance sampling research studies (Buckland et al. 2000). The considering of imperfect detection rate is significantly important in research studies on elusive and rare wildlife species (Thompson 2004) including carnivore species (Long et al. 2010). Furthermore, within studies when probability of detection is estimated, they usually vary over space, time and species (Yoccoz et al. 2001; Pollock et al. 2002). There is possibility of spatial replication, but it needs the similar effort by applying independent replicates such as randomly selected transect, with replacement (Hines et al. 2010). Furthermore, recently established analytical approaches also allow estimation of probability of detection from repeatedly, replicates with spatially autocorrelated, that are not selected randomly (Hines et al. 2010). Under these circumstances, variation in indices could not be explicitly credited to real variations in relative abundance, due to difference in detection likelihood (Pollock et al. 2002; Archaux et al. 2012).

Some species can be detected only in territories where they are existing (MacKenzie et al. 2002). The issues of detectability must be considered to make precise inferences as the species is not detected does not suggest the genuine absence (MacKenzie et al. 2004). The species detection may be affected by biological, physical and anthropogenic factors which include seasonality, weather, topography, biological rhythms and sampling methods (O’Connell 2006). When the probability of detection is not known, counts are generally assumed as indices. Several research studies suggested, indices are vastly linked with population abundance (Hochachka and Dhondt 2000; Wilson and Delahay 2001; Romain et al. 2004), but this also received reproach

(Anderson 2001). The indices are infrequently used for interpretation about the true population size (but before doing so they need calibration) under certain circumstances they can also generate changes in density or abundance (Williams et al. 2002; O' Brien 2011).

There is lack of information on population status of wolves in Pakistan. My objective was to assay the population abundance of grey wolf in Northern Pakistan. This study mainly focused to determine the relative wolf abundance using occupancy-abundance model. Outcomes will help to develop the baseline to assist in population monitoring and to well identify the issues that influence the distribution of wolf. I hypothesize that occupancy-abundance of wolf model would be positively influenced by distance to human settlements, rivers, and road. I also suspect that elevation, slope and Normalized Difference Vegetation Index (NDVI) could negatively influence wolf occupancy-abundance model. These hypotheses help us set up parameterization of models with heterogeneous occupancy probability across space. An understanding of wolf behavior indicates that the probability of encountering wolves on camera traps, given presence could have been a function of the micro-habitat where the camera trap was installed, the altitude and terrain ruggedness. I use these covariates to have potentially influenced the probability of detecting wolves on camera traps.

3.2 MATERIALS AND METHODS

3.2.1 Study Area

The present study was conducted to estimate the occupancy of grey wolf in Northern Pakistan. The study area is described in Chapter 1 (Figure 3.2.1).

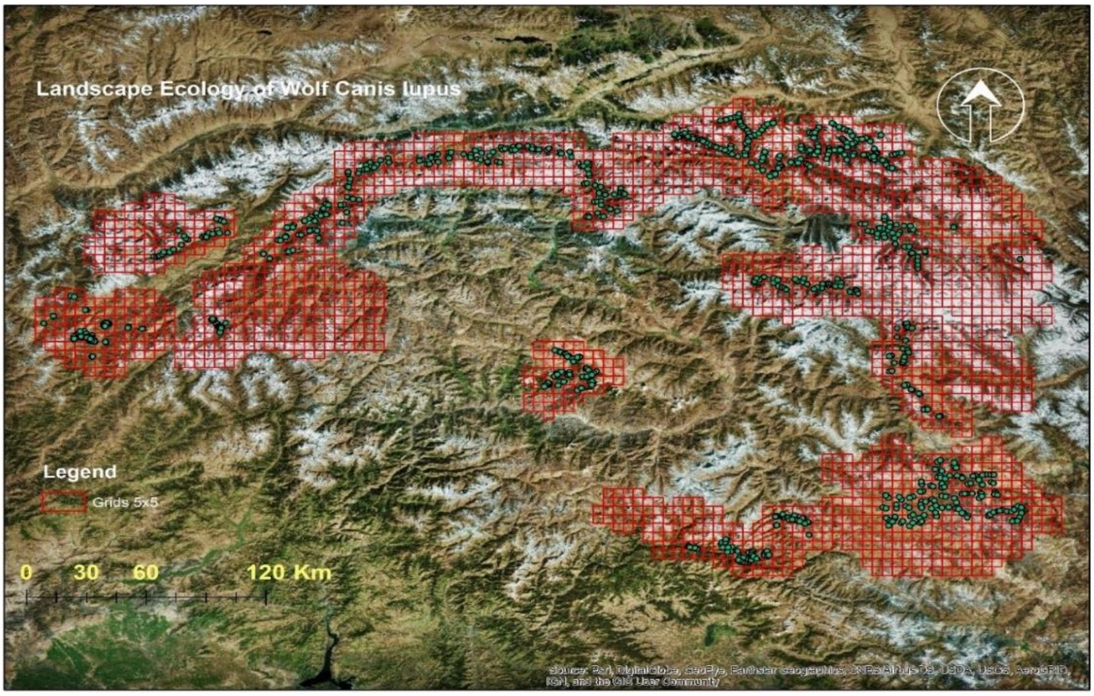


Figure 3.2.1: Map of study area showing areas (red grid) and camera locations (green dots) in Northern Pakistan

3.2.2 Data Collection

3.2.2.1 Camera Trapping

In this study we defined the sampling units as 5 × 5 km^2 cells. Camera traps were installed at 800 locations during the period 2009–2017 and were separated by a horizontal buffer of at least 1 km (Table 1). Non-invasive camera trapping techniques was used, and camera trap locations were identified based on landscapes characteristics—ridges, cliff bases, draw preferred by carnivores and the presence of carnivore signs (Gompper et al. 2006; Jackson et al. 2009; Jackson et al. 2005; Wang and Macdonald 2009; Bischof et al. 2012; Bischof et al. 2013; Ahmad et al. 2016; Kabir et al. 2017). Cameras were located in places identified by expert survey teams as having a high probability of detecting study species—these were generally in valleys along animal trails and small water streams. To confirm the that the installed camera was operational or not pictures of the survey teams were taken during the process of installation and removal of camera traps. A single motion-triggered digital camera with infrared flash (HC500/PC900, Reconyx, Holmen, WI, USA) was deployed at each location on a steel pole (50–60 cm) driven into the ground.

Cameras locations in the different grids are presented on a map in Figure 3.3.1. The location of cameras and their elevation were recorded at each camera trap station with a handheld GPS device. Topographic features in the vicinity of each camera location were categorized into cliff base, draw, ridge and plateau and noted on developed camera trapping data sheets. Camera traps were set to take consecutive images (1-s picture interval) when triggered and were typically kept active at a given location for 10–40 days in different study sites and locations. We developed camera stations in front of each camera and baited them with fish oil to attract the study species. The pictures of bait were taken at the time of camera installation and removal. These cameras remained operational for about 24,000 trap nights. Trap nights per camera were counted from installed-date to taken-down date. The research and wildlife expert team, familiar with the mammalian fauna of the study area, identified the camera trap photographs of species and identified all the photos of wolves. There were some photos which could not be identified due to poor quality were not included in the occupancy analysis.

3.2.2.2 Analytical Approach

I used ‘Presence’ (version 12.7_170921, Hines 2006) software to calculate the occupancy of a species across an area. Presence uses likelihood-based methods on detection non-detection data to guesstimate probability of sites being occupied or used by a species of interest. Due to its replicability, reliability and relatively low cost for implementation, methods of site-occupancy are being used increasingly for monitoring status of wildlife (MacKenzie et al. 2004; MacKenzie et al. 2006). Occupancy, occasionally used as a surrogate for abundance, is estimated from detection/non-detection data, allows gathering of information at a large spatial scale at relatively low costs (Blanc et al. 2014). Since the analysis corrects for flawed detection by estimating detection probability (p), the estimated probability of a site being occupied or used (ψ) is considered to be replicable. Date-wise trap history for each camera station was developed in an excel sheet. Encounter of a species during a 24-hour period was recorded as 1, and non-encounter was recorded as a 0. This helped develop a detection and non-detection trap history for wolves. Occupancy methods allow insertion of covariates that are expected to affect the probability of detecting a species or that of their using a particular site. I tested the effect of different site and survey covariates in the occupancy models to reduce variance in parameter estimates and understand the heterogeneity in detectability and occupancy (MacKenzie et al. 2006).

3.2.2.3 Survey and Site Covariates

Wolves can adapt to a wide range of habitats, making it difficult to identify specific factors that may affect species presence on a site (Mech and Boitani 2010). Covariates were selected based on their applicability across our study area, their relevant predictive power, and their suspected biological significance. I considered the potential effects of roads as a covariate given the fact that they represented accessibility to humans and wolves alike, mainly because most roads in the study area are unpaved with minimum disturbance and traffic pressure, sighting record information and field observation revealed that wolf use these unpaved roads for movement in valleys. In our landscape shepherds prefer moving along roads and streams and established temporary stay which may also attracts wolves to feed on livestock. NDVI is widely used in ecological research and management as a representative of herbivore biomass and feed quality (Pettorelli et al. 2011). Since we don't have information about wild ungulates, such as Himalayan ibex, markhor, blue sheep, etc., the main prey of wolves in northern Pakistan. NDVI value reflects the availability of habitats that can be used by wild herbivores for grazing and their presence may regulate the distribution of wolf. We expected that wolf presence would have been affected by rivers and streams as this area may attract ungulates and other prey species. A site near human settlements and area would have a lower probability of wolf detection in the study area. Being widely known as livestock predator, north Pakistan, we anticipated wolf presence to be affected as a function of distance from human settlements. I used in total eight site covariates such as average NDVI, average slope, average altitude, distance from roads, distance from rivers, distance from settlements, average ruggedness, available habitat (available area-area above 5,000 m), that might outline the wolf dispersal or occupancy (Table 3.2.1). For the plotted grids of 5 × 5 over the study area, i averaged site covariate values in ArcGIS 10.3. by using spatial analyst tool.

I considered parameters that may affect the probability of wolf capture on a particular camera trap as survey covariates. Both the effort the number of days the camera traps remained active and topography were considered as survey covariates, thus affecting the probability of detecting wolf, given presence. We expected to detect wolves in lowland areas such as plateaus, marginal area of valleys near to cliff base which provided enough cover and shelter. Wolves are known to prefer rolling terrain over highly rugged landscapes, which is why i tested the effect of overall slope as a covariate affecting the probability of a wolf using a particular site. The survey covariates, *i.e.* efforts (number of traps nights), and topography (cliff base, draw, ridge and plateau) were used to find what affects the detection probability or occupancy. Wolves do not typically live in altitudes beyond 5,000 m in Pakistan as there is no vegetation, and hence prey at that elevation. Available habitat was calculated by excluding areas higher than 5,000 m in altitude.

Table 3.2.1: Site and Survey Covariates used in Wolf Occupancy Analysis

Abbreviation	**Name**	**Description**
Occupancy covariates		
Alt	Altitude	Numeric
Slop	Slope	Numeric
NDVI	Normalized Difference Vegetation Index	Numeric
Rgdnss	Ruggedness	Numeric
DistRoad	Distance to Roads	Numeric
DistRiver	Distance to Rivers	Numeric
DistSet	Distance to Settlements	Numeric
AvaiHabitat	Available Habitat	
Survey covariates		
Effort	Efforts (number of traps nights)	Numeric
Topography	Topography (cliff base, draw, ridge and plateau)	Categorical

3.2.2.4 Single-Season Occupancy Model

The occupancy model consists of two parameters *i.e.* ψ and "p". Psi is defined as the likelihood, the given area is occupied or used (depending on whether the closure assumption was met for the study period) by a species and "p" is the probability of detecting the species in a sampling unit, given presence (MacKenzie et al. 2002, 2006). I first constructed a general occupancy model that included all possible covariates. A candidate model set was then created with all plausible logical covariate combinations.

The model that best described the variation in probability of occupancy and detection was selected using the minimum Akaike Information Criteria (AIC) (Akaike 1985).

3.2.2.5 Predicting Occupancy of the Entire Study Area

The model has the least AIC value is considered to best explain the variation in detection and site use probabilities. Probability of site use was estimated at the scale of 5x5 km^2 grid representing a sampling unit and using the logistic regression equation for the top model, estimated for sites that were not sampled using raster calculator in ArcGIS 10.3.

3.3 RESULTS

The study area was divided into 1,400 grids of 5 × 5 km^2. Camera trapping was conducted in six protected areas and seven non-protected areas. Two hundred and ninety-nine grids were covered with camera traps and total of 800 camera stations were established. Wolves were photo-captured at 51 different locations across the study areas (Figure 3.3.1).

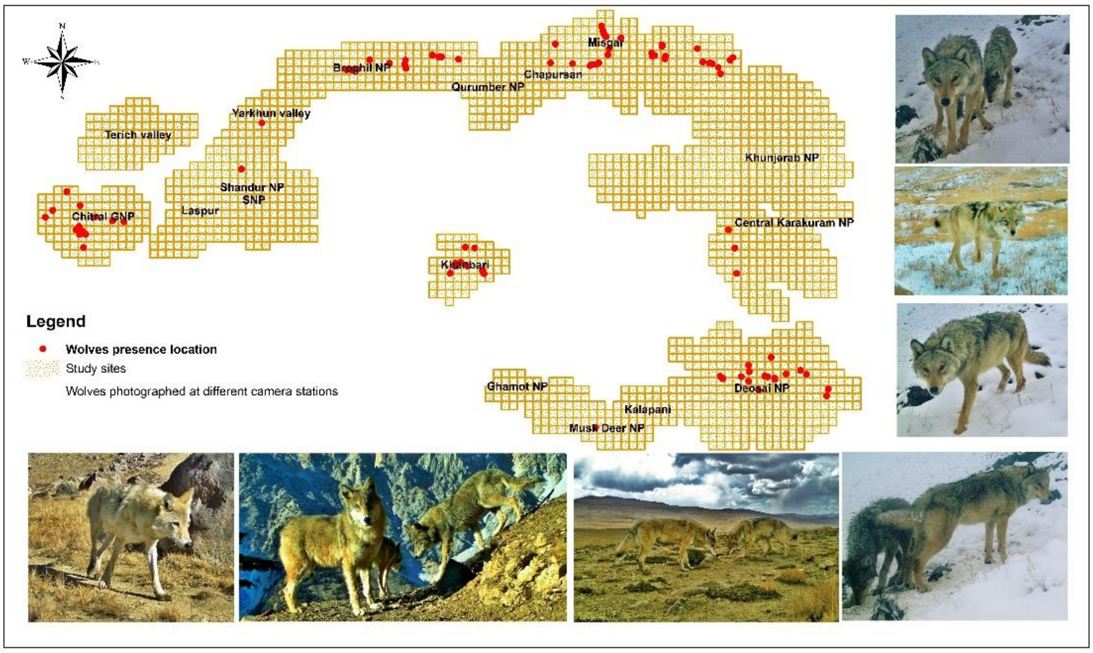


Figure 3.3.1: Locations of wolves' photo-captures in northern Pakistan

Wolves were recorded from inside and outside of the protected areas (PAs). Most of the presence records were obtained from national parks. Presence records along altitudinal gradients range from 3,000 m (Musk Deer National Park) to 4,700 m (Khunjerab National Park). PAs with the highest frequency of presence were Deosai National Park (Himalayan Range), Chitral Gol National Park (Hindu Kush Range), Khunjerab (Karakorum Range) and Broghil National Park (Pamir chain). Outside the PAs, more presence records were obtained from Khanbari Valley in Gilgit-Baltistan. There was no record of presence from Terich, Astor, Misgar, Chipurson, Shimshal and Hisper-Hooper. New record of wolf was obtained from Neelum valley information will be shared with IUCN to revise the distribution map of wolves. Overall, wolf detection was low, suggesting a thin and fragmented population. Studies confirmed the metapopulation of grey wolf in northern Pakistan. We set 800 camera station in northern Pakistan but only ten percent of cameras recorded both wolves and snow leopards.

3.3.1 Single-Season, Single-Species Occupancy

A single-season, single-species occupancy model was conducted for wolf occupancy estimates using the candidate model set (Table 3.3.1).

Table 3.3.1: Summary of top output models run to estimate the effect of different covariates on detection probability (P) and occupancy (Psi) of wolves using PRESENCE (version 12.7_170921) software in northern Pakistan

Model	**AIC**	**deltaAIC**	**AIC wgt**	**Model likelihood**	**-2*LogLike**
"psi(1,DistSettlements,DistRoad,AvlblHabitat),p(1,Effort,Cliffbase,Pleatau)"	288.8	0.0	0.24	1.00	272.79
"psi(1,DistSetlmnts,DistRoad,MeanRgdnss,AvlablHabitat),p(1,Effort,Cliffbase,Pleatau)"	290.7	1.9	0.09	0.39	272.69
"psi(1,DistRiver,DistSetlmnts,DidtRoad,AvlablHabitat,),p(1,Effort,Cliffbase,Pleatau)"	290.8	2.0	0.09	0.37	272.77
"psi(1,DistSetlmnts,DistRoad,MeanRgdnss,,AvlblHabitat,MeanNDVI),p(1,Effort,Cliffbase,Pleatau)"	290.9	2.2	0.08	0.34	270.94

3.3.2 Detection Probability and Occupancy Estimates of Wolves

- The naive occupancy estimate of wolf in the area was 0.13.
- Detection probability for wolves varied across different types of terrain.
- The effect of efforts and plateau were positive whereas cliff base had a negative effect on the probability of detecting wolves on cameras (Table 3.3.2).
- The mean occupancy of wolf across the sampled sites was 0.43 (SE = ±0.09).

3.3.3 Influence of Site Covariates on Occupancy Estimates

- In the top three models, three site covariates including average distance to road, average distance to settlements and available habitat were found collectively affecting the probability of occupancy of wolf (Table: 3.3.3).
- An increase in mean distance to settlements and available habitat increased the occupancy estimates.
- The occupancy reduced with an increase in the average distance to roads.

Table 3.3.2: Effect of survey covariate on detection probability of wolves in northern Pakistan

	Estimate	Std. Error
psi	-0.744283	0.259010
p	-1.238492	0.275867
p.Effort	1.464602	0.388411
p.Cliff_Base	-1.300235	0.580517
p.Plateau	1.636185	0.715947

Table 3.3.3: Effect of site covariate on occupancy of wolves in northern Pakistan

	Estimate	Std. Error
psi	-0.090187	0.476194
psi.Dist_Settlements	3.050208	0.817968
psi.Dist_Road	-1.166542	0.430155
psi.Available_Habitat	1.366171	0.725988

3.3.4 Predicting Occupancy in the Entire Study Area

Given the sheer size of the area, it is nearly impossible to survey the entire north Pakistan for wolf presence and/or distribution. Our surveys however represented a sub-sample of the entire region by covering 25% (10,920 km^2) of total number of grid cells plotted our study area. I used logistic regression equation using the coefficients generated for the top model to predict occupancy estimates in the grid cells that were not sampled. The assumption in estimating probability of occupancy in unsampled regions is that the sampling grids were chosen randomly and represented the variation in covariates (and hence occupancy) in the unsampled grid cells. Based on occupancy modeling, Deosai, Central Karakorum, Khunjerab, Broghil and Chitral Gol National Parks have high relative abundance of grey wolf (Figure 3.3.3).

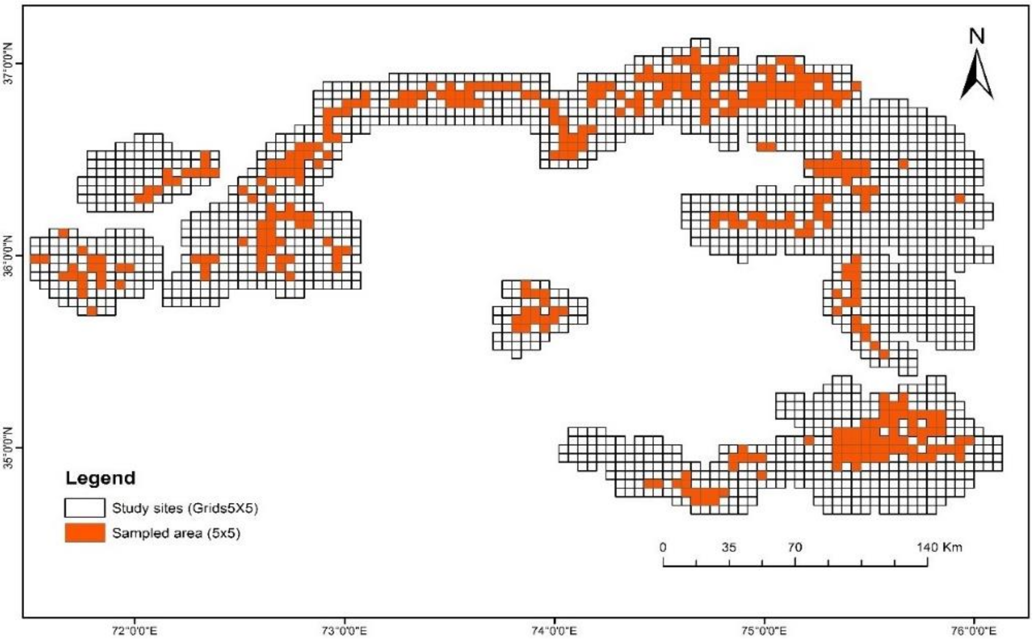


Figure 3.3.2: Study sites (grids of 5 × 5) and sampled areas (red polygons) for monitoring the grey wolf in Northern Pakistan

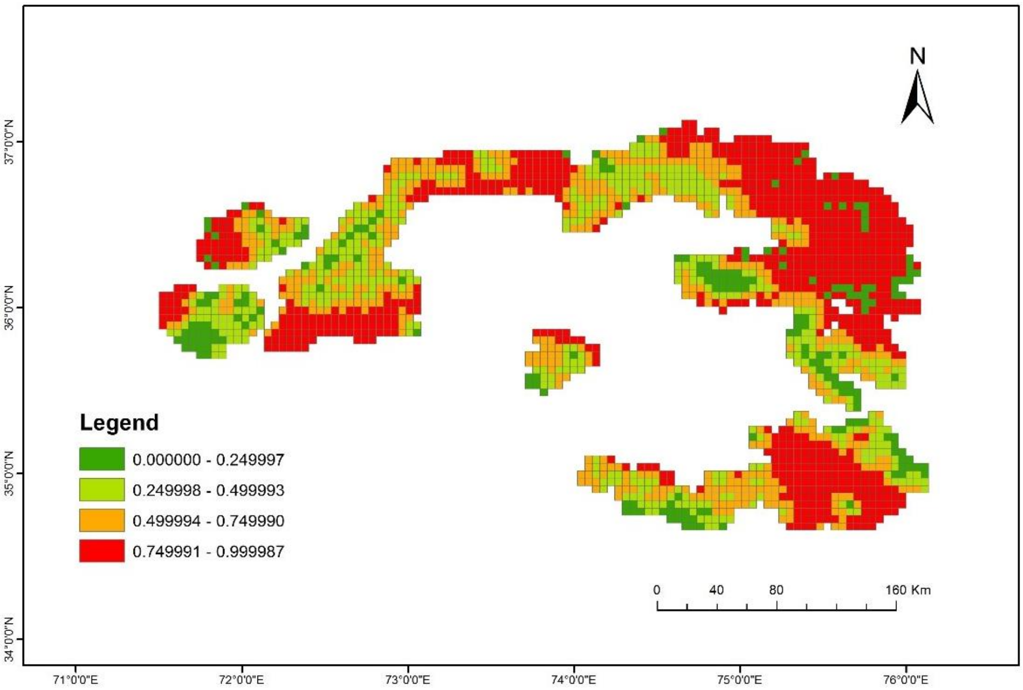


Figure 3.3.3: Spatial pattern of wolf occupancy in northern Pakistan

3.4 DISCUSSION

Wolves are distributed unevenly across spatial and temporal scales on earth. Most of the variation in distribution patterns are attributed to environmental heterogeneity, climatic conditions and anthropogenic pressures. Among the large carnivores, current wolf occupancy in Pakistan is poorly documented. There is limited information on the species occurrence which is largely based on secondary data and anecdotes. Wolves' actual presence across the suspected range is not known, as there has been no scientific research to validate its presence in northern Pakistan.. According to Krebs (2009) *"Ecology is the scientific study of interactions that determine the distribution and abundance of organisms"*, thus understanding species distribution is a fundamental question in ecology. This study primarily focused on the spatial pattern of occurrence and occupancy of grey wolf in northern Pakistan and provided the first-ever information on areas currently inhabited by wolves. I also investigated the influence of socio-ecological variables on wolves' occupancy and detection.

Wolves occupy large areas, and because of their cryptic behavior they are difficult to study across their range. Monitoring populations of large and elusive carnivores such as wolves is difficult and extremely expensive because of their low densities and large and inaccessible areas they occupy (Woodroffe 2001; Gittleman et al. 2001; Galaverni et al. 2011). For unmarked species, camera trapping provides presence-absence data that can be used for monitoring changes in occupancy and informs on distribution (Steenweg et al. 2016). Occupancy can also be used as a surrogate of abundance and is and is relatively cost-effective (Blanc et al. 2014).

The camera trapping is an effective way to study the elusive species like grey wolf (İlemin 2014; Xu et al. 2007; Rovero et al. 2013; Din et al. 2013; Bischof et al. 2014; Wang et al. 2014) and this technique has been used in the current study to add in ecological knowledge in very remote and tough terrain of northern Pakistan, fall in the wolf distribution range illustrated by IUCN. Wolf presence records were obtained from camera trapping with a total effort of 24,000 camera trap nights. The wolf photo-capture records rate varied across the range in northern Pakistan, presumably reflecting variation in species occurrence, abundance, and topographic features. In protected areas, higher wolf photo-captures were recorded from Deosai, Chitral Gol and Broghil national parks. Whereas Khanbari valley in Chitral district had the highest capture success outside the protected areas. Presence records from different locations revealed the adoptability of wolves to various types of habitat. Overall wolf detection rates were very low, and occurrence reflected patchily distributed populations in most remote and border areas of Pakistan (Din et al. 2013; Bischof et al. 2014; Ahmad et al. 2016; Kabir et al. 2017). In northern Pakistan, wolves were relatively flexible in their use of habitat at the landscape scale, as they occupied a wide altitudinal gradient. Their presence was recorded ranging from the moist temperate zone (Musk Deer National Park) up to the alpine zone (Khunjerab National Park) (Kabir et al. 2017). This is in concurrence with the previous studies that suggest wolf is a habitat generalist species and tolerant to anthropogenic factors (Mech and Boitani 2003; MacDonald and Sillero-Zubiri 2004). Due to large home range, low specificity to habitat types and high rate of reproduction, wolves adopt more ecological resilience as compared to other large carnivore species (Carroll et al. 2001; Fritts et al. 2003).

Wolves were photo-captured at 51 locations across Himalaya, Karakorum and Hindukush mountain range. There have been several sightings reports of wolf from Pakistan, but the alpha black male was photo-captured for the first time from Khanbari study site during this study. Majority of wolf photo-captures comprised of 1–2 individuals, packs of four individuals were captured at only two occasions, one from Khanbari and other in Chitral Gol National Park (both lying in the Hindukush range). This information revealed that in fragmented and disturbed habitat wolf preferred to live in small pack around the freely grazing large herd of livestock which make least vulnerable to humans. Mostly snow leopard and wolf overlap habitat across their geographic range but capture events showed that during entire study period and at 800 camera station we rarely photo-captured wolf and snow leopard at same location. Field observation and discussion with local communities also supported this observation. There is no scientific study on ecological interaction between wolf and snow leopard, but our observations indicate wolf may avoid areas occupied by snow leopards (Kumara 2005).

The grey wolf's estimated occupancy was 0.4342, which is low and not uniform across its geographic range. Khunjerab and Deosai national parks represented relatively high occupied within protected areas followed by Broghil and Chitral Gol national parks. The local people believed that wolf populations have disappeared from most of species' historic range in Hoper-Hisper, Shimshal, Misgar, Chapursan and some other valleys of Karakorum range, particularly due to killings in retaliation to livestock depredation. Local people reported higher cases of wolf attacks on livestock as compared to all other large carnivore species found in the area. They also reported that within valleys there is rare sighting of wolf where the areas are known to be occupied by snow leopard. Other possible reason might be all the conservation projects that provide incentives to local communities are directly and indirectly linked with snow leopard conservation in northern Pakistan, so people have some tolerance towards leopards, but they were against wolf. Abbas et al. (2013) reported density of 0.6–1.7 per 100 km^2 in different tracts averaging 1.1±3.9 SEM per 100 km^2 in GB, Pakistan. Din et al. (2013) estimated the Relative Abundance Index (RAI) of grey wolf which was recorded 3.5 in Chitral, Pakistan. Ahmadi et al. (2013) estimated the values 0.53±0.23 SD from random sites for wolf den locations.

3.4.1 Environmental Variables Affecting Wolf Occupancy

There was significant effect of socio-ecological variables on wolf distribution and occupancy in the area. The current study revealed that extensive filed effort and plateau feature of landscape had positive effects on detection probability of wolf in northern Pakistan. The surveys over wide areas with large number of cameras and long duration provided more wolf detection rate. Wolves preferred open and plain habitat and avoided steep slopes and cliff base in our study. In occupancy top model terrain characteristics had significant effect on wolf presence as it prefers to live in open area. Louvrier et al. (2017) described that increased sampling effort is directly proportional to increased probability of detection of the species, consequently highlighting its worth to more accurately explain imperfect detection. Another study supported our results that wolves preferred to live in open areas and wolf-selected open habitats, small distance from road, intermediate elevation range and did not prefer steep slopes (Uboni 2012). Other studies reported that during winter season wolves stay away from steep slopes, avoid heavy snow fall area and use relatively open areas (Ciucci et al. 2003; Hebblewhite and Merrill 2008; Milakovic et al. 2011; Lesmerises et al. 2012).

The pattern of habitat selection is influenced by anthropogenic activities caused by human and usually reported from remote and wilderness areas (Ciucci et al. 2003; Houle et al. 2010; Mech and Boitani 2010). As a consequence, wolf presence and distribution mainly limited to uninterrupted distant areas with scattered population of human (Chapron et al. 2014; Lopez-Bao et al. 2015b). Wolves have been extirpated from their historic range by human activities (Laliberte and Ripple 2004), consequently they became restricted to less populated, remote and undeveloped areas (Paquet and Carbyn 2003; Chapron et al. 2014; Lopez-Bao et al. 2015b). Wolves' behavioral response to human developmental activities (roads, settlements) and their effect on wolf ecology vary according to settlements or roads characteristics, (Jędrzejewski et al. 2004; Blanco et al. 2005; Ahmadi et al. 2014; Zimmermann et al. 2014). I observed that wolf presence was depending upon the type and level of anthropogenic disturbance in the area. Increasing distance from human settlements and intermediate elevation range had positive effects on wolf occupancy. I used human settlements as replicate of human disturbance, which has negative effect on wolf abundance. Wolves preferred to live remote and secluded areas to avoid encounter with human. Roads have positive effect on wolf abundance, as most of the roads are unpaved with low traffic pressures, thus causing little disturbance for wolf and provide an easy movement corridor for safe movement and dispersal. An elevation range between 3,000–5,000 m has a positive influence on grey wolf distribution.

Jędrzejewski et al. (2005) reported that wolves preferred to live in undisturbed areas which are located in tough and mountainous regions across their distribution range. Similar pattern of interaction has been studied by Theuerkauf et al. (2003), where wolves avoided the close surroundings of human settlements. Species distribution models in northern Pakistan revealed that summer huts and grazing pastures restricted wolf distribution (Kabir et al. 2017). Habitat suitability modeling of wolves revealed that altitude, distance to human settlement and roads were the important predictive variables in Iran (Ahmadi et al. 2013). Although human-caused anthropogenic and development activities such trapping, hunting and roads around the protected areas may have negative influence on wolf dispersal ability (Urton 2004). Wolves used secondary gravel or dirt roads to chase their prey due to their increased mobility and increased visibility (Mesler 2015). Wolves select primary roads, either in summer and/or winter with low human use, as paths where locomotion is easier or to enhance the chances of encountering a prey (Houle et al. 2010; Lesmerises et al. 2012). Prey species also use roads for travelling or post-logging flora or roadside for minerals and foraging which may attracts the wolf to chase them (Laurian et al. 2008; Hebblewhite et al. 2009). In some areas, wolves travel on primary roads because roads make movement and prey encountering easier, but this pattern is influenced by human activities (Houle et al. 2010; Whittington et al. 2011; Lesmerises et al. 2012; Lesmerises et al. 2013).

Wolves facing more threats than snow leopards towards extinction. Local communities have negative perception because of livestock loss, and they are actively involved in wolf persecution. Trophy hunting is a conservation-based livelihood source for local community, people are not in favor of wolf presence in community-controlled hunting areas. Anthropogenic development activities such as roads, hydropower stations and dams are major threats affecting prey abundance and wolf movement, thereby contributing to habitat fragmentation and population decline. The dynamic and consequences of feral/sphered guarding dogs and wolf interaction is challenging for wolf conservation. Habitat fragmentation and reported wolf-dog hybridization is serious threats to genetic deterioration to wolf population in Pakistan. With the rapid increase in population of human beings, the effective conservation of large carnivores has become a challenging task (Woodroffe et al. 2005). There is resource sharing between carnivore species and human due to large home range and potentially overlapping and potentially competing over natural resources (Blanc et al. 2013).

3.4.2 Conservation Implications of Wolf Occupancy Modeling

Wolf occupancy was estimated and illustrated on a map for northern Pakistan, this spatio-temporal pattern of wolf occupancy is useful information for the wildlife authorities in decision making. This allows a better understanding of potential sites for planning future research, management of grey wolf in Pakistan. The areas marked with suitable habitat and higher wolf occupancy, probably offer least physiological and biological stress to wolves, thus these should be prime target of conservation. Wolves can be sustained well in these areas with low management costs and conflicts can be resolved with comparative ease. Management plans for grey wolf should involve strategies, based on habitat suitability and occupancy results to lower their costs of survival by improvising improved sustenance with decreased vulnerability. Generally, map-based conflict mitigation actions and wisely devised management strategies can help to promote human-wolf co-existence through the identification of priorities areas where the likelihood of conflict is high. Action against poaching and habitat protection should be implemented in these areas to flourish wild prey populations to reduce carnivore predations over livestock. Sustainable influx of prey can reduce possible human interaction and attacks.

3.4.3 Wolf Occupancy Analysis: Limitations and Recommendations

The implementation of my study has various limitations. Due to harsh weather conditions and tough terrain of Hindukush, Himalaya and Karakorum we could not explore the entire range of wolf in northern Pakistan. The single-season study could not generate the real picture of species occupancy, as it lacks wolves' site use information in other seasons. Climatic factors such as average temperature, humidity, precipitation, etc. should be incorporated in distribution modelling along with biological factors. Improved variables offering comparatively reliable quantification of human disturbances should be used. For instance, using human density instead of settlements and livestock density would perform better. Predators travelling extensively and having large home range sizes, like grey wolf, seem rather attracted to disturbances offering prey (such as roads). Further research is required to pronounce this trend as preference or selection because the identification of limiting factors is important for reintroduction and conservation plans.

Plate 3.1: Wildlife Species Detected while Camera Trapping in Northern Pakistan (2009–2017)

APPENDIX 3.1: SITE OCCUPANCY DATA SHEET

Date:	**Time:**	**Block ID:**	**Point ID:**
Latitude:□□.□□□□□		Longitude:□□.□□□□□	Elevation: m
Topography:	Terrain:	Habitat:	Disturbance:
Observer(s):			

#	Species	Sign type	Sign age	Measurement (LxD) cm	Substrate type	Notes

Key:

Block ID: From map; e.g.: S-01   Point ID: Block-ID-Point; e.g.: S-01-01

Topography: Ridge, Cliff base, Draw, Valley, Saddle, Plateau

Terrain: Brokenness Index, (1–4)

Habitat: Scrub, Forest, Pasture, Barren, Agriculture/Plantation

Disturbance: High, Medium, Low, None

Species: Snow Leopard, Brown Bear, Wolf, Lynx, Ibex, wild ungulate, livestock, etc.

Sign type: Pugmark, Scrape, Feces, Scent, Claw Rake, Digging.

Sign age: <10 days; < 1 month; >1 month

CHAPTER 4

Understanding the patterns of livestock depredation by wolf *(Canis lupus)* in northern Pakistan

4 Understanding the Patterns of Livestock Depredation by Grey wolf *(Canis lupus)* in Northern Pakistan

ABSTRACT

Carnivores have always been considered a serious threat for pastoralists. Understanding the socio-ecological factors that contribute to this conflict is crucial for carnivore conservation. We interviewed 2,317 shepherds to quantify livestock losses due to wolf predation in northern Pakistan. In the study area, 7,583 livestock were killed during 2011–2015 and an average 10 losses were reported from herd size of 49 individuals per year. Small ruminants were more likely to be killed by wolves than large livestock. The extent of livestock losses changed in different seasons, with maximum depredation events occurring in the summers. Wolves preferred to attack small ruminants in open pastures and large ruminants in corrals. A number of factors were positively correlated with livestock predation, including size of livestock holding, mortality due to disease, and occupation (livestock rearing). The negative binomial model suggested that predation incidences tend to decline with increases in the education levels of respondents. Contrastingly, predation counts doubled when livestock holding moved from low to high. Better guarding could prevent wolf attacks, particularly in high-depredation season. Livestock disease was a stronger cause of loss per household with an average of 3.5 animals dying annually. The outcome of this study has policy implications for managing human-wolf conflicts. I recommend the establishment of veterinary centers with the allocation of substantial budgets to reduce livestock mortality to disease. The major obstacle for wolf conservation and extensive killing is livestock depredation in the area. To stabilize the co-existence of wolf populations and livestock wellbeing, i suggest multi-pronged conservation management programs that include sustained compensation schemes, livestock vaccination, and awareness campaigns.

Keywords: *Himalaya-Karakorum-Hindukush, livestock depredation, socio-ecological factors*

4.1 INTRODUCTION

Large carnivores regulate key functions of several ecosystems and are currently threatened across the globe (Ripple et al. 2014). Considering large carnivores' vital ecological role, a decline in their numbers can have a long-lasting effect on the balance and structure of ecosystem functionality (Miller et al. 2001; Miquelle et al. 2005). For example, wolves indirectly promote plant populations by directly controlling the population of herbivores in the Greater Yellowstone ecosystem (Ripple et al. 2001). Global decline in the population of carnivores is occurring due to conflict over livestock depredation (Woodroffe et al. 2005a). In such a conflict, large predators are more prone due to their food requirements and a large home range (Linnell et al. 2001; Macdonald and Sillero-Zubiri 2002). Expanding human population and actions increase the level of competition for resources between humans and carnivores (Graham et al. 2005; Athreyam et al. 2013; Aryal et al. 2014; Kabir et al. 2014). Livestock depredation by carnivores and their retaliatory killings are a conservation concern worldwide (Madhusudan and Mishra 2003; Treves et al. 2006).

Multiple factors contribute to framing this conflict situation across the world. For example, livestock grazing in habitats with carnivore abundance, or in areas of low natural prey and lack of predator avoidance behavior in domesticated animals (Namgail et al. 2007; Suryawanshi et al. 2013; Aryal et al. 2014). Livestock depredation rates are also influenced by local environmental variables, including natural prey abundance (Polisar et al. 2003) and the characteristics of livestock killing sites, *i.e.* villages and corrals (Mech et al. 2000; Ogada et al. 2003). The practice of livestock rearing is the main land use and primary source of rural livelihood in trans-Himalayan pastures (Mishra et al. 2001).

Carnivore populations suffer most from direct persecution due to retaliatory actions by farmers in areas where livestock farming is the predominant land use (Mazzolli et al. 2002). Due to foraging competition, domestic livestock can affect wild ungulate population abundance, which are natural prey for wild carnivores (Sillero-Zubiri and Laurenson 2001). Furthermore, in areas where traditional farming practices have been abandoned and carnivore population abundance has been reduced or eradicated, domestic animals are allowed to graze freely and unsupervised over larger areas (Stahl et al. 2001; Ogada et al. 2003; Madhusudan 2003), which makes them more vulnerable to predation.

The existence of carnivores is a financial risk for local communities (Graham et al. 2005). The loss of domesticated animals is an indispensable worry for the incomes of the pastoralists living in Trans-Himalayan region (Namgail et al. 2007). Peoples' acceptability of large carnivores fluctuates based on many factors, including cultural factors, literacy rates, religious beliefs, livelihood practices, and the characteristics of carnivores (Liu et al. 2011). Negative perceptions of carnivores caused by perceived rate of livestock predation have been identified as a difficult topic for both rural development and wildlife conservation (Woodroffe et al. 2007). The financial cost, preferably especially in developing countries and under-developed rural communities, can be substantial and may result in a lesser degree of tolerance and negative attitudes towards carnivore conservation (Dickman 2010).

Even if the overall public supports large carnivore conservation efforts, local communities will still be reluctant towards it as they must pay the costs of co-existence with carnivores (Muhly and Musiani 2009; Treves and Karanth 2003a). This leads to a conflict that harms conservation efforts and the community involved (Redpath et al. 2015). Therefore, exploring mitigation strategies resulting in a long-term co-existence of globally threatened carnivore species and humans (Mishra et al. 2003) becomes even more urgent and important. Proactive and effective measures are needed to minimize the negative effect of a reactive conflict (Madhusudan and Mishra 2003). Livestock diseases also have economic consequences on livestock farming through the cost of prevention and treatment, reduced production, and disease-caused mortality (Perry et al. 2002; Kock 2005). Loss to disease aggravates the economic hardships of herding communities already suffering from predation losses.

Wolf survival has been threatened by pastoralists' persecution (Mishra et al. 2003; Namgail et al. 2007) in most of its historic distribution range (Fritts et al. 2003). The wolf is one of the most controversial predators because it has come to symbolize wilderness, devastation, destruction, and negative changes (Shelton 2004). Wolves inhabit Central Asian mountain ranges where they live along with a substantial number of livestock (Mishra et al. 2003). The wolf has a broad habitat area, making it more susceptible to conflict with livestock herders who perceive it as destroying farmers' livelihoods (Kaczensky et al. 2008; Conforti and de Azevedo 2003). This is especially true for farmers grazing close to protected areas. The large home range of wolves and conflict over livestock predation trigger a negative public perception towards wolf survival. This consequently results in a dynamic conflict between livestock holders, the carnivores, wildlife managers, and conservationists (Naughton-Treves 2003). In Pakistan, the wolf has disappeared from most of its historical range due to the depredation on livestock and its effect on pastoralists' livelihoods (Roberts 1997; Abbas et al. 2013; Kabir et al. 2017). The wolf is listed as 'vulnerable' but is still heavily persecuted because of predation on livestock. Knowledge of its conflict dynamics in the country is scarce and is limited to a few valleys (Dar et al. 2009; Abbas et al. 2013; Din et al. 2013).

In South and Central Asia, this conflict is mainly developing due to the lack of understanding of the ecological and socioeconomic interaction between threatened species and pastoral communities (Bagchi and Mishra 2006). Most often, there is an existing conflict between competing human priorities and conservation policies (Redpath et al. 2015). Knowledge of key factors responsible for the conflict is essential for conflict management and protection of large carnivores (Dickman 2010; Mateo-Tomás et al. 2012). The identification of ecological dynamics that underlie human-wildlife conflicts is vital, without which the formulation of successful management strategies for large carnivores remains compromised (Bagchi and Mishra 2006). The effect of livestock loss induced by wolves is not a modest problem that can be solved easily—it requires a large and serious effort. Our study is aimed at building a range-wide understanding of livestock depredation pattern by wolves in Pakistan and evaluating socioecological factors in relation to the risk of predation. The results of this study will inform the management of ecologically viable co-existence of wolves and livestock in northern Pakistan.

4.2 MATERIALS AND METHODS

4.2.1 Data Collection

Northern Pakistan is home to over one million people. Livestock holdings represent a significant source of livelihood and are considered to be an asset that farmers can liquidate in times of financial crises (Hussain 2003). Agro-pastoral systems vary along altitudinal gradients and land-use is dominated by livestock rearing and farming. Data collection was carried out during January 2011–December 2015 in different valleys of the study area (Figure 4.2.1). We interviewed people across the study area in the following manner: ten percent of households from each community surveyed were selected and one adult person per household was interviewed.

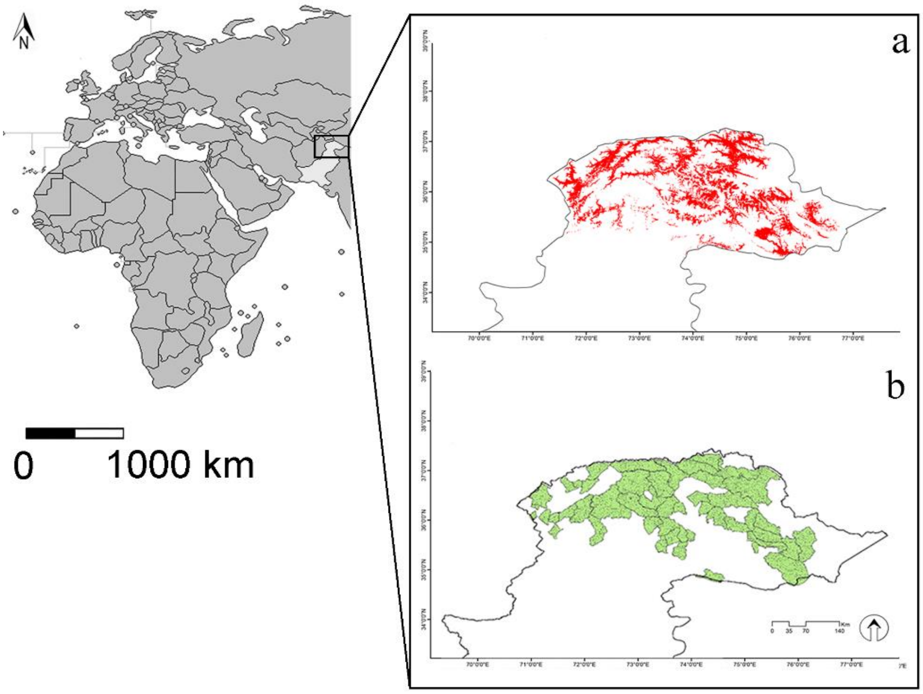


Figure 4.2.1: Study area (light grey) in northern Pakistan showing potential distribution of *Canis lupus* (a) and valleys (b) where we collected information/data on livestock depredation by wolves during 2011–2015.

Efforts were made during the survey to manage interviewees' expectations. I specified that the information was collected independently, without association with local government agencies, and would not lead to special compensation for financial losses due to predation or persecution for wolf poaching. Two approaches were employed. First, qualitative methods included participatory observation, group discussions, unstructured interviews, and group discussions. Second, quantitative methods comprised structured interviews (Appendix 4.1). We asked questions to quantify (i) livestock holdings by type of animal (goat, sheep, cow, yak), (ii) levels of depredation, (iii) social factors (respondents' education, occupation, etc.), (iv) mortality to disease and (v) circumstances.

I categorized the rate of mortality into three categories: nil (= 0), low (< 5), and high (> 5). During the interviews, some wolf attack sites were investigated and the predators' signs were searched. This was done to confirm that only the reliable wolf predation records were considered for further statistical analysis. I used reference photographs of all carnivores found in the study area to minimize local people's mistakes in recognizing depredation cases of wolves, snow leopards, and the Himalayan lynx.

4.2.2 Statistical Analysis: Kriging

Kriging, an advanced procedure of geostatistical analysis in ArcGIS (10.2) was used to extrapolate the rate of livestock depredation over the valley of the study area once the average rate of livestock predation was determined (Figure 4.3.2).

4.2.3 Statistical Analysis

Treating predation count as a response variable, i tested the effects of education, livestock owned, mortality rate (disease caused), circumstances, occupation, season, and valley. The present study involved a random selection of seasons and valleys because all of the seasons and valleys of the study area were not surveyed. These two factors (seasons and valleys) were treated as random variables in the model. The fixed factors tested in the model are described in the Table 4.2.1.

Table 4.2.1: Categories of Socio-Ecological Factors/Variables used in Analysis

	Variables	Categories
1	Education	Uneducated (Nil\< primary), educated (matric), highly educated (graduates)
2	Occupation	livestock rearing, others (govt. and private jobs etc.)
3	Livestock owned	nil (0) low (< 28), high (> 28)
4	Mortality rate (disease)	Nil (0), low (< 5), high (> 5)
5	Circumstances	free grazing, at corral

I constructed separate models for small and large ruminants with mixed-effect nested models using the "lme4" package (Douglas et al. 2015) and the "pscl" package (Jackman 2017) in R (software) (https://cran.r-project.org/). A mixed factor negative binomial regression model was performed as the predation count was over-dispersed (with a mean of 2.99 and variance of 24.45 in small ruminants, and a mean value of 0.28 and variance of 0.64 in large ruminants). Model selection was made through stepwise backward elimination using p-value = 0.05 and effects were plotted using package "effects" (Fox 2016). Variables that made no significant contribution to the model fit were dropped from the analysis. The models were validated through the goodness-of-fit approach, also called the likelihood ratio (McHugh 2009). The model selection was based on AIC and the models with valleys as the random effect were found to be the best. The equations of the best-selected models are below:

$$ln(Small\ Ruminants) \sim Education + Occupation + \ Livestock\ owned + \ Mortality + \ Circumstances + \ 1|Season \ + 1|Valley + Residuals$$

$$ln(Large\ Ruminants) \sim Education + Occupation + \ Livestock\ owned + \ Mortality + \ Circumstances + \ 1|Season \ + 1|Valley + Residuals$$

I also tested how socio-ecological variables frame perceptions. Factors including age, education, earning member, occupation, household size, livestock owned and agricultural land were considered as fixed factors while valley was used as the random variable. The attitude was coded with 0 = "Eliminate", 1 = "Decrease", 2 = "Maintain", and 3 = "Increase". The multinomial model was performed to determine which factors have a modulating effect on attitudes/perceptions (economic status, age, education, earning household members, etc.). The equation of the model tested is presented below:

$$\text{logit}(P(Y_i \le j)) = \beta_0 + \beta_1 (\text{Education}) + \beta_2 (\text{Age}) + \beta_3 (\text{Occupation}) + \beta_4 (\text{Earning Members}) + \beta_5 (\text{Agricultural Land}) + \beta 6 \text{House Hold Size} + \beta 7 \text{Live Stock Owned} + \text{Valley} + \text{Residual}$$

4.3 RESULTS

We interviewed 2,317 individuals from 49 valleys of northern Pakistan, predominantly males (97%). The average age of the respondents was 41 and family size ranged from 5 to 16 members. Some 38% of the respondents were uneducated, 49% educated, and 14% were highly educated. In the study area, 63% of the respondents owned livestock and had adopted agriculture-based livelihood practices as occupations. Among the other sources of income, about 37% of young educated people worked in the tourism and eco-tourism industry. The average number of heads of livestock owned per household was 28. About 53% of households kept 10–50 heads of livestock while the largest herd size recorded from the area was 224 animals. Small ruminants such as goats and sheep comprised the bulk of the livestock population—mean number = 16 and 6, respectively). All households with livestock had sheep and goats whereas fewer owned cows and yaks. For meat, the most slaughtered livestock were goats and yaks.

Some 7,583 heads of livestock were killed by wolves and reported from 49 valleys of northern Pakistan during 2011–2015. From reported cases of wolf attacks, one animal was killed in 67% of the cases, and two or more animals were killed 33% of the time. An average of 10 livestock losses were reported from a mean herd size of 49 heads of livestock. The valley with substantial livestock depredation equally suffered losses due to disease (Figure 4.3.1). The predation of small ruminants (goat and sheep) ranged 0–48 with an average of 3 animals per household killed, and that of large ruminant ranged 0–10 with an average of 0.284 livestock per household killed annually.

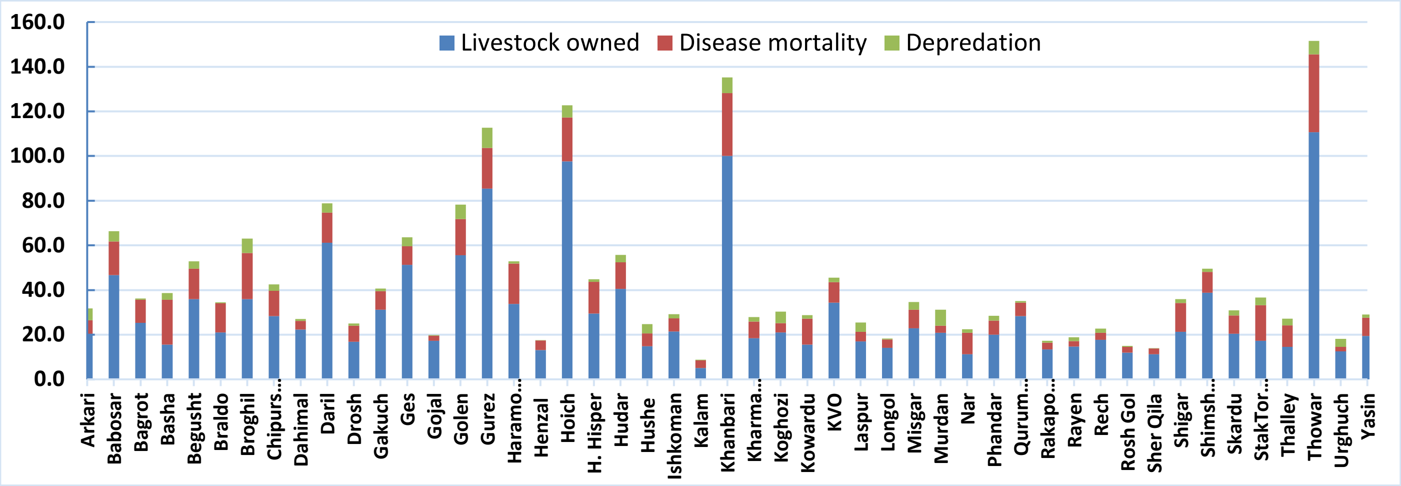


Figure 4.3.1: Valley-wise livestock owned, mortality to disease, and livestock depredation

There was a considerable seasonal disparity in number of livestock depredation events. The incidence of attacks on livestock was more in the summer (57%) and spring (30%), and the lowest in the remaining period. Livestock mortality due to different diseases was commonly reported from the study area. Over 50% of respondents reported a substantial number of mortality incidences due to diseases. Disease-induced mortality was a major loss factor for herders and ranged 0–70 with an average of 3.45 per household annually. Goats (40%) and sheep (27%) were more vulnerable to disease mortality.

The effect of husbandry on depredation in pastures and corrals was also studied. It was reported that 94% of small ruminants were killed in open pastures while grazing and the remaining were attacked in poorly established corrals. Wolf attacks on large ruminants—especially their calves were higher at corrals. Protective measures against the predator were not satisfactory. It was observed that 20% of herders had poorly or partially developed corrals while the remaining kept them open under a poor guarding system. Ten percent of the herders used guard dogs while the majority spent nights on watch duty, protecting the stock by singing or making noises. Residents also built decoys with rocks and human clothes on top of ridges for guarding purposes.

4.3.1 Spatial Pattern of Wolf Depredation

The rate of livestock predation was studied across the valleys and there was a strong relationship between the population status of livestock and the predation rate. Valleys with substantial numbers of livestock depredation suffered equal losses due to disease. The graphical representation of the predation rate is shown in Figure 4.3.1. I have developed habitat suitability model for the wolf in northern Pakistan based on presence points obtained from camera trapping and genetic analysis (Kabir et al. 2017).

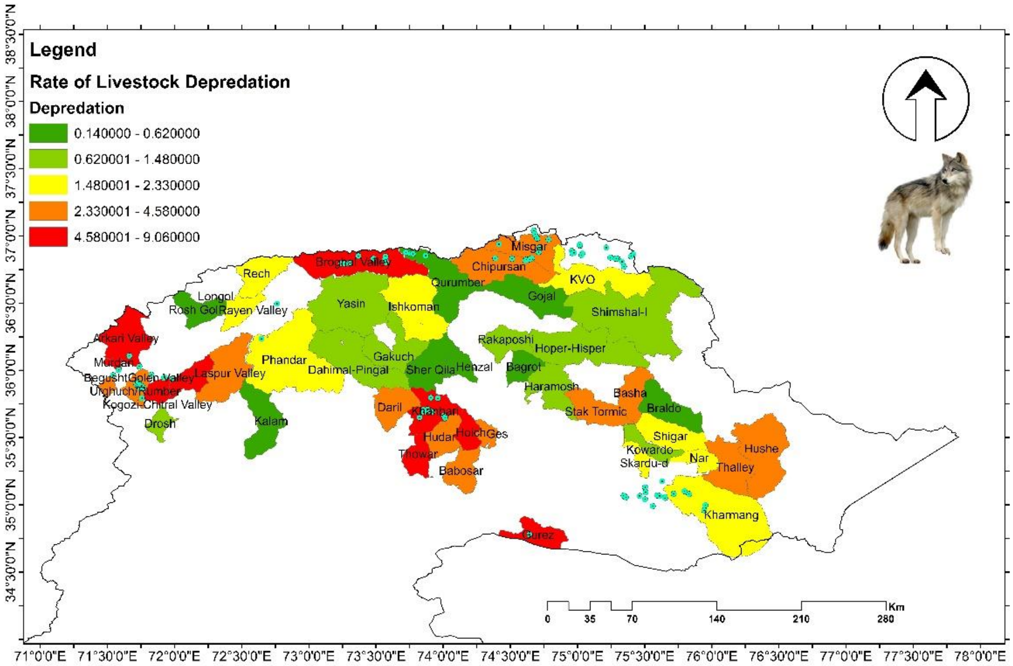


Figure 4.3.2: Spatial pattern of livestock depredation by wolves, extrapolated surface showing rate of livestock depredation in different valleys of northern Pakistan

I compared the rate of predation vis-à-vis availability of suitable habitat. Results demonstrate that areas with suitable habitat had a smaller number of depredation counts with the exception of a few valleys that were located outside the PAs (Figure 4.3.3).

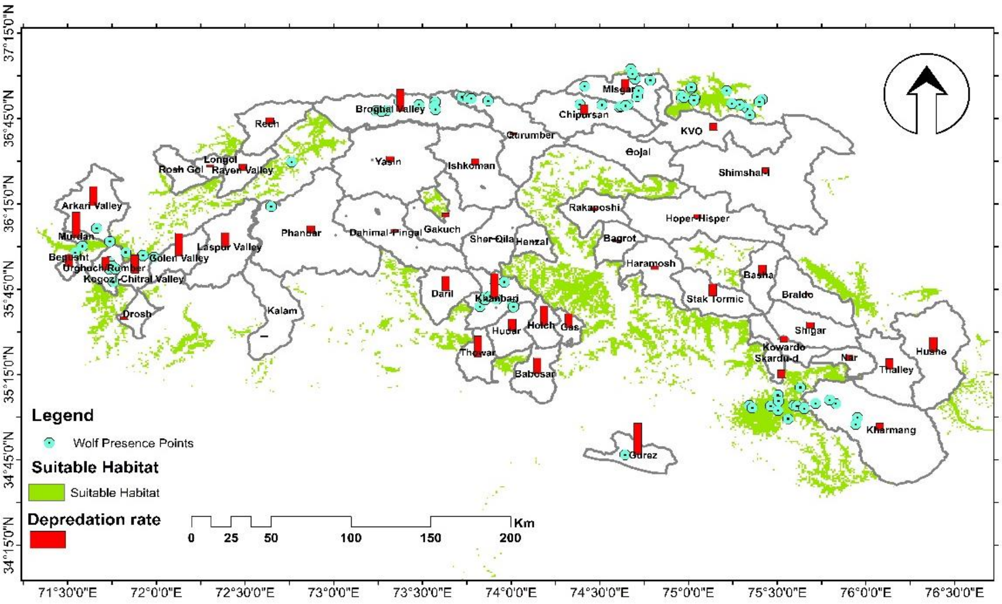


Figure 4.3.3: Wolf depredation in relation to suitable habitat in northern Pakistan

4.3.2 Effect of Socio-Ecological Factors on Predation Count

For small ruminants, three factors including education, livestock owned, and disease-causing mortality significantly explained the variations in predation counts (Figure 4.3.4). The log of predation count decreased by 0.208 when the respondent's education level increased from uneducated to educated. Similarly, the log of predation count decreased by 0.210 when respondents' education levels increased from uneducated to highly educated. The log of the predation count increased by 1.627 with the increase in livestock numbers. Similarly, the log of the predation count increased by 2.052 when livestock numbers increased from nil to large-herd size (Table 4.3.1). Mortality to disease equally contributed to predation, which increased by 0.353 when the mortality rate moved from nil to low. Similarly, the log of the predation count increased by 0.763 when the disease mortality rate moved from nil to high.

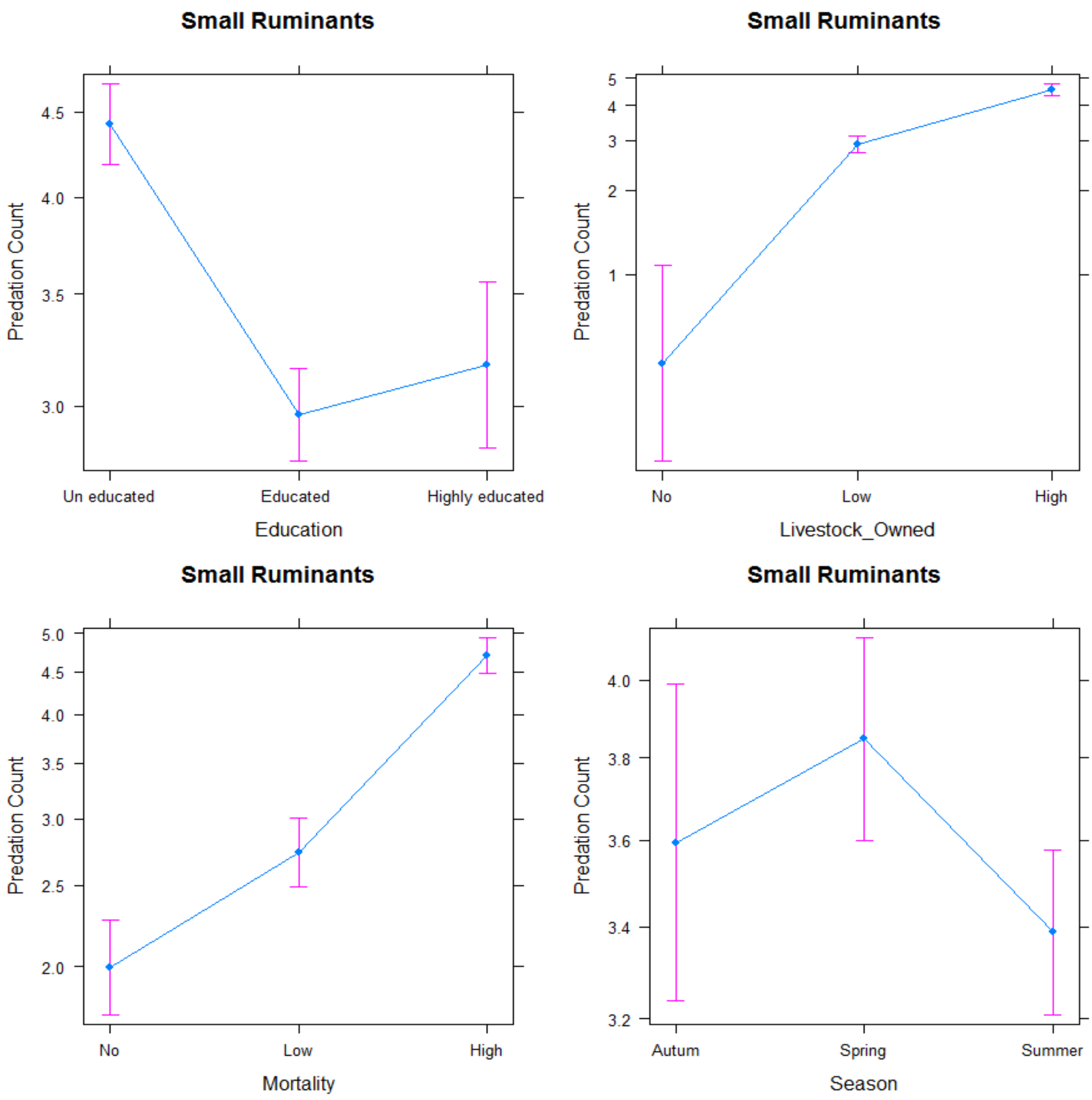


Figure 4.3.4: The predation of small ruminants; a- Education, b- Livestock owned, c- Mortality to disease, d- Season

For large ruminants, three factors viz., education, mortality, and circumstances significantly explained the variations in predation counts (Figure 4.3.5) to further explain the contribution of several factors in predation count. When respondents' education moved from uneducated to educated, the log of the predation count decreased by -0.168 and when respondent education moved from uneducated to highly educated, the log of predation count decreased by - 0.510 (p-value 0.078). When the category of livestock mortality due to disease moved from nil to low, the log of predation count increased at 1.067 (p-value 0.004). When the number of livestock owned increased from nil to high, the log of predation count increased 1.521 with a p-value < 0.001. In the case of large ruminants, predation count decreased by 0.0763 with a p-value of

0.001 in open pastures as compared to predation incidence ‘at corral’ (Table 4.3.1). Model performance was evaluated through AIC. The AIC was 3265 for the final-fitted small ruminants model. It was 1037 for the final-fitted large ruminants model.

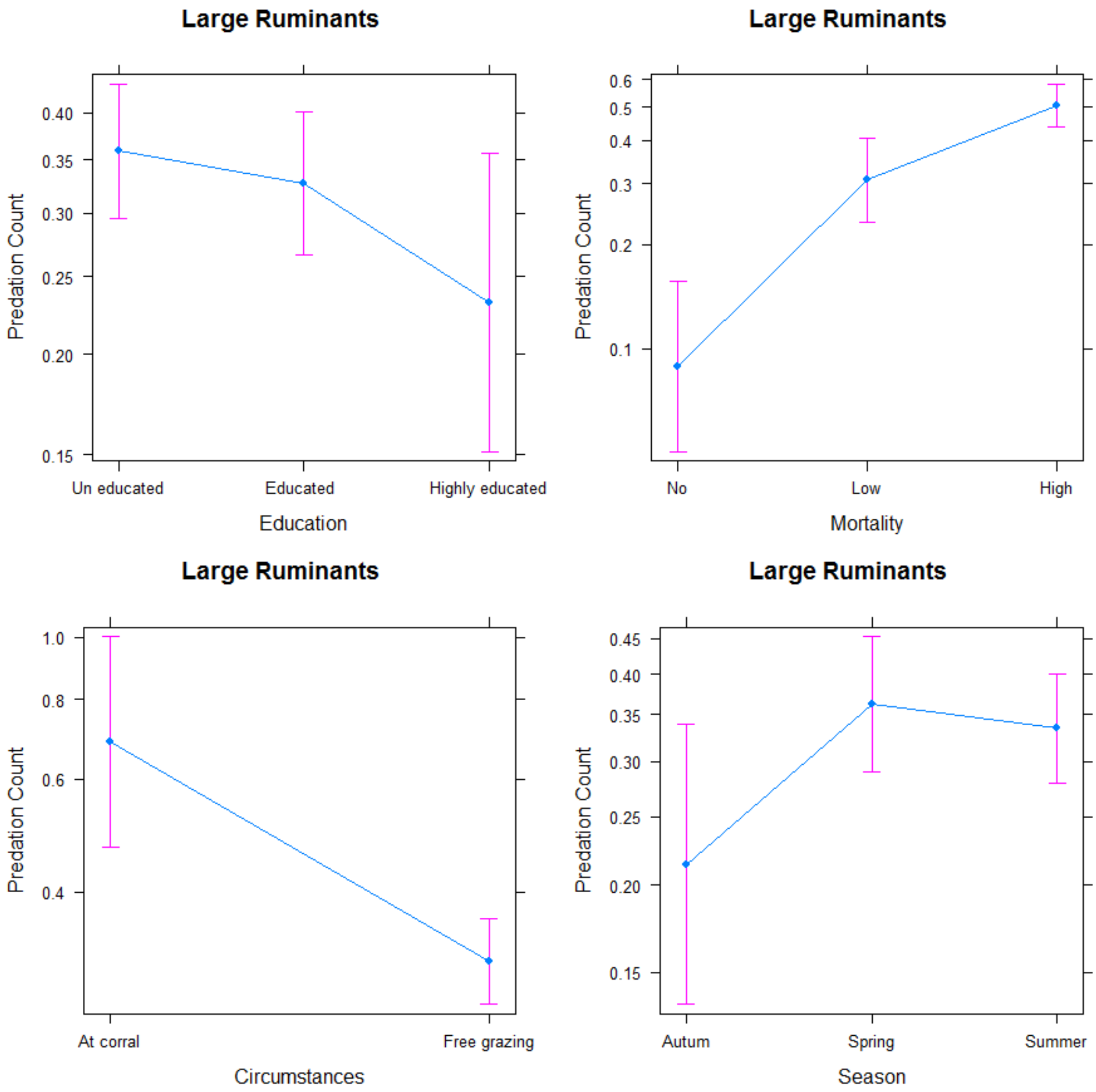


Figure 4.3.5: The predation of large ruminants; a- livestock owned, b- mortality to disease, c- circumstance, d- season

Table 4.3.1: Effect of socio-ecological factors on predation count

Small ruminants		**Estimate**	**Std. error**	**z-value**	**p-value**
(Intercept)		-1.255	0.512	-2.453	0.014
Education	Uneducated	**Reference**			
	Educated	-0.208	0.115	-1.81	0.070
	Highly educated	-0.210	0.158	-1.33	0.184
Livestock owned	Nil	Reference			
	Low	1.627	0.506	3.215	0.001
	High	2.052	0.51	4.023	<0.001
Mortality	Nil	**Reference**			
	Low	0.353	0.167	2.111	0.034
	High	0.763	0.158	4.836	<0.001
Large ruminants		**Estimate**	**Std. Error**	**z-value**	**p-value**
(Intercept)		-0.496	0.43	-1.153	0.249
Education	Uneducated	**Reference**			
	Educated	-0.168	0.174	-0.964	0.335
	Highly educated	-0.51	0.289	-1.763	0.078
Mortality	Nil	**Reference**			
	Low	1.067	0.369	2.894	0.004
	High	1.521	0.342	4.447	<0.001
Circumstances	At corral	**Reference**			
	Free grazing	-0.763	0.3	-2.546	0.011

4.3.3 Public Perception Towards Wolf Conservation

The coefficients for education and livestock owned are positive, indicating that education and large livestock owned increases positive attitudes towards wolf presence (Figure 4.3.6). The results indicate that as education levels move from illiterate to college, respondents are 1.3 times more positive about wolf presence. Similarly, respondents owning a large herd size were 1.3 times more positive about maintaining or increasing the wolf population (Table 4.3.2).

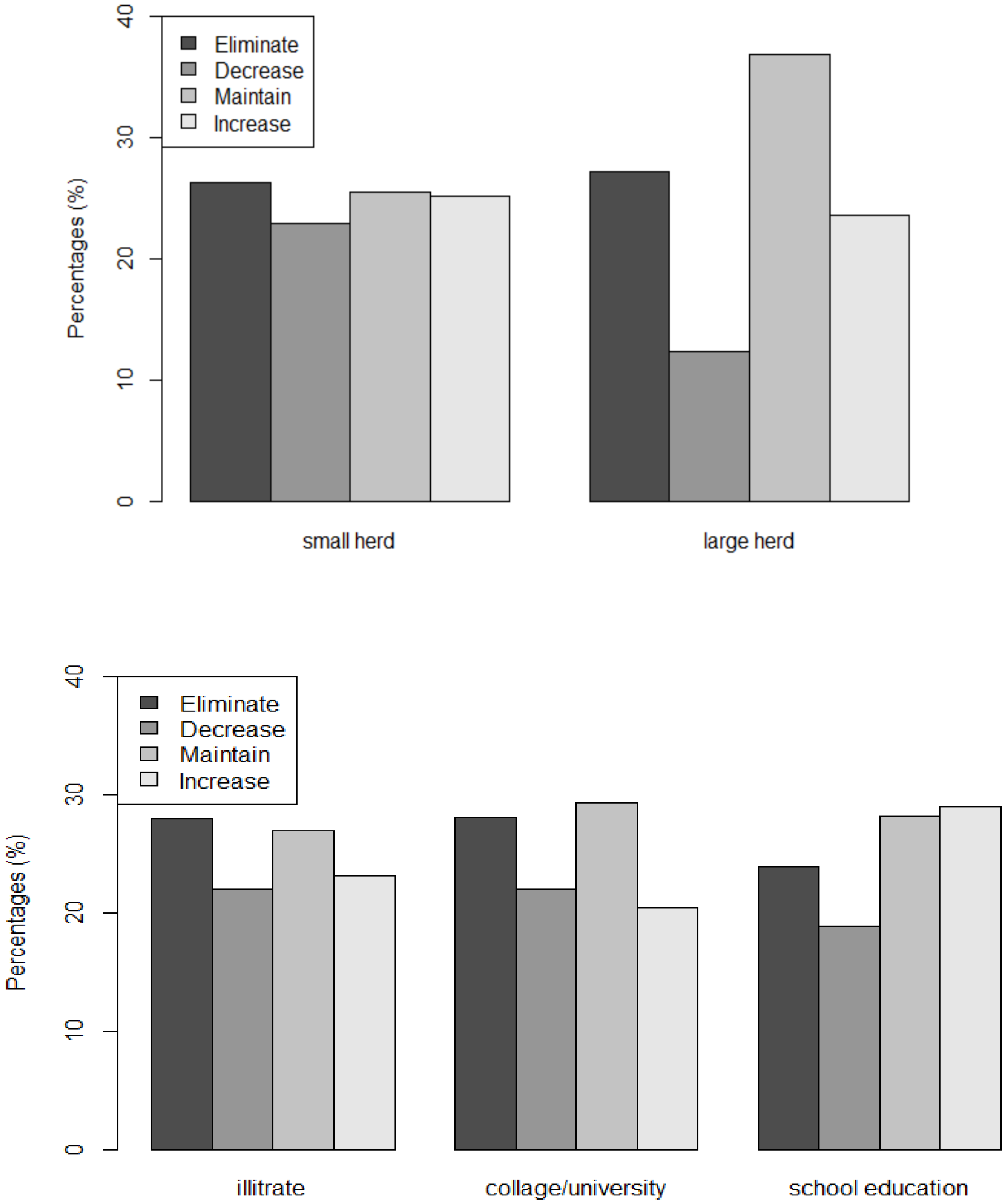


Figure 4.3.6: Socioecological effect of the perceptions of local people towards wolf conservation in northern Pakistan

Table 4.3.2: Effect of socio-ecological factors on perception towards conservation

	Odds ratio	Estimate	Std. error	z value	Pr(>|z|)	
Education: Collage/university	1.31	0.27	0.12	2.24	0.03	*
Education: School education	1.23	0.20	0.09	2.21	0.03	*
Livestock owned large herd	1.23	0.21	0.12	1.74	0.08	.

4.4 DISCUSSION

The global population of large mammalian carnivores is in decline, mainly due to human persecution. The predation on livestock is the most common sources of conflict in the world, although its intensity may vary depending on the natural prey base and abundance of large carnivore found in the area (Inskip and Zimmermann 2009; Li et al. 2013). This puts the survival predators at risk and makes their conservation a daunting challenge (Treves and Karanth 2003b). In Pakistan, the wolf population is fragmented and relatively isolated (Sheikh and Molur 2004). Poaching, land conversion, and habitat degradation cause declines in natural prey and habitat of wolves. These conservation challenges have been negatively impacted the long-term survival of wolves.

This study provided deep insights into the trends of livestock rearing and depredation by wolves in northern Pakistan. Since most people live below the poverty line in northern Pakistan, the only livelihood opportunities available to these socially secluded and remote tribal mountain dwellers are livestock rearing and subsistence farming. They prefer keeping small ruminants such as goats and sheep, as they adapt better to tough terrain and mountain landscapes. Similarly, yaks are the preferred animal among large ruminants, owing to their high adaptability to rough terrain and harsh weather. Yaks comprise the major large ruminant stock and are kept for dairy, milk, meat, and riding. Marketing livestock and livestock products helps people meet the monetary requirements of their families (Bagchi and Mishra 2006).

4.4.1 Circumstances that Promote Wolf Attacks on Livestock

Free grazing large herds of domestic stock leaves them vulnerable to wolf attack. Goats and sheep have been identified as most vulnerable on nearly the complete spectrum of livestock available to wolves. This is consistent with past studies that show that medium- and small-sized livestock were more prone to predation as they were easily killed and dragged away by carnivores (Dar et al. 2009; Kabir et al. 2014; Khan et al. 2014). The main reason for the low depredation of cattle is the fact that local people keep cattle in small numbers. The growing trend of livestock herding in the Himalayan range (Bhatnagar et al. 2006) may develop conflict between rural communities and threaten large carnivore species (Polisar et al. 2003).

I observed that the local community used open pastures even within protected areas to graze their livestock. Livestock overgrazing and removal of vegetation for firewood collection by local people has created pressure on pastureland (Schaller and Kang 2008). Mishra et al. (2004) documented the importance of forage competition amongst herbivores and documented out-competition of wild herbivores by livestock. Overgrazing in pasture lands along with illegal hunting depletes the diversity and density of wild ungulate species (Mishra and Madhusudan 2007), compelling wolves to attack available domestic stock within their habitat. Studies confirmed that depletion of natural prey could be a major contributing factor to high predation on livestock (Woodroffe et al. 2005b; Kolowski and Holekamp 2006). The understanding is not explicit because wolves may also have a high predation rate over livestock in areas where they have little access to large numbers of wild prey (Treves et al. 2004). It was observed that poor herding practices had a significant effect on livestock depredation. In summer pastures, approximately 90% of the pastural communities lacked appropriate stables for corralling their livestock at night. Overall, depredation occurred more often in free grazing pastures as livestock are frequently left unattended and kept in non-predator proof night corrals. Domestic livestock that is also poorly guarded is consequently more vulnerable to wolf attacks (Sillero-Zubiri and Laurenson 2001). Throughout the Himalayan region, poor herding practices are among contributing factors to attacks on livestock (Namgail et al. 2007).

The rate of wolf attacks on livestock was also not proportionally distributed throughout the year. Rather, it peaked in the summers followed by autumn, winter, and spring. People have adopted two types of grazing systems in the area across the range of the wolf in northern Pakistan. They spend the four months from July to October in pastures with their livestock. During winters, they keep their livestock in the village under a strict surveillance and guard system. Predation and seasonal changes are also linked because livestock grazing, and herding are influenced by seasonal changes (Sogbohossou et al. 2011; Dar et al. 2009; Kolowski and Holekamp 2006). During the summer season, higher-altitude grazing lands become accessible and herds are grazed for a longer time. They are mostly looked after by one shepherd or even kept free in the pasture, which increases the chances of encounters with predators (Dar et al. 2009; Kabir et al. 2014).

4.4.2 Local Community Livestock Losses due to Disease

The loss of livestock due to disease was far greater than carnivore-induced losses. Our study revealed that economic loss due to disease is more than double compared with losses due to carnivores. Vaccinations were limited and irregular, and generally not administered unless an animal became ill. There were limited animal health facilities in the area, with the capacity to only provide very basic treatment. Disease-caused mortality of livestock has financial consequences on livestock herding at multiple levels: first, disease-caused mortalities are a direct loss of the animal. Second, through reduced production and the additional economic cost of treatment and prevention (Perry et al. 2002; Kock 2005). Another study makes more evident that livestock losses due to disease were more as compared to predation which is not properly documented in human-wildlife conflicts in research studies. These studies dispel the notion that carnivores are always responsible for livestock loss (Graham et al. 2005; Dar et al. 2009; Nyahongo and Røskaft 2011).

4.4.3 Effect of Socio-ecological Factors on the Rate of Livestock Depredation

A combination of several socio-ecological factors explained predation risk in the area. Uneducated people reported a higher number of depredations than educated people as they have adopted farming and livestock holding as the only source of income. On the other hand, people with education also have other sources of income, and also have knowledge about the role of carnivores in our ecosystem and possess positive attitudes towards carnivores. Sometimes, uneducated people intentionally exaggerated the number of livestock killed by wolves in an attempt to get compensation from the wildlife department and non-government organizations (NGOs) working in wildlife conservation. Across northern Pakistan, high depredation rates were associated with a high density of livestock owned. Similarly, valleys that reported more losses due to disease reported more depredation events. This is because sick and weak animals are more susceptible to wolf attacks as they have a reduced capacity to escape from predators. In the Hindukush and Himalayan range, the increase in the domestic livestock population has been certified as proving the increase in the depredation of livestock by large carnivores (Hussain 2009; Din et al. 2013). Education also plays a significant role in improving attitudes towards carnivores and also can enhance the tolerance level of local communities towards wildlife conservation (Røskaft et al. 2007; Ogada et al. 2003; Lindsey et al. 2005).

4.4.4 Perception and Retaliatory Killing of Wolves

The attitudes of local people towards wolves showed that it was the most hostile animal among all the carnivore species of northern Pakistan. Even though the snow leopard (*Panthera uncia*) is among the top predators in northern Pakistan and causes major losses to local pastoralists, local communities still have a softer perception of this predator. This is because there are many donor-funded projects for snow leopard conservation that provide incentives to local peoples in terms of the development of water channels, predator-proof corrals, and vaccinations. This has contributed to a reduction in local people's negative perceptions of snow leopards. Currently, there is no such program for wolf conservation in Pakistan and local communities do not see any value in conserving wolves. We observed during field surveys that people did their best to hunt and kill wolves upon encounter around the villages or summer pastures. There were many cases of killing of wolves, while the worst one was reported from the Basha valley of CKNP where people put dry wood into a den and blocked any exits with rocks. They then used fire to burn the young puppies. A long-standing tradition of killing wolves still prevails in remote valleys of the Karakorum range where the local community appreciates the act. Hunters kill wolves and bring the dead animals back to the village to receive a kilogram of wheat as a reward. These factors contribute to the expiration of wolves from most of its historic ranges and not all scientific guesses can explain its expiration causes. Urgent steps are required to conserve wolf populations.

4.4.5 Protected Areas are Fundamental to Reducing Depredation Events

The valleys with more depredation records were located in the study area where wolves' presence was confirmed through genetic analysis of scats and camera trapping. Within protected areas (PAs), valleys falling in the most suitable habitat for wolves had low rates of livestock depredation as compared to valleys outside PAs, like Khanbari. I assumed that PAs had sufficient numbers of wild prey and restrictions for free grazing of livestock. Suitable habitats outside PAs provide space for wolves. On the other hand, they are open for free livestock grazing and illegal hunting of wild prey. PAs are fundamental to reducing events of depredation on livestock by *C. lupus.* Human-carnivore interaction is a worldwide problem, but it frequently happened in areas with human communities co-exist and share inadequate resources (Distefano 2005).

4.4.6 Management Recommendations

Human interaction with large carnivores over livestock predation is a key rural livelihood and wildlife conservation concern due to the large number of threatened carnivore persecution in retaliation. The willingness to live with large carnivores depends not only on ecological factors, but also on the political opinions, financial constraints and social and cultural convictions of local communities (Treves 2009). Under the prevailing situation, wildlife managers should prioritize the establishment of PAs covering suitable habitat and movement corridors to extend the PA network for the long-term survival of wolf populations in Pakistan (Kabir et al. 2017). At least within protected areas and buffer zones, there is a need to establish a rotational-based grazing system that would be acceptable to both herders and conservationists. Local communities in the park subsist on low incomes and damage caused by carnivores is hard to absorb economically. Therefore, livestock compensation and insurance programs should be launched in these areas to compensate for economic losses. Improved herding practices and secured corrals may reduce livestock losses to some extent, particularly through building predator-proof corrals in summer pastures. Livestock vaccination to reduce disease-caused mortalities would enable communities to bear carnivore-induced losses. As educated people supported wolves more, education facilities should be improved, and environmental education should be initiated in the area. Communities in those vicinities should be taught that both snow leopards and wolves play a key role in maintaining the local ecosystem.

APPENDIX 4.1: HOUSEHOLD LEVEL – HUMAN-WOLF INTERACTION SURVEY

Enumerator name	**Date**
Respondent name	**Village name**
Education	**Age**
Ethnic background	**Occupation**
How many earning members are there in the household?	
Home much agricultural land does your family own?	**Household size**

Predator status

Did you sight any of following species in past 1 year (January–December)?

	Wolf	Leopard	Snow Leopard	Brown Bear	Black Bear	Lynx
Numbers						
Status (Common/Rare/Absent						

Which species population do you wish to increase/maintain/reduce/eliminate from your area?

↑ / → / ↓/x

Wolf	Common Leopard	Snow Leopard	Brown Bear	Black Bear	Lynx

Which species is the most dangerous for livestock, rate 1–6 (from low to high)

Wolf	Common leopard	Snow leopard	Brown bear	Black bear	Lynx

Livestock

How many heads of livestock does your family own?

Livestock	**Goats**	**Sheep**	**Cattle**	**Yak**	**Other**
Number					
Vaccinated					

Mortality due to disease in one year (January–December 2015)

Livestock	**Goats**	**Sheep**	**Cattle**	**Yak**	**Other**
Number					

Livestock sold in one year

Livestock	**Goats**	**Sheep**	**Cattle**	**Yak**	**Other**
Number					
Total income in Rs.					
Slaughtered for domestic consumption					

Predation losses

Crop damage by wildlife in the past one year (estimated economic loss)

Species	**Winter**	**Spring**	**Summer**	**Autumn**

Any other damage by wildlife: ____

Predation in one year

Predator	**Season/ month**	**Location**	**Prey type**	**Prey sex**	**Prey age**	**Guarded (Y/N)**	**Circumstances**

Seasons: Winter (Dec–Feb), Spring (Mar–May), Summer (Jun–Aug), Autumn (Sep–Nov)

Plate 4.1: Interviews and discussions on livestock depredation with local communities

Plate 4.2: Large herds of freely grazing livestock in different study sites

Plate 4.3: Large herds of freely grazing livestock in different study sites

Plate 4.4: Poorly established night corrals for livestock in different study sites

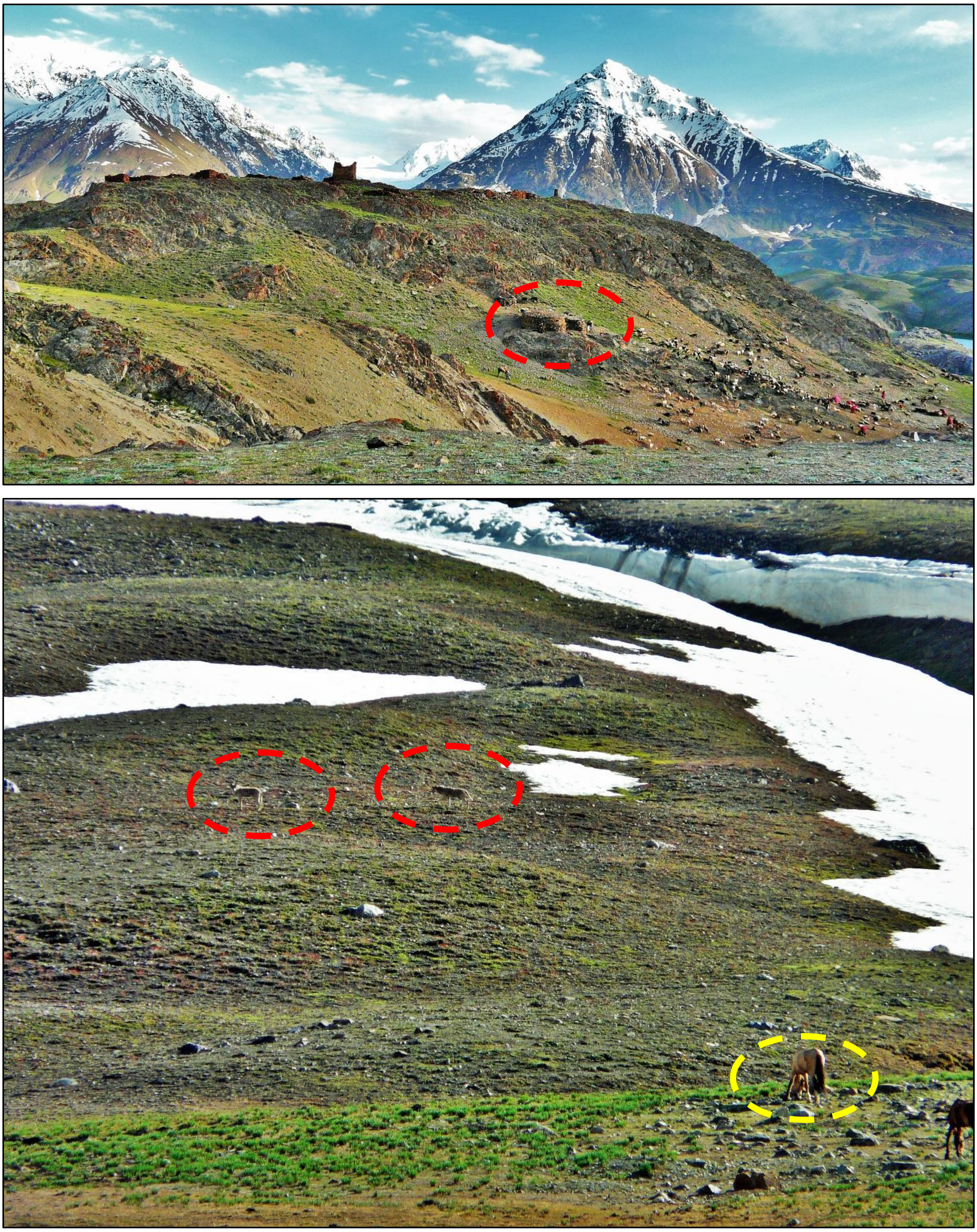


Plate 4.5: Wolves spotted near freely grazing livestock in Broghil National Park

Plate 4.6: Wolves found dead in Chitral, Northern Pakistan

Chapter 5

Feeding Ecology of Grey wolf *(Canis lupus)* in Northern Pakistan

5 Feeding Ecology of Grey wolf (*Canis lupus*) in Northern Pakistan

ABSTRACT

Since wolves are nocturnal and elusive in nature, it is difficult to study their foraging behavior. The study analyzed diet composition and prey selection of wolves in northern Pakistan using scat analysis. We collected 1,186 unidentified scat samples in the suspected range of wolves, covering both the protected and non-protected areas, from 2009 to 2017. Among the samples that yielded DNA in lab analysis, 80 samples were confirmed as belonging to wolves. A microscopic analysis of genetically identified samples indicated that wolves consumed 12 different prey species, including wild and domestic ungulates. Domestic ungulates constituted a substantial part (49%) as domestic goats were the most common prey (19%), followed by sheep and yak (12% each). Wild prey constituted 51% of wolf diet with a major contribution of Himalayan ibex (18%), followed by blue sheep (7%) and Himalayan marmots (7%). A minor fraction of small mammals and birds was also recorded. In terms of biomass consumption, livestock dominated with a contribution of 62.2%, followed by wild ungulates (28.7%) and other wild prey species (9.1%). Higher consumption of livestock by wolf is causing human-wildlife conflict and undermining the support for wolf conservation in Pakistan. This study highlights that livestock loss due to predation by wolves is a serious issue and needs to be addressed to promote acceptance of the latter's presence in Pakistan's rural communities.

Keywords: *Canis lupus*, diet composition, scats analysis, Himalaya, Karakorum, Hindukush

5.1 INTRODUCTION

Mammalian carnivores play a key role in stabilizing the ecosystem through the continuation of energy flow owing to their position in the wildlife community (Ripple et al. 2014; Chetri et al. 2017). Although large carnivores exist in low densities, they play an important ecological role and regulate ecosystems by fulfilling a dual function: they limit the herbivore population through predation, and the meso-carnivores populations through competition, thus maintaining the structure of the ecosystem via a trophic cascade (Carbone and Gittleman 2002; Ritchie and Johnson 2009; Roemer et al. 2009; Wallach et al. 2010; Nakashima et al. 2010; Ripple et al. 2013). Being associated with such crucial ecological processes, top predator elimination can have long-lasting effects on the balance and structure of a whole ecosystem (Miller et al. 2001; Winterbach et al. 2013). Carnivore conservation is, therefore, essential to maintaining smooth ecosystem functioning (Gese 2001). In the 20th century, the globally marked decline in large carnivore populations also necessitated the collection of information on their diet composition and resource selection for their effective management (Schipper et al. 2008; Ripple et al. 2014). Understanding a predator’s diet, its feeding ecology and its effect on population dynamics of prey species is a crucial prerequisite for wildlife managers in areas, where it is the major predator of wild prey species and livestock, to implement effective conservation and management strategies (Oli 1993; Gese 2001; Klare et al. 2011; Derbridge et al. 2012; Chetri et al. 2017).

Mammalian carnivores have large spatial requirements to meet their dietary needs (Ripple et al. 2014). To understand trophic interactions and predator-prey dynamics, it is necessary to determine the diet of wildlife at the ecosystem level (Symondson 2002; Sheppard and Harwood 2005). Similarly, at the species level, an understanding of physiological health, population dynamics and resource selection, predation risk, and dietary habits play an important role in ecosystems (Deagle 2010). Predators may coexist in areas with sufficient prey diversity and numbers by hunting a variety of prey (Karanth and Sunquist 2000; Odden et al. 2010), while predator competition occurs in areas with limited prey species. (Harihar et al. 2011). Diet also has a radical influence on the evolution and ecology of animals and is also essential for studies on large carnivores (Gese 2001; Treves and Karanth 2003). Spatial distribution, hunting success, and prey selection are influenced by the distribution and availability of prey abundance (Henschel and Skinner 1990; Fuller et al. 1992). The feeding pattern can be compared to prey abundance to evaluate functional responses (rate of prey consumption versus abundance) (Bartel and Knowlton 2005).

Knowledge of foraging behavior helps in understanding the ecology of the species and its effect on prey population dynamics (Symondson 2002; Krahn et al. 2007). In some area, prey can be abundant, but the availability of livestock can encourage wolves to attack [livestock] (Hosseini-Zavarei et al 2013). The unavailability of natural prey forces large carnivores to attack domestic animals (Khorozyan et al. 2015). Large predators are more vulnerable to extinction because of their dietary needs (Inskip and Zimmermann 2009) and can become a significant threat to community livelihood and security (Treves and Karanth 2003). Dietary studies tend to investigate carnivore prey profiles and identify predator-induced livestock losses (Treves and Karanth 2003). The damage made by wolves, namely the loss of livestock, remains a point of most contention and thus an examination of diet should be central to scientific research (Spinkyte-Backaitiene and Petelis 2012). Furthermore, persecution of the wolf has been a major issue across its distribution in Pakistan without any detailed assessment of its prey selection but is the reason for its wide-ranging predation on livestock. Studies on diet analysis provide information about prey profile and availability, which also reflects the physiological, morphological, and behavioral adaptation of the predator to successfully pursue, capture, and digest kills (Kok and Nel 2004). It is also a widely applied practice in ecology to understand diet composition, prey-predator interactions, competition between sympatric carnivores, habitat use, and distribution patterns (Klare et al. 2011; Nilsen et al. 2012; Razgour et al. 2011).

Though the importance of diet studies in large terrestrial carnivores is well established, their elusive nature, large home ranges, and rarity makes such studies challenging (Gese 2001; Nilsen et al. 2012; Shehzad et al. 2012). Wolves rely on a wide variety of prey species and their degree of specialization in the diet varies by area, ranging from small to large wild and domestic ungulates, fruits and berries, and even waste (Carnes 2004; Newsome et al. 2016). Wolves are elusive, and, therefore, it is difficult to detect killing sites without the use of a global positioning system (GPS) or radio tracking equipment (Neteler et al. 2012). The wolf’s diet differs both temporally and spatially depending on the seasonal availability of various prey species (Adams et al. 2010). Climatic harshness and tough terrain mostly make it difficult to conduct surveys in the field and to collect information on food availability and habitat quality (Kumar et al. 2011).

Similarly, understanding feeding behavior through questionnaire-based surveys in local communities that share the habitat with the predators lack certainty as results represent subjective public opinion (Mishra 2001; Namgail et al. 2007; Ahmadi et al. 2011). Camera traps have been used to document predation and the behavior of rare, elusive, and nocturnal species, but they have not provided any clue for feeding ecology of wide-ranging predator (Ahumada et al. 2011; Anwar et al. 2011; Espartosa et al. 2011). Therefore, to avoid such complications, diet composition of mammalian predators has been studied by analyzing scats (Oli 1993). It has also been used to find the relative proportion of the main ungulate prey in the diet to analyze the key influence of predators on prey population dynamics (Williams al. 2012; Lafferty et al. 2014), that are difficult to obtain otherwise (Peterson and Ciucci 2003). Conventionally, scat analysis relied on identification of undigested remains such as claws, hooves, bones, hair, and teeth in a predator's scat, as it is the most readily available source of information about its diet and is also accessible to researchers (Symondson 2002; Sheppard and Harwood 2005; Prugh et al. 2005).

Scat analysis is an oft-used non-invasive, relatively inexpensive, and effective approach used for the dietary studies of carnivores (Rana et al. 2005; Edgaonkar 2008; Larter 2013) to generate diet profiles (Klare et al. 2011). In the case of sympatric carnivore species, it has several limitations on reliable identification of scat samples in the field (Ruhe et al. 2008; Farrell et al. 2000). For the diet analysis, experts revealed that where several carnivore species co-exist, sample collection in the field often leads to misclassification (Janecka et al. 2008; Anwar et al. 2011; Shehzad et al. 2012; Wegge et al. 2012; Jumabay-Uulu et al. 2014). Therefore, Jumabay-Uulu et al. (2013) recommend species identification *before* diet analysis as it is the most reliable source of species identification (Lukacs and Burnham 2005; Waits and Paetkau 2005; Lukacs et al. 2007).

The grey wolf is the most controversial predator in the Himalaya-Karakoram mountain ranges, but information about its feeding ecology is limited. It faces several threats, with the main threat to its survival is being retaliatory killing following livestock predation in Pakistan. Knowledge of its foraging behavior is the first step towards solving human-wolf conflicts. Farmers generally exaggerate their losses and managers often do not trust demands’ as actual losses from livestock depredation are difficult to monitor and estimate. The study, therefore, aimed to determine (i) the diet composition of the grey wolf in northern Pakistan (ii) wolves’ dependence on wild animals relative to domestic animals to meet food requirements. It is important to develop a clear understanding of dietary ecology in landscapes with varying levels of human influence to facilitate informed discussions of grey wolf conservation and management.

5.2 MATERIALS AND METHODS

5.2.1 Field Surveys

Field surveys were conducted between 2009 and 2017 in seven national parks in northern Pakistan, including the following protected areas (PAs): Machiara National Park, Musk Deer National Park, Khunjerab National Park, Broghil Valley National Park, Qurumber National Park, Shandoor-Handrab National Park, and Deosai National Park. The surveys were also carried outside the PAs in Phandar Valley, Kotli, Misgar, and Chapursan within the range of wolves (Kabir et al. 2017) in northern Pakistan (Figure 5.2.1). The study covered seven national parks and eight non-protected sites. Survey areas were divided into grids with cells of 5 × 5 km^2 on GIS maps. Survey points were randomly selected within each grid cell and a 50-m radius around each point was searched for wolf and other carnivores' scats. Scats were also searched at camera trap locations and while hiking along livestock and humanmade trails that were known to be used by wildlife (Kabir et al. 2017).

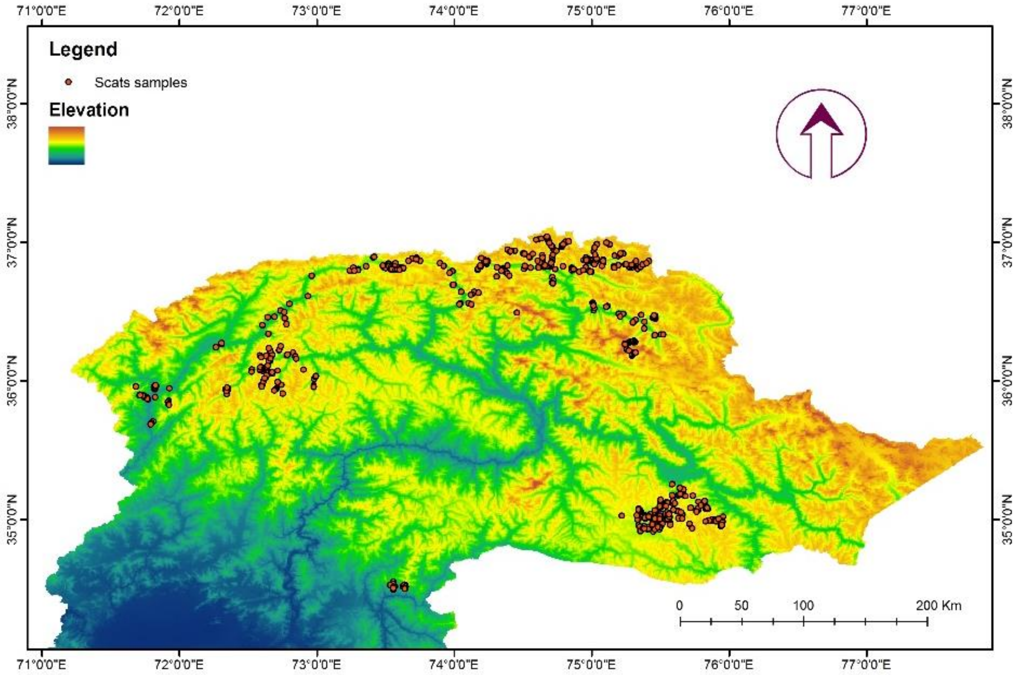


Figure 5.2.1: Locations of scat sample collection in northern Pakistan

5.2.2 Scats Collection and Preservation

Wolf scats were collected following the scat collection and analysis protocol developed by Mukherjee (2001), Odden (2007) and several studies on the feeding ecology of large carnivores (Oli 1993; Mukherjee et al. 1994; Ott et al. 2007; Aryal and

Kreigenhofer 2009; Kabir et al. 2011), with minor modifications. Scat samples from different carnivore species were stored in polyethylene bags which were tagged with dates and GPS coordinates. The substrate, shape (conical and blunt) and color of the scat samples were studied and documented in data sheets (Appendix 5.1). The age of the scat samples was classified as fresh “1 day old”, recent "1–5 days old" and old "older than 5 days". A small portion was stored in 20 ml bottles filled with 95% alcohol. Later in the laboratory, the alcohol was dried, and the samples were preserved to silica gel for further genetic analysis.

5.2.3 Predator Identification

A genetic analysis of collected samples was performed to identify predator species. The Qiagen mini-stool kit was used to extract DNA from scats. DNA was extracted from a 15 mg fecal sample following manufacturer protocols and the DNA was eluted in a final volume of 200 ul. Blank extractions were also performed to examine contamination during the extraction process. A polymerase chain reaction (PCR) was performed on the extracted DNA for amplification of the DNA-specific region of the primer pair V5 template, *i.e.* 12 SV5f (5'-TAGAACAGGCTCCTCAGAG-3 ') and 12SV5R (5'-TTAGATACCCCACTATGC-3 ') and V5 loop of mitochondrial DNA (Shehzad et al. 2012). Sequencing was performed through Next Generation Sequencing (NGS) to confirm the DNA region (amplified deoxyribonucleic acid) (Riaz et al. 2011). OBI tools were used to analyze sequence readings (http://www.prabi.grenoble.fr/trac/OBITools). Of 1,186 analyzed samples of different carnivore species collected from the study area, only 80 were successfully verified. Upon genetic validation of the samples by mitochondrial DNA analyses, genetically confirmed wolf samples were further processed for physical analysis.

5.2.4 Reference Hair Collection (RHC)

Reference hair samples from wild and domestic prey species known to be present in the suspected range of wolves in northern Pakistan were collected in the field and were also provided by the Mammalian Repository of the Zoological Science Division (ZSD), Pakistan Museum of Natural History (PMNH) in Islamabad. A complete tuft of hair from different body parts was collected and verified. Representative samples of all types of hair were collected to avoid distortion of unknown hairs in scat samples. I developed a reference key of 23 prey species found in the study area.

Table 5.2.1: Reference Prey Species Included in Hair Identification Key

	Vernacular name	**Scientific name**
Domestic mammals		
1	Domestic goat	*Capra hircus*
2	Domestic sheep	*Ovis aries*
3	Yak	*Bos grunniens*
4	Cow	*Boss spp.*
5	Dog	*Canis familiaris*
6	Donkey	*Equus asinus*
Wild mammals		
7	Marco Polo sheep	*Ovis ammon polii*
8	Blue sheep	*Pseudois nayaur*
9	Himalayan ibex	*Capra ibex*
10	Urial	*Ovis orientalis vignei*
11	Markhor	*Capra falconeri*
13	Alpine Musk deer	*Moschus chrysogaster*
14	Gray langur	*Semnopithecus schistaceus*
15	Golden jackal	*Canis aureus*
16	Red fox	*Vulpes vulpes*
17	Masked palm civet	*Paguma larvata*
18	Small Indian civet	*Viverricula indica*
19	Stone marten	*Martes foina*
20	Small Indian mongoose	*Herpestes javanicus*
21	Himalayan Marmot	*Marmota caudata*
22	Altai mountain weasel	*Mustela altaica*
23	Cape hare	*Lepus capensis*
24	Kashmir field mouse	*Apodemus rusiges*
25	Royal's pika	*Ochotona roylei*

5.2.5 Scat Analysis

The remains of prey such as hair in scat samples were used for the identification of unknown prey species, using procedure and protocol described by Oli (1993), De Marinis and Asprea (2006) and Kamalakannan (2017). This method is based mainly on microscopic observation of the hair structure, such as the cuticular scale pattern and the medullary structure of the prey hair (De Marinis and Asprea 2006). Scat analysis was performed using the whole mount and scale replication methods. It is a widely used technique for determining the diet composition of various carnivores (Oli 1993; Kaunda and Skinner 2003; Chame 2003; Ott et al. 2007; Aryal and Kreigenhofer 2009).

5.2.5.1 Investigation of Scat Samples

The study used undigested hairs in the scats of wolves to study their diet profile. Genetically identified scat samples were further processed for analysis of wolf diet. The physical analysis started with the separation of undigested materials in scat samples. All scat segments were broken down into small pieces. Undigested remains of prey, including hair, bones, hooves, and nails relevant for identification were separated for further examination. Two sieves, 0.8 mm for large hair and 0.5 mm for short hair, were stacked. Each fragment was segregated apart and washed with slightly warm water and left for 30 minutes to dissolve the mucus. The remains of prey such as teeth, hooves, hair, bones, and feathers were collected using forceps and dried by placing them on blotting paper. The separated remains were then stored in polyethylene bags labeled with the samples' id/tags to avoid confusion among the samples. These remains of undigested scats were then used to make slides and identify prey.

5.2.5.2 Slide Preparation

Slides were prepared using the whole mount and scale replication methods, which are widely used as non-invasive approaches to determine the predator's diet (Oli 1993; Kaunda and Skinner 2003; Chame 2003; Ott et al. 2007; Aryal and Kreigenhofer 2009; Chetri et al. 2017). Slides were made by placing hair in petri dishes that were thoroughly cleaned with 70% ethanol and left for 15–20 minutes to remove mucus and dust particles, and then left to dry completely. Microscopic slides were also cleaned with alcoholic swabs. The study used transparent nail polish as a mounting agent to make slides. Five to seven different hairs from each sample were selected to avoid any bias in the result, *i.e.* slides of all unidentified samples were made. Following the same protocol, slides of reference hair were also developed, which were used as a reference to identify wolves' unknown prey species. The whole mount was prepared by dipping the tuft of hair in 70% ethanol for 15–20 minutes to remove mucus and dust. Permanent slides were prepared using transparent nail polish as the mounting agent. Individual hairs were separated to avoid tangles of hair on the slide. A clean microscopic slide was used to spread hair evenly on the slide mounted with a mounting agent, after which a coverslip was placed on top and allowed to dry.

5.2.5.3 Scale Replication of Hairs

Prey identification was primarily based on the study of cuticular hair scale patterns previously used in several studies (Oli 1993; Kaunda and Skinner 2003; Ott et al. 2007). The clean hairs were placed vertically with respect to the elongated axis of the slides, so that hair protrude from the edge of the slide, further it was effortlessly picked up for the removal. The clean slides were smeared with colorless nail polish (having a refractive index close to glass slides) and the sorted hairs (two to five) were pressed and held straight on the slide using forceps. Hairs were removed once the slides were dry and the casts were observed under the microscope to observe the medullary structure and cuticle. The slides were observed at a zoom level of X10 and X40, X100 to study the structure and pattern of the hair.

5.2.5.4 Hair study and Microphotography

Photographic reference key has been proved to be an easier and more convenient tool for identifying unknown hair in scats (Oli 1993), which was developed to identify prey items from wolves' scats by comparing hair patterns. Slides of hair samples were examined and photographed under the microscope (Nikon, Model ECLIPSE E200 ED MV R) at X10, X40, and X100 magnification, where cortex and medullar patterns were distinct and clearly visible. Microphotographs of the representative scale and medulla patterns were taken to identify the unknown hair obtained from scats. The photographs were taken along the length of reference hair for every sample since scale patterns can vary considerably. The reference key was used for identification of prey species.

5.2.6 Prey Identification

Prey species were identified by comparing the cuticular characteristics of the hairs (Verma and Joshi 2012) such as the position of scale, scale pattern, margin of the scale and the distance between the margins of the scale. Similarly, medullary characteristics like width composition, structure and shape of medullar margins, and shape of hair cross-sections recovered from scats were compared with photographic reference hair key (Malo et al. 2004; Mukherjee et al. 1994). Prey items in the scat samples were identified after a detailed analysis of hair with a photographic key. The key prey species identified through scat analysis were pooled into different categories such as domestic animals, wild ungulates, meso-mammals and small mammals.

5.2.7 Statistical Analysis

As described by Sabrina (2006) and Klare et al. (2011), i calculated both biomass consumption and frequency of occurrence of prey species in the diet.

Frequency of occurrence demonstrates the encounter rate of the prey and calculation of biomass indicates the relative importance of a prey item. Data were tabulated in absolute numbers (AF: number of occurrences of each prey, when present/total number of scats × 100) and relative (RF: number of occurrences of each prey, when present/total number of occurrences of all prey species × 100) frequency of occurrence of each prey species (Lucherini et al. 1995). Ackerman's conversion factor was used to estimate the number and biomass of prey consumed and their proportions relative to total diet (Ackerman et al. 1984; Ramesh et al. 2012; Selvan et al. 2013). Mean body weights of prey species were derived from studies by Sankar and Johnsingh (2002) and Ramesh et al. (2008). Biomass consumption was estimated using the linear relationship developed by Ackerman et al. (1984) as below:

$$Y = 1.98 + 0.035\,X$$

Where:

Y = is weight of prey consumed per scat

X = is average body weight of prey

5.3 RESULTS

5.3.1 Prey Selection

During the survey, we collected 1,186 scat samples of different carnivore species found within the suspected distribution range of wolves and 245 out of the samples were assumed to belong to wolves. Among the successfully amplified samples, a genetic analysis confirmed only 80 (33%) samples with a positive identification of the grey wolf. These samples were further used for diet analysis.

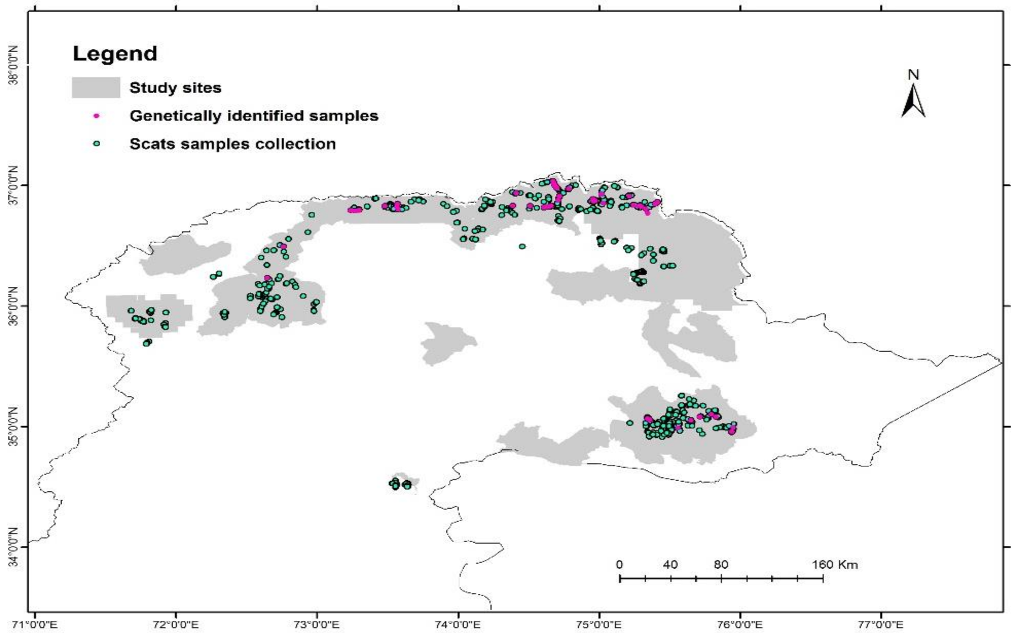


Figure 5.3.1: Locations of genetically identified scats samples of the grey wolf

The identity of the prey species, whose hairs were present in the scat samples, was ascertained by using a photographic reference key of potential prey species found in the range of the grey wolf (Appendix 5.2). The prey profile indicated several prey species. In wolf diet, 12 prey species comprising small mammals (Royle’s pika) to large domestic yak were identified (Table 5.3.1). In terms of frequency of occurrence, domestic animals made up the largest share at 41%, followed by wild ungulates (23%) and other wild prey, 17%. Among wild ungulates, ibex made the largest contribution (11%), followed by blue sheep (7%) and Marco Polo sheep (5%). In domestic animals, goats (19%) were the predominant prey, followed by sheep (15%), yak (12%), and cattle (5%) (Figure 5.3.2). Meso-mammals also constituted an important part of wolf diet, including Himalayan marmot, cape hare, and small Indian civet. Among the meso-mammals, the marmot (7%) was the most frequent prey, followed by cape hare (4%).

Table 5.3.1: Frequency of occurrence of prey in wolf diet in northern Pakistan

	Prey species	**Frequency of occurrence**	**% Frequency**
	Domestic animals	**36**	**51**
1.	Domestic goat	14	19
2.	Domestic sheep	9	15
3.	Yak	9	12
4.	Cow	4	5
	Wild ungulates	**23**	**31**
5.	Marco Polo sheep	4	5
6.	Blue sheep	6	8
7.	Himalayan ibex	13	18
	Other wild prey species	**15**	**17**
8.	Red fox	1	1
9.	Marmot	7	7
10.	Cape hare	3	4
11.	Royle’s pika	1	1
12.	Birds	3	4
13.	Unidentified	9	NA

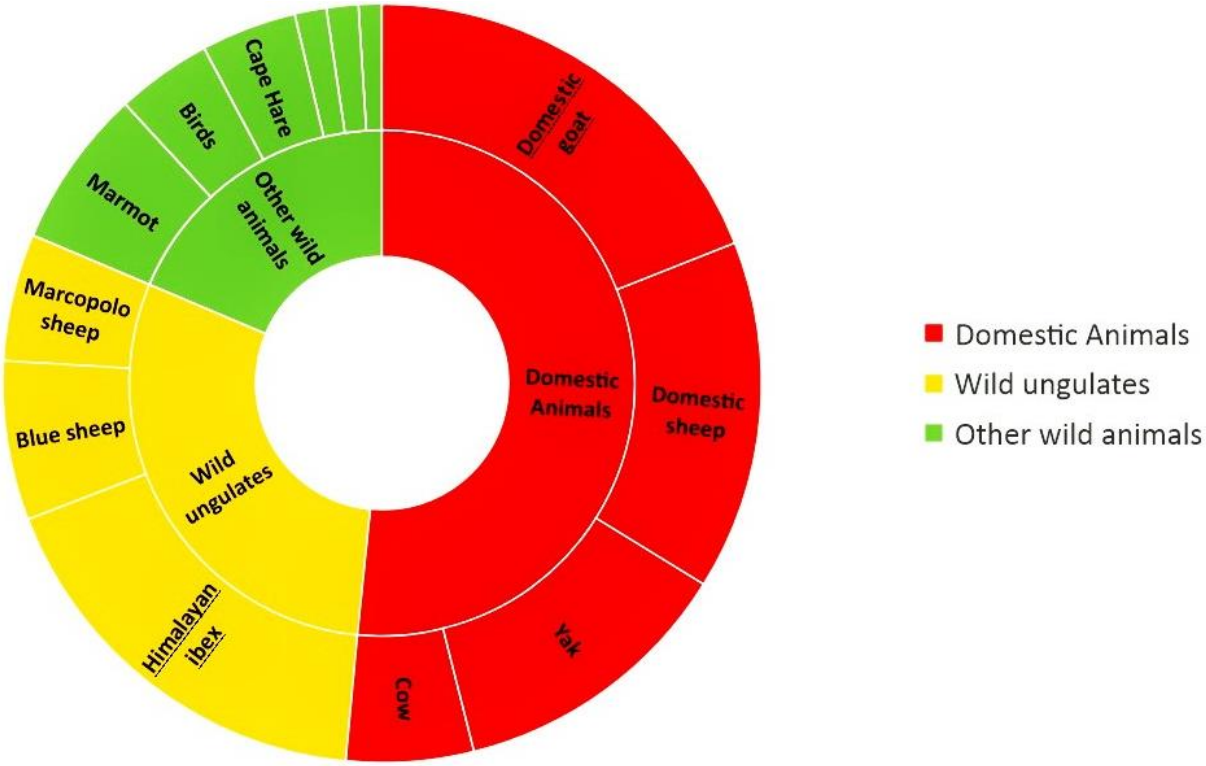


Figure 5.3.2: Contribution of domestic versus wild prey in wolf diet in northern Pakistan

5.3.2 Biomass Consumption by Wolves

The estimated consumed biomass of domestic animals was 62.2%, followed by wild ungulates (28.7%) and other wild prey species (9.1%) (Table 5.3.2.). Among domestic animals, yak was the predominant prey representing 32.4% of biomass consumption, followed by the domestic goat (11.5%) and the cow (10.4%). In terms of biomass consumption of wild prey species, the Himalayan ibex (17.4%) was the most consumed wild ungulate, while the marmot (4.3%) was the dominant contributor in small mammals.

Table 5.3.2: Calculated biomass of prey species in the diet of wolf in northern Pakistan

Vernacular Name	Weight (kg) A	Biomass Consumed per scat (kg) B	No. of scats	Total Biomass Consumed (kg) BC	% Consumption (BC/ S [BC] x 100)
Domestic goat	25	2.86	14	40.0	11.5
Domestic sheep	30	3.03	9	27.3	7.9
Domestic yak	300	12.48	9	112.3	32.4
Cow	200	8.98	4	35.9	10.4
Marco Polo sheep	40	3.38	4	13.5	3.9
Blue sheep	65	4.26	6	25.5	7.4
Himalayan ibex	76	4.64	13	60.3	17.4
Red fox	10	2.33	1	2.3	0.7
Himalayan marmot	4.5	2.14	7	15	4.3
Cape hare	4	2.12	3	6.4	1.8
Royle’s pika	0.2	1.99	1	2.0	0.6
Birds	0.3	1.99	3	6.0	1.7

A = Assumed weight (kg) of the prey species

B = Estimated weight of prey consumed per scat (B = 1.98 + 0.035. A) (Ackerman et al. 1984)

C = Number of scats in which prey species were identified

D = Biomass consumed (BxC)

E = Percentage consumption (BxC/ S [BxC]. 100)

5.4 DISCUSSION

The prey spectrum for wolves in northern Pakistan was found to be as wide as the diversity and availability of prey species in the study area. It is characterized by the occurrence of domestic and wild ungulates and a small fraction of other food categories. Domestic ungulates, principally goat and sheep, constituted a substantial part of wolf diet (both in terms of frequency and biomass) and consumed more than any other category of prey species. Earlier studies on the feeding ecology of grey wolves in Pakistan report similar predation pattern on domestic ungulates (Schaller 1976; Anwar et al. 2012; Shabir et al. 2013). Similarly, other studies in various regions of their geographical distribution such as Afghanistan (WCS 2008), Bhutan (Jamtsho 2017), India (Jhala and Jethva 2003; Maurya et al. 2013), central Iran (Hosseini-Zavarei et al. 2013), Kyrgyzstan (Jumabay-Uulu et al. 2014), and Nepal (Chetri et al. 2017) confirm that wolf diet is dominated by domestic ungulates. The wolf is the most reviled predator in northern Pakistan because of predation on livestock. Public reports also claim massive predation of wolves on livestock (Chapter 04), sometimes even more than other large carnivore species such as the snow leopard *(Panthera uncia)*, Himalayan black bear *(Ursus thibetanus spp.)* and Eurasian lynx *(lynx lynx)* (Mishra 1997; Namgail et al. 2007; Abbas et al. 2013; Khan et al. (2014). In northeastern Iran, wolves kill 5–6 times more livestock than leopards do (Farhadinia et al. 2017).

Wild ungulates were the second most consumed prey by wolves in northern Pakistan. The Himalayan ibex, Marco Polo sheep, and blue sheep were found to be important prey, together with other small to medium sized mammals. The blue sheep was the main prey contributing 50% of wolf diet in Bhutan (Jamtsho 2017). Similarly, wild ungulates formed 42% of wolf diet with the highest contribution of ibex (24%), followed by Marco Polo sheep (18%) diet in Afghanistan (WCS 2008). Wild ungulates also formed a relatively larger portion of wolf diet in the Pamir range of northwest China (Wang et al. 2014). Marmots and hare were frequently encountered small mammals, suggesting the adaptability of wolves to survive when optimal prey was not present. Small prey such as marmots, hares, pika, and birds are also of seasonal importance in the wolf’s diet (Mech and Boitani 2003).

The relative contribution of domestic and wild ungulates revealed that they formed a major portion of wolf diet in northern Pakistan. This indicates the availability of ungulate species to wolves in northern Pakistan. Almost all ungulate prey species are hunted by wolves in their range, and it appears wolves adapt skillfully in each local area to kill particular prey species (Fuller et al. 2003; Sand et al. 2012b). Many studies have been conducted in recent decades to determine the feeding behavior of wolves, and most of them conclude that the wolf's main prey is large- and medium-sized ungulates (Mattioli et al. 2011; Chetri et al. 2017. Newsome et. al (2016) compiled data from 177 published studies and report that large- and medium-sized ungulates form a substantial portion of wolves' global range across Eurasia and North America (5.4.1).

Scat analysis is an integral part of wolf ecological studies and can provide essential or complementary information on wolf diet that is otherwise difficult to obtain (Peterson and Ciucci 2003). For diet analysis, a reasonable, genetically confirmed sample size is essential to explore actual prey profile. Diet descriptions have been based on as few as 10 samples to more than 1,000 (Pontier et al. 2002; Sinclair and Zeppelin 2002; Zabala and Zuberogoitia 2003). In previous studies, samples used for wolf diet analysis vary across different bioregions such as 10 in Baltistan, Pakistan (Anwar et al. 2012), 11 in the Pamirs of northwest China (Wang et al. 2014), 25 in Chitral, Pakistan (Shabir et al. 2013), 36 in Kyrgyzstan (Jumabay-Uulu et al. 2013), 54 in Wangchuck Centiennial National Park, Bhutan (Jamtsho 2017), 57 in Nepal (Chetri et al. 2017), 63 in Chitral and Khunjerab, Pakistan (Schaller 1976), 72 in Turkey (Capitani et al. 2004), 84 in Wahkhan, Afghanistan (WCS 2008), 132 in Iran (Hosseini-Zavarei et al. 2013), 303 in Maharashtra, India (Kamlesh et al. 2011), and 1,246 in Gujrat, India (Jethva and Jhala 2004).

In fact, genetic analysis revealed that the majority of scat samples that were assumed to belong to wolves were poorly identified. The confirmation of scat samples through genetic analysis added credibility to the diet study. Another study in the central Himalayas, Nepal, reports only 57 (24%) were successfully confirmed to be from wolves among the 236 putative wolf scats (Chetri et al. 2017). In more than 90% of earlier studies, samples have been collected and identified based on scat size, color, shape, and other signs, including pugmarks, markings and field experience. Without genetic confirmation of scat samples, several studies on the foraging behavior of large carnivores, their prey preference, conflict, etc. can be doubtful (Weiskopf et al. 2016; Chetri et al. 2017) and consequently, derived prey profile and biomass consumption of predators remains erroneous. The main reasons for the high rate of imperfectly identified samples, such as harsh weather conditions, may sharply change the shape and color of scats in the field. Under other conditions, when the area occupied by several species with similar taxonomic groups such as the fox, domestic dog, and jackal in northern Pakistan, make it more difficult to accurately identify species. Species identification based on field experience, where other species of large carnivores are present, can often be misleading (Janecka et al. 2008).

Feeding ecology studies are a central task to facilitate the conservation of carnivore species in landscapes with varying levels of human influence. Wolves do not suffer solely from significant habitat loss, but also from dwindling prey populations in the remaining habitat, especially outside PAs. It is, therefore, not surprising that wolves feed more on domestic prey than on natural prey in Pakistan. Intensive farming practices have created food competition with wild ungulates and have been recognized as a significant issue of grazing competition, which has resulted in a shortage of food resources, and, consequently a decline in the population of wild prey in the region (Forsyth 2000; Prins 2000; Bhatnagar and Mathur 2001; Ashraf et al. 2014). Wild ungulates usually have a disadvantage compared to domestic livestock as their abundance is often far less than that of domestic animals that are left freely in open pastures for grazing, which results in foraging competition and replaced wild ungulates (Fankhauser 2004; Sillero-Zubiri and Laurenson 2002). Thus, high consumption of domestic ungulates occurs due to the unavailability of wild ungulates (Vos 2000) because in areas where domestic and wild ungulates coexist, predators prefer wild prey as compared to domestic species (Gazzola et al. 2005; Ansorge et al. 2006). Sometimes, although a healthy prey base is available, freely grazing, large-sized herds of domestic animals become easy prey for wolves in the area and certain individuals (problem animals) which can be old, attack livestock intensively. Therefore, the availability of livestock as an alternative food source improves the survival of large carnivores in a human-dominated landscape, though it results in conflict between rural communities and conservationists due to the financial losses involved (Treves and Karanth 2003; Patterson et al. 2004; Shehzad et al. 2015).

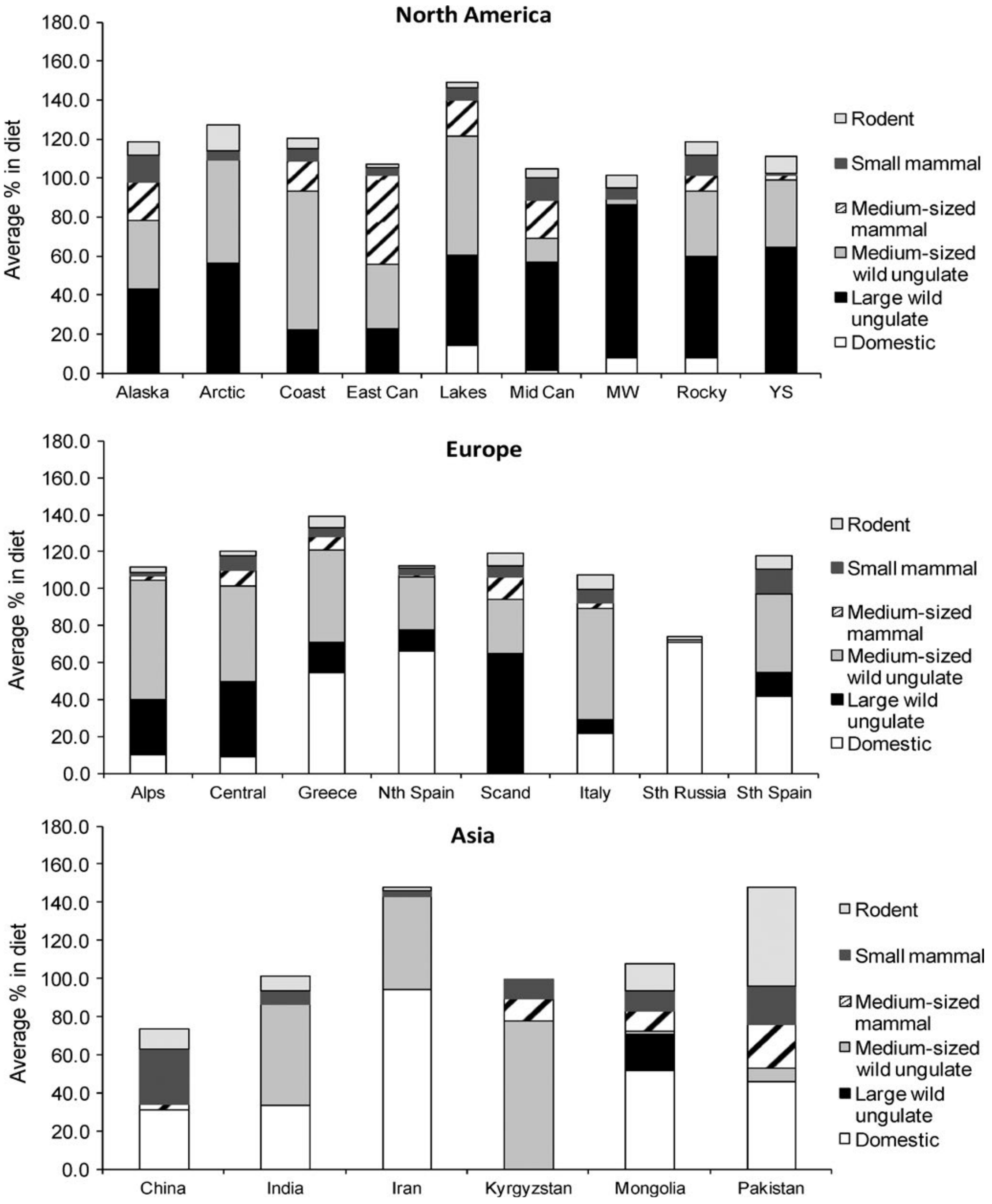


Figure 5.4.1: A summary of 177 studies from different bioregions of three continents, North America (n = 77), Europe (n = 85) and Asia (n = 15) illustrated that domestic and wild ungulates constituted major portion of wolf diet across its range (Newsome et. al 2016).

5.4.1 Implications for Conservation

Understanding the feeding behavior of predators is a key element in the effective management of wild ungulates and carnivores, in understanding predator-prey interactions (Kunkel et al. 2006; Smith et al. 2004). The identified prey profiles confirmed that livestock constituted a significant portion of the wolf's diet, indicating a high level of human-carnivore conflict in the study area. The questionnaire survey also revealed that the wolf is the most damaging predator and is among the most hated wild carnivores in northern Pakistan. Wolves prey on livestock, and, consequently huge economic losses are a major challenge and obstacle for conservation in Pakistan and could potentially create a conflict of interest between farmers and conservation objectives. Furthermore, it becomes important when livestock makes up the bulk of the carnivore's diet while also being the main source of income for farmers.

Depleted prey base, poor livestock husbandry, and improper night corrals are widely seen across the study area. Various studies have revealed that low density of natural prey can contribute significantly to high rates of livestock depredation (Meriggi and Lovari 1996; Woodroffe et al. 2007). When the natural prey population is restored, wolf attacks on domestic animals may decline, when domestic animals are not readily available or abundant (Poulle et al. 1997; Mech and Boitani 2003; Suryawanshi et al. 2013; Hosseini-Zavarei et al. 2013). The availability of wild prey is important for the effective conservation of wolf populations and to ensure coexistence with humans. A prolonged and participatory programme of rotational grazing is required to overcome foraging competition between domestic and wild ungulate species. Livestock removal from their natural habitat also decreases food competition, and it is recommended that the habitat of wolves not be overstocked with domestic ungulates. I also recommend the study of seasonal variation in the diet composition of the grey wolf in Pakistan.

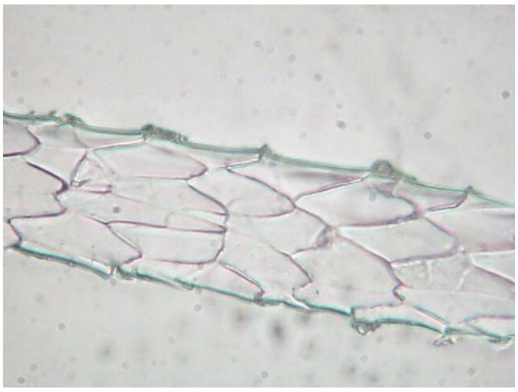
APPENDIX 5.1: DATA SHEET USED IN SCATS COLLECTION

ID:	**Species:**	**Location:**	**Collected by:**
Date:	**Grid:**	**Station/Point:**	**Substrate:**
Latitude (N): ☐☐.☐☐☐☐☐☐		**Longitude (E):** ☐☐.☐☐☐☐☐☐	
Habitat: **(A)**Scrub **(B)**Forest **(C)**Pasture **(D)**Barren **(E)**Agriculture/Plantation			
Topography: **(A)**Ridge **(B)**Cliff base **(C)**Draw **(D)**Valley **(E)**Saddle **(F)**Plateau			
Disturbance: **(A)**None **(B)**Low **(C)**Medium **(E)**High			
Scat collected: **(A)** While in study area **(B)** In buffer area of camera station **(C)** In view of camera (D)In Occupancy Point			
Type: **(A)**Same day **(B)**2–3 days old **(C)**One week Old **(D)**< 1 month **(E)**> 1 month			
Comments:			

ID:	**Species:**	**Location:**	**Collected by:**
Date:	**Grid:**	**Station/Point:**	**Substrate:**
Latitude (N): ☐☐.☐☐☐☐☐☐		**Longitude (E):** ☐☐.☐☐☐☐☐☐	
Habitat: **(A)**Scrub **(B)**Forest **(C)**Pasture **(D)**Barren **(E)**Agriculture/Plantation			
Topography: **(A)**Ridge **(B)**Cliff base **(C)**Draw **(D)**Valley **(E)**Saddle **(F)**Plateau			
Disturbance: **(A)** None **(B)** Low **(C)** Medium **(E)** High			
Scat collected: **(A)**While in study area **(B)**In buffer area of camera station **(C)**In view of camera (D)In Occupancy Point			
Type: **(A)**Same day **(B)**2–3 days old **(C)**One week Old **(D)**< 1 month **(E)**> 1 month			
Comments:			

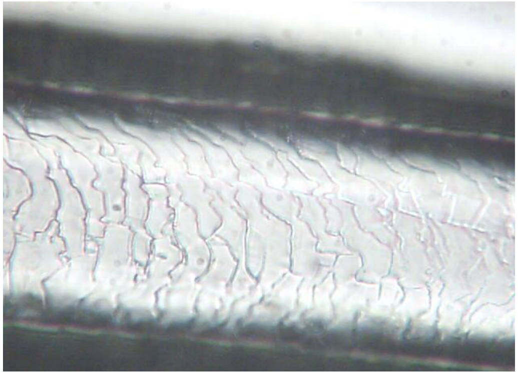
ID:	**Species:**	**Location:**	**Collected by:**
Date:	**Grid:**	**Station/Point:**	**Substrate:**
Latitude (N): □□.□□□□□□		**Longitude (E):** □□.□□□□□□	
Habitat: (A) Scrub **(B)**Forest **(C)**Pasture **(D)**Barren **(E)**Agriculture/Plantation			
Topography: (A) Ridge **(B)**Cliff base **(C)**Draw **(D)**Valley **(E)**Saddle **(F)**Plateau			
Disturbance: (A) None **(B)** Low **(C)** Medium **(E)** High			
Scat collected: (A) While in study area **(B)**In buffer area of camera station **(C)**In view of camera (D)In Occupancy Point			
Type: (A) Same day **(B)**2–3 days old **(C)**One week Old **(D)**< 1 month **(E)**> 1 month			
Comments:			

APPENDIX 5.2: PHOTOGRAPHIC REFERENCE KEY OF PREY SPECIES FOUND IN THE RANGE OF GREY WOLF

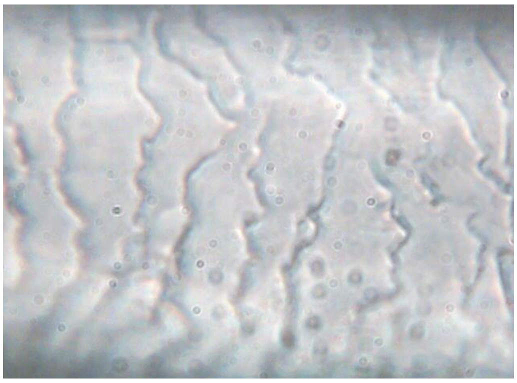


Indian red fox: Scale Replication: negative impression at mid shaft region (40X)

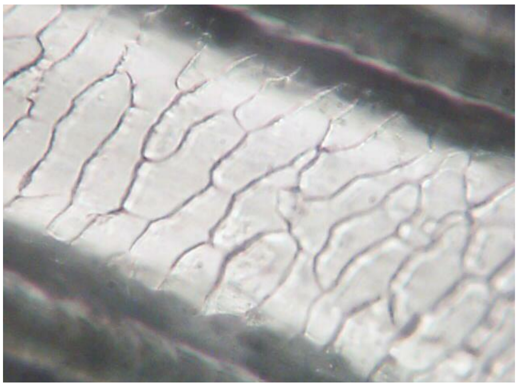
Indian red fox: Whole mount: negative impression at mid shaft region (40X)



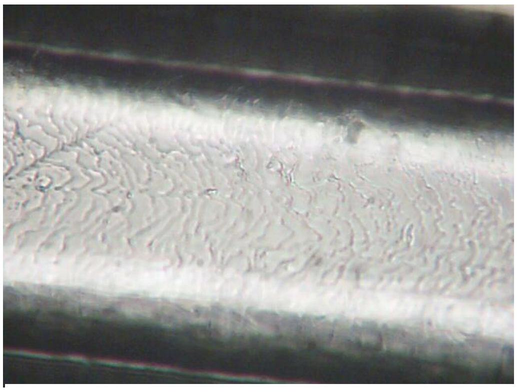
Domestic cow: Scale Replication: negative impression at mid shaft region (40X)



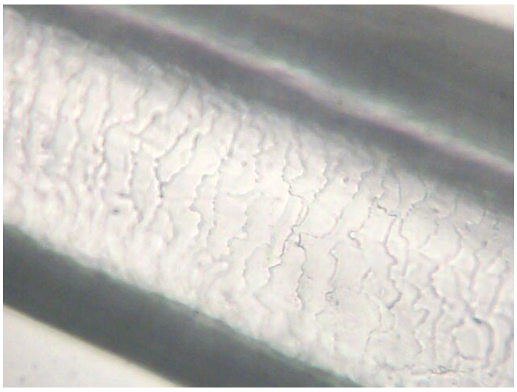
Domestic cow: Scale Replication: negative impression at mid shaft region (100X)



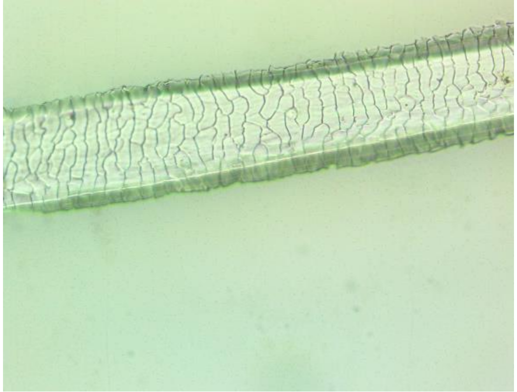
Domestic sheep: Scale Replication: negative impression at mid shaft region (40X)



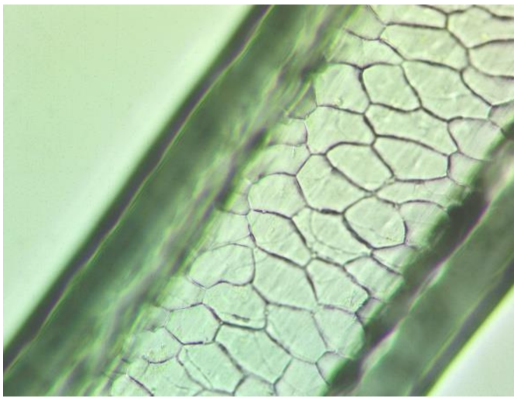
Small Indian mongoose: Scale Replication: negative impression at mid shaft region (40X)



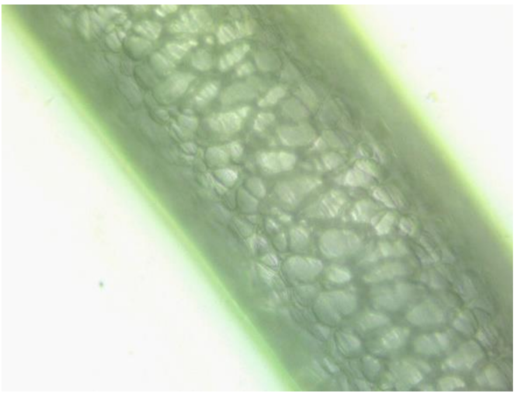
Domestic goat: Scale Replication: negative impression at mid shaft region (100X)



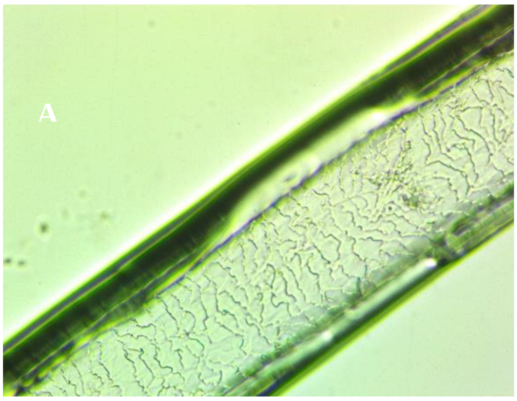
Domestic goat: Scale Replication: negative impression at mid shaft region (40X)



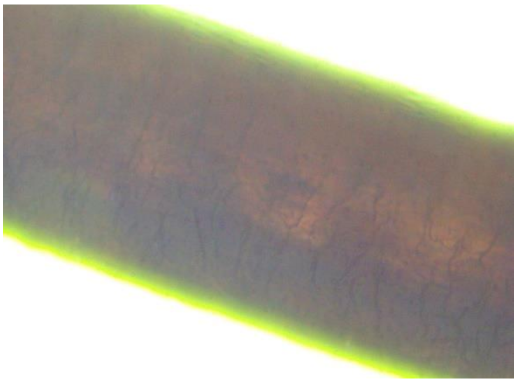
Marcopolo sheep: Scale Replication: negative impression at mid shaft region (40X)



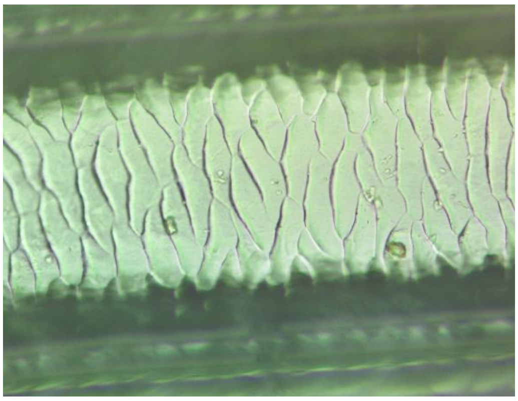
Marcopolo sheep: Whole mount: negative impression at mid shaft region (40X)



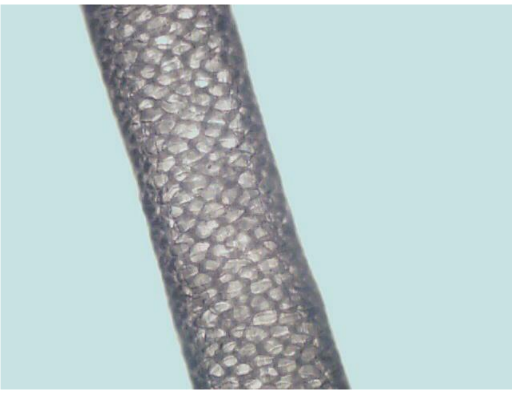
Yak: Scale Replication: negative impression at mid shaft region (40X)



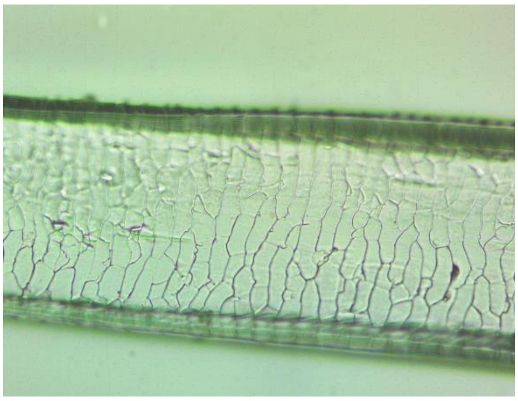
Yak: Whole mount: negative impression at mid shaft region (40X)



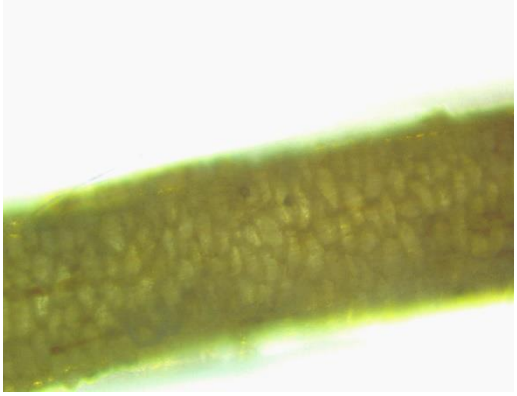
Musk deer: Scale Replication: negative impression at mid shaft region (100 X)



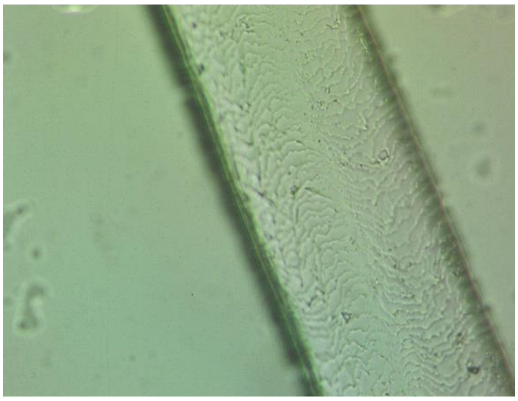
Musk deer: Whole mount: negative impression at mid shaft region (40X)



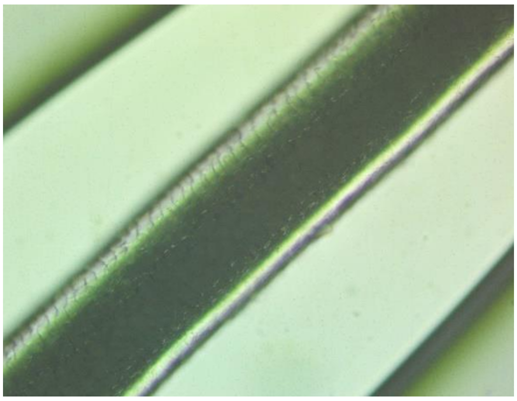
Markhor: Scale Replication: negative impression at mid shaft region (40 X)



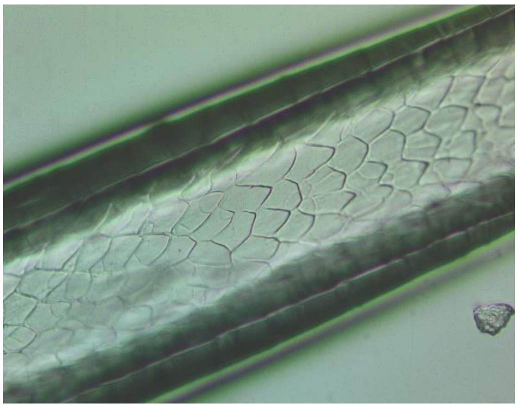
Markhor: Whole mount: negative impression at mid shaft region (40 X)



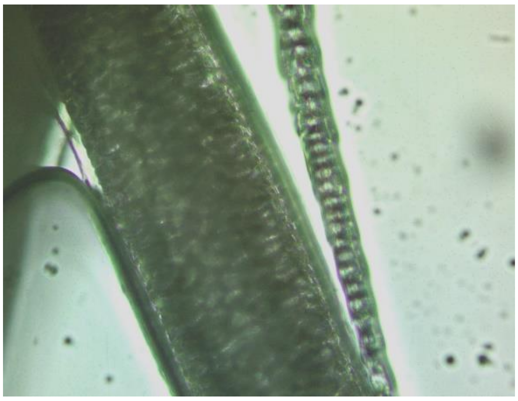
Himalayan Ibex: Scale Replication: negative impression at mid shaft region (40 X)



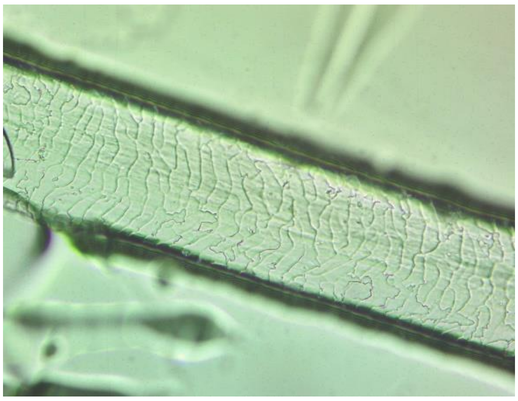
Himalayan Ibex: Whole mount: negative impression at mid shaft region (40 X)



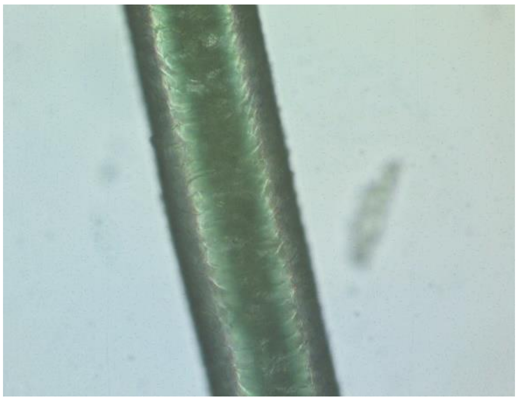
Small Indian civet: Scale Replication: at mid shaft region (40X)



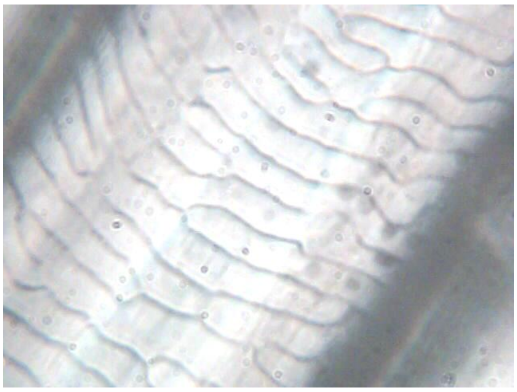
Small Indian civet: Whole mount: negative impression at mid shaft region (40 X)



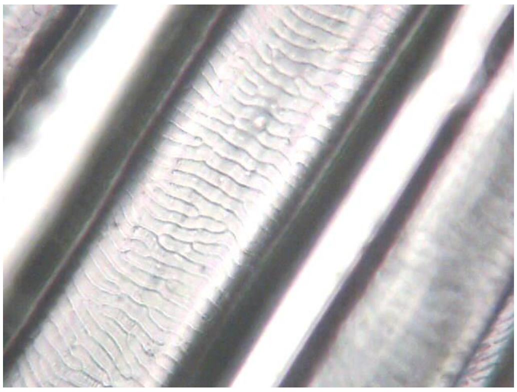
Marmot: Scale Replication: negative impression at mid shaft region (40X)



Marmot: Whole mount: negative impression at mid shaft region (40X)

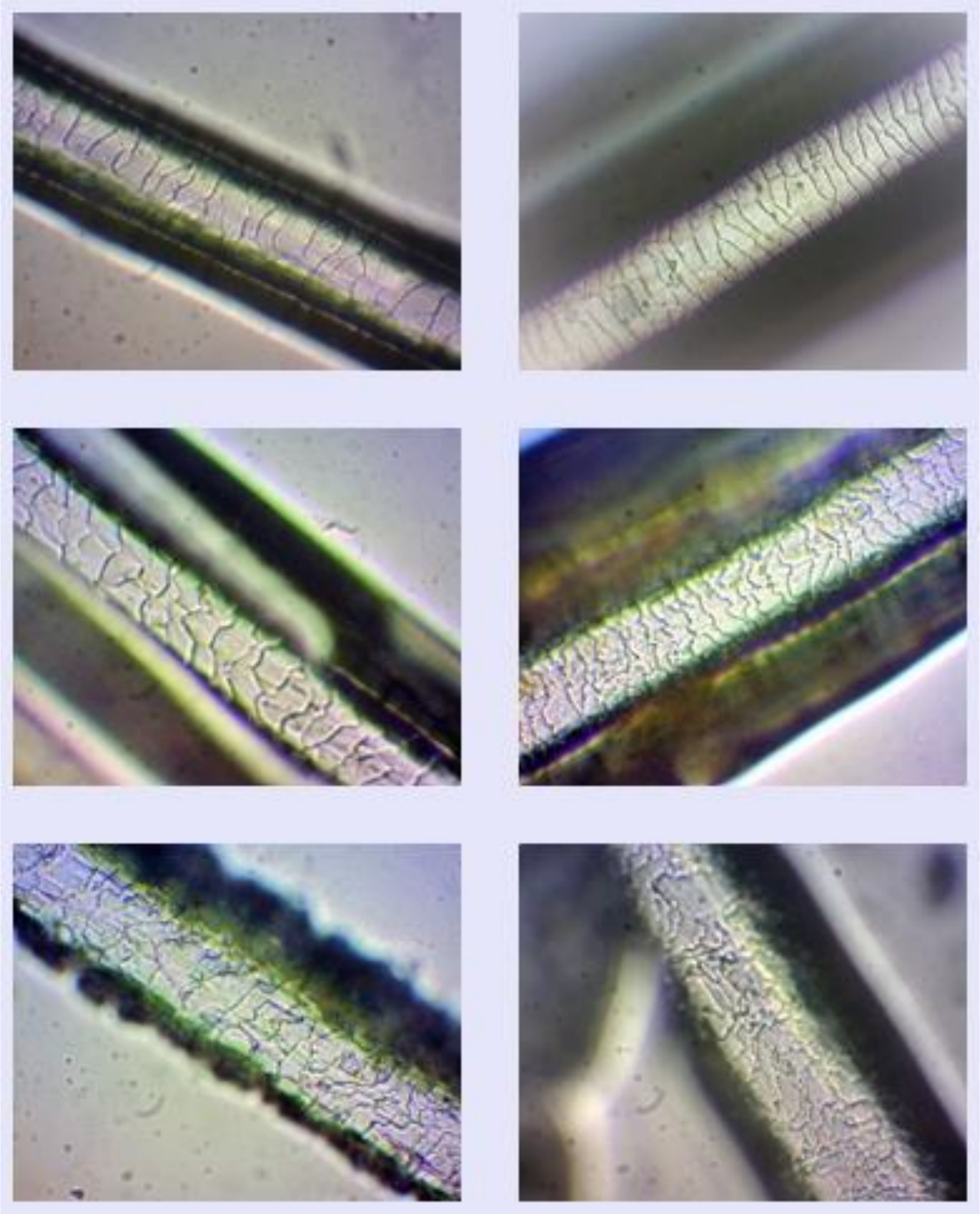


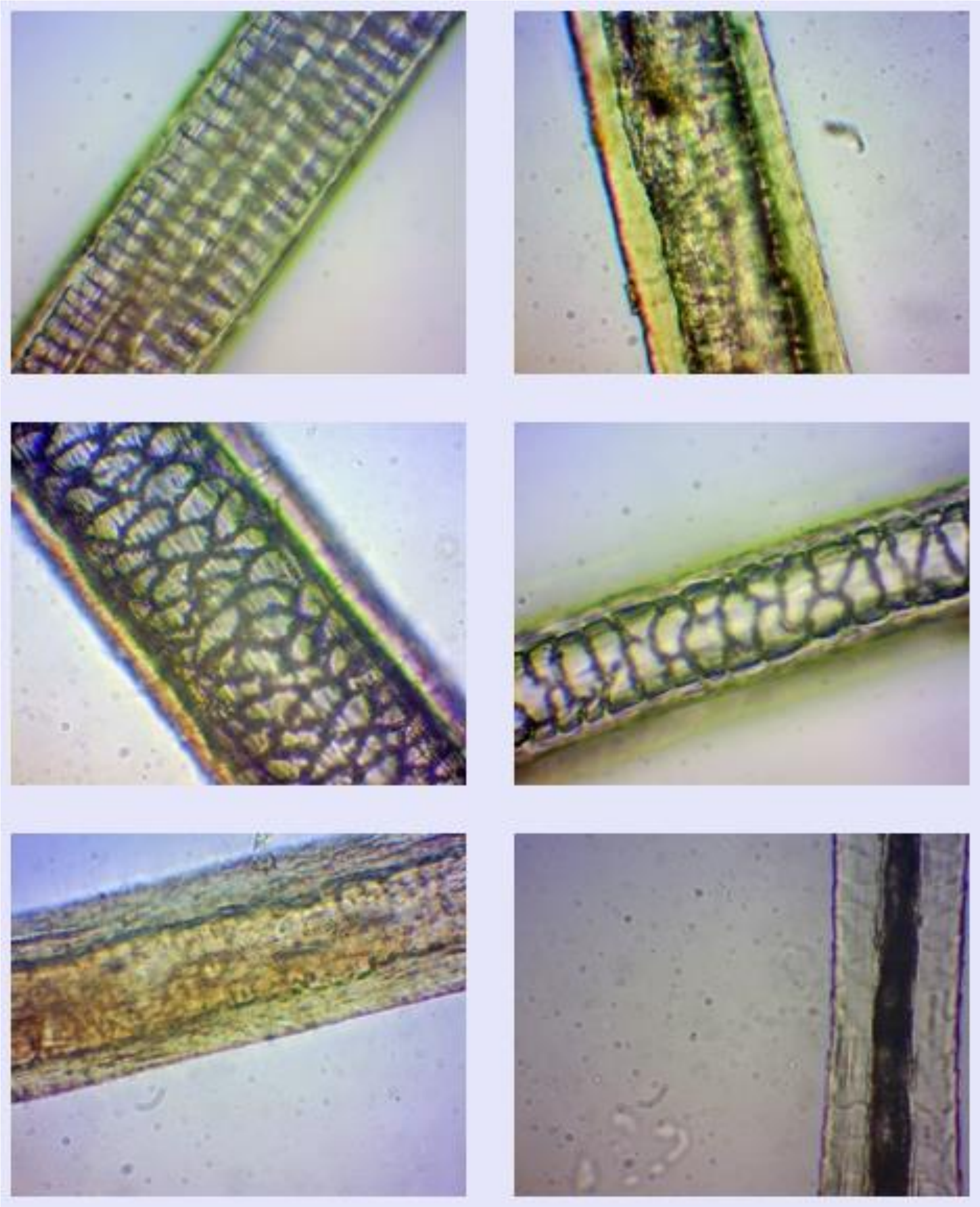
Cape hare: Scale Replication: negative impression at mid shaft region (100 X)

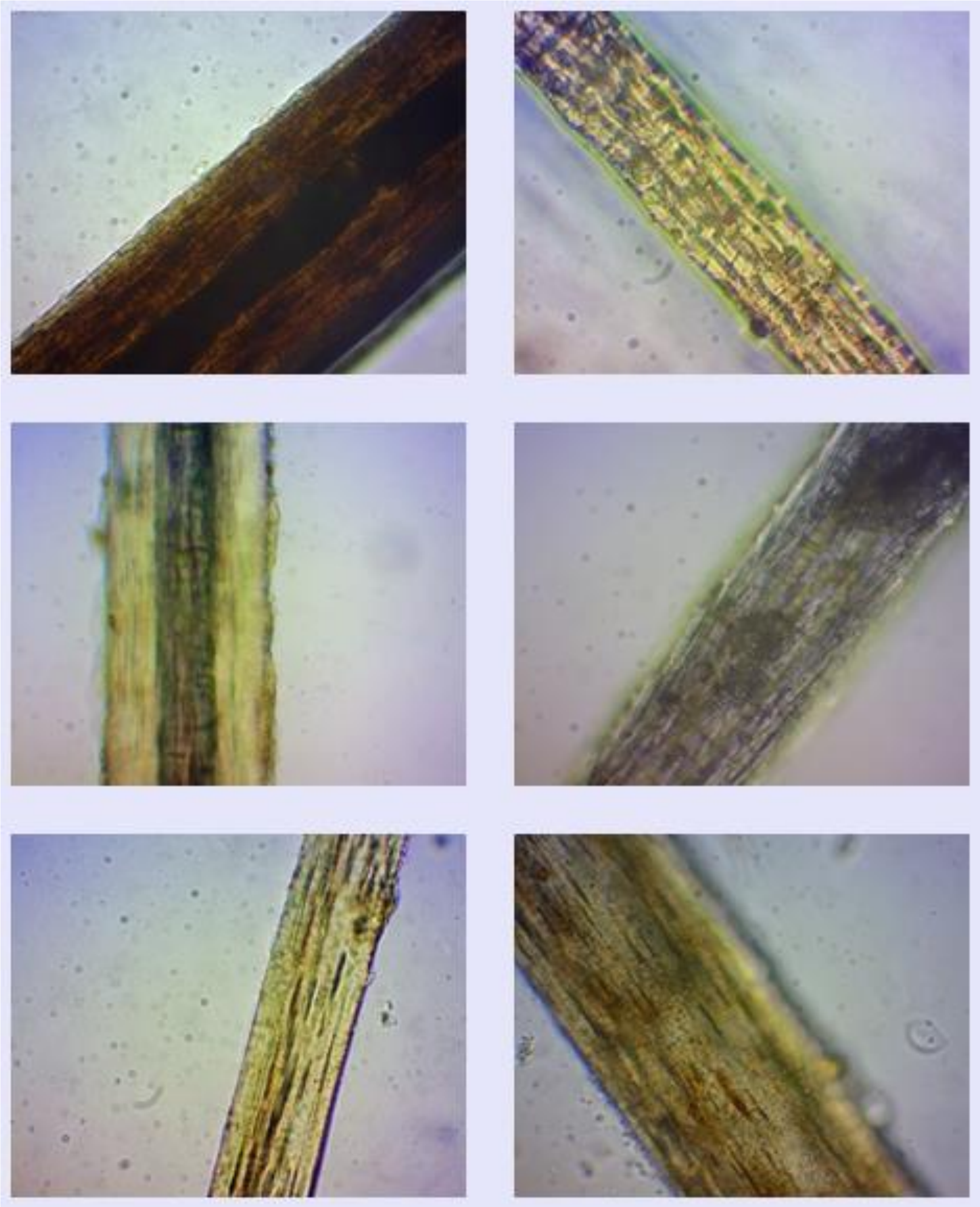


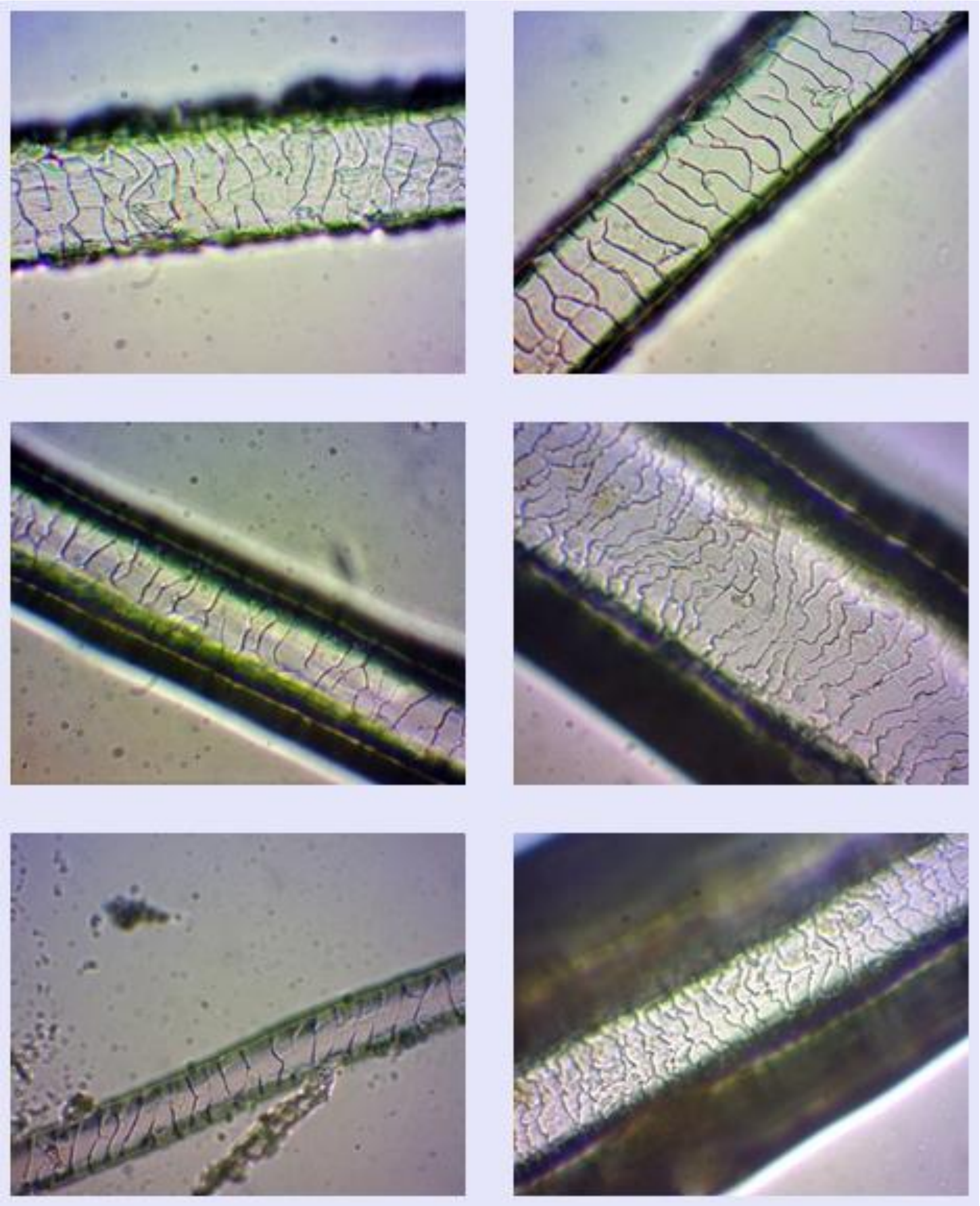
Cape hare: Scale Replication: negative impression at mid shaft region (40X)

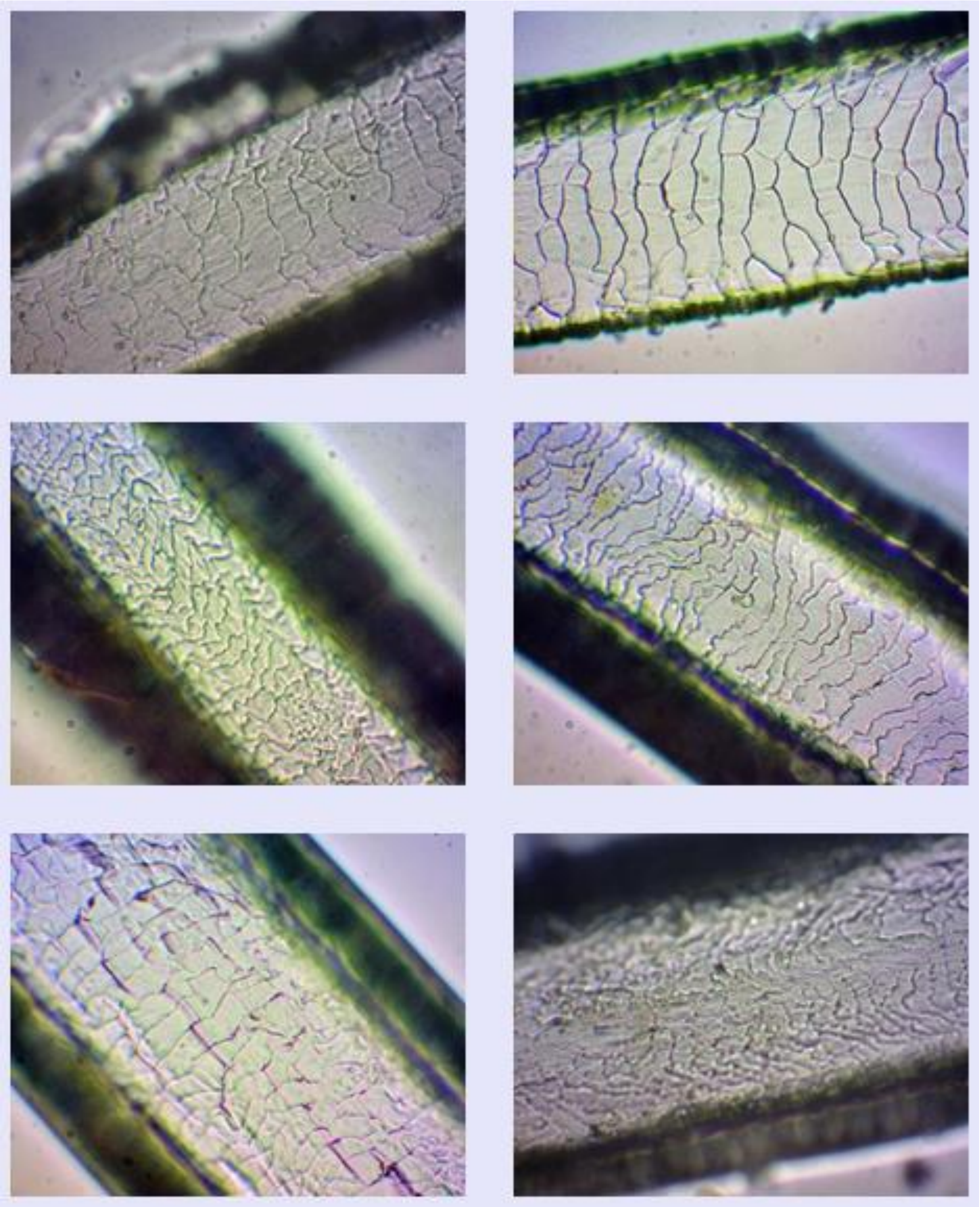
APPENDIX 5.3: PHOTOGRAPHS OF HAIR PATTERN OF UNIDENTIFIED PREY SPECIES OF GREY WOLF











GENERAL DISCUSSION

Large carnivores occupy top ranks in the ecosystems and are used as flagship species in conservation programs in different parts of the world (Poyarkov and Subbotin 2002). Although carnivores are important species, they are suffering from a decline in populations worldwide (Ceballos et al. 2005). The key ecological and biological factors involved in worldwide population declines, which eventually result in extinction, are large home range, body size, high energy demands and slow rates of population growth (Karanth et al. 2011; Marshall 2015). The removal of apex predators from natural habitats adversely affects biodiversity, which often leads to destabilizing the structure of ecosystems and communities by changing the composition of the food chain (Terborgh et al. 2002), affecting interacting species and communities and changing basic ecosystem functions (Duffy et al. 2007; Delibes-Mateos et al. 2008).

In Pakistan, two subspecies of grey wolf (*Canis lupus*) are present, i.e. the Indian wolf (*C. l. pallipes*) and Tibetan wolf (*C. l. chanco*) and both subspecies have been declared an endangered species in Pakistan (Lydekker and Blaine 1907; Sheikh and Molur 2004). Wolf population decline continues in Pakistan (Roberts 1997; Kumar and Rahmani 2000; Jhala 2003). The major threats include negative interaction with local communities, disease transmission from domestic ungulates, poaching, prey depletion, shrinking of habitat, competition with other sympatric carnivore species (Jhala 2003; Irshad 2010). The grey wolf is generally ignored in ecological investigations in Pakistan. The lack of knowledge of the aforementioned ecological aspects of wolves constitute an obstacle in devising effective conservation strategies to cope with threats that wolf populations are facing in Pakistan.

Habitat suitability models are useful to understand species distribution and to guide management and conservation strategies. Habitat suitability modeling of grey wolf in northern Pakistan showed high levels of predictive performances as seen from the values of the area under curve (0.971±0.002) and true skill statistics (0.886±0.021). Wolf habitat selection patterns at a fine scale appear to be influenced by complex interactions between habitat attributes and human disturbances (Ciucci et al. 2003; Houle et al. 2010). Wolves tolerance to human disturbances increased in suitable habitat types (Lesmerises and Dussault et al. 2013). The most suitable habitat in the Hindu Kush range (Khanbari) lacks PAs and has poor connections with other populations. The eastern part of the Pamir range and southern Himalayas are partially connected with the western Karakorum and northern Himalayan populations, respectively. Our study provides a better idea of where wolves may disperse to in case numbers increase in the future, and helps identify priority areas for community engagement, management zones, and proactive planning (Houts 2003).

Wolves are distributed unevenly across spatial and temporal scales on earth. Most of the variation in distribution patterns are attributed to environmental heterogeneity, climatic conditions and anthropogenic pressures. Wolves occupy large areas, and because of their cryptic behavior they are difficult to study across their range. Monitoring populations of large and elusive carnivores such as wolves is difficult and extremely expensive because of their low densities and large and inaccessible areas they occupy (Woodroffe 2001; Gittleman et al. 2001; Galaverni et al. 2011). Camera trapping provides presence-absence data that can be used for monitoring changes in occupancy and informs on distribution for unmarked species (Steenweg et al. 2016). Photographs captured by remote cameras are used to build presence/absence data for species, and this data are entered in various software programs to model site occupancy and habitat suitability (MacKenzie et al. 2002; MacKenzie and Royle 2005; Sharma et al. 2014).

Occupancy can also be used as a surrogate of abundance and is relatively cost-effective (Blanc et al. 2014). The site occupancy study technique has been developed on the principle that the variation in the amount of available habitat occupied by a species is linked with the fluctuation in population size of that species (Royle and Nichols 2003). Occupancy gives a realistic measure for status estimation and changes, and also provides a cost-effective, credible alternative to assessment over large scale, multi-species monitoring programs (Mackenzie et al. 2002; Pollock et al. 2002). Data on species occupancy can be used to develop vigorous inferences about many variables like resources selection, distribution, population size, species interaction and metapopulation dynamics (MacKenzie and DeLuca 2006).

Human interaction with large carnivores over livestock predation is a key rural livelihood and wildlife conservation concern due to the large number of threatened carnivore persecution in retaliation. The practice of livestock rearing is the main land use and primary source of rural livelihood in trans-Himalayan pastures (Mishra et al. 2001). Marketing livestock and livestock products helps people meet the monetary requirements of their families (Bagchi and Mishra 2006). During the summer season, higher-altitude grazing lands become accessible and herds are grazed for a longer time. They are mostly looked after by one shepherd or even kept free in the pasture, which increases the chances of encounters with predators (Dar et al. 2009; Kabir et al. 2014). The willingness to live with large carnivores depends not only on ecological factors, but also on the political opinions, financial constraints and social and cultural convictions of local communities (Treves 2009). The damage caused by wolves, namely the loss of livestock, remains a point of contention (Ogada et al. 2003; Spinkyte-Backaitiene and Petelis 2012). Multiple factors contribute to framing this conflict situation across the world. For example, livestock grazing in habitats with carnivore abundance, or in areas of low natural prey and lack of predator avoidance behavior in domesticated animals (Namgail et al. 2007; Suryawanshi et al. 2013; Aryal et al. 2014).

Knowledge of species' dietary habits is crucial for the study of complex ecosystem processes (Treves and Karanth 2003), predator-prey dynamics and trophic interactions such as interspecific resource partitioning (Symondson et al. 2002; Sheppard and Harwood 2005; Klare et al. 2011), resource selection, population change, and physiological health (Deagle et al. 2010). Scat analysis is an integral part of wolf ecological studies and can provide essential or complementary information on wolf diet that is otherwise difficult to obtain (Peterson and Ciucci 2003). For diet analysis, a reasonable, genetically confirmed sample size is essential to explore actual prey profile. The confirmation of scat samples through genetic analysis added credibility to the diet study. Another study in the central Himalayas, Nepal, reports only 57 (24%) were successfully confirmed to be from wolves among the 236 putative wolf scats (Chetri et al. 2017).

To facilitate a informed discussion on grey wolf conservation and management, it is important to develop a clear understanding of dietary ecology in landscapes with varying levels of human influence. The prey spectrum for wolves in northern Pakistan was found to be as wide as the diversity and availability of prey species in the study area. Earlier studies on the feeding ecology of grey wolves in Pakistan report similar predation pattern on domestic ungulates (Schaller 1976; Anwar et al. 2012; Shabir et al. 2013). Similarly, other studies in various regions of their geographical distribution such as Afghanistan (WCS 2008), Bhutan (Jamtsho 2017), India (Jhala and Jethva 2003; Maurya et al. 2013), central Iran (Hosseini-Zavarei et al. 2013), Kyrgyzstan

(Jumabay-Uulu et al. 2014), and Nepal (Chetri et al. 2017) confirm that wolf diet is dominated by domestic ungulates.

Understanding the feeding behavior of predators is a key element in the effective management of wild ungulates and carnivores, in understanding predator-prey interactions (Kunkel et al. 2006; Smith et al. 2004). Many studies have been conducted in recent decades to determine the feeding behavior of wolves, and most of them conclude that the wolf's main prey is large- and medium-sized ungulates (Mattioli et al. 2011; Chetri et al. 2017. The availability of wild prey is important for the effective conservation of wolf populations and to ensure coexistence with humans. The identified prey profiles confirmed that livestock constituted a significant portion of the wolf's diet, indicating a high level of human-carnivore conflict in the study area. Wild ungulates usually have a disadvantage compared to domestic livestock as their abundance is often far less than that of domestic animals that are left freely in open pastures for grazing, which results in foraging competition and replaced wild ungulates (Fankhauser 2004; Sillero-Zubiri and Laurenson 2002).

GENERAL CONCLUSIONS

The scientific information is necessary to inform practical conservation strategies, which are unfortunately lacking across the distribution range of the grey wolf in Pakistan. The study demonstrates that non-invasive study techniques such as camera trapping, genetics sampling, and interviews in combination with statistical and analytical tools is a promising approach to understand landscape ecology of threatened carnivore species. The study represents the first large-scale assessment of wolf distribution, habitat suitability, and movement in Pakistan. The habit suitability model predicted potentially suitable for the grey wolf and main predictors for habitat suitability included the distance to road and rivers and the mean temperature of the wettest quarter. The corridor modeling generated estimates of habitat connectivity among scattered wolf populations in northern Pakistan. The mean occupancy of wolves across the sampling sites was low. In the top three models, three site covariates including average distance to road, average distance to settlements, and available habitat were found to collectively affect the probability of wolf occupancy.

A major obstacle for wolf conservation and extensive killing is livestock depredation in the area. Wolves preferred to attack small ruminants in open pastures and large ruminants in corrals. Several factors were positively correlated with livestock predation, including the size of livestock holding, mortality due to diseases, and occupation of the owner (livestock rearing). The negative binomial model suggests that predation incidences tend to decline as the education level of respondents increases. Diet analysis confirmed that wolves consumed both wild and domestic ungulates. Increasing population of livestock is fostering conflicts and undermining support for wolf conservation in Pakistan. The outcome of this study has policy implications for managing human-wolf conflicts.

SUMMARY

Wolf population decline continues in Pakistan and lack of scientific knowledge is the most pivotal issue for wolf conservation. Although field research on the grey wolf is challenging, the scientific information is necessary to inform practical conservation strategies. Habitat suitability models are useful to understand species distribution. We detected suitable habitat for the grey wolf using a maximum entropy approach (Maxent ver. 3.4.0) and identified suitable movement corridors using the Circuitscape 4.0 tool. Our model showed high levels of predictive performances as seen from the values of the area under curve (0.971±0.002) and true skill statistics (0.886±0.021). The main predictors for habitat suitability for *C. lupus* were distance to road, mean temperature of the wettest quarter, and distance to river. The model predicted ca. 23,129 km2 of suitable areas for the wolf in Pakistan, with the majority of suitable habitat in remote and inaccessible areas that appeared to be well-connected through vulnerable movement corridors.

After the identification of a suitable habitat, the study investigated local sites used by wolves through occupancy modelling using camera trap data. Occupancy based modeling provides a framework for monitoring of population structure and territorial range. Different combinations of survey and site covariates were tested using the Presence software (version 12.7_170921, Hines 2006) to describe variation in the probability of detection and markability and occupancy of wolves at the site level. In the top three models, three site covariates (distance to road, distance to settlements and available habitat) were retained. The effect of efforts and plateau was positive whereas cliff base had negative effect on probability of detecting wolf on cameras. Single-season single-species occupancy model yielded mean occupancy of wolf across the sampled sites to be 0.43 (SE = ±0.09).

The third aspect of the study related to understanding the socio-ecological factors that contribute to human-wolf conflicts. Interviews with 2,317 local people were held and a mixed-effect nested negative binomial regression model was constructed using the “lme4” package and the “pscl” package in R (software) (https://cran.r-project.org/) to determine the influence of socio-ecological factors on livestock losses due to grey wolf predation. Once the average rate of livestock predation was estimated for different valleys, it was extrapolated to entire study area using geostatistical analysis in ArcGIS (10.2). In the study area, 7,583 livestock were killed during 2011–2015 and an average

10 losses were reported from herd size of 49 individuals per year. The extent of livestock losses changed in different seasons, with maximum depredation events occurring in the summers. Wolves preferred to attack small ruminants in open pastures and large ruminants in corrals. A number of factors were positively correlated with livestock predation, including size of livestock holding, mortality due to disease and occupation (livestock rearing). We searched and collected 1,186 unidentified scats samples belonging to different carnivore species found in the suspected grey wolf range, in both protected and non-protected areas. Upon genetic validation of the samples through mitochondrial DNA analyses, confirmed wolf samples were further processed for physical analysis. A microscopic analysis of genetically identified samples indicated that wolves consumed 12 different prey species, including wild and domestic ungulates.

The identified movement corridors suggest that wolves can potentially expand their range in northern Pakistan in the presence of a conducive environment. The outcome of this study has policy implications for managing human-wolf conflicts. Increasing population of livestock is fostering conflicts and undermining support for wolf conservation in Pakistan. The study highlights livestock loss by the wolf as a real and serious problem and this issue needs to be addressed to promote wolf acceptance in the rural communities of Pakistan. The study suggests multi-pronged conservation management programs that include sustained compensation schemes, livestock vaccination, and awareness campaigns to sustain the co-existence of wolf populations with rural communities.

FUTURE PERSPECTIVES

The study demonstrates that non-invasive study techniques such as camera trapping, genetics sampling, and interviews in combination with statistical and analytical tools is a promising approach to understand landscape ecology of threatened carnivore species.

- With this study, we hope to inspire fellow Pakistani wildlife scientists to initiate camera trap surveys as well. Further ecological studies, targeting threatened wildlife species are urgently needed to acquire baseline data on their status and conservation needs in Pakistan.
- The habitat suitability map provided by this study can inform future management strategies by helping authorities identify key conservation areas.
- Wolf occupancy was estimated and illustrated on a map for northern Pakistan, this spatio-temporal pattern of wolf occupancy is useful information for the wildlife authorities in decision making. This allows a better understanding of potential sites for planning future research and monitoring grey wolf population in Pakistan.
- For instance, using human density instead of settlements and livestock density would perform better. Predators travelling extensively and having large home range sizes, like grey wolf, seem rather attracted to disturbances offering prey (such as roads). Further research is required to pronounce this trend as preference or selection because the identification of limiting factors is important for reintroduction and conservation plans.
- Wolves can be sustained well in these areas with low management costs and conflicts can be resolved with comparative ease. Generally, map-based conflict mitigation actions and wisely devised management strategies can help to promote human-wolf co-existence through the identification of priorities areas where the likelihood of conflict is high. Action against poaching and habitat protection should be implemented in these areas to flourish wild prey populations to reduce carnivore predations over livestock.
- The major obstacle for wolf conservation and extensive killing is livestock depredation in the area. The outcome of this study has policy implications for managing human-wolf conflicts. To stabilize the co-existence of wolf

populations and livestock wellbeing, i suggest multi-pronged conservation management programs that include sustained compensation schemes, livestock vaccination, and awareness campaigns. I recommend the establishment of veterinary centers with the allocation of substantial budgets to reduce livestock mortality to disease.

- As educated people supported wolves more, education facilities should be improved, and environmental education should be initiated in the area. Communities in those vicinities should be taught that both snow leopards and wolves play a key role in maintaining the local ecosystem.
- A prolonged and participatory programme of grazing is required to overcome foraging competition between domestic and wild ungulate species. It is recommended that the habitat of wolves not be overstocked with domestic ungulates. I also recommend the study of seasonal variation in the diet composition of the grey wolf in Pakistan.

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