# SNOW LEOPARD STATUS IN BHUTAN 

## National Snow Leopard Survey Report 2022-2023



Nature Conservation Division
Department of Forests and Park Services Ministry of Energy and Natural Resources

Royal Government of Bhutan

Report prepared by:

1. Letro, Nature Conservation Division
2. Tashi Dhendup, Nature Conservation Division
3. Pema Dendup, Jigme Dorji National Park
4. Ugyen Penjor (PhD), Flora and Fauna International, UK
5. Tandin, WWF Bhutan (Former National Coordinator for NSLS 2022-23)
6. Sonam Wangdi, Nature Conservation Division

Data analysis:
Letro, Nature Conservation Division
Ugyen Penjor (PhD), Flora and Fauna International, UK

Report reviewed by:
Dr lan Durbach and Dr Koustubh Sharma, PAWS Initiatives, GSLEP

Field coordination and data management:

Ugyen (JKSNR), Pema Rinzin (DFO Paro), Ugyen Dorji and Sonam Lhaki Dema (DFO Thimphu), Pema Dendup (JDNP), Karma Wangdi (WCNP), Karma Chorten Dendup (JSWNP), Sangay Drukpa (BWS)

Overall Guidance:
Lobzang Dorji, Director, DoFPS

Layout and design by:
Letro, Nature Conservation Division

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## TABLE OF CONTENTS

TABLE OF CONTENTS ..... II
LIST OF TABLES ..... IV
LIST OF FIGURES ..... IV
LIST OF ACRONYMS ..... V
FOREWORD ..... VII
ACKNOWLEDGEMENT ..... IX
EXECUTIVE SUMMARY ..... XI
CHAPTER 1 INTRODUCTION ..... 1
1.1. Global Status and Conservation significance ..... 1
1.2. Snow leopard conservation in Bhutan ..... 3
1.3. National snow leopard survey 2022-2023 ..... 4
CHAPTER 2 MATERIALS AND METHODS ..... 5
2.1. STUDY AREA ..... 5
2.1.1. Bhutan: geographic location ..... 5
2.1.2. Species diversity ..... 6
2.1.3. Survey area and population demography ..... 7
2.2. POPULATION SURVEY DESIGN ..... 8
2.3. Training of the field surveyors ..... 10
2.4. CAMERA TRAP FIELD SURVEY AND MONITORING ..... 10
2.5. SCAT COLLECTION ..... 12
2.6. CAMERA TRAP DATA MANAGEMENT ..... 13
2.7. DATA ANALYSIS ..... 14
2.7.1. Spatially-Explicit Capture Recapture (SECR) modelling ..... 14
2.7.2. Habitat suitability modelling ..... 18
2.7.3. DNA ANALYSIS OF SCAT SAMPLES ..... 19
CHAPTER 3 RESULTS ..... 21
3.1. Photographic Captures ..... 21
3.2. SNOW LEOPARD INDIVIDUALS ..... 23
3.3. PhOTOGRAPHIC CAPTURE-RECAPTURE AND ACTIVITY PATTERNS ..... 23
3.4. Estimates Of SNOW LEOPARD ABUNDANCE AND DENSITY. ..... 27
3.5. SNOW LEOPARD INDIVIDUALS FROM THE SCAT ANALYSIS ..... 30
3.6. Predicted snow leopard habitats ..... 31
3.7. OthER RECORD OF MAMMALS ..... 32
CHAPTER 4 DISCUSSION AND MANAGEMENT RECOMMENDATIONS ..... 33
4.1. BhUTAN: A SNOW LEOPARD STRONGHOLD RE-CONFIRMED ..... 33
4.2. SNOW LEOPARDS IN NEW TERRITORIES ..... 34
4.3. NON-INVASIVE GENETICS: A POTENTIAL TOOL FOR SNOW LEOPARD MONITORING IN Bhutan 354.4. CARNIVORE COMMUNITY AND RICH PREY35
4.5. HUMAN-SNOW LEOPARD CONFLICT ..... 37
4.6. Key recommendations ..... 37
APPENDICES ..... 46
Appendix I: Abundance and density estimate as obtained from Maximum Likelihood APPROACH. ..... 46
Appendix II: Images of identified snow leopard individuals with their activity centre
MAPS ..... 47
Appendix II: CARNIVORES CO-OCCURRING WIth SNOW leopard CAPTURED DURING the SURVEY ..... 77
Appendix IV: National snow leopard survey teams ..... 79

## LIST OF TABLES

Table 1: Number of grids assigned to each field offices for stationing camera traps in the survey area ..... 10
Table 2: Number of snow leopard individual captured in each division.
Recaptured snow leopard individuals in more than one field office are highlighted in bold texts. ..... 23
Table 3: Posterior parameter estimates from Bayesian Model with $2.5 \%, 50 \%$ and $97.5 \%$ quantiles ( $95 \%$ credible intervals) ..... 28
Table 4: Snow leopards identified among scat sampled in Bhutan using 7- microsatellite genotypes. ..... 30
Table 5: List of predictor variables used for modelling snow leopard habitat suitability and their contributions ..... 31
LIST OF FIGURES
Figure 1: Administrative map of Bhutan with the protected area network. ..... 5
Figure 2: Map showing 310 survey grids across the survey area ..... 9
Figure 3: Space mask ( 20 km buffer with 1 km spacing) on an irregular study area. ..... 17
Figure 4: Total number of camera stations and number of camera stations that captured snow leopards. ..... 22
Figure 5: Graph showing the elevation range and snow leopard capture frequencies ..... 22
Figure 6: Record of individual snow leopard capture with regard to capture frequency and captures in different camera stations ..... 25
Figure 7: Temporal activity pattern of snow leopard and blue sheep. ..... 26
Figure 8: Histogram of overall snow leopard population abundance in Bhutan. Thevalues in parenthesis represent $95 \%$ credible interval. The dashed black lineindicates the observed individuals ( $n=96$ )27
Figure 9: Sex-specific population abundance of snow leopards in Bhutan. The values in parenthesis represent $95 \%$ credible interval ..... 27
Figure 10: Baseline detection probability (p) of snow leopards in Bhutan estimated from the Bayesian spatial capture-recapture model. Figure 11: Posterior distribution of Activity centres of individual snow leopards ( $n=96$ ) across the suitable habitat in Bhutan. ..... 29
Figure 12: Map of the study area showing the posterior density estimate from the Bayesian model ..... 29
Figure 13: Habitat suitability map of snow leopard as determined by MaxEnt modelling based on current presence evidences. ..... 32

## LIST OF ACRONYMS

AKRA:
BFL:
BSL:
BWS:
CFO:
Cl :
CITES:
CMR:
CTFM:
DFO:
DoFPS:
FD:
GPS:
GSLEP:
HWC:
ID:
IUCN:
JDNP:
JKSNR:
JSWNP:
KPI:
m.a.s.I.:

MCMC:
MLE
NCD:
NKRA:
NSB:
NSLS:
PAs:
PAWS:
RGoB:
SECR:
SLIMS:
WCNP:
WWF:

Agency Key Result Areas
Bhutan for Life
Bhutan Snow Leopard
Bumdeling Wildlife Sanctuary
Chief Forestry Officer
Credible Intervals
Convention on International Trade in Endangered Species of Wild Flora and Fauna
Capture Mark Recapture
Camera Trap File Manager
Divisional Forest Office
Department of Forests and Park Services
Forest Division
Global Positioning System
Global Snow Leopard Ecosystem Protection Program
Human-wildlife conflic $\dagger$
Identification
International Union for Conservation of Nature
Jigme Dorji National Park
Jigme Khesar Strict Nature Reserve
Jigme Singye Wangchuck National Park
Key Performance Indicator
Meters above sea level
Markov Chain Monte Carlo
Maximum Likelihood Estimation
Nature Conservation Division
National Key Result Areas
National Statistics Bureau
National Snow Leopard Survey
Protected Areas
Population Assessment of the World's Snow Leopards
Royal Government of Bhutan
Spatially-Explicitly Capture-Recapture
Snow Leopard Information Management System
Wangchuck Centennial National Park
World Wildlife Fund


Department of Forests and Park Services

## FOREWORD

† gives me immense pleasure to write the foreword for the National Snow Leopard Survey Report 2022-23, which provide an updated status of snow leopards in the kingdom of Bhutan. The national snow leopard survey is one of the major activities undertaken by the Department of Forests and Park Services during the $12^{\text {th }}$ Five-Year Plan. Snow leopards adorn the highland ecosystem of northern Bhutan and are the reining carnivore of our alpine landscapes.

Snow leopards are threatened by habitat degradation, prey depletion, conflicts with humans, and climate change across its range. However, in Bhutan, the species continue to thrive with a healthy and viable population. This is attributed to the sound environmental policies, the overwhelming support of the Royal Government of Bhutan, conservation partners, donors, and the efforts of extraordinarily dedicated team of forestry professionals, mainly the frontline rangers.

This report is an outcome of the concerted effort of our rangers over the last 10 months in setting up, monitoring, and retrieving camera traps from 310 camera stations, data management and sorting of over a million images, and analyzing the data. Since snow leopard habitats are located in some of the most remote and rugged terrains above 4500 m.a.s.l, successfully executing the fieldwork without any mishap is a great feat. More than 80 rangers have been engaged in the fieldwork. In this regard, I congratulate Nature Conservation Division and all the field offices involved in the snow leopard survey for their dedicated efforts and outcomes.

I hope the report will be of immense value to all working towards safeguarding and conserving our pristine natural environment in general and snow leopards in particular. Taking inspiration from this report, I hope our policymakers, conservation partners, and all relevant stakeholders will further affirm their commitment to conserving our iconic snow leopards for their long-term survival.

Thank you and Tashi Delek


## Lobzang Dorji <br> Director



## ACKNOWLEDGEMENT

The successful completion of the second national snow leopard survey (NSLS-2022-23) is a collective effort of many individuals and organizations. In this regard, the Nature Conservation Division would like to acknowledge everyone who has been part of our journey from the inception of the National Snow Leopard survey plan till the final production of this report.

Firstly, we would like to congratulate all the crew members of the national snow leopard survey for successfully completing this mammoth task of installing, monitoring and retrieving the camera traps in the most remote and rugged terrains with full dedication. We recognize the level of effort, sacrifices and commitment you put forth to collecting this invaluable data on the snow leopard population in the country. We also thank all the chiefs and focal officers for their leadership and coordination at the field level.

We would like to thank the Bhutan for Life programme for its generous financial support of Nu. 17.8 million, without which such a resource-extensive exercise would not have been possible. We would also like to thank WWF Bhutan for their support with field gears provided for our field crew which enabled our rangers to comfortably conduct the survey.

We thank Dr lan Durbach, Dr Koustubh Sharma, and Dr Justine Shanti Alexander of the PAWS initiatives under the Global Snow Leopard and Ecosystem Protection Program for their invaluable contribution to designing the survey and reviewing the report. We thank Dr Jan Janecka, Duquesne University, US, for the support in genetic analysis of the snow leopard fecal samples. We would also like to thank Mr Tandin, the former National Coordinator for NSLS 2022-2023, for his dedication and effort towards planning and initiating the survey. We are greatly thankful to Dr Tshering Tempa of the Bhutan Ecological Society and Dr Ugyen Penjor from Flora and Fauna International for their support in analyzing the data. We thank Mr Lhendup Tharchen for his feedbacks.

Lastly, we would like to express our deep gratitude to everyone who contributed to the National Snow Leopard Survey 2022-2023 in one way or the other. We look forward to your kind support in the future.

## Nature Conservation Division



## EXECUTIVE SUMMARY

The snow leopard (Panthera uncia) has a wide habitat range spanning over 2.8 million $\mathrm{km}^{2}$ across 12 range countries in south and central Asia. The species is globally threatened, yet there isn't a robust global population estimate. The difficulty in obtaining a reliable population estimate is attributed to the secretive nature of the species, low population densities, and remoteness of the mountainous terrains that they occupy. The global snow leopard population is estimated to be between 3,920 to 7,500 individuals, though these numbers vary between different assessments.

Snow leopards, being an apex predator of the alpine landscape, represent the ecosystem's overall health. However, large carnivores such as the snow leopard are globally threatened by habitat loss and fragmentation, prey depletion, retaliation in response to livestock depredation, poaching for illegal trade, human-induced climate change and anthropogenic disturbances. This calls for conservation actions from all government agencies, nongovernment conservation organizations, conservationists and local communities to unite and save the species.

In Bhutan, snow leopard finds a haven across the wide expanse of pristine alpine habitats, less inhabited by humans. The species is revered by the local communities, and it is strictly protected under forest and nature conservation laws. Bhutan started the first snow leopard survey in the 1980s, but the first robust estimate was reported only in 2016 using remote camera traps. The National Snow Leopard Survey (NSLS) 2022-2023 was conducted with the main objective of estimating the current population of snow leopards in Bhutan, including its distribution pattern. It is expected to contribute towards the global goal of assessing the global snow leopard population.

The NSLS report 2022-2023 provides an updated status of the snow leopard in Bhutan. The report is an outcome of the camera trapping survey spanning five protected areas namely Jigme Khesar Strict Nature Reserve, Jigme Dorji National Park, Wangchuck Centennial National Park, Bumdeling Wildlife Sanctuary and Jigme Singye Wangchuck National Park and two Divisional Forest Offices of Paro and Thimphu.

A total of 310 camera stations were set up across the field sites that accumulated a survey effort of 22,636 trap nights, with over 10,000 images of snow leopards. A careful review of 476 images identified 96 adult individuals
and 10 cubs across the landscape. Bayesian-based Spatially Explicit Capture Recapture (SECR) modelling estimated 134 snow leopards, as compared to the 2016 baseline of 96 individuals. Snow leopard density was estimated at 1.34 snow leopards per $100 \mathrm{~km}^{2}(1.2$ to 1.48 individual snow leopards per $100 \mathrm{~km}^{2}$ at $95 \% \mathrm{CI}$ ), with possibility of higher density in western Bhutan than in central and eastern Bhutan. Habitat distribution modeling also suggests more suitable habitats in western Bhutan as compared to other regions.

DNA analysis of 184 carnivore fecal samples collected during the survey also yielded 71 positive detections for snow leopards of which 31 were successfully genotyped and yielded 22 snow leopard individuals. Through improved sampling design for fecal sample collection, non-invasive
genetics can be a potential tool for snow leopard monitoring in Bhutan.

An estimated increase in snow leopard numbers from the 2016 baseline indicates that conservation efforts in the country are having a positive impact. It also re-affirms Bhutan as a stronghold for snow leopard conservation. To ensure that the species thrives in the face of emerging threats and challenges, snow leopard population needs to be monitored periodically at the national scale, patrolling and surveillance needs to be strengthened, and the degrading habitats should be managed regularly. There is also a need to enhance scientific knowledge on the species, and incidences of human-snow leopard conflicts need to be addressed to ensure harmonious co-existence between snow leopard and people.


## CHAPTER 1 <br> INTRODUCTION

### 1.1. Global status and conservation significance

snow Leopards (Panthera uncia) are one of the world's most elusive felids with legendary secretiveness and camouflage (Hunter and Jackson 1997). Its range of occurrence extends from the Himalayas in the south, across the Qinghai-Tibet Plateau and the mountains of Central Asia to the mountains of southern Siberia in the north, covering an estimated area of 2.8 million $\mathrm{km}^{2}$ (McCarthy et al. 2017). Snow leopards are mostly found in the higher elevations of the Himalayas and the Tibetan Plateau from 3,000 to over 5,000 m.a.s.l, but they also occur as low as 500 m in the higher latitudes of the Altai region (Snow Leopard Network 2014). Snow Leopards occur in 12 countries: Afghanistan, Bhutan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia, Tajikistan and Uzbekistan. Wild caprids
are the principal natural prey of snow leopards, and species include blue sheep (Pseudois nayaur), Siberian ibex (Capra sibirica), Argali (Ovis ammon), markhor (Capra falconeri), etc... depending on the locality (Lyngdoh et al. 2014; Thinley et al. 2016; Shrestha et al. 2018; Holt et al. 2018). Wild prey abundance is one of the main determinants of snow leopard abundance (Wegge et al. 2012; Suryawanshi et al. 2013).

The snow leopard is globally threatened, yet it is one of the least studied large carnivores (Snow Leopard Network 2014). While enormous progress has been made in estimating its populations at local scales, the regional and global population estimates remain ambiguous (Suryawanshi et al. 2021). The global snow leopard population size is estimated to be between 3,920 to 7,500 (Mccarthy and Chapron 2003; Janečka et al. 2008; Snow Leopard


Working Secretariat 2013), with 2,710 to 3,386 adults (McCarthy et al. 2017). The secretive nature of the species, low population densities, and sparse and sporadic distribution in remote mountainous terrains contribute to the difficulty in obtaining reliable populations. Moreover, the surveyed areas and sample sizes are usually small, making extrapolating the density estimates to non-surveyed areas difficult (McCarthy et al. 2017). However, the global population is projected to decline by at least $10 \%$ over 3 generations ( 22.62 years), and the species is listed under 'Vulnerable' category of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (McCarthy et al. 2017). Snow leopard is also listed on the Appendix I of the Convention on International Trade in Endangered Species (CITES), therefore, trade in the animal and its body parts is strictly prohibited.

The declining population of the snow leopard is due to threats which can be broadly categorized into three types; 1) decline in prey due to competition from livestock and habitat degradation (Shrestha and Wegge 2008; Aryal et al. 2014); 2) retaliatory killing of snow leopards as it preys on livestock (Wegge et al. 2012; Suryawanshi et al. 2013) and; 3) poaching of the animal for illegal trade in furs, bones and other body parts (McCarthy et al. 2017). Other emerging threats include climate change and land use change due to
global warming and burgeoning infrastructure development (McCarthy et al. 2010; Aryal et al. 2016; Penjor et al. 2020).

The snow leopard is an iconic species and an ideal flagship for conserving the high mountain ecosystems; therefore, protecting the species has great conservation significance. Concerted efforts have been put in place by the governments of 12 range countries and global conservation organizations. Snow Leopard Information Management System (SLIMS) was developed to monitor the status and distribution of snow leopards and their prey during the 1990s. The SLIMS assesses relative snow leopard abundance through repetitive standardized sign surveys (Hunter and Jackson 1997). In 2002, the Snow Leopard Survival Strategy was developed by the Snow Leopard Trust to streamline Snow Leopard conservation efforts. In 2013 governments of the snow leopard range countries initiated the Global Snow Leopard Ecosystem Protection

## "Snow leopards are

Vulnerable on the IUCN Red List
and the global population is
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10\% over 3 generations ( 22.62
years)" - IUCN

Program (GSLEP) to combat threats and strengthen transnational collaboration for snow leopard conservation (Snow Leopard Working Secretariat 2013). At the International Snow Leopard and Ecosystem Conservation Forum 2017 in Bishkek, the range country governments formally endorsed a plan to develop a global snow leopard population assessment initiative called Population Assessment of the World's Snow Leopards (PAWS), which aims to produce a robust estimate of the threatened cat's population status within the next 5 years (GSLEP 2017).

### 1.2. Snow leopard conservation in Bhutan

Bhutan is one of the 12 snow leopard range countries, and the northern alpine ecosystems of Bhutan provide a safe haven for this charismatic species. In Bhutan, the snow leopard is known by different local names, such as gangzig, chenzig, chenggu and tsagay. Its range starts from Jigme Khesar Strict Nature Reserve (JKSNR) in the west to Bumdeling Wildlife Sanctuary (BWS) to the east, with the majority of the prime habitats located in Jigme Dorji National Park (JDNP) and Wangchuck Centennial National Park (WCNP) (DoFPS 2016). Divisional Forest Office (DFO) of Paro and Thimphu are the areas outside the protected landscape where snow leopards are present. A lone snow leopard was recorded once in Jigme Singye Wangchuck National Park
(JSWNP) in central Bhutan (Letro et al. 2021), which is essentially disconnected from the primary snow leopard habitats of northern Bhutan. The snow leopard is revered as a mountain deity by yak herders and mountain communities (NCD 2019) but is in jeopardy and conflict with humans due to predation on livestock (mostly calves) (Lham et al. 2021b).

Until 2016, there was limited knowledge of the population and distribution of snow leopards in Bhutan. The first snow leopard survey was initiated in the 1980s using sign and anecdotal information (Jackson et al. 2006). Nevertheless, the snow leopard being classified as endangered globally in the past; the species was strictly protected by the national laws since 1995 by listing under 'Schedule I' category of the forest law (RGoB 2023). Any illegal act of capturing, or killing the species is a felony of fourth degree. Most of the past conservation works on snow leopards were confined to protected areas like JDNP and WCNP. For example, Snow Leopard Information Management System (SLIMS) training

## "Snow leopard is listed

under Schedule I of the Forests
and Nature Conservation Act
2023, and illegal act against it is
an offence of fourth degree

## felony"

was delivered only in JDNP in 1997 and 2000 (Jackson et al. 2000) and snow leopard-focused camera trapping was conducted in JDNP in 2012 (Thinley et al. 2014) and in WCNP in 2014 (WCNP and WWF 2016).

The nationwide snow leopard survey 2015-2016 using both sign survey and camera traps, for the first time, has helped the country to generate a robust population estimate of 96 (SE $\pm$ 8) individuals, with an estimated density of 1.08 (SE $\pm 0.09$ ) animals per 100 km² (DoFPS 2016). Subsequent analysis of the data has helped ascertain the potential snow leopard habitats in the country, where Lham et al. (2021a) estimated $7,206 \mathrm{~km}^{2}$ of Bhutan as suitable for the snow leopard: $3,647 \mathrm{~km}^{2}$ as highly suitable, $2,681 \mathrm{~km}^{2}$ as moderately suitable, and $878 \mathrm{~km}^{2}$ as marginally suitable. Fortyeight per cent of the total suitable habitat consists of protected areas.

Today snow leopard conservation is one of the priorities of the Department of Forests and Park Services (DoFPS) and conservation efforts are guided by Snow Leopard Conservation Action Plan. Bhutan, as a member country to the GSLEP, is also mandated to contribute towards achieving the mission of identifying and securing at least 20 snow leopard landscapes across its range by 2020 - "Secure 20 by 2020". As such, most conservation actions and development works in the
snow leopard habitats of Bhutan are linked towards securing their habitats and maintaining a viable population.

### 1.3. National snow leopard

 survey 2022-2023The National Snow Leopard Survey (NSLS) is one of the key progress indicators under the National Key Result Areas (NKRA) and Agency Key Result Areas (AKRA) of the 12th Five Year Plan of the erstwhile Ministry of Agriculture and Forests, Royal Government of Bhutan (MoAF 2019) and the indicator requires Bhutan to maintain the 2016 baseline of 96 snow leopard individuals by the end of $12^{\text {th }}$ FYP. The conservation Milestone 7 of Bhutan for Life project requires the country to maintain a stable population of snow leopards by year seven, i.e., 2024 (BFL 2015). To achieve these national objectives and to contribute towards the global goal of assessing the global snow leopard populations, the NSLS 2022-2023 undertaken.

The key scientific objectives of the NSLS 2022-2023 were to:
a. Estimate the current population of snow leopards in Bhutan,
b. Understand the distribution pattern of snow leopards across the country.

## CHAPTER 2

## MATERIALS AND METHODS

### 2.1. Study area

### 2.1.1. Bhutan: geographic location

Bhutan is a landlocked country between latitudes $26^{\circ} \mathrm{N}$ and $29^{\circ} \mathrm{N}$, and longitudes $88^{\circ} \mathrm{E}$ and $93^{\circ} \mathrm{E}$, bordered by the Tibet Autonomous Region of China to the north and Indian states of Arunachal Pradesh, Assam, West Bengal, and Sikkim to the east, south and west. The country has a geographical area of $38,394 \mathrm{~km}^{2}$, and is divided into twenty administrative districts. Most of the country is characterized by mountainous terrain, with forests
encompassing $69.7 \%$ of the land (FMID 2023). More than half the country is protected through a network of protected areas in the form of National Parks, Wildlife Sanctuaries, Strict Nature Reserve, Botanical Park and Biological Corridors (Figure 1).

Located in the eastern Himalayas, Bhutan is part of the Himalayan biodiversity hotspot (Myers et al. 2000), a biogeographic region where exceptional concentrations of endemic species are undergoing critical loss of habitat. There is also an extreme variability in elevational


Figure 1: Administrative map of Bhutan with the protected area network.
gradient with southern foothills going as low as 90 m.a.s.l to the highest peak, Gangkhar Puensum, at 7,570 m.a.s.l in the north, making an abrupt rise in elevation within a short aerial distance of less than 170 kms . The annual temperature ranges between $15^{\circ} \mathrm{C}$ and $30^{\circ} \mathrm{C}$, with annual precipitation between 300 millimeters and 7800 millimeters across Bhutan (NSB 2021). These factors create enabling conditions for different ecosystems to thrive with great diversity of wild fauna and flora, making Bhutan a biologically diverse country.

### 2.1.2. Species diversity

Over 11,200 species have been recorded in Bhutan (NBC 2019). Many of these organisms fall under the

threatened category of the IUCN "Red List of Threatened Species" and are of global conservation significance. Approximately 200 mammals are expected in Bhutan (Wangchuk et al. 2004), and 27 of the recorded mammals are globally threatened. Some of the threatened species include the Royal Bengal tiger (Panthera tigris tigris), common leopard (Panthera pardus), Asian elephant (Elephas maximus), snow leopard (Panthera uncia), red panda (Ailurus fulgens), golden langur (Trachypithecus geei), Asiatic black bear (Ursus thibetanus), and the onehorned rhinoceros (Rhinoceros unicornis). Bhutan takin (Budorcas taxicolor whitei), which is also globally threatened, is the national animal of Bhutan. The critically endangered Chinese pangolin (Manis pentadactyla) and pygmy hog (Sus

salvanius) are also reported in the country. Bhutan is also the hotspot of wild felids (Tempa et al. 2013), with tigers and snow leopards co-existing together in one landscape (Dendup and Lham 2023).

### 2.1.3. Survey area and population demography

Snow leopards in Bhutan are confined to the alpine mountains of Northern Bhutan, and the National Snow Leopard Survey II encompassed $\sim 10,000 \mathrm{~km}^{2}$ focusing primarily on potential snow leopard habitats within protected areas of Jigme Khesar Strict Nature Reserve (JKSNR), Jigme Dorji National Park (JDNP), Wangchuck centennial National Park (WCNP), and Bumdeling Wildlife Sanctuary (BWS). Amongst the Forest Divisions, the survey was conducted in potential snow leopard habitats of DFO Paro and Thimphu. The survey also covered the alpine landscape of Jigme Singye

Wangchuck National Park (JSWNP) in central Bhutan, which is geographically isolated from the northern snow leopard landscape (NCD 2022).

With a total population of 763,249 and a mean population density of 19.89 people per $\mathrm{km}^{2}$, Bhutan is one of the least densely populated countries in Asia (NSB, 2022). About $62 \%$ of the country's population are rural people who depend on agriculture, animal husbandry, and forests as major sources of livelihood (RGoB 2017). Communities residing in the snow leopard habitats are traditionally nomadic pastoralist rearing yaks and frequently interacts with snow leopard, occasionally coming in conflict due to livestock depredation. Nevertheless, there is no official record of retaliatory killing of snow leopards by yak herders in Bhutan (Thinley et al. 2014). However, social surveys have shown that people are becoming
intolerant when livestock depredation incidences increase, leading to changes in peoples' traditional lifestyle with many people abandoning the practices of nomadic herding (Wangchuk and Wangdi 2015; Jamtsho and Katel 2019). In recent times, the upland people's income is largely supplemented by the sale of medicinal plants and fungi, such as the highly-priced and sought-after medicinal fungus Yartsa Guenbub (Ophiocordyceps sinensis), the collection of which was legalized in 2004 (Wangchuk et al. 2015). Nevertheless, the collection season of medicinal plants in summer poses risk of disturbance to snow leopard and blue sheep habitats, and their ecology.

### 2.2. Population survey design

Camera trap surveys have become a method of choice for estimating and monitoring the population of snow leopards across its range (Jackson et al. 2006; McCarthy et al. 2010). Bhutan's national snow leopard survey of 2016 was also conducted using camera traps (DoFPS 2016). The NSLS of 2022-2023 was also planned to be executed using remote camera traps as the primary method. However, scat sampling for noninvasive genetic analysis was also incorporated to test the potentials of this tool for monitoring snow leopards. In order to decide on the camera trap locations, the potential snow leopard
habitats in the elevation range of 3400 to 5200 m.a.s.I were first mapped, and the inaccessible areas within this potential habitat range were identified and excluded from the sampling area. The inaccessibility of the area is either due to geopolitical reasons or because of the difficult terrain. The potential and accessible snow leopard habitats were then divided into high-density areas and low-density areas based on the snow leopard density map generated from camera trapping during the 2016 snow leopard survey (DoFPS 2016; Lham et al. 2021a).

Across the accessible potential habitats, the nationally adopted $4 \times 4$ km biodiversity monitoring grids, which were divided into $2 \times 2 \mathrm{~km}$ grid cells were overlaid using QGIS. The grid cell size for this survey was reduced to $2 \times 2$ km from $4 \times 4 \mathrm{~km}$ employed in the last snow leopard survey of 2016. This simplifies fieldwork (since cameras are placed in the best location within each grid cell) and increases the expected number of photographic recaptures at multiple locations, which are important for the spatially

## "Camera trapping is a

state-of-the-art approach to
monitor rare and elusive
species, such as the snow
leopard"
explicit capture-recapture (SECR) model. Reducing the grid size does not compromise comparability with the 2016 survey or bias results, because SECR models are continuous in space and use exact distances e.g., between camera locations, regardless of grid cell size. The only potential effect is improved precision in density estimates arising from greater numbers of recaptures on different camera. The potential snow leopard habitat was then used as a mask layer to generate locations of camera stations in SECR study design simulation (Durbach et al. 2021). The simulation was performed in the SECR design app to identify grid cells to establish camera stations. For the simulation, a total of 303 camera trap
stations (detectors) and a density of 1 per $100 \mathrm{~km}{ }^{2}$, lambda0 (baseline encounter rate) of 0.8 , and sigma (movement parameter) of 3000 m were specified. The sigma values are lower than the 8000 m observed in the previous survey in Bhutan to reduce the distance between camera traps and increase recaptures. The result from the simulation exercise was then used to carryout ground-truthing by each field office to verify and assess the actual accessibility of grids. A few grids that were either inaccessible or located in very low probable areas were shifted to nearby potential snow leopard habitat locations. A total of 310 grids of $2 \times 2 \mathrm{~km}$ were finalized for stationing camera traps.


Figure 2: Map showing 310 survey grids across the survey area.

9

Table 1: Number of grids assigned to each field offices for stationing camera traps in the survey area.

| Survey area | Number of grids |
| :--- | :---: |
| Jigme Khesar Strict Nature Reserve | 31 |
| Divisional Forest Office Paro | 29 |
| Divisional Forest Office Thimphu | 14 |
| Jigme Doriji National Park | 111 |
| Wangchuck Centennial National Park | 87 |
| Bumdeling Wildlife Sanctuary | 32 |
| Jigme Singye Wangchuck National Park | 6 |
| Total | $\mathbf{3 1 0}$ |

### 2.3. Training of the field surveyors

Once the sampling grids were finalized, the field staff from the snow leopard range offices were trained to conduct the fieldwork. The fieldwork comprises installation, monitoring and retrieval of the camera traps, and the identification and collection of snow leopard fecal samples.

A total of 24 teams consisting of three members in each team were formed to implement the NSLS 2022-2023. The allotment of the team for each field

division was based on the number of camera trap stations that needed to be installed, which directly correlates to the extent and distribution of the survey area. The training was provided to standardize survey techniques based on the field manual for National Snow Leopard Survey (2022-2023) developed by NCD (NCD 2022). In addition to the technical components of the survey, such as camera trapping and scat collection, significant attention was devoted to prioritizing health and safety during the field survey. This was especially crucial due to the remote and highaltitude terrains, most of which lacked cellular network coverage and were far from the nearest human settlement.

### 2.4. Camera trap field survey and monitoring

At each camera station, two Reconyx ${ }^{\circledR}$ (HyperFire) camera traps in "medium-high passive infrared (PIR) sensitivity" mode, with a photo burst of

5 photos per trigger were set up on either side of the trail on which a snow leopard was likely to pass. Two cameras were setup $30-50 \mathrm{~cm}$ above the ground and pointed at $45^{\circ}$ to $90^{\circ}$ to capture the flanks, hind (tail) or front (face and forelimbs) portions of adult snow leopards, for easy identification of the individuals. The placement of camera stations inside the selected grid cell was left to the survey team to decide. Nonetheless, camera traps were installed in areas where snow leopards were expected to frequent such as the ridge lines, valley bottoms, caves, overhung rock face, etc.

To prevent the concentration of cameras at a location, the minimum distance between two camera stations was set at one kilometer. A specific camera number was assigned to each camera trap, and location data, including geocoordinates, habitat type, and signs of prey, were recorded through epicollect5 forms.

Jackson et al. (2005) reported the deployment of camera traps for 35 days based on the cumulative capture curves. However, a greater number of days (45-50 days) is recommended to ensure adequate recaptures. DoFPS
(2016) recommended more than 100 days of camera trap deployment in the field based on the availability of funds. For the NSLS 2022-2023, camera traps were initially planned for a duration of

90 days deployment, but due to the nature of field accessibility and camera functionality, a uniform 90 days could not be maintained.

The camera trap deployment began soon after the training, and the first camera was stationed on $4^{\text {th }}$ August 2022 in JDNP and was completed by $8^{\text {th }}$ September 2022 in WCNP. Camera retrieval started on $4^{\text {th }}$ November 2022 in WCNP, and except for a few

camera traps that became inaccessible due to snow, the last camera was retrieved on 29 th December 2022 from DFO Thimphu. The total time duration of deployment across the entire study area was 148 days.

The timeframe for camera trapping was maintained considering the following reasons;

- The timing lies between the peak monsoon and peak winter seasons, which is most favourable for conducting field surveys.
- During the peak summer and winter, incessant rainfall and heavy snowfalls make the terrain unfavourable for fieldwork.

While efforts were made to monitor camera traps once after 45 days of installation, monitoring could not be carried out in a few isolated and distant locations. Monitoring of camera stations started on $1^{\text {st }}$ October 2022 and was completed on 10th November 2022. During the monitoring period, memory cards were changed, batteries were replaced, disoriented cameras were repositioned, non-functional cameras were replaced, camera lenses were cleaned, and obstructing objects between the camera lens and animal paths were removed. For the stations where camera traps were lost, new
cameras were set up to record the data for the remaining survey period.

### 2.5. Scat collection

Putative snow leopard scats were collected during the installation, monitoring and retrieval of camera traps. Scats were collected along the trails and at the camera traps sites. Once sighted, a small piece, usually from the lower part and the tip, was collected and stored in a plastic vial containing silica. Contamination was minimized during scat collection using new gloves and twigs for each fresh scat. Information, including date of collection, geo-referenced locations, and conditions of the scats, among others, were also recorded. The

samples were stored in a deep freezer (-4C) before transferring to a lab for analysis. Once at the lab, the samples were transferred to $70 \%$ ethanol for long-term storage and to inactivate potential viruses, parasites, and bacteria.

### 2.6. Camera trap data management

Data processing and management are critical steps in camera trapping studies. Each camera trap yields a vast amount of photographic data, which needs to be processed and labelled before any formal analysis can be undertaken. Traditionally, processing camera trap data has been performed manually by entering data into a spreadsheet. This is time-consuming, prone to human error, and data management may be inconsistent. After the retrieval of the memory cards from the camera, images have to be retrieved and stored securely. Secondly, files need
to be organized and labelled. Third, and often most time-consuming, is image content identification and information management, which is also called image interpretation or annotation and extraction of metadata in the form of .csv (comma-separated value) files from images. This whole process is called "data management".

The preliminary image sorting and data management were done by respective field offices based on the training delivered before the start of the survey. Camera Trap File Management (CTFM) tool was used to sort the images and extract metadata of the images in a csv file. A data management workshop was organized to further refine the sorted data and to identify the individual snow leopards following a uniform standard protocol across different field divisions. Large carnivores such as the tiger, common leopard and snow leopard are naturally marked

with distinct pelage patterns on the flanks, limbs, rumps, thighs, and tail (dorsal side), making identifying unique individuals possible. Using these markers, individuals were identified to avoid double counting of the same individual to obtain reliable estimates of capture probabilities and population abundance (Karanth 1995; Jackson et al. 2005). The presence or absence of the scrotum was used to determine sex, as male snow leopard have noticeable testicular protrusions. Snow leopards were divided into two age groups based on their level of independence from their mothers; adults and cubs, with cubs being always recorded with their mother.

Most of the cameras didn't take pictures as desired; hence, a combination of images from the tail, forehead, thighs, and limbs was used for the unique characterization of individuals. The identified individuals were thoroughly compared with the rest of the individuals to avoid double counting. Each individual was assigned a primary feature (the most clear and distinct body part with a unique rosette or spot pattern) and a secondary feature (an additional body part with a somewhat distinct rosette or spot pattern and other features such as a number of accompanying individuals; Appendix 1).

Besides the unique markers on the individuals, attributes such as time,
date and location of the capture were also used to differentiate between the individuals. In isolated grids, capturing two or three individuals in an image led us to identify two or three distinct individuals. Images not assigned primary and secondary features were discarded and treated as noncapture.

Based on the frequency of images captured during different times of the day, activity pattern of snow leopards was computed through nonparametric kernel density estimation using 'overlap' package (Meredith and Ridout 2021) in R.

A capture history was built using the date stamp on the images and the location IDs (camera station codes). Each day was considered a single occasion, and multiple captures of an individual in a single trap location during the same sampling occasion were treated as one capture incident (DoFPS 2023).

### 2.7. Data analysis

### 2.7.1. Spatially-Explicit Capture Recapture (SECR) modelling

There are two model-based approaches to estimating animal abundance and density from capture-recapture studies; the likelihood estimate (Borchers and Efford 2008) and the Bayesian
framework for inference (Royle and Young 2008). These models are known as Spatially-Explicit CaptureRecapture (SECR or SCR) models (Efford 2004; Borchers and Efford 2008; Royle and Young 2008; Royle et al. 2009), and they explicitly account for the spatial variation in abundance by deriving from the estimated location of all individual activity centres across the available habitat. The area calculation in the SECR method is also spatially explicit without relying on the archaic method of estimating area based on the movement distance of individuals, which is often prone to errors. SECR models how an animal's distance from a detection device which affects the likelihood that an individual in a population will be captured.

While there are advantages and drawbacks to both methods, for the NSLS 2022-2023, the Bayesian approach using data augmentation (Royle and Young 2008; Royle et al. 2009) was used to estimate the number and density of snow leopards

considering the following advantages;

- Bayesian inference allows the direct use of probability to characterize information about unknown model parameters;
- posterior inferences can be made which are valid to any sample size which is crucial for ecological studies of rare and elusive animals, such as snow leopard, where sample sizes are often very small (Royle et al. 2013).
- the Bayesian approach to analysis that makes use of data augmentation to estimate the density of animal in the study area (Royle and Kéry 2007) allows for inclusion of animals that were not captured during our survey.
- this was also the method used during the snow leopard survey of 2016 and the same method was selected to ensure comparability of the results.

Taking into account the data lost due to camera theft, dysfunctional camera, and delayed re-installations, the actual active operational days for each camera was during the study period was taken into account during the analysis. A single-season (i.e., demographically closed) spatially explicit SECR hierarchical model with two hierarchical levels was built: 1) a point process ecological model that describes the spatial distribution of individuals (density that is the main
ecological process of primary interest) and, 2) an observation model accounting for imperfect detection and variability in snow leopard detectability that described the observation of individuals in camera traps (Royle et al. 2009).

The SECR model estimates the following three main parameters: 1) the baseline detection probability $p_{o}$, which describes the detection probability at a camera station located at an animal's activity centre $s_{i}$ - a latent variable representing the expected location about which an individual is expected to use space during the sampling period, 2) the spatial variance (scale) parameter of the detection function sigma ( $\sigma$ ), and 3) the number $N$ of snow leopard activity centres within the available habitat $S$ (i.e., the detector grid with a buffer of some distance), which can also be used to derive density $D$. Additionally, the SECR models can also accommodate the covariate effect on density and detection parameters, hence can also estimate the effects (regression coefficients) of spatial and individual covariates in detection probability and density.

To model the probability $p$ of detecting an individual $i$ at camera station $j$ as a decreasing function of the distance $d$ between the camera trap and the individual's activity centre s, the half-normal detection function was used. By convention,
detection probability can be expressed as

$$
p_{i j}=p_{0_{i j}} \exp \left(-\frac{d_{i j}^{2}}{2 \sigma^{2}}\right)
$$

The detection function is assumed to reflect individual space use and can directly be linked with the home range concept (Royle et al., 2014). Data-augmentation approach was used to include individuals that may be present but were not exposed to sampling. The detection of an individual is conditional on the individual state $\mathrm{zi}_{\mathrm{i}}(=1$ if an individual $i$ is a member of the population $N, 0$ otherwise) and is governed by the inclusion probability $\psi$ such that $z_{i} \sim \operatorname{Bernoulli}(\psi)$. The population size can then be derived by summing the $z_{i}$ values,

$$
N=\sum_{i=1}^{M} z_{i}
$$

where $M$ is the data-augmented population size (Royle et al., 2007) and represents the maximum number of snow leopards in the habitat $S$.

The ecological model describes the number and distribution of all snow leopards present in the population (i.e., observed and augmented (undetected) individuals). In the data augmentation approach, the superpopulation size $M$ was chosen at 200, sufficiently larger than $N(=96)$. Given relatively high photographic captures of snow leopards during the survey, an augmentation factor of 2 was chosen to facilitate the analysis
by Markov Chain Monte Carlo (MCMC).

The sex model $p_{o}$ (sex). $\sigma($ sex) was implemented to estimate variation in the probability of capture ( p 0 ) (when the distance between activity centre and camera trap is zero) and ranging scale parameter $(\sigma)$ separately for male and female. The models were fitted in JAGS (Just Another Gibbs Sampler) program (Plummer 2003) using MCMC algorithm in $R(R$ Development Core 2023), using the "jagsUl" package (Kellner 2018). A uniform prior distribution on population size and the distribution of
activity centre was used. The superpopulation ( $M$ ) and population size $(N)$ are related by parameter $\psi . \psi$ is the probability that an individual on the list of size $M$ is a member of the population of size N that was exposed to sampling by the trap array (Royle and Young 2008). The models were run using three parallel chains of 10,000 iterations each, discarding the first 2000 iterations as burn-in and thinning at the rate of 10 . The model convergence was assessed by visually inspecting trace plots and histograms for each parameter and ensuring that the Gelman-Rubin statistics (R-hat) values were <1.1.


Figure 3: Space mask (20 km buffer with 1 km spacing) on an irregular study area.

The model convergence was assessed by visually inspecting trace
plots and histograms for each parameter, and ensuring that the

Gelman-Rubin statistics (R-hat) values were <1.1. The mean, median, and $95 \%$ credibility intervals from these converged samples for the model parameters were computed. These models are computationally expensive so multiple covariate models could not be run during this analysis using the Bayesian framework. For instance, model with sex covariate took $\sim 9$ hours to run 100 iterations. So, adding more parameters such as covariate or goodness-of-fit considerably increases the computation time. However, we used maximumlikelihood (MLE) approach to perform model selection and estimated density for comparability (Appendix I). but we report results from the Bayesian framework. The posterior predictive density of the animal location was produced based on the bivariate-normal model of animal movement. The values of the pixels are the expected number of animals in the pixel at a given moment in time.

### 2.7.2. Habitat suitability modelling

- Species occurrence points

Species occurrence points for snow leopard comprised of camera trap locations that captured the snow leopard during the NSLS 2022-2023 and location of snow leopard scats which resulted in positive identification of snow leopard during
its genetic analysis, collected during the survey. A total of 215 occurrence points were obtained (144 camera stations and 71 scat collection sites), which were rarified to assign only one record per $\mathrm{km}^{2}$ to reduce spatial autocorrelation. A total of 192 occurrence points were retained for MaxEnt modelling.

## - Variables for the MaxEnt model

Different predictor variables were used for modelling snow leopard distribution and habitat suitability in Bhutan. Topographic variables such as elevation and slope were derived from the 12.5 m resolution Digital Elevation Model raster downloaded from the USSS (USGS 2018). Land use and land cover variable representing the different forest types and land use types of Bhutan was obtained from the Department of Forests and Park Services (FRMD 2017). Climatic variables were downloaded from the WorldClim data hub (Fick and Hijmans 2017) and from the 19 bioclim variables available, two were used (total annual precipitation and annual mean temperature) as they were found to have more influence in similar studies (Rashid et al. 2021). Variables exhibiting anthropogenic influences such as the human settlement and roads were obtained from National Statistics Bureau (NSB 2005; NSB 2022)and raster file with Euclidean distance were processed in ArcGIS 10.8.1 for these two
variables. The format of the predictor variables was changed from raster to ASCII format in Arc GIS 10.8.1 (ESRI 2020).

## - Maxent modelling

MaxEnt software version 3.4.3 (Phillips et al. 2020) was used to execute the maximum entropy algorithm (Phillips et al. 2006) with $70 \%$ training and $30 \%$ test data and a logistic output format was set up as it describes the probability of snow leopard presence, with continuous habitat suitability ranging from 0 (unsuitable) to 1 (most suitable). The pixel value equals to or higher than the tenpercentile training presence threshold were considered as the suitable habitat. The model performance was assessed using AUC (Area under the Receiver Operating Curve) whereby values $\leq 0.5$ indicate very poor fit, $>0.5$ indicate good fit, and equal to 1 indicate perfect fit (Fielding and Bell 1997). From the suitability map, value $>0.7$ of the predicted habitats were identified as the prime habitats in the study landscape while value from 5-7 were identified as moderately suitable habitats (Kalashnikova et al. 2019; Rashid et al. 2021).

### 2.7.3. DNA analysis of scat samples

DNA was extracted from all samples using the Qiagen QIAamp Fast DNA

Stool Mini kit using the manufacturer's protocol (Hacker et al. 2022). The mean DNA concentration was 18.85 ng/ul, and a 260/280 ratio was 1.81 . This is a reasonable concentration from scat samples, and the 260/280 ratio indicates pure DNA. Most of the scat had intact DNA; of the samples with visible DNA on a SybrGreen stained agarose gel, $80 \%$ showed a single high-molecular weight band indicating intact DNA, whereas 20\% had a smear indicating DNA degradation.

Microsatellite analysis was performed on snow leopard PCR-assay identified scat in triplicate with a panel of 7 microsatellites using the Qiagen Type-it Microsatellite PCR and $55^{\circ} \mathrm{C}$ annealing temperature. The primes were labelled with FAM, NED, PET, and VIC and amplified in two multiplexes: Plex 1: PUN1157 FAM, PUN1262 NED, PUN834 PET, Plex 2: PUN100 FAM, PUN894 VIC, PUN124 NED, and PUN225 PET (Janečka et al. 2008; Janečka et al. 2011; Janecka et al. 2017). Negative and positive controls were included in all PCR plates. PCR products were visualized on a SybrGreen stained agarose gel to verify amplification. Amplicons were diluted 1:10, then 1.5 ul was placed in 8.7 ul of Formamide with 0.3 Ul of ABI 500LIZ size standard.

These plates with amplicons and 500 LIZ in formamide were submitted for fragment analysis on the ABI3730 instrument. After the injections, the
electropherograms for each PCR reaction were downloaded and analyzed in GeneMarker 2.6 software. The allele sizes were determined based on the peak heights and profiles. The genotypes were scored for each triplicate, and consensus genotypes were reconstructed. Sex identification was performed with PCR assay using felidspecific primers that amplified the 100-bp portion of the SRY using the Qiagen Type-it Microsatellite PCR kit. Each snow leopard scat sample with
a 7-microsatellite genotype profile was tested with the SRY marker in triplicate to determine sex. The snow leopard was identified as a male if there was a 100-bp band in 2 or 3 PCR replicates. If there was no amplification, it was identified as a female. If there was amplification in 1 of 3 replicates, it was scored unknown sex. The sex for the female samples was also verified using AMELY PCR assay (Janečka et al. 2008; Janecka et al. 2014).

## CHAPTER 3

## RESULTS

### 3.1. Photographic captures

Atotal of 310 camera stations were deployed across potential snow leopard habitats in the country. JDNP had the highest number of stations at 111, followed by WCNP with 87 stations. JSWNP has the lowest number, with six camera stations. Of them, data from one camera station was completely lost due to the loss of the camera and yielded only two trap nights. Some of the cameras were retrieved earlier due to the risk of inaccessibility caused by snow cover.

Of the 310 stations, snow leopards were photo captured at 144 camera stations yielding a naïve occupancy of 0.46. JDNP hadxf the highest number of stations with snow leopards (66), followed by WCNP (30). Only one camera station captured snow leopards in BWS, while no snow leopards were captured from JSWNP and Dagala region (six stations) under DFO Thimphu (Figure 4). The highest elevation at which the snow leopards were captured was 5090 m.a.s.l under WCNP while the lowest elevation was 3634 m.a.s.l under DFO Thimphu. Majority of the snow leopards were captured by camera stations between the elevation of 4251-5000 m.a.s.l (Figure 5).



Figure 4: Total number of camera stations and number of camera stations that captured snow leopards.


Figure 5: Graph showing the elevation range and snow leopard capture frequencies.

### 3.2. Snow leopard individuals

The 310 camera stations yielded a survey effort of 22,636 trap nights and captured over 10,000 images of snow leopards. We used 476 images to identify 96 adult individuals, of which 45 were identified as male, and 51 as female (Table 2; Appendix II). Snow leopard images from 13 stations were not used during the abundance and density analysis due to poor quality and ambiguous
identity to match the identified snow leopards.

Individual identification of snow leopard was made carefully by observing the rosette pattern on both flanks, face, limbs, torso and tails. Many images look like unique individuals, but due to a lack of multiple reference points for comparison, they were not classified as unique individuals, and, therefore, were not included in the analysis. These images include the capture of only one flank, part of the limbs, odd facial angles, or tails.

Table 2: Number of snow leopard individual captured in each division. Recaptured snow leopard individuals in more than one field office are highlighted in bold texts.

| SI. No. | Name of Field <br> Office | No of Adult <br> Individuals | Adult Snow leopard ID | No. of Cubs <br> Identified |
| :---: | :--- | :---: | :--- | :---: |
| 1 | JKSNR | 12 | BSL_01-BSL_12; BSL_05 | 0 |
| 2 | DFO Paro | 11 | BSL_13-22; BSL_05 | 2 |
| 4 | DFO Thimphu | 4 | BSL_23-25; BSL_27 | 0 |
| 3 | JDNP | 53 | BSL_26-78; BSL_27 | 8 |
| 5 | WCNP | 17 | BSL_79-95 | 0 |
| 6 | BWS | 1 | BSL_96 | 0 |
| 7 | JSWNP | 0 |  | 0 |
|  | Total | $\mathbf{9 6}$ |  | $\mathbf{1 0}$ |

### 3.3. Photographic capturerecapture and activity patterns

Photographic capture-recapture patterns show that most of the
identified individuals have recaptures at more than one location. Individual BSL_01 (Bhutan Snow Leopard 01) had the maximum number of recaptures at 16 camera stations under JKSNR. BSL_02 was
captured at 12 different camera stations, and BSL_87 was captured at seven different camera stations. Two individuals were captured by six different stations, three individuals at five locations, 11 individuals at four locations, and 12 individuals at three locations. BSL_05 was captured by cameras in both JKSNR and DFO Paro, and BSL_27 was captured in adjoining camera stations of JDNP and DFO Thimphu.

Thirty-three individuals were captured at two different camera stations, and 32 individuals were captured at only one station. BSL_01 also had the maximum capture frequency captured at 59 different times. This was followed by BSL_02, captured 22 times, and BSL_27,
captured 16 times. The majority of individuals were captured between one to 13 different times. These capture-recapture patterns show a highly localized distribution of individuals except for a few individuals that covered a vast area.

Three camera stations (JDNP_026, JDNP_051, PFD_001) captured five different snow leopard individuals each, followed by eight stations that captured four individuals, and sixteen camera stations captured three different individuals. Thirtyeight camera stations captured two different individuals, and the remaining 65 stations captured only one individual snow leopard during the survey period (Figure 6).



Figure 6: Record of individual snow leopard capture with regard to capture frequency and captures in different camera stations.

The activity pattern on a 24 -hour time scale showed that snow leopards are mostly active at dawn and dusk with greater peak of activities in the evening hours, exhibiting a crepuscular behaviour. Activity decreases to its minimum during the mid-day, though some degree of activities were observed during all time
periods of the day. Blue sheep on the other hand was most active throughout the day starting as early as 06:00 hours to 17:30 hours (Figure 7). The activity overlap between snow leopard and its prey blue sheep was also highest during the morning and evening hours (Snow leopard-blue sheep Dhatl $=0.49$ ).


Figure 7: Temporal activity pattern of snow leopard and blue sheep.


### 3.4. Estimates of snow leopard abundance and density

The Bayesian SECR model yielded a total estimated number of snow leopards in Bhutan at 134 individuals (SD $\pm 7.06$ ). The estimated range of snow leopard abundance was between 121 and 148 individuals at
$95 \% \mathrm{Cl}$ (Figure 8; Table 3). This is an increase of snow leopard population in Bhutan by $39.5 \%$ from the baseline population of 2016 where 96 (SD $\pm 8$ ) individuals were estimated.


Figure 8: Histogram of overall snow leopard population abundance in Bhutan. The values in parenthesis represent $95 \%$ credible interval. The dashed black line indicates the observed individuals ( $\mathrm{n}=96$ ).


Figure 9: Sex-specific population abundance of snow leopards in Bhutan. The values in parenthesis represent 95\% credible interval.

The density estimates varied from 1.2 individuals to 1.48 individual snow leopards per $100 \mathrm{~km}^{2}$ (at $95 \% \mathrm{Cl}$ ) with a mean density estimate of 1.34 snow leopards per 100 km² (SD $\pm 0.071$ ) (Table 3; Figure 9, 12). The posterior density map predicted higher density
of animals in western Bhutan (JDNP, JKSNR, DFO Paro) as compared to similar habitats in central and eastern Bhutan. The density estimate for female was marginally higher than male (Table 3).

Table 3: Posterior parameter estimates from Bayesian Model with 2.5\%,50\% and $97.5 \%$ quantiles ( $95 \%$ credible intervals).

| Parameters | mean | sd | $\mathbf{2 . 5 0 \%}$ | $\mathbf{5 0 \%}$ | $\mathbf{9 7 . 5 0 \%}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $p_{\text {ofemale }}$ | 0.011 | 0.002 | 0.009 | 0.011 | 0.014 |
| $p_{o_{\text {male }}}$ | 0.008 | 0.001 | 0.006 | 0.008 | 0.009 |
| $p_{\text {o overall }}$ | 0.008 | 0.001 | 0.007 | 0.008 | 0.009 |
| $\sigma_{\text {female }}$ | 2.917 | 0.14 | 2.646 | 2.917 | 3.21 |
| $\sigma_{\text {male }}$ | 4.773 | 0.174 | 4.454 | 4.759 | 5.114 |
| $\sigma_{\text {overall }}$ | 4.161 | 0.127 | 3.921 | 4.157 | 4.414 |
| $N_{\text {overall }}$ | 134.667 | 7.062 | 120.725 | 134 | 148.275 |
| $N_{\text {male }}$ | 62.993 | 4.441 | 56 | 63 | 73.55 |
| $N_{\text {female }}$ | 71.673 | 6.386 | 61 | 71 | 86 |
| $D_{\text {overall }}$ | 1.344 | 0.071 | 1.205 | 1.338 | 1.48 |
| $D_{\text {female }}$ | 0.716 | 0.064 | 0.589 | 0.709 | 0.829 |
| $D_{\text {male }}$ | 0.629 | 0.044 | 0.559 | 0.629 | 0.719 |
| $\Omega$ | 0.672 | 0.047 | 0.581 | 0.671 | 0.758 |
| $p_{i}$ | 0.471 | 0.056 | 0.358 | 0.476 | 0.577 |

$p_{o}=$ baseline detection probability/encounter probability; $\sigma$ - scale parameter in $\mathrm{km} ; \mathrm{N}=$ abundance, $\mathrm{D}=$ density per $100 \mathrm{~km}^{2} ; p_{i}=$ sex-ratio of the population; $\Omega=$ data augmentation parameter

Baseline detection probability differed between sexes with higher probability for females (median and $95 \% \mathrm{Cl} p_{o}=0.011,0.009-0.014$ ) than males (0.008, 0.006 - 0.009). The overall (without sex differentiation) baseline detection probability was similar to that of male ( $0.008,0.007$ -
0.009). However, the movement (or scale) parameter ( $\sigma$ ) was opposite: significantly higher for males (4.759 $\mathrm{km}, 4.454 \mathrm{~km}-5.114 \mathrm{~km}$ ) than females ( $2.917 \mathrm{~km}, 2.646 \mathrm{~km}-3.210 \mathrm{~km}$ ). The overall movement parameter estimate was $4.157 \mathrm{~km}(3.921 \mathrm{~km}$ 4.414 km ) (Table 3).


Figure 10: Baseline detection probability ( p ) of snow leopards in Bhutan estimated from the Bayesian spatial capture-recapture model.


Figure 11: Posterior distribution of Activity centres of individual snow leopards ( $\mathrm{n}=96$ ) across the suitable habitat in Bhutan.


Figure 12: Map of the study area showing the posterior density estimate from the Bayesian model.

### 3.5. Snow leopard individuals from the scat analysis

A total of 184 putative snow leopard scats were collected from various field offices, of which 71 samples tested positive for snow leopards. Thirty-one samples were successfully genotyped (42.3\%, Table 4), and consensus genotypes were used to determine the profile at each of the seven loci. The combined 7microsatellite genotype profile had PID-unr of 0.000046 and PID-sib of 0.012 . The genotype profiles were then used to identify individual snow leopards, assign unique identification
numbers, and determine in which scat each snow leopard was observed.
There were 22 snow leopards observed among the 31 successfully genotyped scats, of which nine were male and 13 were female (Table 4). Nine snow leopards were observed in multiple scats, and 13 snow leopards were observed only once. One snow leopard was observed in scats from DFO Paro and JDNP, indicating that the specific sites where they were collected are close enough to be used by the same individual. For the remaining snow leopards detected in multiple scats, all of them were only observed in the same survey are

Table 4: Snow leopards identified among scat sampled in Bhutan using 7microsatellite genotypes.

| Survey Site | Total number <br> of scats <br> collected | PCR Assay <br> - Uncia <br> Scat | Uncia Scat <br> Successfully <br> Genotyped | \% Uncia Scat <br> Successfully <br> Genotyped | Identified Snow <br> Leopards (F - <br> female, M - male) |
| :--- | :---: | :---: | :---: | :---: | :---: |
| JKSNR | 53 | 16 | 7 | $43.8 \%$ | $3(2 \mathrm{~F}, 1 \mathrm{M})$ |
| DFO Paro | 31 | 11 | 3 | $27.3 \%$ | $2(2 \mathrm{M})$ |
| JDNP | 53 | 32 | 14 | $43.8 \%$ | $10(6 \mathrm{~F}, 4 \mathrm{M})$ * |
| DFO | 1 | 0 | 0 | n.a. | 0 |
| Thimphu | 0 | 0 | 0 | n.a. | 0 |
| JSWNP | 40 | 11 | 6 | $54.5 \%$ | $6(4 \mathrm{~F}, 2 \mathrm{M})$ |
| WCNP | 5 | 1 | 1 | $100.0 \%$ | $1(1 \mathrm{~F})$ |
| BWS | 184 | 71 | 31 | $43.7 \%$ | $13 \mathrm{~F}, 9 \mathrm{M}$ |
| Total: |  |  |  |  |  |

*One individual was common between JDNP and DFO Paro. In this table, the individual is included under JDNP

### 3.6. Predicted snow leopard habitats

The variables with the highest contributions in the MaxEnt model were land use land class and annual mean temperature, and distance from road, while elevation and annual mean temperature has the highest permutation importance (Table 5).

The predictions show the relationship between snow leopard presence data and the environmental variables defined in the study area. An AUC value of 0.95 and 0.94 were obtained for training and test data respectively. With AUC value of greater than 0.8, the results exhibited high accuracy of the models as AUC
value of greater than 0.80 indicates that for $80 \%$ of the time, a random selection from the positive group (sensitivity) will have a score greater than a random selection from the negative class (specificity) (Sharma et al. 2020). Hence, the results from this model can accurately predict habitat suitability for snow leopards in the study area.

Predicted map (Figure 13) shows that western Bhutan has higher habitat suitability as compared to northcentral and eastern Bhutan. Certain patches of higher elevated mountain landscapes in central Bhutan and far east also exhibited potential suitable habitats for snow leopard, though no records were made during the NSLS 2022-2023.

Table 5: List of predictor variables used for modelling snow leopard habitat suitability and their contributions.

| Factors | Predictor <br> variable | Original <br> spatial <br> resolution | Percent <br> contribution | Permutation <br> importance |
| :--- | :--- | :---: | :---: | :---: |
| Topography | Elevation | $12.5 \times 12.5 \mathrm{~m}$ <br> Slope | $12.5 \times 12.5 \mathrm{~m}$ | 4.2 |
| Climate | Total annual <br> precipitation <br> Annual mean <br> temperature | $800 \times 800 \mathrm{~m}$ | 7.2 | 60.9 |
| Land use types | $30 \times 300 \mathrm{~m}$ | 21.3 | 8.2 |  |
| Land cover | Lanthropogenic | 14.6 |  |  |
| Distance from <br> settlement <br> Distance from <br> road | $30 \times 30 \mathrm{~m}$ | 45.1 | 1.8 |  |
|  |  | 2.5 | 3.8 |  |



Figure 13: Habitat suitability map of snow leopard as determined by MaxEnt modelling based on current presence evidences.

### 3.7. Other record of mammals

During the survey period, camera traps also recorded many other species. The rare and elusive Pallas's cat (Otocolobus manul) was captured at two stations; one from WCNP and one from JDNP. The camera traps also recorded a widespread distribution of Himalayan wolf (Canis lupus chanco) across the snow leopard landscape of JKSNR and Paro FD, JDNP and WCNP. The snow leopard survey also, for the first time, captured a deer species, the

Thorold's deer (Cervus albirostris) from DFO Paro, which is a new species record for the country (see Appendix III). Asian badger (Meles leucurus), which was never recorded in the past in JKSNR, was also captured at a highelevation station for the first time. Tiger and snow leopards were also captured at the same camera trap station in two locations, one each in DFO Thimphu and WCNP. A camera station under DFO Thimphu also recorded common leopard and snow leopard at the same location.

## CHAPTER 4 <br> DISCUSSION AND MANAGEMENT RECOMMENDATIONS

### 4.1. Bhutan: A snow leopard stronghold re-confirmed

The national snow leopard survey 2016 highlighted that Bhutan is a stronghold of snow leopards, with an estimated 96 individuals. It was for the first time that the snow leopard population in the country was declared, establishing empirical evidence that Bhutan has a healthy and viable population of snow leopards (DoFPS 2016). The NSLS 20222023, reporting 134 individuals, reaffirms Bhutan as a conservation stronghold for snow leopard in the Eastern Himalayas.

The snow leopard density was found higher in the western region as compared to the central and eastern regions, with a strong possibility for population connectivity. The DNA analysis of scat samples also suggested relatively higher snow leopard abundance in JDNP ( $\mathrm{N}=10$ ) than other sites (JKSNR N=3, DFO Paro $N=2$, WCNP $\quad N=6, \quad$ BWS $\quad N=1)$. Nevertheless, this interpretation regarding abundance needs to be treated with caution because it does not consider sampling effort or spatial distribution of scats within the survey area. The capture of snow leopards with cubs at many locations in DFO

Paro and JDNP also suggests the population is viable and breeding.

With most of the snow leopard habitats being closer to international borders, population dispersal to potential habitats across the border to India and China is high and possible. With continued conservation efforts, Bhutan can certainly be a safe haven for the snow leopards to thrive and serve as a source population in the region.

The increase in snow leopard numbers from the 2016 baseline indicates that conservation efforts in the country are having a positive impact. The department has been strengthening the anti-poaching activities through patrolling, and alpine habitat management works have been continuously pursued through the support of the Bhutan for Life project. It is also important to note that survey area has been increased during this national survey by filling up gaps left during the past survey. During the 2016 survey, only 223 camera station were set up whereas 310 stations were set up during this survey.

### 4.2. Snow leopards in new territories

Snow leopards have expanded into new territories with records from places where the species was previously not recorded or were rare. During the last national survey in 2016, snow leopard was not recorded from BWS. A camera trap survey in the following years did record the species at few locations, and current survey captured the species in one station. Although present, it appears that the snow leopards are rare and found in lower densities in the area. The NSLS 2022-2023 expanded the study area in DFO Thimphu including the Dagala regions and snow leopards were captured in many stations under DFO, Thimphu. Unlike in 2016 where the BWS area was excluded for density estimation, the entire surveyed areas have been accounted during the analysis this time. Movements of individuals within and among populations at larger landscapes is expected to help
maintain genetic variability and population viability (Riordan et al. 2016). Image captures from DFO Paro and Thimphu landscape is a clear indication of the importance of securing conservation beyond protected areas and maintaining functional biological corridors that provide safe and contiguous landscape for the movement of species between our protected areas.

While snow leopard was not captured in camera traps set up in the Dagala region under DFO Thimphu and Black Mountain region of JSWNP, possibilities of snow leopard presence in these areas cannot be ruled out as Black Mountain once reported a rare capture of snow leopard (Letro et al. 2021) and snow leopard was captured by camera traps set for national tiger survey 2022 in the Dagala region. With evidence of snow leopards recorded in forested habitats (Bandyopadhyay et al. 2019), and species habitat and

behavior alteration in the face of climate change (Forrest et al. 2012; Watts et al. 2019; Kalashnikova et al. 2019), breeding snow leopards inhabiting these isolated high-altitude habitats in the future is probable. Snow leopard habitat suitability modeling also yielded potential habitats in these landscapes and high-altitude regions on Sakteng Wildlife Sanctuary in the far east.

### 4.3. Non-invasive genetics: A potential tool for snow leopard monitoring in Bhutan

Non-invasive genetics is a popular alternative to camera trapping for estimating the abundance and density of wildlife populations. The data obtained from the DNA can be brought into a capture history format similar to the camera traps as required for SECR models. During the current survey, the number of samples collected were lower as it was done opportunistically while carrying out the camera trap survey. Nevertheless, the results from the analysis showed that non-invasive scat survey can be a viable option for snow leopard monitoring in Bhutan as most of the samples that genotyped successfully yielded promising results in individual identification. Several reports indicate that the method is cost-efficient and could be tested in Bhutan's context. The main limitation for Bhutan is the lack of a well-
equipped lab that can do complex genetics analyses.

The current fecal sample analysis also found that more than half of the putative snow leopard scats belonged to other species. Therefore, cautionary measures should be taken ii using sign surveys for snow leopard monitoring and dietary analyses as the results show a significant misidentification of samples. The species identification must be confirmed with genetics first. Genotyping with more microsatellites will also yield more information on the genetic diversity, which is vital information for ensuring long-term population viability. This analysis is currently underway and the results should provide further insights into population structure and dispersal.

### 4.4. Carnivore community and rich prey

Of the 310 camera stations, 185 stations captured blue sheep, the primary prey species of the snow leopard. More than 60,000 images of blue sheep were photographed during this survey, indicating prey richness. A healthy population of prey is an important determinant for carnivore population viability (Carbone and Gittlemann 2002), abundant blue sheep means the snow leopard have access to abundant and easy wild prey, obviating the need to hunt for domestic livestock, eliminating
conflict with humans and thus supporting a thriving snow leopard population. Studies across the range found that there is positive correlation between snow leopard and wild ungulate density, and this underlines the need to conserve the ungulate prey population. Its abundance and body size makes the blue sheep an ideal prey for snow leopards (Lyngdoh et al. 2014).

Other potential prey species captured by the camera trap are Himalayan musk deer (Moschus chrysogaster), marmot (Marmota himalayana), goral (Naemorhedus goral), serow (Capricornis thar). These species have been described as prey species besides the main prey species the blue sheep in other similar regions (Aryal et al. 2016; Holt et al. 2018; Suryawanshi et al. 2021; Lham et al. 2021b).

Intriguingly, current survey showed that there is potential habitat overlap
large carnivores such as tiger and common leopard, besides its sympatric carnivores such as dhole, Himalayan wolf, and red fox. Two camera stations captured both snow leopard and tiger while one station captured snow leopard and common leopard. Earlier studies in Bhutan found strong evidence of tigers and leopards occupying sites together when prey species such as gaur was abundant (Penjor et al. 2022). While the current records may be occasional overlaps without much interspecific interactions, long-term overlap of habitat between these top carnivores may result in either resource exploitation and behavioral interferences (Donadio and Buskirk 2006) or co-existing through habitat separation and exploitation of different prey species (Lovari et al. 2013). Maintaining diverse and healthy prey population is therefore vital (Karanth et al. 2004; Lovari et al. 2013; Shrestha et al. 2018; Suryawanshi et al. 2021). between snow leopard and other


### 4.5. Human-snow leopard conflic $\dagger$

Human-wildlife conflict (HWC) is a major conservation challenge (Madden 2004) and predation of livestock by large carnivores is one of the serious issues impacting both rural livelihoods and conservation (Karanth and Sunquist 2000; Kissui 2008; Wegge et al. 2012). Predation of livestock by snow leopard is quite common across its range (Wegge et al. 2012; Suryawanshi et al. 2013; Alexander et al. 2015) and it is no exception in Bhutan. Yak (Bos grunniens) herding is very common in all the snow leopard habitats across the country and in recent years, people seeking government interventions for snow leopard predation were high from the highland areas of JKSNR, DFO Paro and JDNP. Increased population of snow leopard will definitely put pressure on the herding communities as snow leopard will prey on the yak calves. In the snow leopard abundant places like JKSNR, past studies accounted $78.86 \%$ of livestock kills to snow leopard (Dorji and Powrel 2022), while in WCNP in central Bhutan, predation of livestock by snow leopard was found to be $24 \%$ (Jamtsho and Katel 2019). Lham et al. (2021) reported that across the snow leopard landscape, yaks constituted $14 \%$ of the snow leopard prey.

### 4.6. Key recommendations

Bhutan is inarguably one of the important snow leopard conservation strongholds among the snow leopard range countries. Limited anthropogenic pressure, abundance of prey species, and strong conservation policies and actions have resulted in thriving population of snow leopard in the country. In the last five years, snow leopard population has increased and with records of many snow leopards with cubs, the future is only promising. To have continued long-term viable snow leopard populations in the country, the following recommendations are proposed:

## - Conduct periodic population survey

Successful conservation and charismatic large carnivores such as the snow leopard relies on the effectiveness of monitoring programmes (Alexander et al. 2016; Suryawanshi et al. 2021). With annual monitoring of snow leopard populations going to be more difficult due to huge cost implications and limited human resources, it is vital that periodic population surveys are conducted at least once in five years. Nevertheless, intensive monitoring of smaller areas on annual basis is recommended to have better insights into population processes.

## - Strengthen patrolling and surveillance

While there are no reports of snow leopard poaching in the country, the prevalence of illegal market for wildlife parts in the region may prompt local people to kill snow leopards to sell their parts. Besides, snow leopards preying on domestic animals will also increase the risk of retaliatory killing by the livestock owners. Therefore, patrolling needs to be strengthened and upscaled to reduce dangers of snow leopard killing and poaching. Patrolling can also curb other illegal activities taking place in snow leopard habitats.

## - Habitat assessment and management

Carnivores play a key role in regulating the terrestrial ecosystems, but their prevalence is also a result of complex interactions between food and habitat availability. For the snow leopard to thrive, there should be a healthy prey population, which in turn depends on good natural habitats. Snow leopard being an elusive species, anthropogenic disturbances should also be minimized. Prime snow leopard habitats must be protected as core zone and critical conservation areas. Therefore, periodic assessment of alpine habitat and placing management intervention where necessary is important. Studies should be done for a better understanding of the
carnivore intraguild relations to inform management and conservation actions in areas where there is a habitat overlap between snow leopards and other carnivores.

## - Habitat use and connectivity

The possibilities of snow leopard population dispersal from high density areas to other potential habitats are high in Bhutan. In order to ascertain these dynamics, scientific studies should be continued, and snow leopard movement should be tracked through GPS collaring snow leopards or by using non-invasive genetic sampling.

## - Human-snow leopard conflict management

As a predator that occasionally kills domestic animals, the conflict between local highlanders and snow leopards is heightening. In order to ensure positive interaction between snow leopards and the people sharing the same landscape, it is vital that livestock insurance schemes are established. Implementing interventions, such as providing coral fencing to yak herders has proven effective in reducing snow leopards preying on yak calves. Consequently, it is essential to sustain and enhance these interventions for continued positive outcomes.

- Community-based wildlife tourism

An innovative intervention to reduce the impact of snow leopard predation onto highlander's livestock population would be through the introduction of community-based wildlife tourism in areas with high snow leopard density which consequently also experience high livestock predation rates. These areas are coincidentally also popular trekking destinations thereby offering high potential of success of such interventions.

- Conservation action plan

To effectively prioritize conservation efforts to preserve snow leopard habitats and safeguard their prey species, it is crucial to establish pragmatic conservation action plans.

Given that the existing national snow leopard conservation action plan has expired, it is imperative to develop new action plans to identify conservation management requirements and secure sufficient funding for their successful implementation.

"Introduction of<br>community-based wildlife<br>tourism in areas with high snow leopard density in an opportunity"

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## APPENDICES

Appendix I: Abundance and density estimate as obtained from Maximum Likelihood approach.

Overall population estimate

| Estimate | SE | Lower CL | Upper CL | $\mathbf{n}$ | Area ( $\left.\mathbf{k m}^{\mathbf{2}}\right)$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 128.5602 | 13.29682 | 105.0272 | 157.3661 | 96 | 9979.598 |

Overall density estimates per $100 \mathrm{~km}^{2}$

| Estimate | SE | Lower CL | Upper CL |
| :---: | :---: | :---: | :---: |
| 1.28823 | 0.13324 | 1.052419 | 1.576878 |

Sex specific estimates
Male population estimate

| Estimate | SE | Lower CL | Upper CL | $\mathbf{N}$ | Area (km ${ }^{\mathbf{2}}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 61.1795 | 8.642293 | 46.44699 | 80.58501 | 45 | 9979.598 |

Density estimate per 100 km ${ }^{2}$

| Estimate | SE | Lower CL | Upper CL |
| :---: | :---: | :---: | :---: |
| 0.6130457 | 0.08659961 | 0.4654195 | 0.8074976 |

Female population estimate

| Estimate | SE | Lower CL | Upper CL | n | Area (km ${ }^{\mathbf{2}}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 67.22891 | 10.27853 | 49.90777 | 90.56157 | 51 | 9979.598 |

Female Density estimate per 100 km ${ }^{2}$

| Estimate | SE | Lower CL | Upper CL* |
| :---: | :---: | :---: | :---: |
| 0.6736635 | 0.1029954 | 0.500098 | 0.9074671 |

Appendix II: Images of identified snow leopard individuals with their activity centre maps


BSL_01


BSL_02


BSL_03



BSL_09


BSL_10


BSL_11


BSL_12


BSL_13


BSL_14


BSL_15


BSL_16


BSL_17


BSL_18


BSL_19


BSL_20


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BSL_26


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BSL_31


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BSL_37


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BSL_39


BSL_40


BSL_41


BSL_42


BSL_44


BSL_45


BSL_46


BSL_47


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BSL_75



BSL_76

BSL_77


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BSL_79


BSL_80


BSL_82


BSL_83


BSL_86


BSL_87


BSL_88


BSL_89


BSL_90


BSL_93


BSL_94


BSL_96

Appendix II: Carnivores co-occurring with snow leopard captured during the survey



Asiatic golden cat (Catopuma temminckii)


Pallas's cat (Otocolobus manul)


Himalayan wolf (Canis lupus chanco)


Asiatic wild dog (Cuon alpinus)


Tibetan red fox (Vulpes ferrilata)

Yellow-throated marten (Martes flavigula)


Asiatic black bear (Ursus thibetanus)


Red panda (Ailurus fulgens)


Record of new deer species for the country Thorold's deer (Cervus albirostris)

Appendix IV: National snow leopard survey teams


Team JDNP and JSWNP during the training workshop at Punakha


Team WCNP and BWS during the training workshop at Bumthang


Team JKSNR, DFO Thimphu and DFO Paro during the training workshop at Haa


Training opening session at UWIFoRT, Bumthang

