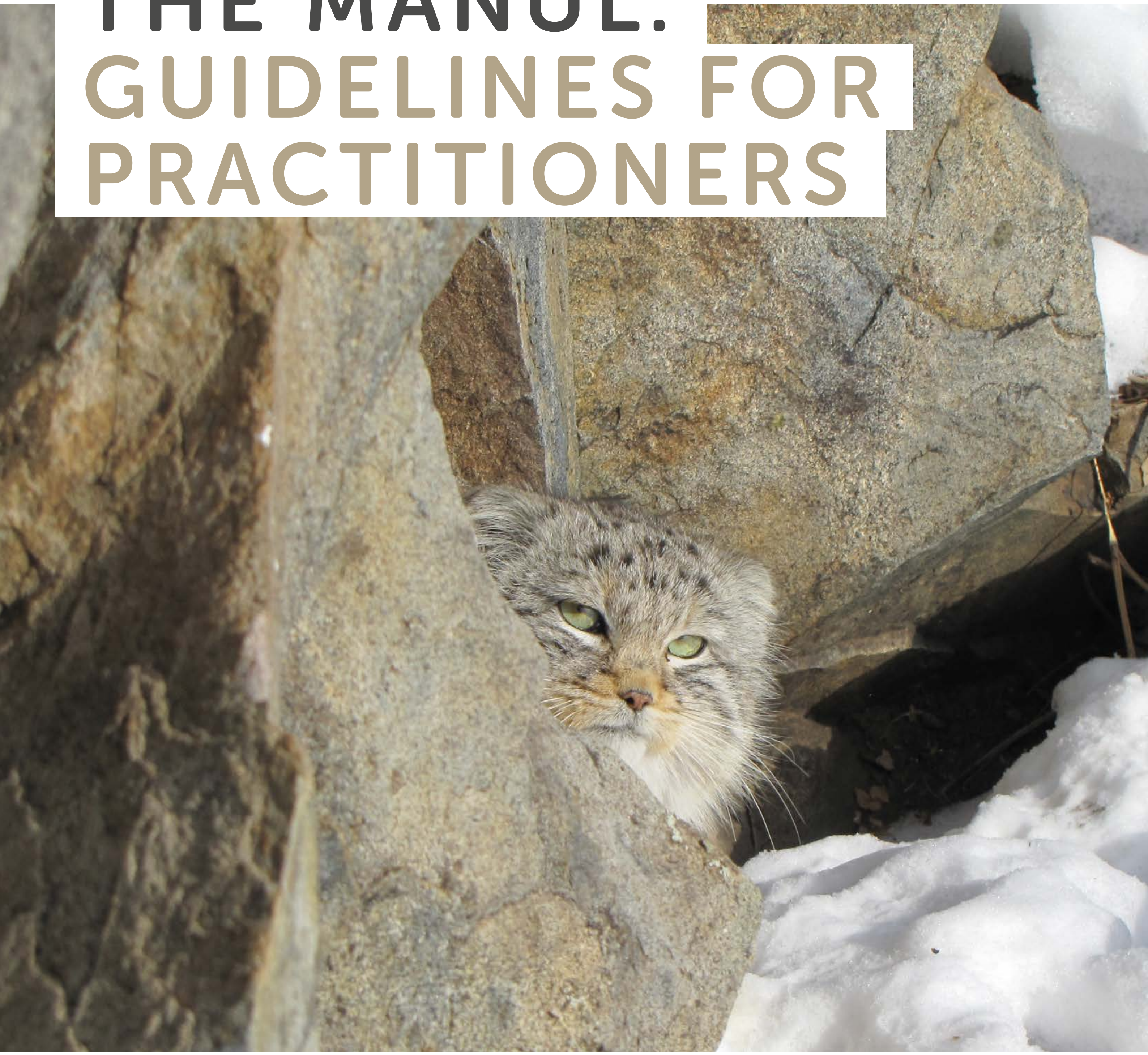
MONITORING THE MANUL: GUIDELINES FOR PRACTITIONERS



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CONTENTS

FOREWORD

Field monitoring of wild animal species is rarely accomplished without challenges. Logistical, environmental, and ecological factors dictate the need for appropriate sampling regardless of location, taxa, or objectives. With regards to felids there is no questioning their popularity when it comes to field research. Their role and impact on the ecosystems combined with their "hypercarnivore" lifestyle and cultural significance have resulted in an extensive and historical resume of field studies across the globe. Despite the vast number of studies on felids, there is a significant skew toward the big cats with the scientific knowledge base for small-bodied cat species, including the Pallas's cat or manul (*Otocolobus manul*), much smaller. Given the solitary and elusive nature of most small cats, like the manul, that inhabit remote environments, it is not difficult to understand this gap in field research.

Recognising this gap and following years of field research and conservation, the Pallas's cat International Conservation Alliance (PICA) and their conservation partners identified the need for targeted and easy to follow guidance on best practices for monitoring the manul in the wild. This guide details an extensive compilation of data collection methods and monitoring techniques for the manul that will help practitioners deliver more effective conservation and research efforts.

This guide was edited by Ehsan Moqanaki and Gustaf Samelius that, in close collaboration with a number of co-authors, have brought together a wealth of knowledge on surveying and monitoring manul populations. Each chapter compiles tried and tested techniques from a range of carnivore research projects over the last few decades, with a focus on the manul. In doing so this guide provides a detailed insight into the most effective data collection methods to enhance future conservation and research efforts for the species. We are grateful to all involved in the development of this book and hope that it serves as a valuable practical guide to current and future conservation and researcher efforts, while contributing to long-term conservation actions for this amazing small cat.

The Pallas's cat International Conservation Alliance

PREFACE

A researcher installing a camera trap for manul monitoring
Photo: A. Antonevich

GLOSSARY

PICA:	Pallas's cat International Conservation Alliance (https://pallascats.org/about-pica/)
MWG:	Manul Working Group (https://savemanul.org/), previously known as the Pallas's Cat Working Group (PCWG)
IUCN:	The International Union for Conservation of Nature (https://iucn.org/)
Cat SG:	IUCN SSC Cat Specialist Group (http://www.catsg.org/)
Red List:	IUCN Red List of Threatened Species (https://www.iucnredlist.org/)
Conservation Strategy:	A range-wide conservation strategy for the manul that was released as part of a Special Issue in Cat News in 2019 (http://www.catsg.org/index.php?id=711) and was the result of a collaborative strategic planning process during the Global Action Planning Meeting at Nordens Ark, Sweden, in 2018.
Global Action Planning Meeting:	A meeting that was organised by PICA in November 2018, where participants (1) reviewed and assessed the knowledge and conservation status of the manul, (2) updated the species' historic and current distribution range, and (3) developed a range-wide Conservation Strategy for the manul that is also part of the Special Issue in Cat News.
Status Review:	A review of the knowledge and conservation status of the manul that was published as part of a Special Issue in Cat News in 2019 (http://www.catsg.org/index.php?id=711).

PREFACE

The Pallas's cat or manul *Otocolobus manul* is a small wild cat native to Western Asia from Iran and the Southern Caucasus in the southwest, through Central Asia and the Himalayas, to Mongolia, China, and Russia in the northeast. Although the manul was downlisted from Near Threatened to Least Concern by the IUCN Red List in 2020, the latest assessment was based on the manul's large-scale distribution, which indicates that, at the global level, the manul has a low risk of extinction. However, at the local and regional level, the manul faces multiple anthropogenic threats and are at risk of reduction in both numbers and distribution, resulting in several populations being in immediate need of conservation actions. In November 2018, a Global Action Planning Meeting was organised by PICA at Nordens Ark, Sweden, to develop a Conservation Strategy for the manul. Twenty-eight participants attended this meeting, including experts from Cat SG, MWG, and researchers from eight range countries. This collaborative strategic planning process resulted in Status Review and range-wide Conservation Strategy for the manul that was published as a Special Issue in Cat News in the Spring of 2019 (http://www.catsg.org/index.php?id=711).

The Conservation Strategy calls for a document on best practices on how to monitor the manul that can be used by practitioners throughout the range. Therefore, PICA initiated the development of this guide on practical advice on how to survey and monitor the manul efficiently. Standardisation of the methods is important to allow comparison of monitoring data across the species range. A lack of reliable monitoring techniques was identified as one of the main challenges for the research and conservation of the manul in the Status Review. This guide is intended to aid conservation and research on the manul by providing standardised methods to monitor the species and thereby enable better prioritisation of populations in the greatest need of conservation action. This is in line with the fact that the lack of suitable monitoring methods is hindering effective conservation of the manul. This guide is the result of discussions on these topics during and after the Global Action Planning Meeting. One important aspect of monitoring the manul that has emerged through these discussions is the lack of practical knowledge of space use and movement patterns of the manul that is hindering effective data collection and where to sample; where to look for scats for non-invasive genetic monitoring and where to put remote cameras for photographic sampling. This guide therefore has a whole chapter (Chapter 2) that focuses on signs and sampling locations that we think will be very useful to help improve surveying and monitoring the manul.

This document is a practical guide about techniques for surveying and monitoring the manul in its natural habitat. Our target audience are practitioners, such as researchers and wildlife managers, who may be familiar with the species and with some, but not all, the methods available. The emphasis is on non-invasive data collection methods that their applications range from detecting presence and understanding habitat use to study occupancy and population size. Developing this guide was challenging for two main reasons. First, the manul occurs in many different habitats across the range and the techniques required to monitor it effectively therefore must be robust and applicable in a diversity of habitats. Second, the manul is a poorly studied species with few publications to inform the monitoring of the species.

This is perhaps not surprising for a small wild cat, as these species generally receive much less attention than the more charismatic "big cats". Yet, the manul is exceptionally poorly studied even when comparing to other small wild cats of Asia. As a result, this guide involves techniques that, even if tested and applied on other felids, have not yet been systematically tested and applied to monitoring the manul (but see case studies cited in the References). We are also highlighting the possibility to use by-catch data from surveys and monitoring other species, such as large carnivores, to be used for monitoring the manul. However, this should be done with care as by-catch data were collected with other species in mind and the sampling design may thus not be appropriate for all questions.

Numerous books, book chapters, and guidelines have been published on survey and monitoring methods of rare or elusive species that are relevant for the manul. This guide does not intend to replace or revise previous work. Rather, our intention was to review these guidelines and present appropriate monitoring tools based the collective experience of the co-authors of this guide to provide an evaluation of the applicability and feasibility of these methods for surveying and monitoring the manul in its natural habitat. We therefore do not provide a complete coverage of the technical aspects of each technique, but rather introduce the different techniques and explain what information they can contribute and how they can be used to monitor the manul. Each chapter also highlights the pros and cons of the different techniques for surveying and monitoring the manul, makes recommendations on what they are best suited for, and provides sources on where to find additional information. New techniques are constantly being developed and the best practices on how to survey and monitor wildlife is therefore changing too. We refer the readers to key publications for further details, many of which are cited in this guide. Likewise, this guide alone cannot help practitioners to master the monitoring techniques brought up in this guide effectively and we therefore encourage the readers of this guide to consult and collaborate with experts in each field to apply these techniques more effectively for surveying and monitoring the manul.

Practitioners must rely on their practical experiences from their study sites when using the information described in this guide and be mindful of their own safety and the safety of others, including the study species. Studying the manul in its natural habitat requires receiving necessary permissions from the authorities in the range countries where the procedure may vary from country to country. In addition to following these rules and regulations, we encourage manul practitioners to review the latest ethical guidelines and protocols available for studying rare or elusive species. This is especially important when using invasive research tools that are only briefly mentioned in this guide, such as GPS-collaring. We urge manul researchers to follow relevant protocols and seek adequate training before embarking into any activities that may involve the manul or its habitat. Likewise, there are important ethical considerations in working with people in public surveys and practitioners should consult with social scientists and follow the guidelines for socio-ecological research to refine their protocols.

PREFACE

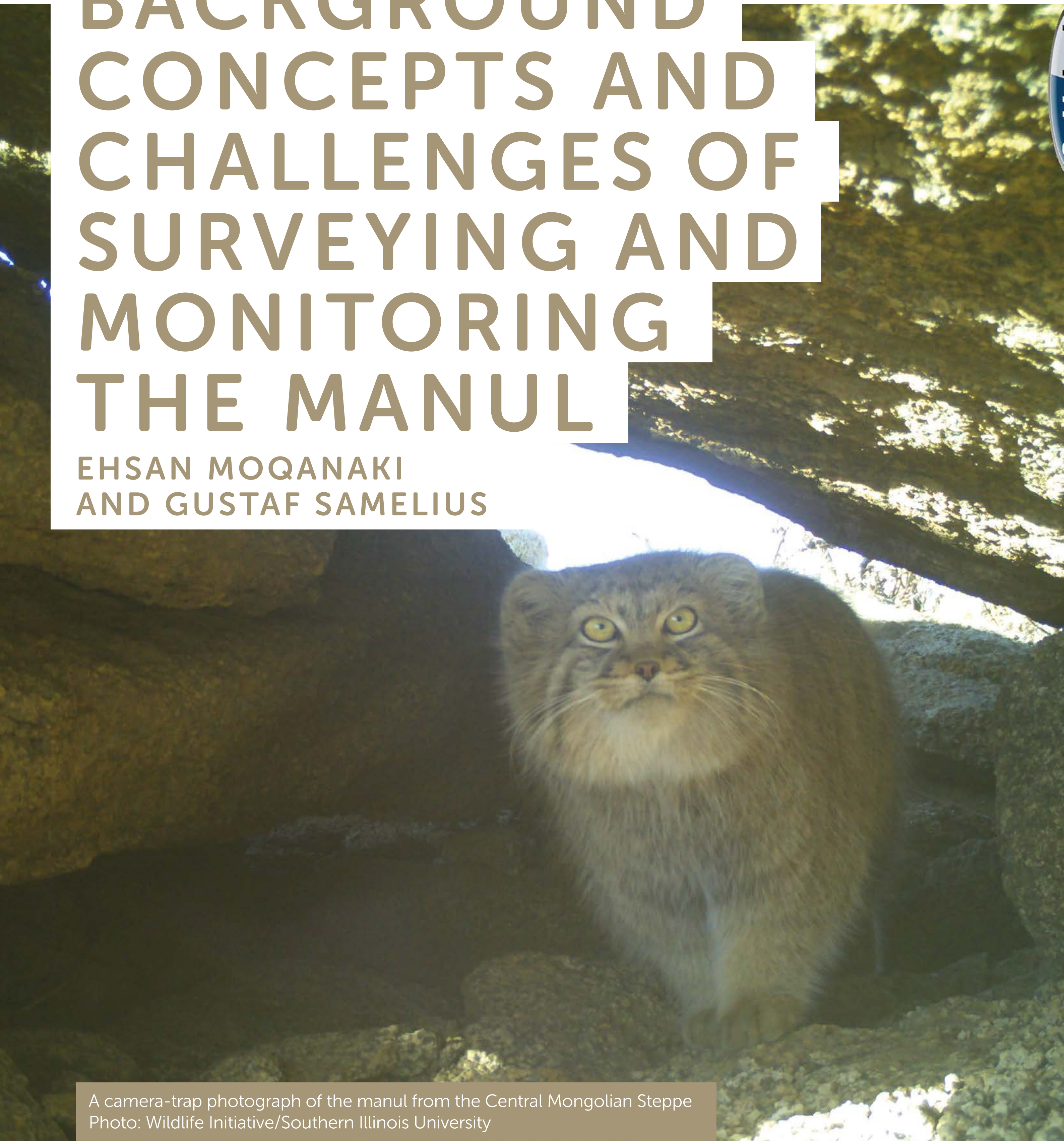
The development of this guide has been a collaborative effort by a large group of co-authors with various expertise and background in manul research and conservation. Many people have contributed to this guide, and we are grateful for their support, in particular Emma Nygren, David Barclay, and Katarzyna Ruta for their administrative and scientific management as well as polishing our English. We are indebted to all the people who have shared photographs and camera-trap images with us (credited in the figure captions). Many thanks to our co-authors for their feedback on Chapter 1, and to Mahdieh Tourani, Fridolin Zimmermann, and Juliette Young for their review of some chapters of this guide. The preparation of this guide was supported financially by Fondation Segré through a grant to PICA.

Ehsan Moqanaki and Gustaf Samelius

CHAPTER 1

BACKGROUND CONCEPTS AND CHALLENGES OF SURVEYING AND MONITORING THE MANUL

EHSAN MOQANAKI
AND GUSTAF SAMELIUS



A camera-trap photograph of the manul from the Central Mongolian Steppe
Photo: Wildlife Initiative/Southern Illinois University

GLOSSARY

Abundance:	The number of individuals from the target population inhabiting the study area during the sampling period.
Density:	The number of animals per surface area.
Distribution:	A measure or description of where the animals occur in.
Occupancy:	The proportion of sites within a given area in which the species occurs.
Population dynamics:	Variation in population size, distribution, and population structure (e.g., age and sex) across time and space.
Survey:	A one-time effort to gauge the state of a population where we focus on occupancy and population size in this guide. A survey can focus on other aspects of the state of the population, such as threats to the population and human dimensions (e.g., attitudes and tolerance). A survey may include repeated sampling (e.g., several field visits) but is still a one-time effort in that only one estimate of the status of the population is derived.
Monitoring:	Repeated efforts to gauge how the status of a population is changing over time, where we focus on occupancy and population size in this guide. Other aspects of the state of the population, such as age structure and threats to the population, and changes in human dimensions related to the population can be also monitored. Monitoring can thus be seen as several surveys repeated over time.
Non-invasive sampling:	Sampling animals from the target population without having to physically capture, restrain, or even see them.
Camera trap:	Or trail camera, typically consists of a camera and a sensor that is triggered by heat and motion, which is used to capture images of wildlife with minimal human interference.
Non-invasive DNA:	Collection of DNA present in naturally shed cells found in biological substances, such as hair or scats (i.e., faeces).
Imperfect detection:	We never see or detect all the animals present in an area, which means that the probability of detecting the target species is always less than one regardless of sampling effort.
Hierarchical model:	A statistical model consisting of multiple levels to model processes that are linked to one another. In analysing survey and monitoring data of wildlife, hierarchical models are useful to disentangle observation noise (e.g., bias and errors) from the ecological process of interest.

1.1. INTRODUCTION

Monitoring is often defined as the process of examining or gauging how the state of a population varies over time and is the results of repeated surveys of the population (Yoccoz et al. 2001, Nichols and Williams 2006). Monitoring of wildlife populations often focuses on distribution and population size, but it can also investigate other aspects of the state of the population, such as sex ratios, age structure, threats to the population, or human dimensions related to the population (e.g., attitudes or conflict). Monitoring forms the basis of many management and conservation efforts as knowing how populations fare over time and how they respond to various conservation actions lie at the heart of such efforts (Yoccoz et al. 2001, Nichols and Williams 2006, Jones et al. 2013). An important aspect of all monitoring is that it is based on scientifically sound methods to allow for informed evaluation of previous management and conservation strategies.

The main threats to manul survival include habitat loss, degradation, and fragmentation (Ross et al. 2020). These threats mostly result from a growing human population that exerts pressure on manul habitat by unsustainable livestock grazing, agricultural and infrastructure expansion, and extraction of natural resources (Ross et al. 2019a). In areas where these threats are acting in concert, immediate actions are required if remaining manul populations are to be conserved (Moqanaki & Ross 2020). Whether the first step is to collect baseline information on the status of these populations or act with the best data available is debated (Chadès et al. 2008, McDonaldMadden et al. 2010, Jones et al. 2013). For small, isolated, and highly threatened manul populations, we recommend focusing on immediate conservation actions with the best data available. However, the advantage of establishing population monitoring is that managers can reliably evaluate the effectiveness of the conservation measures. Surveying and monitoring manul populations are also important for the identification of conservation priority areas, development of conservation strategies for mitigating threats, and balancing whether we need more research or direct conservation actions.

1.2. SURVEYING AND MONITORING THE MANUL

There are some distinctions between wildlife surveys and monitoring, mostly regarding the duration and the questions asked. We define a survey as a one-time effort to gauge the state of a population, where we focus on occupancy and population size in this guide. However, a wildlife survey may focus on other aspects of the state of the population, such as population structure or threats to the population, or human dimensions related to the population (e.g., attitudes and tolerance). For example, a manul survey can be simply confirming the presence of the manul in a protected area during the sampling period. A more complex survey may target estimating the population size of the manul in the same area. A survey may involve replicated sampling (e.g., multiple visits of the study area), but it is still a one-time effort in that we derive only one estimate on the status of the population. Monitoring, however, is a repeated effort to gauge how the state of a population is changing over time. Monitoring can thus be seen as several surveys repeated over time and monitoring has a temporal depth that is not present in surveys.

Survey and monitoring protocols should aim to gauge the status of the target population while at the same time also optimising the time, cost, and labour required to collect the information. For manul survey and monitoring, standardised approaches must be cost-effective, logistically feasible, and robust to different sources of error and bias. The rugged and mountainous habitats inhabited by the manul, as well as the low density and elusiveness of the species, prevent the application of sampling methods based on direct observations. Methodological innovations have provided us with several methods to survey and monitor manul populations (Fig. 1). However, we need to define clear objectives for our sampling and that we have the financial and logistical resources needed to collect the information properly.

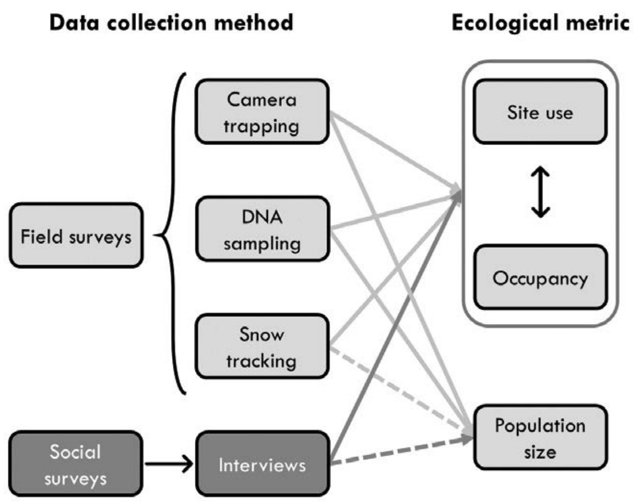


Figure 1: Data collection methods (left) that have high potential for surveing and monitoring manul populations (right). In this guide, we focus on four non-invasive data collection methods: (1) camera trapping; (2) DNA (or genetic) sampling; (3) snow tracking; and (4) interview-based surveys. The relevant ecological metrics from these methods are occupancy (or, in some cases, site use), and population size (i.e., abundance and density). In some cases, relative abundance or proxy-based measures of density can be obtained (illustrated by dashed lines), as estimating population size can be a challenge when working with rare or elusive species like the manul. Practitionares may also combine different data types from multiple sampling methods (e.g., camera trapping and DNA sampling) to improve inferences on manul populations (arrows not shown here).

In this guide, we define non-invasive data collection as those that do not involve capturing or handling of the animals (Zemanova 2020). Using one or a combination of these data collection methods, practitioners can collect the necessary information to answer questions about manul distribution and population size. Table 1 summarises pros and cons of the different data collection methods included in this guide. Our aim in this guide is to provide practitioners with an overview of the basic concepts on how to survey and monitor manul populations using the non-invasive data collection methods described in Figure 1 and Table 1. The guide is structured so that each method is described by its own chapter which includes the basic concepts of the methods and provides readers with references for additional information (Chapter 3-6). We have also included a chapter with tips on manul signs and where to conduct sampling that we think will be very useful for surveying and monitoring manul populations given that there is currently very limited information on this for the manul (Chapter 2).

Table 1: Comparison of non-invasive data collection methods for surveying and monitoring manul populations in their natural habitat.

Method: Camera trapping	
Pros	**Cons**
• Many commercial options • Straightforward sampling protocols that do not require much training or manpower • Continuous, autonomous monitoring for long time • Reliable species identification and potentials for individual identification • Recording date and time of detections • Several open-source and user-friendly software for data management and analysis • Additional information (e.g., reproduction and behaviour, co-occurring species) • Useful for education and outreach	• May require additional permits in some areas • Expensive when conducted over large areas • Cheaper cameras are prone to malfunction and errors • Difficult to purchase in or import to some countries • Losses are relatively high (e.g., theft, flood, wildfires, damages by animals) • Missed detections are potentially higher for small cats like the manul • Automated species and individual identification are still under development • Thousands to even as many as millions of photos and videos to manage and process

Method: Faecal-DNA sampling	
Pros	**Cons**
• Straightforward sampling protocols and potentials for training and involving non-professionals • Relatively low cost to collect the samples in the field • Several hundred samples can be processed quickly • Reliable species and individual identification • Additional information (e.g., genetic relatedness, population structure, gene flow, diet, diseases)	• Additional permits are required for sampling • Difficult to find fresh manul scats in systematic sampling • Longevity of target DNA and thus success of analyses varies with environmental conditions (e.g., exposure to rain or sun) • Potentially low DNA amplification success and high genotyping errors • Some media for sample preservation can be challenging or hazardous to carry in the field (e.g., ethanol) • Unknown gap between collection time and the time of defecation (i.e., unknown timespan for the study) • Restrictions on transport of biological samples to and from some countries • Expensive lab procedure • Specific lab skills required to process data and interpret results

Method: Snow tracking	
Pros	**Cons**
• Low cost • No special equipment is needed • No additional permissions are required • Potential for combining with non-invasive DNA sampling (e.g., scat, hair, urine)	• Restricted to suitable weather conditions (i.e., after a good snowfall) and substrates • Logistically challenging during difficult weather • Relatively high errors in species identification even for experienced practitioners, especially in the presence of other small cats • Individual identification is not possible • No precise date and time from tracks, although it can be gauged based on the time from the last snowfall

Method: Interviews	
Pros	**Cons**
• Relatively low cost • No special equipment is needed • Potentials to engage with and involve members of the public • Opportunities for collaboration with social scientists (i.e., interdisciplinary research) • Possibility to study past population trends • Can provide additional information (e.g., attitudes, perceptions, trade, conflict claims)	• Time is usually limited for building trust • Difficult to vet the data • High risk of bias and errors • Risk that private and sensitive information are not kept confidential if the information is not treated properly

1.3. ACCOUNTING FOR IMPERFECT DETECTION

Surveying and monitoring animals are challenging as we never see or detect all the animals in the study area; a fact that is referred to as imperfect detection (Kellner and Swihart 2014, Kéry and Royle 2016). For example, we may not see or detect all the animals in the area because some of the animals may not pass by our cameras, or because the quality of some of the photos or DNA samples are too poor. Surveying and monitoring manul populations is challenging also by their shy and elusive behaviour, the fact that they often occur at low densities, and that manuls tend to live in rugged habitats which often result in their low detectability (Ross 2009, Ross et al. 2012, 2019b, 2020). It is also difficult to find their signs and scats in the field, let alone to identify manul individuals to study populations (Zhao et al. 2020, Anile et al. 2021, Hacker et al. 2022).

To design survey and monitoring programs for the manul, practitioners should consider the sampling effort required to meet the study objectives while accounting for different sources of error and bias. At the same time, practitioners need to optimise field-efforts based on operational costs, logistical realities, and local conditions. There are analytical frameworks that account for imperfect detection during the sampling to provide reliable estimates of ecological metrics (Kellner and Swihart 2014, Kéry and Royle 2016). When analysing survey and monitoring data of wildlife, the most popular analytical frameworks are occupancy and capture-recapture (MacKenzie et al. 2002, MacKenzie and Nichols 2004, Efford 2004, Royle et al. 2014). Occupancy models use detection and non-detection data (i.e., site detection histories) for the sampling sites to estimate the proportion of area occupied or used by the target species (MacKenzie et al. 2002, MacKenzie and Nichols 2004). Capture-recapture models also use detection and non-detection data for the individual animals encountered in the survey (i.e., individual detection histories) to estimate abundance and density. One of the more recent extensions of capture-recapture are spatial capture-recapture models that incorporate the location of the detections and thereby provides spatially explicit estimates of population size; thus, these models are advantageous to classic capture-recapture models (Borchers 2012, Royle et al. 2014, 2018, Tourani 2022). Individually identified data can be difficult to achieve for the manul and model extensions such as spatial mark-resight might be also useful to obtain population size estimates (Gilbert et al. 2021).

In this guide, we chose not to delve into analytical details, since we focus on the practical aspects of data collection for surveying and monitoring manul populations. Rather, we highlight important aspects of survey design relevant to the manul and provide references to the literature for further reading. Different analytical frameworks make specific assumptions that must be met and considered when sampling manul populations and analysing the resulting data. For example, when estimating population size in single-season capture-recapture models, the population is assumed to be demographically closed, i.e., no births, deaths, immigration, or emigration occurs during the sampling (Lukacs and Burnham 2005). Likewise, in occupancy modelling, we assume that occupancy across the study area does not change during the sampling period (MacKenzie et al. 2002, Rota et al. 2009).

Consideration of model assumptions can be particularly important when working with wildlife for which much of the ecology is unknown, such as for the manul. For example, in occupancy modelling, the recommendation for the spacing between the traps or the detectors (e.g., camera traps) is that it is large enough relative to the average home range of the target species to reduce the risk of an animal being detected by more than one detector (MacKenzie et al. 2002, O'Connell and Bailey 2011). However, since published information on the home-range size of the manul are limited, some guesswork is needed to decide on appropriate spacing between the detectors given the study questions. Based on the available data from Mongolia and Russia, the average home range size of adult manuls is between 27 and 99 km^2 for males, and between 10 and 23 km^2 for females (Ross 2009, Kirilyuk and Barashkova 2011, Ross et al. 2012, 2019b). Considering this available information, the spacing for a large-scale occupancy-based survey would be about 8 - 10 km. However, shorter distances can also be used when working at local scales (app. 1000 km^2) because the choice of the sampling design is also dictated by the questions asked. In contrast, to estimate population size using spatial capture-recapture models, detections of individuals at multiple detectors are needed to inform about the individual home ranges (e.g., multiple camera traps within the home range; Borchers and Efford 2008, Borchers 2012, Royle et al. 2014). For example, Anile et al. (2021) used camera trap spacing of about one km in their study to estimate density of a manul population using spatial capture-recapture.

1.4. TEMPORAL CONSIDERATIONS OF SAMPLING

Occupancy and capture-recapture frameworks are usually based on repeated visits of the study sites (called sampling occasions) that are used to create detection histories for these analyses (Williams et al. 2001, Mackenzie and Royle 2005, Royle et al. 2018). Sampling occasion can be as fine as one day to several weeks. For example, in snow tracking of the manul, one day of tracking can be considered as one sampling occasion. Detection histories show whether manul presence at a location was detected during that sampling occasion or not. Some sampling methods, such as camera trapping, are continuous processes, where the traps operate and collect data continuously throughout the sampling period. The sampling duration is usually divided into several sampling occasions of appropriate length by the research team based on the study objectives and, to some extent, the resulting data (e.g., number of detections). It is therefore common to decide on the length of the sampling occasion after the data have been collected. As a rule of thumb, on the one hand, sampling occasions should not be too short, as this may result in detection histories with too few detections to analyse the data. On the other hand, sampling occasions should not be too long either as this does not leverage the data to its full potential, because the number of detections in each sampling occasion is reduced to detections and non-detections and thereby, we may lose data by merging several detections into one. For a relatively high-density manul population (15 ± 5 manul individuals/100 km^2) with high detectability (3.1 ± 0.44 manul detections per camera trap), Anile et al. (2021) used one day as the length of the sampling occasions. Such high resolution might not be attainable in low-density populations surveyed by only a few camera traps, where one or two weeks may be more appropriate length of the sampling periods. Thus, the sampling period should be long enough to allow detection of the manul in multiple occasions (e.g., 60 to 120 days), while at the same time not being too long as this may violate some of the model assumptions (Lukacs and Burnham 2005, Rota et al. 2009). As an alternative for analysing long-term data, dynamic occupancy and open-population capture-recapture models have been developed (Royle et al. 2014, Kéry and Royle 2016).

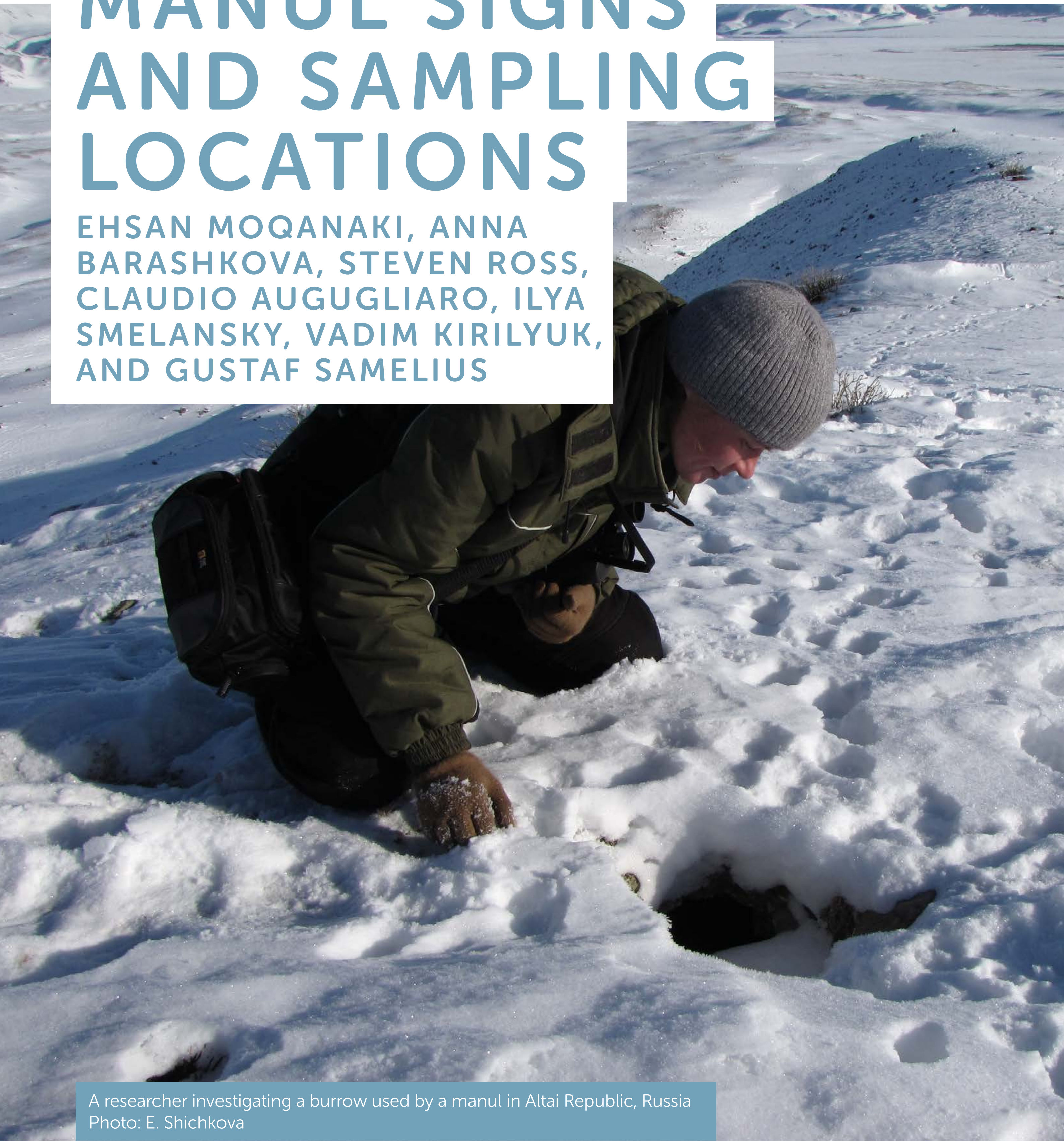
1.5. SPATIAL CONSIDERATIONS OF SAMPLING

The size of and the distance between the sampling sites are important in both occupancy and capture-recapture frameworks. While the spatial aspect of the sampling differs somewhat for the two approaches, it is important that the sampling is conducted so that we have a reasonable chance of detecting the animals (MacKenzie et al. 2002, O'Connell et al. 2011, Rovero and Zimmermann 2016). For capture-recapture studies, this means that we should have at least one sampling site (e.g., a camera trap or scat sampling station) per animal home range and ideally a few more. For occupancy studies, sampling sites should be spaced far enough so that it is unlikely for one individual to be recorded in more than one sampling site during a given sampling occasion. Such spatial design will also reduce spatial autocorrelation in occupancy modelling (Johnson et al. 2013). When studying occupancy of the manul at large spatial extents (over 10 000 km^2), we recommend sampling sites of around 64 - 100 km^2 based on the reported home range sizes (Kirilyuk and Barashkova 2011, Ross et al. 2012, 2019b). Sample size greatly influences the precision and accuracy of estimates of population size and occupancy. Similar to the temporal considerations of sampling, a fine-resolution spatial sampling design may lead to too few detections to estimate occupancy and population size and are usually logistically challenging. To collect data sufficient for analyses, there is a trade-off between surveying more sites with fewer number of visits (extensive) vs. using longer sampling of fewer sites (intensive) but the consequences can be different for occupancy vs. population size estimates (Steenweg et al. 2018).

CHAPTER 2

MANUL SIGNS AND SAMPLING LOCATIONS

EHSAN MOQANAKI, ANNA BARASHKOVA, STEVEN ROSS, CLAUDIO AUGUGLIARO, ILYA SMELANSKY, VADIM KIRILYUK, AND GUSTAF SAMELIUS



A researcher investigating a burrow used by a manul in Altai Republic, Russia
Photo: E. Shichkova

GLOSSARY

Signs:	Any kind of sign, trace, or trail of an animal, including a set of pugmarks or footprints (i.e., tracks) and scents. Also known as spoor.
Snow track:	An animal track left in snow
Scat:	The excrement or faecal dropping of a wild animal (i.e., faeces)
Latrine:	A place where carnivore species habitually defecate and urinate.
Marking site:	A location used for scent marking by animals (e.g., urinating or defecating) for intra- and interspecific communication, such as territorial marking. Sometimes referred to as a signpost.
Den:	A shelter or lair of a wild animal. A den may be used temporarily or year-round, as a safe resting location, anti-predator cover, refuge against unfavourable weather, or to give birth and raise offspring during the maternal period.

2.1. INTRODUCTION

Identifying manul signs in the field is crucial to increase the efficiency of surveys and monitoring programs using non-invasive data collection methods covered in this guide. A knowledge and ability to identify signs left by the manul, its habitat preferences and movement and scent-marking behaviours provide valuable information that will increase sampling efficiency when surveying the manul. Such knowledge can also be used to increase detection of the manul and other co-occurring carnivores' and for assessing factors related to its presence (Fig. 1). Detecting and recording manul signs accurately contribute towards estimating the spatial distribution and relative abundance of the manul using the analytical methods mentioned in Chapter 1. The main advantage of using sign surveys is the ability to sample large spatial extents with relatively low cost. Sign-based survey and monitoring of wildlife may, however, be challenging in certain situations due to low detection rate. Confusion with signs from other similar species may also affect data collection and inferences, and so must be done with care (Harrington et al. 2010, Monterroso et al. 2013, Morin et al. 2016). In addition, individual identification of manuls is not possible using signs alone. Special training is required to search, detect, and reliably identify signs at the species level. In this chapter, we describe the primary habitat and signs of the manul that may be used in the field to improve manul sampling efforts using sign surveys, camera trapping and non-invasive DNA sampling.

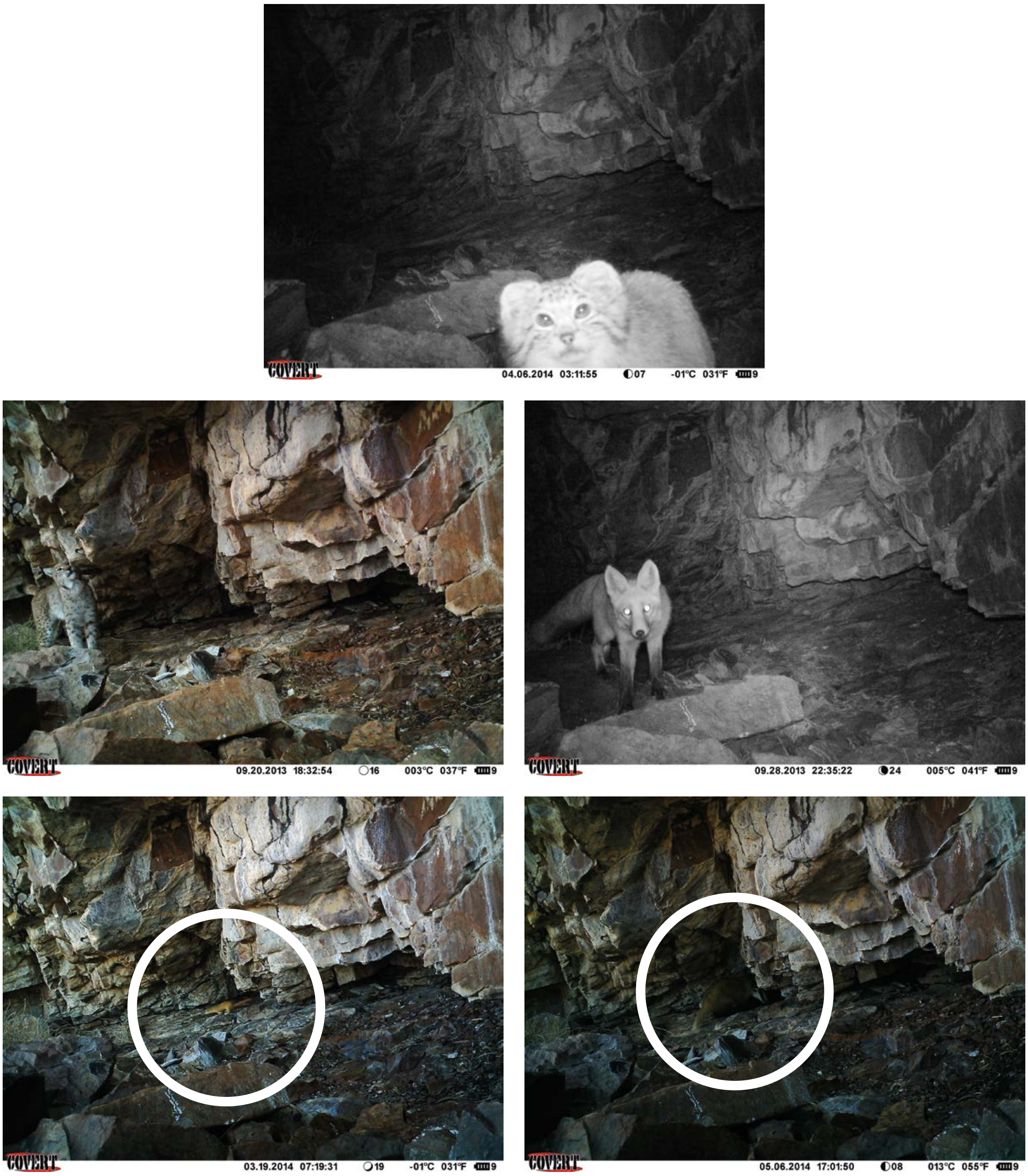


Figure 1: An optimal sampling location can provide valuable information on not only the manul (top) but also the diverse group of terrestrial mammal species co-occurring with it. Clockwise from top left (second and third rows): Eurasian lynx *Lynx lynx,* red fox *Vulpes vulpes,* Asian badger *Meles leucurus,* and the Altai weasel *Mustela altaica* camera-trapped at a camera station in Central Kazakh Upland, Kazakhstan. Photos: Sibecocenter

2.2. HABITAT

Montane grasslands, shrub steppe, hill slopes, and semi-desert foothills characterise the manul's primary habitat (Ross et al. 2019; Fig. 2). At the home-range level, radio-tracking has revealed the manul's preference of habitats that provide suitable cover, such as rocky outcrops, ravines, and other disruptive cover (Ross 2009, Ross et al. 2010a, Barashkova & Kiriliuk 2011; Fig. 3). In addition, sites that provide refuge or dens for manuls are crucial for the species survival (Ross et al. 2010b). Dens protect manuls from their natural predators, unfavourable weather, and provide cover for raising offspring (Ross et al. 2010b). As den-like cavities are used by the manul on a daily basis, they are always found in habitats occupied by the species (Figs. 3-5; see Section 6.6). Marmot burrows, rock crevices, den sites of sympatric carnivores (e.g., badgers *Meles* spp. and foxes *Vulpes* spp.), and tree cavities are known to be used by manuls (Ross 2009, Dibadj et al. 2018, Ross et al. 2019). As the manul has a requirement for denning habitat, targeting den habitats for sign surveys and as sampling locations may help increase the detection of the species.

Figure 2: The manul occurs in a range of habitats across a broad geographic distribution. Optimal locations for non-invasive sampling of the manul may therefore vary across habitats. Photos from top, left to right: eastern Mongolia (A. Barashkova); Mongolian Altai (I. Monti); Gobi Altai, Mongolia (N. Battogtokh); central Mongolia (I. Monti); Qinghai-Tibetan Plateau, China (B. Weckworth/Panthera); Qilian Mountains, northern China (S. Dazhao/Chinese Felid Conservation Alliance)

Figure 2: (continued) More examples of manul habitats across its global geographic range. Photos from top, left to right: Buryatia Republic, Russia (A. Barashkova); Russian Altai (A. Barashkova); Russian Dauria (A. Barashkova); Tyva Republic, Russia (A. Barashkova); northern Balkhash, Central Kazakh Upland (A. Barashkova); Tayathan, western Kazakh Upland (I. Smelansky)

Figure 2: (continued) More examples of manul habitats across its global geographic range. Photos from top, left to right: Manrak, eastern Kazakhstan (A. Barashkova); Sarychat-Ertash Reserve, Kyrgyzstan (K. Zhumabai uulu); Humla, Nepal (G. Werhahn/ Himalayan Wolves Project); Changthang, India (N. Mahar); Bamiyan Plateau, Afghanistan (N. Jahed/WCS Afghanistan); Khosh Yeylagh, north-central Iran (M. A. Adibi)

2.3. FOCAL POINTS

There are four main microhabitats that are often suitable for finding manul signs and obtaining camera-trap photographs and, possibly, DNA samples:

(1) Marking sites and latrines: Manuls check and probably scent-mark conspicuous objects in rocky habitats and hilly steppes, particularly at junctures of two major valleys or passages. Examples are vertical and overhanging rocks, shelters under boulders, and rock crevices (Li et al. 2013; Figs. 4-5). In addition, manuls also create and use latrines. These consist of a collection of manul scats in a small area (1 x 1 m) with loose dirt. Latrines are often found close to a manul den site (S. Ross, pers. obs.). Although these latrines may be visited and sniffed by other manuls, evidence suggests only one manul uses the latrine at any given location (Ross 2009, Ross et al. 2010a). Other manuls may visit the latrine to smell and gain information about the latrine holder, for example reproductive condition, as has been observed in other carnivore species (Macdonald 1980, Allen et al. 2016). Thus, these locations should be inspected for potential manul signs and can be selected for camera trapping and non-invasive DNA collection of putative scats and hair.

Figure 3: In areas with scattered rocky habitats, conspicuous habitat features that potentially provide a refuge for manuls should be targeted for non-invasive data collection methods described in this guide. Photos: C. Augugliaro/A. Barashkova/I. Smelansky

Figure 4: Rock crevices with single or double-access passages are hotspots for camera-trapping manuls (top and bottom right). Latrines (bottom left) are also excellent places to pick up signs and collect putative scats for DNA analysis. Photos: C. Augugliaro (top), Wildlife Initiative/Southern Illinois University (bottom)

Figure 5: Sheltered cavities and edges of rocky habitats that are colonised by rock-dwelling pikas or rodents are good places for searching for manul signs. Each row shows one good sampling location from two viewpoints. Photos: A. Barashkova/I. Smelansky/M. Gritsina

(2) Pika and rodent colonies: The manul mainly feeds on small lagomorphs and rodents (Ross et al. 2010a, 2019; Fig. 6). As the manul lives in temperate to semi-arid environments with long cold winters, it is particularly dependent on prey that are available year-round. Thus, non-hibernating colonial rodents and lagomorphs are key prey for the manul, particularly over winter. Among them, pikas *Ochotona* spp. are preferred, followed by voles (specifically *Alticola* spp. and *Lasiopodomys* spp.; Fig. 6). Areas with high densities of these prey species in mountainous terrain, such as rockslides, talus slopes with large boulders, ravines with patches of vegetation nearby, and open grasslands hosting large colonies of pika and rodents, are visited frequently by manuls (Ross 2009; Figs. 5, 7-8). Manuls depend significantly less on hamsters and gerbils (Ross et al. 2012, 2019, Zhao et al. 2020, Baatargal & Suuri 2021), which occur at low density in the manul's prime habitat.

Habitats hosting pika and vole colonies vary across the manul's range. Colonies of rock-living pikas are usually identifiable by small stones and soil near small burrows at the edge of rock piles, presence of small, dark, oval or spherical droppings, and vegetation caches left for drying known as hay piles (Fig. 6). Some species of pika have gerbil-like settlements, or like those of Brandt's vole *L. brandtii*, ones that are a series of burrows in open areas of slopes or on flat areas (e.g., in depressions), including those overgrown with bushes. Pikas rarely travel far from their burrows for foraging, which results in distinctive grazing lines near their colonies by the end of summer. Colonies of different species of voles and pikas can look very similar. For example, rock-living voles establish large colonies in the crevices of rocks and can be easily detected by the emissions of small stones and droppings that usually appear as black oblong excrements (Fig. 6). The flat-headed vole *A. strelzowi* often makes fence-like structures out of pebbles, droppings, grass, and mud, which protect the entrance to its shelter from small predators. The same arrangements are observed in the mountain silver vole *A. argentatus* (Sludskii 1978; A. Barashkova, pers. obs.). In rocky habitats, the Kazakh pika *O. opaca* similarly blocks the space between the stones, and there are the same emissions of stones near the rock plates under which it settles (Fig. 6). Kazakh pika may also dig holes among bushes. The same is true for the Mongolian pika *O. pallasi;* the species usually burrows, and where there are rocks with crevices, it can settle within them. Colonies of gerbils and Brandt's voles are very similar; they can burrow in almost every available substrate.

Figure 6: Small, non-hibernating lagomorphs and rodents are manul's main prey. Photos from top, left to right: Mongolian pika (also known as the Pallas's pika *O. pallasi*); Afghan pika *O. rufescens* (A. Taktehrani); Daurian pika *O. dauurica* and an example of its hay pile; Kazakh pika *O. opaca* (previously considered as *O. pallasi)* at the surface entrance to its burrow and its small, oval-shape droppings.

Figure 6: (continued) More examples of manul prey and their colonies and signs (from top, left to right): flat-headed vole *Alticola strelzowi* and its burrows and pellets in rockslides; Brandt's vole *Lasiopodomys brandtii;* and Mongolian gerbil *Meriones unguiculatus* - Photos: A. Barashkova/A. Lissovsky/A. Tomilenko/V. Kirilyuk

Figure 7: Manuls frequently visit pika and rodent colonies and settlements for hunting. When installing a camera trap, care must be taken to position the camera to minimise triggering by small non-target mammals. Here, a manul is waiting outside an active colony of the flat-headed vole *Alticola strelzowi* in a rocky outcrop. A vole emerged from the hideout and was ambushed by the manul but the vole apparently escaped. Photos: Sibecocenter

Figure 8: Manuls either take their prey into a safe place, such as dens or shelters (top left: Daurian ground squirrel *Spermophilus dauricus*) and under rock shelters (top right), or consume prey at the capture site (bottom). These locations can be selected for camera trapping or collection of DNA samples. Photos: V. Kirilyuk (top left), Wildlife Initiative/Southern Illinois University (top right), A. Barashkova (bottom)

(3) Predator cover: Due to manuls being at risk of predation from other larger sympatric terrestrial and aerial carnivores, they use the landscape and disruptive cover to evade predation while moving and foraging (Ross 2009, Ross et al 2010b). A common feature of landscapes inhabited by the manul is a mix of rugged, rocky disruptive cover, hills or mountain steppe, shrub steppe, ravines, and flat, open grassland steppe habitat. The combination of disruptive features provides the manul with a network of predator cover habitat. The species generally stays in close proximity to these cover features while moving, or accessing more open habitats for prey (e.g., the Daurian pika in grassland steppe). Key features used by the manul while moving or foraging include narrow passages through rocky outcrops, shelves on steep slopes and rocky crests, ridges, edges of rocky habitats, shrub steppe and ravines (Figs. 3-5). Typically, the manul prefers to move through areas with a high proportion of rocky and ravine habitats, and surveys are likely to observe more signs by focusing in or near to these habitats (Fig. 9). Although currently we lack sufficient data, in more open habitat that lack good cover it is likely that any form of disruptive cover, such as tall grass, depressions, or changes in topography, will be used by the manul as part of their home range. In periods with good snow cover on the ground, snow tracking is very useful to detect such movement paths (Figs. 10-12; see Chapter 5).

(4) Dens: Potential refuges and den sites, such as marmot burrows and rock crevices, are another good sampling location for the manul (Figs. 8, 12; see Section 6.6). Manul hair is quite distinctive and therefore checking the top of a burrow or crevice entrances for hair might provide a good means of identifying the inhabitants, whether it is a fox, badger, manul, or marmot. Manuls use middens (i.e., latrines), where they accumulate scats, while they are at their den. The latrines are often within 30 m of the den sites (S. Ross, pers. obs.). These sites can be targeted for scat DNA collection (Chapter 4).

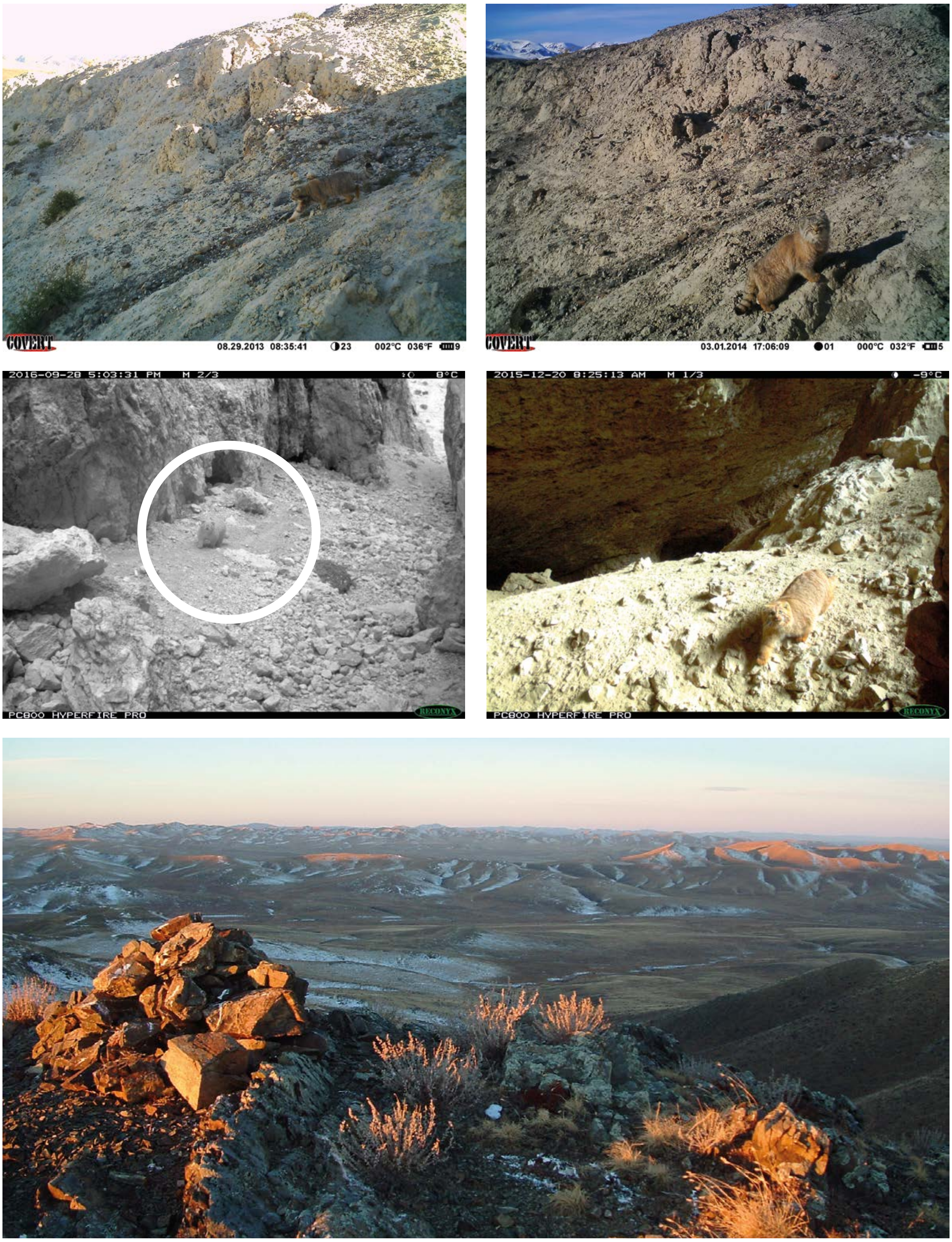


Figure 9: The manul has a preference for certain micro-habitats within a site, such as movement paths that passthrough ridge lines, ravines, rocky areas, and other disruptive cover. Manul habitat generally has a network of cover habitats that allow safe movement and access throughout their home range (bottom). To maximise manul detectability, these locations should be targeted during the sampling. Photos: Sibecocenter (top), WCS Afghanistan (middle), S. Ross (bottom).

2.4. TRACKS

Footprints of the manul are similar to those of other felids. The paws have four toes that form a shallow, asymmetric arc or teardrop-shape (Fig. 10). The four toes almost always register in both front and hind tracks and claws usually do not show. The largest toe is medial and the smallest lateral. Each foot has an interdigital pad called a plantar pad. Two lobes are present on the anterior edge of the interdigital pad (Fig. 10). The larger prints are from the front feet. The front tracks may appear wide or round, while the hind tracks are more rectangular. Based on snow-tracking surveys in Russia and Kazakhstan, in good conditions the length of the manul's footprint, i.e., the distance from the bottom of the pad to the front edge of the front fingers, does not exceed 4 cm (A. Barashkova, pers. obs.). The size of the track is, however, influenced by the depth that the foot sinks into the surface, resulting in larger footprints in soft substrates (e.g., snow, mud). Thus, measurements from the same animal in different substrates may vary, and using visual impressions of track size to assess age and sex can be misleading.

Figure 10: Although footprints form the basis for species identification from tracks, finding a clear print is not always possible. It is therefore important to inspect the entire scene, searching movement paths forward and backward as far as possible to improve the identification, and document the detected tracks properly. Photos: M. A. Adibi (top), A. Barashkova (bottom)

Figure 11: Manul tracks and other signs can be found in rock shelters, under boulders, and other crevices - Photos: A. Barashkova

Figure 12: Manul tracks near active burrows of non-hibernating lagomorphs can be more easily found after a good snowfall.
Photos: A. Barashkova

2.5. SCATS

Manul scats are morphologically similar to those of other felids: usually compact, cylindrical, and segmented, and often containing hair from their prey (Chame 2003, Hunter 2019, Baatargal and Suuri 2021; Fig. 13). However, animal faeces vary individually depending on their contents, defecation-specific conditions, age of the scat and exposure to the environmental factors (e.g., rain, humidity), as well as animal age and health. As a result, even experienced field biologists cannot reliably distinguish scats from different mesocarnivores (Harrington et al. 2010, Monterroso et al. 2013, Moqanaki 2018). For the manul, in addition to misidentifying scats with those of other co-occurring wild, feral, or domestic cats (e.g., *Felis* spp., Chinese mountain cat *F. bieti,* Eurasian lynx *Lynx lynx*, and even snow leopard *Panthera uncia* and the Persian leopard *P. pardus tulliana/ saxicolor*), they are also morphologically similar to those of other mesocarnivores, such as red fox *V. vulpes* and corsac fox *V. corsac* (Baatargal and Suuri 2021). Scat collection from known manul dens and latrines (Ross et al. 2010a, Baatargal and Suuri 2021) combined with camera trapping (Fig. 14) can increase the likelihood of correctly sampling manul scats. However, DNA analysis to genetically determine the species of origin is the most reliable technique to verify the scat is indeed from a manul (Zhao et al. 2020; see Chapter 4).

Figure 13: Scats which probably belong to the manul, found in Altai Republic, Russia. Note the manul's snow-tracks near the defecation location. Photos: A. Barashkova

Figure 14: Carnivores conspicuously deploy their faeces in certain spots as visual or olfactory cues for communication. These latrines or signposts are usually visited by different species and individuals and can be targeted for camera trapping and non-invasive DNA sampling of the manul. Photos: Wildlife Initiative/Southern Illinois University (top), WCS Afghanistan (bottom)

2.6. DEN SITES

Dependence of manuls on den sites and refuges is well documented (Ross et al. 2010b). Depending on their availability, any environmental features that can provide the required cover for resting, feeding, predator avoidance, and raising offspring may be used by manuls. Good examples are marmot burrows and abandoned dens of sympatric mesocarnivores (e.g., badgers and foxes), and rock cavities and crevices (Figs 8, 11-12, 15-19). Manuls have also been recorded using hollowed trunks of aged trees (Dibadj et al. 2018) and abandoned human-made structures, such as ruined and uninhabited buildings, old haystacks, military bunkers, and abandoned farm machinery (Fig. 20). The use of dens varies across seasons, as rock-den locations are preferred during the summer and for raising young, whereas deeper dens of marmot or sympatric carnivores are preferred during the winter, presumably due to their insulation from the cold (Ross et al. 2010b; 2019). Manuls do not use a non-breeding den for more than a few consecutive days, and they tend to use different refuges when moving across their habitat.

When sampling near den sites, extra caution is required not to disturb the manul and the location. This is especially important during the maternal period when kittens are in the den (April to August; Fig. 19); researchers should minimise leaving human and strong scents as much as possible at that time. It is crucial that the researchers do not get too close to the den and do not touch or move the kittens for photography; they should always keep a safe distance. Likewise, whilst setting up a camera trap near a den, the disturbance caused by installing the camera and potential effect of the camera's flash should be minimised. Setting up camera-trap stations under or in front of rock shelters (instead of den) is more appropriate for manul survey and monitoring (Figs 1 & 4). Nonetheless, we recommend using infrared-flash cameras when monitoring den-like locations, and the camera should face the likely path to and from the den rather than the den entrance itself.

Figure 15: Typical dens used by the manul: marmot dens (top) and a rock den used for raising young (bottom) - Photos: S. Ross

Figure 16: A rock cavity under a boulder was used as a den during the maternal period of raising offspring by a female manul. Photos: V. Kirilyuk/The Living Steppe

Figure 17: Identifying den sites and refuges used by the manul is easier during snow-tracking surveys. Here, manul tracks from and to the marmot burrow the manul uses are visible in the surrounding area. Photos: A. Barashkova

Figure 18: A manul den (possibly burrowed by a badger or fox) during winter in a sand massif in Tyva Republic, Russia. Note the manul tracks near the den. Photos: A. Barashkova

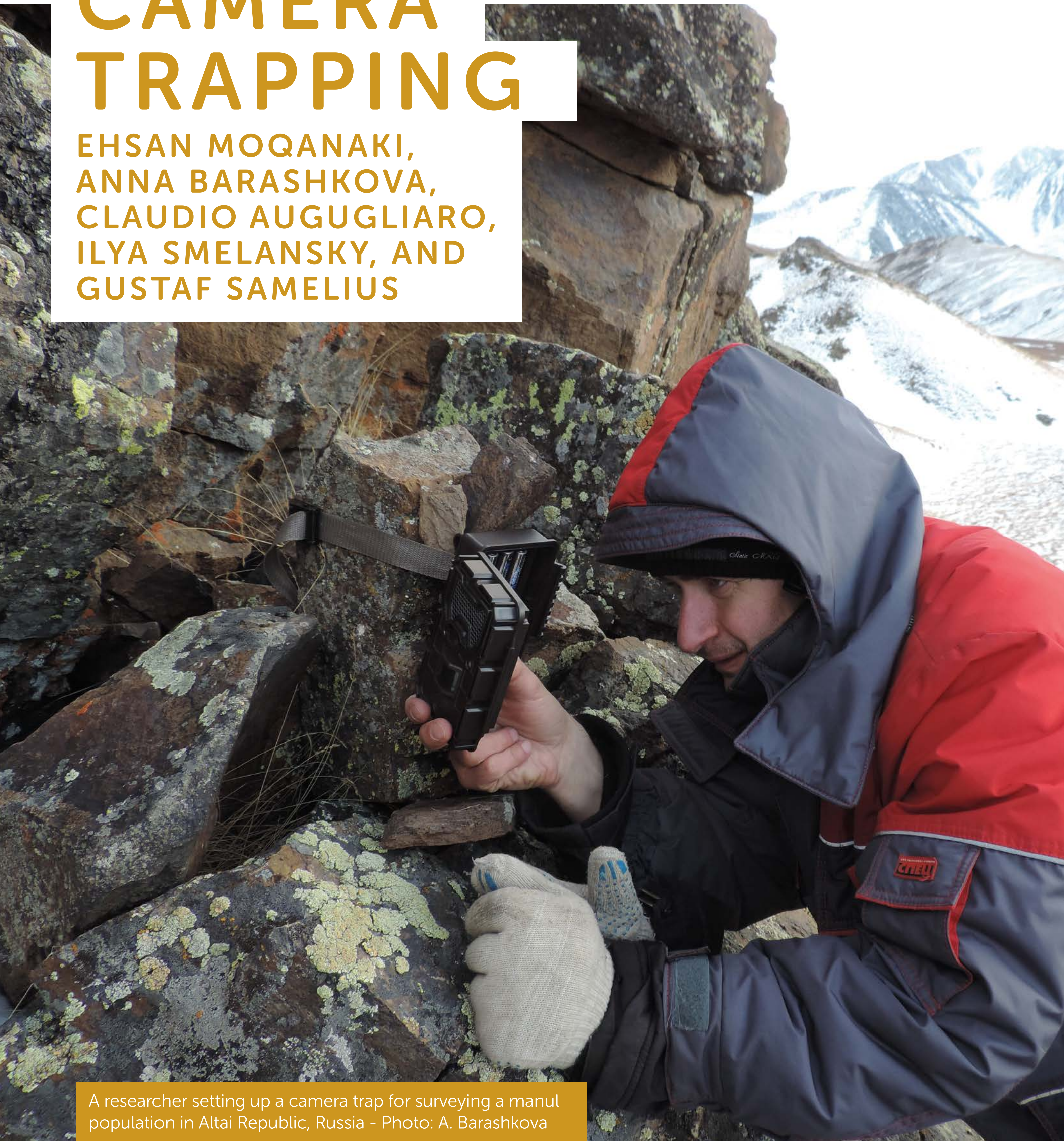
Figure 19: Finding manul's rock dens is very difficult. When inspecting known or suspected dens to record the den characteristics and litter size, always keep a safe distance and do not disturb the location. Photos: WWF-Russia (top left), A. Baranova (top right), Shuanglong/Horseback Planet Society (bottom)

Figure 20: Abandoned human-made features may be used by manuls as den sites or temporary refuge. These locations may also be occupied by manul prey, such as rodents. Top: an abandoned, Soviet-time, military bunker in Russian Dauria and manul kittens inside it; Bottom: abandoned farm machinery in Daursky Reserve, Russia, and a photo-trapped female manul with prey inside one of the combine harvesters - Photos: V. Kirilyuk, I. Smelansky

CHAPTER 3

CAMERA TRAPPING

EHSAN MOQANAKI, ANNA BARASHKOVA, CLAUDIO AUGUGLIARO, ILYA SMELANSKY, AND GUSTAF SAMELIUS



A researcher setting up a camera trap for surveying a manul population in Altai Republic, Russia - Photo: A. Barashkova

GLOSSARY

Camera trap:	Automatically triggered wildlife camera. Modern, commercially available digital camera traps are triggered by heat and motion. Other names may also be used, such as motion-sensitive camera, remote camera, trail camera, game camera, or scouting camera.
Detectability:	The probability that the focal species is detected and photographically recorded at a camera location. The term is sometimes used interchangeably with detection probability.
Camera location:	Or camera station. The physical position of a camera-trap device in space (i.e., latitude and longitude) to detect wildlife.
Camera deployment:	The placement of a camera trap in the study area.
Sampling days:	The number of 24-hour days a fully functional camera trap is used to record wildlife. The number of sampling days can be used as a measure of sampling effort during each camera deployment.
Occasion:	A subset of sampling effort at each camera location (e.g., a week) as defined by the user for a specific analysis.
Sampling effort:	The total number of 24-hour days that the functional cameras were used during the sampling period.
Photographic record:	A photo or video captured by a camera trap, containing wild and/or domestic animals and other non-blank (e.g., humans) and blank images.
Capture event:	The photographic capture of an animal that forms the basis of most analyses. The term is sometimes used interchangeably with independent detection.
Independent detection:	Temporally independent photographic records of a given individual or species at a camera deployment. Multiple non-independent images are often condensed into a single independent detection. This includes, for example, several images of the same animals within a few seconds (i.e., several pictures from the same visit condensed into one observation). It may also include what is judged to be the same animal visiting the same camera within a few minutes up to as much as an hour. An independent detection is often referred to as a capture event.
Photographic rate:	The number of independent detections of the focal species at a camera deployment divided by the length of time the camera was active, which can be then multiplied by 100. This measure is sometimes referred to as Relative Abundance Index (RAI). However, photographic rate can be interpreted as an index of animal activity, rather than an index of abundance.

Blank images: Empty or “ghost” images and videos with no animals in them, because of: (1) animal moving too fast in the camera’s field of view or due to slow trigger speed; (2) non-target objects, such as moving vegetation, in the detectors’ field of view, or (3) shaky cameras; (4) Positioning the camera towards direct sunlight resulting in unusable images; and (5) camera malfunctioning.

Field of view: The horizontal extent of a scene that is visible in camera-trap images, which usually varies from 35° to 100°. The field of view of a camera is determined mostly by the focal length of its lens. A large field of view resulting from wide-angle lenses allow a larger area to be seen in the captured images, with the caveat that the focal animal will be smaller in the frame. Not to be confused with detection zone.

Detection zone: The maximum distance that a sensor can detect a subject (i.e., detection distance; ≈ 10 - 30 m) and detection angle (≈ 15° - 75°) determine the size of the camera’s detection zone. The size of the focal animal and its speed in entering the detection zone influence the detection distance and angle; thus, the size of the detection zone is relative to the focal species. The area that has been sampled across all the cameras is determined by the detection zone. Depending on the field of view of the camera, wide detection angles may lead to more blank images.

Resolution: The visible resolution of an image, typically presented as the number of megapixels (MP), which is determined by multiple elements, such as the pixel resolution of the sensor and quality of the lens. The higher the image resolution, the slower the shutter speed, which can result in blurred images in low-light conditions. Therefore, a compromise between resolution and shutter speed is needed to produce the sharpest images.

3.1. INTRODUCTION

Camera trapping is considered as a non-invasive (or rather, minimally invasive) data collection method to study wildlife populations using a class of remotely triggered cameras. Detailed description of how camera traps work is provided, among others, by Swann et al. (2011), Rovero and Zimmermann (2016), and Apps and McNutt (2018). Camera traps have been used in a wide range of study systems, and often outperform, or are as effective as, alternative sampling tools in terms of quality and quantity of the resulting data (Wearn and Glover-Kapfer 2019, Leempoel et al. 2020). Modern digital camera traps can be deployed in almost any field conditions to study rare, elusive, or threatened species, as the device can be operational 24 hours a day and left unattended for several months. Although camera traps are more commonly used in terrestrial systems to record large and medium-sized mammals (Burton et al. 2015, Delisle et al. 2021), there are also applications for species survey and monitoring in arboreal and even semi-aquatic environments (Gregory et al. 2014, Moore et al. 2021).

By recording the date and time of each photographic record (Fig. 1), camera traps can be used as point-based detectors across multiple sites to construct detection and non-detection histories (often referred to as capture histories) of the target species (O'Connell et al. 2011). By repeating the camera trapping in space or time, we can account for imperfect detection to reliably estimate occupancy, abundance, density, and vital rates (Linkie et al. 2010, Rovero and Zimmermann 2016, Sollmann 2018; see Chapter 1). The camera-trap data can be then used to explore activity patterns and spatio-temporal trends in distribution and population dynamics (Ridout and Linkie 2009, Sollmann 2018). In camera trapping, individuals from the target population can be followed over time in situations where the animals can be identified by unique markings. The individual-based data can then be analysed using spatial capture-recapture methods to estimate density and vital rates (Royle et al 2014, Tourani 2022). There are also applications for using partially marked or unmarked individuals (Gilbert et al. 2021).

For manuls, camera traps not only reveal the species' lesser-known behaviours, but also have the potential to provide solid data on presence, activity patterns, occupancy, habitat use, and even abundance and density (e.g., Shrestha et al. 2014, Anile et al. 2021, Augugliaro et al. 2021). In addition, by depicting the manul in its natural habitat, camera-trap photographs can help raise awareness and investment in manul research and conservation. Nonetheless, camera trapping for manul surveys and monitoring is a challenging endeavour, which requires considerable resources, expertise, and fieldwork in logistically demanding conditions. The main challenges faced by manul researchers to effectively study the species using camera traps are the limited knowledge about the manul ecology, insufficient resources, and low density and low detection rates in many areas across its range. Sparse data resulting from spatially and temporally limited surveys lead to several analytical challenges that must be addressed depending on the study questions (Shannon et al. 2014, Gerber and Parmenter 2015, Gálvez et al. 2016). Given the cost and effort involved in camera trappings, manul researchers should follow standard sampling design and reporting guidelines to maximise the value of the data obtained.

There are many valuable resources for optimal camera-trap based sampling, data management and analyses, and reporting that can be used for manul survey and monitoring as well (e.g., O'Connell et al. 2011, Cadman and González-Talaván 2014, Fleming et al. 2014, Meek et al. 2014, Gillespie et al. 2015, Rovero and Zimmermann 2016, Wearn and Glover-Kapfer 2017, Abrams et al. 2018, Sollmann 2018). Following a standardised framework will also facilitate follow-up surveys, larger-scale analyses, and comparison between studies, and often result in less biased and more precise estimates of the parameters of interest.

The main steps for conducting a camera-trapping study are: (1) determining study goals and objectives, (2) developing field methodology and protocols, (3) data collection, (4) data extraction, storing, and management, and (5) data analysis, interpretation, and reporting. In this chapter, our main objective is to describe the basic steps for data collection and management for manul surveys and monitoring across its range. In addition, we briefly review some of the practical aspects and relevant analytical pathways for using camera-trap data but refer readers to the references provided in this chapter for more details about the topic.

Figure 1: Most digital camera traps provide date, time, moon phase, temperature, and a unique identifier (i.e., camera ID) on the images. These metadata are also stored on the memory card and can be extracted and used during the analysis. Photos: Wildlife Initiative/Southern Illinois University

3.2. CAMERA-TRAP SPECIFICATIONS

A wide range of commercially available camera traps are suitable for manul survey and monitoring. The market is developing rapidly and, like other electronic devices, various products with different makes, functions, and prices are constantly introduced. Because of this, there is no ultimate model, and any list of top models would be quickly outdated. Manul camera-trappers should search for the latest products and reviews and seek feedback by fellow colleagues about different models. The optimal camera trap and its specifics depend on the objectives, study design, and logistical considerations (e.g., budget, climate, terrain, and country restrictions). Sharing knowledge and experience is a good practice and helpful for disseminating the knowledge and there are websites and pages on social media that contribute to the dissemination of information. For example, see reviews, guides, and community discussions available at https://www.trailcampro.com/, http://www.emammal.si.edu/, and https://wildlabs.net/.

Camera traps are different in many features, including their trigger speed, detection zone, image sensor, imaging capabilities, field of view, flash type, battery life, video capability, and housing and security features (Wearn and Glover-Kapfer 2017, Palencia et al. 2022). Regardless of the brand, main features to consider in purchasing camera traps are the camera's (1) sensor, (2) trigger speed, and (3) flash type. To trigger a camera, heat and motion detectors are often used to respond when an object with a surface temperature different from the background's moves in the camera's field of view. Most camera traps in the market come with a passive infrared heat-in-motion sensor that is triggered when a warm-blooded animal walks through the sensor's field of vision (Apps and McNutt 2018). Currently, high quality, passive-infrared sensors can detect almost all animals as small as 100 g, ideally when they walk past the camera within about two metres (Wearn and Glover-Kapfer 2017). Models with active-infrared sensors (i.e., triggered whenever an object breaks the sensor's beam) are also available but to our knowledge have been used less frequently in camera-trapping of rare or elusive species. These cameras are more difficult to work with since they require communication between a transmitting and a receiving unit on opposite sides of the focal point.

A camera's trigger speed is how fast it responds to an animal moving in front of the camera and records an image or video. Most cameras in the market range from 0.1 to 4 seconds in their trigger speed (Wearn and Glover-Kapfer 2017, Palencia et al. 2022). Cameras with a trigger speed greater than one second are generally considered to be slow, because they function relatively poorly when installed perpendicular to wildlife trails and are more prone to missing animals that move quickly. When the camera is set to record a sequence of photos in each detection (e.g., for capturing cryptic behaviours, group sizes, or improving individual ID), the recovery time between the initial trigger and the sequential images is also important. The recovery time for different brands and models range from 0.5 second to as much as one minute (Wearn and Glover-Kapfer 2017).

Figure 2: Xenon and LED white-flash cameras produce full-coloured images at day and night (top). Standard infrared cameras take black-and-white photographs in low-light conditions (bottom). If the animal is moving quickly, the images by infrared cameras are often blurry (bottom). Photos: Wildlife Initiative/Southern Illinois University (top), Sibecocenter (bottom)

Figure 3: A white-flash camera may result in overexposed images if the animal is within 1.5 m of the camera (top right). Overexposure can also happen when using infrared cameras when the animal gets too close to the camera (bottom). Photos: Sibecocenter

Commercially available camera traps are broadly classified based on the flash type as (1) white-light flash, including Xenon white flashes and white LED, and (2) infrared (IR) flash, including no-glow ("IR black flash") and low-glow (or red-glow) near-IR flash (Rovero and Zimmermann 2016, Wearn and Glover-Kapfer 2017). White flashes provide coloured pictures in both day and night (Figs. 2-3). In contrast, IR cameras produce monochrome (black-and-white) images in low-light conditions, which often result in lower quality and resolution of night-time images (Figs. 2-3). Depending on the model and settings, daytime images can be either monochrome or colour. Xenon white flash produces sharp images of passing animals as it effectively freezes any movement. LED flashes are not as strong as Xenon flashes and may result in motion blurred images if the passing animal is walking fast. A sharp capture is specifically important for individual ID using pelage pattern or any other natural markings.

The main disadvantage of white-light flashes is that animals can see the flash, which may induce behavioural responses in some species (e.g., avoidance of the camera; Wegge et al. 2004, Meek et al. 2014, Caravaggi et al. 2020), making these camera traps potentially more invasive than cameras with IR flashes. As a result, white-flash cameras may deter some animals or individuals from entering the camera station. In addition, people can also see white-light flashes, which can alert people to the camera and therefore these cameras are more likely to be stolen or vandalised in areas with high human presence. IR flashes are invisible to humans and most animals and therefore often more useful depending on the specifics of the study system (see discussions in Meek et al. 2014, Rovero and Zimmermann 2016). A white-flash camera, especially Xenon white-flash, also has higher battery consumption and usually requires a few seconds to recharge; thus, often have slower recovery time, which leads to longer intervals between subsequent photo captures. More recent models with LED white flash do not appear to be limited by long recovery times (Wearn and Glover-Kapfer 2017). To avoid overexposed images caused by white-light flashes (Fig 3), we recommend placing cameras a bit farther away from main trails or scent-marking stations even if our recommendations otherwise is to place the cameras close to these features (Figs. 4-5; see Chapter 2). The intensity of a Xenon flash can be reduced manually by putting opaque tape over the flash, if the flash intensity cannot be modulated via the settings of the camera trap (Rovero and Zimmermann 2016). The same can be done with infrared LED flashes (Rovero and Zimmermann 2016).

Figure 4: Rock crevices and shelters with single- or double-access trails are ideal places for recording manuls. However, the focal point is often very close to the camera and the risk of animal avoidance of white-flash cameras is high. This manul does not seem to be affected much by the camera as the cat (identified by the spots in its face) returned to the location after a few weeks. Photos: Wildlife Initiative/Southern Illinois University

IR cameras are advantageous if behavioural responses or interference by humans are of concern. Further, lower battery consumption means that the camera will remain functional several weeks longer than white-flash cameras, particularly compared to Xenon white-flashes, which is important for hard-to-access locations where regular checking is not practical. The disadvantage of IR cameras is that the resulting images at twilight and night, or when the animals is passing by the camera quickly, can be blurry, making species and individual ID difficult (Figs. 2-3). Partial solutions are to program the camera for a sequence of images per detection (i.e., choosing "burst" modes) and using attractants to make the animal stay longer in front of the camera (Polisar et al. 2014, Tourani et al. 2020).

The cost of camera traps ranges from as low as about 50 USD per camera to over 1,000 USD per unit. The price of higher quality camera traps, however, typically ranges between 150 and 600 USD per unit (not including memory cards and batteries). Common problems with lower-quality models are poor durability and poor performance under field conditions (e.g., slower trigger speed and recovery time), fewer customizable settings, and poor-quality images (Wearn and Glover-Kapfer 2017). Thus, for large-scale or intensive camera trapping, purchasing cheap cameras is generally a poor investment. Although the start-up cost is high when working with quality cameras, it is generally a cost-effective approach in the long run given their durability and high-quality images (Abrams et al. 2018).

Some camera-trap models, sometimes referred to as networked cameras, are equipped with wireless technology with the option to send the images, as well as memory and battery status, over mobile phone or wireless networks to a central base station, email address, or phone number. Then, there is no need for physically retrieving the photos from the camera's memory card or physically checking the battery and memory status, and in cases of potential vandalism, resulting images will not be lost. Nonetheless, these models are usually expensive (> 500 USD), limited to sending only smaller files (e.g., no videos), require 3G or higher network coverage that is usually not available in remote locations, use more battery power which impacts battery life, and there are additional costs for sending and receiving data (Rovero and Zimmermann 2016). Also, the resolution of the images that are stored on the SD card are often of higher quality than those sent by the wireless networks and recovering the SD card can thus still be important for identification of individuals.

The most straightforward answer to the question of how many cameras is needed for a camera-trap study is to buy as many cameras as possible. The minimum number of cameras needed to achieve a study objective depends on many factors, such as the questions asked, study design, the size of the study area, the density of the target species, logistical considerations (e.g., site accessibility, time to set up each station, budget), and the time it will take to complete the study (Wearn and Glover-Kapfer 2017). Simulation-based studies and power analysis can help researchers to explore the minimum number of units and sampling effort required (e.g., Galvez et al. 2016, Efford and Boulanger 2019, Dupont et al. 2021, Durbach et al. 2021, Morin et al. 2022). However, it is often difficult to estimate the optimal number of cameras needed and the optimal sampling effort and a basic rule-of-thumb is therefore that more cameras are better. It is also a good practice to have some backup cameras to replace malfunctioning or stolen cameras. In our experience, although depending on the type of camera, season and field conditions, a failure rate of about 10 - 20% of the units (including theft and vandalism) is to be expected for over 12-month trapping effort.

Figure 5: Passages through boulders and cliff crevices are optimal locations for manul camera-trapping. These features are distributed unevenly across the manul habitat, which needs to be considered during the design of the camera trapping. Photos: C. Augugliaro (top), Wildlife Initiative/Southern Illinois University (bottom)

3.3. STUDY DESIGN AND PROTOCOLS

A sampling protocol should be developed before starting the camera trapping. The conventional analytical approaches we briefly introduced in Chapter 1 make specific assumptions about the sampling design and resulting data. Thus, optimal camera-trap design for population size estimation can be different from that of occupancy modelling, and using data collected with one question in mind may therefore not be suitable for other type of questions as it may violate some of the key assumptions of the different estimations (O'Connell and Bailey 2011). Integrating the knowledge of people who are familiar with the study area (e.g., local rangers) is always recommended.

Opportunistic camera trapping can be used to confirm manul presence in one site, especially when few camera-trap units are available (Moqanaki et al. 2019), but this design is not appropriate if the study objective is to estimate probability of occurrence or density using occupancy and spatial capture-recapture models, respectively (Chapter 1). To analyse the camera-trap data using these methods, usually a systematic design should be followed. Depending on the study objectives and realities of the fieldwork, different systematic designs may be considered. A common approach at the landscape-level is the so-called grid-based design, where the study area is overlaid by a grid in the size of the average home range of adults from the target population or the best data available (e.g., 5 × 5 or 10 × 10 km^2 for manuls, depending on the study questions and the total extent of the study area). In manul surveys at regional or local levels, smaller cell sizes should be considered (Anile et al. 2021). For occupancy analysis, a single camera trap then will be placed at approximately the centre-point of each grid cell, or a suitable spot identified by the investigator within each cell. For estimating density, a nested grid design within each grid can be used to allocate two or more cameras in each cell .

The length of the sampling period depends on the study question, the study species and logistical realities. As a rule of thumb, the sampling period should be relatively short as most conventional capture-recapture models assumes demographically closed populations (i.e., no additions or deletions of animals during the study), but long enough to collect the necessary information needed and considering the logistical challenges to deploy and collect the cameras that are often considerable in the rugged landscapes where the manul lives (Fig. 5). In other words, try keeping the sampling period as short as possible while still allowing for enough detections. For carnivore species like the manul, an intensive sampling period of about 60 to 90 days is recommended (Polisar et al. 2014, Abrams et al. 2018). Nonetheless, long-term camera trapping throughout the year, even extensively, may provide new insights into the species' ranging behaviour and variation in site use and detection probabilities, and can be used to improve future sampling design (Harmsen et al. 2021) and in awareness raising programs (V. Kirilyuk, unpublished data).

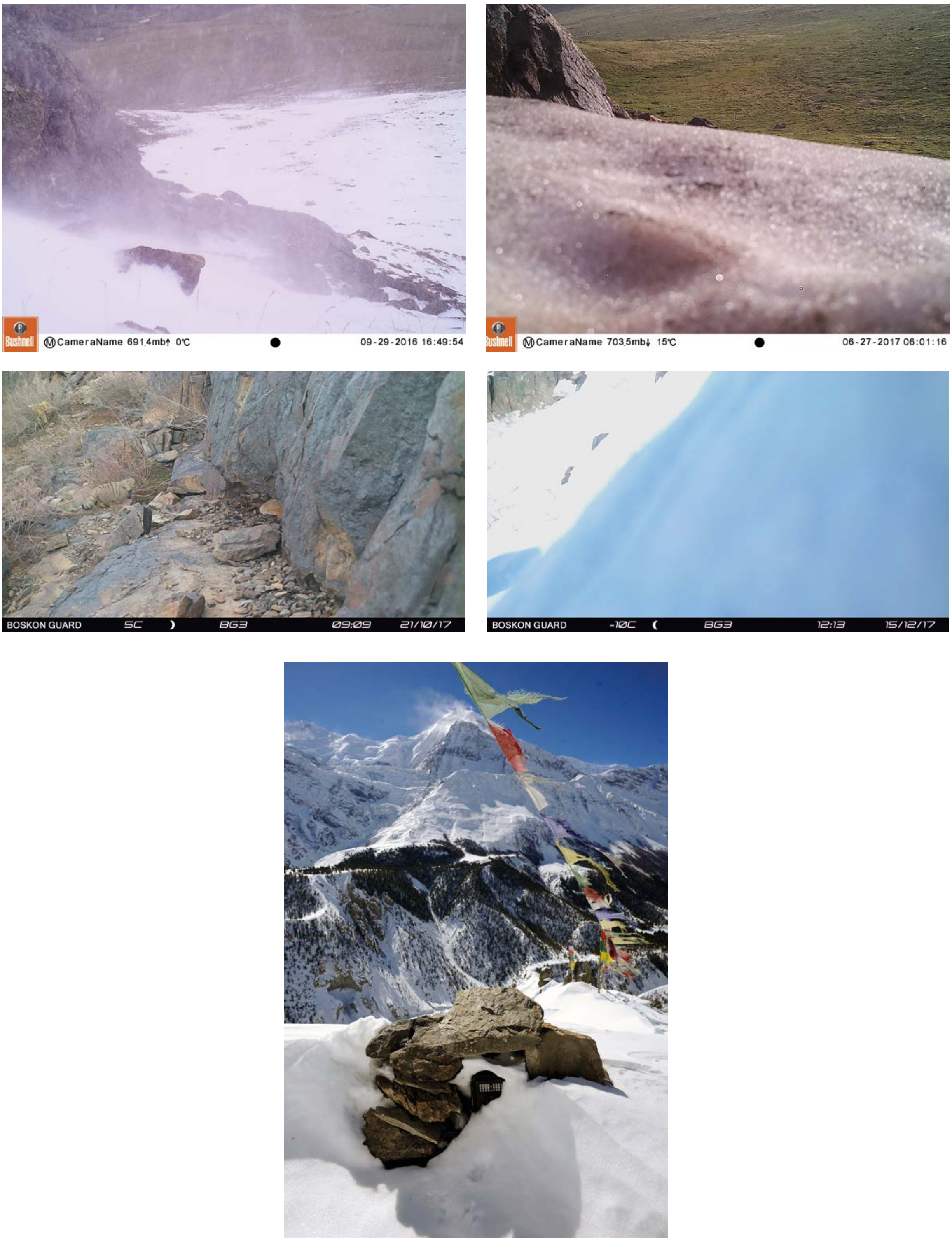


Figure 6: Camera trapping in snowy regions can be challenging, particularly near steep cliffs, where strong winds and accumulation of snow may affect the camera's performance. Top: A camera was installed in a location where snow was accumulated (left), where the camera was eventually buried in snow for more than six months (right). Middle: A camera was installed at the bottom of a cliff (left) and was then completely buried under snow within less than two months (right) - Photos: Sibecocenter. The photo in the bottom shows a camera station buried in snow in Annapurna, Nepal's Himalaya (Photo: G. R. Regmi)

For the manul, we recommend conducting the camera trapping over the course of the warm season (April/May - August) to avoid logistical challenges, such as harsh weather and heavy rains and snow during other times of the year (Fig. 6), and because the manul's prey availability and biomass are the highest during the warm months (Ross 2009, Ross et al. 2019b). In August and September, young manuls start moving away from their natal areas and start to establish their home ranges (V. Kirilyuk, unpublished data) which is likely to increase detection. At the same time, female manuls with kittens reduce their movement during the first months after birth (from about July as observed in the Russian Dauria; Ross et al. 2019b) which, in turn, may reduce detection. An alternative to sampling in the warmer time of the year, depending on the study objectives, is to sample during the peak of the mating period (December - February; Ross et al. 2019b) as movement and activity is high and manul detectability therefore likely high. However, intensive camera trapping during this period can be logistically challenging in some areas (Fig. 6). During heavy snowfalls and blizzards, manuls appear to reduce their activity and may stay very close to their dens for several days until favourable conditions emerge (V. Kirilyuk, unpublished data).

Camera traps provide data for multiple species simultaneously. Given the many camera trapping projects on the big cats inside manul habitat, in particular the snow leopard *P. uncia* (Wong and Kachel 2016; Fig. 7) and the Persian leopard *P. pardus saxicolor/tulliana* (Moqanaki et al. 2019), the data obtained in these surveys and monitoring programs can provide by-catch data regarding the manul with no or little additional costs. However, camera-trapping studies that are not specifically targeting manuls in optimal sampling locations may result in poor detection and incomplete sampling of the species' habitat. Efficient camera-trapping of the manul requires a targeted sampling design with cameras placed at optimal sampling sites as described in Chapter 2, such as rock cavities, marking sites, and under rock shelters, to maximise detection probability (Figs. 4-5) and to assure representative sampling of all habitats used by the manul. Previous studies have shown that camera-trap surveys conducted in the same areas, with comparable sampling effort and during partially overlapping periods, may give substantially different results in terms of detection of the manul because of camera trap placement, where cameras in one study was set to detect large mammals (Augugliaro et al. 2020) and in the other study were set to detect manuls (Augugliaro et al. 2021). Thus, sharing knowledge and data and communication between projects are crucial. Therefore, although tempting, using by-catch data but not accounting for the sources of bias may provide misleading results.

Figure 7: There are many camera-trap surveys conducted within the manul's habitat to collect information on other species, especially for the snow leopards (top-left). These studies usually collect large volumes of records of the manul (bottom-left) and other co-occurring species and can provide important baseline information if used properly. Photos: Snow Leopard Foundation, Kyrgyzstan

3.4. EQUIPMENT

3.4.1. Battery

All camera traps require good quality batteries, and most cameras use between 8 and 12 AA batteries. There are three main types of batteries for camera traps (Rovero and Zimmermann 2016): (1) alkaline, (2) lithium, and (3) rechargeable (nickel–metal hydride [NiMH] and nickel–zinc [NiZn] batteries). Which batteries to use depends on the camera used and climatic conditions but one general recommendation is to avoid mixing different types of batteries in one unit. We also recommend using proven brands that should be available in most of the manul's range countries. Cheaper, locally available brands may also be used, although poor-quality products generally result in poor camera performance and may even harm to the device. It is a good practice to test the durability of the batteries under natural field conditions before purchasing large quantities and embarking into the field. Different camera traps drain batteries at different rates, but for most digital models, a set of alkaline AA batteries will generally last for several thousand images, but much less when recording video, especially for white-flash cameras. Using alkaline batteries in white-flash cameras may result in the flashlight going dim over time when the batteries begin to wear down. This slow but continuous loss of voltage will also affect the flash range, effective detection distance, trigger speed, and recovery time after each photographic event (Wearn and Glover-Kapfer 2017).

Since manul surveying and monitoring often target remote locations with harsh climate, such as extreme temperatures during winter, it is important to strive to reduce battery failure. In manul's northern distribution range (Ross et al. 2020), where camera traps are exposed to relatively long periods of low temperature during the winter (Figs. 6 and 8), it is advisable to use lithium batteries. Although reduction in discharge rate is expected under such situations, in our experience quality lithium batteries are almost unaffected by sub-zero temperatures as low as -40 degrees Celsius (Fig. 8). In contrast, alkaline batteries may cease to work in cold environments at around -10 degrees Celsius. In such situations, when the temperature goes up during the day, the camera may start working again for some time. If the camera setting does not involve taking a picture every day to ensure functionality, it will be impossible to detect and account for these days of inactivity during the analysis. Thus, for hard-to-access locations, where regular checking of the camera is not possible, using lithium batteries is preferred as they may keep the camera station operational for several months and even up to a year. The caveat is the upfront cost, as lithium batteries normally cost about two to three times as much as alkaline batteries, especially when purchased in small quantities.

Figure 8: Lithium batteries keep camera stations operational for long periods even at extreme sub-zero temperatures, like these two locations in Russia's Altai Republic (top) and Kazakhstan (bottom). Note the temperature stamps on the photos showing -27 and -42 degrees Celsius, respectively. Photos: Sibecocenter

Chargeable batteries can be also used and despite their higher up-front costs, they may be cost-effective in large-scale camera trappings as they can be used several times. NiMH rechargeable batteries perform reasonably well in cold conditions. Compared to the single-use disposable batteries, using rechargeable batteries can be better for the environment as well, if they are used to their full potential. Quality rechargeable batteries have up to five years usable life. However, some rechargeable batteries have a lower voltage rating than single-use batteries, which may affect a camera's performance. Many cameras require a charge of 1.5V for optimal operation, while some of the rechargeable NiMH batteries available in the market only have a charge of 1.2V. NiZn batteries typically have a slightly higher voltage compared to non-rechargeable batteries. Thus, compatibility and optimal performance must be ensured before purchasing NiMH batteries. Rechargeable batteries also often have a quicker depletion rate and depending on the usage, usually last only about two months in the field, compared to between two and nine months for most single-use batteries based on our experience. Another potential problem with rechargeable batteries is that they may become weak and unpredictable with age, plus that they generally have to be cycled (i.e., used) a few times before they reach their full capacity (i.e., they may not perform as well the first few uses). In addition, charging all batteries can be time-consuming (especially for large camera-trapping efforts), may involve human errors (e.g., forgetting to charge some of the batteries), and require several dozen good-quality battery chargers. This means additional front costs and added work to charge the batteries and good organisation must be in place before, during, and after the fieldwork. In our experience, finding quality rechargeable batteries and chargers in some of the manul's range countries can be difficult compared to purchasing single-use batteries and, hence, do not recommend using rechargeable batteries and especially not for winter work. If using rechargeable batteries, make sure that they do not go below 30% of their capacity after a sampling session as this may affect how well they will recharge. Do not rely on the battery indicator of the camera unit. Batteries that are no longer suitable to be used in camera traps but are not dead can be still used in handheld GPS units or low-tech devices (e.g., headlights). Non-functional batteries should be disposed properly and not with regular waste. However, access to recycling may vary in the range countries and users should follow the local guidelines and recommendations.

3.4.2. Memory card

Each digital camera trap may photograph thousands of photos and hundreds of videos (if the option is available and activated) between each check. Almost all commercial camera traps on the market accept an SD card with a minimum storage capacity of 16–32 GB, which should suffice for a couple of months in most situations. SD cards also come in different speed ratings, which is the time it takes to write or store data on the card. This is an important consideration especially when using higher specification cameras that have higher resolution images and video. We recommend using a minimum of Class 6 or Class 10 cards, which have write speeds of 6MB/s and 10MB/s, to reduce the risk of any missed data due to slow write speeds.

The compatibility of the SD card with the camera should be checked before going to the field. We strongly advise the users to program each camera with a unique camera ID number, which is a function that is available in virtually all modern cameras. The ID number should be set to appear in the image stamps and in the metadata, as this is very useful when processing the images for analysis. Beside a unique ID, if a site is to be named (e.g., after a landmark or local geographic names), the name should be unique, short, informative, and easily recognizable by all members of the team. It is also a good practice to label each SD card, with labels that match the camera ID. However, handle SD cards carefully. Avoid putting your fingers on the contacts and avoid contact with mobile phone or magnet. Ideally, it is a good practice to use plastic cases or specially designed boxes to store the SD cards. These cases are designed to protect the SD cards from moisture, scratching, pressure, and electrostatic charge. After downloading images to your computer, always reformat the memory cards to clean them completely. There are more than one way to reformat a memory card and we suggest to use quick reformatting in general but to use full reformatting if the SD card has problems.

3.4.3. Camera accessories

Camera-trap accessories, such as metal boxes, cable locks, and cords, to secure cameras and reduce theft can be also considered. We believe features from the manul habitat (e.g., rocks, stones, deadwood) provide enough material for setting up a camera station (Fig. 9), but if setting up a station at a certain height is necessary or a lure station is planned to be established, additional material will be required, such as wooden sticks, pieces of cotton or fibre, attractant, single-use gloves to prevent getting attractant on places not intended, hammer, screwdriver, and headlight. Metal boxes, locks, and bungee cord are not a 100% deterrent of vandalism and can be heavy to carry in the field and are also relatively costly. In areas where camera-trap losses due to theft is likely, using camera traps with camouflage patterns or cameras with infrared flashlight are recommended (Meek et al. 2019, Haines 2022). Camouflage meshes and material from the surrounding environment can be also used to disguise the camera trap, such as wood bark, branches, and leaves, but the investigator must make sure that using such material does not influence the sensor or field of view of the camera. To hold a camera in place, mounting straps or cables can be also used (Fig. 9). However, tie-down straps gradually lose their strength over time when exposed to sun, wind, and temperature fluctuations. We have also experienced gnawing of the straps by rodents when cameras were installed close to rodent colonies (A. Barashkova, pers. obs.). Thus, it is better to hedge and secure the straps with an additional metal cable or wire. The camera's stability must be monitored during long-term surveys and monitoring of the manul.

Figure 9: Elements from the environment can be used to set up a camera station at the height and angle of interest. Rust-resistant, heavy duty mounting straps and cable locks are useful to tighten up the camera around a rock, tree, or pole. However, these attachments may loosen over time and should be monitored during long-term studie. Photos: I. Smelansky and A. Barashkova

3.4.4. Miscellaneous

Handheld GPS devices are required to record the coordinates of each camera station and, if required, survey routes and search tracks. Smartphone apps exist for recording coordinates, and they can be used instead of handheld GPS units in areas with good mobile phone coverage. However, using handheld GPS units is preferable since tracking apps are often power-hungry and the phone battery may drain very fast, particularly in cold weather conditions. Every team member should record the coordinates using the same system and this should be recorded in the data sheets. We suggest using the geographic coordinate system (latitude and longitude in decimal degrees), especially across large spatial extents that may expand over several UTM zones. The geographic coordinate system can then be easily converted to the desired projected coordinate system for more advanced analyses.

A digital camera can be very useful to take photos of the camera station, check the camera-trap photos taken by inserting the SD card from your camera trap into your hand-held camera to assure that the camera is angled as intended, and quickly go through the photos from the camera trap during the checks. Recording the characteristics of each camera station can be useful for identifying important explanatory variables in data analyses. Before checking the photos from the camera trap in your digital camera you should check that the digital camera can read the image format of the camera trap and does not damage the images. If the camera trapping is done in areas used by humans (e.g., used by livestock herders, farmers, and hunters), establishing contacts with the community to explain the project objectives and even engaging them in the process is recommended. Putting contact information and information about the study at the back or top of the camera or even inside the camera should be considered but this depends on the level of trust between the investigator and the community (Sharma et al. 2020). It can also be a good practice to add a note on the camera to discourage people to remove the camera. Some cameras have the option to password-protect the photos and videos obtained. If this option exists in the settings, we suggest using it.

3.5. DATA COLLECTION

3.5.1. Camera settings

Check all the cameras before moving them to the field to ensure that the cameras are fully functioning and that they have fresh batteries and empty SD cards. Check and, if required, update the software of the camera and we recommend to test this for one camera first to assure that the update does not have a bug or other problem that will make all your cameras non-functioning. Pack the cameras carefully and, if possible, individually to minimize any damages during the transport (Fig. 10). Settings should also be checked prior to placing a camera trap in the field. Most importantly, check: date and time (all cameras should follow the same format), the photograph mode (photo, video, or hybrid), minimum delay between photographs, sensitivity (high, medium, or low), number of photographs or length of video to be taken per trigger, picture interval, and that each camera is programmed to have a unique identifier (i.e., camera ID). We recommend using the date format YYYY-MM-DD and 24-hour clock (military time). Depending on the model, other options in the setting, such as time lapse, image size or photograph resolution, and night mode can be specified. All information about the cameras should be recorded and later entered in a common database. Examples of well-structured data sheets are available elsewhere (e.g., Kelly et al. 2013, Van Berkel. 2014, Gillespie et al. 2015, Molloy and Cowan 2018) and they can be modified based on the specifics of your study. During camera checking, if a unit is not working (e.g., malfunction, dead batteries, out of storage space, vandalised, destroyed by animals or floods; Figs 6 and 11), this should be recorded and addressed during the analysis.

Figure 10: Accessing camera locations for the manul may come with logistical challenges and the need to transport cameras long distances. When transporting cameras, handle cameras with care and clean, dry hands or gloves, pack them individually to minimize potential damage to the cameras, and pack them separate from food and strong odour. Photos: A. Barashkova

Some cameras can record video. In the settings, depending on the unit, the user can either select between photo and video modes, or if this option is available, choose the hybrid mode, in which the camera will take a photo and then start the video-recording. However, there is often a bit of delay between the photo and the start of the video in hybrid modes, so that some of the videos will be empty. We therefore recommend setting the camera to either video or image mode but not to hybrid mode. Videorecording by camera traps may provide interesting insights into the manul's behaviour and material for education, awareness, and fundraising. However, depending on the camera brand the video mode can be slower than the image mode and therefore there is a higher risk of missed detections (Wearn and Glover-Kapfer 2017). In addition, recording videos uses much more memory on the camera's SD card. In our experience from working with typical commercial brands available in the market, a camera deployment that is capable of recording over 10,000 photos may be able to record only about 500 short videos. Battery consumption is also considerably higher for video recording. Further, working with videos during the data processing and analysis is difficult because of their large sizes and that their metadata are not standardised and sometimes date and time information is not stored properly. Many of the freely available tools and software packages used for camera-trap data management cannot handle videos (Young et al. 2018, Glover-Kapfer et al. 2019, but see Bubnicki et al. 2016). Reviewing videos to assign species and individual ID is also a laborious process with higher risk of human errors (e.g., missed detections) as the resolution of the videos are generally lower than that of the photos. We therefore do not recommend video trapping in manul surveys and monitoring. A compromise would be to record a sequence of photos with short intervals ($\leq$ 1 second), if the camera's setting allows that, and then the resulting images can be stitched together as a video of joint photographs (Vogt et al. 2014, Wearn and Glover-Kapfer 2017).

3.5.2. Camera location

The process of searching for an optimal camera trap location starts when the investigator is in the field near the planned location. This is a time-consuming process, and several factors are needed to be considered to ensure the best results. If the survey team consists of more than one investigator, a good practice is to split up and search the surrounding for manul signs and most optimal locations with the highest likelihood of visits by the manul. A description of manul signs and good sampling sites for the species is provided in Chapter 2. Micro-habitats containing features that funnel manuls across rocky outcrops are often good sites to yield manul detections. Specifically, features close to rocky terrain and cavities should be targeted (Figs. 4-5, 12, 14-16). In habitats where rock outcrops are less abundant or are too small and at low elevation, manul detectability by camera traps are probably lower. Nevertheless, some of the hotspots of manul occurrence do not contain rock outcrops and instead are characterised by rolling hills, such as the Russian part of Dauria and the Northern Balkhash region in Kazakhstan (Barashkova et al. 2019; see Chapter 2). In such habitats, finding suitable locations for installing camera traps may take more time and requires creativity. In areas and time periods where flooding may occur, avoid placing the cameras in drainages and swampy areas. Likewise, avoid camera trapping in avalanche zones and areas exposed to wildfires, or where strong winds could form snow drifts (Figs. 6 and 11). Information available from previous field surveys (e.g., tracks, droppings) can be very useful and save time, and we recommend engaging with local people and investigators who have experience of working in the study area.

Figure 11: Camera losses and malfunction due to natural or human-made accidents are sometimes inevitable. However, susceptible locations must be avoided as much as possible. Placing cameras in barren rocky areas, away from bushes, and clearing vegetation around the camera location reduce the risk of damage to cameras in fire-prone areas. Photos: Sibecocenter

Figure 12: Manuls regularly use trails and narrow passages on steep slopes, ravines, and ridges. At these locations, make sure that the camera's field of view is wide enough by increasing the distance between the camera and the focal point. Taking test images helps refining the camera's position and field of view. Photos: A. Barashkova (top)/Sibecocenter (bottom)

Figure 13: In long-term camera trapping, some stations may attract non-target animals. If the animal stays in the camera location, the memory will be filled up quickly and the station will no longer be operational after a few days. Top row: These two cameras were functional only for a few days after the arrival of rosy starlings (*Pastor roseus*). Bottom left: This camera was installed near a site that was later occupied by a fox (*Vulpes vulpes*) family and the memory was filled within four days. Bottom right: A fledging of the Eurasian eagle owl (*Bubo bubo*) lived near the camera for several days, resulting in thousands of photos of the bird within a few days. Photos: I. Smelansky (top left)/Sibecocenter (top right and bottom)

Figure 14: In mountainous habitats, the talus provides cover against the manul's natural predators (e.g., raptors), while the rocks provide a cooling effect in the summer and, with snow over it, insulation and warmth during the winter. Such locations are optimal for both the manul and the non-hibernating pikas and rodents it preys on. In these habitats, camera traps installed on a pile of rock fragments or cliffs near mountain meadows should have a wider field of view, as finding trails that canalise manul movement can be difficult. Photos: N. Jahed (top left), WCS Afghanistan (top right and bottom)

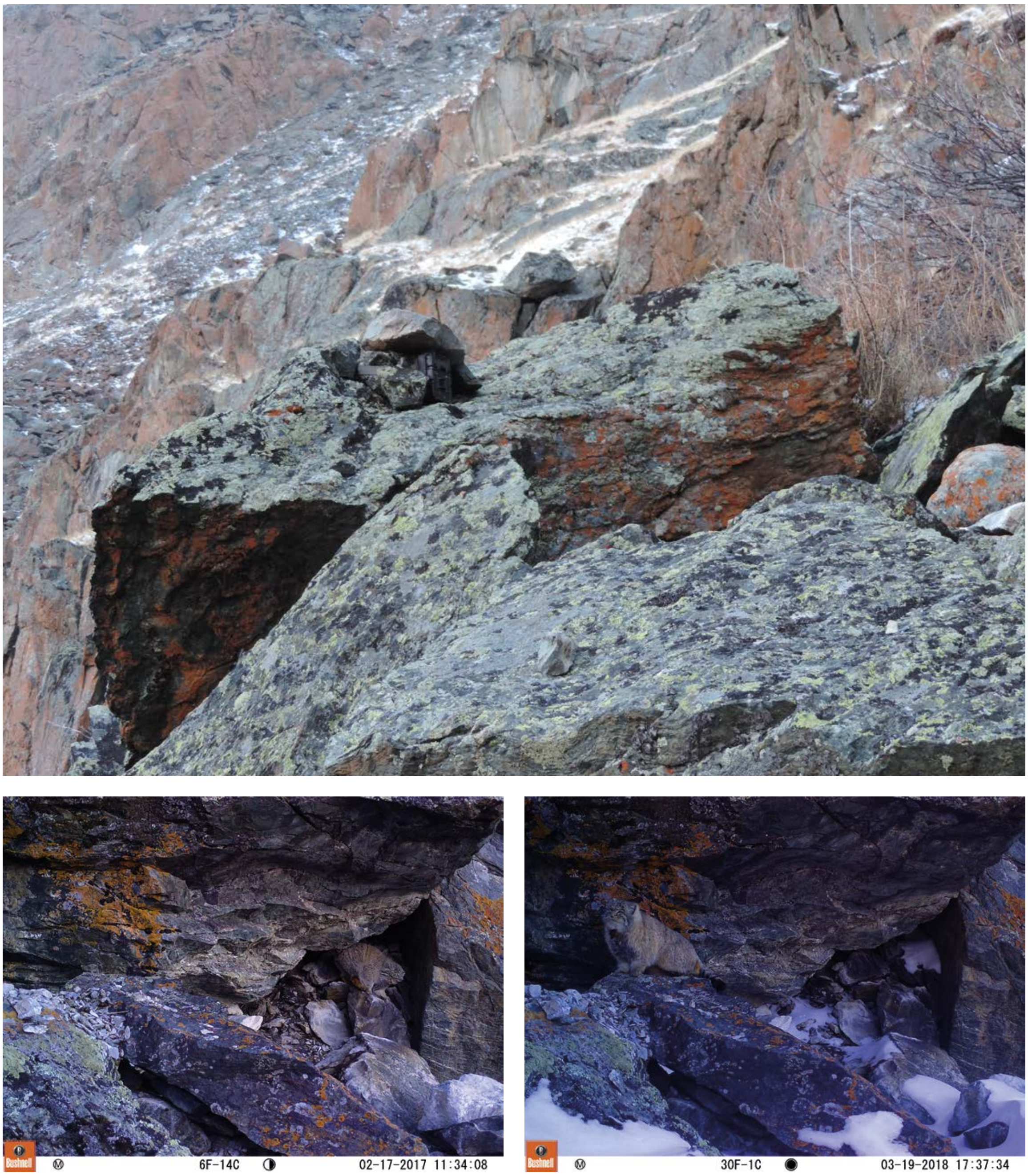


Figure 15: Manuls often use shelves on steep slopes and narrow passages between a collection of rocks to move across the landscape and for hunting. Setting up a camera station in such locations can be challenging as there is little flexibility in mounting cameras in the optimal height and angle. Orienting camera sensors almost perpendicular to the target trail should still be followed as much as possible. Photos: A. Barashkova (top), Sibecocenter (bottom)

Installing cameras near colonies of pikas (*Ochotona* spp.) and voles can improve manul detectability (Augugliaro et al. 2021; Figs. 15-16). These areas are inspected by the manul and other mesocarnivores, which makes the prey colonies a suitable location for camera trapping (Chapter 2). Many camera trappers target wildlife trails and undisturbed dirt roads for recording large carnivores (Kolowski and Forrester 2017, Hofmeester et al. 2021). There is evidence that manuls use such trails; for example, dirt roads in undulating habitats and ungulate trails on talus (Barashkova et al. 2019), and some of the bycatch photographs obtained from trails during surveys of big cats are of manuls (e.g., Shrestha et al. 2014, Moqanaki et al. 2019). Using wildlife or human-made trails helps manuls to move easier and faster from one location to another and allows the cats to avoid walking in deep snow or in tall grass. If targeted for camera trapping, focusing on these trails may increase detectability of manuls during certain periods (e.g., winter). However, if the trails are used by humans and livestock, the risk of vandalism or theft is higher, plus that the memory card can get filled up with non-target images (Fig. 13).

Figure 16: A collection of good locations for photo-capturing manuls in their natural habitats (left column) and the resulting photographs of the manul (right column). The camera at the top was placed at a trail that passed through a collection of rocks. The cameras in the middle and at the bottom were placed by pika and rodent colonies in rocky outcrops.

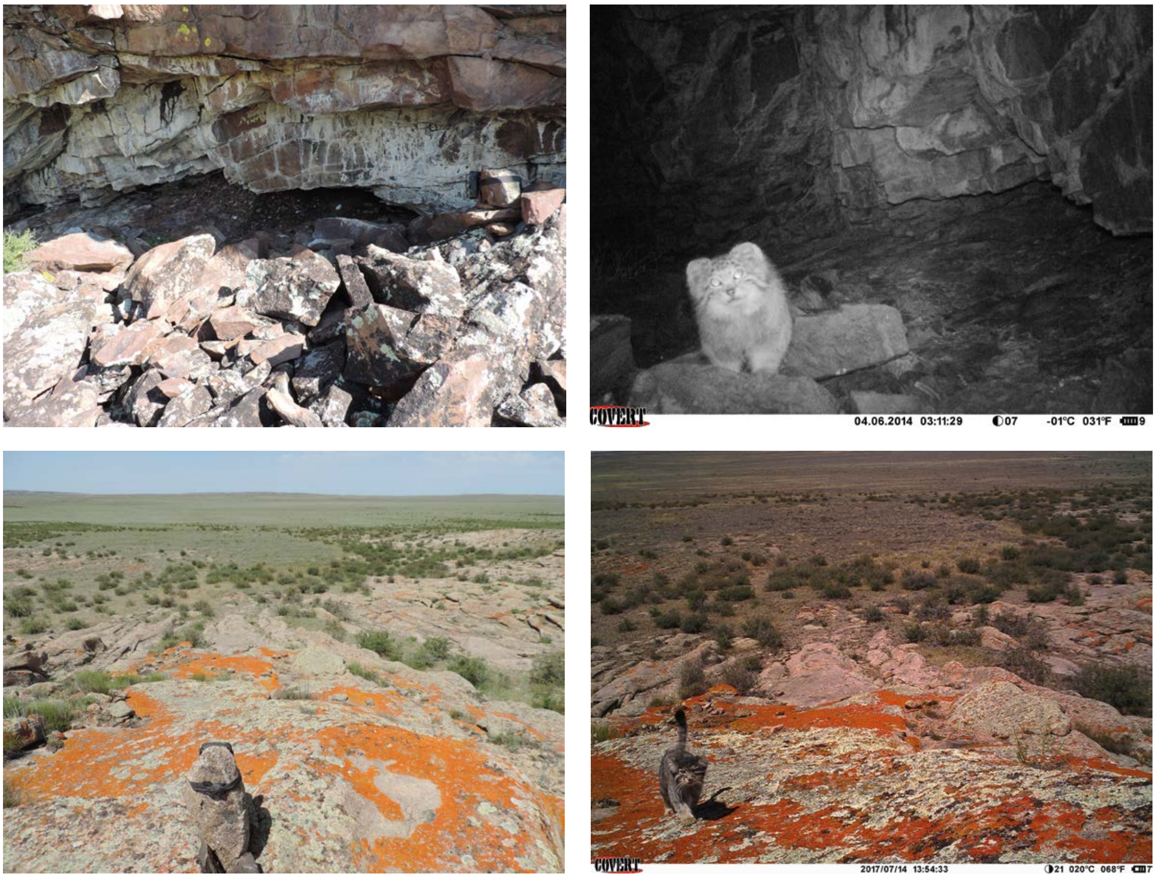


Figure 16: (continued) More examples of good camera sites for phoptographing the manul. Sheltered rock cavities and signposts on the top images are inhabited by voles, which are visited by a manul. The bottom photos show a rocky outcrop with natural pathways in Eastern Kazakhstan, used by a manul. Photos: Sibecocenter, Manul Working Group, I. Smelansky, T. Kisebayev, A. Barashkova

3.5.3. Camera placement

Several studies have evaluated the trade-offs of structured vs. semi-structured and randomised camera-trap placement (e.g., Wearn et al. 2013, Cusack et al. 2015, Fonteyn et al. 2021, Hofmeester et al. 2021). Regardless of the design, placing the camera traps at predefined coordinates is not always possible or even desired. The camera-trap locations should be selected based on the research questions in mind, specifics of the field site (Figs. 6, 11-13), and how to maximise detectability of manuls (Figs. 4-5; Chapter 2). Although discrepancies between the planned and actual locations of camera traps are expected ($\leq$ 500 m), the approximate minimum distance between the camera stations should be respected to meet the assumptions of the analytical framework in mind (Chapter 1). When multiple individuals and teams are responsible for setting up the cameras, communication between team members during the fieldwork is important to ensure that minimum distances are maintained.

The characteristics of the camera location will dictate the camera placement to some extent. A good practice to optimise the camera's field of detection is to place the unit on flat terrain as much as possible (Fig. 16). The camera's area of high sensitivity is a straight horizontal plane extending out from the camera's sensor; thus, the sensitive area should be parallel with the ground (Abrams et al. 2018, Palencia et al. 2022). In steep terrain, which is commonly encountered in manul habitats, adjusting the camera angle given the ground of the target area may be required for optimal camera sensitivity (Figs. 12, 15). Such adjustments depend on the elevation gap from the plateau or valley bottom and camera location. It is very challenging to get the camera to sit securely in place on rugged terrain (Fig. 9). A ground slope upwards or downwards will directly impact the camera's sensor, so the motion sensor will point either down towards the ground or up towards the sky, both resulting in a higher proportion of missed detections (Fig. 20). Nonetheless, cameras can be placed slightly off perpendicular to the focal trail to increase the portion of the focal point covered; thus, increasing the detection probability (Rovero and Zimmermann 2016; Fig. 12).

Place cameras at a height of about 20-30 cm (below knee height), between 0.5 and 2 m from the focus area depending on the focal length of the camera and the flash type (Fig. 17). On the one hand, a camera too close to the focal point will result in close-up photos, where only a part of the animal is visible, which makes the identification difficult, and the flash by the camera may white out the subject, resulting in poor-quality images (Fig. 3). On the other hand, placing a camera too far from the target area would result in photos that are too dark and low in quality that may make species and individual ID difficult or even impossible. Further, a location too high above the ground may result in missed detections or missing portions of the manul body and non-detection of cubs or juveniles accompanying the mother. The latter, however, would not influence occupancy or density estimation since data on juveniles are usually not included. Placing cameras at an optimal distance of 0.5-2 m from the point, where the manul is expected to travel, will result in a large enough field of view for the camera and, therefore, good-quality pictures (Figs. 16, 18).

Avoid spots and angles that receive direct sunlight, since excessive heat on the camera will reduce the sensitivity of the sensors to passing animals and result in false triggers (e.g., when clouds block the sun). Likewise, do not point the camera at objects from the environment that absorb or reflect the heat (e.g., large rocks or sunlit streams). If two cameras are placed in a station (Fig. 18), they should be faced in different directions, so one camera's flash does not affect the images taken by the other one. Taking test images during the placement of the camera to refine the field of view is a good practice (Rovero and Zimmermann 2016).

Figure 17: Linear features, such as existing passages at rock outcrops, tend to funnel the manul movement in more predictable ways and increase the likelihood of detection by camera traps. To minimise the chances of missing detections, the camera's trigger should be tested by walking and even crawling past the camera in every direction possible. Note that this camera's viewshed has changed slightly during checking. Photos: I. Smelansky (top), Sibecocenter (bottom)

Figure 18: Paired cameras opposing each other should be directed towards the same focal point but not facing each other directly, otherwise they may trigger each other or result in overexposed images when the flashes are activated. Photo: S. Spitsyn/Altai Biosphere Reserve

3.5.4. Viewshed obstruction

Vegetation cover is usually not a problem in placing cameras in barren or rocky habitats. However, vegetation can be a considerable problem if (1) it exists directly in front of the camera; (2) when installing camera traps before the start of vegetation period where new plants may grow in front of the camera; and (3) where the plants moving in the wind can trigger a camera and fill up a memory card in a day or two (Fig. 19). It is therefore important to clear the vegetation, such as grass, hanging branches or leaves, that may interfere with the camera's performance or affect the quality of resulting images. Vegetation in front of the cameras not only results false triggers or prevent the camera's sensor from triggering, but also can make the species and individual ID difficult. Vegetation may reflect the camera's flash, which is more likely in IR units, and result in white-out images (Fig. 3). In cameras in which the sensitivity of the sensor can be reduced or by programming the camera to operate outside the hot hours of the day, the problem with heated vegetation should be less of a problem, although this also results in lower detectability during the day.

Clear the vegetation only in the camera's field of view and take test photos to make sure that no vegetation or landscape feature is obstructing the field of view (Figs. 19-20). Another benefit of clearing vegetation is to funnel the animal towards the camera to increase detectability. This can be done also in winter when snow covers the ground, to clear a travel route passing the camera, where manul movement is less constrained. However, a path in the snow will be blown in very quickly when the wind picks up in open landscapes where the manul lives. In locations where complete coverage of a trail or road is not possible, natural features from the environment, such as rocks and tree trunks, can be placed at one side of the trail as obstructions to increase the likelihood of the manul walking past the camera. This trick can be also used to deter the manul and co-occurring animals from passing too closely to the camera when the camera has to be placed close to the target area because of field conditions in that spot.

Figure 19: Vegetation at ground level in the immediate vicinity of the camera can lead to many "empty" images on hot or windy days (top left). Vegetation can also cause problems with obstructing the camera's viewshed (top right). In warm seasons, plants grow quite tall in just a few days and the camera's susceptibility to the moving vegetation that is heated by sun often result in many blank images (bottom). Photos: Sibecocenter

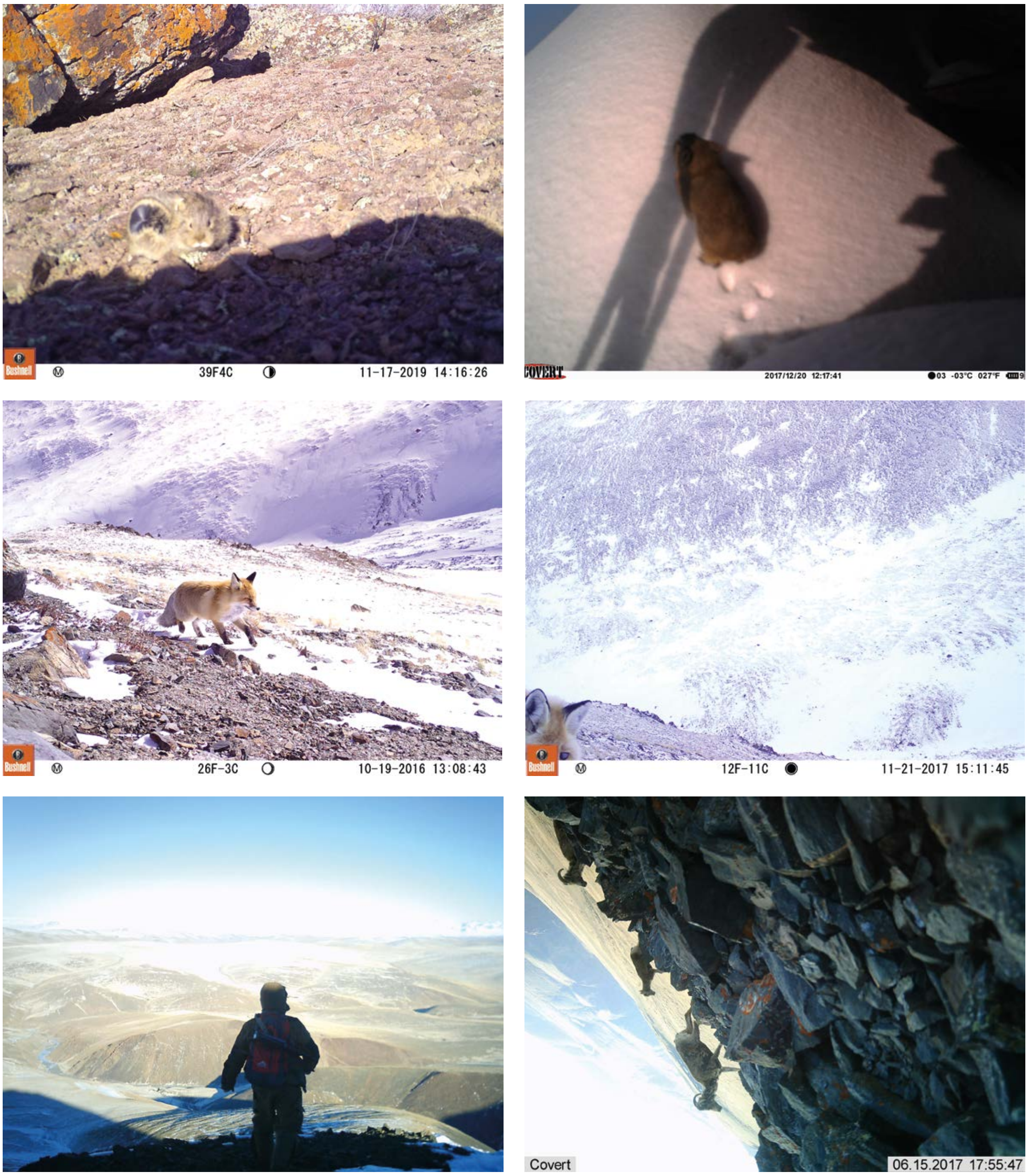


Figure 20: Examples of cameras' viewshed that were moved during the trapping session but the camera remained, to some extent, operational and recorded wildlife. In such situations, the camera station is less efficient than the original setting. In the top photos, the cameras were not secured and tilted downward. The viewshed of the camera in in the middle was changed unintentionally when checked and pointed upward with only a small part of the passing animal captured in the right photo. The camera at the bottom was not secured and eventually fell over.

Figure 20: (continued) Examples where the set-up of the cameras was not ideal and the resulting obstructed viewshed affected the quality of the images obtained. Top left: The camera was not levelled with the ground and the viewshed was mostly above the focal point on the trail. Top right: The viewshed of camera was blocked, in part, by a large boulder and the passing badger (*Meles leucurus*) was therefore partly blocked. Bottom left: The viewshed of the camera was compromised by rocks used to secure the camera. Bottom right: The viewshed of the camera included some large boulders close to the camera that could result in overexposed photos when using white flash cameras. Photos: Sibecocenter

3.5.5. Attractants

Attractants include lures with scent (e.g., plant- or animal-based extracts, a perfume), an object, sound or food that is inaccessible to the animal, or baits as food rewards (Rovero and Zimmermann 2016). Attractants do not necessarily draw animals from far distances (i.e., more detections), but they may increase the likelihood of animals to spend more time in front of the camera; thus, may result in more quality photos from different angles (Tourani et al. 2020). Attractants, if correctly selected and implemented, may be useful to lure manuls to camera locations and increase their detectability and decrease time to first detection. However, we are not aware of published studies demonstrating the effectiveness of attractants for surveying the manul. In an experimental study at Moscow Zoo, no effects of valerian were observed on captive manuls (A. Barashkova, unpublished data) and the effectiveness of scent and vocalization in a field study in Kyrgyzstan were inconclusive because of small sample size (the Pallas's Cat International Conservation Alliance, unpublished material). For other small cats, castoreum (American beaver *Castor canadensis*) scent, valerian oil or tincture, and catnip (or catmint *Nepeta* spp.) extract are found effective at increasing capture success (e.g., McDaniel et al. 2000, Monterroso et al. 2011, Maronde et al. 2020, Tourani et al. 2020). Scent lures in different formulas and combinations are commercially available (e.g., www.fntpost.com). They can be sprayed on natural features or used with lure sticks placed in front of the camera. Scent lures need to be replaced frequently during the sampling period, especially in wet or humid environments and the tropics, as scents tend to wear off over time. Replacing the lures can be logistically challenging and costly to do and may even introduce some levels of disturbances at camera locations. Novel objects, such as a wooden stick or hanging compact disks, can also act as a visual attractant (Cove et al. 2014). Camera locations themselves may attract the manul and other species when the station is recently established (Fig. 22). In addition, targeting some natural features in the manul habitat for camera trapping may increase their detectability as the species check these locations more frequently or spend more time there (Figs. 14, 16, 21).

Using attractants is not always favourable in analysing camera-trap data since it may violate some of the model assumptions given the analytical framework, such as effective sampling area and site independence (Gerber et al. 2012, Burton et al. 2015; Chapter 1). The use of attractants may lead to variable behavioural responses in animals, both within and between species. The consequences of using lures on the detectability of wildlife may vary across species, space, time, and even individuals from the same population (Mills et al. 2019, Tourani et al. 2020). There are contradictory results that increasing detectability of some species may come at the cost of decreasing detectability of other species (e.g., Rocha et al. 2016, Fidino et al. 2020). Thus, whether to use an attractant or not depends on the study aim and objectives. Specifically, if an attractant is used on some but not all camera stations, it should be recorded during the survey and its effects quantified in the analysis (Meek et al. 2014, Braczkowski et al. 2016, Rovero and Zimmermann 2016, Tourani et al. 2020).

Figure 21: Carnivores deposit their urines and scent-gland secretions near prominent rocks and cliff-faces. Camera traps can be installed near such features, particularly the vertical face of a potential scent-marking rocks or cliffs. This manul was photographed at a communal signpost used by snow leopards in the Chikhachev's ridge of Altai Republic, Russia. Photo: S. Spitsyn/Altai Biosphere Reserve

Figure 22: Camera traps, as novel objects in the environment, may attract manuls to investigate them. Photos: S. Spitsyn/Altai Biosphere Reserve

3.5.6. Test mode and arm

Most camera traps have a test mode to check that the camera is capturing the intended focal area. When in test model, usually a light will start blinking when there is movement (and heat) in the detection zone of the camera. This feature helps final adjustment of the camera, where one should adjust the direction and angle of the camera (Fig. 23), particularly if the camera does not indicate detection (i.e., does not blink) when the investigator moves in the intended focal area. It is good practice to repeat the test at close (about one metre) and farther ($\geq$ 3 m) distances in every conceivable angle at which the manul may pass the camera, to check the camera's detectability at varying distances (Polisar et al. 2014, Abrams et al. 2018). For units without this feature, turn on the camera and take some test images by mimicking an animal passing by the camera's field of view; for example, moving through the camera by walking in a crouch or crawling on knees and hands (Fig. 17). Then, test images can be viewed and checked that the camera captures the intended focal area by inserting the SD card into a tablet, laptop, or digital camera that has been already checked that would not damage the SD card or images takes.

Figure 23: Before leaving the camera station, make sure to check that the camera is placed at the right distance and angle from the focal area and that the camera's viewshed is not obstructed. Placing the camera almost parallel to the focal trail is the best option in most situations for maximising manul detections. However, in some locations the camera needs to be oriented on an angle of less than 45° relative to the focal point for better results. Photos: A. Antonevich, I. Smelansky, A. Barashkova

Figure 24: In wet environments or humid occasions, waterproofing might be an issue with some camera brands. Condensation may be also built up on the camera's lens as seen in this photo. Regularly check for moisture during the camera checking, make sure that the camera is sealed up, and use silica desiccants inside the camera unit if it is required. Photo: Sibecocenter

After the test images, check one more time that the camera is placed at the right distance and angle from the focal area and that the camera's viewshed is not obstructed before leaving the area and that the camera is sealed up. Then, turn on the camera and record this final step in the data sheet. It is also a good practice to hold the data sheet with the camera ID, date and time, written down in front of the camera to take a few photos of the sheet, as this can work as a backup if the internal storing of camera ID or other metadata fails. This same technique can be used when cameras are checked or retrieved, as a backup for metadata fails (e.g., date and time). Use large and clear fonts so the sheet will be readable in the resulting images. Do not leave the batteries and SD memory cards inside a camera after retrieving the camera at the end of the field season. Check the cameras before their storage to make sure that they are clean and dry, and carefully pack them to avoid damage. Inspect battery terminals for any evidence of corrosion and regularly check for moisture (Fig. 24). When many cameras are available, it is good practice to have a database in which all the devices are listed and run an inventory so that any difficulties encountered during the sampling are described at the end of each session.

3.6. DATA EXPORTS AND MANAGEMENT

3.6.1. Software and database

Good camera-trap data management must provide consistent data structure but be flexible towards different study designs and, ideally, allow for the creation of input data-files for further analyses (Forrester et al. 2016). Detailed descriptions and comparisons of software packages for camera-trap data entry and management are provided by many sources, such as Niedballa et al. (2016), Scotson et al. (2017), Wearn and Glover-Kapfer (2017), Ramachandran and Devarajan (2018), Young et al. (2018), and Glover-Kapfer et al. (2019). For most manul researchers, free and open-source software packages are the best options for data entry, sharing, and analyses (see the references above). Some of these software packages are R-based (https://www.r-project.org/), which means that an intermediate level of understanding the R environment and coding skills are required (e.g., camtrapR; Niedballa et al. 2016). There are also general-purpose, easy-to-use toolkits with user-friendly interfaces that do not require coding skills (e.g., Camelot; https://gitlab.com/camelot-project/). Some examples of such software are provided and compared by Young et al. (2018) and Glover-Kapfer et al. (2019). Some international projects also offer global camera-trap data repositories for data management and sharing (e.g., Wildlife Insights: https://www.wildlifeinsights.org/), which involve using machine learning models to automate flagging blank images, identifying species, counting individuals, and describe behaviour (Norouzzadeh et al. 2018, Willi et al. 2019, Whytock et al. 2021). Below, we describe a simple workflow of extracting and storing the raw data to prepare the input files for subsequent analyses.

Camera trapping at multiple sites results in large amounts of data, i.e., hundreds of thousands to even millions of individual photos, including photos of non-target wildlife, domestic animals, humans, and empty photos. Thus, it is important to properly store and manage the resulting data as soon as possible, during or soon after conducting the fieldwork. For each survey, two databases are typically created: (1) camera-trap records, where each row in the database is one unique photographic record; and (2) survey-related information, where each row is one independent camera station. These two databases should contain matching columns (e.g., station and survey IDs and date) so they can be merged for different purposes during the data analysis.

The first step in every data management workflow is extracting and storing the camera-trap images from SD memory cards on a computer's hard disk or internet cloud. Consider a folder structure based on data sharing policies and practicalities accepted by the survey team. We recommend storing the images from each camera in their own folder that is labelled by the camera ID and survey date (e.g., year), or alternatively as subfolder for each study area and year where each subfolder is labelled by the camera ID. Almost all software programs available offer tools to automate this step by using metadata from images (e.g., camera ID, date, and time). This step also helps species assignment and individual ID for tabulating the records and preparing input data-files for further analysis. User-defined tags for each image can be set to include, but not limited to, camera ID, coordinates, study site, species ID, individual ID (if possible), flank (right or left), sex (if possible), age class (juvenile, sub-adult, or adult), behaviour (e.g., scent marking, walking, resting), and group size (number of individuals in the record, number of juveniles).

In addition, it could be worthwhile to differentiate between the number of individuals on the picture from the number of individuals detected during the triggering event or subsequent triggering events (if the delay is very short). By using software tools, this information can be written into the image metadata. Almost all the freely available data management software packages use Exiftool (www.exiftool.org) to read and write metadata from and to images. Automating the data management procedure maximises the level of information obtained from camera-trap records, while reducing the time and effort required to enter and tabulate the resulting data (Whytock et al. 2021). Specifically, time-consuming and error-prone steps, such as the assignment of relevant information to each image and creating input files for further analyses (e.g., the creation of encounter histories that require starting and finishing dates and occasion length) will be greatly facilitated.

3.6.2. Species ID of manuls

All images of target species should be entered into a comprehensive database, in which each line contains the information (e.g., station ID and coordinates, date, time, species, and individual ID) for one independent detection (capture) event. Think of an Excel spreadsheet file, where each row represents one camera-trap photo, and each column contains the relevant information for that row. This basic format can then be easily converted to encounter histories for each species or individual that provides flexibility for further processing, such as adjusting the length of sub-sessions within each camera trapping session. Most software packages introduced in the previous section have automated data management workflows that makes data management more efficient as manual labour is generally very time-consuming and prone to human errors.

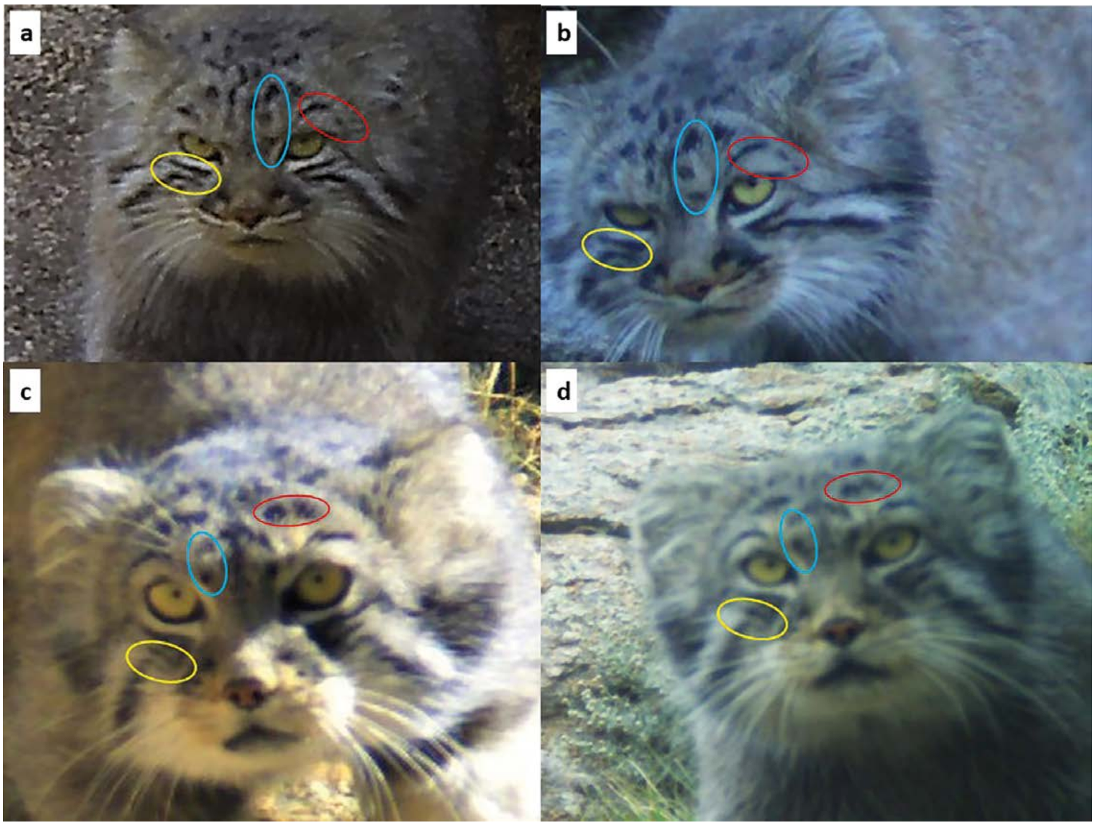
Manuls have rather distinctive morphological characteristics that make their identification at the species level in good-quality images easy. These include broad and flattened head with the ears on the sides (rather than on the top of the head like most other felids), spotted forehead with horizontal black and white stripes that run from the eyes to the cheeks, round pupils, short legs, striped tail, and thick fur (Ross et al. 2019b). In lower-quality photographs, when only a part of the animal body is visible or at night photos where the animal is far away from the camera (Fig. 25), manuls can be misidentified and confused with small wild cats, such as wildcats *Felis silvestris* or *F. lybica*, Chinese mountain cat *F. bieti*, and domestic or feral cats *F. catus*, or even other small and medium-sized carnivores, depending on the study area.

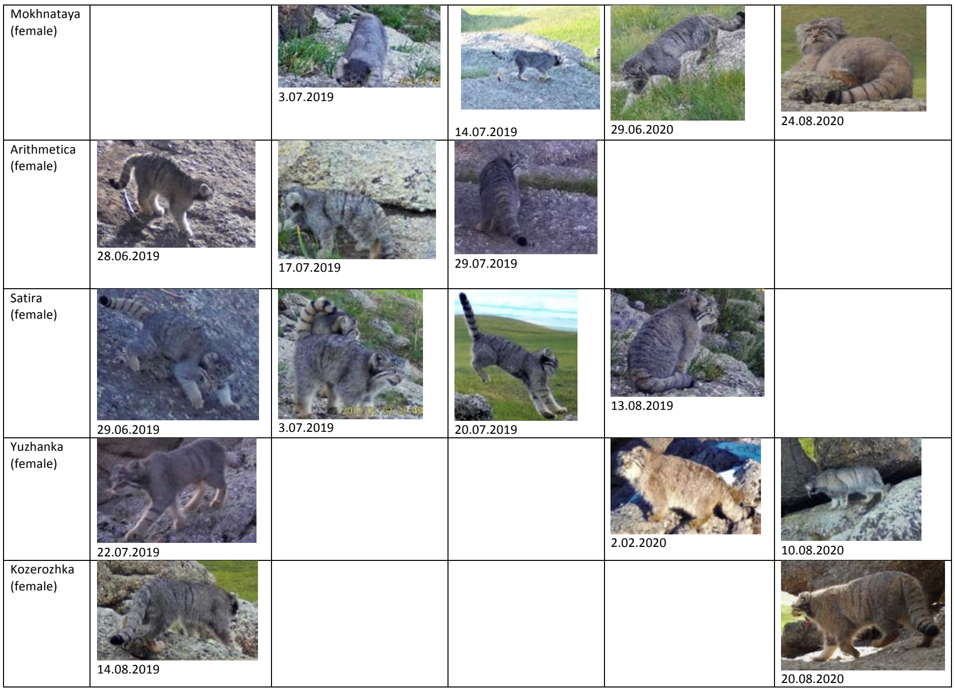
Figure 25: Although identifying manuls from camera-trap images are generally straightforward, in poor-quality images or when only part of the animal is visible, caution is needed. Both these images are of the manul, even if somewhat difficult to say because of the low position of ears in the top photo and stocky body and stiped tail in the lower photo. Photos: M. Gritsina/ Manul Working Group (top), Sibecocenter (bottom)

3.6.3. Individual ID of manuls

Although manuls' coat is marked by faint striping and there is great variation in coloration across regions and seasons (Ross et al. 2019b), the small black spots on the forehead and the stripes and marks on the cheeks and face appear to be individually unique (Anile et al. 2021; Fig. 26). Using the unique markings in the face provides an important opportunity for individual identification that allows for more detailed analyses, such as population density estimation and survival analyses (Williams et al. 2002, Royle et al. 2014). We are, however, aware of only one study to date that has used these marking patterns to identify manul individuals from camera-trap photographs (Anile et al. 2021; Fig. 26). By targeting marking sites and other optimal sampling locations described in Chapter 2, the probability of capturing manul photos with visible forehead towards the camera is higher than for other camera placements. Such photos allowed Anile et al. (2021) identification of individuals from some of the camera-trap photos and thereby estimate population density (Fig. 26). The manul's tail is also distinctly banded, with narrow stripes ending in a dark tip (Ross et al. 2019b), and in combination with the lacerations around the mouth and stripes on the front legs, can be further used to improve the individual identification (Anile et al. 2021). Assigning sex to the manul individuals photographed by camera traps is often very difficult except for females accompanied by kittens (Fig. 26), or when, in rare occasions, the scrotum is visible in the image.

Despite these unique markings, individual ID can be very challenging and only possible for a portion of photographs because the most reliable results will be obtained when the animal is looking directly at the camera. For example, Anile et al. (2021) could identify manul individuals in 20 out of 54 (37%) and 42 out of 99 (42.4%) detections in two sampling periods. In addition, individual ID errors in camera-trap studies can be relatively high, even for those species that are commonly considered to be easily identifiable using unique coat pattern (e.g., snow leopard *Panthera uncia*; Johansson et al. 2020). A good practice is therefore to quantify the ID errors (i.e., observer error) by doing a blind test, where multiple investigators independently go through the photographs and identify them to individuals. Then, the results can be compared, and any discrepancies should be checked, decided, and reported to minimize misidentification, although it is important to realize that some of the individuals may still be misidentified as the true answer is not known (Johansson et al. 2020). We recommend following Choo et al. (2020)'s guidelines, and in every report provide details of the individual ID process and a list of photographs for each individual as supplementary material. This will also facilitate data sharing and collaborations in the future by shared processing and format.





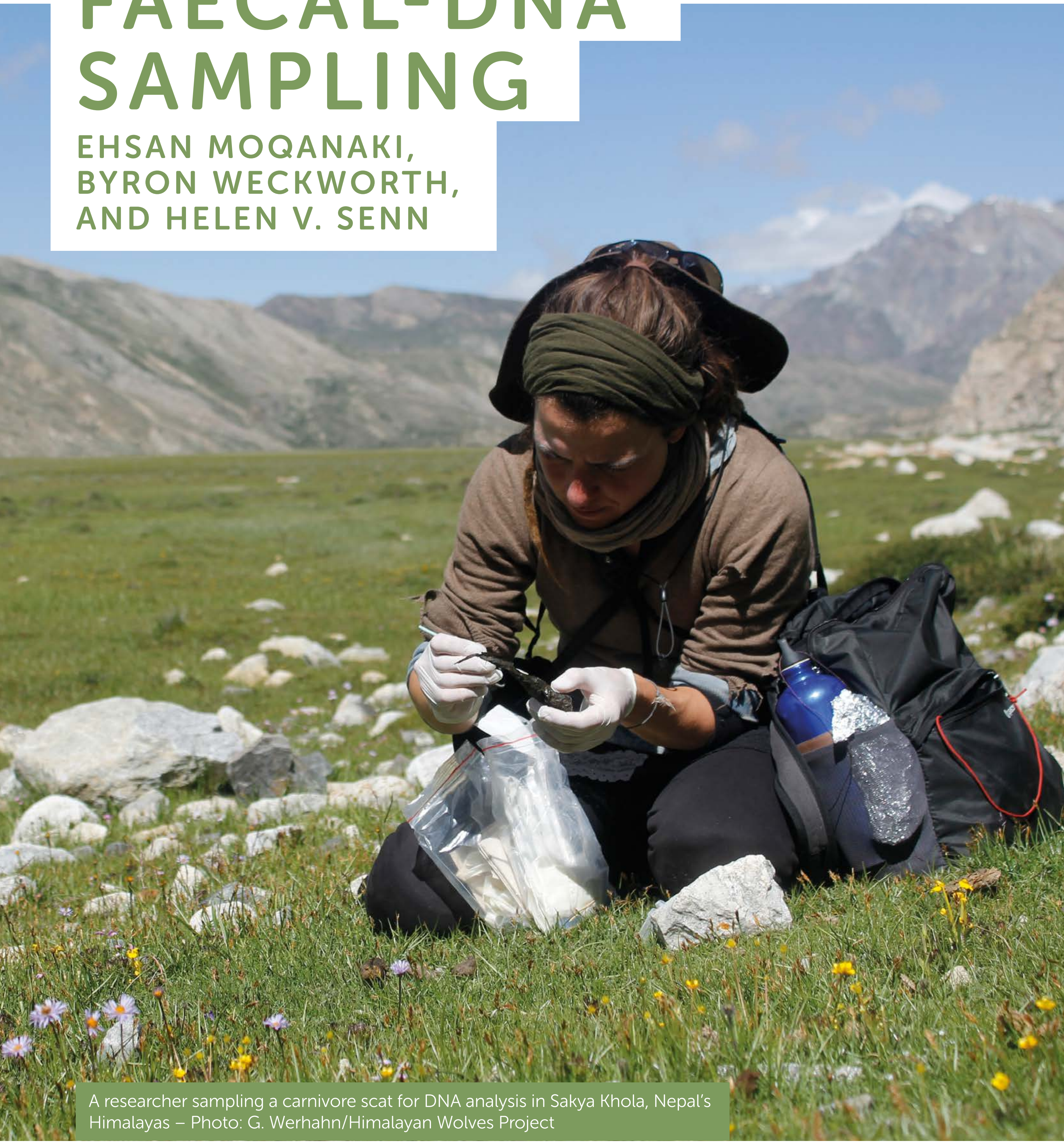
Mokhnataya (female)		3.07.2019	14.07.2019	29.06.2020	24.08.2020
Arithmetica (female)	28.06.2019	17.07.2019	29.07.2019		
Satira (female)	29.06.2019	3.07.2019	20.07.2019	13.08.2019	
Yuzhanka (female)	22.07.2019			2.02.2020	10.08.2020
Kozerozhka (female)	14.08.2019				20.08.2020

Figure 26: In good-quality photos of the manul when the animal is facing the camera, the natural markings of the face can be used for individual identification (top). Figures (a-b) and (d-c) are two different individuals and each colour corresponds to the same unique spot used for individual identification. Creating capture history for each manul individual is also possible in long-term studies (bottom). Photos: Anile et al. (2021) (top four images), V. Kirilyuk (bottom diagram)

CHAPTER 4

FAECAL-DNA SAMPLING

EHSAN MOQANAKI,
BYRON WECKWORTH,
AND HELEN V. SENN



A researcher sampling a carnivore scat for DNA analysis in Sakya Khola, Nepal's Himalayas – Photo: G. Werhahn/Himalayan Wolves Project

GLOSSARY

DNA:	Deoxyribonucleic acid is basically the hereditary material in almost all living organisms.
Genotype:	The unique genetic makeup of a particular individual - i.e., the specific alleles at a locus.
Genetic Marker:	A variable DNA sequence at a known location on the chromosome.
mtDNA:	Small, circular DNA found in the mitochondria of the cell that is maternally inherited.
PCR:	Polymerase Chain Reaction is a technique to amplify small segments of DNA.
DNA Metabarcoding:	The determination of multiple different species in a given sample via sequencing of a DNA barcode.
DNA barcode:	A short DNA sequence commonly used in DNA metabarcoding. The primer annealing region is conserved for all species of interest, but the internal region is variable enough to discern species based on its nucleotide order.
Microsatellite:	A repetitive sequence of nucleotides that is often non-coding and often highly variable between individuals of the same species. Also known as short tandem repeat.
Genotyping error:	When the observed genotype does not correspond to the true genotype.

4.1. INTRODUCTION

Target DNA that can be isolated from the faeces and hair collected in species' natural habitats are usually termed non-invasive samples. These samples are either naturally left in the environment (e.g., species depositing faeces), or collected via fixed devices stationed in the environment (e.g., lured barbed wire used for hair snagging). Non-invasive sampling for wildlife survey and monitoring has evolved almost in parallel with the use of camera traps during the past three decades. Overviews of different DNA sources, applications, and limitations for wildlife studies have been published elsewhere (e.g., Waits and Paetkau 2005, Beja-Pereira et al. 2009, Kelly et al. 2012, Carroll et al. 2018, Taberlet et al. 2018, Beng and Corlett 2020, Lefort et al. 2022). When done properly, researchers can use DNA from non-invasive samples and appropriate genetic markers to reliably identify species, as well as individuals within a species and their sex. Genetic markers used need to be appropriate to the species and the question being asked. This information can then be applied in detecting species presence, quantifying occupancy or habitat use, and estimating abundance and density at small or large spatial extents.

With adequate sampling, DNA-based species and individual ID of wildlife can be even more reliable than camera trapping (Leempoel et al. 2020, Sales et al. 2020). This could also be relevant for manul survey and monitoring when quality of images are low or individually unique patterns do not exist or are ambiguous. However, it is currently very difficult to find manul scats in a systematic manner that outperforms camera trapping. DNA sampling could potentially also be more practical in areas with high risk of camera-trap vandalism. Further, genetic analyses of non-invasive faecal samples may provide additional information on diet, kinship, and population structure. Given the ongoing development of fast and cheap portable DNA tool kits (e.g., Wimbles et al. 2021), DNA-based survey and monitoring of wildlife will, in the near future, be made available to a wider audience. Nonetheless, best practice guidelines are rarely available for DNA sampling despite its popularity. This is in part because of the multiple interacting factors influencing DNA quality and quantity in biological samples recovered under field conditions, which are often system specific (Broquet et al. 2007, Woodruff et al. 2015). The low quality and quantity of target DNA in non-invasive samples require particularly rigorous efforts in enriching the DNA fragment or region of interest, which is done by Polymerase Chain Reaction (PCR). PCR from non-invasive samples can be prone to several risks and errors that can result in both false negative and false positive errors (Taberlet et al. 1999, Pompanon et al. 2005, Beja-Pereira et al. 2009, Lampa et al. 2013, Lahoz-Monfort et al. 2016). Thus, conducting pilot surveys before initiating large-scale DNA sampling to optimize the protocols is always recommended to assess cost-efficacy trade-offs (Janečka et al. 2008, Wultsch et al. 2015).

CHAPTER 4:
FAECAL-DNA SAMPLING

In survey and monitoring of terrestrial carnivores, scats are the most common source of DNA (Kelly et al. 2012, Rodgers and Janečka 2013). Carnivore scats can be found in relatively large quantities, particularly outside wet or tropical systems, but recovering them under field conditions and extracting usable DNA in the presence of decomposers, PCR inhibitors, and non-target DNA can be very challenging (Beja-Pereira et al. 2009, Goossens and Salgado-Lynn 2013, Beng and Corlett 2020). In this chapter, we focus on a scat sampling protocol for manul survey and monitoring to maximize DNA yield in the lab (i.e., high PCR successes and low genotyping errors). We do not intend to review and describe all possible DNA sampling methodologies and refer the reader to the references provided in this chapter. We also do not provide lab protocols because we presume that most manul researchers would submit their samples for processing to dedicated DNA laboratories who would work up appropriate protocols. Further examples are provided elsewhere (e.g., Cancellare et al. 2021). Note that it is recommended to engage with a DNA lab and associated experts to clearly agree on the cost and methodological aspect of your project prior to initiating the DNA sampling. If lab work is not scheduled to follow quickly after sample collection, then further provisions may be required to ensure samples are adequately prepared for long-term storage and viability. This may include storage at -20 degrees Celsius in a university lab or national or international biobank. It is of critical importance to appreciate that the choice of sample collection and storage methods can make the difference between the success and failure of a project and consultation with molecular genetics experts is strongly advised prior to sample collection. We also advise that, in selecting a lab to work with, you consider whether the lab has had past experience of working on non-invasive (or low quality) samples and wildlife species since the appropriate analytical methods can be less straightforward to develop and apply than in medical and agricultural contexts. This is usually because of a lack of previous background genetic research on the species. We review the basic principles of DNA sampling for species and individual ID through a simple and practical sampling strategy, and best practice guidelines for DNA preservation and storage until shipment to a molecular lab.

4.2. NON-INVASIVE DNA SOURCES

4.2.1. Overview

All organisms shed DNA into the environment, and it can be collected directly from scat, hair, saliva, and urine (traditional non-invasive samples), as well as from soil and water (environmental DNA or eDNA; Rees et al. 2014, Cristescu and Hebert 2018) and even by filtering the air (Clare et al. 2022). For manul survey and monitoring, we believe non-invasive sampling of scats are the best source of DNA for most purposes. It is possible that eDNA approaches could add benefits for rapid occupancy survey and monitoring efforts (Franklin et al. 2019, Leempoel et al. 2020). Development of eDNA methods for manul is ongoing (Franklin et al. unpublished), and still requires field validated proof of concept, but the potential is promising.

The target DNA in environmental samples (both non-invasive and eDNA) is usually degraded and persists in low quality and quantity. Specific DNA markers and amplification procedures have been developed to target short, highly variable and informative DNA fragments (80 to a few hundred base pairs) for sequencing, making the extraction of usable DNA from even historical samples possible (Waugh 2007, Valentini and Pompanon 2009, Chaves et al. 2012). Nonetheless, as a rule of thumb, the lower the quality of a sample, the less likely there is to be successful in DNA amplification. Thus, faecal sample collection should target getting as fresh samples as possible to increase the PCR success and reduce potential errors. It should be noted that the environmental conditions that the faeces are found in will have a considerable impact on the rate and extent of DNA fragmentation (Santini et al. 2007, Panasci et al. 2011, Vynne et al. 2012). Samples from dry, cold, low-UV conditions will degrade at a reduced rate compared to wet, hot, high-UV conditions. One of the most important factors is the presence of environmental moisture. This makes work on faecal samples from the tropics highly challenging as the samples degrade rapidly (Goossens and Salgado-Lynn 2013), while the arid, high-altitude montane areas where the manul occurs are more beneficial. Nevertheless, adequate desiccant to dry out collected scats (especially fresh ones) is always essential to ensure optimum DNA quality.

Many studies include a short single mitochondrial or nuclear DNA marker for species ID. Short regions of DNA are used to maximize the chances of amplification, despite the inevitable process of DNA fragmentation due to the degradation of the sample (see above). Mitochondrial DNA markers are often preferable because they are present in higher copy number per cell than nuclear DNA, meaning the DNA is more abundant and easier to detect within poor-quality samples. The use of multiple genetic markers can be advantageous to improve the accuracy of species and individual ID. Reliable species ID using non-invasive or eDNA samples requires access to reference databases of previously identified sequences from reliable sources. At least for some of the mammalian vertebrates, Barcode of Life Database (BOLD) based on the DNA barcode region is available (Ratnasingham and Hebert 2007). There are also non-curated databases, such as GenBank (https://www.ncbi.nlm.nih.gov/genbank/), that are widely used by researchers and perfectly adequate, but there are occasionally issues of quality control or validation of sequence veracity.

It is always good to check where the sequenced specimen comes from within the species range, and this may sometimes require searching for additional information within an associated published paper or contacting the researcher directly. Reference data is sometimes generated from zoo animals and in this case the geographic origin can be difficult to obtain. When it comes to wildlife species, these databases often provide refence data for a limited set of genes on a limited number of individuals. It is not always possible to find representative population-level samples from across a species range or make comparison with gene regions of interest. Thus, embarking on a wildlife DNA project often involves generating relevant reference datasets denovo, for example by collecting and analysing samples from across a species range (e.g., from museum archives) so that comparisons can be made with the target population of interest. Needless to say, it is a great help to other researchers in your field if you can deposit any genetic dataset generated with as much information about the samples as possible (particularly a precise geographic origin and date of sampling). Most genetic publications today will require the associated data to be published on the relevant public archive (e.g., https://www.ncbi.nlm.nih.gov/genbank/). Doing this as comprehensively as possible is a great help to species conservation.

4.2.2. Manul scats

A description of manul scats and where to search for them in the field is provided in Chapter 2. Paying attention to the morphological characteristics and characteristics of the defecation location can increase the likelihood of manul scat sampling and reduce the cost of lab analyses (Fig. 1). Depending on the study aims and objectives, however, it is possible to target sampling of more than one species and provide valuable information on the entire carnivore community. We therefore recommend the collection of all putative, intact carnivore scats that are encountered within the manul habitat.

Figure 1: Putative manul scats encountered in a snow-tracking survey in the Altai Republic, Russia - Photos: A. Barashkova

4.3. PERMITS

Manul researchers should be aware of and obtain all the necessary research permits for DNA sampling and transfer of the genetic material. This might consist of permission to conduct sample collection in the country and region being targeted. If analysis is being conducted in a different country, then permission may be required to export samples from the country of collection. Another permission may also be required to import the samples into the country of analysis (e.g., veterinary health permits). The manul is listed on Appendix 2 under CITES legislation and any blood and tissue samples will require these permits. Faecal samples do not generally require CITES permits (although interpretations on this can vary by country); however, DNA samples are still genetic sources and regardless of their actual or potential values are subject to the Nagoya Protocol (https://absch.cbd.int/en/; see Abrams et al. 2018). Export should involve the drafting of an access and benefits sharing agreement with the receiving party . Whenever handing samples over to another laboratory (even in the same country as collected), it is advisable to put in place a Material Transfer Agreement, which defines the ownership of the samples and what downstream research can be conducted on them. It may be advisable to loan samples to a lab for a specific analysis, but not to transfer ownership. It is also advisable to split samples, or collect duplicates in the field, and have a back-up on hand in the case that samples get lost in postage or are accidentally destroyed. Other permitting regulations vary from country to country and across time, and it is the responsibility of the investigators and their respective organizations to familiarize themselves with the latest information before conducting DNA sampling. In technical reports and scientific publications, the authors should also demonstrate that their study was conducted in full compliance with the local and national laws and international agreements.

4.4. SAMPLING DESIGN

The optimal sampling period varies from region to region and depends on logistical realities and available resources. The manul's geographic range predominantly overlaps with the continental climate that is characterized by aridity and large variations in annual temperature (Ross et al. 2019b, 2020). An arid climate, especially during warm months with little precipitation, is optimal for scat collection as environmental conditions that allow for quick drying help reduce the rate of DNA degradation. However, direct UV radiation also degrades DNA. During cold and dry winters freezing reduces the rate of DNA degradation, but multiple freeze-thawing cycles or wet snow could decrease the quality of DNA. Sampling during winter months is also more challenging in the predominantly mountainous and rugged habitats of the manul. Published studies are not conclusive on optimal sampling periods and related PCR success. Werhahn et al. (2018) sampled the highlands of Nepal in May-June and in Qinghai–Tibetan Plateau, Zhao et al. (2020) and Hacker et al. (2022) successfully conducted scat collections between June and July and from October to November, and between April and October, respectively. A pilot study in the study area to compare PCR successes in different sampling periods would be useful to guide the design of a large-scale DNA survey and monitoring efforts.

The optimal frequency for DNA sampling depends on the study questions. For both occupancy analysis and density estimation using spatial capture-recapture methods, multiple visits are typically required to construct a detection history (see Chapter 1). However, there are also examples of incorporating data from single visits (e.g., Moqanaki et al. 2018, Lauret et al. 2021). For estimation of abundance and density, a general rule of thumb is to have several recapture events by collecting samples about 2 – 3 times the presumed size of the focal population, which might not be achievable in DNA surveys of the manul. Simulation-based studies can help identify optimal scenarios of DNA sampling, considering genotyping success and errors (e.g., Lonsinger et al. 2021). Based on our experience, a PCR success of about 40 – 60% for species ID can be expected in most carnivore surveys and monitoring studies using non-invasive DNA from scats. If sampling methods are optimized and only fresh samples collected, the PCR success could be much higher (Skrbinšek et al. 2010), but the sampling effort to accumulate an adequate sample size would be considerably greater and perhaps prohibitive. For identification of individuals and sex, other markers such as microsatellites and single nucleotide polymorphisms (SNPs) are more sensitive and typically have lower PCR success than mitochondrial markers (see above). This field is constantly evolving, and new techniques and lab protocols are introduced to increase PCR and genotyping success and reduce errors.

4.5. SCAT COLLECTION

To maximize the detection of manul scats, collection efforts should target optimal manul habitats, such as montane grassland and shrub steppe with rocky outcrops (Ross et al. 2019b), in particular where pika *Ochotona* spp. or other rodent colonies exist. Further details on good sampling location for manuls is provided in Chapter 2. In the Qinghai-Tibetan Plateau, all of the genetically confirmed manul scats were found in alpine meadows and screes at 3800 m above sea level (Zhao et al. 2020). Likewise, Hacker et al. (2022) genetically identified manul scats collected at elevations above 3600 m in different mountainous sites across the Qinghai-Tibetan Plateau. The single genetically confirmed manul scat from the Nepalese Himalaya was found in a barren rocky boulder field (Werhahn et al. 2018).

To date, few studies successfully recovered large quantities of manul scats in its natural habitat for DNA sampling. There is anecdotal evidence that manuls may sometimes bury their scats. In a pilot DNA sampling of highland carnivores in the Himalayas of Nepal, Werhahn et al. (2018) could genetically assign only one of the scats found to the manul after about 810 km of walking transects. Zhao et al. (2020) genetically assigned 14 scats to the manul, out of 100 putative carnivore scats collected during about eight months of sampling from Gongga Mountain in the Qinghai-Tibet Plateau, China. However, Hacker et al. (2022) successfully identified 88 manul scats from four different sites across the Qinghai-Tibetan Plateau. We therefore recommend collection of all putative carnivore scats that are not degraded. In the scheme of things, a DNA extraction and genetic identification of species is relatively cheap nowadays, while getting to the field and finding usable scats is time intensive and expensive. However, exposure to rain and direct UV radiation and decomposer activity (e.g., fungi and invertebrates) increases the degradation of the target DNA in the scat. A method to approximate the age of a carnivore scat in the field is to check its amount of moisture and, more importantly, odour (Fig. 2); an odourless and weathered scat is probably too old to be collected (Vynne et al. 2012). Thus, it is advisable to focus on collecting putative carnivore scats (Chame 2003, Hunter 2019) that are intact, with no sign of mold, insect, or worm presence, preferably with odour and shiny appearance when it is broken into pieces. When embarking on scat collection, it is first useful to think about where the best source of DNA is likely to be within the scat (Stenglein et al. 2010, Wultsch et al. 2015). If the researcher would like to know something about the animal that deposited the scat (species or individual ID), then the most useful DNA will likely be contained on the outer surface of the scat, where cells that have been scraped or sloughed from the digestive tract will accumulate. If the researcher is interested in the diet, then sampling an entire cross-section of the scat is required.

Figure 2: Ageing samples in the field is not reliable using morphological characteristics only. However, some features, such as the odour and colour, can be used to inform the sampling. Here are examples of snow leopard *Panthera uncia* scats in different conditions (from top to bottom): A fresh scat is shiny outside and soft inside with strong odour. Intermediate age is when outer surface of the scat is not shiny, but it is still soft inside and with odour when broken. A relatively old scat is when both outside and inside is dry, usually turn into white, and there is no odour. Photos: G. Samelius

4.5.1. Equipment

Scat collection requires a variety of essential equipment, including a GPS unit within each sampling team (with spare batteries) for recording the latitude and longitude coordinates of sample locations and search tracks. A digital camera is often used to document whole scat morphology and defecation sites. Collection materials include preferably either 15mL plastic capped tubes, or 50mL (or larger) specimen collection tubes or cups (Fig. 3). Unbleached paper bags can also be used, depending on what is available and what type of sample is needed to meet the project objectives (Fig. 3). We do not recommend using plastic or ziplock bags. Plastic tubes filled with drierite (silica desiccant) provide the easiest option that preserves sample quality, minimize the risk of contamination, and protects sample integrity during transport. Other preservatives, such as ≥95% ethanol or commercially sold buffer solutions can also be used but have their drawbacks (Tende et al. 2014). Ethanol can leak and erase the writing on tubes, and is also often difficult to carry in the field and ship by postal service or on commercial flights. The purpose of desiccant and ethanol is to pull the moisture out of the collected scat and arrest enzyme and microbial activity that further degrades the DNA. Many desiccants change colour as they become saturated with moisture and so it can be refreshed to maintain sample preservation. It is not possible to know when ethanol has become saturated and is no longer optimizing sample preservation. Thus, careful consideration should be made when deciding on the sampling materials based on available space, shipping needs, access to materials, and experience of the field teams. Additional field sampling equipment includes single-use gloves, permanent markers, pencils, specific data sheets, razor, and lighter.

4.5.2. Sample Collection

If survey teams consist of more than one investigator, we recommend they divide work with one person as the recorder and the other as the collector. This also ensures a standardisation of methodology across the sampling effort. When a sample is found, there are a number of steps to take before even touching the scat.

The first step is sample labelling. The collection tube, cup, or paper bag should be labelled using proper nomenclature (e.g., Year-Month-Day, collectors initials, scat number). This nomenclature should be defined ahead of time with rules for how scats are numbered, how multiple scats at the same location (e.g., latrine) are indicated, and how these numbers change across sample teams, locations, and dates. Additionally, each unique sample should have a traceable waypoint location marked via GPS. A well-designed and thought-out nomenclature will ensure that each scat has its own unique field name and provide the basic information for reference later on, such as collector and date. A good practice is to label both on the cap and the tube. This way, no information will be missed out and consistency is assured. In particular, when more than one sampling team is involved, no sample will receive identical ID numbers. Poorly planned and executed sample labelling can lead to significant errors in the lab and waste of field collection efforts.

Figure 3: Single-use gloves (top), a freshly cut wooden twig or branch (bottom), or even a piece of rock can be used to individually place the scat into sampling tubes or paper bags without touching the sample or the risk of contamination. Use each glove or twig for one sample only and discard it immediately. Photos: A. Moharrami (top)/B. Ghorbani (bottom)

The second step is filling out the sample sheet. Each sample should be recorded on a corresponding pre-made sample sheet (see Cancellare et al. 2021 for an example). The survey team should have received training well before conducting the surveys, and a specific data sheet to describe each step of data collection should be provided. The sample sheet should at the very least allow for documenting the same nomenclature as on the collection container, as well as other useful information such as GPS location, elevation, transect number, nearest landmarks, nearby sign, substrate-specific information, or anything else that could help describe the putative species presence point and be useful for analysis and interpretation later. Finally, after the sample container is permanently labelled and the sample sheet is filled out, a photo of the scat with the container can be taken to provide further documentation (Fig. 4). A photo of the datasheet at the end of every day is also an easy way to backup information in case a data sheet is lost or accidentally destroyed. All recorded information should be transferred to secure, electronic formats as soon as possible.

With sample sheet and container now ready, the scat can be carefully handled. It is imperative that bare hands do not touch the scat (Figs. 3-4). This could lead to contamination of the next samples collected, ruining the validity of the samples, and wasting the entire effort. It is also an important human health and safety issue due to the risk of zoonotic infections from the scat (Reynolds and Aebischer 1991). A new tool for collection should be used for every scat sample. Disposable gloves, tweezers, or even rocks and sticks can be used. In the case where only the host DNA is desired, an alternative method is using a disposable collection tool, such as toothpicks or cotton swabs soaked in ethanol or the buffer agent, to scratch or swab the surface of the scat and increase the proportion of sloughed gut cells and minimize the unintentional sampling of prey items (Lampa et al. 2013, Miles et al. 2015). This approach has been used successfully on carnivore scats collected in manul habitats in the Himalayas (G. Werhahn, pers. comm.). However, collection of an entire cross-section of the scat is preferable as that sample can be used both for host DNA and diet analysis (Klare et al. 2011). Even if diet analysis is not of immediate need, long-term storage of the sample allows future research efforts to take advantage of the sampling effort. Using the disposable gloves, tweezers, or other collection tools, break off or cut a full cross-section of the scat. In most cases, chunks of scat (full circumference and diameter) that appear freshest and the least dry are best as the DNA is less likely to be degraded. Quality of the target DNA may also vary depending on the sampling location within a scat (e.g., sampling from the side vs. tip of the scat; Stenglein et al. 2010, Wultsch et al. 2015). If an entire scat is collected and sampling will be done later on because of logistics or time constraints, we recommend marking the top of the scat (i.e., the outer surface) with fingernail polish, and making a note on the collection sheet, to provide information to orient lab staff and allow processing of the scat on a part that had not been in contact with the ground (C. Hacker, pers. comm.).

When using desiccant, the tubes should be prefilled with desiccant and collecting larger volumes of faecal material than is needed should be avoided. A rule of thumb is to fill each tube 1/3 to 1/2 full of desiccant prior to collecting the sample. It is easiest to do this while in the field camp or even before travel to the field sites, and to prepare many tubes at once for later use in the field. Place scat into the tube and securely place the cap. Do not overstuff the tube. Make sure there remains airspace between the scat and the lid and sides of the tube. This allows the desiccant to work at properly drying the sample. Gloves should only be used once per sample and carried out by the survey team to be disposed properly. Wooden sticks or rocks should also be disposed immediately and used only once to ensure no sample cross-contamination. If using tweezers, sterilize between samples with fire by using a lighter to burn off any scat or hair on the tweezer for at least five seconds (let cool before packing up tools). Do not touch the end of the tweezers with bare hands at any time. Avoid collecting the entire scat if not necessary to maintain the chemical and visual cues that scat provides intra-specific communication within the habitat.

In the case that sample tubes are not available, brown (unbleached) paper bags half-filled with silica beads can be used (Fig. 4). However, samples should be transferred to a more rigid container (i.e., tubes) with proper storage media as soon as possible before shipping. When tubes are not readily available, avoid using plastic or ziplock bags for storing scats. Paper bags are more environmentally friendly and are usually made from renewable material. Using paper bags can also help to dry out fresh samples in dry conditions (e.g., semi-arid habitats). We recommend cross- or rectangular-bottom paper bags that are at least 14×20 cm. Storing samples in paper bags is less practical in a wet environment, or in case there are bone fragments or hard parts (e.g., stones) in the scat. To reduce the risk of cross-contamination or tearing off the bags, a sample can be double bagged with the top folded or rolled and securely fastened. Faecal samples in bags must be checked regularly, topping up with silica if there is any sign of moisture. Regardless of the material, each sampling container should be individually labelled with appropriate nomenclature (as above).

Figure 4: Taking a photograph of each sample during the scat collection is good practice. The ID number and a proper scale should be always present to track the sample when it is needed. Try using permanent, pre-defined and consistent labelling (top). Photos: B. Weckworth (top), E. Moqanaki (bottom)

4.5.3. Sample Preservation and Storage

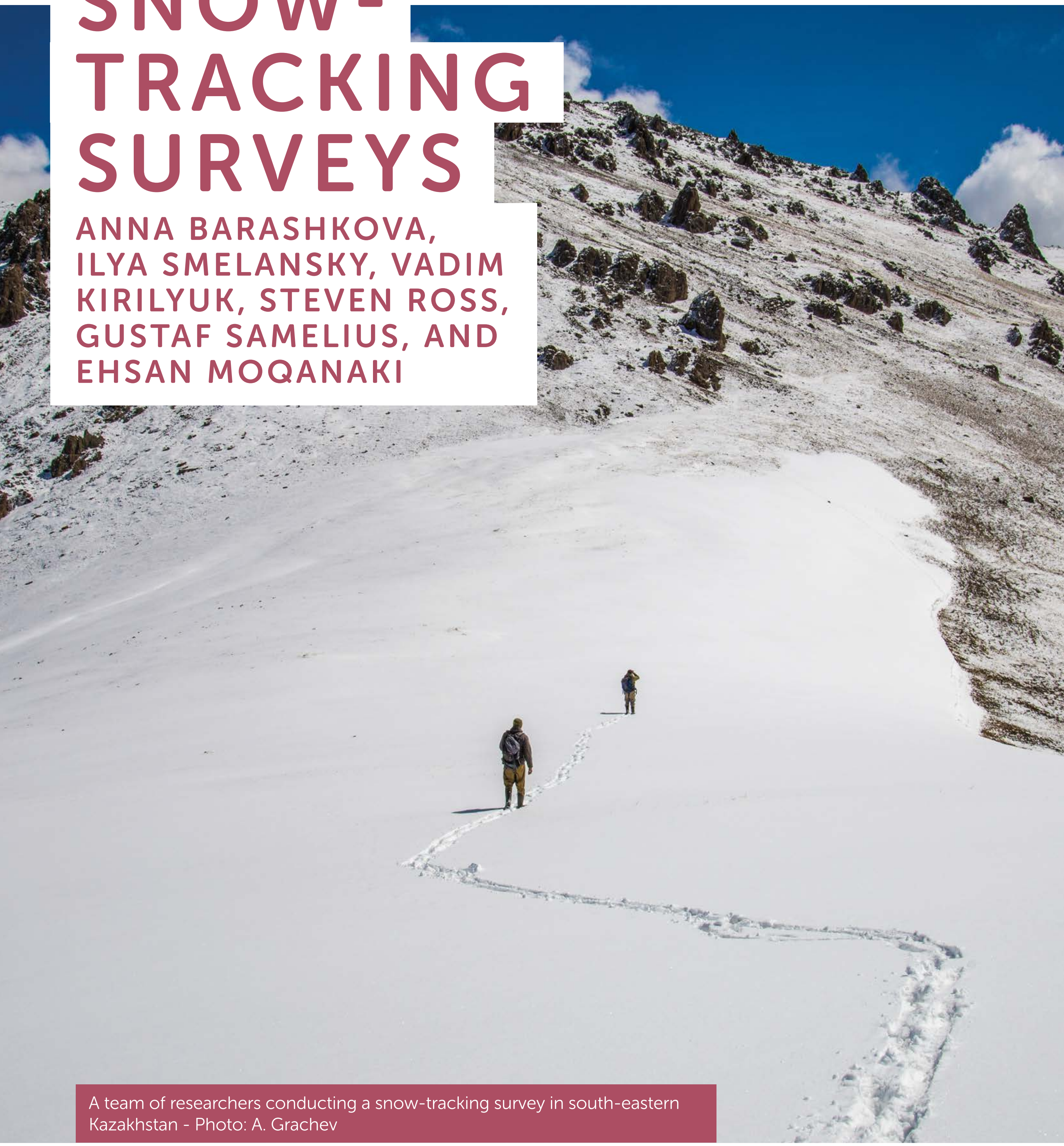
Several studies have compared different mediums and storage methods for the preservation of faecal samples and there is no ultimate guide (Tende et al. 2014). The efficacy of the storage mediums and methods should be tested for the specific environmental conditions and focal DNA samples. Communicate with the partner DNA lab on their preferred medium for the storage of faecal samples; different labs have their own protocols of sample storage, and we advise following the specific guidelines developed by your lab. For animals with strictly carnivorous diet, it appears that a high concentration of ethanol ($\geq$ 95%) is a good medium (Panasci et al. 2011, Wultsch et al. 2015). A sample can be individually stored in a capped or centrifuge tube filled with ethanol or a commercially sold buffer agent (ratio of 1 sample : $\geq$ 4 medium). Using ethanol can be advantageous because it is relatively cheap and can be purchased in almost all the manul's range countries. However, carrying a flammable medium such as ethanol in the field can be potentially hazardous. There is also the risk of spills, which may wipe out the labelling information. In some of the manul's range countries, purchasing large volumes of highly concentrated ethanol can be difficult because of the prohibition of alcoholic beverages. Ethanol is also occasionally mixed with methanol (also known as methyl alcohol or methyl hydrate) and additive colours to prevent its consumption, which may have consequences for DNA preservation as well. Buffer agents are an alternative medium and using them may also eliminate a step in the laboratory analysis, but they are comparatively more expensive than ethanol and may not be easily purchased in all the manul's range countries. Faecal samples can be stored in desiccant long-term at room temperature in a cool and dark environment once they are sufficiently dried and as long as the temperature is stable. Some labs might prefer to transfer samples to a different preservation medium as soon as possible. There are also multi-step approaches where more than one preservation method is combined; for example, placing the sample in ethanol for 24 hours and then replacing it with silica desiccant (Renan et al. 2012). Freezing samples at or below -20 degrees Celsius as soon as possible is another option when feasible. However, care must be taken to minimize frost-thawing events and avoid storing samples in freezers with self-defrost functions (such as most household freezers; Kelly et al. 2012).

Labels written with permanent marker or even pencil will be erased if ethanol spills on the labels. Alternatively, a piece of paper marked with pencil in a plastic bag around the sample tube can be used. The key is to test any system in advance since materials can vary. A good practice for labelling the samples is to use a thermal printer with good, water- and freezer-proof, ribbon-like labels as described by Karamanlidis et al. (2009). These types of labels are more durable, even when the sample is stored in a freezer or if ethanol spills. Using a thermal printer, one can print unique barcodes and human-readable codes for each sample. Different barcode fonts are freely available on the internet to download and install. In choosing a labelling system, try coming up with an informative combination of letters and numbers, and use zeros to ensure that the sample code is the same length (as this makes databasing easier); for example, combination of initials of the study site followed by a unique combination of letters and numbers (e.g., NEP0001, CA0102). Avoid using very long sample codes and combinations than can be mis-read during data entry (e.g., 5 and S/s or O/o and 0; Karamanlidis et al. 2009).

CHAPTER 5

SNOW-TRACKING SURVEYS

ANNA BARASHKOVA, ILYA SMELANSKY, VADIM KIRILYUK, STEVEN ROSS, GUSTAF SAMELIUS, AND EHSAN MOQANAKI



A team of researchers conducting a snow-tracking survey in south-eastern Kazakhstan - Photo: A. Grachev

5.1. INTRODUCTION

Snow tracking is the process of studying the ecology and behaviour of animals by following their tracks in the snow. Snow tracking has been used for studying the manul across its northern range in Russia and Central Asia, where the technique is also known as “winter transect census” or “snow-tracking census” (Kirilyuk & Puzansky 2000; Barashkova et al. 2008, Barashkova & Smelansky 2011, Barashkova 2012, Kirilyuk & Barashkova 2011, 2016). However, in this guide we refrain from using “census” (as in, total counts), because no data collection method can successfully detect all individuals from a population (see Chapter 1 for a discussion on imperfect detection – i.e., when detection probability is less than one).

Snow tracking depends on reliable and predictable snow cover (Fig. 1), which for the manul may vary greatly across different regions and over time. Snow tracking is not a practical method for surveying the manul in arid and semi-arid areas, such as the Gobi Desert or many habitats in Southwest Asia (Moqanaki et al. 2019, Barashkova et al. 2019, Ross et al. 2020; Fig. 2). While lack of snow can limit the utility of snow tracking, heavy snow cover and very cold conditions reduce the effectiveness of the method in other areas, despite suitable tracking conditions. In addition, snow tracking by inexperienced personnel and using poor study designs can result in unreliable data and wrong conclusions. However, if properly designed, implemented and analysed, snow tracking can provide useful information about space-use, distribution, and relative abundance and density of the manul. Non-invasive DNA sampling of manul scats and even paw prints can also be integrated into snow tracking (Franklin et al. 2019; see Chapter 4).

Figure 1. Snow tracking is time-consuming and labour-intensive. Nevertheless, over large spatial extents when other data collection methods, such as camera trapping or non-invasive DNA sampling are not practical, snow tracking can be used to provide valuable information about the basic ecology and behaviour of the manul. Photos: A. Barashkova

Figure 2. The manul is very well camouflaged and difficult to see in its natural habitat (top photo where the manul is in the centre of the photo). It is easier to detect the manul against the snow (bottom). Photos: M. A. Adibi (top), A. Barashkova (bottom)

Snow tracking allows several species of terrestrial mammals and ground birds to be simultaneously surveyed. Manul tracks are usually difficult to reliably distinguish from those of other small cats (Fig. 3; Chapter 2); thus, misidentification errors in areas where other small, wild or feral, cats co-occur can be high. We therefore do not recommend using snow tracking in areas where the manul overlaps considerably with other small cats, such as the Asiatic wild cat *Felis lybica ornata* or the Chinese mountain cat *F. bieti,* and areas with permanent occurrence of the sand cat *F. margarita*, the leopard cat *Prionailurus bengalensis*, or the jungle cat *F. chaus*. Otherwise, when other non-invasive data collection methods, such as camera trapping, are not practical or possible, snow tracking can be used to monitor species occurrence and even provide relative abundance and density estimates across large spatial extents as baseline information (Kojola et al. 2014, Kuzyakin 2017, Rozhnov et al. 2019, Franklin et al. 2019). Estimating relative abundance and density from snow tracking is based on the assumption that the number of animal tracks crossing the survey line is proportional to the population density of the species, and that the detection probability of animal tracks is the same between the two periods or locations of interest. The number of tracks observed depends on (1) the average daily distance travelled by the animal of interest (i.e., an increase in the daily movement of the animal increases the probability of crossing the survey line); (2) survey-specific conditions (e.g., personnel skills, snow conditions, logistics).

Figure 3. Examples of the manul's tracks in the snow – Photo: A. Barashkova

5.2. LOCAL CONDITIONS

5.2.1. Time of year

The optimal season for snow tracking of the manul depends on local conditions, logistics, and the capabilities of the survey team. In many areas across the northern range of the manul, snow-tracking conditions are generally better during the first half of winter when the snow is soft. At the end of winter, the snow often gets hard from the wind and snow-free areas are more common, which results in the tracks becoming more difficult to see and identify. When the temperature decreases significantly, the manul tends to be less active and may only travel short distances from its den (Ross et al. 2019b), which would result in few or no tracks. Furthermore, when the snow becomes hard from the wind, very few tracks are left as the manul tends to move on top of the snow with no detectable tracks. The snow should be deep and soft enough that footprints remain identifiable (Figs. 3-4). Thus, the weather conditions have a large impact on how successful snow tracking is for detecting the manul. If obtaining relative measures of abundance or density is intended, it is important that the snow conditions are similar, otherwise the indices of relative abundance and density may not be comparable.

5.2.2. Time of monitoring in relation to previous snowfall

The optimal time for snow tracking is on the second day after a good snowfall, when the weather is calm and clear, and the temperature is between -5 and -20 degrees Celsius; colder weather causes manuls to remain in their shelters. Conducting snow tracking during the second day after a solid snow fall increases the chances of the tracks being clearly identifiable, reduces the risk of misidentification of tracks, and ensures that there has been enough time for the tracks to accumulate (Halfpenny et al. 1997). Waiting longer than two days after a solid snow fall may result in unfavourable weather conditions, making identification of the tracks more difficult. Tracking during snowfall or strong winds is not recommended since the tracks will quickly be obscured, and field conditions may also not be safe for the survey team. On south-facing slopes, care should be taken to ensure that the sun has not melted the snow, which will make tracking difficult or even impossible. There are also methods attempting to standardise the time for tracks to accumulate (Kojola et al. 2014), including: (1) pre-checking of the transect line: all existing animal tracks should be covered by snow or clearly marked. Then, one or two days later during the actual survey, all new crossings by the focal species are recorded. (2) No pre-checking: in cases where a good snowfall has completely covered all the older tracks one or two days before the survey, no pre-checking is required.

Figure 4: When snow depth is about 10 cm and the snow is soft, snow-tracking conditions are optimal. At slow travelling speeds, the hind foot of the manul registers behind the front foot. Photos: A. Barashkova (top), V. Kirilyuk (bottom)

5.3. OPTIMAL CONDITIONS FOR SNOW TRACKING

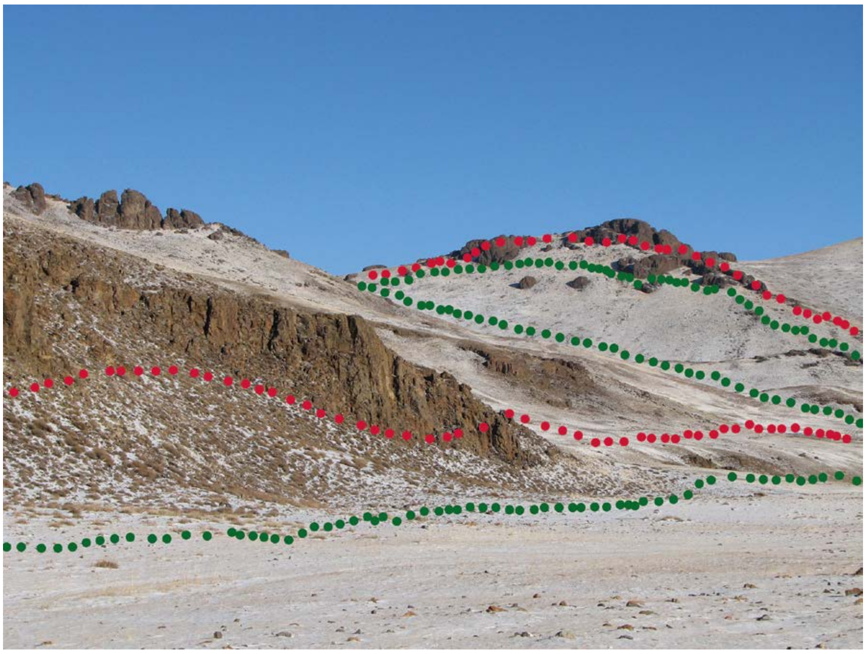
Snow tracking for manul survey and monitoring should be carried out in the presence of complete or almost complete snow cover, otherwise one may get biased results from sampling only part of the area (but see below for targeted sampling; Figs. 1 and 5-6). Areas that meet such conditions exist throughout most of the northern part of the manul's range, from central Kazakhstan in the west, through the Russian part of the range and the north and centre of Mongolia, to the Dauria region in Russia, Mongolia, and China (Barashkova et al. 2019; Ross et al. 2020). In general, any amount of snow can help in finding signs of the manul. However, a minimum of two-to-five centimetres of snow that is soft enough to leave animal tracks is usually required to provide reliable results (Halfpenny et al. 1997), especially when estimating relative abundance or density is the main study objective. The tracks of the manul are found more often in areas close to natural refuge and topographic features that provide cover and connectivity between rocky habitats, such as valley bottoms, saddles, and ridges (Figs. 5-6). However, as a general rule, sampling should be conducted evenly throughout the study area as much as possible. Incomplete snow cover and recurring melting and freezing are usually less of a problem on north-facing slopes, and we recommend focussing on these areas where sampling of the whole study block is not required. Good sampling locations to search for manul signs are described in Chapter 2.

Figure 5. Mountain ridges and saddles, especially where they intersect multiple terrain, are good locations to search for manul tracks, where the whole study block does not need to be sampled. Photos: A. Barashkova

5.4. DESIGNING SNOW-TRACKING SURVEYS FOR MONITORING THE MANUL

The design of snow tracking surveys will depend on the question asked and what aspect of the ecology is being monitored. Depending on the study questions and whether repeated sampling is required (e.g., multiple visits for occupancy studies), one may have to search the sampling sites several times during winter. Alternatively, replicates can be, for example, multiple winters in consecutive years or segments of transects in each sampling site (Hines et al. 2010, Gopalaswamy et al. 2012, Crosby & Porter 2018). For monitoring the manul, it is important to carry out annual surveys at the same time-period and with similar weather and snow conditions. If seasonal changes are of interest, we recommend conducting one survey at the beginning of the winter and one survey at the end of winter. Data from both the start and end of the winter has been used in assessing the survival of local manul populations during harsh winters. However, snow tracking has a lot of associated variability and is generally not suited to assess mortality, as manuls may shift their home range or change their behaviour when faced with unsuitable conditions; thus, absence may be due to other reasons.

As a starting point for snow tracking to monitor the manul population, we recommend using a grid-based approach wherein investigators should attempt to search transects of similar lengths within the grids. The size and number of sampling grid cells depends on study questions and whether the survey is conducted at a local or regional level (Chapter 1), as well as the snow tracking conditions and logistics. For local surveys, smaller sampling cells (e.g., 1 x 1 or 2 x 2 km) should be considered. Based on the logistics and practicalities of fieldwork, sampling cells may be selected randomly or in a stratified fashion and then searched for tracks (Fig. 6). In regional surveys, the size of the sampling cells can be selected based on the average size of annual or winter home-ranges of adult manul females (10 - 25 km^2; Barashkova and Kirilyuk 2011, Ross et al. 2012). An alternative approach is to adapt the Finnish wildlife monitoring scheme, where three straight transects of four kilometres form sides of a triangle (Helle et al. 2016). Searching of each triangle should be performed within one day (Helle et al. 2016). In this scheme, relative abundance is calculated as the number of crossings per 10 km and within 24 hours (Kojola et al. 2014). In occupancy studies, usually all grid cells will be searched, regardless of their potential to contain optimal sites to leave manul tracks. To collect data for estimating relative abundance or density, a targeted searching of preferred habitats can be considered (i.e., preferential sampling design; Conn et al. 2017) and snowless areas or rugged terrain, where the likelihood of encountering tracks is generally very low, can be avoided (Fig. 6).



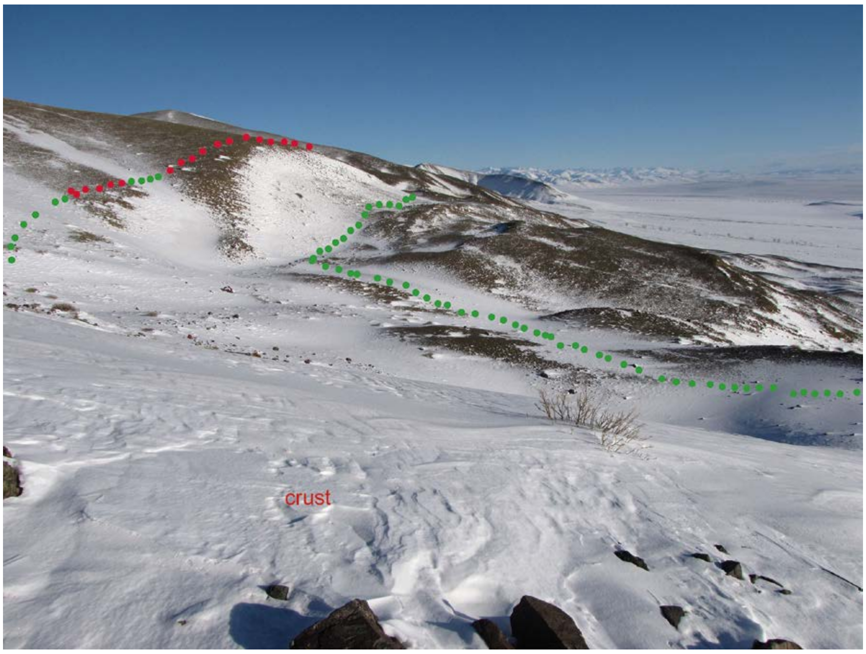


Figure 6: Search tracks are often determined before conducting the surveys, but they can be adjusted on the spot based on the local conditions. In these photos, green dotted lines illustrate optimal search tracks with snow, and red dotted lines show areas with little snow or unsuitable rocky areas that can be avoided depending on the study questions. Photos: A. Barashkova

There is a trade-off between the number of sampling sites and the effort needed to achieve an acceptable level of error for estimates of occupancy and abundance (DiRenzo et al. 2022). The minimum sampling effort (e.g., length of search tracks) required in each sampling cell depends on snow conditions, size of the sampling unit, and the abundance of the manul in the area. For rare and elusive species, such as the manul, typically a dozen of kilometres of tracks should be searched in each sampling site to have a probability of 50% or more to detect the species when monitoring abundance or change in abundance (Moqanaki et al. 2018, Bischof et al. 2020). In occupancy-based snow tracking when detection and non-detection data of the manul is collected using spatial (e.g., multiple lines of transects) or temporal (searching the sampling site multiple times) replicates, as soon as the manul is detected the search can be concluded in that sampling site before the whole survey track is completed. This is because the detection of the manul is already confirmed, and it will not change for that survey track if the species is detected more than once. However, we recommend even sampling effort across the sampling sites as much as possible. Search tracks should be GPS-recorded, so they can be mapped, measured and sub-sampled later based on the specifics of the sampling site. The length of search tracks can be used as a measure of sampling effort to account for variability in detection in the analysis, depending on the question asked.

The calculation of relative density and abundance from snow tracking of the manul in Russia is based on (1) the average number of track crossings of the manul per transect length (e.g., 10 km of search tracks) and (2) the average movement distance of the manul per day based on a conversion factor that matches with the local conditions during the survey. This conversion factor typically relies on quite a few assumptions that require detailed knowledge; for example, travel distances by the focal population of the manul, the detection process during the survey, and time passed since the last snowfall. We note that relative abundance and density indices are often unable to account for imperfect and variable detection (Sollmann et al. 2013). Thus, like other data collection methods described in this guide, it can be challenging to compare relative abundance or density estimates of the manul when the survey conditions (e.g., snow cover, personnel experience and skills) are not the same.

5.5. FIELDWORK

5.5.1. Equipment

To conduct the snow tracking within a relatively short period of time, quick and safe transport between the sampling sites is important and appropriate vehicles should be used. Within each sampling site, snow tracking can be conducted on foot when the snow is not too deep (less than 30 cm; Fig. 7). In general, in the habitat of the manul where flat areas constantly alternate with rocky features, using snowshoes or skis is not practical. If available, snowmobiles can be used for transportation and sometimes for tracking instead of walking, if driving at low speeds such as 10 km per hour. However, using snowmobiles may not be practical in the relatively steep and rugged terrain that is typical across the manul's range, and is likely to result in lower detectability of tracks compared to walking, skiing, or snowshoeing (Fig. 7). Thus, snow tracking done on foot is often more suitable. Handheld GPS units (including spare batteries that work in cold conditions), binoculars, digital cameras, a track identification guide, rulers or meter sticks, and communication devices are also among the basic equipment items required for snow tracking.

Figure 7: Planning for transportation between the sampling sites is crucial during snow tracking. In many areas, snow-tracking must be conducted with skis or snowshoes. Snowmobiles are usually not practical for snow tracking of the manul because they can only be used in relatively flat areas, which is generally rare in most parts of the manul's range. In addition, snowmobiles generally require relatively deep snow which is not optimal for snow tracking of the manul. Photos: A. Barashkova

5.5.2. Survey team and typical characteristics of manul tracks

Special attention should be given to the training of personnel in the biology and behaviour of the manul, the skills of working safely in winter conditions, and identifying the tracks, scats, and other signs of the manul (Chapter 2). The manul footprint and trail (Figs. 3 and 5) can be easily confused with those of similar-sized felids and canidae species. The main distinguishing feature of a cat track is a round footprint with relatively small toe pads, clearly imprinted pads, the absence of claw marks, and the asymmetry of the footprint with one leading toe (i.e., one of two middle toes protruding a bit forward; see Chapter 2). The claw marks of foxes, in contrast, are always clearly visible in deep snow, and their footprints are more diamond-shaped compared to that of cats whose footprints are symmetrical with the middle toes on the same line. In addition, the manul rarely runs in snow, usually puts its feet in a mismatch when walking (i.e., sometimes the back foot is in front of the front foot and vice versa), and their paws often leave a drag-mark in deep snow (Figs. 4-5). However, large adult manul individuals at a fast pace may leave tracks in a straight line in a similar fashion as a red fox would do. Thus, it is important to check the tracks by carefully examining paw prints.

We recommend that two observers simultaneously search the trails to reduce misidentification. As soon as a potential track of the manul is sighted, the trail must be examined carefully to ensure that it is actually from a manul. Optimal locations to search for manul's snow-tracks are not evenly distributed across the land (Figs. 7-8). During the surveys, all tracks encountered should be recorded and multiple photos with proper scale should be taken for further validation (Fig. 3). Recording signs of other medium-sized and large mammals will provide useful information for understanding the occurrence of these species, as well as the spatial relationships between species and their co-occurrence,

Figure 8: Manuls generally avoid open flat areas and instead, tend to walk on the edges of rocky habitats, which may result in no or little snow-tracks in the topographically complex habitats they occupy. Photos: I. Smelansky (top), E. Balakina (bottom)

5.5.3. Survey protocol

We recommend the following steps for snow tracking of the manul:

1. Prepare a protocol for keeping records (see number 2 below for suggested information to record) and select travel routes to transfer to your GPS unit or to a map. Bring a digital camera, track identification guide, and a ruler or meter stick to help identify and photograph the tracks. Ensure the digital camera time matches that of the GPS so that records from both devices can be synchronized. Using a digital camera with an integrated GPS-receiver can be particularly useful but is not necessary. Software packages installed on smartphones or tablets that are developed specifically for recording survey lines and observations, such as SMART (https://smartconservationtools.org/), can be used to help with the data recording, but are not a pre-requisite for successful monitoring. Such platforms allow scientists, managers, and rangers to easily collect, visualize, store, analyse, report, and act on a wide range of data relevant for monitoring and studying wildlife, although wildlife can be monitored successfully without them For more information on such platforms, see the example of WWF's snow leopard monitoring program: https://smartconservationtools.org/ or https://nextgis.com/nextgis-collector/

2. Record general characteristics of the sampling site, such as: habitat type, weather conditions (e.g., temperature, precipitation, wind, cloud cover), snow depth, snow texture and percent of snow cover, days since last snowfall, time of the day, and potential disturbances.

3. Transfer the transect lines and borders of the sampling sites to the GPS for easy navigation, although you may also use regular maps. Ensure the GPS tracklog is activated to record the survey effort (e.g., length of search tracks), or alternatively mark these on the map. Record all manul tracks that cross the survey line when monitoring relative abundance or density. Similarly, record all intersections of animal tracks with the transect line, even if visually it can be interpreted as originating from the same individual. Preferred habitats away from the survey line can be also examined for occupancy studies. Mark the start and end of the animal tracks encountered and record all tracks and other signs of the manul crossing the transect line.

4. Make detailed notes in the protocol about the signs encountered and take photos of all manul tracks encountered (i.e., footprints and trail with proper scale) and other signs (e.g., urination and defecation points, scratches, prey remains). These photos will help to improve the species identification of the tracks and other signs and will allow sharing of information with collaborators and peers.
5. Mark the areas devoid of snow or with a hard snow crust in which animal tracks are not visible, by using a GPS or on your map to exclude the area from the analysis if this is required by the study questions and analytical framework (Fig. 6). Preferred habitats away from the survey line can, as mentioned in the number 3 above, be searched for occupancy studies (e.g., near pika colonies; Fig. 9), but tracks found away from the survey line typically should not be included for estimation of indices of abundance or density.

Figure 9: Pikas, such as these Daurian pikas *Ochotona dauurica* (top-right and bottom), do not hibernate and are active all winter long, especially during sunny days. The manul and its tracks can be often found near such pika's burrows. Photos: V. Kirilyuk (top-left), A. Barashkova

CHAPTER 6

INTERVIEW SURVEYS

EHSAN MOQANAKI, JUSTINE SHANTI ALEXANDER, AND GUSTAF SAMELIUS

Interview with a community member in Eastern Kazakhstan to learn about the manul status - Photo: R. Nefedov

GLOSSARY

Welfare:	Welfare is a wide concept that ranges from economic and spiritual well-being of people to ethical consideration of treatment of animals.
Human well-being:	Psychological, spiritual, and economic well-being of people, including perceptions of safety, spiritual or religious connection with their surroundings, and local livelihoods.
Community:	A group of people within a bounded geographic territory, such as a neighbourhood or village, who actively interact with one another. The community's members usually share common values, beliefs, and behaviours.
Community science:	The collection and analysis of ecological data (e.g., species occurrence and status) accumulated, in part or in whole, by members of the public. Sometimes referred to as citizen science.
Traditional knowledge:	The cumulative body of ecological knowledge, skills, practice, belief, and perception embedded in the traditions of indigenous, local, or regional communities. Also known as indigenous knowledge or native science.
Interview:	An attempt to elicit information or expressions of perception, opinion, or belief from another person as a data collection method.
Informant:	A member of the public who voluntarily participate in an interview and gives a piece of privileged traditional ecological knowledge. Also known as key informants or local experts in human-wildlife interactions literature. In the context of community science, we define an informant as a person from whom we obtain information about local wildlife, which is different from how the term is usually used within the politics or law-enforcement world.
Questionnaire:	A set of predetermined questions, often with a choice of answers, devised for the purposes of the study.

6.1. INTRODUCTION

There is an increasing call for integration of social science in species conservation and management (Sandbrook et al. 2013, Bennett et al. 2017, Niemiec et al. 2021). Humans are the key force driving and shaping the Earth's ecosystems, and almost every aspect of wildlife management and conservation centres around human impacts on wildlife and their ecosystems (Fryxell et al. 2014). The most common method of data collection in conservation-related social science research is conducting interviews (Newing et al. 2010). Interview surveys attempt to elicit information or expressions of perception, opinion or belief from members of the public (Newing et al. 2010, Young et al. 2018). Interviews are commonly used to collect socio-economic and ecological data relevant to the human dimensions of wildlife. Examples include, but are not limited to, exploring local perceptions and attitudes towards wildlife, drivers of wildlife damage, intensity of conflict claims, evaluating locally feasible solutions for coexistence, effects of protected areas on human societies or wildlife populations, and decision-making and governance processes (Newing et al. 2010, Conrad et al. 2011, Dickinson et al. 2012, Lepczyk et al. 2020). In addition, the expert knowledge of respondents, in particular community members who are knowledgeable about the study area and target wildlife (Fig. 1), can also be used to study species occurrences and richness, probability of occupancy or site use, and sometimes even relative abundance and population trends (Karanth et al. 2009, Zeller et al. 2011, Pillay et al. 2014, Young et al. 2018, Altwegg and Nichols 2019). Thus, interviews can be used as both a qualitative (e.g., content analysis) and quantitative research method (e.g., occupancy analysis).

An interview is a mutual learning process that occurs between those who conduct the interview and those that are interviewed (Newing et al. 2010). There are methodological and ethical challenges in conducting interviews and using the data collected appropriately. As a result, there are several sources of information on how to conduct and use interviews for wildlife research and conservation (e.g., Newing et al. 2010, Drury et al. 2011, St. John et al. 2014, Crandall et al. 2018, Young et al. 2018, Brittain et al. 2020a). These resources highlight how important it is that interviews are conducted in a systematic and structured way to minimise biases and respect the integrity of participants.

There are several techniques to interview respondents about wildlife, ranging from face-to-face verbal or group exchanges to mail, telephone, or internet surveys (Newing et al. 2010). Depending on the study objectives, an interview may be done once or repeated over time, and last from a few minutes to several hours. Although commonly used in wildlife studies and potentially perceived as simple and straightforward, conducting interviews requires adequate training to acquire a new set of skills and follow effective sampling protocols (Newing et al. 2010, St. John et al. 2014, Young et al. 2018). Interviews are usually conducted by people who come from a different social context and usually have different cultural norms, which can result in misunderstandings between the interviewer and interviewee (Brittain et al. 2020a). Even published research may suffer from poor implementation and reporting of interviews and some may have ethical issues (Young et al. 2018, Ibbett & Brittain 2020). In this chapter, we focus on conducting interviews to collect data for estimating the occurrence of the manul. Nevertheless, these techniques can be extended to study changes in the distribution of the manul (Chimed et al. 2021), as well as trends in the abundance and manul-human interactions.

Figure 1: People with varying social and cultural backgrounds share the habitat with the manul across its broad geographic range. In designing and conducting interviews, taking the diversity in human societies into account is important. From top, left to right: Eastern Mongolia (A. Barashkova), Tyva Republic of Russia (N. Goreva), Nepal's Himalaya (G. Werhahn/Himalayan Wolves Project), Altai Republic of Russia (M. Ushakova), Qinghai Province of China (B. Weckworth), and the Iranian Caucasus (E. Moqanaki).

6.2. ETHICAL CONSIDERATIONS

Wildlife surveys and monitoring that involve people require adequate ethical rigor (Newing et al. 2010, Young et al. 2018, Brittain et al. 2020a). This is especially important for the collection of personal information and when sensitive topics, such as poaching, are investigated (Ibbett & Brittain 2020). An interview must not compromise the relationships within the target community (Mishra et al. 2017). Extra care must also be taken when working with marginalized and socially excluded individuals, groups, or communities, who have been influenced by injustices and tend to lack trust in government representatives and people from outside the community (Brittain et al. 2020a). Therefore, before starting any work that involves people, discipline-specific guidelines and codes of conduct should be considered to inform an ethical research practice, and ethical approval should be obtained from relevant ethical boards and committees (Ibbett & Brittain 2020). This process may vary across countries and involve granting specific permits from different local, national, or international institutions.

Participants must be provided with all the information they need to make informed and independent decisions about whether to participate in the study. For an informed consent to participate in an interview, the goal of the study, methods, and intended use of data must be described in a language that is understandable to participants (Young et al. 2018, Ibbett & Brittain 2020). Assuring anonymity (i.e., participants remain unidentified) and confidentiality (i.e., the personal information will be protected from being disclosed to others) is an important consideration, especially when interviews include collecting sensitive information (St. John et al. 2014). It is therefore important to abide to privacy standards and relevant laws regarding the processing of personal data. There are guidelines available (e.g., Data protection in the European Union: https://ec.europa.eu/info/law/law-topic/data-protection_en) that can be adapted to fit local conditions.

6.3. DESIGNING INTERVIEW SURVEYS

Designing an interview survey includes the following main steps (Young et al. 2018, Niemiec et al. 2021, also see: https://snowleopardnetwork.org/module-4-social-research/): (1) identifying research questions, (2) selecting interview method and target respondents, (3) preparing the initial questionnaire and conducting pilot interviews to refine the questionnaire, (4) conducting interviews, and (5) data preparation, analysis, and reporting.

6.3.1. Identifying research questions

The first question to ask is whether the interview is the most appropriate method to obtain the data needed. This step involves evaluating possible approaches and solutions to identify targets, threats, and opportunities, as well as skills of the project team (Niemiec et al. 2021). The study objectives must be clear, realistic and specific enough to be researched, and they must address an aspect of the biology, ecology, or conservation that the local community is expected to know (Newing et al. 2010). In this guide, we provide an example where the aim is to assess the distribution and trend of the manul in different areas across its geographic range. The study objectives presented in the following sections are therefore selected accordingly to address this aim. We recommend researchers review the pros and cons of the different data collection methods discussed in Chapter 1 to decide on whether an interview survey is the best approach. Interviews can also be used in combination with other data collection methods described in this guide (e.g., camera-trap data; Huang et al. 2020).

Interview surveys of the manul can be designed to meet different objectives. The surveys we specifically consider in this guide are those attempting to estimate distribution, occupancy or site use, and relative abundance of the manul. Interviewing people knowledgeable about wildlife occurrence are a particularly useful tool to study distribution on large spatial scales (also known as key informant sampling in the context of citizen or community science; Karanth et al. 2009, Zeller et al. 2011, Pillay et al. 2014, Young et al. 2018). Like other data collection methods described in this guide (Chapter 1), we recognize that given the rarity and elusiveness of the manul, the probability of detecting the species by respondents (e.g., direct observations or finding signs of occurrence) is likely less than one at any given time in most survey sites regardless of the level of knowledge and engagement of the respondent and survey effort or method adopted (Kellner and Swihart 2014, Altwegg and Nichols 2019). Thus, a combination of adequate sampling, efficient interview methods, and proper analysis to accommodate imperfect detection is required. If a survey design cannot accommodate imperfect detection, then making inference on relative abundance can be considered. However, such measures of relative abundance are controversial as they ignore detection bias and assume it is constant across space and time (Boitani et al. 2012, Sollmann et al. 2013).

Interviews that collect manul presence and absence (or detection and non-detection) data from knowledgeable members of the public (Chimed et al. 2021; see the next section on sampling) can answer questions related to manul occurrence and distribution using occupancy modelling, including assessing: (1) distribution or probability of site use; (2) change in distribution between two or more time periods; (3) effect of certain variables on the detectability, distribution or probability of site use. Such different objectives may require different sampling designs, different types of data, and even different sampling efforts (Boitani et al. 2012, Altwegg and Nichols 2019). The survey objectives must always be kept clearly in mind; goals and objectives come first, as they drive the design and field work. Thus, realistic yet functional objectives should be chosen for interview surveys of the manul.

6.3.2. Selecting interview methods and target respondents

An optimal interview is easily replicated and administered, whilst retaining standardisation (Newing et al. 2010). The key elements to consider for designing the sampling scheme are: (1) sampling-specific details of the study area; (2) interview method and sampling approach; and (3) statistical considerations, if relevant. An adequate sampling scheme must address the specifics of the study location and the survey's objectives, the biology and behaviour of the manul, its local distribution and abundance, and the resources and time available. Thus, an interview design that works for the manul in one region may not be suitable in other areas.

When designing interview surveys and deciding on who and where to interview, we specifically consider three main areas that are important for assuring that adequate and representative data is collected: (1) how to select who to interview, (2) coverage and where to sample, and (3) replicated sampling. In interview surveys, sampling is about selecting who and where to interview and what places to visit, events to attend, and people to talk to (Newing et al. 2010). There are different ways of selecting who to interview and which approach to use will be driven by the study question. For example, we may select a cross-section of the community (sometimes referred to as representative sampling) or we may choose to focus our interviews on community members who are judged to have more knowledge about the research question than the rest of the community (e.g., rangers or managers). Cross-sectional sampling of the community is more common when human perceptions and attitudes towards the manul is studied. Another approach is to use a stratified-like sampling approach, where members of the target community are divided into sub-groups (i.e., strata) based on specific characteristics that they share and the survey team is interested in (e.g., occupation, education level, gender).

When doing an interview study, we must decide on whether to interview individuals, households, or groups of people (e.g., groups of herders or rangers). An individual is the most common sampling unit in most studies and an important part of the study design is therefore how to select who to interview and how many individuals to interview (Newing et al. 2010). Individuals are generally the sampling unit in studies to assess public attitudes and perceptions of animals. Other studies may choose households as the sampling unit where you may choose to interview the head of the household or the person in the household that spend the most time on the land depending on the research question (Newing et al. 2010). Group interviews can be especially informative when complex issues are examined or when you may be interested in additional questions that may help increase the understanding on distribution or trend.

Interviews should be conducted at a scale (i.e., geographic extent) and resolution (i.e., grain) that is representative of the study landscape. In surveys to determine the probability of occurrence or site use of the manul using occupancy models, we recommend using a grid-based approach, where the study landscape is covered by a network of grid cells representing large-enough areas depending on the study objectives and practical, as well as analytical considerations (Karanth et al. 2009, Zeller et al. 2011, Pillay et al. 2014; Chapter 1). Within each grid cell, multiple potential respondents should then be identified, approached, and interviewed. The number of interviews required per spatial sampling unit depends on the aims of the study and the research design, as well as practicalities of the fieldwork. There are methods to determine the required sample size given the accepted confidence interval and level. As a general rule, the greater the precision and level of confidence required, the larger the sample size must be (Newing et al. 2010). Occupancy-based simulations can also be used to study the trade-offs between the sampling effort and the level of confidence (e.g., Pillay et al. 2022). In deciding on the target sample size, non-response rates must also be considered. For example, out of the total number of questionnaires distributed among the participants, only a portion of them will be returned fully completed and usable. This rate varies greatly based on the interview methods used and is generally lower for interview studies than for surveys which are distributed among the target community without meeting and talking to the respondents and it may be as high as 50% for the latter (Newing et al. 2010). Response rate should be closely monitored during the survey to adjust the sample size accordingly.

Quantitative analysis of interview data (e.g., using occupancy models) require replicates in each sampling area. Depending on the study question, replicates can be either interviewing the same respondents at different times (i.e., longitudinal design; Newing et al. 2010) or interviewing multiple respondents within each sampling unit. In conventional occupancy analysis to estimate probability of site use, the latter is required.

6.3.2.1. Interview methods

Interviews we consider for the manul survey and monitoring can be classified into three main groups: (1) structured interviews, (2) semi-structured interviews, and (3) unstructured or open-structure interviews. Structured interviews typically follow a fixed set of predetermined questions that are generally close ended, where the same interview script is used in each interview. A close-ended question can be answered with a predetermined categorical response, such as a "yes" or "no", or a scale (e.g., 1-5) and typically does not require a longer response, contrary to open-ended questions. This approach allows for close comparison between different interviews but does not allow respondents to shape the discussion (Newing et al. 2010). Unstructured interviews, in contrast, do not force the answers to follow a predetermined script but are instead based on the responses of the respondents themselves, and questions are asked spontaneously based on the answers received and, thus, open-ended by default (Newing et al. 2010). While the unstructured approach allows for an in-depth understanding of questions and limits pre-conceived researcher bias in shaping the interview, such interviews typically offer little assurance that all relevant issues will be covered and sometimes present a problem for comparative and quantitative data analysis (Newing et al. 2010). Semi-structured interviews combine elements of structured and unstructured interviews and rely on asking a combination of questions within a predetermined thematic framework and questions that are not predetermined (e.g., open-ended question).

There are advantages and disadvantages of each of the three interview techniques for manul survey and monitoring, depending on the aims and objectives. Researchers in wildlife studies often prefer to use semi-structured interviews because they allow the interviewer to ask additional questions if an interesting or new line of enquiry develops during the interview. This flexibility is important for investigations of complex issues, which analyse complex processes that can rarely be foreseen (Rose et al. 2016). In addition, the combination of closed and open-ended questions enables both quantitative and qualitative analyses of the data, strengthening the results by drawing on the information obtained from each of these question types (Brittain et al. 2020b). Our focus in this guide is on surveying and monitoring of manul distribution and occurrence and thus a structured approach is recommended since the investigator needs to collect specific information on manul detection and non-detection and covariates of occupancy and detectability (Altwegg and Nichols 2019, Chimed et al. 2021). The most common method is using questionnaires, where a set of standardised questions are asked in the same way and in the same order for all respondents (Newing et al. 2010). Questionnaires typically use a cross-sectional design in which we collect data from many different individuals at a short period of time, they can be sent to participants (rather than relying only on face-to-face interactions), and these are almost always analysed quantitatively.

6.3.2.2. Target respondents

Participants must be identified, selected, and approached carefully (Fig. 2). Note that respondents may or may not share the same values related to manul research and conservation as us, and it is important that such differences do not affect the relationship between the interviewer and respondent. In social surveys for wildlife, questions are typically formulated to cover the full range of opinions and perceptions that exist in the target community (Crandall et al. 2018). Accordingly, interviews can play a central role in documenting local perceptions and attitudes towards the manul, including hunting and trade, various threats, and other human-manul interactions (Barclay et al. 2019, Pallas's Cat Global Action Planning Group 2019). In such studies, it is therefore advisable to target a representative sample of the target community. However, in conducting interviews for assessing the distribution of the manul, we may specifically target knowledgeable members of the community (Young et al. 2018) as we are interested in obtaining reliable, spatially referenced information about the manul (Karanth et al. 2009, Zeller et al. 2011, Chimed et al. 2021). Suitable respondents are community members and professionals that are knowledgeable about the manul and the survey area and are willing to share their knowledge and expertise. In this context, we are particularly interested in those individuals who possess in-depth knowledge about the manul occurrences and population status in the survey area through their personal practices (Pillay et al. 2014, Nelson et al. 2018, Chimed et al. 2021). Depending on the setting and survey objectives, these local experts can be those who spend a significant amount of their time in manul habitats (e.g., hunters, livestock herders, shepherds, farmers), or community leaders and village council members who act as conduits of information (Figs. 1-2).

There are several different methods for identifying potential participants in interview surveys. We identify two general approaches for manul survey and monitoring:

(1) Passive approach: In situations where the survey team has already started interacting with members of the target community (e.g., a village), respondents can be identified passively through these informal interactions (Crandall et al. 2018). In rapid interview surveys, when each target village or human settlement is visited only once, investigators can search for individuals at work (e.g., livestock pastures) or popular meeting places (e.g., village shops or local cafes) and opportunistically look for evidence of expertise and relevant skills in the residents (Fig. 3).

Figure 2: When studying the manul distribution, suitable respondents are community members of the public that are likely to have more experience with the manul occurrence and other wildlife in general. Examples of such members are village council members, rangers, herders, hunters, farmers, environmental agency personnel, and hikers. Photos (from top, left to right): M. Ushakova, G. Werhahn/Himalayan Wolves Project, R. Nefedov, B. Weckworth, and E. Moqanaki

(2) The snowball technique: At the end of each interview or through local stakeholders, ask for potential respondents with the desired experience or expertise: "who else with similar experience or expertise would you recommend us to talk to?". Propose it in a way that you do not question the performance of the respondent during the interview, but you are looking for more perspectives by similarly experienced members of the community that may or may not agree with the respondent's views. This technique is particularly useful when finding local experts is difficult, for example, across large spatial extents or sparsely populated landscapes. The advantage of this technique is the potential to create a network of community members by being connected with more individuals through members of the target community. This may even help to build trust, especially when the survey team is introduced by well-respected members of the public (Fig. 3).

Figure 3: Local gathering locations, such as village shops, cafes, religious sites, or even road junctions, are good places to search for and identify potential respondents for interviews. Photos (from top, left to right): F. Jafarzadeh, O. Chimed, B. Weckworth, R. Nefedov, E. Moqanaki, and G. Werhahn/Himalayan Wolves Project

6.3.3. Preparing the initial questionnaire and conducting pilot interviews

For interview surveys of the manul, we rely on questionnaires. Questionnaires are basically a series of specific and often closed questions to be answered by the respondents (i.e., using a structured interview approach), either on their own or by the interviewer. Having a questionnaire helps to structure the interview as a question-and-answer session and not an informal conversation (Newing et al. 2010). In addition, following a fixed order of similar questions and answers that are collected in a similar fashion help to collect targeted data and compare the responses and study the patterns quantitatively. This is the case when all questionnaires are completed by respondents only, or all by the interviewer, otherwise we will get variations by mixing the two approaches. Well-prepared questionnaires are specifically useful in interviewing many respondents over large geographical areas within a reasonable time. The key is to balance the number of questions with the amount of data targeted to be collected. Thus, involving a pilot test of the initial questionnaire, ideally with members from the target community or focus groups, to optimise the questions and the protocol to ask the questions is highly recommended.

The first step in preparing the questionnaire is to define the subject matter. Write down what information we would like to collect. Dividing the questionnaire based on the different types of information targeted helps to find the structure. We identify three main sections in questionnaire used for collecting detection and non-detection data of the manul: (1) Information on the knowledge of the respondent: species biology (e.g., unique morphological characteristics), specific local names, and ability to correctly identify the species using photos provided; (2) Information on species occurrence: observation claims by the respondent, including the date and location of each event; and (3) Information on the respondent's attributes that can explain the socio-demographic characteristics that may have influenced the responses (e.g., age, occupation, past experience).

After defining different sections of the questionnaire, think about the variables that are to be answered in each section. For each variable, one specific question should be prepared. Keep the questions clear, specific, and short enough; too short a question is likely to result in a short and less informative answers (Newing et al. 2010). Simple "how", "what", and "why" questions will unlikely result in in-depth answers. For detailed answers, longer questions are required to make the point clear. At the same time, questions that are not specific enough or are lengthy and ambiguous will confuse the respondents and result in poor-quality answers. The wording of the questions is important because together with the way the questions are asked and the way that the interview is moderated by the interviewer, it influences the quality of the answers received. Coding can be used, as long as the criteria are simple, informative and interpretated similarly by members of the survey team. Otherwise, we recommend closed questions with closed checklists to be selected and answers that can later be coded for the analysis. This way, the original answer will always be available to be checked. If a rating scale is used (e.g., "how abundant is the manul in this area?"), a numerical scale that is easy to understand for the respondent can be used.

Number the questions and provide enough space for the answers, if check boxes are not used. We recommend always including a "don't know" option in the answers as this helps later in the analysis to differentiate lack of information from lack of data (i.e., when a specific question was not administrated for a given reason and should be considered as NA in the associated data set). We recommend to verbally ask the questions and the answers to be recorded by the interviewer. However, if the questionnaire is going to be self-administrated, the layout should be easy to follow for respondents with different educational backgrounds. The layout should also be prepared in a way that processing the data will be straightforward; think about how the data is going to be extracted. We suggest limiting the number of questions to one paper (i.e., two pages) and number each page (1/2 and 2/2) and print the interview number as the unique identifier on each page.

After drafting the first version of the questionnaire, check it with a small number of people to provide feedback. This step can be done by colleagues, or even friends and family. The important aspect is that the test person should come fresh to the questions so any ambiguities or other problems that might have been overlooked can be pointed out (Newing et al. 2010). After this step, the questionnaire should ideally be refined one more time (or piloted) with a small group of people from the target community under field conditions. However, field piloting may not be always achievable when the target community lives in difficult to access locations or when multiple trips to the study area are not possible. Nonetheless, at least one formal round of testing the questionnaire with people representing similar background or characteristics with the target respondents should always be considered. The information obtained at the end of this test has the aim of improving the actual interview and the resulting answers should not be included in the analysis.

Figure 4. Carrying good-quality photos of the manul, and multiple photos from different angles, helps to assess the knowledge of respondents and evaluate the reliability of their observation claims. Photos: A. Barashkova (top), E. Nygren (bottom)

Besides the questionnaire, additional material may be used to accompany the interview. For assessing the knowledge of the respondents, it is always good practice to carry multiple photos of the focal species. Prepare good-quality photos of the manul and co-occurring species that it may be mistaken with to be used during the interviews (Fig. 4). Using these photo-guides not only helps to assess the knowledge of the respondent (e.g., scoring their knowledge of the manul from high to low), but also usually increases their willingness to participate in the interview and opens interesting conversations. Use good-quality materials to print the photos to increase durability of the pictures under field conditions. A section of the questionnaire should be allocated to record the performance of the respondent in correctly identifying these photos. It is good practice to carry gift card and photos with the target species and give it to the respondents if they showed interest in having similar photos of the manul. Such gifts can have educational values but are unlikely to influence the outcome of the interview, especially when given at the end of the interview.

6.3.4. Conducting the interview

6.3.4.1. Interview types

Once the type of interview is decided and the questions are defined, the data can be collected through different methods, such as (1) face-to-face, or personal, interviews; (2) group-interviews, where several people from the target community are answering the questions as a group (Fig. 5); (3) self-administrated questionnaires, where the questionnaire is provided or sent to the respondent and the interviewer is not present during the answering; (4) telephone interviews; and (5) online surveys. We recommend personal or group interviews for assessing the manul distribution and occurrence probability. In our experience, this method generally provides greater participation, and it is also important for other aspects of a conservation-oriented study, such as local engagement, as well as the opportunity to gather additional information about the manul and its habitat (Young et al. 2018).

6.3.4.2. Initiating interviews

Select target locations for searching for potential respondents from the target community beforehand. Consulting with people who are familiar with the target community and area is always appreciated. Attend the interview in a professional yet friendly fashion. For example, in most situations it is better to not be accompanied by or appear to be representing governmental officials and staff (Fig. 6). Once the respondent is identified and approached, the interviewer should greet the person, introduce the interview team, and state the aim of the interview. The respondent should verbally consent to participate in the interview, after the goal of study and how the collected data will be used is explained in a language that is easy for the respondent to understand. If the interviewer's mother tongue is different from those of the respondents, an assistant who is familiar with the language of the respondents should be present. This assistant should have received training in not influencing the interview and act as a translator and if it is required, such assistance can be a local guide to facilitate building trust and improve the quality of the interview. Always thank the respondents for their participation in the study, even if they decide to leave or interrupt the interview. We do not recommend recording the interview as in many situations it may negatively influence the willingness of the respondents to participate. However, if required, first ask for their permission and assure their anonymity. If necessary, for example when sensitive information such as poaching activity is discussed, confidentially of the participants should be assured.

Figure 5. Group interviews can provide detailed and high-quality data as the information is extracted from a larger body of knowledge. However, conducting group interviews can be challenging because participants may have different perceptions of a question and contradictory opinions, and influence each other's responses (i.e., audience effect bias). Photos: F. Jafarzadeh

Figure 6. In most situations, it is important that the survey team does not appear as working for or associating with government agencies, as it may influence willingness of respondents to participate in the study and impact the quality of answers obtained. Use private vehicles commonly used by the locals and if it is not necessary, no government personnel should accompany the team during the interviews. Photos: N. Goreva (top), E. Moqanaki (bottom).

Try to create a friendly environment when conducting the interview. Balance the level of formality based on the situation and study questions. The interview location can greatly influence the atmosphere during the interview; a meeting at the office of an environmental agency leader is different from interviewing a group of farmers in a local café (Figs. 4-5). Familiarize yourself with the target community; relaxing socially to create a friendly environment does not mean to ignore the norms and cultural background of the target society. Embrace the fact that different societies have different cultures in what is acceptable. If working in a culture that is not your own, observing how people interact in everyday conversation helps to fit in with the cultural norms (Newing et al. 2010). However, always be professional and focus on your study. While we encourage listening to the respondents' stories that might not be relevant to the study, avoid engaging in any social controversies. If the questionnaire allows it, try to be flexible and be prepared to make decisions during the interview or change the direction of the interview. Depending on the design of the questionnaire, if an opening question leads to a very lengthy answer that is not relevant to the study objectives, the interviewer should respectfully change the direction of the interview as soon as possible. Overall, it depends on how much time we have, whether what the respondents are saying is relevant to the study, and whether we will have a chance to interview the respondents again if time runs out before all the planned points have been covered. It also depends on how long we can concentrate for and whether we can remain patient. Stop the interview at once if you are getting irritated. If you have all evening ahead of you and will be able to talk to the same person at another time, if necessary, then there can be an argument for just sitting back and listening, even if much of the material is irrelevant. In such situations, you will get to know each other, and they will probably be more open with you in subsequent meetings. However, if time is limited you may have to interrupt them politely and bring them back to the subject with a more specific question. A useful way to do this without causing offence is to say, for example, "that's very interesting, and I'd like to come back to it another time. But first, can I ask you ...". You can always make an appointment for an informal chat or second interview if necessary.

6.3.4.3. Conducting interviews

In face-to-face interviews, it is essential to treat the respondents with courtesy. Listen carefully, make eye contact (if culturally acceptable), and unless necessary, do not interrupt the respondent. The most important skill in conducting interviews is to encourage participants to open up and to talk about the things and with the level of detail we are interested in (Newing et al. 2010). It is good practice to start with more general and uncontroversial questions and increase the degree of complexity and controversy through the interview and make decisions based on the performance and willingness of the respondent. The interview usually becomes easier as it goes on. Thus, the interview should gradually narrow down to the subject area. Keep the personal questions (e.g., respondent's age, gender, education, socio-economic characteristics) to the end of the interview. Letting the interviewee tell a story about themselves or an interesting incident they had with local wildlife helps to put the respondent at ease and lets the conversation flow, so higher quality answers can be obtained later when asking about controversial subjects (Newing et al. 2010).

To collect detection and non-detection data of the manul, each observation claim by the respondent must have a date and location, in which geographical coordinates can be extracted. If grid cells are the spatial unit, it is often enough that the observation can be assigned to one of the pre-defined grid cells. However, it is good practice to extract the approximate geographical coordinates of each observation, in case the sampling unit would be modified for practical reasons during the analysis (e.g., when smaller sampling units are used in a hierarchical nested approach), or the data is going to be used in other analytical frameworks (e.g., ecological niche models that require fine-resolution occurrence data with high spatial accuracy; Elith and Leathwick 2009). The approximate location of manul detections can be found on local maps together with the respondent during the interview (Fig. 7). However, reading maps is not always easy and it is a time-consuming step that may affect the willingness of the respondents. Alternatively, a triangulation approach can be used where the respondent is asked to provide the approximate distance of the incident to several local landmarks (e.g., the respondent's village, a lake or spring, mountain, and forest). Then, the approximate location of the observation can be extracted by the survey team during data entry.

Figure 7. The spatial reference of the respondents is often based more on local names and landmarks than geographical areas on a map. We therefore recommend using a map with local names, such as human settlements and natural features. The coordinates can then be extracted using this spatial reference (top). Logistical constraints and time limitations may prevent using maps. As an alternative, the respondents can be asked to provide the approximate distance and direction of their knowledge area or specific observations. Photos: F. Jafarzadeh (top), E. Moqanaki (bottom)

For questions that require more in-depth answers, preface the questions with some additional comments or background information. Alternatively, several shorter questions can be asked about the same thing but worded differently. How to word the question will affect the results. For example, the question "do you think wolves belong in the mountains where you live" and "do you think wolves should not be part of the area in which you live" will likely give different results although they are aiming at the same subject. In a similar vein, avoid interviewer bias as much as possible (Fig. 8). This happens during the interview when the interviewer's expectations or opinions may somehow cause distortion of responses and affect the outcomes (Newing et al. 2010). An example relevant to this guide is when the respondent is pushed or specifically encouraged to provide records of the manul occurrence. Then, the respondent may provide poor-quality records or inaccurate answers to please the interviewer. Always remember we are interested in high-quality detection and non-detection data of the manul and not just more records of the species occurrence at any cost.

It can also be useful to evaluate the respondents' expertise as part of the interview by including questions in the questionnaire about their experience and familiarity with the area (e.g., years as shepherd in the survey area), and score their knowledge about the manul biology or wildlife in general using, for example, yes–no or true–false questions about the biology of the manul or the survey system at large (Crandall et al. 2018). Such information can be also incorporated in assessing detection in the analysis. The interviewer should not react to the respondents' answers, even if they are perceived to be wrong; do not correct the respondent even if they claims do not match with your knowledge of the manul and the study area. Instead, make comments and note in the questionnaire form as much as possible. Providing check boxes ensures standardization of the questionnaire but in cases where notes should be made, write in a manner that someone else can easily read the writing. When making many notes, even the interviewer may forget the context after several days and it is therefore good practice to extract the information from the questionnaires as soon as possible and it is better to be done by or in presence of the same person who conducted the interview.

During interviews, there is the potential to collect by-catch data on different aspects of the study system, such as co-occurring species presence and distribution, human-wildlife conflict, and socio-economic information of the target community. Although we value such by-catch data and they can be collected whenever it is possible, we recommend using a simple questionnaire with a series of short questions that is specifically designed for collecting manul presence and absence (or rather, detection and non-detection) data. We are specifically interested in eliciting the respondents' observations of the manul across the study landscape during the time of interest. Thus, when it comes to by-catch data, do not ask too much from the respondents as it may have a negative impact on the quality of the data obtained and affect the respondents' willingness to participate in the survey. It is good practice that the questionnaire allows for skipping the sections that are not crucial to the study based on the assessment of the interviewer during the interview. The interviewer should finish the interview when enough information has been collected, when the conversation begins to flag, or when either the interviewer or the interviewee is losing concentration.

Figure 8. It is important to respect the respondents and not to judge their answers. Likewise, the interviewer must have received training to deal with different situations that may occur in the field. For example, when asking sensitive questions or encountering activities that may be illegal (e.g., poaching) or differ from the opinion of the interviewer, it is important that the interviewer does not judge the answers to maintain and build trust, which is important for obtaining reliable results. Photos: R. Nefedov (top), F. Jafarzadeh (bottom)

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Photo: A. Barashkova

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