

APPENDIX: CASE STUDY

Assessing Distribution and Abundance of Three Small Felid Species in Royal Manas National Park, Bhutan

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Introduction and Study Objectives

Wild felids are among the most biologically threatened taxa on earth, and many species are believed to be threatened with extinction in their natural environment (Swanson 2005). Although it is a small country, Bhutan is home to 11 of the world's 36 felid species (Wang 2008, Wangchuk et al. 2006). Tigers (*Panthera tigris*), leopards (*Panthera pardus*), snow leopards (*Uncia uncia*), clouded leopards (*Neofelis nebulosa*), and leopard cats (*Prionailurus bengalensis*) are warranted full protection as Schedule 1(A) species in Bhutan (MoA and RGoB 2002). In contrast, Bhutan's six other felid species have not yet been granted conservation status due to lack of information on their distribution, abundance, and population trends.

This case study draws on many of the topics from this book to develop an example of how one might actually implement a scientific study of Bhutan's small felid species. The objectives of this example case study proposal are: 1) compare three non-invasive methods—remote camera trapping, scat collection (for genetic analysis), and hair collection (also for genetic analysis)—for surveying small felid species potentially differing in abundance, home range area, and habitat use; and 2) determine the distribution and abundance of three small felid species—leopard cats, marbled cats (*Pardofelis marmorata*), and golden cats (*Pardofelis temminckii*)—in Royal Manas National Park, Bhutan, where camera trapping recently confirmed their presence.

In the rest of this Appendix chapter, we will write as if this were an actual proposal to do this work, providing a “hands-on” example for how the topics of this book might be applied to conduct wildlife research in Bhutan. Objective 1 will be addressed in a pilot study on one intensive 5X5 km survey unit. In this pilot study we will use capture-mark-recapture techniques to determine abundance of the three small felid species, and compare probability of detection, species- and habitat-specific biases, and relative cost of each survey method for achieving study objectives. For Objective 2, we will use an occupancy modeling framework to determine probability of occupancy and proportion of area occupied across the landscape for each target species within the Park.

Study Area

Covering 1057 km², Royal Manas National Park is Bhutan's largest representative habitat of tropical and sub-tropical ecosystems (Figure A1). It is located in south central Bhutan and is bordered on the north by Jigme Singye Wangchuck National Park (1723 km²) and on the south

by India's Manas Tiger Reserve World (360 km²). RMNP's broad altitudinal range (129 m–2124 m above sea level) encompasses a diversity of ecosystems, including sub-tropical moist broadleaf forests, warm broadleaf forests, cool broadleaf forests, subtropical dry chir pine habitats, temperate meadows and grasslands, and freshwater and wetlands ecosystems. The Park is home to thousands of animal and plant species, many of which are globally endangered. Royal Manas National Park is not only the most biologically diverse protected area in the Kingdom of Bhutan, but is also a hotspot for global biodiversity (Tempa et al. 2013).

The Park is administratively divided into three blocks—Gomphu, Manas and Umling—to ensure effective delivery service to people residing within the Park. The blocking is not based on habitat types and each block has a separate staff for research with research priorities being the same in all blocks. The park is inaccessible during the summer months (July–September) due to heavy rains and frequent roadblocks.

Target species

This study will evaluate the distribution and abundance of three small felid species known to occur in Royal Manas National Park: the leopard cat, marbled cat, and golden cat. Information on habitat, home range, and behavior are provided in Table A1 and images of the species are provided in Figure A2. To date, there have been no studies on these cats within Bhutan.

Timeline

This study will establish guidelines for implementing non-invasive studies of small felid species in similar habitats of Bhutan and will generate baseline data for long-term monitoring of population trends for three small felid species in RMNP. Prior to implementing a pilot study, we will conduct reconnaissance and mapping of major foot and game trails and access points in RMNP. A comprehensive trail map will facilitate planning and implementation of the Park-wide occupancy survey and subsequent long-term monitoring. Potential target species scat and hair samples collected during reconnaissance will be used, in conjunction with known-species zoo samples, to optimize extraction and amplification of DNA for species (and potentially, individual) identification.

In Year 1, we will conduct a pilot study (Objective 1) to evaluate the effectiveness of three non-invasive survey techniques for achieving study objectives. The pilot study for abundance/density estimation will be implemented at one intensive minimum 5X5-km survey unit located in an area of high trail density within RMNP (see Figure A1 for suggestion). We suggest a spacing of 1 km between cameras but admit that survey size may need to increase to 1.5 km spacing if we do not achieve enough captures and recapture of different individuals. The pilot study will be conducted over two 3-month sessions, e.g., April–June (Session 1) and October–December (Session 2), with results compared between sessions to explore for seasonal differences in detections and abundance/density estimates. The October–December time frame is probably best due to the weather and hence the first survey should begin at this time.

We will use findings from the pilot study to inform design of the larger-scale occupancy survey, which will be implemented in Years 2 and 3. The occupancy survey will be conducted by RMNP

administrative block, beginning with the Manas block. In total, we plan to survey 35-40, 3X3-km units, 3-4 times each, distributed over the three administrative blocks over a 2-year period. Occupancy estimates from surveyed units will be combined with GIS landscape and field-collected habitat data to predict occurrence of target species across the Park.

Pending outcome of the initial 3-yr study, we suggest expanding, in Year 4, to conduct annual abundance/density surveys via remote camera traps for monitoring long-term population trends in the original (pilot) target block noted in Figure A1 and in at least 2 other blocks for comparison to the original survey block. These survey sites should be similar in size and will be conducted once per year. This long-term monitoring program will allow the accumulation of data that will enable estimation of trends in abundance through time (i.e., stable, increasing, or decreasing) and eventually allow for calculation of yearly survival rates for the felids. This information, currently unknown for these wild felid species, is important for assessing population status and health of the felids. Positive population trends and high survival rates could indicate healthy or persistent populations while negative population trends and poor survival would indicate a potential problem with long term species survival.

Used in combination with the occupancy surveys (see below), we can determine where across the park the small cats are present, and then, using our estimates of density from the long-term monitoring, extrapolate density estimate to estimate total number of felids on a park-wide basis.

Pilot Study

The pilot study will evaluate feasibility and cost-effectiveness of three detection methods (remote cameras, hair rub pads, and scat transects for DNA sample collection) for the Park-wide occupancy study. Specifically, for each survey method, pilot study results will be used to: 1) optimize survey protocols, e.g., determine appropriate spacing of camera and hair rub pad stations; 2) estimate target species 'capture' rates; 3) identify species-habitat relationships that may warrant habitat stratified sampling; 4) identify seasonal biases in species detection and occurrence; 5) calculate minimum cost-per-unit for occupancy detection; and 6) estimate minimum cost-per-unit for estimating abundance of common target species.

Remote camera trapping

We will follow standardized camera trapping protocols developed for other species of small cats such as ocelots (Dillon and Kelly 2007, 2008), Geoffrey's cats (Cuellar et al. 2006), and bobcats (Heilbrun 2006). Cameras will be placed in a grid-like formation with a spacing of 1-1.5 km between camera traps based on the small home range sizes of the leopard and marbled cats. This spacing should ensure no holes in the grid large enough for an entire home range and hence each individual should have a probability of being captured (Otis et al. 1978). We do note however, that this spacing may be too close together for the golden cat, which has a much larger home range and therefore our grid may not be large enough to accurately assess golden cat abundance (Maffei and Noss 2008).

We will use a minimum of 25 camera stations and each station will have 2 cameras mounted on opposite sides of a road or trail to photograph both flanks of the passing animal for positive ID.

Cameras will be placed in areas that are natural funnels (e.g. trails, roads, newly cut trails, etc.) at 20–30 cm in height and will be operative for 24 hours per day. Cameras will be checked for proper functioning, downloading images, and for battery and memory card levels approximately every 10 days. Stations will be operational for 70–90 days to ensure enough captures and recaptures for mark-recapture analysis.

Both leopard cats and marbled cats have unique coat patterns allowing for individual identification necessary for mark-recapture analysis. Golden cats have subtle markings that may allow estimation of abundance following the methods of Kelly et al. (2008). We will compile capture histories for each individual animal and analyze the mark-recapture data for each species in Program CAPTURE (Rexstad and Burnham 1991) to estimate abundance. We will also estimate density using the classic $\frac{1}{2}$ mean maximum distance moved ($\frac{1}{2}$ MMDM) method originally developed for small mammals (White et al 1985) and modified for tigers (Karanth 1995, Karanth and Nichols 1998). Finally, due to the recent development of spatially explicit models for analyzing camera-trap, mark-recapture data, we will also estimate density directly through Program DENSITY (Efford 2004; 2007). Both CAPTURE and DENSITY are available as free software downloads.

For species that are not individually marked (i.e., the golden cat or any prey species we are interested in), we will calculate trap success as the number of photo captures per trap night. A photo capture will consist of any distinct individual photo captured within a 30-minute time period. Trap success can be used as an indication of activity level at each particular camera station, and has been used as an index of abundance (O'Brien 2003) but this is controversial (Carbone et al. 2001, 2002, Janelle et al. 2002). At the very least trapping rates can identify areas in the study site with high versus low animal activity and animals “captured” can be used to compile a species inventory for the park (as in Tempa et al. 2013).

DNA collection

Hair rub pads

We will examine the effectiveness of hair rub pads for detecting target species. In the pilot study within the remote camera trapping grid, we will place rub pad sets along surveyed game and foot trails, halfway between camera sites. A rub pad station will consist of 4 rub pads spaced along the trail at 10 m intervals. Rub pads will be nailed to trees at target species' shoulder height, with visual attractants (pie pans) overhead. A detailed description of hair rub pad construction, set-up, and choice of scented lures can be found in Long et al. (2008). Rub pads will be checked and rebaited every 10 days while checking camera traps for proper functioning. If rub pads prove to be cost effective for detecting target species, their use as a survey tool of the Park's small felid species will be expanded in the Park-wide occupancy study.

Scat collection

Scat collection will be conducted on foot during the checking of the camera stations and hair rub pads within the pilot study unit. In addition, researchers will explore other likely felid movement corridors such as game trails, ridge lines, and riverbanks. In all cases researchers will note type of trail, weather conditions, scat color, scat degradation category, presence of mold, etc., following Wultsch (2009) (and this book Chapter 3). Researchers will record distance

travelled on scat transects to assess the efficiency of scat collection techniques (scats collected per km walked)—much like a photographic trap success rate, which can also be used later to assess activity levels and potentially relative abundance.

Scat samples are known to degrade due to environmental factors. Data collected regarding scat quality can be compared to amplification success to determine how to identify high quality scat samples in the field for more efficient data collection and analysis. If this approach proves difficult or inefficient, an alternative is to pre-clear scat transects, then resurvey transects at a later date (e.g., 10 days after clearing, concurrent with checking camera and hair rub pad stations) to collect newly deposited scats.

Appropriate sampling duration (i.e., time between clearing a transect and resurveying for fresh scats) depends on 'capture' rate, DNA degradation rate, and logistical issues of site access and coordination with concurrent survey methods. A longer sampling duration may increase sample size, but may reduce DNA quality. DNA degradation rate may vary with season, with faster degradation during warm, wet seasons. If a sampling duration of 10 days yields low genotype success (e.g., successful DNA amplification from <70% of collected scats), a maximum sampling duration for scat transects can be determined by clearing felid scat from transects, and resurveying transects every 2 to 3 days for several weeks. When a new felid scat is found, the date of first discovery should be recorded and a portion of the scat collected during each subsequent survey until no sample remains. Thus, each scat will yield samples of various ages (i.e., exposure to DNA-degrading field conditions) for estimating season-specific DNA degradation rates. These data can be used to determine the relationship between number of days passed and number of new target felid scats deposited per km transect, and DNA amplification success as a function of sample age.

Individual identification

The DNA from both the scat and hair collection techniques can be used to identify individuals through microsatellite analysis, but identifying individuals from genetic samples is more costly and time-intensive than identifying species (Chapter 3). If funding permits individual identification, mark-recapture statistics can be used following similar methodology as for remote camera trapping (see above and previous chapter this book) to estimate abundance and density within the pilot camera trapping grid. This would provide tremendous insight into which technique is most efficient and economical for abundance estimation.

Habitat assessment

For the three methods described above, capture rates can be linked to specific habitat variables collected at the camera station level (or for scat, within a specified radius of scat locations) to enhance understanding of habitat features that influence animal presence and activity across the grid (Davis et al. 2011). Therefore, we will collect habitat data surrounding each station/scat location following Davis (2009) and Davis et al. (2011). Please see Chapter 4 for habitat data collection protocol surrounding camera stations. Similar measures can be taken surrounding each scat sample. In addition, if detailed GIS maps are available, habitat variables can be extracted from GIS layers in circular buffers surrounding camera traps or scats following Holub and Kelly (2008) to further examine relationships between capture rates and landscape

features. If the pilot study identifies some target species as habitat specialists, subsequent surveys will use a stratified sampling design for detection surveys.

Occupancy Survey

After completion of the pilot study, we will expand our approach through the use of detection/non-detection surveys to predict occupancy of target felid species across the entire Park (see Chapter 2). We will use a combination of all three types of detection methods for this study. Typically, repeated visits to a site are used to create a detection history for each site (like a capture history for each individual animal in mark-recapture) and to estimate detection probability, site occupancy, proportion of area occupied, and to model the covariates that influence occupancy. Occupancy data can be analyzed in the free software Program PRESENCE (MacKenzie et al. 2006).

For our study, we have placed a 3X3 km grid across the entire RMNP (Figure A3). We suggest surveying a random (or stratified random) subset (30 to 40) of these cells. The final sampling protocol for the occupancy survey, particularly the most cost-efficient combination of methods and their implementation (number and distribution of stations, sampling duration, etc.), will be based on results of the pilot study. For example, we may use 5 remote cameras for 2 weeks in each grid cell placed in likely locations for our target species. During initial set-up of camera stations and the follow-up site visit to retrieve photos we may survey a minimum of 5 km of transects for scat collection within each grid cell. If a particular target species proves elusive to camera trapping and scat surveys but effectively detected by hair rub pads, we may distribute hair rub pads in habitat types frequented by that species. Each cell will be surveyed a minimum of three times to create a detection/non-detection history for each grid cell.

For each searched grid cell we will extract GIS information on important habitat and landscape features to be used as covariates in predicting occupancy across the landscape. Such covariates may include slope and elevation (ruggedness), habitat type, % available water, distance to nearest road, road density, distance to nearest village, human use pattern, etc. In this way, these patch-occupancy models (MacKenzie et al. 2006) will allow us to use detection/non-detection surveys, combined with spatial modeling, to estimate and predict species occurrence across a landscape. As an example, Linkie et al. (2006) conducted repeated sign surveys for tigers (tracks and scat) in Sumatra, combined with data layers from GIS, to model tiger presence and predict probability of occurrence across the landscape. They found that tiger occurrence was predominantly influenced by distance to public roads, and identified four core areas for tigers.

Anticipated Results

We anticipate estimating abundance and density for the leopard cat, marbled cat, and possibly the golden cat. We will also provide a species inventory for all species captured via remote camera photographs providing a baseline of information on predators and prey species. We will assess factors such as optimal trap spacing and optimal combination of detection devices resulting in the largest number of species detections. Habitat data collection surrounding each camera trap station will be used to model the factors that influence trap success giving us valuable information on habitat preferences. In addition, trap success of predators relative to

other predators, prey, and humans will give us insight into other biological factors influencing target species activity rates within the camera grid.

We also anticipate completing a feasibility study designed to evaluate the cost-efficiency of the three survey methods for achieving project objectives. Criteria for comparison will include (scaled to method-specific costs): 1) number of each target species detected as a function of survey duration; 2) time to first detection of each target species; 3) proportion of each target species detections accounted for by each device type; and 4) rate of trap "failure".

After the initial pilot study, expansion of trapping grids and stratifying by habitat will allow determination of whether abundance/density of target species varies by habitat type. Occupancy surveys will result in estimates of detectability, occupancy rates, and proportion of area occupied. We will assess species occurrence over an entire landscape through sampling only a portion of that landscape. And finally we will determine the landscape factors most important in determining occupancy over a broad scale, giving us tremendous insight into the ecology of the target species.

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Table A1. Three small felid species surveyed in this case study.

Target Species	Habitat	Home Range	Distinctive Markings	Behavior	Additional Information
Leopard cat (<i>Prionailurus bengalensis</i>) ^{1,2,3}	Occurs in a broad spectrum of habitats, from tropical rain forest to temperate broadleaf and coniferous forest, and shrub and grasslands.	4.8-5.1 km ²	Patterned face and body	Solitary; Primarily nocturnal; hunts in trees and on ground.	Opportunistic hunter. Preys on hares, rodents, reptiles, moles, insects, amphibians, game birds, fish, mouse deer, eggs, domestic poultry, and aquatic prey.
Marbled cat (<i>Pardofelis marmorata</i>) ^{3, 4}	Moist tropical forests.	5.3km ²	Patterned face and body. It looks like clouded leopards but smaller in size.	Believed to be purely arboreal and primarily nocturnal.	Rarely seen in wild. Primarily preys on birds, but also eats squirrels, rats and other rodents, lizards, insects and frogs. Hunts mostly in trees. Status: CITES Appendix I
Golden cat (<i>Pardofelis temminckii</i>) ^{3, 4}	Tropical and subtropical moist evergreen and dry deciduous forests up to 3000m elevation; occasionally seen in more open habitats.	32.6-47.7km ²	Facial stripes; body is lightly patterned.	Solitary; believed to breed in tree hollows.	Primarily preys on rodents, small deer, reptiles, birds and amphibians. Status: CITES Appendix I

¹ Austin et al., 2007; ² Izawa et al., 2009; ³ Wangchuk et al., 2006; ⁴ Grassman Jr. et al., 2005

Table A2. Some considerations for applying remote camera trapping and genetic methods in surveys of species distribution and abundance.

	Remote Camera Trapping	Non-Invasive Genetics	Combination Camera and Genetics
RESEARCH OBJECTIVES	Presence/distribution/occupancy for target "un-marked" species and abundance/density for target individually marked species	Presence/distribution/occupancy and abundance can be determined for all target species.	Increase the detection rates for presence/distribution/occupancy. Allow comparative analysis of photographic vs. genetic mark-recapture
PRIMARY ADVANTAGES	Set up is relatively easy. Can gain distributional information on many species and can simultaneously target several carnivores for abundance estimation.	Can get individual ID. Field effort relatively low and data can be collected in one or a few site visits (field component of study can be completed quickly), depending on question.	Can better estimate probability of detection for the method (which will differ by species & habitat).
PRIMARY DISADVANTAGES / CAVEATS	Up-front cost for purchasing cameras can be high; requires maintenance, so may not be applicable for remote sites	Laboratory costs can be high, especially if ability to identify species scat or target species for hair snares is low (i.e., end up collecting many non-target species scat and hairs). Difficult to apply in scat-genetics in wet climates/habitats (high rate of DNA degradation). Closure issues for scat collection—not knowing how old the scat is.	Need to ensure additional cost/effort of applying both methods is outweighed by the benefits over using just one method.
SPECIES	Especially useful for carnivores because they readily use trails. More powerful for species with distinct coat patterns allowing for individual identification and subsequent abundance and density estimation.	Most applicable to species that deposit scat along trails or in easy-to-find "latrines", or species that will rub on baited hair snares. For species with little prior genetic data, additional time/expense/expertise will be necessary to identify species-specific primers.	Pilot studies—it is unclear which method may have higher detectability when you do not know much about the species. Using both approaches can increase detectability for a suite of species and evaluate most efficient method for each species.

	Remote Camera Trapping	Non-Invasive Genetics	Combination Camera and Genetics
HABITAT / TERRAIN	Especially useful in forested habitat types where trail provide natural “funnels” for animals	Can be applied at any sites that can be accessed by foot, where scat may be found relatively easily	Certain habitats may be more accessible by one technique vs. the other increasing detection probability through the use of both.
CLIMATE / SURVEY SEASON	Year-round, but remote cameras function better in dryer conditions. Providing cover and protection for cameras can mitigate this problem.	Scat stays viable in field longer in cold, dry climates. Degradation rate varies by habitat—field samples may be “good” for anywhere from a few hours to several months after deposition	Both techniques are easier and more efficient in dryer conditions.
SITE ACCESSIBILITY / CONDITIONS	Can be applied anywhere that can be accessed by foot. If no trails exist, new trails can be cut through forest and animals will come to use them relatively quickly, increasing capture probability.	Can be applied at any sites that can be accessed by foot, where scat may be found relatively easily or hair snares can be attached. For hair snares, would need to visit sites at least twice; for scat sampling, can visit sites just once, depending on the question.	Certain sites may be more accessible by one technique vs. the other increasing detection probability through the use of both.
PROJECT BUDGET	Initial camera costs are high and high quality batteries are always needed.	Laboratory costs can be high, especially for individual identification; species identification is cheaper.	Costs for the field team can be shared by both techniques.
AVAILABLE MANPOWER & SKILLSET	Manpower can be periodically high when attempting to check all camera stations in a short time. Skills in trouble shooting electronic equipment and minor programming of cameras are needed. Data entry and analysis is substantial.	Low manpower requirements for field work; need a trained laboratory technician (or send samples to a contract laboratory)	Can conduct both techniques simultaneously with the same team trained in both scat searching and camera set-up/checking techniques.
SPECIAL EQUIPMENT /RESOURCES	Remote cameras, memory cards (at least 2 per camera), batteries, card reader.	Access to genetics laboratory	Will need both a genetics lab and a computer lab (or laptop computer at a minimum) for data analysis.

	Remote Camera Trapping	Non-Invasive Genetics	Combination Camera and Genetics
SAMPLE / DATA STORAGE REQUIREMENTS	Card reader to download photographs from memory cards. Laptop, desktop or external hard drive needed to store large number (thousands) of photographs.	In dry climates, can air dry samples in paper bags but best to store samples in silica dessicant until they can be frozen in -20C freezer. Best to extract DNA from samples soon after sample collection. Extracted DNA can be stored long-term (but needs to be in -20C freezer?) Need to be careful about sample cross-contamination in field & laboratory.	Will need large area to store scat samples especially if scats will later be used for diet analysis. For photographic data a large amount of computer storage space will be needed for photographs.
STUDY DURATION	Approximately 3 months per abundance/density survey to avoid violations of closed population assumptions. Variable duration for occupancy surveys.	Need to avoid violations of closed population assumptions.	Can conduct both studies simultaneously.
ADDITIONAL USES FOR DATA	Photographs are excellent for garnering public interest / conservation marketing; can obtain data on other species in area (with minimal additional cost/labor). Repeated surveys over time at the same sites can provide survival, recruitment, population growth rates, etc.	Can be used in studies of inbreeding, gene flow (connectivity)—incorporated into larger-scale genetic studies	Combining demography from long term camera studies with gene flow and connectivity from genetic study can provide a much more complete picture of overall population status, trends and health.

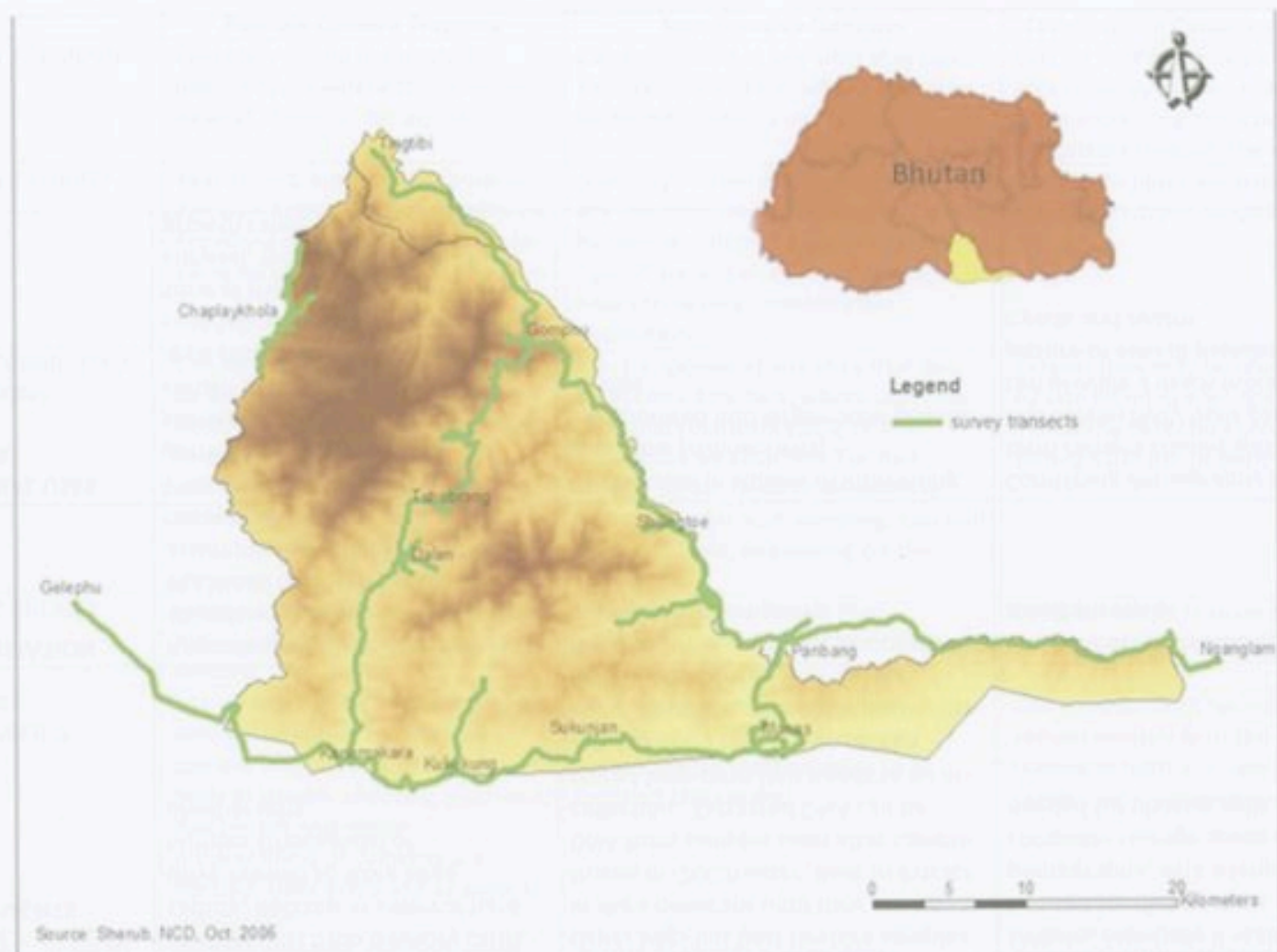


Figure A1. Royal Manas National Park. The black box (5X5 km) denotes the suggested location of the pilot camera trap grid centered on a network of trails. The suggested distance between traps of 1 km will result in a grid of 25 camera stations with 2 cameras each for 50 cameras total.

a)



b)



c)



Figure A2. Target species for this study; a) leopard cat b) golden cat and c) marbled cat. Photos courtesy of RMNP/NCD/WWF 2009.

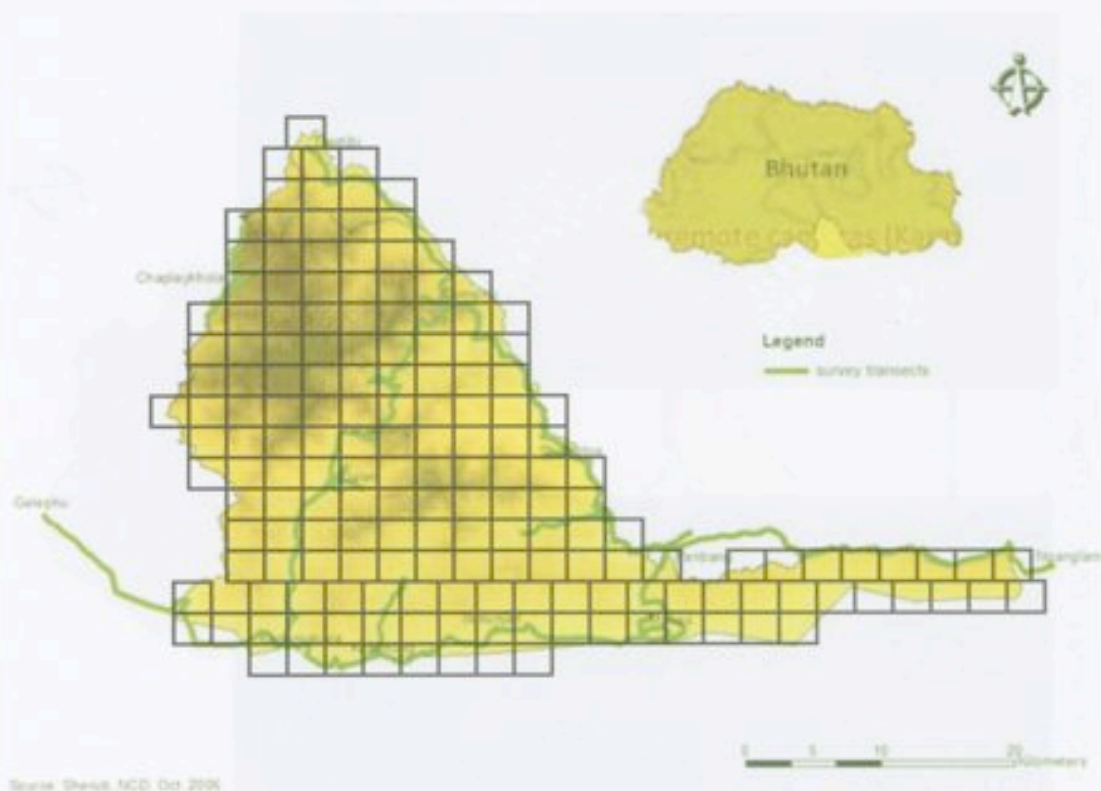


Figure A3. A hypothetical example of an occupancy estimation grid placed across RMNP. Each of the 174 cells is 3 X 3 km. A random (or stratified random) subset of cells can be searched for sign (scats, tracks, hair, and with remote cameras) a minimum of 3 times to create a detection history for each searched cell. This information can be used to predict occupancy across the entire park for the unsearched cells based on the habitat and landscape factors that most influence occupancy for the target small felid species.