Original Research Article

Estimating snow leopard density using fecal DNA in a large landscape in north-central Nepal

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ABSTRACT

Although abundance estimates have a strong bearing on the conservation status of a species, less than 2% of the global snow leopard distribution range has been sampled systematically, mostly in small survey areas. In order to estimate snow leopard density across a large landscape, we collected 347 putative snow leopard scats from 246 transects (490 km) in twenty-six 5×5km sized sampling grid cells within 4393 km^2 in Annapurna-Manaslu, Nepal. From 182 confirmed snow leopard scats, 81 were identified as belonging to 34 individuals; the remaining were discarded for their low (<0.625) quality index. Using maximum likelihood based spatial capture recapture analysis, we developed candidate model sets to test effects of various covariates on density and detection of scats on transects. The best models described the variation in density as a quadratic function of elevation and detection as a linear function of topography. The average density estimate of snow leopards for the area of interest within Nepal was 0.95 (SE 0.19) animals per 100 km^2 (0.66−1.41 95% CL) with predicted densities varying between 0.1 and 1.9 in different parts, thus highlighting the heterogeneity in densities as a function of habitat types. Our density estimate was low compared to previous estimates from smaller study areas. Probably, estimates from some of these areas were inflated due to locally high abundances in overlap zones (hotspots) of neighboring individuals, whose territories probably range far beyond study area borders. Our results highlight the need for a large-scale approach in snow leopard monitoring, and we recommend that methodological problems related to spatial scale are taken into account in future snow leopard research.

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1. Introduction

Snow leopards *Panthera uncia* are sparsely distributed over the mountainous regions of twelve countries in Central Asia (McCarthy et al., 2016). Although widely distributed, world-wide population numbers are believed to be quite low. Main threats to the species are retaliatory killing due to livestock loss, poaching, and degradation of habitat due to developmental

activities (e.g. mining, dams, roads, railway lines etc.) and global climate change (Maheshwari and Niraj, 2018; McCarthy et al., 2017). The snow leopard is listed as vulnerable in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (McCarthy et al., 2017). It is also listed in Appendix I of the Convention of International Trade in Endangered Species (CITES).

The global snow leopard population size is estimated to count between 4678 and 8745 individuals with a mean density of 0.9−1.8 leopards per 100 km^2 (McCarthy et al., 2016). However, this estimate may be inaccurate, as the remote and rugged mountain habitat of the species have rendered population surveys challenging. To date, less than 2% of the global snow leopard distribution range has been sampled systematically, and surveys have often focused on prime habitats. The Bishkek Declaration 2017 and the Kathmandu Resolution 2017, both endorsed by governments of all the 12 snow leopard range countries, identify the need to improve global snow leopard population estimates (GSLEP, 2018). A collaborative initiative, the Population Assessment of the World's Snow Leopards (PAWS), is currently being implemented by various organizations and government agencies across the snow leopard range.

Sign surveys using Snow Leopard Information Management System (SLIMS) have been widely used to estimate snow leopard abundance and distribution (Jackson et al., 2006). However, sign surveys alone were deemed unfit to derive quantitative population estimates (McCarthy et al., 2008). The development of capture-recapture analyses based on identification of individuals within a population was a marked improvement (Karanth et al., 2006), and non-invasive sampling methods including camera trapping and genetic sampling by DNA analysis of scats are now widely applied (e.g. Ale et al., 2014; Jackson et al., 2006; Janečka et al., 2011; Sharma et al., 2014; Waits and Paetkau, 2005; Wegge et al., 2012). Obtaining DNA from scats is particularly well-suited for snow leopards as they are extremely elusive, inhabit dry habitats where scats are often well preserved, and have reasonably predictable marking sites (Jackson and Ahlborn, 1989). However, although non-invasive scat sampling may enable large-scale distribution and abundance surveys of felids at low costs, identifying individuals from DNA samples is sometimes challenging due to poor DNA quality (Janečka et al., 2011).

Nepal plays an important role in snow leopard conservation by providing connectivity between populations in India, China and Bhutan. High snow leopard densities were estimated in different parts of this country based on surveys of sign (scats and scrape marks) and radio telemetry data: a) 5−10 snow leopards/100 km^2 in Langu Valley, Dolpa (Jackson and Ahlborn, 1989) and b) 4.8−6.7 snow leopards/100 km^2 in Manang of Annapurna Conservation Area (Oli, 1997). Similarly, relatively high densities were later reported from studies using camera trapping and DNA analysis of scats (DNPWC, 2017). However, the previous studies were conducted in relatively small areas, i.e. smaller than the average home range size of GPS collared snow leopards in Mongolia (Minimum Convex Polygons 95% = 503 km^2 ± 286 SD, Local convex hull 95% = 179 km^2 ± 80 SD, Johansson et al., 2016), thus compromising the validity of the conventional capture-recapture methods that were used. Conventional capture-recapture analysis typically requires that sampling is conducted in areas that are much larger than the average home range size to prevent positive bias and violation of the assumption of geographic closure (Foster and Harmsen, 2012). In order to obtain a snow leopard density estimate on a larger spatial scale, we sampled DNA from scats across a large area of more than 4000 km^2 in the Annapurna-Manaslu Landscape in Nepal. Using recently developed spatially explicit models, we investigated how heterogeneity in snow leopard density and detectability across space was influenced by habitat structure, elevation and topography.

2. Methods

2.1. Study area

The study area in the Annapurna-Manaslu landscape (28−29°N, 83−85°E) falls in the rain shadow of the Trans and semi-Trans Himalayas, Nepal. The northern part of the landscape adjoins the vast Tibetan Plateau (Fig. 1). The major proportion falls within the two conservation areas - the Annapurna Conservation Area (ACA) and the Manaslu Conservation Area (MCA); a smaller proportion is located in the Bhimthang valley between these two protected areas. We defined the borders of our study area by delineating a minimum convex polygon around all scat sampling transects (Fig. 1), except in the northern section, where we used the Nepal-China international boundary. The size of the total area covered by the polygon was 4661 km^2. However, the habitats of snow leopards lie above the tree line at an elevation ranging from 3000 to 6000 m (Snow Leopard Network, 2014). Hence, we removed the area that was either above or below this elevation range, after which the size of the area of potential snow leopard habitat was recalculated to 4393 km^2. Climate in the region is highly seasonal, and the area harbors several important species of global significance. Examples are Himalayan wolves (*Canis lupus chanco*), brown bear (*Ursus arctos*), Tibetan sand fox (*Vulpes ferrilata*), Eurasian lynx and several species of weasel (*Mustela* spp.) and marten (*Martes* spp.). Important ungulates species include bharal (*Pseudois nayaur*), Himalayan tahr (*Hemitragus jemlahicus*), Tibetan argali (*Ovis ammon hogdsoni*), kiang (*Equus kiang*), alpine musk deer (*Moschus chryogaster)* and Tibetan gazelle (*Procapra picticaudata*). The landscape is inhabited by agro-pastoralists, and accessible areas are used for livestock grazing. A detailed description of the study area is given in Chetri et al. (2017).

2.2. Field sampling

We sampled scats mainly within 26 grid cells of 5 × 5 km that were distributed across the landscape with 5−10 km between nearest neighboring cells. This represented about 15% of the total study area. However, we also collected scats along
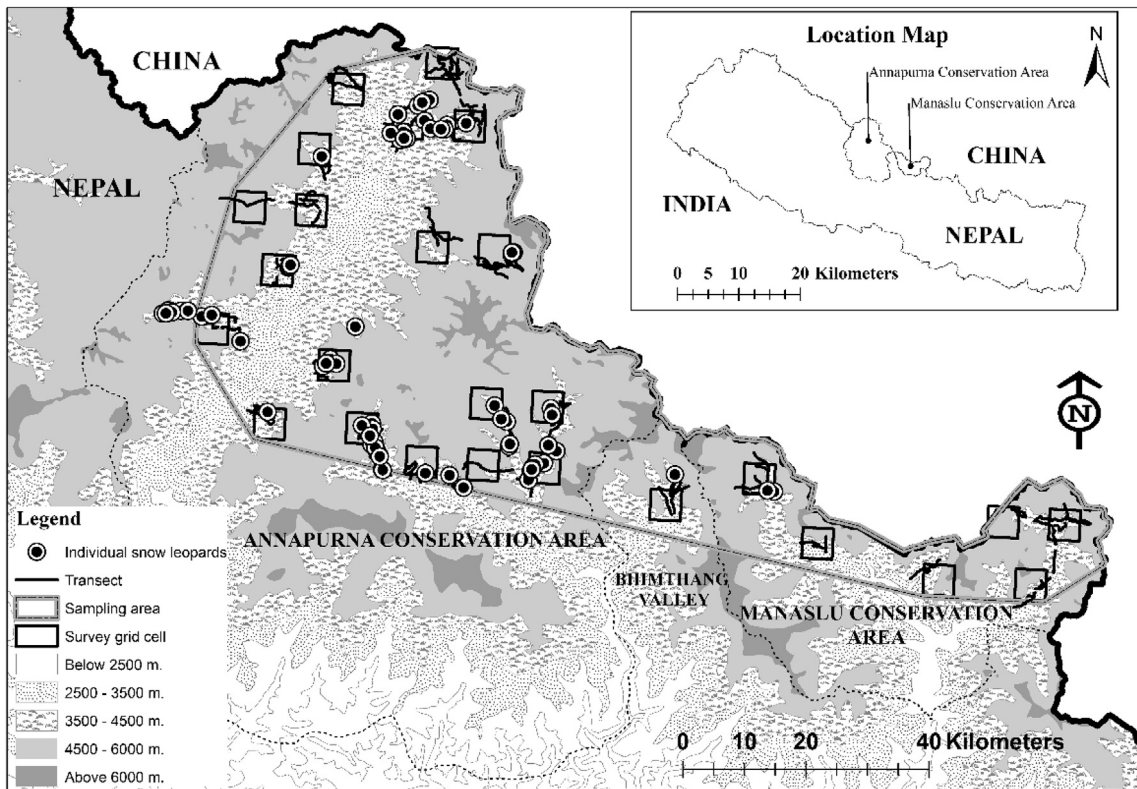
Fig. 1. Study area with location of grid cells, transects and genetically verified individual snow leopards.

trails connecting the cells and along some trails extending beyond grid cell borders (Fig. 1). We avoided placing grid cells in areas that were inaccessible due to high elevation or ruggedness, areas falling in and around larger settlements, areas with cultural restrictions and near main trekking trails and roads. Details concerning sampling design were described by Chetri et al. (2017). All the selected cells were located within the distribution range of snow leopards (McCarthy et al., 2005; Oli, 1997). Transects were positioned along trails, mountain ridges, river beds and mountain passes (Jackson and Hunter, 1996). Selected transect locations covered all the accessible snow leopard habitat types and available habitat features in the region. We broadly classified each transect into four main habitat categories: i) grassland-dominated by *Carex* and *Kobresia* spp. ii) scrubland-dominated by *Caragana* and *Rosa* spp. and sometimes mixed with Juniper scrub iii) mixed scrubland-grassland, and iv) barren land (loose soil, gravel or boulders with scarce vegetation). We also classified transects locations into four main topographic categories: i) open livestock trails (in open landscapes encompassing grassland, scrubland, mixed scrubland and alpine meadows); ii) rugged livestock trails (passing through broken cliffs), iii) dry riverbeds and iv) ridgelines.

Once a putative snow leopard scat was encountered, a small portion of the outer dried layer was extracted using twigs or sharp-edged grits and then preserved in a plastic tube with silica desiccant for DNA fecal analysis (Janečka et al., 2008). Altogether, 573 putative snow leopard scats were collected throughout the survey period from November 2013 to September 2014. At each scat location, we left nearly half of the scat in order to avoid influencing the territorial marking behavior of the snow leopards (Lovari et al., 2009). We revisited 108 of these sites after an interval of 205 ± 10 days (SD) in order to assess scat degradation over time. All but one scat had disappeared during this period, thus suggesting that most of the collected scats were less than ca 6 months old during the time of collection. Therefore, although the sampling extended to nearly a year, evidently none of the scats encountered during the transect surveys were likely to be more than a few months old. The effect of possible violation of closure of populations can be assumed to be minimal, considering that the survey was conducted across a very large area. Assuming the overall population did not undergo substantial changes during a particular season, the overall density represents average density across the period.

2.3. Molecular genetic analysis

The collected samples were submitted to the Center for Molecular Dynamics Nepal for individual identification. Due to economic limitations, the samples were screened before submission to the laboratory as described by Chetri et al. (2017). The

screened scat samples (n = 347) were analyzed to identify species following the methods described by Karmacharya et al. (2011). In total, 182 (52.4%) samples were successfully verified as snow leopard scats (Chetri et al., 2017).

The verified scats were analyzed further for individual identification using a panel of six microsatellite markers specifically designed for snow leopard (Janečka et al., 2008). Two multiplex panels of PCR amplification were used, i.e. (1) PUN124, PUN229, PUN1157 and (2) PUN132, PUN894, PUN935, in 10 μl reactions with PCR conditions and profiles described in Karmacharya et al. (2011). All genotyping reactions were run in three independent replicates. Three identical homozygote profiles or two identical heterozygote profiles were required for acceptance of single-locus genotypes, which is in accordance with Janečka et al. (2008). As an additional quality control, we calculated the quality index (QI) described by Miquel et al. (2006) for all samples. Following the recommendations of Miquel et al. (2006), we discarded all samples with a quality index of <0.625 from the data set. We also discarded all samples with less than five successfully genotyped loci.

Of the 182 verified snow leopard samples, 86 samples had one or more locus missing. In total, 81 samples gave reliable results according to the above-mentioned criteria. The quality of these 81 samples was high with an average QI of 0.92, ranging from 0.73 to 1.00. Accordingly, the genotyping error rate was low with an average allelic drop-out rate across samples and loci of 2.9%. The presence of false alleles was even lower and was detected only 6 times (<0.5%). Based on the observed allele frequencies (Supplementary data, Appendix S1) of the 34 different snow leopard individuals that were identified in the study area, the probability of identity (PI) were estimated to 3.3×10^{-5} for unrelated individuals and 1.5×10^{-2} for siblings. Siblings could be distinguished with >95% probability from only four loci (PI = 3.6×10^{-2}). Among the 34 identified snow leopards, 20 pairs of individuals (3.6% in a matrix of 561 possible pairs) showed genotypes that differed by less than three alleles; 4 pairs with one mismatch and 16 pairs with two mismatches. Altogether, these figures strongly suggest that different individuals could be distinguished reliably in the study area (Waits et al., 2001).

2.4. Data preparation

Spatial capture recapture analyses require data about where identified individuals were located during the sampling period. While the capture file provides information about the captures and recaptures of identified individuals, the trap file contains information about sampling effort and locations of traps. In our case, since captures were made in the form of genetic samples collected on transects, we used data input formats for transects as recommended by Efford (2018). Each capture was listed with information about the individual ID of the snow leopard, along with coordinates that corresponded with the coordinates of the transect line. We plotted GPS track data of each transect in ArcGIS version 10.3 and calculated the length of each surveyed transect. The trap file was created by listing each transect as a set of two or more vertices. Each vertex or location where a scat was located was listed as a new row on the trap file. Transect length in meters was included as effort for each transect (see Supplementary data, Appendix S2-S3). Snow leopards are known to use habitat non-uniformly within their home ranges, making the probability of capturing them (in this case their scats) heterogeneous. Accordingly, we included detection covariates (e.g. topography and habitat) in the trap file that were likely to affect the probability of snow leopards depositing scats and that of the researchers detecting them (see above). For covariates affecting density, we used elevation from the 30 × 30 m Digital Elevation Model from Aster Global Digital Elevation Model data, and estimated terrain ruggedness index from the elevation data using the terrain analysis plugin in the Quantum GIS 2.14 software. No scats were collected for analysis from outside the surveyed transects.

3. Data analysis

We used the package SECR (Efford, 2018) in R to estimate density using maximum likelihood based spatially explicit capture recapture models. SECR combines a state model and an observation model where the former describes the distribution of animals' activity ranges in the area of interest, and the latter relates the probability of detecting an individual at a particular location to the distance of the detector from the center of an animal's activity range. The distribution of activity centers in the population are treated as homogeneous Poisson or inhomogeneous Poisson distribution, depending on the need to evaluate effects of habitat types on density (Borchers and Efford, 2008). The detection model describes the decline in detection probability with distance from the activity center. The integration space (also known as mask) represents habitat in the vicinity of traps that is potentially occupied by the species of interest, and is close enough that animals occupying it may be detected on the traps. This region can sometimes be defined by hard boundaries such as fences, high ridgelines, etc. The integration space (mask) should be large enough that no animal with an activity center outside it could be detected by a camera trap.

The mask boundary in our case was defined by a polygon drawn with a buffer of 20 km from all transects that were sampled to collect genetic data. Additionally, hard boundaries were defined in areas that were impenetrable due to fences or geographic features such as high ridgelines. The assumption was that animals with activity centers beyond the mask boundaries would have negligible probability of visiting and depositing scats in transects that were sampled. We used a pixel size of 1 km × 1 km to estimate the density distribution of activity centers of individual ranges of snow leopards within the entire mask. We excluded areas above 6000 m and below 3000 m above sea level (Snow Leopard Network, 2014). These areas represented glaciers and forested habitats that are not used by snow leopards, and hence there was a negligible probability of snow leopard activity centers being located there.

Multiple models were compared to estimate density and detection probability as functions of ecologically meaningful covariates. For detection probability, we parameterized the expected encounter rate at distance zero from the activity center ($\lambda 0$). We tested the effect of topography and elevation on the encounter rate at the activity center, and we assumed that the average ranging parameter (σ) was robust across the sample size of encountered snow leopards and the study area. A set of 9 candidate models were run with density as functions of elevation and ruggedness. Effects of covariates such as elevation, topography and habitat type on detection probability were also tested (see Table 2). Each candidate model represented a specific hypothesis examining the relationship between snow leopard density, detection and explanatory variables. We used the Akaike Information Criteria (AIC) for model selection (Burnham and Anderson, 2002). All SECR analyses were implemented using a multi-core computer to facilitate parallel processing and hence speed up the analysis from a mask that spread across 14,174 km^2. We used model-averaging of the density surfaces from the top models to estimate the most plausible abundance and density in the study area.

4. Results

We identified 34 different individuals from the fecal DNA extraction and analysis, of which 20 were males and 14 were females (Table 1). Only two different individuals were identified in the eastern section of the study area, i.e. Bhimtang valley and Manaslu, while 32 were in the western part, Annapurna. The total number of animals that were recaptured one or more times was 23. Among recaptured individuals, the maximum relocation distances averaged 6.3 km (SE = 1.0 km, min = 0.2 km, max = 16.2 km). The top model of snow leopard density had an AIC weight of 0.33, and the next two models were close contenders with AIC weights of 0.26 and 0.22 respectively. Ninety-five% of the AIC weights were distributed across five of the nine models (Table 2), and we therefore used model averaging to estimate snow leopard density and abundance in the entire landscape (Fig. 2).

As per the top model, the encounter rate at activity centers for the study duration was estimated to be 6.6×10^{-4} (95% CI 4.07×10^{-04}-1.07×10^{-3}). The ranging parameter sigma, also explained as half the radius of the activity range of the snow leopards during the sampling period, was estimated to be 3.82 km (95% CI 3.15km-4.63 km) for the top model and the mean density of snow leopards across the entire region (mask) was estimated to be 0.94 (95% CI 0.63–1.42) snow leopards per 100 km^2.

The total number of snow leopards estimated in the entire area of 14,174 km^2 included in the SECR analyses was 144 (SE = 28.2, 95% CL = 101.1–214.3). The model averaged density of snow leopards for this entire area was 0.95 animals per 100 km^2 (SE = 0.19, 95% CL = 0.66–1.41) with predicted densities varying between 0.1 and 1.9 snow leopards per 100 km^2 within the area (Fig. 2), thus highlighting the heterogeneity in densities as a function of habitat types (elevation and topography). Region-wise, the density in Annapurna was predicted to be 1.07 animals/100 km^2 (95% CL = 0.71–1.62) and 1.16 animals/100 km^2 (95% CL = 0.73–1.87) in Bhimthang-Manaslu. Density as the quadratic function of elevation scored as the top model, indicating that it increased with elevation until a certain threshold and declined for elevations further up. The second and third best models estimated density as a function of elevation, and as a constant respectively. The probability of detecting snow leopard scats was highest at dry riverbeds, followed by ridgelines and livestock trails.

5. Discussion

Our overall density estimate of 0.95 (SE 0.201) snow leopards per 100 km^2 is lower than most recorded estimates from snow leopard ranges (see e.g. (Alexander et al., 2015; Alexander et al., 2016; Chen et al., 2016; DoFPS, 2016; Jackson et al., 2006; Janečka et al., 2011; Kachel et al., 2017; McCarthy et al., 2008; Suryawanshi et al., 2017; WCNP, 2016). The lowest snow leopard density of 0.15 individuals/100 km^2 was reported from a camera trap survey in SaryChat, Krygyzstan (McCarthy et al., 2008). Another camera trap survey conducted by Jackson et al. (2006) estimated the highest density of 8.49/100 km^2 in Hemis National Park, Ladakh, India. Previous high density estimates could be an artifact from sampling in small study areas of very good snow leopard habitat. The ungulate prey density in our landscape (Chetri et al., 2017) was relatively high compared to other study areas where both snow leopard and prey densities have been recorded (Kachel et al., 2017; McCarthy et al., 2008; Suryawanshi et al., 2017). The variation in predicted snow leopard density of more than one order of magnitude

Table 1
Number of individuals, sex and capture rates of snow leopards identified by fecal DNA analysis of scats collected in the central Himalayas, Nepal.

Number of individuals	Sex		Number of captures
	Male	Female	
11	8	3	1
13	7	6	2
5	1	4	3
2	2	0	5
2	1	1	6
1	1	0	7

Table 2
Candidate spatial capture recapture models of snow leopard density based on fecal DNA collected in the central Himalayas, Nepal.

Model	npar	Log Likelihood	AIC	Delta AIC	AIC weight
D ~ StdElv + I(StdElv2)lambda0~Topographysigmā1	8	−955.955	1927.910	0	0.330
D ~ StdElvlambda0~Topographysigmā1	7	−957.195	1928.389	0.479	0.260
D ~ 1lambda0~Topographysigmā1	6	−958.341	1928.681	0.771	0.225
D ~ StdRgd + StdElvlambda0~Topographysigmā1	8	−957.173	1930.346	2.436	0.098
D ~ StdRgdlambda0~Topographysigmā1	7	−958.34	1930.680	2.770	0.083
D ~ 1lambda0~stdElvsigmā1	4	−964.151	1936.301	8.391	0.005
D ~ 1lambda0~Habitatssigma~1	6	−967.036	1946.072	18.162	0
D ~ 1lambda0~1sigmā1	3	−975.316	1956.631	28.721	0
D ~ StdElvlambda0~1sigmā1	4	−974.971	1957.942	30.032	0

Elv = Elevation of the point in the mask for which density is estimated; Elv^2 = Square of Elevation (Combination of Elv and Elv^2 used to test an increasing and then decreasing relationship between snow leopard density and elevation); Rgd = Ruggedness of the point in mask for which density is estimated; D = Snow leopard density; lambda0 = encounter rate of snow leopard at distance zero from activity center (surrogate for detection probability); sigma = Spatial scale of detection function for snow leopards.
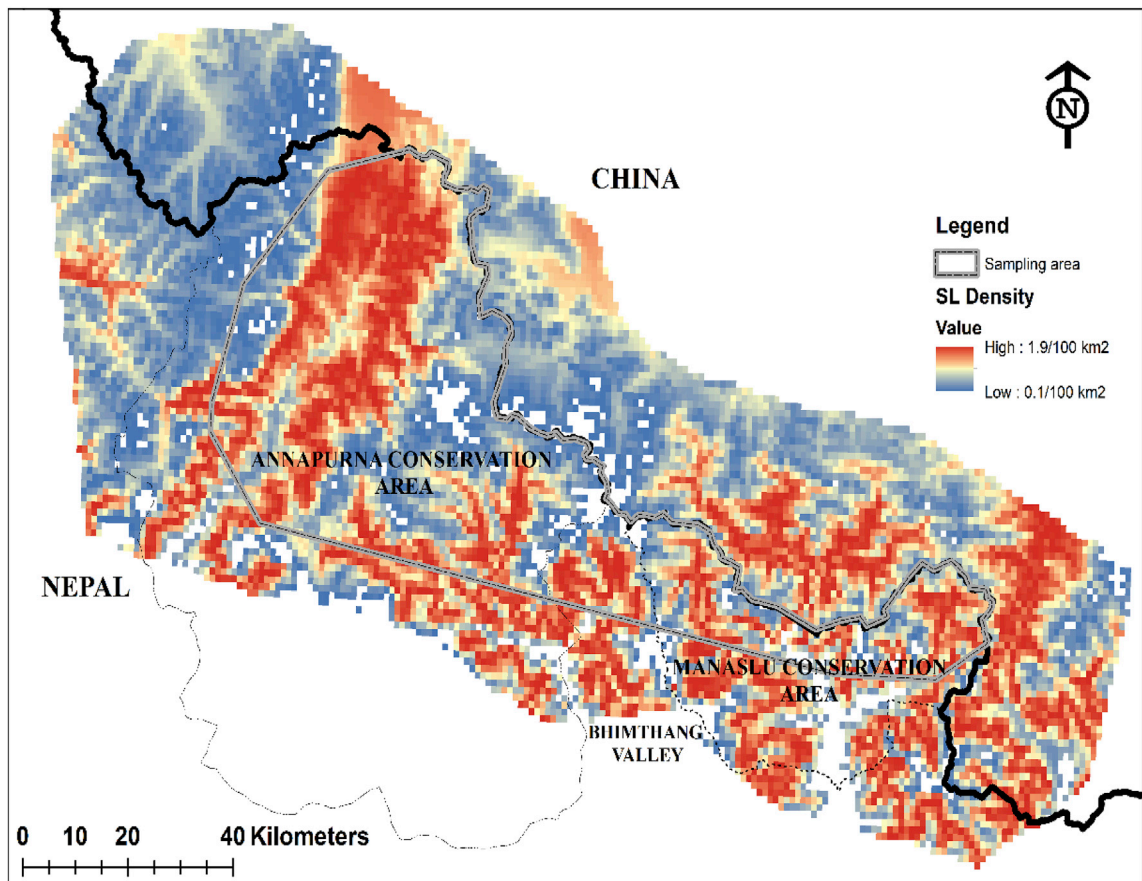

Fig. 2. Predicted snow leopards density in the landscape based on the top model as a quadratic function of elevation.

within our study area entails that small areas may end up with high densities unless chosen randomly. It is noteworthy that positive bias in density estimates from small study areas has also been observed in several other species (see e.g. (Cuellar et al., 2006; Dillon and Kelly, 2007; Zimmermann et al., 2013).

Using GPS-telemetry, Johansson et al. (2016) demonstrated that snow leopard home range sizes were between 6 and 44 times larger than previous VHF-based estimates. The average 95% MCP home range estimates from Johansson et al. (2016) were ca 500 km^2, thus larger than most study areas where snow leopard densities have been estimated previously (Alexander et al. 2015, 2016; Chen et al., 2016; Jackson et al., 2006; Janečka et al., 2011; Oli, 1994; Suryawanshi et al., 2017). Home ranges in our study area may have been somewhat smaller than those reported from Mongolia due to a high prey density (Chetri et al., 2017). Nevertheless, the new insight into the spatial ecology of snow leopards entails that the appropriate scale for snow leopard monitoring areas is probably in the order of several hundreds of km^2. Because snow leopards are rare, and

researchers have often relied on preliminary surveys to identify appropriate sites to conduct thorough surveys, smaller study areas are more likely to be located in relatively prey rich "hot spots". To address this bias, it is important that either the inherent variability in density as a function of habitat is modeled, or sampling areas are large enough to reduce bias.

Although most scat samples were deposited within a shorter time period, some might have been up to six months old. This raises the question of population closure; some animals might have died during this period, and scats from some pre-dispersing sub-adults might have been included in the analysis. However, owing to the large spatial scale of our study, such factors - and any migration in and out of the sampling area or long-distance movements of non-residents (transients) - have probably had minimal effect on the density estimate (Royle et al., 2016).

There are obvious logistic constraints associated with intensive sampling of large areas, particularly in snow leopard habitat. A fundamental question is thus how large areas need to be surveyed in order to obtain reliable density estimates? According to Bondrup-Nielsen (1983), sampling grids should be at least 16 times larger than the average home range size to avoid positive bias. A more conservative recommendation of study areas three to four times larger than average home ranges was suggested by (Maffei and Noss, 2008). However, while Maffei and Noss (2008) used conventional CR, a comparable simulation study concluded that SECR models are far more flexible regarding sampling design, and that models performed well as long as trapping arrays were similar to or larger than one average home range (Sollmann et al., 2012). An important benefit of SECR, particularly for wide ranging animals like snow leopard, is that it estimates density and properly accounts for movement of animals beyond the sampling grid, and, that it accounts for heterogeneity in exposure to trapping due to where individual home ranges are located. Regarding model flexibility, SECR does not require that the capture probability of all individuals should be greater than zero, which is a fundamental assumption in conventional CR models (Borchers and Efford, 2008; Efford, 2004; Karanth and Nichols, 1998; Royle et al., 2009). SECR may therefore perform better in large sampling areas, as these models are less sensitive to bias caused by scat survey transects or camera trap positions being spaced too far apart to cover the home ranges of all inhabitants. Furthermore, in elusive and wide ranging species such as snow leopards, SECR may perform better, as these models are less sensitive to low capture probabilities (Blanc et al., 2013; Sollmann et al., 2012).

In this paper we demonstrate the importance of considering spatial scale in future monitoring of snow leopards. Moreover, we show that non-invasive genetic sampling can be used to estimate snow leopard populations at a large spatial scales. Our study contributes to PAWS initiative not only by adding data points to this global initiative, but also by providing valuable protocols to estimate and monitor snow leopards using genetic sampling across large spatial scales. A consequence of our study is that the abundance of snow leopards in Nepal, and perhaps elsewhere, may be over-estimated. Given that our large scale study provided a lower density estimate than the relatively small scale studies that was the basis for the country-wide assessment in Nepal (DNPWC, 2017), the abundance of snow leopards in Nepal is likely to be lower than previously assumed. These insights can have serious consequences for the conservation of snow leopards and underscore the need for better, systematic surveys using refined sampling and analytical methods.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00548.

References

Ale, S.B., Shrestha, B., Jackson, R., 2014. On the status of snow leopard Panthera uncial (Schreber, 1775) in Annapurna, Nepal. J. Threat. Taxa 6, 5534–5543.

Alexander, J.S., Gopalaswamy, A.M., Shi, K., Riordan, P., 2015. Face value: towards robust estimates of snow leopard densities. PLoS One 10 e0134815.

Alexander, J.S., Zhang, C.C., Shi, K., Riordan, P., 2016. A granular view of a snow leopard population using camera traps in Central China. Biol. Conserv. 197, 27–31.

Blanc, L., Marboutin, E., Gatti, S., Gimenez, O., 2013. Abundance of rare and elusive species: empirical investigation of closed versus spatially explicit capture–recapture models with lynx as a case study. J. Wildl. Manag. 77, 372–378.

Bondrup-Nielsen, S., 1983. Density estimation as a function of live-trapping grid and home range size. Can. J. Zool. 61, 2361–2365.

Borchers, D.L., Efford, M., 2008. Spatially explicit maximum likelihood methods for capture–recapture studies. Biometrics 64, 377–385.

Burnham, K.P., Anderson, R.D., 2002. Model Selection and Multimodel Inference, a Practical Information-Theoretic Approach, second ed. Springer Verlag, New York.

Chen, P., Gao, Y., Lee, A.T., Cering, L., Shi, K., Clark, S.G., 2016. Human–carnivore coexistence in Qomolangma (Mt. Everest) nature reserve, China: patterns and compensation. Biol. Conserv. 197, 18–26.

Chetri, M., Odden, M., Wegge, P., 2017. Snow leopard and Himalayan Wolf: food habits and prey selection in the central Himalayas, Nepal. PLoS One 12 e0170549.

Cuellar, E., Maffei, L., Arispe, R., Noss, A., 2006. Geoffroy's cats at the northern limit of their range: activity patterns and density estimates from camera trapping in Bolivian dry forests. Stud. Neotrop. Fauna Environ. 41, 169–177.

Dillon, A., Kelly, M.J., 2007. Ocelot Leopardus Pardalis in Belize: the Impact of Trap Spacing and Distance Moved on Density Estimates.

DNPWC, 2017. Snow Leopard Conservation Action Plan (2017-2012). Department of National Parks and Wildlife Conservation, Kathmandu, Nepal.

DoFPS, 2016. National Snow Leopard Survey of Bhutan 2014 − 2016 (Phase II): Camera Trap Survey for Population Estimation. Department of Forests and Park Services, Ministry of Agriculture and Forests, Thimphu, Bhutan.

Efford, M., 2004. Density estimation in live-trapping studies. Oikos 106, 598–610.

Efford, M., 2018. Secr 3.1 - Spatially Explicit Capture−Recapture in R. https://cran.r-project.org/web/packages/secr/vignettes/secr-overview.pdf.

Foster, R.J., Harmsen, B.J., 2012. A critique of density estimation from camera-trap data. J. Wildl. Manag. 76, 224–236.

GSLEP, 2018. Global Snow Leopard & Ecosystem Protection Program. http://www.globalsnowleopard.org/blog/category/press-release/.

Jackson, R., Ahlborn, G., 1989. Snow leopards (Panthera uncia) in Nepal: home range and movements. Natl. Geogr. Res. 5, 161–175.

Jackson, R.M., Hunter, D.O., 1996. Snow Leopard Survey and Conservation Handbook. International Snow Leopard Trust, and US National Biological Service, Seattle, USA.

Jackson, R.M., Roe, J.D., Wangchuk, R., Hunter, D.O., 2006. Estimating snow leopard population abundance using photography and capture-recapture techniques. Wildl. Soc. Bull. 34, 772–781.

Janečka, J.E., Jackson, R., Yuquang, Z., Diqiang, L., Munkhtsog, B., Buckley-Beason, V., Murphy, W.J., 2008. Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. Anim. Conserv. 11, 401–411.

Janečka, J.E., Munkhtsog, B., Jackson, R.M., Naranbaatar, G., Mallon, D.P., Murphy, W.J., 2011. Comparison of noninvasive genetic and camera-trapping techniques for surveying snow leopards. J. Mammal. 92, 771–783.

Johansson, Ö., Rauset, G.R., Samelius, G., McCarthy, T., Andrén, H., Tumursukh, L., Mishra, C., 2016. Land sharing is essential for snow leopard conservation. Biol. Conserv. 203, 1–7.

Kachel, S.M., McCarthy, K.P., McCarthy, T.M., Oshurmamadov, N., 2017. Investigating the potential impact of trophy hunting of wild ungulates on snow leopard Panthera uncia conservation in Tajikistan. Oryx 51, 597–604.

Karanth, K.U., Nichols, J.D., 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79, 2852–2862.

Karanth, K.U., Nichols, J.D., Kumar, N.S., Hines, J.E., 2006. Assessing tiger population dynamics using photographic capture-recapture sampling. Ecology 87, 2925–2937.

Karmacharya, D.B., Thapa, K., Shrestha, R., Dhakal, M., Janecka, J.E., 2011. Noninvasive genetic population survey of snow leopards (Panthera uncia) in Kangchenjunga conservation area, Shey Phoksundo National Park and surrounding buffer zones of Nepal. BMC Res. Notes 4, 516–524.

Lovari, S., Boesi, R., Minder, I., Mucci, N., Randi, E., Dematteis, A., Ale, S.B., 2009. Restoring a keystone predator may endanger a prey species in a human-altered ecosystem: the return of the snow leopard to Sagarmatha National Park. Anim. Conserv. 12, 559–570.

Maffei, L., Noss, A.J., 2008. How small is too small? Camera trap survey areas and density estimates for ocelots in the Bolivian Chaco. Biotropica 40, 71–75.

Maheshwari, A., Niraj, S.K., 2018. Monitoring illegal trade in snow leopards: 2003–2014. Global Ecology and Conservation 14, e00387.

McCarthy, K.P., Fuller, T.K., Ming, M., McCarthy, T.M., Waits, L., Jumabaev, K., 2008. Assessing estimators of snow leopard abundance. J. Wildl. Manag. 72, 1826–1833.

McCarthy, T., Fuller, T., Munkhtsog, B., 2005. Movements and activities of snow leopards in Southwestern Mongolia. Biol. Conserv. 124, 527–537.

McCarthy, T., Mallon, D., Jackson, R., Zahler, P., McCarthy, K., 2017. Panthera uncia. The IUCN Red List of Threatened Species 2017: e.T22732A50664030. https://doi.org/10.2305/IUCN.UK.2017-2.RLTS.T22732A50664030.en. (Accessed 3 January 2018).

McCarthy, T., Mallon, D., Sanderson, E.W., Zahler, P., Fisher, K., 2016. What is a snow leopard? Biogeography and Status Overview. In: McCarthy, T., Mallon, D. (Eds.), Snow Leopards: Biodiversity of the World: Conservation from Genes to Landscapes. Elsevier Academic Press, Amsterdam, The Netherlands, pp. 23–41.

Miquel, C., Bellemain, E., Poillot, C., BessiÈRe, J., Durand, A., Taberlet, P., 2006. Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. Mol. Ecol. Notes 6, 985–988.

Oli, M., 1997. Winter home range of snow leopards in Nepal. Mammalia 61, 355–360.

Oli, M.K., 1994. Snow leopards and blue sheep in Nepal: densities and predator: prey ratio. J. Mammal. 75, 998–1004.

Royle, J.A., Fuller, A.K., Sutherland, C., 2016. Spatial capture−recapture models allowing Markovian transience or dispersal. Popul. Ecol. 58, 53–62.

Royle, J.A.N., J.D Karanth, K.U., Gopalaswamy, A.M., 2009. A hierarchical model for estimating density in camera-trap studies. J. Appl. Ecol. 46, 118–127.

Sharma, K., Bayrakcismith, R., Tumursukh, L., Johansson, O., Sevger, P., McCarthy, T., Mishra, C., 2014. Vigorous dynamics underlie a stable population of the endangered snow leopard Panthera uncia in Tost Mountains, South Gobi, Mongolia. PLoS One 9 e101319.

Snow Leopard Network, 2014. Snow Leopard Survival Strategy. Revised 2014 Version Snow Leopard Network, pp. 1–145. Seattle, Washington, USA.

Sollmann, R., Gardner, B., Belant, J.L., 2012. How does spatial study design influence density estimates from spatial capture-recapture models? PLoS One 7, e34575.

Suryawanshi, K.R., Redpath, S.M., Bhatnagar, Y.V., Ramakrishnan, U., Chaturvedi, V., Smout, S.C., Mishra, C., 2017. Impact of wild prey availability on livestock predation by snow leopards. Royal Society Open Science 4, 170026.

Waits, L.P., Luikart, G., Taberlet, P., 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol. Ecol. 10, 249–256.

Waits, L.P., Paetkau, D., 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J. Wildl. Manag. 69, 1419–1433.

WCNP, 2016. Population Status and Distribution of Snow Leopard in Wangchuck Centennial National Park in Bhutan. Department of Forests adn Park Services and WWF.

Wegge, P., Shrestha, R., Flagstad, Ø., 2012. Snow leopard Panthera uncia predation on livestock and wild prey in a mountain valley in northern Nepal: implications for conservation management. Wildl. Biol. 18, 131–141.

Zimmermann, F., Breitenmoser-Wursten, C., Molinari-Jobin, A., Breitenmoser, U., 2013. Optimizing the size of the area surveyed for monitoring a Eurasian lynx (Lynx lynx) population in the Swiss Alps by means of photographic capture-recapture. Integr. Zool. 8, 232–243.