

PAWS

GUIDELINES





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PAWS Process Outline

Snow leopards are the icon of high mountains of Asia and represent habitats that provide water to large parts of Asia. Their ecosystem services benefit more than a billion people, and sustain unique high-altitude cultures. The species is threatened by poaching and illegal trade, retribution killing by local people due to predation on livestock, climate change, mining, and large-scale infrastructure development. Robust population estimates about the species are available only from less than 2% of their entire distribution range, which are also biased in resulting from sampling mostly best habitats. This makes any projections about the populations across the landscapes, countries or range unreliable.

Governments from the snow leopard range countries identified the need for more robust and expansive monitoring of snow leopard populations. This was reflected in the Bishkek Declaration 2017, Kathmandu Resolution 2017, Issykkul Statement 2018 and Shenzhen Consensus 2018 related to [the Global Snow Leopard and Ecosystem Protection Program \(GSLEP\)](#)¹.

The International Snow Leopard and Ecosystem Conservation Forum 2017 (SL Forum 2017) in Bishkek set the goal of developing a robust estimate of the global snow leopard population. At a high-level meeting of officials, range country governments decided that this effort to end the uncertainty surrounding the size of the world's snow leopard population was to be given high priority by the GSLEP program.

Taking into account the amount of effort and resources that are already being invested in estimating snow leopard populations at local sites where various national and international conservation organizations are operating, and the need to maintain a statistical rigor, follow up meetings have been conducted with participation of scientists and conservationists from over 20 organizations and different countries. PAWS or Population Assessment of the World's Snow Leopards, may take up to five

¹ www.globalsnowleopard.org

years to be completed. The process is envisaged to generate additional benefits than just population estimates. These include the most reliable ever distribution map of snow leopards, spatial mapping of threats faced by the species across different parts of its range, capacity building of young conservationists and local champions, and identification of potential refugia for snow leopards in response to various climate change projections.

To obtain reliable snow leopard population estimates, a two stage process has been identified by the technical and oversight panel that was set up by the GSLEP Steering Committee. These two stages involve various activities including training and capacity building workshops, periodic interaction with specialists supervising the effort, fundraising, procurement of equipment, field surveys, analysis of data, and setting up of national and regional leads and partner organizations.

While both stages are important, the proposed design does not require completion of the first stage prior to initiation of the second stage. A continuous review and feedback mechanism is built in to facilitate course correction and adaptive improvement of the study design.

The primary two stages, as identified by the technical oversight and support panel are:

1. Assessing snow leopard distribution as a function of one or more habitat covariates

- a. Method(s): Site occupancy modeling
- b. Data required: Interview, sign survey and/or camera trapping
- c. Sampling unit: 100-200 sq km grid cells that can potentially be represented through the sampling effort
- d. Workshop needs: Up to five workshops focused on planning, designing, field training, pilot surveys feedback, and analysis
- e. Human Resource requirements: Field teams to collect data in the field, research support to design, coordinate and monitor implementation, analyze data and interpret results.
- f. Indicative Timelines: For each survey, 1 month for planning and logistics, 1 month for pilot data collection, 15 days for pilot data analysis, 1-2 months for main data collection, 1-2 months for analysis across a study area covering

10,000-50,000 sq km

- g. Outcomes: A GIS surface denoting probability of site use by snow leopards

2. Estimating abundance as a function of heterogeneous density across space

- a. Method(s): Spatial Capture Recapture modeling
- b. Data required: Camera trapping or genetic sampling
- c. Sampling unit: 500-5,000 sq km of a coverage with clusters of camera trapping/genetic sampling
- d. Workshop needs: Up to six workshops focused on planning, field training, data collection and organization, analysis, feedback and course correction, and stratification and selection of sites
- e. Human resource requirements: Field teams to install cameras or collect genetic data in the field, research support to design sampling, coordinate and monitor implementation, analyze data and interpret results
- f. Indicative Timelines: For each survey, 1 month for planning and logistics, 1 month for setting up cameras or collect genetic data, 2 months for data collection and organization, and 2 months for analysis. The intensive sampling may need to be conducted in several sites depending on the sampling requirements for a desired level of precision
- g. Outcomes: Snow leopard density and abundance estimates

The stage 1 (occupancy) surveys will help develop a surface that provides probabilistic maps of survey units used by snow leopards. These probabilistic maps can then be used to stratify and identify sites for intensive sampling (stage 2). It is important to note that completion of stage 1 is not mandatory to initiate stage 2 sampling, as the latter can be initiated using appropriate selection of sites based on available knowledge. Each intensively surveyed location will eventually contribute to the spatial representation of the global effort. An indicative workflow provides a graphical representation of the entire PAWS process (Fig. 1).

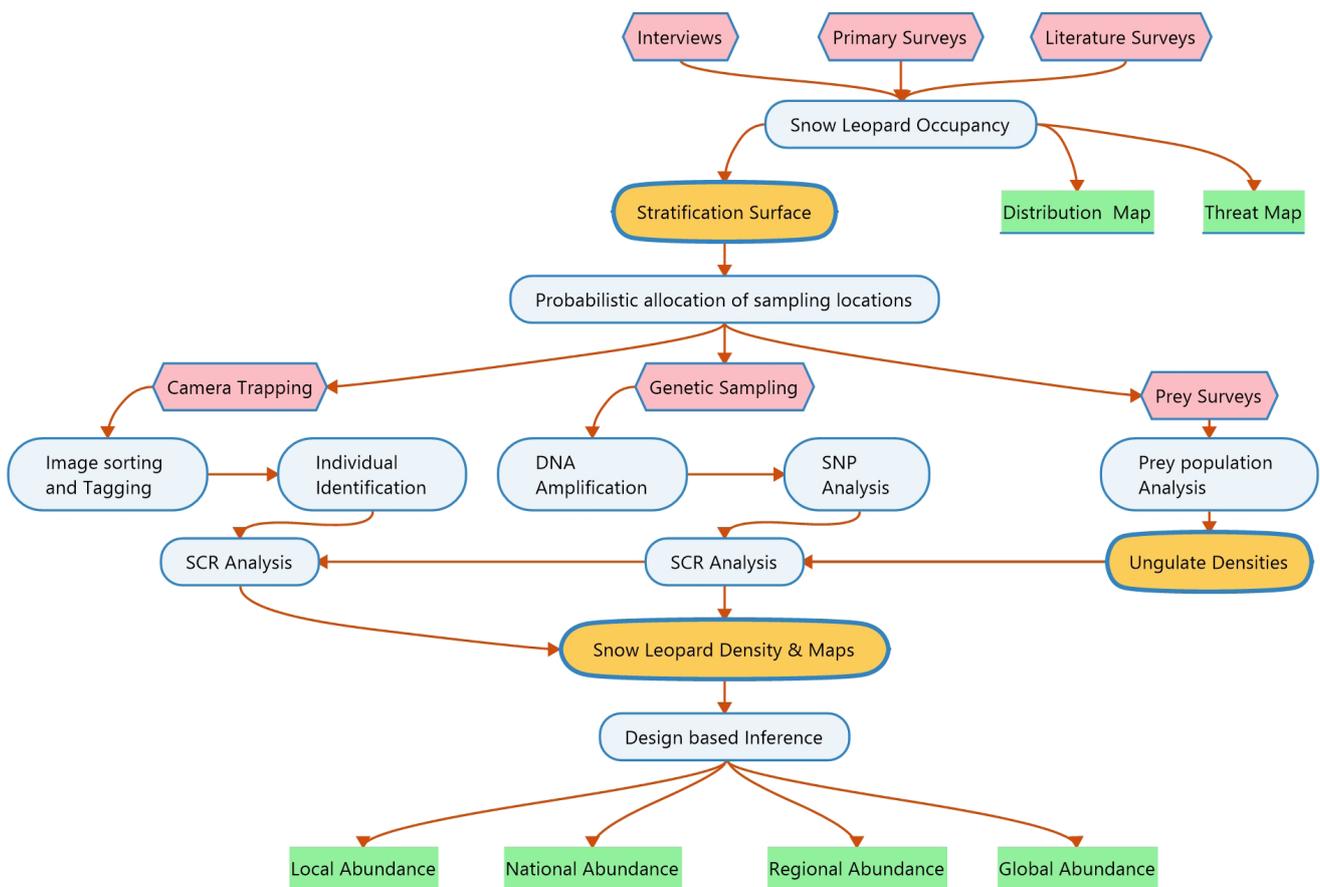


Figure 1. The suggested workflow to estimate robust snow leopard abundance in large landscapes using a design-based inference framework. PAWS proposes occupancy-based distribution modelling to help with stratification, and intensive sampling in selected locations to estimate abundance using spatial capture recapture modelling.

Introduction and Overview

It is critical to understand the spatial population ecology of animals and plants for effective management and conservation of biodiversity. Accurate estimates of ecological state variables such as animal occurrence and density provide key metrics for monitoring population changes over time in response to changes in environmental conditions or as a result of conservation actions. A popular method called capture-mark-recapture (in short, capture-recapture) was widely used for big cats for several years where probability of detection and abundance of animals were estimated from the capture (detection) histories of individuals in a particular area of interest. The method relied on identification of individuals captured (e.g. on cameras or through genetic samples), over a period of time (e.g. [1]–[4]) to generate capture histories. The unique spot patterns on snow leopards make them individually identifiable [5]. The capture protocol for identifying and recapturing individuals was fairly detailed and requires several considerations (see [6] for similar protocols to monitor tigers). In conventional capture recapture, the study area is required to be large enough to encompass many home ranges of the target species so as to avoid overestimation of abundance or density due to the edge effect [7], [8]. The biggest challenge with conventional capture recapture was ad hoc estimation of the effective sample area making it difficult to estimate density with any level of confidence.

Spatial Capture Recapture (SCR) methods estimating wildlife population density and abundance were first introduced by Efford [9], and have developed rapidly since [10], [11]. Although Royle et al. [12] provide a detailed review and introduction to SCR methods, and Borchers and Fewster [13] provide an updated review and speculation on future developments, there are few guidelines available to field practitioners that offer step by step guidance to collecting relevant data, especially for snow leopard populations.

Spatial capture recapture (SCR) methods require that animals are sampled so that there is a high probability of them being encountered at more than one location. At the

same time, closed population spatial capture-recapture methods require populations to be closed to changes, thus making it mandatory for the sampling to be done in a period that is short enough to assume closure. The SCR methods are founded on the principle that space use by animals is heterogeneous, typically with more time spent in or near the activity centers, and a decline in space use further away from the activity center. This can, in turn, lead to greater probability of individual animals being captured (or photographed) close to the centers of their activity ranges as opposed to their fringes. The spatial data on animals encountered multiple times at different locations during the survey provide the required data to estimate the detection function and the number of animals that may not get captured (hence enumerated), to ultimately estimate the population density from a survey.

Estimates of population abundance and density are intuitive for managers, researchers, donors, politicians, and members of local communities. The number of individuals (abundance) is the most widely accepted state variable for monitoring most species across the world. However, the high cost of implementation, and violation of assumptions are potential obstacles in estimating number of individuals in a population. Low densities of snow leopards in their habitat and large home ranges potentially lead to low probability of being encountered on camera traps or in genetic samples. Low number of recaptures increases the variance and the size of confidence intervals therefore reducing power to detect changes. Spatial Capture Recapture provides an opportunity to estimate more than one state variable for monitoring. At best, it allows estimation of abundance and density of snow leopard populations in a landscape, and an understanding of population dynamics over a period of time, but the methods also help understand habitat use at different scales. The data can be used to answer questions about impact of conservation actions in sites with different levels of conservation action.

Although spatial capture recapture analysis can be used to estimate abundance from reasonably large landscapes, there are significant costs associated with covering large areas with either camera traps or transects to collect capture-recapture data of individual animals. This is where methods of site occupancy [14] can help determine the patterns of snow leopard space use across relatively large landscapes. Occupancy methods are widely used to determine probability of occurrence, site use, local colonization/



extinction, and range contraction/expansion with the help of indirect surveys such as interviews (e.g. [15], [16]) , or direct surveys such as those recording signs or camera trap encounters. The flexibility of occupancy methods, which can be implemented with a variety of sampling methods, spatial extents, effort levels and kinds of data makes them a valuable tool for developing distribution maps and monitoring changes over large time frames. Occupancy, or probability of site use, as a function of habitat can also help define strata for which specific intensive sampling strategies (for spatial capture recapture analysis) can be developed.

These guidelines follow standard procedures for data collection, statistically valid designs based on compliance with assumptions, and data analysis frameworks based on published literature and data from different species including snow leopards. We have divided the guidelines into the following three sections for convenience of use:

1. Planning
2. Sampling design
3. Data collection, management and analysis

It is important to clearly state the purpose and objectives in advance and conduct a thorough situation analysis, especially since any snow leopard population assessment exercise is resource intensive and requires basic to advanced skills at different levels of its implementation. Often the exercise can be coupled with a capacity building exercise for frontline staff, data managers and analysts to ensure reliable statistical inference from the data.

Planning

Identification of region of interest, goals, costs and resource availability

a. Identify region of interest

Identifying the region of interest is the first step towards reliable estimation of snow leopard population occupancy, density and abundance. From defining the resources needed to conduct sampling, to determining where survey effort will be located and what information will be obtained, the region of interest has significant consequences for the survey in terms of cost and expected outcomes. Smaller areas of the order of a few hundred square kilometers are straight forward to sample, but recent studies show density of snow leopards is not homogeneous varies by habitat [17]. Unless modeled as a function of habitat covariates, or selected using a randomized statistical design, small study areas run the risk of misrepresenting the density of the larger distribution area if the smaller areas have higher or lower density than the average in the larger area. A recent analysis of population studies on snow leopards [18] indicates that smaller study areas tend to be selected in regions with high snow leopard density (Fig. 2). The same can be true if large study areas of the order of several home range sizes are selected. In this case, unless a large number of samples are collected with adequate spatial randomization, or the estimates are modeled using appropriate habitat covariates, the density estimates will be positively biased if high density regions are sampled too heavily and negatively biased if they are sampled too lightly.

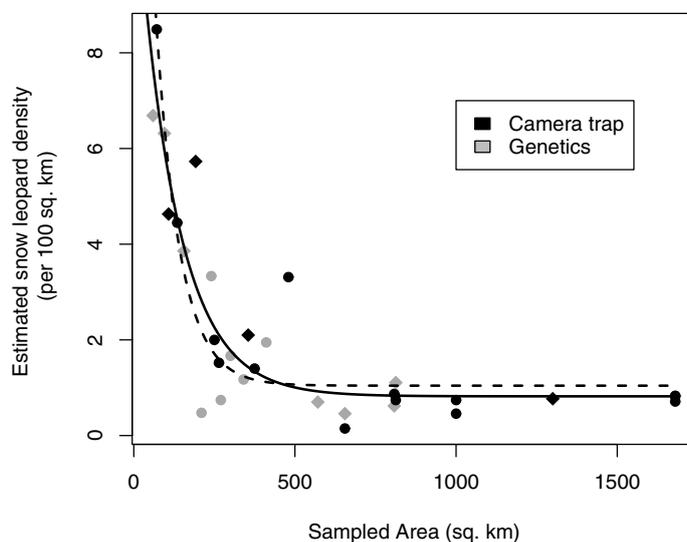


Figure 2. Snow leopard population density plotted against the size of sampled area. Black dots represent studies that used camera trapping ($n = 18$) while grey dots indicate molecular genetics ($n = 13$) sampling. The diamonds indicate studies which only estimated the abundance of snow leopards and had not attempted to estimate density ($n = 11$), while circles represent studies that estimated density of snow leopards ($n = 20$). The continuous line is the negative exponential model fitted to the entire dataset while the dotted line is the model fitted to only those studies that estimated density. (credit: Suryawanshi et al. 2019; Population Ecology)

Resources usually constrain the extent of habitat that can be sampled using camera traps or genetic data sampling. Larger areas such as those representing entire provinces, landscapes or countries need to be sampled programmatically using an appropriately randomized design to subsample the entire area of interest, possibly within appropriately identified strata. When strata are placed in such a way that there is low expected variation in density within strata and higher variation in expected density between strata, one can substantially reduce variance in overall abundance estimates. This increases the power of the exercise to detect changes, hence making it valuable for monitoring.



Some study areas may have hard boundaries whereas others may be permeable, with high or low resistance (e.g. porous fences, highways, canals, high ridgelines etc.). Regions of unusable habitat or beyond non-permeable boundaries (e.g. altitudes beyond permafrost, thick forested habitats, water-bodies, steppe, settlements, in other words habitat types where snow leopards cannot have an activity center) should be excluded from the survey region if possible. This however does not preclude the need to sample areas that may still be used by snow leopards albeit with a much lower probability.

b. Identify goals

The goals of any population assessment exercise usually include estimation of the range of the species (which parts of space are occupied by the species), estimation of density (how many individuals there are per unit area in the occupied parts), and how the occupancy and density change over time. Density estimation has greater data demands than does occupancy estimation, and data for occupancy estimation are easier and cheaper to gather than those for density or abundance estimation:

i. Estimating abundance and/or density, and comparing it across space and time:

Estimation of snow leopard density and abundance requires data collection where individual animals can be identified whenever they are detected. Using sophisticated modeling through maximum likelihood [10] or Bayesian spatial capture recapture analyses [19], one can then estimate the probability of detection at any point in space. Density and detection probability can be modeled as functions of spatial or non-spatial covariates [10].

ii. Estimating proportion of area being used and monitoring changes across time in the form of local colonization and extinction.

In situations where it is impossible to identify individuals or the scale is too big to sample adequately for abundance or density estimation, indirect surveys using evidence of presence such as signs or interviews of locals for recent encounters can be used to obtain reliable and replicable information about species distribution using the occupancy framework. The data can also be used to monitor changes in occupancy or site use over time by estimating probabilities of local colonization and extinction [20]. Models using covariates to define detection probability and probability of occupancy (or site use) from large areas can be used to create strata that can then be sampled intensively using stratified sampling for population estimation.

c. Identify resources vs costs:

Monitoring biological populations can be resource intensive, especially for rare and elusive species. Since reliability and replicability are important in assessing and monitoring animal populations, it is important to estimate costs and then invest time and resources only if one is able to secure sufficient resources. Local travel, accommodation, meals, global positioning system receiver, data sheets, stationery

and field gear are some of the essential costs for each field survey. Given that surveys need to be designed carefully to adhere to statistical and ecological assumptions, it is always valuable to invest resources and taking support from experts in planning, designing and analyzing surveys.

Camera trapping and genetic sampling are two widely used and accepted methods for collecting data on animals that can be individually identified. Research on snow leopards has indicated that 30-50 camera traps are often adequate to sample for snow leopard abundance in 500-2,000 sq km. Camera traps rank differently on their performances. So far Reconyx™ have been found to be most reliable in the snow leopard habitat, with researchers managing to identify more than 90% of the encounters as individuals [21]. With their longevity making them last many field seasons (some units being used in the field for 10 years continuously), their initial costs soon even out when compared with other cheaper camera traps. Recent experiments also indicate that the process of identifying snow leopard individuals may not be error-free, and requires reporting of an individual's or a team's identification skills to reduce subjectivity.

Genetic samples on the other hand can be reasonably cheap to collect in the field and be used to sample areas between 500-5,000 sq km for abundance. Mostly collected as fecal matter, only storage equipment such as vials or zip lock bags with silica and gloves are required to collect scats from the field. Given a high rate of inaccuracies in identification of scats belonging to particular species, a large number of samples get discarded from the snow leopard data during genetic screening, thus adding marginally to the cost. While currently the most widely used method to identify individuals from amplified DNA screened as a species are micro-satellite based, recent developments are indicating a possible shift towards whole genome based methods for individual identification in the near future. Analysis of each sample incurs cost, and results have been found to vary among laboratories and lab technicians with different levels of expertise. Since a small variation in the number of individuals and their associated detection histories can lead to substantial biases in population estimates, it is prudent for the analyses to be cross verified if possible to minimize the chances of misidentification.

Sign surveys produce useful data that can help identify spots for setting up camera traps and collect data that can be used to determine species distribution, habitat use and/or

occupancy. No additional equipment is required as long as field personnel are able to accurately identify signs belonging to the species of interest along specific transects. An efficient method to collect reliable species distribution data not only from the current period, but from the past as well is that of interviewing key people who have been using habitat in different capacities over the years (e.g. [15], [16]). These can include hunters, herders, rangers, tour guides, transporters, etc. Interviewing people systematically about reports of personal observations or sightings in the recent time periods, within their areas of knowledge, does not require much resources in addition to the essential commodities listed above but only obtains data that can generate coarse models of current and past distributions (probabilities of sampling units being used by a species).

Sampling Design

Monitoring snow leopard populations in small sampling areas of a few tens of square kilometers through camera trapping is often of limited value for monitoring populations. Camera trapping requires several camera traps to be set out in the field for a specific duration of time, typically short enough to assume population closure (no changes in the population due to mortality, recruitment or migration during the sampling period), but long enough to produce recaptures of individuals within the population on more than one camera trap. Cameras should be spaced such that individuals have high probability of being detected on more than one camera and low probability of being detected on all cameras.

Genetic sampling on the other hand requires several samples collected on transects or search blocks that represent a cross section of the population. These samples can be collected on systematic transects, trails or block searches representing the area of interest in such a way that same individuals can be detected on more than one point in space. Alternately, genetic data can be collected from hair snares installed strategically and visited periodically to collect follicles of snow leopards that can eventually be analyzed and determined to belong to different individuals.

To generate data that allow estimation of parameters with satisfactorily low variance, a reasonable number of animals need to be captured and recaptured at different locations. At minimum, two sets of data are required for analyses. These are data on trapping locations (camera trap or transects) and capture events (which individuals were captured or recaptured on which camera or transect segment).

Spatial Capture Recapture analyses allow estimation of how density varies in space within the sampled study area, as well as how detection probability varies with site, spatial variables, and importantly with distance from an activity center (Fig. 3). This flexibility allows for the application of sophisticated models that address inherent variation in snow leopard density and detection probability within and between study areas.

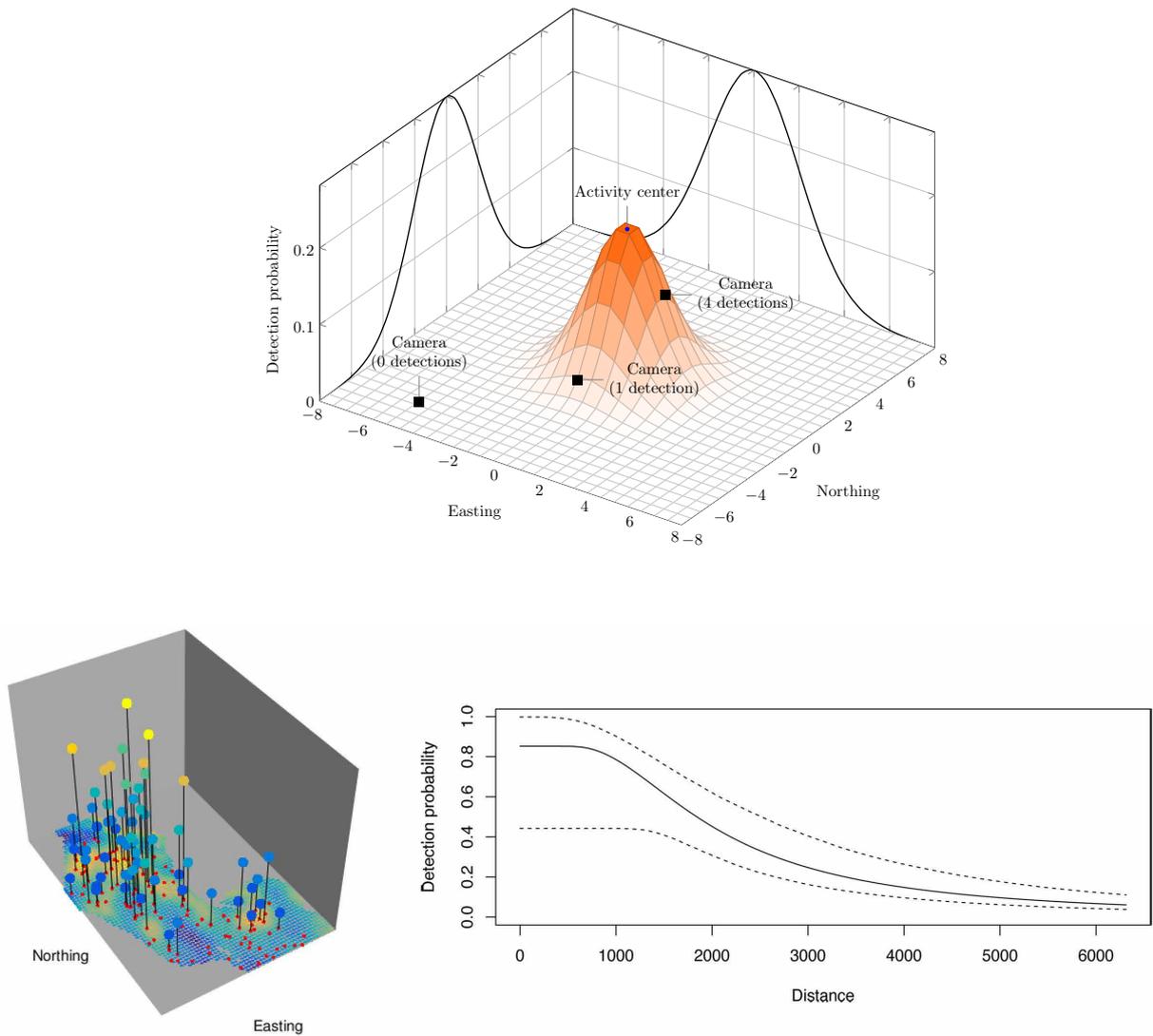


Figure 3. Schematic depiction of (a) detection curve of an animal around its activity center with number of hypothetical encounters at traps set up at different distances; (b) a two dimensional depiction of detection probability as a function of distance from the activity center; and (c) number of encounters on different cameras set up across a study area with non-uniform animal density. Colours on the base represent a habitat suitability variable (blue=low suitability, yellow=high suitability); red dots are traps; “pins” show numbers of detections at each trap: longer pins with yellow heads indicate more detections, while shorter pins with blue heads indicate fewer detections.”

Spatial Capture Recapture analyses are an effective way to estimate and monitor snow leopard populations and perform spatial as well as temporal comparisons. The methods can be valuable especially in situations where the extent of suitable habitat available for snow leopards may vary between study areas or periods, thus making it difficult to estimate the true effect of a specific conservation action. Models that include interaction terms between site and conservation action type can be developed in spatial capture recapture framework to estimate the efficacy (or lack) of conservation efforts.

a. Macro-level Survey design: Sampling in 10,000 sq km or more

Snow leopard range is expected to be nearly 2 million sq km. To generate a better understanding about the global snow leopard populations, it is imperative to estimate their populations from large tracts of their distribution range represented by GSLEP landscapes, provinces, country boundaries or mountain ranges. However, intensive sampling in areas larger than 2,000 sq km has rarely been achieved, primarily owing to logistical and equipment related constraints.

Snow leopard density can be estimated in two ways. Model-based inference builds a model relating environmental covariates to density, and uses this model to extrapolate throughout the range. Design-based inference selects a random sample of points throughout the study region and infers overall density by averaging over the survey points.

Model-based inference depends critically on developing a reliable model of how environmental variables affect animal density. Over extremely large areas such as the snow leopard range this is extremely difficult and perhaps impossible. The range of potential habitats that would need to be sampled is large, the relationship between covariates and density may vary from place to place, and which are important explanatory variables may also vary from place to place. We therefore propose to use design-based inference to estimate snow leopard density over large spatial scales, leaving open the option of using model-based inference at smaller spatial scales (the scale of a single array of camera traps, for example). The proposed design uses the following three steps, after identifying suitable large-scale spatial strata:

1. Select a number of survey “points” within a stratum, using a suitably randomized design.
2. Estimate snow leopard density and associated uncertainty at each of these points. In practice, this means placing an array of camera traps approximately centered on the point and surveying an area around the selected point, using the methods described in the following Section b (typically sampling an area of around 500-5,000 sq. km).
3. Use design-based inference, based on the design in 1. above, and using the estimates from 2. above, to obtain a single mean snow leopard density and

abundance estimate with associated estimates of uncertainty, within a stratum. These stratum-specific estimates can then be combined to obtain a single global estimate of snow leopard density or abundance, with associated estimates of uncertainty.

We refer to the selection of survey points in step 1 above as the “macro-level” design, and the design of the survey at each of the macro-level survey points as the “micro-level” design.

Strata should be defined in advance. We anticipate that a single stratum will not span more than one country and within each country, further stratification may occur, stratifying further by region and/or expected snow leopard density. Strata can be defined using estimated occupancy probabilities or expert knowledge and should be fixed in advance of the survey. The survey effort required to obtain a given level of precision with each stratum will be estimated in advance, to try to minimize the resulting uncertainty associated with density and abundance estimates within strata and over all strata. This typically involves allocating more survey points to areas where snow leopard density is expected to be higher.

Within each stratum, we propose to randomly select survey points using Balanced Acceptance Sampling, described below, to generate co-ordinates of the survey points. The number of survey points in each stratum will be chosen so that the expected within-stratum coefficient of variation for estimated snow leopard density is less than 30%. Reasonable effort should be made to design the micro-level survey (for example, positioning an array of camera traps) so that the provided sample co-ordinate lies in the interior of the survey area, as close to the center of the array as is feasible.

Estimates of density and abundance obtained using design-based inference will typically be more efficient, with lower variance, if survey points are approximately evenly distributed across the study region. This is because they better sample the average density in the stratum than would points that may be clustered in certain parts of the region.

A survey made up of points that are approximately evenly distributed in space is known as spatially balanced. A regular grid of survey points is one example of a spatially

balanced sample. Often, however, using a regular grid is not feasible, because some of the survey points fall in areas that are inaccessible or because the study region is highly irregular.

Several approaches have been developed that construct grid-like designs that are flexible enough to avoid the problems that are encountered by perfectly regular grids (local pivotal methods [22]; generalized random tessellation stratified designs [23]; balanced acceptance sampling [24]). We use balanced acceptance sampling here, on the basis that it is easy to implement and use, and that one does not need to know the number of survey points in advance, so additional survey points can be added at any later stage while retaining good spatial balance.

Balanced acceptance sampling generates a deterministic sequence of points called a Halton sequence. The sequence is deterministic and consists of points that are evenly distributed over the interval 0-1. For example, the first 10 numbers in the one-dimensional Halton sequence are $\{1/2, 1/4, 3/4, 1/8, 5/8, 3/8, 7/8, 1/16, 9/16, 5/16\}$. Each point is mid-way between two points generated earlier in the sequence. The key property of the Halton sequence is that any subset of the sequence is also evenly distributed, provided the subset consists of points that are next to each other in the sequence. This design allows additional survey points to be added whenever desired, or infeasible points to be removed, without compromising the key properties of the design. Halton points can be generated in two or three (or more) dimensions (Fig. 4).

The large-scale design and stratification depends on having reliable knowledge of which areas are occupied by snow leopards (and ideally, which areas have higher and lower density). Estimates of occupancy can be obtained by conducting preliminary surveys, using interview or sign based occupancy methods. Interview based sampling can be conducted across large regions by dividing it into several sampling units (grids or watersheds) that may or may not be larger than the typical home range size of the snow leopard, which, based on recent studies is about 250-300 sq km of usable habitat (e.g. excluding high and low altitudes and other areas used exclusively by humans). Each sampling unit can be surveyed for occupancy indirectly by independently interviewing more than one person who has current (and preferably also past) knowledge about it. Each report of snow leopard presence can be recorded as detection of the species,

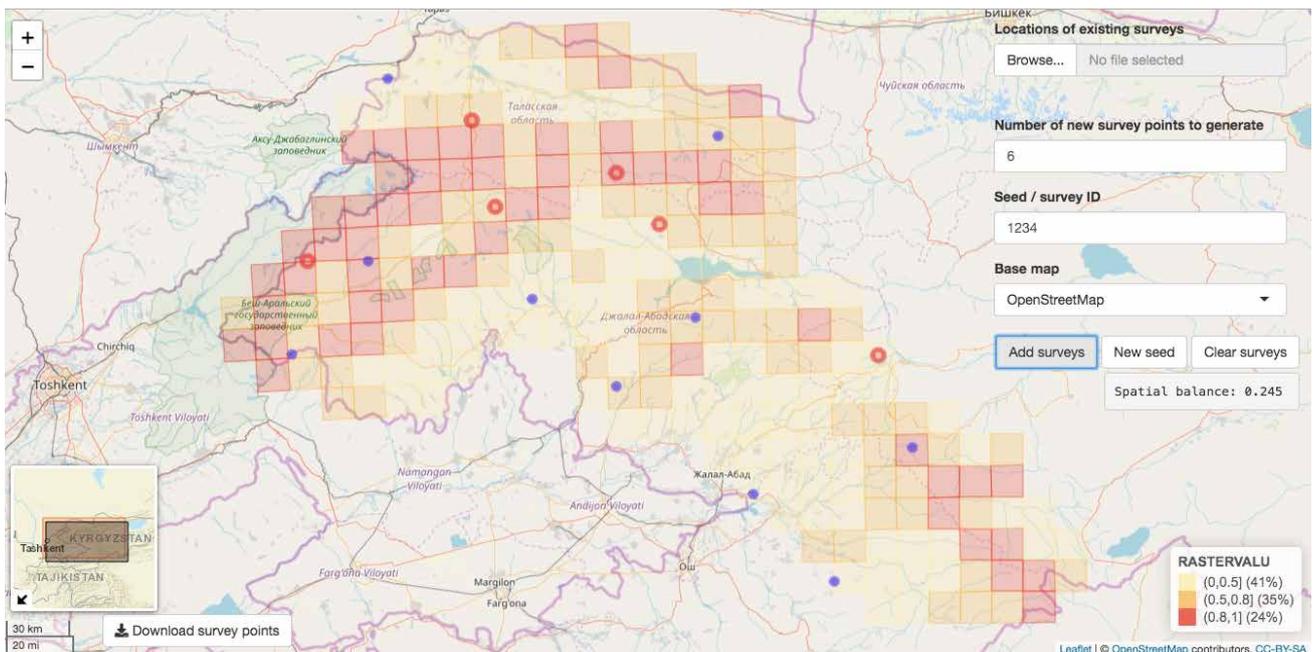


Figure 4. Screenshot of software for generating survey site locations. The study region has been stratified by predicted snow leopard occupancy probabilities (yellow = low, orange = moderate, red = high). The legend in the bottom right of the screen shows the name of the stratification variable, the cutoffs used, and the proportion of survey effort allocated to each stratum. Blue and red circles denote existing and proposed new survey sites respectively. These denote the approximate centre of any camera trap survey area, with the exact locations of camera traps within the survey area to be determined by some micro-level survey design.

whereas lack of reports do not necessarily indicate absence, and hence must only be treated as non-detections, not absences. This information may be collected for the current period, and also for a certain number of years in the past, as long as the time period is clearly annotated in the data. Details about each person interview, such as her/his profession, age and familiarity with the sampling unit that they report about must be recorded so these can be used to model the variation in detection probability. The information collected can then be reorganized into uniform sampling units where each interview provided reasonable representation of detection or non-detection of the species of interest in the sampling unit (grid cell) that it represents. Data from multiple interviews can be analyzed in the occupancy framework by modeling the variation in each sampling unit being used by snow leopards as a function of habitat types or other covariates. With good design and analysis where detection is modelled with appropriate covariates to account for heterogeneity, the method helps generate reliable maps of snow leopard distribution within the survey region (e.g. Fig. 5). The probabilities of site use can be used to generate strata that can then be used to choose sampling units for intensive sampling using a stratified sampling design. A representative selection of sampling units can then be surveyed intensively to obtain precise abundance estimates by camera trapping or genetic data collection.

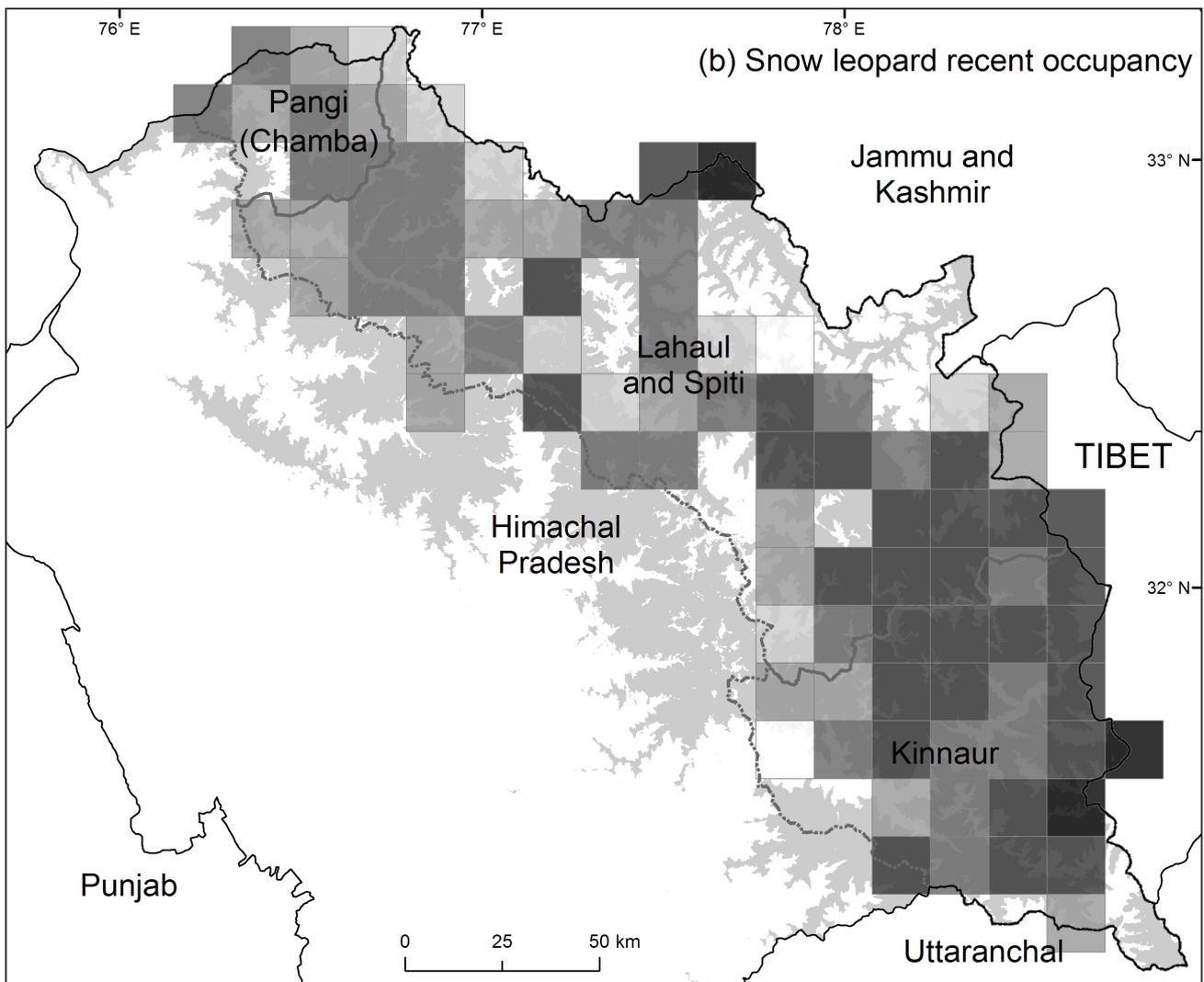


Figure 5. Probability of site use (occupancy) by snow leopards across the Greater and Trans-Himalaya mountains of Kinnaur, Lahaul, Spiti and Pangi, Himachal Pradesh, India, estimated using interview-based site occupancy surveys. (credit: Ghoshal et al. 2017; Oryx)

b. Micro-level design: Sampling in 500-5,000 sq km

Areas between 500 sq km to 5,000 sq km are typically the size of sites that are sampled with 30-50 camera traps or surveyed for collection of genetic data. Conventionally, the study areas for capture-recapture were expected to be many times bigger than the home range size and have no holes (areas with snow leopards but no sampling). These assumptions have been relaxed considerably in the spatial capture recapture framework as long as certain other assumptions are met with (see Sampling Design for more details). Recent studies (e.g. [25]) indicate that spatial capture recapture models perform well with relatively smaller sampled areas, as long as they are similar to, or larger than the extent of individual movement during the study period. However, as observed from available information, animal density is seldom constant across

space, and is often a function of habitat covariates. In such situations, more often than not one ends up sampling an area that has higher density, which can in turn bias the results [18]. Often these smaller sampling sites are chosen in areas that are most likely to record snow leopards (high encounter rate of snow leopard signs and scrapes), which are likely to produce positively biased results. This can be addressed to some extent by modeling density, detectability and ranging parameters as functions of habitat types, but this requires representative sampling even from areas that are likely to have low probability of detection or density. Typically, camera trapping studies have been designed keeping in mind the recommendation of at least two cameras within each potential home range. Some of these design constraints are relaxed in planning spatial capture recapture surveys in lieu of other requirements, such as getting an adequate number of recaptures of snow leopards on multiple cameras. While more specific sampling recommendations are currently in development, in general the most efficient model for obtaining camera trapping data is that of setting up cameras in an array so that as many snow leopards as possible get an opportunity to be photographed on more than one camera. A networking design where each new unit is installed within 3-5 km of another unit typically works, though data quality may improve considerably if cameras are set in clusters [25], [26]. In SCR, one has the opportunity of setting up more than one camera in close proximity to another if there is more than one promising location. In fact, setting up a few cameras within close vicinity of each other may help inference. It is important to collect location specific habitat data (e.g. topography, altitude, terrain, presence of waterbody etc) from each of the sites where a trapping station is installed so that one can use it to model the variability in detection probability as a function of habitats or other relevant covariates.

Spatial capture recapture methods may assume closure of populations, which is why it is important to collect data from a session for a period short enough that an assumption of closure is likely to be met. Our experience from various sites indicates that for a trapping area greater than 500 sq km sampled for 2-3 months with 30-40 camera stations typically generates reasonable data for spatial capture recapture inference. Spatial capture recapture analysis allows the possibility of moving cameras in this period as long as times of installation and removal are recorded with each camera location. If one is able to install a greater density of traps within the study area, this can provide higher quality of data for estimation of the shape of the detection

functions. This, however affects the spatial extent that can be covered during a sampling effort. Recent recommendations [25]–[27] propose a denser trapping grid in a relatively smaller area (provided it is large enough to encompass a reasonable sample of snow leopard activity ranges for the sampling period) as opposed to a rarefied trapping grid in a large area. One important assumption is that all habitat types being represented in the study area are covered by the sampling design. Ideally one would sample all potential habitat types even if some are less likely to produce snow leopard captures. However, if certain habitat types are not being sampled and there is no way to claim representation of this habitat type in the current sampling design, it is best to exclude them from the survey region during analysis (see Data Analysis below) than make projections about the animals' density in areas with no representative sampling.

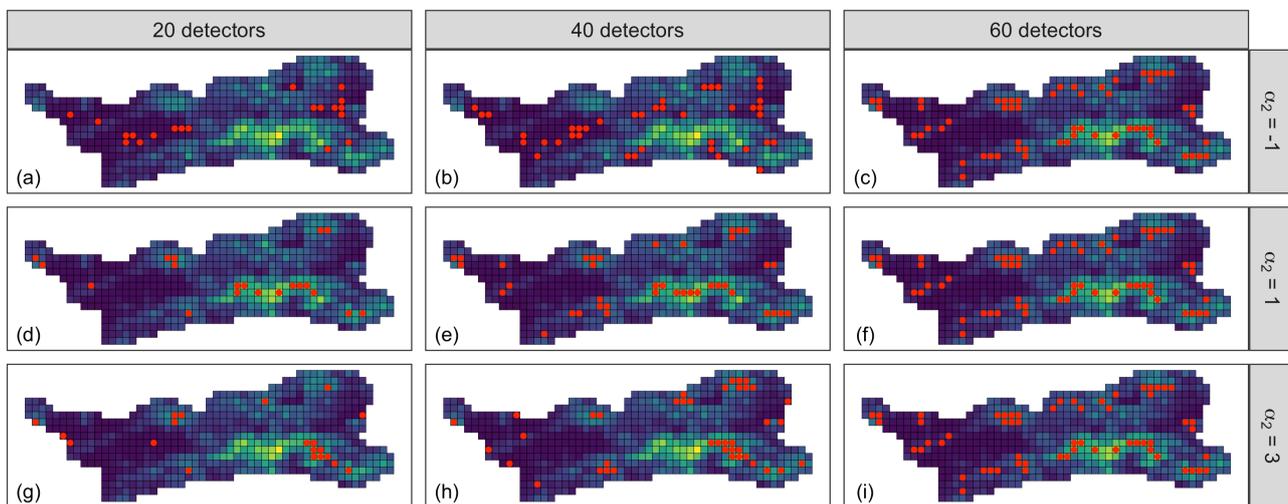


Figure 6. Optimal survey designs where connectivity depends on a particular covariate, either through a negative (first row) or positive (second and third rows) relationship. Lighter grid cell colors denote higher values of the covariate and darker colors denote lower values. Detectors are placed so that they maximize the likelihood of recaptures, but small clusters of detectors persist throughout the survey region as a means of ensuring that individual animals are recaptured on multiple detectors.

Similar principles apply in case of using genetic data in spatial capture recapture framework. However, in case genetic samples such as fecal material are collected on transects, the data are treated differently as each transect can be considered as multiple detectors (by cutting the transect into multiple segments, each of which is then considered as a detector), with a strong assumption that all scats being collected belong to a short enough period of time that there are no mortalities or births during this period. The most effective means is that of dividing the study area into manageable strata (grids or watersheds) that are smaller than the activity range of the animal. Typically surveys have been conducted within 5x5 km sized

grids based on known ranging patterns of snow leopards. Each of the grids that can be physically accessed (without risking life) is surveyed by walking one or more transects within. Each transect or each point within a transect where purported snow leopard feces are searched for may have its own characteristics such as terrain, topography, substrate type etc. that can in turn affect the probability of the snow leopard leaving (or not leaving) feces there and that of the field personnel detecting them. Sometimes weather conditions can also have strong effects on the teams' ability to detect feces. It is valuable to collect this information that can in turn help model the detectability as a function of one or more of these covariates.

c. Intermediate-level design: Sampling in 5,000-10,000 sq km

As briefly discussed above, larger study areas, even though they offer the potential to sample a greater number of individuals, are often logistically more difficult to sample adequately if one wishes to maintain the same sampling intensity over the whole area as is used in smaller areas. Because more detectors are required to sample a larger area without reducing sampling intensity (number of detectors per unit area), surveyors are often faced with a difficult tradeoff between expanding the study into larger areas with reduced sampling intensity or sampling smaller areas with greater intensity. A negligible fraction of studies have sampled areas larger than 2,000 sq km intensively for snow leopard abundance estimation. It is possible to estimate snow leopard abundance in areas between 5,000 and 10,000 sq km by collecting genetic data on transects using similar methods to those discussed above (e.g. [17]). Since SCR analyses allow holes within the study area (areas with no sampling within a study area despite potential ranges within), it is possible to survey a much larger area than can be covered intensively, by sampling smaller sub-regions within the larger region and by using an appropriate design to select the sampled sub-regions, and estimate density and abundance across the whole survey region – see “Macro-level design” section above.

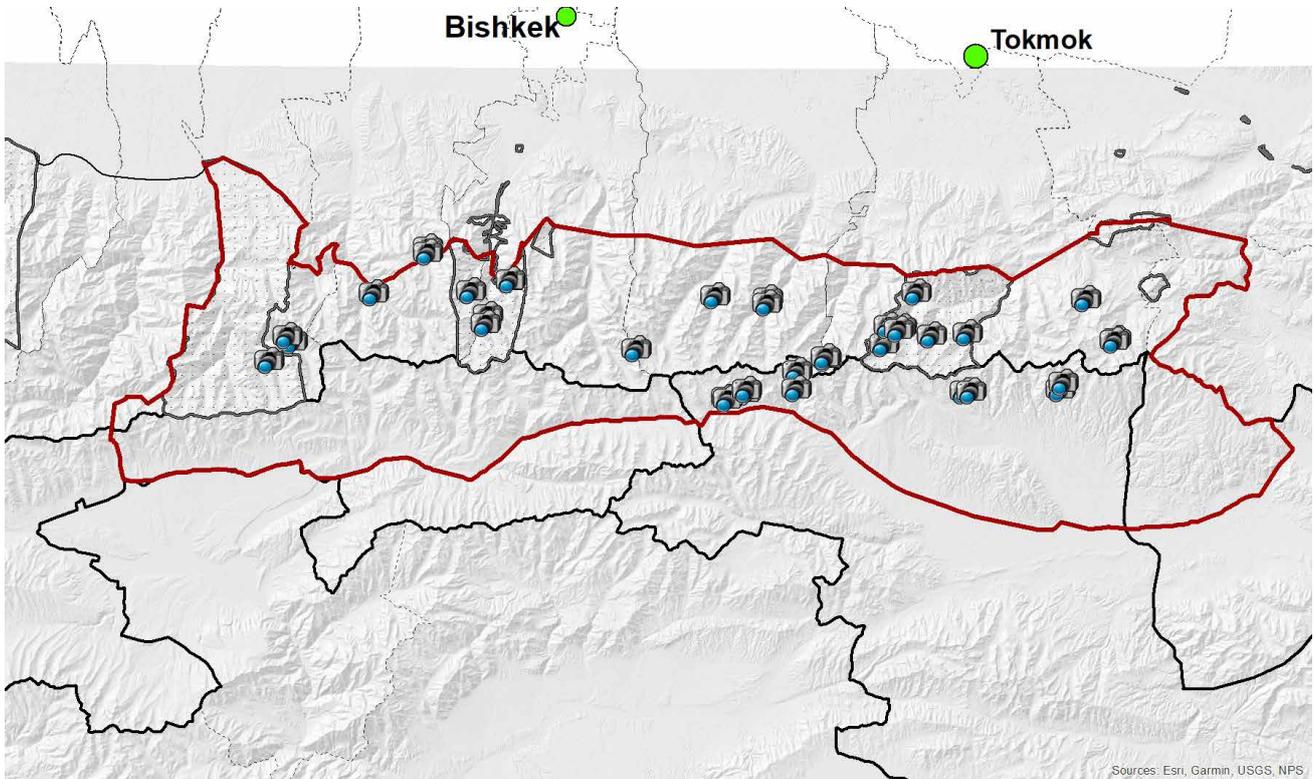


Figure 7. Snow leopard trapping design using clustered (a) sampling design that was used recently for a camera trapping session in the Kyrgyz Ala Too Range.

Data collection, management and analysis

a. Data collection

Data collection for snow leopard abundance and distribution can be classified into five types, i.e. camera trapping, GPS tagging, genetic sampling, interview based occupancy surveys and sign or camera trapping based occupancy surveys. Although with some overlap, data are collected differently for each of the types, and these field methods can have substantial implications on the quality of the data available for analyses and, eventually, the estimated parameters. Each survey requires resources in terms of money and time, making it important that data collection protocols are planned carefully.

i. Camera trapping

Scraping sites, overhanging rocks, saddles on ridgelines, cliff bases and water-bodies are often considered to be excellent sites for setting up camera traps. Once the camera location has been identified by surveying a particular area, the cameras can be set up along a rock or a tree at an appropriate height and angled so that they detect and photograph the entire snow leopard whenever the animal moves in front of it. Ideally the cameras should be set up in a way that the animals walk between 2 and 10 meters from it. Detailed investigation using data from multiple years and locations reveals that flanks are the most identifiable parts of a snow leopard, even though it is possible to identify them based on their facial and tail patterns as well [28]. Since we notice similarity in some patterns between siblings and parent-offspring pairs, it is highly recommended that at least three differences or similarities are identified between any two sets of images to determine whether they belong to different or same individual. To improve the chances of getting good images with identifiable patterns, cameras are usually set in a way that they take at least 5 rapid-fire pictures at each encounter. This improves the chances of getting sufficient images from each encounter to be able to identify individuals based on their patterns. Cameras today are equipped to take several thousand images without running out of memory or battery. Therefore, we propose that they

are set at high sensitivity and allow no delays between encounters. With 32GB high speed SD cards and Nimh AA sized batteries, some of the high quality camera models (e.g. Reconyx™ Hyperfire500) are known to be operational for more than 6 months, sometimes even a year, without requiring replacement of either the memory card or batteries. They use semi-infrared illumination in the night, have a long battery life that can sustain more than 2,000 encounters, are capable of taking multiple images with little blurring at each encounter, have the capacity to store more than 30,000 images in a 32GB card, use a combination of heat and motion sensors to minimize false triggers, have a tough casing to sustain physical and climatic abuse in the field, and are well camouflaged making them less visible to animals and humans alike.

While literature on tigers and other felids recommends use of two cameras at each station, data collected on snow leopards suggests that a single unit at each camera station can generate usable data where more than 95% of the encounters can be identified into individuals [21]. This is primarily because when set at scraping sites or waterholes, often the cat spends a few minutes sniffing and walking around at the spot, and on many occasions reveals both its flanks or a good view of its forehead and/or tail. Once more than one side of a snow leopard are recorded, it gets catalogued for even those encounters where it might just be passing through a camera. Setting up single cameras at each station allow doubling of the number of cameras for sampling, leading to substantial benefits in analyses. However, it is important to note that captures of animals identified with only one flank should not be compared with animals identified by other flank (e.g. an animal with only left known flank should not be treated as an individual unless all identified animals' left flanks are known and vice versa). It is important to note that with small number of individuals in a sampled population, misidentification can be a serious issue and lead to strong positive bias in estimated abundance.

The limitations of camera trapping include high equipment and logistical cost, risk of theft or damage to expensive equipment due to landslide or animal bite, and restriction on the extent of an area that can be sampled in each sampling occasion due to limited equipment and resources. Low densities of the target species can result in few images and hence poor estimation, but it is nevertheless important to

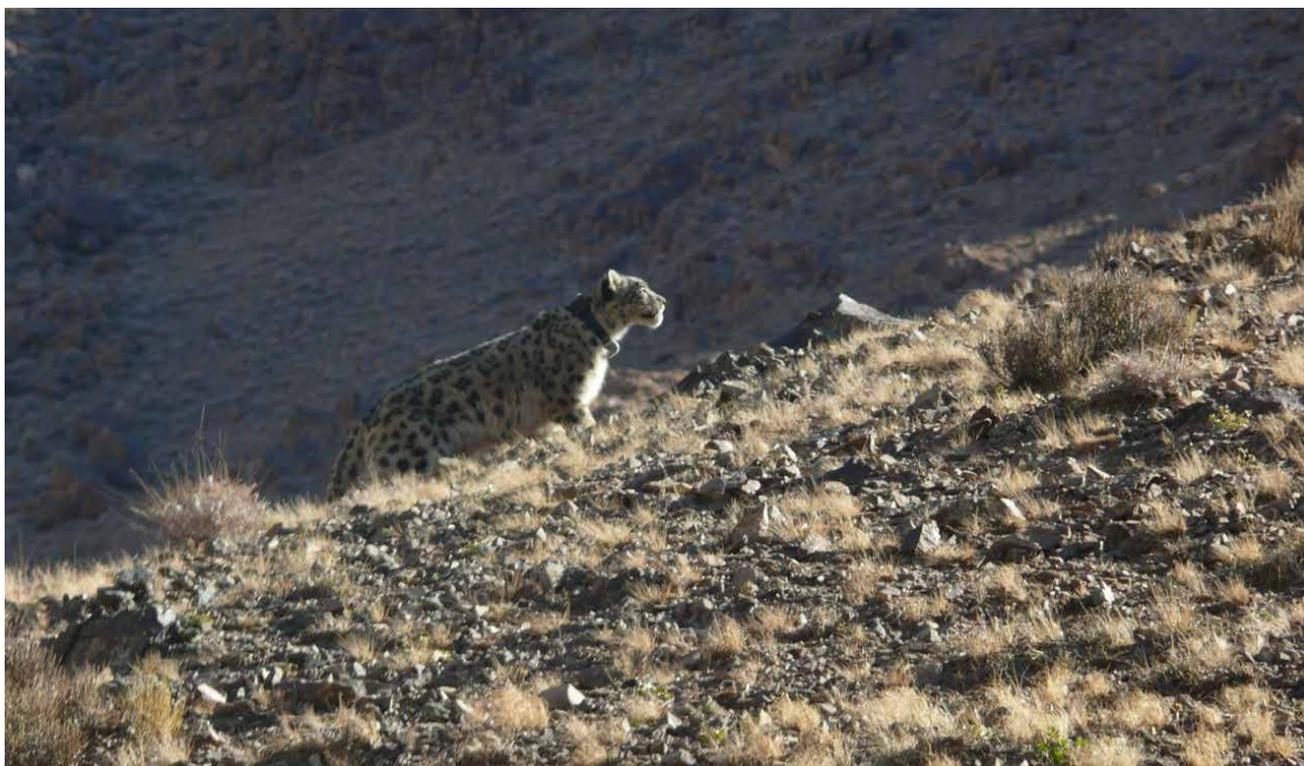


Camera traps are typically installed at locations that have high chance of snow movement of snow leopards such as saddles on ridgelines, cliff bases, overhanging rocks and canyons.

sample such areas if the goal is to estimate snow leopard populations across large landscapes or ranges. A moderate level of skill is required to install and monitor camera traps, which can be provided through one or two days of training for the field staff.

ii. GPS tagging (Telemetry)

GPS telemetry data can provide rich information on animal movement, ranging, and habitat use. While these data on their own are of limited use for density and abundance estimation, they are potentially extremely useful when combined with survey data from camera traps and/or spatial sampling of genetic data. This is because movement and habitat use must be (and is) implicitly estimated from spatial camera and genetic sampling data, in order to estimate density. However, the information about movement and habitat use that is contained in camera trap and genetic data is often sparse. Supplementing these data with much more precise and detailed information about movement and habitat use that is contained in GPS telemetry data, has the potential to not only provide better estimates of density than can be obtained from camera trap or genetic data alone, but also to give better estimates of habitat use in a snow leopard population than can be obtained from either telemetry, or camera trap/genetic data alone.



GPS telemetry provides high resolution data about animal movement, behavior and activities.

iii. Genetic sampling

Genetic data can be collected at a relatively smaller investment from much wider areas that would be required for sampling with camera traps. Genetic data can be collected using scats detected from transect surveys, or hair snares set up at points. Collecting hair snare data requires regular site visits to each snare to reduce data losses and this can be resource intensive, but if collected correctly, the data generated are known to be superior to that from feces, which may be much older or have degraded genetic data. However, most data published or analyzed using genetic sampling for snow leopard population have been collected using fecal DNA. Transects can be positioned covering trails, mountain ridges, river beds and mountain passes covering a fair representation of all habitat types available to the snow leopard. Feces are typically found on cliff bases, dry riverbeds, ridgelines and animal trails. Each vertex or point of interest in a transect should be recorded (using GPS trail feature) and can be further classified based on its characteristic features. On encountering a purported snow leopard scat, a small portion of its outer dried layer should be extracted using a knife and preserved either in a plastic tube with silica desiccant or a high quality zip lock bag for DNA analysis. Each preserved sample should be annotated properly to provide its precise location and date of collection and the relevant information about its age and micro-habitat characteristics around the collection site.

The limitations of genetic sampling include high cost of data analysis at genetic laboratories and high variability found due to unknown factors in lab specific decision points often resulting in to identify individuals. Given the strong dependence of spatial capture recapture analyses on identified individuals, any uncertainty in identification, particularly false positives (samples from the same individual classified as multiple individuals) can lead to substantial biases in the results. Some researchers have also reported grafted data from zoos and other areas being presented as genetic samples for population analyses, thus resulting in inflated populations. A relatively low level of skill is required to conduct genetic sampling transects, which can be achieved through a half-day long pre-survey training.



Genetic sampling requires field personnel to conduct surveys looking for feces. Once collected, feces need to be genetically screened to identify species that they belong to as they can often be confused with other predators including wolves and dogs.

iv. Interview-based occupancy surveys

Interview-based data are best collected by first creating a grid or watershed-based map of the entire area of interest and then identifying places where key informants can be met with and interviewed in a relaxed environment. Each set of interview data can be collected on a form with clearly marked sampling units so the interviewee can indicate the cells for which they have knowledge about. Conversations about the sighting of snow leopard or its evidence often reveal the accuracy of the observation. Verification kits such as images of snow leopards, their pugmarks, scrapes and kills can be used to ascertain the ability of the interviewee to identify and report the presence accurately. It is important to collect information from more than one informer about each sampled unit (grid cell). This requirement however can be relaxed for a small fraction of sampling units as long as there are enough sampling units with two or more informants reporting either detection or non-detection of snow leopards within. Interviewing more than one person together should be avoided to prevent non-independence of data points.



Interview based surveys using topographic sheets and GPS provide valuable data that can be analyzed in the site-occupancy framework

v. Sign or camera trapping based occupancy surveys

It is likely that in some parts of the snow leopard range, one may not find sufficient number of reliable informers. In such cases, interview-based distribution mapping becomes unfeasible. Such situations can be addressed by creating a grid based map of the entire area and identifying specific locations (potential marking sites) within these that can be surveyed for snow leopard signs or camera trapped. It is important for the surveys to represent the grid cells in such a way that they represent the probability of the entire grid-cell being used (or not) by snow leopards. Sign surveys can be conducted by walking across transects looking for snow leopard signs, or looking for snow leopard signs at specific marking sites such as overhanging rocks, cliffs, ridgeline saddles etc. Camera trapping data can be collected by setting up 2 or more cameras for a few weeks, or a single camera for several weeks so there is ample opportunity for the cameras to detect snow leopards in the sampling unit that they represent, given presence. Information about the weather conditions, length of transects (if used) substrate (if doing sign surveys), topography, terrain and presence of humans/livestock can be recorded as these are likely to affect the probability of detecting snow leopards if they are using the sampling unit (grid cell). Other subtleties such as season length, lifespan of sign available for detection in different climatic conditions and area represented by camera traps should also be addressed at the time of designing surveys.



Presence of snow leopards can be detected through scrapes on the ground, urine markings or hair stuck on overhanging walls where they typically rub their cheek to leave a scent.

b. Data Management

Data once collected requires to be reorganized into a format that can be used on specific analysis tools. These formats may vary between methods and tools that are employed to analyze the data, and hence it is important to archive raw data separately for reprocessing, should it be required under special circumstances later. Multiple methods can be employed to facilitate easy management and sharing of data in raw and processed formats.

i. Camera trapping

1. Creating trapping data and covariates

SCR methods require at least two datasets to estimate detection functions and snow leopard densities and their respective dependence on spatial (and/or temporal) covariates: data on trap arrays and data about individuals captured and recaptured on these trap arrays (Fig. 8). If density or detection probability is to be modelled as a function of spatial variables (e.g. habitat types), they also require data from which the spatial variables at every point on the landscape (for density) and every camera location (for detection). Since existing spatial capture recapture software does not handle spherical coordinate systems (degree, minutes, seconds), all location data should be projected to UTM or a similar projection system (such that distances between projected points are proportional to distances between points on the ground). Given the vast expanses of habitat and their relative accessibility, some cameras may stay in the field longer than others. This in turn reflects on the effort being made by each trapping station in encountering snow leopards (the longer the camera is deployed, the greater the survey effort). Therefore, installation and removal dates must be recorded and attached to the data describing the trapping array. Each camera trap may have micro-habitat characteristics such as its topography, terrain ruggedness, presence of waterhole, or proximity to human settlement that can in turn affect the traps' ability to detect a snow leopard in addition to their distance from an animal's activity center. Recording these location specific habitat characteristics can be used to model variation in detection probabilities or rates (or effective ranging area). The trap array data typically consists of a trap id (name), its x and y coordinates, effort, and specific covariates that can be categorical as well as continuous.

Minimum data			Usage information				Covariates	
Detector ID	x coord.	y coord.	Binary	Numeric			V ₁	V ₂
A ₁	0	0	101	1.0	1.0	1.5	26.1	11
A ₂	100	0	111	1.0	1.2	1.0	20.7	10
	⋮			⋮			⋮	
C ₂	100	300	111	3.0	3.5	2.0	18.5	13
C ₃	200	300	001	0.0	0.5	1.0	23.0	11

Figure 8. Snippet depicting typical capture data on camera traps. Individual ID represents individual snow leopards that are captured on one or more occasion during the sampling period, Detector ID represents the unique identifier to each camera trap and should match the IDs provided in the trap data; Occasion represents the number of distinct occasions (e.g. days) the traps have been kept operational (can be left to 1 if the effect of time on detection probability can be assumed to be constant); Covariates denotes animal specific covariates that could affect their probability of being captured on particular cameras or the size of the activity range.

2. Listing encounters of Snow Leopard Trast and other wildlife

Any sequence of photos of a snow leopard by a camera trap separated by some suitable time (e.g. 1 hour) such that encounters are far enough in time that they can be considered independent, is usually defined as an encounter. While it may take several hours to classify snow leopard photographs and other non-target species, it helps in future analyses and even other studies to have a detailed encounter data of all species recorded on the camera traps. Usually done manually, there are outline tools available now (e.g. snow leopard image sorter on thr GSLEP website powered by Microsoft and Snow Leopard Trast) that help extract snow leopard images using artificial intelligence-based algorithms, thus reducing the desk time for researchers (Fig. 9). Each encounter of snow leopard can be recorded with information about camera id, date, time and number of images for that particular encounter. The camera ids listed against the encounters must match exactly with the camera ids mentioned in the trapping array data.

3. Identifying individuals

Individual snow leopards can be identified based on their unique pelage patterns. Each snow leopard encountered can be compared to a catalogue of identified snow leopard photos and assigned a known id if there is a match, or entered as a new individual if there is not. We have noticed that some patterns are often shared between siblings and parent-offspring pairs. To avoid misidentification, it is important that at least three differences or similarities

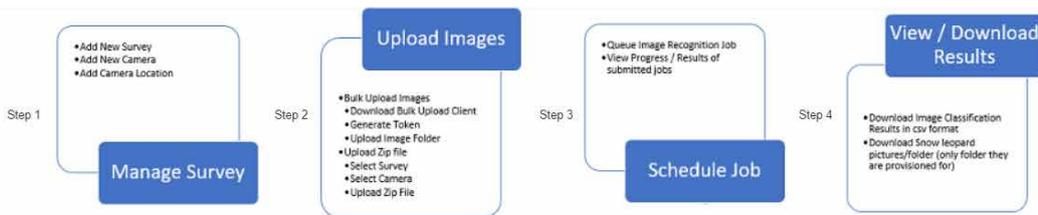


Figure 9. The Artificial Intelligence (AI) based tool developed by Microsoft in partnership with Snow Leopard Trust has been trained to identify photographs with snow leopards from the thousands of photographs of non-target subjects such as moving vegetation, livestock, people and other wildlife. A database is developed where images are allocated probability of containing snow leopards, and those likely to have snow leopards can be downloaded along with the database. Once segregated, the photographs can be used for classifying into individual IDs that can subsequently be used for spatial capture recapture analyses.

are identified between any two sets of images to classify them as different or same cat. Images where up to three patterns cannot be compared (e.g. those which are too close, too far, too dark, too bright or too blurry) must be discarded for analyses. Most snow leopard encounter data can be classified into three categories, easily identifiable (clear, ample images of body parts to facilitate identification), difficult angles (oblique angles, swift movement, only one flank or face or tail photographed), and impossible to identify (blurry, odd lighting, too close or too far). It takes relatively less time to quickly go through easily identifiable images and they can be quickly assigned names on the encounter data sheet, making sure that each identified individual is listed against a camera trap id described in the trapping array. The difficult angles usually require consultation between two or more people with different levels of experience in identifying individuals. Lack of up to three similarities or differences are discarded and saved as impossible to identify images, but kept for future reference, sometimes even in the subsequent years.

Recent experiments indicate that the ability to identify individuals varies across observers and is often also a function of cumulative effort. A tool to

estimate an observer's ability to identify individual snow leopards is available on the GSLEP website (camtraining.globalsnowleopard.org). A self-evaluation session followed by reporting of individual identification scores from this online tool (Fig. 10) can help validate identification skills of the observers, and also add appropriate uncertainty to the estimated densities once specific methods are developed.

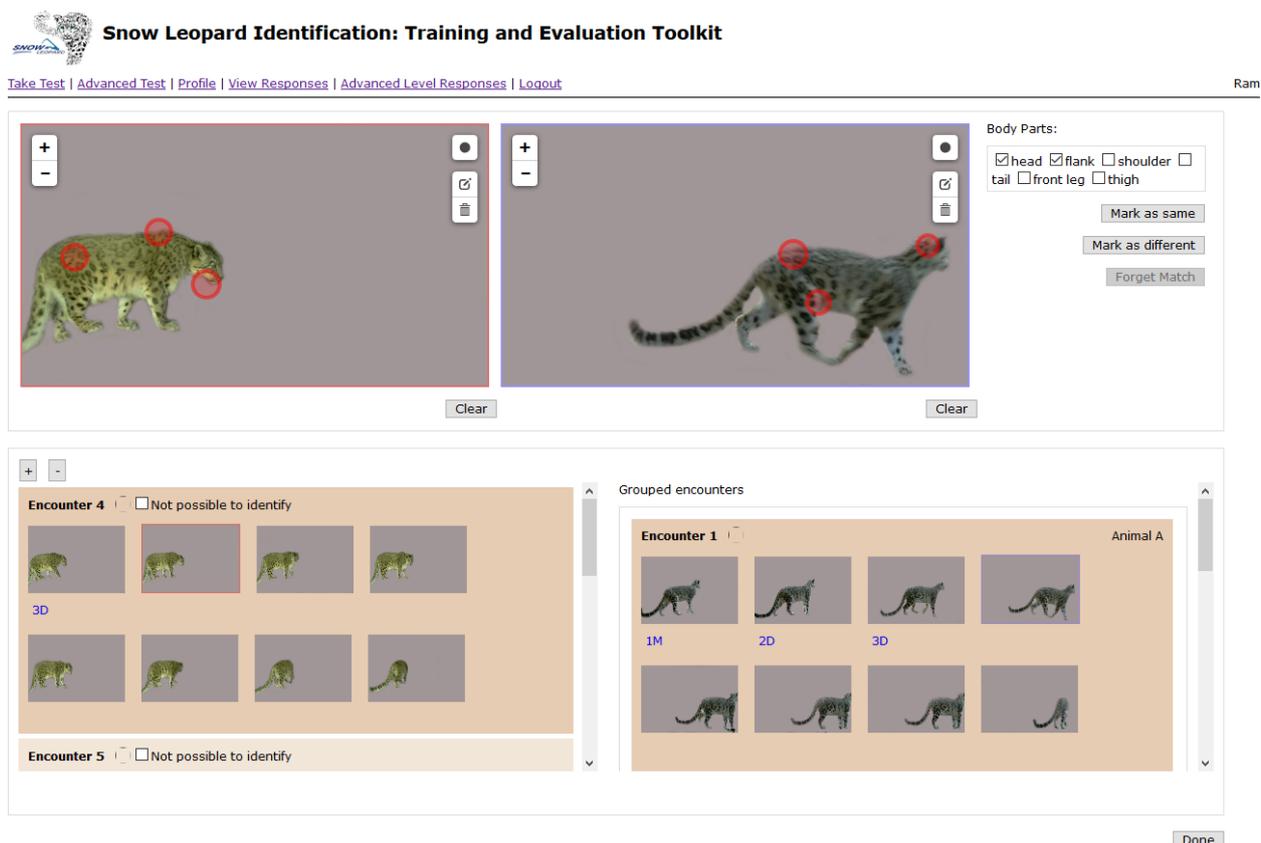


Figure 10. The snow leopard identification training and evaluation tool-kit helps train, practice, evaluate and report researchers' skills in identifying snow leopards as individuals based on their spot patterns. The tool can be used at basic and advanced levels to cater to specific requirements of researchers working on individual identification of snow leopards for population estimation using spatial capture recapture analysis.

4. Creating capture histories

Various formats are used for the encounter data, though count summary data (secr) are some of the most widely used given much faster processing. This format lists session (periods of survey effort or trap arrays between which recaptures cannot occur usually because they are too far apart), name of the

snow leopard, occasion (Each session may have one or more occasions) and trap ID which should be the same as listed on the trapping data (Fig. 11). More details about the data formats can be found on tool specific vignettes on data formatting. Additional information about the age class or sex of the identified individual can be incorporated to model age/sex specific differences in density and detection function.

Minimum data			Covariates		
Individual ID	Detector ID	Occasion	V_1	...	V_c
1	A_3	1	51.0	...	Male
1	A_3	3	51.0	...	Male
2	B_1	2	49.8	...	Female
2	B_1	2	49.8	...	Female
3	A_1	5	33.2	...	Male
⋮	⋮	⋮	⋮	⋮	⋮

Figure 11. Snippet depicting formatted data for camera traps. Detector ID represents a unique identifier to each camera trap; x and y coord. represent easting and northing coordinates (to be specified in projected coordinate format only); Usage Binary can be a single number representing number of days a trap has been operational; or a combination of 1s and 0s representing days when the traps were non-operational; covariates represent variables that are likely to affect the probability of detecting the animal of interest on that particular camera.

5. Developing spatial GIS layers

Spatial capture recapture analyses produce estimates of density without having to estimate the sampling area using ad hoc methods such as Mean Maximum Distance Moved (MMDM) or $\frac{1}{2}$ MMDM. However, it is generally useful to bound the extent of the analysis to prevent unreasonable projections in areas that may not be represented in the sampling. The integration space (also known as mask or state space) represents habitat in the vicinity of camera traps that is (a) potentially occupied by the species of interest, and (b) close enough that animals occupying it might get detected on the camera traps (e.g. Fig. 12). This region can sometimes be defined by hard boundaries such as fences, high ridgelines, steppe, etc. However, in most situations camera traps are placed in continuous habitat without hard boundaries where probability of detecting an animal reduces with distance from its activity center. The integration space should be large enough that no animal with an activity center outside it could be detected by a camera trap, transect or

a hair snare. Packages such as `secr` [29] in R provide tools to check whether the integration space is large enough. The integration space is also used to distinguish snow leopard habitats from non-habitats (e.g. areas with very high or very low altitudes). Suitable habitat comprises of those points that have the potential to be occupied. Treating non-suitable habitat as suitable habitat can cause density in suitable habitat to be underestimated.



Figure 12. Regions of unusable habitat with hard boundaries defined by steppe in this case from South Gobi, Mongolia (also fences, high ridgelines, water-bodies or other physical features in other cases) shall be defined as inaccessible to snow leopards and excluded from the survey region assuming that snow leopards cannot have an activity center within those.

In addition to defining habitat boundaries, habitat covariates for spatial models of density can be stored with the integration space points. Covariates for modelling a density surface can be provided for each point by exporting and adding covariates using a third party GIS software or using spatial tools in R. Density and abundance can be estimated outside the camera trapping array on the basis of the randomized survey design, or using a model (as long as the habitat within the camera trapping arrays are representative of habitat in the areas outside of it).

ii. Genetic sampling

1. Creating transect data and its covariates

Genetic sampling data are either collected on hair snares or by conducting active searches for feces in designated areas. Transect searches are different

than camera trapping in that each detection may have different coordinates (few chances of two encounters on the same location) and these points can be anywhere in a continuum of linear space (defined by transect). Capture data in case of transects needs to be in XY format of density with one row per record. Records are automatically associated with transect lines entered as a series of X and Y coordinates denoting several vertices (start and end points). Usage data can be incorporated the same way as before, only that here it denotes the length of the entire transect instead of the number of days in case of camera traps. As with camera traps, covariates affecting probability of detection of feces can be a function of one or more covariates that can be categorical as well as continuous (Fig. 13)

Detector ID	Vertex	x coord.	y coord.	Usage	V ₁	V ₂
1	v1.1	200	200	101 /	26.1	A
1	v1.2	200	500	- /	-	-
1	v1.3	400	500	- /	-	-
1	v1.4	400	200	- /	-	-
1	v1.5	200	200	- /	-	-

Figure 13. Snippet of typical transect data used to collect samples for genetic analysis of individuals. Detector ID denotes unique id for each transect; vertex denotes two or more vertices that define the transect; x and y coord denote the easting and northing coordinates of each vertex of a particular transect; Usage represents the overall effort or length of the transect; and V₁ and V₂ represent covariates that are likely to affect probability of detecting snow leopards on a particular transect. Each transect may only have a single value of usage and covariates irrespective of the number of vertices.

2. Screening for snow leopard feces

DNA extracted from scat samples can be used as templates for species identification. PCR amplification can be done with mitochondrial DNA (mtDNA) cytochrome-b segment based specific primers of expected product size ~150 bp. Snow leopard specific PCR primer set (CYTB-SCT-PUN F and CYTB-SCT-PUN R) can be used for specific identification of Snow leopards [30].

3. Identifying individual snow leopards using microsatellite

A set of polymorphic microsatellite markers are used to generate multilocus genotypes that are used to match individuals. The number of microsats depends on the available resources, but 6 has been deemed sufficient to

give an adequate Probability of Identity (PID) value in the past [30]. Samples can be replicated for at least three times for microsatellite PCR. Once the microsatellite PCR products are run in ABI genetic analyzer and analyzed by Genemapper 4.0 under specific parameters, allele calling done on the basis of appearance of specific patterns of peaks within expected product range across all six loci. The combination of allele call data in six loci gives a unique genotype of an individual. This is done for all three replicates of each sample. The consensus genotype data for each sample is eventually determined on the basis of quality and pattern of microsatellite peak and its consistency throughout replications. From the consensus genotype data, number of unique individuals is determined by carrying out genotype matching across six loci using commonly used microsatellite data analyzing program called GenAEx 6.41.

4. *Creating capture histories*

Each location where individually identified snow leopard feces were collected is recorded in the capture data as a new record with name of the snow leopard and the location where that particular sample was collected. Unlike camera trapping data, one does not need to provide individual transect names, but actual locations where the scat was collected.

5. *Developing spatial GIS layers that are likely to affect densities*

The GIS layers used in the case of genetic sampling are similar to the ones prepared in case of the camera trapping and follow the same general principles as described above.

iii. Future developments

1. *Genomic framework for the snow leopard across its range*

A set of low-cost, non-invasive genomic tools that can be applied in the field by conservationists is currently being developed. The development of these tools will allow conservationists to identify the species, individuals, and even reconstruct family relationships based on genetic signatures in fecal, hair, saliva, or even environmental DNA samples at substantially low costs and much greater reliability. The genomic and metadata can be aggregated and analyzed on an intelligent cloud platform to give both conservation

practitioners and policy makers an unparalleled insight into the health of populations and species such as information about reproductive success, diet, resistance to disease, human-wildlife conflict, illegal wildlife trade and other key indicators and will provide the basis for data-driven management and policy decisions..

The development of snow leopard genome using single nucleotide polymorphism (SNP) panels will allow genotyping additional individuals at a very low cost from low-quality DNA material. This will in turn result in development of effective, efficient, and affordable DNA field-kits using multiplex PCR with the SNP panels for local, low-cost and rapid processing of DNA collected through non-invasive sampling or wildlife trade samples. A user-friendly intelligent cloud-based database in conjunction with the field-kits will ensure simple, rapid in-country DNA analysis of genomic data for population assessments, management and for forensics in the wildlife trade. The interface will be suitable for individuals with minimal genetic training and provide a robust and easy to use analysis platform which can be applied to a variety of questions.

iv. Occupancy surveys

1. *Cataloguing interview and sign survey forms into detection-non detection data and detection covariates*

Occupancy modelling detection data should typically be formatted in such a way that each row represents a sampling unit and each column represents a survey or sampling effort (Fig. 14). The exact nature of what is considered a repeated survey of a sampling unit will depend on the type of data collected and employed survey methods. For example, in an interview survey, the repeat surveys would be the different interviewees that have indicated knowledge of that cell, however using camera traps, the repeat surveys can be the periods the camera was active for in a sampling unit. If snow leopards were detected in a survey, the data is entered as a 1, and a 0, if snow leopard were not detected. Where an unequal number of surveys conducted at the sampling units, a '-' can be used to indicate that no data was collected for that survey occasion for that unit.

Each interviewee in a survey can have information about more than one sampling units within a survey area. The survey form shall collect data about the areas that an interviewee has visited within the specified time frame irrespective of whether s/he has seen a snow leopard there or not. For areas that the interviewee reports knowledge about, an encounter can be registered as a 1 and non-detections shall be registered as a 0. For situations where informants do not have any knowledge about presence of the species of interest, or if there is no informant, , a '-' shall be inserted instead, denoting no data. Each entry in the detection-non detection matrix must correspond to one or more tables of the same dimensions denoting age, experience, profession, and any other detection covariates that could affect probability of detection and retention of the observation information by the informant. In case informants have also provided information about the species' detection in a particular predefined period from the past, the information can be included in the table (Fig. 15).

Seen SL evidence?	Identified SL?	Detection Past	Village	Interview Id.	Reliable (r) / Unreliable (ur)	Age	Gender	Old profession	Current profession	Time of activity (months)	Duration of time spend per year (months/day)	Total duration of familiarity with the area	Familiarity_zs core
yes	yes	1	Salarang	rbs1	r	28 m		forest guard	forest guard	may to august	2	4	-1.54438801
no	yes	0	Rokcharang	rbs2	r	60 m		farmer	anti-poaching	may to august	1	30	0.210927033
no	no	0	Salarang	rbs3	r	55 m		herder	farmer	june to august	3	15	-0.80175473
			Salarang	rbs3	r	56 m		herder	farmer	june to august	3	15	-0.80175473
			Salarang	rbs3	r	57 m		herder	project labour	june to august	3	15	-0.80175473
no	no	0	Salarang	rbs4	r	40 m		herder	school employee	june to august	3	12	-1.00429108
no	yes	1	Rokcharang	rbs5	r	48 m		herder	bar member	june to november	6	3	-1.61190013
no	yes	0	Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
			Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
			Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
			Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
			Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
			Yangpa	bhaba1	r	54 m		herder	porter	June to september	4	8	-1.27433955
yes	yes	1	Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
yes	yes	1	Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
yes	yes	1	Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
			Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
			Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
			Yangpa	bhaba2	r	55 m		herder	tourist guide	June to september	4	20	-0.46419414
no	yes	1	Yangpa	bhaba3	r	57 m		herder	orchard	June to september	4	25	-0.12663355
no	yes	1	Yangpa	bhaba3	r	57 m		herder	orchard	June to september	4	25	-0.12663355
no	yes	1	Yangpa	bhaba3	r	57 m		herder	orchard	June to september	4	25	-0.12663355
no	yes	1	Yangpa	bhaba3	r	57 m		herder	orchard	June to september	4	25	-0.12663355

Figure 15. Raw interview data representing interviewees reporting about detection or non-detection of snow leopards from various sampling units. Also shown are data collected on age, gender, profession and familiarity or experience to be used as potential detection covariates.

Each transect or its segment, individual camera trap, or an array of short periods (few days or weeks) during which a camera trap was recording images can be treated as a survey replicate. In some cases, multiple methods can be used to collect data, and these need to be collated specifically noting which dataset represents which method. A typical survey form for sign surveys is

often also valuable for identifying best locations to set up camera traps for intensive sampling. Exploratory camera trapping helps determine probability of presence (hence occupancy) of snow leopards in large sampling units. Each detection in the form of a sign or photograph of a snow leopard can be registered as a 1, and days, camera traps, transects (or transect segments), or survey points that were sampled but did not detect presence of the snow leopard are registered as a 0. Locations that are not surveyed, or periods when no data is collected during a sampling period are registered as a '-' denoting no data. Each entry in the detection-non-detection matrix must correspond to one or more tables of the same dimension denoting covariates that are likely to affect probability of detection of the species.

2. *Developing spatial layers that are likely to affect snow leopard distribution*

Occupancy surveys are divided into several sampling units defined by grid cells or watersheds covering the entire length and breadth of the area of interest. Information about detection or non-detection of snow leopard is collected from each unit using the methods described above. Probability of use by a species of interest can depend on one or more covariates including geological, habitat and anthropogenic features. These covariates are assumed to characterize the entire sampling unit during the sampling period unlike survey covariates that can vary for each sampling unit as defined by individual key informants or micro-habitat and weather conditions. Continuous covariates such as altitude, ruggedness, distance from settlements, prey densities, livestock densities, etc need to be estimated for the entire sampling unit to be used effectively as site covariates. Once the occupancy analyses are run, the top model or model-averaged estimates of the probability of a site being used can be mapped by assigning the values to their corresponding sampling units.

c. Data Analysis

i. Spatial Capture Recapture

1. *Identify candidate model sets*

Spatial capture recapture analyses allow both detection probability and density to depend on variables associated with traps, occasions and location

in space respectively. Heterogeneity in capture probabilities due to factors other than distance from snow leopard activity center can be addressed by modeling them as a function of one or more of these variables. Neglecting heterogeneity can result in negatively biased estimates of density and hence abundance.

Individual heterogeneity refers to differences in capture probability due to variables that are specific to individuals (e.g. sex, age class). Models are available for dealing with individual heterogeneity whose source is not observed (e.g. if sex was not recorded and detection probability depends on sex).

Similar to detection, variation in animal density within the sampling area can also be modeled as a function of one or more covariates (e.g. habitat, elevation, water, etc.).

As the first step in running an analysis considering effects of covariates on density and detection, a candidate set of models should be prepared based on an ecologically meaningful set of potential relationships between detection probability (or encounter rate) and density, and their respective covariates. Different models can be used to explore complex relationships by using additive or interaction effects of two or more covariates. Density is often assumed to be constant (homogeneous) within an entire study area. The ability to model density as a function of one or more covariates allows one to investigate whether differences between two sampled areas are because of a specific conservation/protection regime or just because one area has a better gradient of more suitable habitats to support higher densities, for example.

2. *Develop SCR codes*

To analyze the spatial capture recapture data, one needs to first load all relevant data about traps, captures, extent of study area (integration space or mask), trap covariates and density covariates on the R platform. Standard data formats and codes informing the software about the formats being loaded can be found on R package specific vignettes. Alternately, sample exercises

and model formulations are available on the GSLEP website's resource center that can be used to rewrite codes to analyse data from a new area. All trap specific covariates are read at the time the trap information is being loaded by clearly highlighting the particular columns in the trap data listed after '/' as covariates.

The mask can be made by uploading a shapefile defining the extent beyond which there is negligible likelihood of the trapped animals being captured. The integration region can be constructed by specifying a 'buffer' region around the detectors. Typically, a buffer is defined to be bigger than known radius of snow leopard home ranges and checked for bias using built-in secr functions. Spatial covariates can be included in the integration area (mask) either by adding covariates from R objects uploaded as shapefiles or rasters. Alternately one can use a process that requires a few additional steps, but can be executed faster using GIS software where the mask is exported as a csv file, opened as a spatial dataset in a third party GIS application, covariates are added using standard point overlay features, and saved as a csv before reading the csv file as a mask object.

It is advisable to centre and scale continuous covariates to have a mean of 0 and a standard deviation of 1. This standardization, also known as z-score transformation of data, is known to make model fits more stable, and makes coefficients comparable.

It is also advisable to plot integration area (mask), spatial covariates, traps and encounters to visually verify that all relevant information is loaded correctly.

Model codes can be written by using secr vignettes for heterogeneous density and detection. Specific examples of models testing various scenarios based on recent studies on snow leopards can be found on the GSLEP's resource center.

To speed up analyses, secr allows use of more than one core of the processing unit, where available on computers. The cores can however only be accessed

by each model one at a time, meaning a single model estimating density and detection in a single area can only access one core. Multiple cores can only be utilized to improve speed when comparing with more than one site or session. Alternately, if the entire candidate set of models is ready, one can run the whole set by threading all models into a single code block in series and then utilizing one core for each model. This approach can speed up the overall analysis by a number of times equal to the number of cores available on the CPU.

3. *Model averaging (if needed) and presenting density and abundance*

Models are ranked based on minimum AICc (or AIC), which balances the improved fit due to use of more parameters against the increased variance due to use of more parameters [31]. Coefficients of model parameters can be used to determine the direction and intensity of the effects of covariates (on density or detection probability). The AIC weight of each model is an indication of the level of support in the data for each model. If the top model has model weight close to 1, it is simplest to explain density and detection based on the coefficients defined by that model. However, it is possible at times that models will have small AIC or AICc differences (e.g. differences of less than 2). In this case, Buckland et al. [32] recommend use of model averaging techniques.

ii. Occupancy Analyses

1. *Identify candidate model sets*

Similar to SCR analyses, occupancy estimation deals with 2 or more unknowns. The basic models estimate probability of a site being occupied, and probability of detection of a species given presence. Multi-season models estimate other unknowns such as probability of species becoming locally extinct or colonizing new sites. Each of the above unknowns can be tested as a function of one or more covariates. The detection-non detection data, and site and detection covariates as discussed in the data management section can be used, and a candidate set of ecologically reasonable potential relationships can be listed before running the models. Site covariates affect the probability of a species of interest occupying (or using a sampling unit),

whereas detection covariates affect detection probability. It is possible for modeled parameters to have additive or interactive effects of more than one covariate, which can be formulated using standard modeling procedures.

2. *Run models*

The formulation of models varies between PRESENCE [33] and R packages such as RPresence and Unmarked [34], but conceptually both use the same approach. Details about developing specific models can be found in [35] and [36]; User manual for Presence; or the vignette for the R packages Rpresence and Unmarked. A wide range of interactive, full identity, relational models is possible to develop both in PRESENCE and RPresence and Unmarked. Models are ranked based on AIC (or AICc), and can be used to determine the best model balancing between number of parameters and fit.

iii. **Model averaging results**

As in case of Spatial Capture Recapture, it is possible that more than one models explain the variability in detection and probability of occupancy (or use). In case the AIC weights are spread over more than one model, it is best to model-average estimates and report the model averaged occupancy and detection probabilities. Model averaged (or top model in case of single model ranking high) results providing probabilistic estimates for each sampling unit being occupied or used by snow leopards can be plotted to provide reliable distribution maps of snow leopards (or any other species of interest). These modeled probabilities of sampling units being occupied provide valuable stratification to help allocate efforts for intensive camera trapping or genetic sampling for abundance estimation.

PAWS Technical Oversight and Support Panel

Population size of snow leopards in the wild is poorly understood. Population abundance studies using scientifically valid techniques and sampling cover a fraction of their entire distribution range, with the majority of studies being biased towards best habitats. Projections of the global snow leopard population based on these data are not reliable. Governments of all snow leopard range countries have therefore strongly emphasized the need for more robust and expansive monitoring of the world's snow leopard populations, and stated this need in the Bishkek Declaration 2017.

Population assessment of the world's snow leopards (PAWS) is an ambitious initiative that fosters collaborations between governments, ecologists, statisticians, programmers and data scientists. To facilitate such collaborations, select panels and committees have been formed. The exercise is being conducted under the overall oversight of the GSLEP Steering Committee comprised of Environment Ministers of all 12 snow leopard range countries, and coordinated by the GSLEP Secretariat.

An expert panel, comprising of leading snow leopard scientists, statisticians, and a representative of the GSLEP National Focal Points supervises and provides scientific guidance to PAWS. A separate coordination and delivery committee includes organizations and institutions that work closely with the range countries to deliver on the specific goals of executing surveys and helping with subsequent data analyses.

The expert panel is responsible for providing scientific oversight and guidance. This includes development and approval of methods and sampling strategy standards for PAWS, providing support and guidance to implementing partners, and supervising data analyses and interpretations. Additionally, the panel ensures generation of periodic reports and provides a brief progress update every 6 months. The panel meets at least once a year, preferably on the sidelines of the GSLEP Steering Committee Meeting. The panel's term is set for a period of 2 years following which the panel's membership is reviewed and reconstituted. Members may be re-invited or replaced depending on their other time commitments and feasibility to contribute to the PAWS process.

Members of the first panel include:

1. Prof. David Borchers, Head-Statistics, Centre for Research in Ecological and Environmental Monitoring, University of St. Andrews, Scotland, UK (Co-Chair)
2. Prof. Lu Zhi, Centre for Nature and Society, Peking University and Steering Committee Chairperson, Snow Leopard Network (Co-Chair)
3. Mr. Ashiq Ahmad Khan, former Steering Committee Chairperson, Snow Leopard Network
4. Dr. Charudutt Mishra, Scientific and Conservation Director, Snow Leopard Trust, Senior Scientist, Nature Conservation Foundation, and Executive Director, Snow Leopard Network
5. Dr. Chris Sutherland, Assistant Professor, Department of Environmental Conservation, University of Massachusetts-Amherst, USA
6. Dr. James Nichols, Scientist Emeritus, United States Geological Survey, USA
7. Prof. Sandro Lovari, University of Sienna, Italy and Steering Committee Member, Snow Leopard Network
8. Dr. Som Ale, Clinical Assistant Professor, Biological Sciences, University of Illinois and member, Snow Leopard Network
9. Dr. Thomas McCarthy, Head, Snow Leopard Program, Panthera
10. Mr. Wali Modaqiq, Deputy Director General, National Environment Protection Agency and GSLEP National Focal Point, Afghanistan
11. Dr. Darryl Mackenzie, Head, Proteus, New Zealand (invited on special request to strengthen delivery on occupancy design, analyses and training)



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