EXTERNAL SCIENTIFIC REPORT

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# Guidance on estimation of abundance and density of wild carnivore population: methods, challenges, possibilities

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## Abstract

This guidance reviews the methods for estimating relative abundance and density in nine large European wild carnivore species, some representing relevant health concerns and provides insights on how to obtain reliable estimations by using those methods. On a local scale, the appropriate method should take into account the characteristics of the study area, the estimated survey efforts, the expected results (i.e. a measure of true density or just an index of abundance to monitor the trend in space and time) the level of accuracy and precision, and a proper design so to obtain a correct interpretation of the data. Among all methods, the camera trapping (CT) methods, especially those recently developed, are the most promising for the collection of robust data and can be conducted in a wide range of species, habitats, seasons and densities with minimal adjustments. Some recently developed CT methods do not require individual recognition of the animals and are a good compromise of cost, effort and accuracy. Linear transects, particularly Kilometric Abundance Index (KAI) is applicable for monitoring large regions. A large challenge is compiling and validating abundance data at different spatial scales. Based on ENETWILD initiative, we recommend developing a permanent network and a data platform to collect and share local density estimates, so as abundance in the EU, which would enable to validate predictions for larger areas by modelling. It would allow to identify gaps in the data on wild carnivores (including the species not assessed in the present report) and to focus on these areas for improving predictions. This platform must facilitate the reporting by wildlife policy makers and relevant stakeholders, but also citizen science initiatives. Also, there is need to improve the reliability of local density estimations by developing practical research on methods able to derive densities in untested species and situations, making the application of methods easier for local teams.

* ENETWILD Consortium: www.enetwild.com

**Key words:** Abundance, camera trap, density, direct counts monitoring, genetic CMR, indirect methods population estimation, wild carnivores, Wolf, *Canis lupus*, Golden jackal, *Canis aureus*, Brown bear *Ursus arctos*, European badger, *Meles meles*, Eurasian lynx, *Lynx lynx*, Iberian lynx, *Lynx pardinus*, Northern raccoon,*Procyon lotor*, Raccoon dog, *Nyctereutes procyonoides*.

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## Summary

Carnivores are specialized predators with a relevant ecological role inside the trophic cascade, influencing lower trophic levels by regulating the structure and functioning of many ecosystems. In total there are 35 species ("IUCN Red List of threatened species," 2012) of carnivores in Europe including native and exotic ones. Several wild carnivore species are widely distributed across Europe and they use different habitat types, whereas the distributions of others are more restricted or associate to certain regions or habitats. Most species require conservation strategy in Europe, and some promote conflicts in local situations and/or suppose a risk for livestock and/or public health. Therefore, we need to know the abundance and distribution of carnivores across Europe for decision-making processes for conservation, to reduce conflicts and the epidemic risks. Similarly to other wildlife in Europe, pathogen surveillance schemes in wild carnivores lack integration with appropriate population monitoring. Given the diversity of species, their ecology, management, available methods and the geographical diversity of Europe, methodological harmonization of monitoring techniques is duly needed, if possible, using multi-species cost-effective, practical reliable methods. The estimation of carnivore population density is a difficult task. They are normally elusive animals, many of them are nocturnal and live at low densities and are therefore difficult to be observed. The available methods for population estimation are not always fully reliable or at least have not been evaluated comparatively among species, habitats and/or regions.

On the basis of these considerations, this guidance aims to give an overview of the methods used to estimate relative abundance and population density of large wild terrestrial carnivore species over Europe. We point out the drawbacks and the advantages of each technique aimed at obtaining information on the distribution, abundance and demographic trends, and provide some recommendations to select the methods to estimate the population size or density. This guidance deals with medium to large terrestrial European carnivores, and evaluated the methods used in nine large wild carnivore species widely distributed across Europe and/or representing relevant sanitary concerns: red fox (*Vulpes vulpes*), wolf (*Canis lupus*), golden jackal (*Canis aureus*), brown bear (*Ursus arctos*), European badger (*Meles meles*), Eurasian lynx (*Lynx lynx*), Iberian lynx (*Lynx pardinus*), Northern raccoon (*Procyon lotor*), raccoon dog (*Nyctereutes procyonoides*). The contents are organized by species, so the reader can access to a comprehensive review and evaluation of the taxa of interest.

We evidenced the different approaches that have been used to assess wild carnivore population size over Europe. However, not all of them, even those having potential to be used in multi-species designs, have been tested in all species, with particularly emphasis for recently developed camera trapping (CT) methods. As a result, comparisons among different areas and species are often complex to be conducted. It is impossible to highlight the best method for each species (or a universal method for all) or for each environment. Every method on estimating wild carnivore populations has its own logic, depending on the geographical, geomorphological, vegetational, and land use conditions, as well as species biology, which create a plethora of situations where carnivore each species respond with different distribution patterns and diverse population dynamics.

The **general recommendations** for practical implementation of methods to estimate wild carnivore relative abundance and density (see the previous guidance for wild ungulates) are:

- A sampling strategy that optimizes accuracy while limiting the bias of density estimations. No method will provide perfectly unbiased, accurate, precise data if the design of the

study (sampling strategy) is not representative and if the efforts to implement the method are insufficient.

- In order to produce comparable data, a method that provides density estimate rather than abundance should be used if possible.
- The method should be used in a harmonized way: we provide detailed instructions for the design of most recommended methods, but specific protocols must be specifically adapted to local conditions.

On a **local scale** (e.g. in hunting management units), our evaluation of the *pros*, *cons*, accuracy and reliability offer guidance to users to select the methods that best fits their circumstances, and related practical recommendations. We present some basic recommendations, general and specific to species, for the practical use to estimate abundance. The selected method must be accurate and reliable, have the potential to be used for cross validation with other methods, have moderate costs, and be able to adapt to local conditions. Much thought should be given to choosing a method appropriate to the characteristics of the study area (habitat, infrastructure, unpaved roads, etc.), the estimated efforts and the expected results, the need for density or just an index of abundance to monitor the trend in space and time, the necessary level of accuracy and precision; and design of the sample size to obtain a correct interpretation of the data. With repeated sampling over time, both relative indices and absolute estimates of animal abundance can be used to monitor population trends. However, CT methods, especially those recently developed, are promising and can be conducted in a wide range of species, habitats and densities with minimal adjustments, and at any time, to collect robust data. Non-invasive genetic approaches are also becoming more cost-efficient. Non-invasive genetics sampling produce estimates with high level of accuracy and precision and moderate reliability but it is expensive and therefore difficult to apply on a large scale. Telemetry can be applied in all habitats and produce estimate with high level of accuracy and precision (only for territorial species), but the disadvantages are the high cost and effort to catch animals and applicability limited to a local scale only. Overall, the best suggested method to estimate the real population density in several species, unless until some recently developed CT methods are tested, is probably the combination of trapping, marking, telemetry and camera traps. After evaluating the cost, effort, and accuracy, we concluded that the most (potentially) effective method is CT, which has a moderate/high cost and moderate effort, high accuracy and precision, also in low densities, and can be used in most conditions. CTs can allow individual recognition as well and subsequent capture-recapture models. However, high effort is required to catch, mark and follow the animals. Capture- mark-recapture (CMR) produce estimate with high level of accuracy and precision but requires the capture and marking of the animals, greatly increasing the costs and sampling efforts. However, methods that use camera-trap and not require individual recognition have recently been developed and are a good compensation of cost, effort and accuracy. Spatial capture-recapture (SCR and its extensions) are in continuous development, and they also allow for the estimation of home-range centres within the sampled area. However, methods that use camera-trap and not require individual recognition have recently been developed and are a good compensation of cost, effort and accuracy. Random Encounter method (REM, and its extensions) and potentially distance sampling with CT, have been used for some carnivores and promising results has already been obtained to estimate population density without individual recognition. REM requires prior knowledge of average individual speed and activity parameters of the studied population, while Random encounter rate and staying time (REST) does not. For both methods, all needed parameters can be calculated from camera trapping without need of marking or capturing individuals. In the context that CT are becoming more popular and useful for multispecies, a framework based on a stratified design of study sites and application of CT would be an excellent to monitor trend of densities and to be used as a benchmark and calibration for indirect methods and relative abundance. Distance sampling on transects is a cost effective alternative, specially at medium-high densities and where good detectability is good. It can be applied also to calculate relative abundance. The negative effect of vegetation on visibility can be a limitation in areas with a large proportion of the land with forest cover, where this methodology requires high efforts.

In relation to **relative abundance estimates at local scale and large scale**, it can be calculated with several methods that focus on finding signs of animal presence, which can also be used also to calculate local density. The latter often needs calculating local parameters which cannot be extrapolated to other populations or seasons. Indirect methods that rely on counts of the signs of the animals (e.g. droppings, breeding refugia) are less expensive than direct methods and can be applied to the range of habitats found in Europe. Despite the relatively low costs and ease of learning, these methods may have low reliability, accuracy and precision and may depend on the season and often are applied at local scale. However, at present, these indirect methods can normally be used only to measure relative changes in animal density in the same region over time (and among regions if methodology is harmonized), and there are few evidences to indicate that they can be used to compare between regions or to obtain quantifiable estimates of animal density. When possible, they should be applied with direct methods to confirmed data. Good results can be obtained by genetic capture-recapture, but genetic analyses of samples can be expensive. Linear transects are cost-effective method to calculate relative abundance because they can be applied at local and large scale, with high accuracy, moderate reliability and moderate cost and efforts. This approach includes several techniques for detecting carnivores and are adaptable according to the ecology of the animal and the climatic and environmental conditions. Particularly, Kilometric Abundance Index (KAI) uses transect to calculate relative abundance. It is applicable at spatial scales of several hundred $km^2$ and useful for monitoring large regions after stratification of sampling for widely distributed species (e.g. red foxes, badgers). Random placement of transects should be repeated over years for comparisons purposes along time, and it should be tested whether stratification allows for similar sampling effort and bias in each habitat class. Hunting bag statistics deliver large but inaccurate data amounts with low effort, and only for some, non-protected species (e.g. red fox). Hunting data may be sufficient to provide information on large-scale density and relative abundance, long-term trends and in specific hunting areas. However, not all species are hunted, and methods are diverse. Hunting statistics are irregularly and not-completely collected, and in most cases, not possible to be compared. It is necessary to harmonize the hunting data collection frameworks to make them usable and comparable. Vocal methods in big canids have been so far some the most diffused way to perform census and relative abundance estimation, however, they have some important disadvantages: they can just be performed seasonally, only territorial groups can respond, and differentiating individuals requires sophisticated digital sound analysis techniques.

In conclusion, apart from the elaborated methods for estimating wild carnivore relative abundance and density, there is **need for compiling and validating abundance data at different spatial scales**. To be able to receive enough data, we request wildlife managers and hunters to use a method with which an estimation density is performed, which should be accompanied by the collection of detailed hunting statistics for species like the red fox. Based on ENETWILD initiative, we recommend developing a permanent network and a data platform to collect and share local density estimates, so as abundance in the EU, which would enable to validate predictions for larger areas by modelling. It would allow to identify gaps in the data on wild carnivores (including the species not assessed in the present report) and to focus on these areas for improving predictions. This platform must facilitate the reporting by wildlife policy makers and relevant stakeholders, but also citizen science initiatives (e.g. MammalNet[2]) and available open data (as the Global Biodiversity Information Facility[3]). A relevant challenge to improve the reliability of local density estimations are developing practical research on methods able to derive densities in untested species and situations, making the application of methods easier for local teams. For that purposes, the training of local teams is essential. For huntable species, e.g. red fox, it is needed to improve the estimates of populations at large scale by means of hunting data collection frameworks, which should be harmonized to validate and make them comparable across Europe. It is essential to involve the national and regional administrations in data collection and sharing, as well as the hunter and conservationist associations.

[2] www.mammalnet.com

[3] www.gbif.org

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor

This guidance is a deliverable of the specific contract 7 related to the framework contract "Wildlife: collecting and sharing data on wildlife populations, transmitting animal disease agents" (Contract number: OC/EFSA/ALPHA/2016/01 - 01) awarded by EFSA to Universidad de Castilla-La Mancha. The deliverable is indicated in the signed amendment of the specific contract 7 as follows:

- Terms of reference: Provide practical guidance on how to calculate reliable estimation of wild carnivores and presence and abundance from census data, harvest data and/or other type of population estimates
- Deliverable: Guidance for wild carnivores (written report)
- Deadline: 31 Mar 2020

## 1.2. Introduction and scope of the report

Carnivores are specialized predators with a relevant ecological role inside the trophic cascade, influencing lower trophic levels by regulating the structure and functioning of many ecosystems (Ripple et al. 2014, Wikenros 2006). Within the mammalian group, the order Carnivora comprises more than 270 different species which present considerable differences in size, morphology and behaviour (Hunter 2019). In the course of their evolution they have adapted to live in all the terrestrial environments, from Antarctica to the vast deserts of Sahara, in the great tropical forests, in freshwater and saltwater. The carnivores of Europe are largely derived from the Eurasian and African biogeographic zones and therefore exhibits relatively low levels of endemism, as most species tend to have very wide ranges. The order Carnivora includes 12 families, 8 of which live on land, and 8 of which are present in Europe: Canidae (wolves, foxes and related species), Felidae (cats, lynxes), Ursidae (bears), Procyonidae (raccoons and related species), Mustelidae (martens, weasels, badgers, otters, and related species), Herpestidae (mongooses) and Viverridae (genets and related species). There are three aquatic families: Otariidae (sea lions and fur seals), Phocidae (true, or earless, seals), and Odobenidae (the walrus). These aquatic families are referred to as pinnipeds. In total there are present 35 species ("IUCN Red List of threatened species," 2012) of carnivores in Europe including autochthonous and exotic ones. Some of the most popular domestic animals derived from wild members of this order: the domestic dog and cat. Several wild carnivore species are widely distributed across Europe and they use different habitat types (e.g., red fox, badger), whereas the distributions of others are more restricted or associate to certain regions or habitats (e.g. the European mink or the Iberian lynx). This guidance deals with medium to large terrestrial European carnivores, excluding small carnivores (martens and smaller), so as arctic (arctic fox *Alopex lagopus* and polar bear *Ursus maritimus*) and subarctic-boreal (wolverine *Gulo gulo*) species.

Unfortunately, due to significant environmental and climate changes, there has been a high loss of habitats (Ripple et al. 2014) which, in addition to human pursuits, have meant that many species, particularly large carnivores, have been included in the red lists of IUCN (IUCN Red List of Threatened Species. Version 2020-1, https://www.iucnredlist.org). The conflict between carnivores and man is in fact another very delicate issue. As a result of the loss of habitats, animals have been forced to live in areas that are increasingly close to human habitats, resulting in more and more conflicts over damage caused by attacks on livestock and in the beekeeping sector but also to the numerous road accidents (Bartol et al. 2019). In Europe, habitat loss and degradation is the greatest threat to terrestrial mammals, followed by human disturbance, pollution, accidental mortality, overexploitation and invasive species. The majority of European wild carnivores are protected due to it conservation status. However, some of them are huntable (Treves 2009, Packer et al. 2009).

Another threat to biodiversity and ecosystems is the entry of invasive alien species which, as well as causing damage to the environment with consequences for the use of ecosystem services (Pejchar & Mooney 2009), compete with the other native species and in some cases cause their extinction, in fact those invasive species that are able to settle in an area turn out to have adaptive characteristics and resistance (Kolar & Lodge 2001, Park 2004, Tallis & Kareiva 2006, Carrete & Tella 2008, Crowl et al. 2008, Gómez-Aparicio & Canham 2008, Kenis et al. 2009). Among the invasive species in Europe we find the racoon (*Procyon lotor*) and the American mink (*Neovison vison*), carnivores and native to North America, and the raccoon dog (*Nyctereutes procyonoides*) and the small Indian mongoose (*Herpestes auropunctatus*) native to Asia. Other species are present due to historical introductions: the genet (*Genetta genetta*) and the Egyptian mongoose (*Herpestes ichneumon*). For instance, the raccoon has been recently included by the European Commission in the list of invasive alien species of Union relevance (Regulation 1143/2014), which requires each Member State to prevent this species from being presented in a state of natural freedom. This justifies even more clearly how important it is to study and monitor carnivore populations, and to use effective methods of collecting reliable data.

Carnivores may also represent reservoir species for diseases with potential to be shared with domestic animals and/or humans. Specially, member of the Canidae family represent and are responsible for the maintenance of the infectious cycle and hence for the presence, for instance, of rabies. In Europe, the elimination of wildlife rabies using oral rabies vaccination of foxes for more than 30 years has been a success story (Muller et al. 2005). Foxes are the only known reservoir for rabies in Europe, and raccoon dogs are important transmitters, while other carnivores play a less important epidemiological role (EFSA 2015). The demographic expansion of raccoon dogs and their movements after hibernation are risk factors for rabies recurrence. The combined densities of foxes and raccoon dogs, which often share the same habitats, could allow rabies epizootics to persist. The transmission rate of rabies (the average number of susceptible animals infected by each rabid animal) is determined by population density, home range overlap, activity and habitat use of vector species (Wandeler 1980, Holmala & Kauhala 2006, Singer et al. 2009). For instance, Carnivores, particularly the badgers (*Meles meles*) may play also a role for the maintenance and transmission of animal tuberculosis (TB) (caused by the *Mycobacterium tuberculosis* Complex, MTBC). In continental Europe, badgers infected with TB have been reported in both France and Spain (Gortázar et al. 2012) and have become the focus of epidemiological studies in certain areas (Barbier et al. 2016, Payne et al. 2013, Acevedo et al. 2019). In both the UK and Ireland badgers have been implicated in the spread of the MTBC to cattle and in acting as a wildlife reservoir for bovine tuberculosis (Corner et al. 2011). The UK and Irish governments spend millions each year attempting to eradicate bovine tuberculosis (bTB), in part through culling badgers (DAFM, 2016). Carnivores are reservoir of some parasites of zoonotic concern, for instance humans may become infected by hand-to-mouth contact after exposure to a contaminated environment (e.g., *Echinococcus* spp). Human activities and politics (e.g., fragmentation of the environment, land use, recycling in urban settings) have consistently favoured the encroachment of urban areas upon wild environments, ultimately causing alteration of many ecosystems with changes in the composition of the wild fauna and destruction of boundaries between domestic and wild environments (Otranto & Deplazes 2019). Therefore, the exchange of parasites from wild to domestic carnivores and *vice versa* have enhanced the public health relevance of wild carnivores and their potential impact in the epidemiology of many zoonotic parasitic diseases. Risk of transmission of zoonotic nematodes from wild carnivores to humans exists via food, water and soil (e.g., genera *Ancylostoma, Baylisascaris, Capillaria, Uncinaria, Strongyloides, Toxocara, Trichinella*) or arthropod vectors (e.g. genera *Dirofilaria* spp.).

Similarly to other wildlife in Europe, pathogen surveillance schemes lack integration with appropriate population monitoring (i.e. the denominator data). Integrated monitoring means combining population and disease monitoring. Given the diversity of available methods and the geographical diversity of Europe, methodological harmonization of monitoring techniques is duly needed (Ryser-Degiorgis 2013). Determining species distribution range and population abundance is necessary since these patterns represent key information for decision-making processes. Therefore, we need to know the abundance and distribution of carnivores across Europe for conducting efficient population management and to reduce the epidemic risks. The knowledge of carnivore distribution, abundance and density is essential when evaluating risks, adequately managing the risk of shared pathogens (under a One Health perspective) and implementing control activities (e.g. oral vaccination in the case of rabies).

Carnivores are elusive animals, many of them are nocturnal and live at low densities and are therefore difficult to observe. The available methods for population estimation are not always fully reliable or at least have not been evaluated comparatively among species, habitats and/or regions. The population density is the measurement of population size (absolute abundance) per area unit, while the population relative abundance is the relative representation of a species in a particular ecosystem. These measures are calculated by means of methods that give a kind of proxy of the population size (e.g. camera trap). The estimation of carnivore population density is a difficult task. Reportedly by recent scientific literature, the methods traditionally used by wildlife manager are neither precise nor accurate enough to be considered as a gold standard. Many approaches have been used to assess population size in order to increase the detection of each target species (Gros et al. 1996, Forsyth et al. 2019, Mumma et al. 2015). As a result, comparisons among different areas are often complex to be conducted. Another consequence is that it is impossible to highlight the best method for each species or for each environment because the geographical, geomorphological, vegetational, and land use conditions create a plethora of situations where carnivore each species respond with different distribution patterns and diverse population dynamics.

With repeated sampling over time, both relative indices and absolute estimates of animal abundance can be used to monitor population trends. Abundance estimation requires a consistent and standardised application of a technique to be able to detect changes or differences with a certain degree of accuracy, validity and reliability. The methods used may be indirect or direct:

- Indirect methods are those based on the study of animal traces (e.g. interviews, snow and mud tracks, genetic analyses of excrement and hair, wolf howling).
- Direct methods involve the observation of the animal, in some cases, also capturing (e.g. photo traps, radio telemetry, censuses, transects). Most methods being able to estimate the number or density of a population are direct, they can give information on the movements and population structure.

On the basis of these considerations, this guide aims to give an overview of the methods used to estimate relative abundance and density of large wild terrestrial carnivore species over Europe. Some of them are of sanitary concern. The list includes red fox (*Vulpes vulpes*), wolf (*Canis* *lupus*), golden jackal (*Canis aureus*), brown bear (*Ursus arctos*), European badger (*Meles meles*), Eurasian Lynx (*Lynx lynx*), Iberian Lynx (*Lynx pardinus*), Northern raccoon (*Procyon lotor*), raccoon dog (*Nyctereutes procyonoides*), pointing out the drawbacks and the advantages of each technique aimed at obtaining information on the distribution, abundance and demographic trends. The contents are organized by species, so the reader can access to a comprehensive review and evaluation of the taxa of interests.

# 2. Data and methodologies

The guidance is based on literature review performed on literature databases owned by ENETWILD. Additional knowledge on recent literature as well as experiences on the presented census methods came from own experiences and advices from experts within the ENETWILD consortium and from external experts. We present an evaluation of different census methods by species according to the desirable characteristics for monitoring populations in local management units, practicability, applicability and accuracy, and a final summary (Tables 11 and 12) and discussion. The evaluation is based on expert knowledge from experts of ENETWILD consortium.

## 2.1. Definition of wild carnivore population parameters

Wildlife managers have to choose between two main options when trying to assess the wild carnivore population dynamics: i) estimate the absolute population size/density; or ii) estimate a relative index of annual variation in population size/density (or even only monitoring damages caused by wild ungulates as an indirect index of abundance). It is required the knowledge of carnivore population parameters to prepare management and conservation plans. Although a complete glossary of the main population parameters is provided at the end of the report, next we introduce some key concepts related to the correct study design to estimate density and abundance while meeting assumptions of data representativeness and sufficient sampling effort:

**Population size or absolute abundance (N):** it is the size of the population. It can be a known or estimated number, expressed in number of individuals. When related to area unit, it gives the absolute population density.

**Relative abundance or abundance index:** it refers to the relative representation of a species in an ecosystem or study area. Relative abundance can be calculated by different methods (either direct or indirect). Over the years the relative abundance reflects the temporal N or density (d) of a population but does not directly estimate these parameters. Since relative abundance increases with d, it is useful for monitoring animal populations over time, as well as for conducting large-scale studies on the factors that determine the abundance of species. Nonetheless, this relationship cannot be linear (Figure 1). Sometimes, due to financial, logistical, or time constraints, wild carnivore surveys can only deliver relative abundance, instead of total population size or density estimates.

**Population density (d):** it is a measurement of population size per area unit, i.e., population size divided by total land area. The absolute density usually is expressed in heads per 100 ha or km$^2$. It can be calculated by different methods (either direct or indirect).

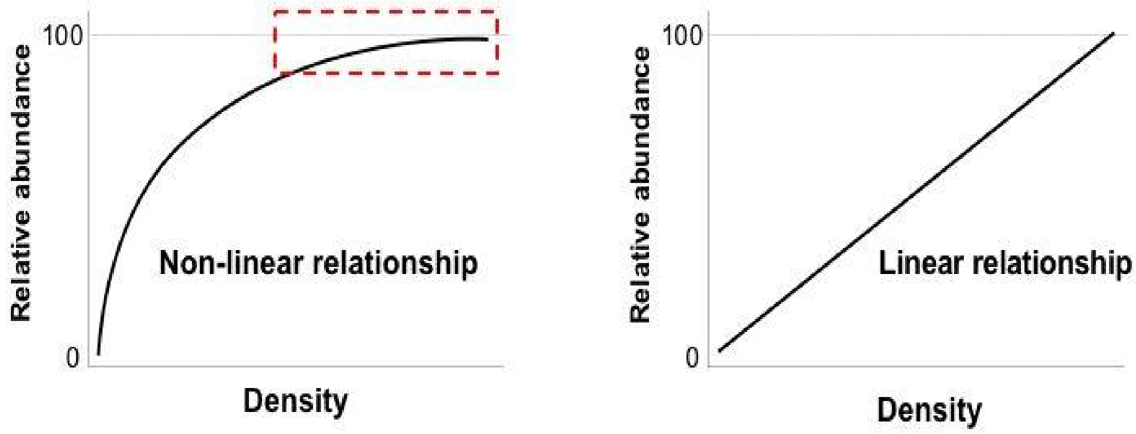


**Figure 1.** The best indices of relative abundance are those that have a linear relationship with the population density (right) in a given area, but often, these relationships lead to saturations for large abundance values (left). The Y-axes indicate the relative (expressed from 0 to 100) value of relative abundance.

## 2.2. Criteria for evaluation of wild carnivore population estimations methods

In order to **compare and make use** of wild carnivore population estimates, we require accuracy, precision and reliability of such population estimates, which must to be expressed in the same (comparable) units (density or relative abundance) or scale (e. g. absent, low, medium, high):

- The **accuracy** of estimations of relative abundance or density refers to the degree to which a measurement represents the true value (i.e. how close a central measurement is to the true value).
- The **precision** of the estimations refers to the degree of resemblance among study results or samples, were the study to be repeated under similar circumstances, that is, how close the repeated measurements are to each other.
- **Reliability** of density and relative abundance depends on the previous concepts, and in this report considers (i) how trustable estimation is when repeated exactly the same way (high precision), and (ii), what is the difference between the mean estimated relative abundance or density and the true value (accuracy or bias, which is useful for comparisons within and among studies). Unbiased data is required to detect true changes in population size. Bias results from poorly measuring the relative abundance or density. For example, when the survey staff is poorly trained, camera trap to quantify wild animal malfunctions and are not checked, measuring too low, or when hunting data represent a biased sample of a population.

We use reliability to evaluate the different methods for estimating density and relative abundance of wild carnivores. High accuracy of average values (Figure 2, left) allows for comparative purposes along time for a given population and for spatial comparisons among populations.

Biased estimations (Figure 2, right) would only allow for comparative purposes when population estimates are considered as a relative index (even when the goal was estimating a density) and bias is normalized (e. g. comparisons along time for a given population).

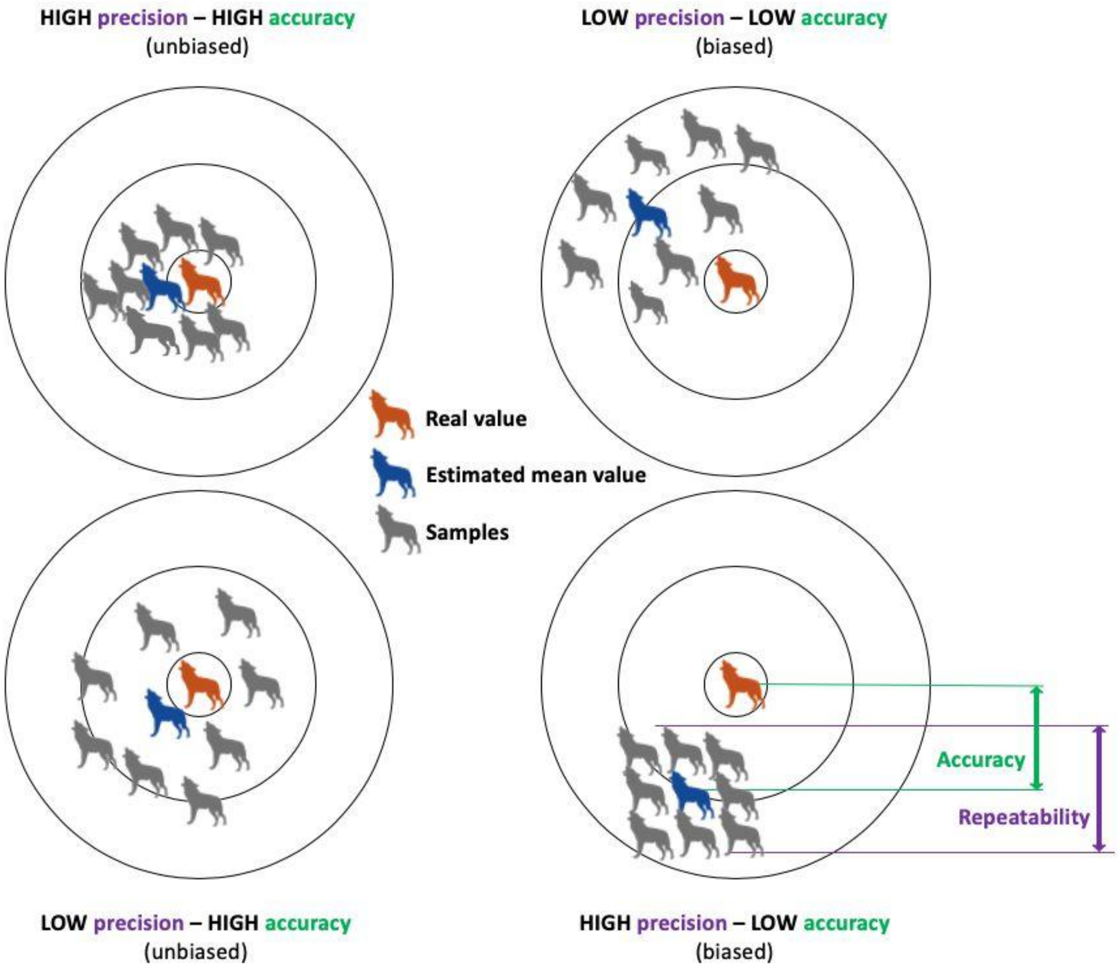


**Figure 2**. Accuracy, precision, and bias of population estimates. The first situation (top left) can be labelled as reliable.

The design of the study and the sampling strategy are essential to correctly estimate precise unbiased (and therefore reliable) density and abundance estimates at local scale, which, in turn, will make data comparable across areas. *No method will provide accurate (unbiased), precise data if the design of the study (usually sampling) is not representative and the effort insufficient. This is especially true for species with an aggregated pattern of spatial distribution and marked habitat selection.*

**Sampling** is used when calculating population parameters on large areas inhabited by wild carnivores, in this case. Getting an estimation of a large area from a sample is useful because it is often impossible to get a measurement from every single animal (or their signs) that we are counting. For this, it is necessary to select some plots/proportion of surface in which density and/or relative abundance are estimated. To optimize sampling protocols, the previous definition of study regions or areas based on the distribution of environmental features and/or populations is recommended. The results of these estimates give rise to an average that will be extrapolated to the whole area of study. A correct study design means avoiding bias during sampling and applying enough effort to estimate precise reliable estimations of density and relative abundance.

Spatial distribution of several carnivores is clamped and clustered as a result of their spatial ecology, land use and distribution of resources, among other factors as well. The most common distributions (Figure 3) are contagious, so the greater the aggregation, the lower the precision of the abundance and density estimations. Therefore, we need to have notions of the distribution of the population in the territory in order to make a good study design.

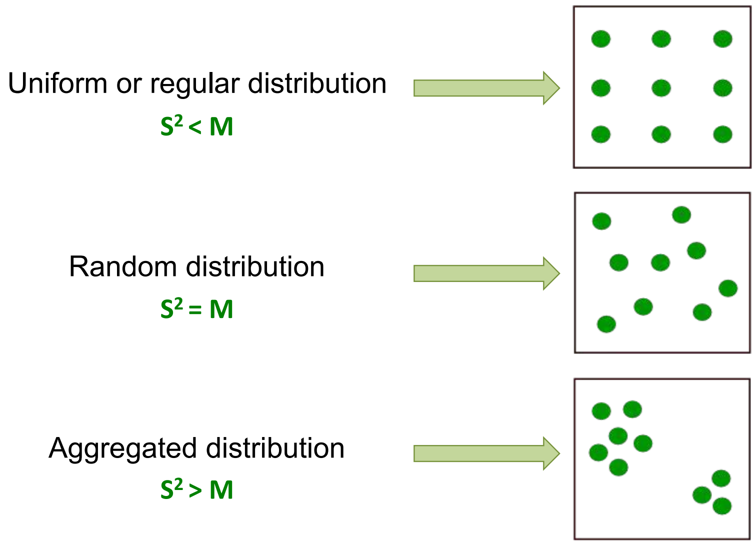


**Figure 3**. Patterns of distribution of individuals, which affect the final population size estimation, for which a correct study design is needed. The aggregated distribution represents a contagious spatial distribution, typical of some wild carnivore species ($S^2$=variance of data, M= mean value).

Since such individuals tend to be aggregated and not randomly distributed, transects, sampling plots, camera trap placement, hides, etc., should be stratified by habitat type, avoiding roads and other singular features (see e.g., Figure 4). All relevant environments within the study area, which may impact wild carnivore distribution, must be considered for the design of a sampling. Since we are sampling, the recommendations to deal with some assumptions are:

- The sample does represent the whole study area. Therefore, we should make sure there are enough samples to be representative. Take a sample from each proportion of the study area, whatever is feasible, but the more the better.
- The sample can be:

- randomly chosen in the best way to fairly represent the characteristics of the study area, and when done in its simplest form, this method is called simple random sampling;
- collected using systematic random sampling. Systematic random sampling is when samples are taken at fixed, predetermined intervals (e.g. a camera trap every 1.5 km). What makes this type of sampling random is that the start point is randomized. A transect line is laid along an environmental gradient and samples are taken at predetermined intervals;
- collected using stratified sampling when it is better to divide a study area into smaller zones with similar habitat or land use and sample within those. When possible, stratifying by (relative) abundance can increase the precision because equivalent encounter/trapping rate (e.g. camera traps) would be achieved within strata (Figure 5). This approach allows us to randomly select from different categories (e. g. habitats), or strata. For example, if the individuals of the population you are sampling in a study area select more a particular kind of vegetation cover (Figures 4 and 5), instead of randomly sampling points transects or plots, you might want to divide the study area into zones of similar vegetation cover and sample within those divisions.

- Transects, plots, and camera trap sites must be placed using fine scale maps of the study area and should be stratified while also considering the description of the habitat composition.
- The sampling effort must be quantified per habitat type (e.g., as the proportion of transects or plots across the different habitat types).
- It should be tested whether stratification allows for similar sampling effort and bias in each habitat class, which requires an *a priori* knowledge of the distribution of habitats in the study area. This is even more important when comparing different methods in each area (no bias should occur due to different sampling effort in each habitat type by the different methodologies).

The result of a given procedure when a stratified design is not performed is a biased estimation of relative abundance and density towards those habitats that are over-represented in the sampling. This would thus cause low precision and incomparability with other values obtained from different study areas.

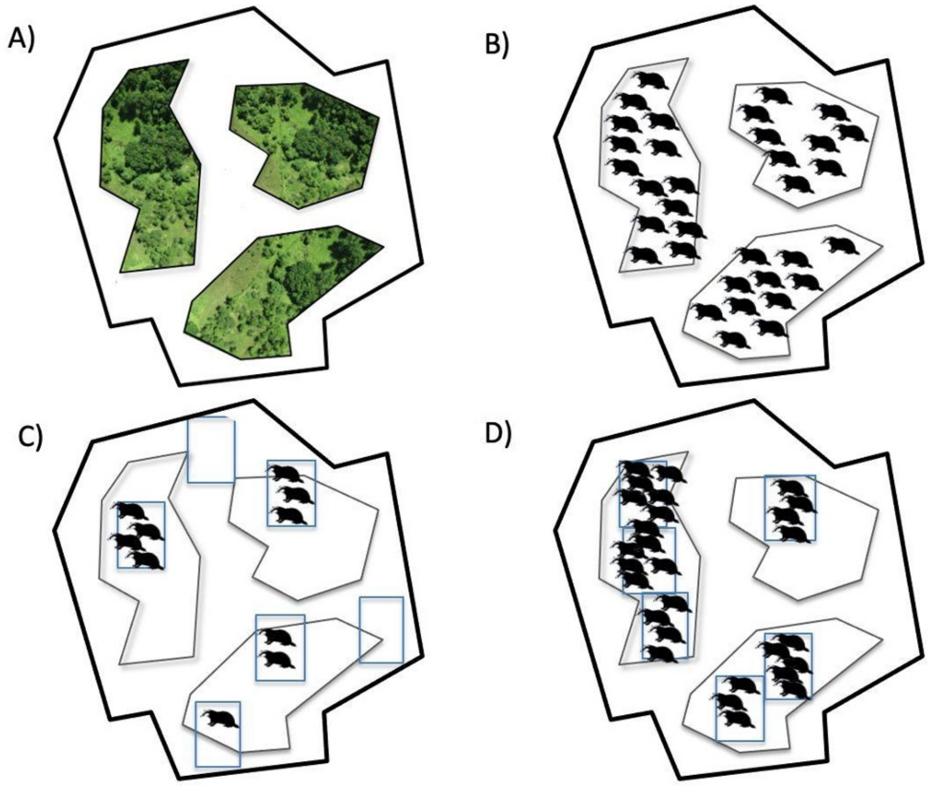


**Figure 4**. Area of habitat used by the species (A, green areas), animal distribution (B). A random sampling (C), a stratified sampling (D). In this case, a stratified design increases accuracy and precision of density estimation (and therefore total population) once relativized to the sampling area (stratum habitat). Modified from Tellería (1986). The outer black line is the total management or ecological unit we pretend to census, in which the animals preferably inhabit or uses the green zones (A).

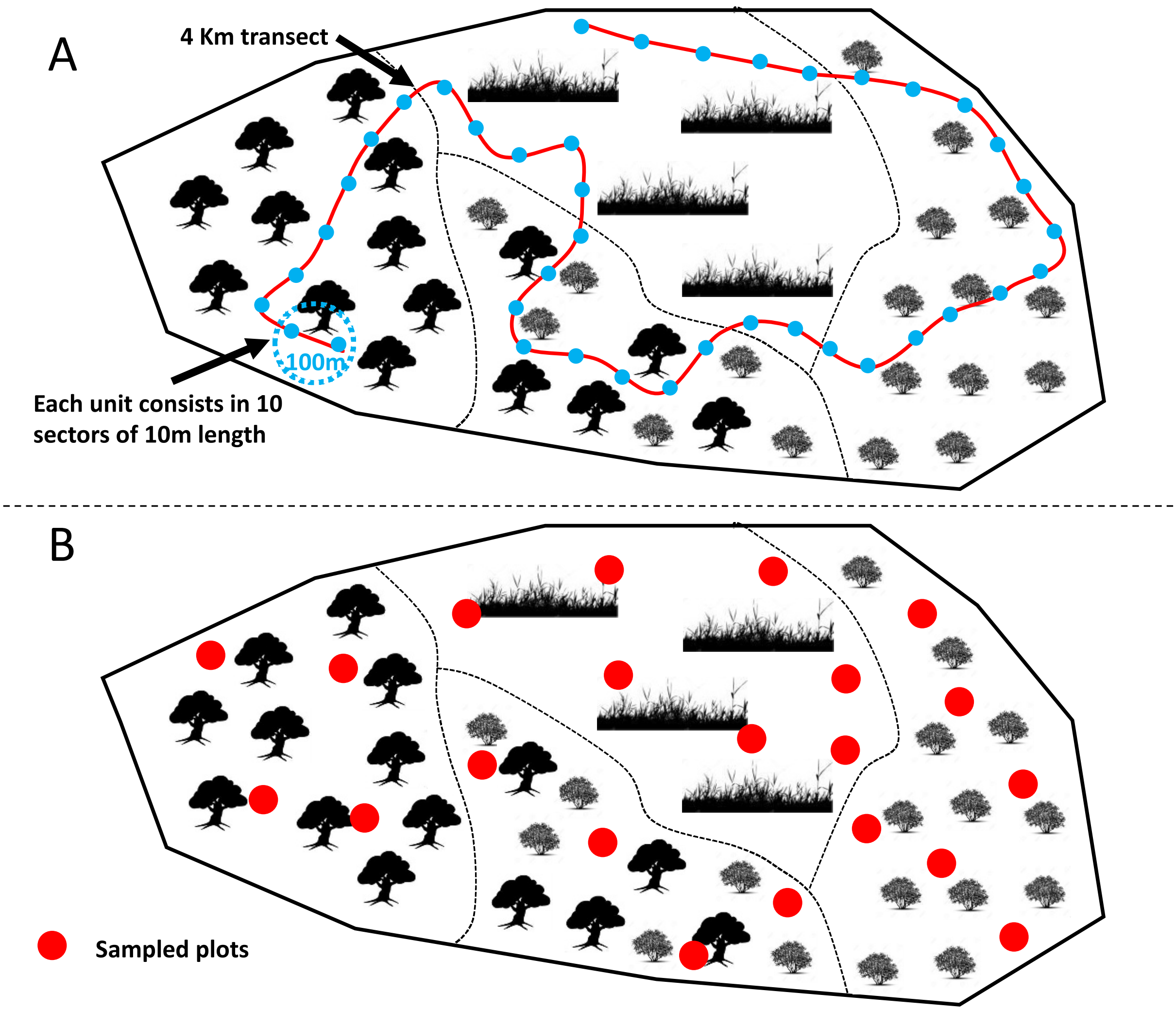


**Figure 5**. Schematic representation of a transect design for (A) faecal pellet frequency counts considering woodlands, scrublands, and open habitats, and (B) signs of presence within plots of excrement counts (with and without cleaning), hides, beat areas or camera trap placements.

# 3. Available methods for wild carnivore population estimation

An overview on the methods for estimation of population density and relative abundance in wild carnivores was published by Gese (2001) (see Iijima 2020, for wildlife in general). Additionally, as a data basis, we used some national reports and international publication in peer reviewed journals (see reference list), the expertise of the ENETWILD consortium members and of 18 experts, which delivered recent insight in national appliance. Similarly, previous reports within the ENETWILD project realised an analysis of the available methods to estimate abundance and other population parameters in wild boar and wild ruminants.

As a general organisation, the authors proposed a classification of the methods based in two main groups: direct and indirect methods.

As indicated, **direct methods** are based on the direct observation of animals (visual contact with animals); therefore, they depend on their rhythm of activity/seasonality. A critical point is that they are generally not applicable on a large scale due to the high costs (but see Sobrino et al. 2009) and they are not readily applicable to all habitats (which is not the case of camera traps, see below). Moreover, they require the presence of expert personnel. There are long term monitoring regional programs based on direct methods (e.g. Sobrino et al. 2009). However, density estimates derived from direct methods (capture-mark-recapture and radiotracking methods) offer data necessary for other studies on the estimate of the abundance (Sadlier et al. 2004). However, density estimates derived from these methods are likely to represent the benchmark against which other estimates of abundance are measured.

**Indirect methods** rely on counts of the signs of the animals (e.g. droppings, dens) and are normally less expensive than direct methods and can be applied to the range of habitats found in Europe. However, the major factor currently limiting the use of indirect methods is that their relationship with population density has not been validated in most cases (Figure 1). Indirect signs can be found in a wide variety of habitats, and, with practice, they are readily identifiable, and less labour intensive. However, at present, these indirect methods can only be used to measure relative changes in animal density in the same region over time, and there are few evidences to indicate that they can be used to compare between regions or to obtain quantifiable estimates of population density.

For each of the nine species in this document, both direct and indirect methods have been analysed.

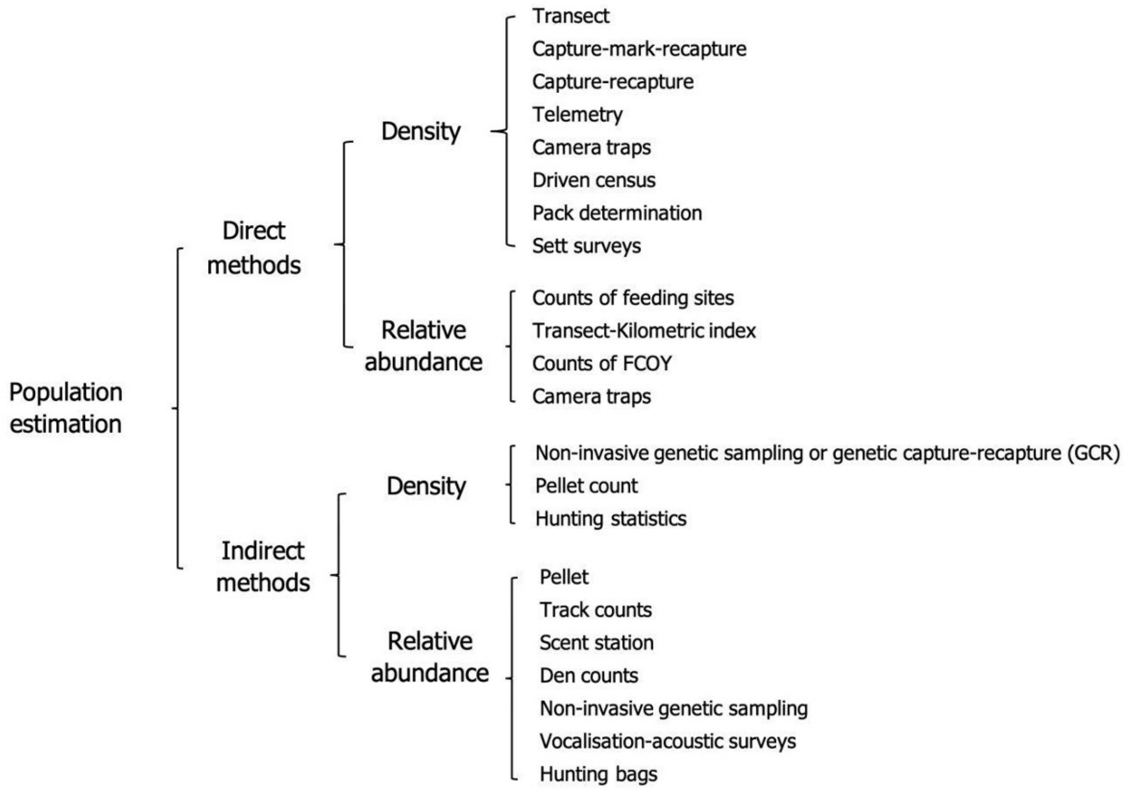


**Figure 6**. Classification of available methods for estimating of wild carnivore population density and relative abundance. Direct methods: methods based on the direct observation of animals, Indirect methods: methods based on the detection of presence signs, see the text for more details. Note that some authors classify set surveys as indirect. FCOY: counts of females with cubs of the year. Camera traps (as a tool rather than a method) include a number of methods to estimate population size, such as the application of capture-recapture, or more specific camera trap methods, such as the Random Encounter Model (REM) and its extensions. As a simplification, camera trap-based methods, which are direct, are illustrated as a single item.

The comparisons of several methods have taken place, in almost all cases, analysing the results in terms of precision (more often) and accuracy (rarely found in literature) of the results in one species only. Additionally, they have also been considered the monitoring efforts - in terms of working days and the costs of equipment - necessary to collect and to analyse the data. In the following sections, we will only describe methods recommendable in terms of sufficient reliability and affordable effort by species, and a specific section is included for some species regarding methods that have not been tested yet but are promising or potentially suitable. An evaluation of each method in term of accuracy and precision, cost and effort and suitability to derive population density or relative abundance is presented for each species, and a final section addresses a common discussion and present the conclusions.

# 4. Description and evaluation of available methods by wild carnivore species

## 4.1. Red fox (*Vulpes vulpes*)

The red fox is a canid of the *Vulpes* genus and is the most widespread terrestrial carnivore mammal species. It is distributed throughout the northern hemisphere, from the Arctic Circle to southern North America, Europe, North Africa, Asian steppes, Northern India, Japan and China; and they were brought to Australia approximately in the 1840s where they have played a major role in the decline of a number of species of native animals. It is not present in Iceland, the Arctic islands (including Greenland) and some parts of Siberia (Hoffmann & Sillero-Zubiri 2016). It is well adapted all types of habitats, from mixed deciduous and coniferous forests to cultivated areas, river areas, prairies and to urban and peri-domestic environments, spreading from sea level to 4,500 m a.s.l. (Lovari & Riga 2016, Hoffmann & Sillero-Zubiri 2016).

The morphology of the red fox can vary according to the geographical area and the environment frequented. It is the largest species of the true foxes (the ones belonging to genus *Vulpes* ); the length of the body varies between 46-70 cm and that of the tail between 30 and 60 cm; in general, males reach larger dimensions than females. The fur varies from dark brown to beige on the upper parts, while the lower parts are white (Lovari & Riga 2016).

The red fox is omnivorous and has no specific food needs. Being a highly adaptable carnivore, its home range varies in size from 10 to over 5000 ha, proportionally to the availability and quality of food resources (Lucherini et al. 1995). The availability of food resources also influences social organization (Zabel & Taggart 1989). Within the family areas, each male can reproduce with 2-4 females, or it may happen that a dominant male and female form a monogamous pair. There may also be individuals on the territory which do not reproduce. The mating is in January-February and the gestation lasts about 50 days. The size of the litter is 4-5 puppies, more numerous litters (1-14) occur in case of high human-induced mortality. The births occur inside dens, from late January to late May, where the puppies stay for 10-15 weeks. Their weaning is around 6 weeks and independence at 4 months, for safety reasons, the mother can move the puppies to other dens (Lovari & Riga 2016). The red fox is considered a game species in most European countries, and their populations are normally "controlled" by hunting or trapping, although they have natural predators, such as golden eagle or some mammal top predators. It is reservoir to relevant pathogens, even zoonosis, such as *Echinococcus multilocularis* or rabies in central and eastern Europe (Freuling et al. 2013, Oksanen et al. 2016). They are opportunistic predators (i.e. on small game) and scavengers. Therefore, the estimation of red fox abundance is of great interest from ecological, conservation, sanitary and management points of view.

**Direct methods**

**1. Linear transects**

<u>Objective:</u> By observing individuals / groups along transects, the objective is to estimate the local density or relative abundance.

Methodology: The animals can be detected by using linear transects or point transect. In this last case, the observations of individuals / groups take place from fixed points (e.g. from hide-outs or high seats) and, using distance sampling (see below), by recording the distances from the observer and to the animals (Ruette et al. 2003). Transects can be carried out by using a vehicle to monitor large areas, or alternatively on foot, but it may be demanding in terms of sampling effort and it is difficult to apply at spatial scales of several hundred km$^2$. Transect methods can be implemented during the day or during the nightlight by using spotlights to detect the individuals and binoculars to confirm the species (Sobrino et al. 2009). Spotlighting are recommended to commence 1 h after sunset and normally they are conducted from the back or on top of the car, at approximately 10 km/h (Sharp et al. 2001). The presence of a reflecting *tapetum lucidum* increases the probability to detect individuals during nocturnal spotlight counts.

Practical guidelines:

- An autumn census could be used to detect the increase in fox numbers due to recruitment and dispersal, while an early spring estimate would comprise largely breeding individuals.
- A correction factor can be calculated by radio-tracking local home range of red foxes, and determining the mean percentage of time spent by several individuals in open habitats, where they are detectable by spotlighting (Beltrán et al. 1991).
- Within each season, spotlighting is conducted several nights (Sharp et al. 2001). High occupancy levels and / or low detectability will tend to increase the number of repetitions required. In case of low density it is necessary to make long trips or numerous aftershocks on different days to detect presence (Mahon et al. 1998).
- An alternative to the spotlights for some nocturnal species is the use of infrared, but in the case of the fox it has been found that it cannot improve significantly the number of animals observed (Focardi & De Marinis 2001).
- In the study design phase, it is important that a subsample of sites is visited multiple times to perform an analysis of detectability. These precautions can serve to avoid the effects of false absences generated by low detectability and allow obtaining more reliable estimates.
- A disadvantage of using spotlights is that its application depends largely on the availability of passable tracks or roads in vehicles. Therefore, it is not applicable in regions with limited vehicle access.
- In areas where animals are hunted, they may exhibit flight behaviours when perceiving the **proximity** of the vehicle, so the detection probability will be lower.

Transect methods include different techniques to detect animals and to estimate population density or indices of abundance:

*Linear transect: KAI (Kilometric Abundance Index)*

Transects have been used to obtain index of relative abundance known as KAI (Kilometric Abundance Index) of red foxes on a regional scale for instance, in Italy (Beltrán et al. 1991), Australia (Kay 2000), France (Stahl 1990, Stahl & Migot 1990), Great Britain (Reynolds 1995), Spain (Sobrino et al. 2009) and North America (for foxes of San Joaquin, Ralls & Eberhardt 1997). KAIs can be obtained from the total number of foxes counted each night and is calculated by dividing the number of observations (individuals or groups) between the total number of kilometres travelled.

$$KAI = \frac{N°fox}{N°\,nights \cdot N°\,km/night}\ or\ \frac{N°fox\,(total)}{Nº\,km\,(total)}$$

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: Three samples per site will approximate the optimum to minimize the variance of the estimates of occupation (Mackenzie & Royle 2005) and 2-3 samples per site will maximize the power to detect trends (Field et al. 2005). However, high levels of occupancy and / or low detectability will tend to increase the number of repetitions required. This confirms the importance of performing several replicas in each sampling, which allow obtaining more reliable estimates.

- Reliability: reliable but need for assumption

  - animals in the vicinity of the transect are detected
  - do not move before being detected
  - each individual is detected only once
  - the detection of each individual is independent of the other detections
  - the influence of variables such as the observer, station or the weather can be considered negligible

- Cost and effort required: Relative low cost and effort, the advantages of this method are its simplicity and speed of obtaining.
- Spatial scale: local but applicable at spatial scales of several hundred $km^2$ (e.g. (Sobrino et al. 2009), and useful for monitoring large regions after stratification of sampling.
- Comparability: spotlight counts to index red fox abundance was assessed in an arid environment through a comparison with a scat deposition index (active attractant) (Sharp et al. 2001), however comparisons require specific evaluations for each situation and method. Useful for multi-species monitoring.

Practical guidelines:

- Consider the principles of sampling (summarized at https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1449) when calculating population parameters on large areas inhabited by red fox. It is necessary to select some proportion of surface in which density or relative abundance is estimated by different transects. The results of these estimates give rise to an average that will be extrapolated to the whole area of study. A correct study design means minimising bias during sampling and applying sufficient effort to produce precise reliable estimates of density or relative abundance. Red fox spatial distribution is a result of its spatial ecology, land use and distribution of resources, as well as other factors. We therefore need to have notions of the distribution of the population and habitat selection in the territory in order to make a good study design.
- Field operations developed during the same season

- Random placement of transects, at least repeated over years for comparisons purposes along time.
- Transects should be stratified by habitat type, avoiding roads and other singular features. All relevant environments (different habitat which similar use for red fox or characteristics may be grouped) within the study area (which may impact red fox distribution) must be taken into account for the sampling design.
- It should be tested whether stratification allows for similar sampling effort and bias in each habitat class. This is even more important when comparing different methods in a given area (no bias should occur due to different sampling effort in each habitat type by the different methodologies).
- Transects must be placed using a fine scale maps of the estate and should be stratified while also taking into account the description of the habitat composition.
- The sampling effort must be quantified per habitat type (e. g. as the proportion of transects across the different habitat types).
- The result of a given procedure when stratified design is not performed is biased estimations of relative abundance and density towards those habitats that are over-represented in the sampling. This would thus cause low precision and incomparability with other values obtained from different study areas.
- Spotlight counts are done from 10 p.m. to midnight, which is the period coinciding with the peak of fox activity.
- For each transect, the team should consist of tat least two people: a driver and an observer; each transect is covered three to five times per year.
- Use of a thermographic camera (TI) is optional.

*Linear transects: fixed width band*

Objective: By observing individuals / groups along transects and within a pre-defined detection band, the objective is to estimate the population density.

Methodology: The transects, usually by spotlighting, are used to estimate the densities by defining a band on the sides of the transect in which all the individuals present are assumed to be observed. The width of this band is defined by the distance perpendicular from the travel line where visibility is close to 100%. Through this method (Beltrán et al. 1991) calculated densities (range 2.01 to 4.3 foxes / km$^2$) considering a fixed observation band 150 m wide. Ruette et al. (2003) reported few differences among the effective strip width estimates between the 12 sites (range 191-286 m). In an agricultural area of western Poland, Panek & Bresiński (2002) used transects linear fixed band with 200 m width (densities ranged from 1.02 in spring to 1.63 foxes/km$^2$ in winter).

Evaluation:

- Appropriateness to estimate density or abundance: density
- Accuracy and precision: medium, need good visibility
- Reliability: medium
- Cost and effort required: Relative low cost and effort, the advantages of this method are its simplicity and speed of obtaining
- Spatial scale: local

- Comparability: comparisons still require specific evaluations for each situation against other methods. Useful for multi-species monitoring.

Practical guidelines:

- Scott et al. (2005) in the arid region of Jordan estimated that at least 15 repetitions are necessary to estimate the density of foxes accurately (in total 150 km) and that there is no significant effect of the moon phase on the number of foxes observed.
- In order to obtain reliable estimates of density at least 30 observations are necessary.

## 2. Distance sampling

*Distance sampling on transects*

Objective: By observing individuals / groups along transects and their group size and position relative to the observer, the objective is to estimate the density after modelling the probability of detection in relation to the distance to the observer.

Methodology: By observing individuals / groups along transects and recording the perpendicular distance between the observer and the observed animal, the objective is to obtain a detectability function with which to estimate the local density of the red fox population. The "distance sampling" techniques (Buckland et al. 1993) have been used to determine the density of foxes and the usefulness of this method to estimate densities in different environments at moderate to high densities (e.g. Heydon et al. 2000, Ruette et al. 2003).

The distance sampling method is based on the probability of observing an animal decreases with increasing distance to the observer. The study of distribution of these observation distances allows to obtain a function of detectability that serves to estimate the density of the observed species. In addition to the number of observations it is necessary to write down the perpendicular distance of each observation to the route line, in order to obtain the detectability functions. From the observation distance and angle respect to the line of progression of the observer, it is possible to calculate by trigonometry the distance to the observer.

To select the most appropriate model, the Akaike information criterion (AIC) is used or, if this is similar between the models, likelihood-ratio tests. In order to obtain reliable estimates of density at least 30 observations are necessary (ideally 60-80), so in the case of species such as the fox it is often necessary sampling efforts of the magnitude of tens or even hundreds of kilometres in one transect, or, if not possible, repeat it a number of nights until the necessary number of observations are collected. The adjustment of detectability functions and density calculations can be performed through computer programs such as "DISTANCE" (for Windows; http://distancesampling.org; Thomas et al. 2010).

One drawback of the distance sampling method is the effect of vegetation on visibility which ncrease the required effort to obtain observations. Distance sampling is able to model the probability of detection as a function of the distance in close vegetation. Different studies indicate that during the night, the main period of activity of the foxes, open zones are preferably used. This may be a limitation in areas with a large proportion of the land with forest cover, where this methodology requires high efforts (Heydon et al. 2006). Therefore, stratified design of sampling and analysis of data in relation to habitat are required to avoid sampling bias. The location of transects along roads and in open habitats probably induced biased results. Thermographic cameras are an option to increase detectability and contact rates.

Evaluation:

- Appropriateness to estimate density or abundance: population density.
- Accuracy and precision: medium-high.
- Reliability: need for assumptions: (i) all animals on the transect are detected; (ii) the distances are measured accurately and (iii) the animals are detected at their original location.
- Cost and effort required: The high effort required is compensated by low cost, as trained volunteers are often available (experienced hunters). Nonetheless, volunteers have to be motivated.
- Spatial scale: local.
- Comparability: A minimum number of transects must be sampled depending on the distribution of red fox in the study area, requires areas of good visibility and stratified design to be comparable among sites.

Practical guidelines for distance sampling:

- Consider the practical guidelines above regarding KAI, and particularly, stratification of design, which is even more relevant for distance sampling to produce reliable and comparable results.
- It is recommended to use a rangefinder to estimate the distance because the estimation of distances by direct observation is subject to errors, especially at night and they can also vary considerably between observers (Blackwell et al. 2004).
- Infrared technology can help to detect the foxes (Blackwell et al. 2004).
- As potential food resources changes with season (e.g., ripening mast [*Quercus* spp.], corn [*Zea mays*]), the frequency in which animals are observed on or close to the survey route can decrease (Blackwell et al. 2004).
- Need of at least 30 sightings (ideally 50-60).

*Distance sampling as "random" point counts (e.g. from hide-outs)*

Objective:

By observing individuals / groups from fixed points and recording the perpendicular distance between the observer and the animal, the objective is to obtain a detectability function with which to estimate the local density of the red fox population. To our knowledge, there was just one attempt using this method for red fox (Ruette et al. 2003).

### 3. Capture-Mark-Recapture (CMR)

Objective: To identify part of the animals of the population since the proportion of animals "recaptured" during the samplings is known. The local population size (density) can then be estimated.

Methodology: Capture-Mark-Recapture (CMR) methods include a large family of methodologies reviewed in (Buckland et al. 2000). This method needs trapping and marking (Chandler & Andrew Royle 2013), or at least, individually identifiable animals (e.g., through CT by means spotted fur or morphological signs present on the animal's body that allow identification (Karanth & Nichols 1998, Sarmento et al. 2009). Animals of a population are captured, individually marked or photographed to allow identification, and released at the capture site. The frequency of marked individuals observed in subsequent capture sessions is then used to estimate population size. Capture and release for scientific purposes may require licensing in some countries. Recapturing is highly affected according to the first capture and experiences of individuals and it may increase the potential risks of injuries, also lethal, for the recaptured individuals. There are different mathematical models for closed populations (without births / deaths or emigration / immigration) and for open ones. If there is no certainty about the condition of the population then it is better to use models for open population. Numerous mathematical models have been developed to estimate the population size based on CMR data and there are computer programs specific for this (e.g. CAPTURE, Rextad & Burnham 1991; and *R* packages like "unmarked").

Due to its high effort and local conductibility, this method may only be used for scientific purposes, knowing that better methods exist. Recently, spatial capture-recapture models (SCR), in a broad sense, are replacing traditional CMR for wildlife monitoring (Royle et al. 2013, Jimenez et al. 2019).

For red fox, CMR has been conducted with recapturing with re- sightings by camera traps (marking or individually identifiable animals are needed) has been performed (Sarmento et al. 2009, Dorning & Harris 2019). There are survey techniques which facilitate individual identification of subtly-marked species like red fox (Dorning & Harris 2019), evidencing that accuracy is enhanced by camera-trapping techniques that yield large numbers of high resolution, colour images from multiple angles taken under varying environmental conditions.

Spatially explicit capture-recapture (SCR) models (or spatially explicit mark-resight, SEMR models) can be used for abundance and density estimation, frequently through individual identification of target species using camera-trap sampling. This is achieved by modelling the number and positions of the individual centres of activity as well as distance-related heterogeneity in capture probability (Chandler & Royle 2013).

Generalized spatial mark-resight (Gen-SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks (Gen-SMR is included in the section "Camera trapping without individual recognition"). For indirect CMR see "Genetic analyses of pellets (genetic CMR)". Research suggests that the use of effective attractants in CT sites can significantly increase the odds of detection. Captive research reports that to increase the probability of detection of the red fox the best lure is the combination of lynx urine and valerian solution (Ferreras et al. 2018), but is also possible to use bait as live birds, domestic cat urine or mean beat (Ferreras et al. 2018, Barea-Azcón et al. 2007, Díaz-Ruiz et al. 2016, Sarmento et al. 2009).

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high.
- Reliability: reliable but need for assumptions:
    - the population is closed (without immigration or emigration) and there are no births or deaths between captures
    - that all individuals have the same probability of being captured
- Cost and effort required: Although it is very precise and accurate, it is expensive and requires high effort It is necessary to mark a significant proportion of the population.
- Spatial scale: local.
- Comparability: While the use of effective attractants can significantly increase detection probabilities, they may differently affect red fox detection probabilities in CMR studies by CT; and therefore, it is needed to assess the effectiveness of several attractants to evaluate their usefulness for non-invasive survey methods.

Practical guidelines:

- Individual identification of subtly marked species may be facilitated from carefully designed survey techniques, accuracy is enhanced by large numbers of high-resolution colour images, from multiple angles taken in varying environmental conditions.
- Younger foxes should be excluded because they have few distinguishing features.
- With photo identification systems many photos may be deleted due to image quality or individual's lack of identification, risking the loss of important information in population studies, and are likely to lead to erroneous conclusions in a number of behavioural studies.
- Sarmento et al. (2009) estimated abundance using the heterogeneity (Mh) model of the software program CAPTURE.
- Cameras approximately at 2-4 m from the lure, are attached at a 40-50- cm height (Swann et al. 2004) and are arranged in order that also lateral views of foxes are photographed, thus detecting most diagnostic features.

### 4. Telemetry

Objective: Radio-tracking, or wildlife telemetry is the technique of determining information about an animal through the use of radio signals from or to a device carried by the animal. Marking with radio-emitters the individuals of a population, estimate the density assuming that each individual contributes to density with equal proportion to the proportion of their home range included within the catch area.

Methodology: The main type of radio-tracking in use today for red fox is global positioning system (GPS). Thanks to radio-tracking, it is possible to identify the home range and their use by radio-followed animals (Van Etten et al. 2007a). Density can be estimated indirectly from radio-tracking data based on the inverse relationship between the size of the home range and the population density (Trewhella et al. 1988). Marking with radio-emitters most of the population and tracking them for a period of at least one year is currently considered one of the most reliable method to determine the density of territorial carnivores, which is often combined with other methods such as CT studies (Jimenez et al. 2019) and can be used as a reference to contrast results obtained by other methods (Schauster et al. 2002).

However, this method indicates only the minimum density since it assumes that each home range is used exclusively by a single animal, which is never true. The density is estimated in the area where there is certainty of having marked most of the existing individuals, which is usually defined by the polygon that encompasses all catches ("catch area"). It is assumed that each individual contributes to density with equal proportion to the proportion of their area of champion included within the catch area. The density is calculated by adding the contributions of different individuals.

Evaluation:

- Appropriateness to estimate density or abundance: density
- Accuracy and precision: high (but it assumes minimum density)
- Reliability: reliable but need for assumption
- Cost and effort required: Expensive costs, high effort, difficult to catch animals. Due to its high effort and local conductibility, this method may only be used for scientific purposes.
- Spatial scale: local, can be applied in all habitats
- Comparability: It is necessary to mark a significant proportion of the population

Practical guidelines:

- Trapping efforts are conducted mostly in areas where red foxes or red fox tracks have been observed (Silva et al. 2009).
- Applying colourful ear tags can help identify animals caught (NASCO Farm & Ranch, Fort Atkinson, Wisconsin) (Van Etten et al. 2007).

## 5. Camera trapping (CT) without individual recognition

Objective: Converting trap rates into population density without the need for individual identification and/or where animals are unmarked.

Methodology: Most density estimators used for population monitoring of carnivores are based on CMR data that are often based on the individual recognition, implying invasive costly marking techniques and individual tagging. Beyond CMR, a number of models to estimate the size of a population where animals are unmarked and/or without the need of individual identification has been published (Rowcliffe et al. 2008). Recently, promising results in other species were obtained applying camera trapping to estimate density without the need for individual recognition (Palencia et al. 2019, 2020).

*Random encounter model (REM)*

This method was developed and tested in several species (Rowcliffe et al. 2008, Rovero & Marshall 2009, Rovero et al. 2010, Rowcliffe et al. 2011, Rovero et al. 2013, Rowcliffe et al. 2013) and some carnivores (Cusack et al. 2015, Tosh & Twining 2017) and it is based on the behaviour of gas molecules (Rowcliffe et al. 2008). This method rescales the trapping rate (y / t) to population density using the day range (DR, i.e. daily distance travelled by an individual), group size (i.e., the mean number of animals in a group), and camera-related parameters (radius and angle of camera detection).

$$D\ (density) = \frac{y}{t} \cdot \frac{\pi}{DR \cdot r \cdot (2 + \propto)}$$

Where $\propto$ is the angle and *r* the radius of detection of the cameras, *DR* is the daily range of displacement. The model assumes the following:

- Camera traps can capture animals in any direction, and animal signals are detectable from any

direction.

- Animals are in a homogeneous environment and move in straight lines of random direction with speed.

- Camera traps can capture animals at a detection distance r and that if an animal moves within this detection zone, they are captured with a probability of one.

We recommend ≤ 10 m, even less for small species, which makes comparisons between sites possible, assuming there is equal detectability for most camera trap models and species. However, variability among CT models and species (the body size influences the probability of detection as a function of the distance and must be tested.

The DR is the parameter most costly and time-consuming to be measured, but it can be estimated from photo trapping data (Rowcliffe et al. 2016) rather than relying on fine resolution GPS or radio-tracking data. However, comparative studies to finely describe the distances travelled among different regions, habitats and seasons are required to evaluate the potential practicability of REM. The procedure to calculate DR is based on the key assumption that all individuals in the sampled population are active at the peak of trap rate, and then, that the trap rate at a given time of day is proportional to the level of activity in the population.

*Random encounter rate and staying time (REST)*

REST is an extension of REM (Nakashima et al. 2018). The speed parameter is not necessary, and it is substituted by resting time in the CT detection area. It still has not been published in red fox to our known. However, unpublished data indicate that REM, REST and distance sampling with CT have shown good agreement for red fox in South Spain (Palencia et al. 2020).

*Point transect using camera traps (CT-DS)*

It consists of an adaption of point transect methods (distance sampling) to camera traps (CT-DS) (Howe et al. 2017). This approach has not yet been validated against other methods for red fox and other species (e.g. ungulates). Similarly, it still has not been tested in red fox to our known.

*SCR for unmarked*

To estimate density or population size for explicit spatial regions, can be used Spatial capture-recapture (SCR) models (Royle, 2004; Forsyth et al. 2019; Jimenez et al. 2019; Chandler & Andrew Royle, 2013;) through individual identification of target species using camera-trap sampling, as indicated in the section above. These are an extension of CMR without capturing for unmarked or unidentifiable individuals (Chandler & Royle,2013, Ramsey et al. 2015). SCR is spatially explicit and therefore it uses capture histories for each geolocated CT, while CMR uses the overall history of capture withing a given study area. This is achieved by modelling the number and positions of the individual centres of activity as well as distance-related heterogeneity in capture probability. Because the models are spatially explicit, a by-product of the estimation process is the likely locations of home-range centres within the sampled area. Spatial mark-resight (SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks. However, in many cases, it is not possible to read the marks in camera-trap pictures, even though individuals can be recognized as marked. A new extension, Gen-SMR, has been applied to red fox density estimation that allows for this type of incomplete identification, demonstrate good performance (Jimenez et al. 2019). Gen-SMR is broadly applicable as it addresses the common problem of incomplete identification of marked individuals during resighting surveys.

*N-Mixture models*

The number of observed individuals by any method rarely represents a reliable estimation of the number of individuals occurring in a given area. In the last years formal approaches have been proposed to estimate animal abundance from repeated counts at fixed sites, without marking individuals to identify them (e.g. Belant et al. 2016, Ketz et al. 2018, Gomez et al. 2018). The number of individuals detected in a given site is counted using standard monitoring techniques (CT in this case), and each site is generally surveyed in multiple occasions. The repeated counts in a given site are then used to jointly estimate the detection process (imperfect detection) of individuals and population size based on N-mixture models (Royle 2004). As they do not require capture or manipulation of individuals, such models might allow collecting abundance information over larger areas compared to traditional approaches. N-mixture models are increasingly used to estimate population size on the basis of species counts, although no experience is available in red fox.

*CT in general*

Evaluation:

- Appropriateness to estimate density or abundance: density (but possible relative abundance).
- Accuracy and precision: High (also in low densities), although do not always provide appropriate confidence intervals.
- Reliability: quite reliable, different analysis-methods adaptable to local conditions, practicable, conductible (for several species at the same time), need for assumptions and previous information. For instance, the REM and spatial explicit methods, respectively, require knowledge of average speed and home-range size parameters of the studied population (however, the first can be derived from CT data, and if the number of recaptures is enough, you do not precise home-range estimations).
- Cost and effort required: those without the need of capture (non-invasive) are affordable, moderate costs, need for CT, theft of cameras in some locations, medium effort, manpower required to analyse photo/video material, photos might be collected via online-photo-databases.
- Spatial scale: local, adaptable to regional studies.
- Comparability: Evaluate missing detections with your specific camera model and under your study circumstances (camera did not release although animal was there, Amelin 2014, Fischer 2018).
- All assumptions should be stated in literature.

Practical guidelines:

- Since different CT methods are available, we will focus on those most practical, previously classified as of relative medium effort, and able to generate reliable data over a wide range of situations across Europe. Random encounter model (REM and its extensions) does not require individual recognition, whereas Spatial mark-resight models (SMR) work recognizes only a part of individuals. We do not exclude the use of other CT methods, but we recommend those, already tested in similar species, in terms of practicability as they can provide an excellent balance between the required effort (and level of knowledge) and reliability.
- Field operations developed during the same season (optimally during summer, pre-hunting) in order for density estimations to be comparable across sites and time.
- Random placement of CTs (recording coordinates) to obtain a regular uniform distribution: a minimum 5 x 5 CTs (to guide the location using a buffer of 100 m around the nodes of the grid) at 1.0 Km x 1.0 Km or less for SMR, 1.5 Km x 1.5 Km for REM.
- Use a grid of CTs covering all the habitats of the study area, stratifying (at least one grid / 5000 ha) according to habitats, so that no of CTs in each habitat are finally proportional to habitat availability.

- Infrared CTs located on posts or vegetation 30 cm above the ground. Use sticks to reference the animal captured by the camera-trap in REM method as indicated in (Keuling et al. 2018).
- Infrared CTs set 24h per day and to take up to three (or more) consecutive pictures at high sensitivity, selecting the minimum time lap between bursts (1 second if possible).
- The date and time of capture automatically stamped onto each image or recorded as metadata
- CTs checked weekly to change batteries and memory cards, as well as ensure proper functioning and baiting.
- Overall sampling period minimum of 1 month.
- Key CT settings have to be known: angle of detection and effective range must be known to determine the surveyed surface.
- For REM, average daily travelled distance can be calculated from trapping records, which requires indicating distances in a blank picture, signalized as follows to describe the speed of passes (after taking this blank picture, signals can be removed).

## 6. Driven census

Objective: The animals are driven into a delimited area by trained people, sometimes assisted by hounds, and counted as they cross the line of observer to determine density.

Methodology: This method can be used in woodland or dense vegetation cover (Goszczyński et al. 2008). For example, Telleria and Sáez-Royuela (1984) estimated densities of fox by sampling in plots of 75 hectares of oak hardwoods. Collective hunting events may provide indications on abundance and may be used for obtaining density estimation of red fox.

Evaluation:

- Appropriateness to estimate density or abundance: density and relative abundance
- Accuracy and precision: low to moderate. The accuracy of the results will be positively correlated with the number of beaters, and negatively correlated with the size of area sampled.
- Reliability: moderate, adaptable to a wide range of local conditions, practicable, conductible, possibility to obtain simultaneous data on other animals, particularly game species. This is probably not an ideal method to use in nature reserves due to perturbation issues.
- Cost and effort required: high, a large number of observers and beaters must be mobilized at one. However, this problem can be solved by profiting from drives organized for hunting (Sáez-Royuela & Telleria 1988). The time spent in organizing a sufficient number of helpers (observers, beaters, and their hounds, etc.) should be considered when planning a drive census.
- Spatial scale: local, and regional if good data hunting activities are recorded under an organized framework.

- Comparability: low.

Practical guidelines:

- Each forest compartment to be censused is surrounded by observers who must maintain a separation between them (50-100 m), enough to maintain visual contact.
- The whipped area is defined by the total travelled distance by the line of beaters and the distance between both ends of the line.
- This method can be done also in areas with low visibility such as forests or scrub areas dense.
- Drive counts during hunts can be used to estimate winter numbers of red fox. In that cases, the number of beaters should be almost equal to the number of observers (hunters) participating in the counts.
- Each observer records on an observation form the species and number of individuals if grouped.
- A coordinator collects the same information on animals seen by the beaters. After beating each block, the coordinator collects information from all observers and immediately resolves any possible inconsistencies in order to minimize the likelihood of double counting.

**Indirect methods**

**1. Non-invasive genetic sampling**

Objective: Determining the effective population size by genetics (individuals or pellets).

Several conceptually different types of $N_e$ (the effective population size) can be distinguished, but the most commonly used is the number of individuals in a population who contribute offspring to the next generation (Royle 2004). The estimates of $N_e$ tend to provide a lower number than an actual population size. As indicated for CTs, genetics is a tool to generate data, and subsequently, population size estimations are derived by implementing other approaches (such as capture-recapture).

Methodology: Non-invasive genetic sampling (NGS) is a safe and cost-effective alternative to physical capture and can be used to obtain information on sex ratio and home range of individuals in a population (Kohn & Wayne 1997). By genetic analysis of polymorphic mini or microsatellites extracted from excrement it is also possible to identify individuals and thus obtain population estimate (Beltrán et al. 1991). This molecular technique can be used for faeces and hair from which DNA can be extracted (Kendall & Mckelvey 2006). Specifically, faeces contain a small amount of DNA on their surface coming from its intestinal wall of the animal that produced them (Kohn & Wayne 1997). Scats can be collected systematically across an area and a trained scat detection dog can be used to locate scats (Mumma et al. 2015); carnivores often defaecate along trails or territorial boundaries, this allows you to find it easily.

Hair can be collected from den sites, snow track routes, or other areas frequented by the species of interest , Kendall & Mckelvey 2006). It is also possible to use an odorous attractant to attract animals to a sampling point where a hair collection device will be present (Kendall & Mckelvey 2006). To collect hair, you can use barbed wire, or glue or adhesives or brushes; the devices will be effective if hair is caught and hair samples containing useful DNA are obtained (Kendall & Mckelvey 2006).It’s possible to do a genetic analysis of pellets (genetic CMR) marking of faeces with radioactive isotopes (Beltrán et al. 1991).

Evaluation:

- Appropriateness to estimate density or abundance: density and individual presence.
- Accuracy and precision: High, particularly for capture-recapture approaches.
- Reliability: High, however determining the $N_e$ (local) by genetics is possible if appropriate sampling and corrections based on population dynamics parameters are applied.
- Cost and effort required: mainly in winter, high effort, expensive, need for assumptions, hardly applicable in low densities, still needs to be combine with other sampling techniques in most cases. This approach is time demanding and relatively expensive (Luikart et al. 2010).
- Spatial scale: local.
- Habitat: all, performs well in forest areas.
- Recommendations to improve comparability and accuracy: these techniques have the molecular modelling part (modelling of marking and recapture has been already discussed before). The following is regarding the molecular side:
  - Make robust designs for DNA sampling; avoiding biases in the collection of data associated with the behaviour of animals or habitat selection.
  - Assesses the probability of capture locally to consider the necessary effort.
  - Standardize and test laboratory protocols.
  - Collect information on hunted (sampled) animals.
  - Make a good selection of quality samples.
  - Optimize the use of genetic markers (within and between laboratories).

Practical guidelines for faeces:

- To estimate absolute abundance, individual identification using microsatellite is less efficient than capture-recapture in cameras trap (Harrison et al. 2002).
- For a program at regional level of study of populations of carnivores like the fox, genetic analysis methods are not recommended for the high cost, although it will decrease in the future.
- In a case study in which there are two different fox species, it is recommendable to investigate if there is a seasonal difference in frequency between them. Moreover, seasonality and species can have an effect among relative altitude and sampling locations.

Practical guidelines for hair:

- DNA degradation may be rapid in warm, wet environments and hair snares may become snow covered in the winter.
- DNA from hair is often less degraded than DNA extracted from scat, and generally provides more consistent results at far lower cost.
- Given that the use of a bait is based on a behavioural response from the animal it is essential to know biology and understand the behaviours to be successful in the sampling.
- Hair collection can survey large, remote areas and locate rare, cryptic animals. Also, it allows discrimination between closely related species, individuals, gender.
- The collection structure must permit or promote use by the animal.
- For most species, hair sampling is currently less effective for estimating population size than for estimating occupancy (Kendall & Mckelvey 2006) because hair collection methods may not be efficient enough to provide a capture-recapture sample size sufficient for meeting this objective. Moreover, extracted DNA may be of too low quality to reliably identify individuals.
- When (Harrison et al. 2006) sampled kit fox hair in cubbies, saw that dog brushes snagged more hair during molting, but lint roller tape was better at sampling hair from winter coats.

## 2. Pellet counts

Objective: Record the number / frequency of red fox droppings per unit of effort to calculate local density or a relative abundance.

Methodology: Pellet counts are frequently used to monitor wildlife species. There are lots of methods based on counting the number of pellets or their frequency along transects or in plots (Webbon et al. 2004, Kolb & Hewson 1980, Beltrán et al. 1991). It allows comparisons of abundance relative to over time, or between different areas, although it requires trained personnel in the search and identification of excrement. It can be used also in heavily wooded areas, where den counts and spotlighting are not suitable (Cavallini 1994). To apply this methodology, February - March represents a period of relative stability within fox populations; it is towards the end of the dispersal period, before the peak period of births, and towards the end of the main culling period (Webbon et al. 2004). However, seasonal variations due to climate constraints may determine the best period. In addition to the abundance of species, the number of faeces found is determined not only by the abandonment of the species but also by the rate of defecation and by the rate of disappearance. The defecation rate can be estimated in tests with captive animals, although variations may exist due to sex, age, social status, type of food and different behaviours of marking that may vary throughout the year. On the other hand, the disappearance rate can be very variable depending on various factors of both climate type (temperature, humidity, rain, Cavallini 1994). A strong limitation when comparing fox abundance in different seasons is rainfall effect (Kolb & Hewson 1980). Without knowledge of rainfall pattern, seasonal variations in the index may be erroneously interpreted (Cavallini 1994).

*Pellet counts with cleaning*

Objective: Starting from the estimate of the accumulation rate of excrements per unit of time and surface unit (Putman, 1984), and relating them to the defecation rate per individual and unit of time, calculate population density (or a relative abundance) (Putman 1984, Schauster et al. 2002, Webbon et al. 2004).

Methodology: Unlike the pellet count without cleaning, all transects within the study area are initially crossed to remove all previously existing scat (cleaning). After 2-6 weeks walking the same transects a second time and counting the number of accumulated droppings between the two visits; if the land is flooded or if there is snow on the ground not include the area in any analyses (Schauster et al. 2002, Webbon et al. 2004). It can be estimated an accumulation rate of excrement per day and unit of surface, and related to the daily defecation rate per individual (Schauster et al. 2002). Several factors can affect the use of this method of monitoring fox abundance, like the daily defecation rate, but it can be estimated from captive tests (Webbon et al. 2004).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance and population density.
- Accuracy and precision: low (in most cases can just be used as an index of abundance).
- Reliability: low. Requires experienced staff for recognition. Parameters to estimate population density (defecation and sometimes disappearance rates) must be locally calculated and cannot be taken from literature).
- Cost and effort required: low to medium costs/efforts. For density, local parameters (defecation and disappearance rates are values) requires performing *in situ* experiment, which increase the effort required. The clearance and estimation of defecation and disappearance rates require lot of work.
- Spatial scale: local.
- Comparability:
    - To estimate density, calculate local defecation rate and dung persistence rate for that population during that season and for the year.
    - Design must be adapted to aggregation of faecal pellets distribution.

Practical guidelines:

- Observers should be given a guide describing the morphological characteristics and odour of fox faeces in comparison with those of other species. They must record information like the transect route, the position of scats found on both visits and habitat information.
- The use of 2 observers is recommended to minimize the chance of missing or misidentifying scat along the route (Schauster et al. 2002).
- Animals are motivated to deposit "new" scats when "old" ones are encountered. Knowlton (1984) noted that removal of scats may slightly reduce deposition rate in subsequent days. He noted also that up to 30% of individual scats were routinely missed each time transects were walked, regardless of the observer, and suggested that transects be walked twice (Schauster et al. 2002).
- Extraction of DNA from faecal samples may enable comparisons to be made over small spatial scales (if you also wish to apply genetic approaches, see above).
- The use of non-random transects associated with linear features vs. random transects data may have possible bias although some studies suggest that non-random transect placement may not unduly affect density estimates (Walsh & White 1999) but may

significantly reduce the logistical and cost implications of large-scale surveys (Webbon et al. 2004).
- All habitat information can be analysed in GIS for measurement of areas and lengths, habitat diversity (e.g. Simpson's diversity index) to examine the effect of strata, survey protocols and culling and habitat-related variables on relative fox density
- A minimum of 30 sites may be required to obtain a consistent estimate of relative fox density (Webbon et al. 2004).

## 3. Track counts

Objective: To record the number / frequency of snow tracks per unit of effort to calculate an abundance index for a local population. In general, by recording the number of any tracks left by red fox in the sampling effort unit, indexes of relative abundance ca be calculated (Indices can be calculated as number of set of tracks per day, presence/absence of tracks or either length of interval between sets of footprints).

Methodology: Snow tracking have been used to calculate abundance indexes based on counts of tracks detected per unit of sampling effort. Indices can be calculated from the total number of sets of tracks per transect per day, or the presence or absence of footprints in each length of transect, or the length of interval between sets of footprints (Wilson & Delahay 2001). It may be sufficient to simply count sets of tracks to determine presence or absence and derive a crude estimate of population size when the target species is rare and wide ranging (Wilson & Delahay 2001). It is performed in the winter season, usually one day after a snowfall (Kurki et al. 1998) to minimise the effects of freezing, thawing and windy conditions (Wilson & Delahay 2001).

Snow track surveys are conducted by walking along the transects that usually are divided into shorter segments, such as kilometres, which constitute the units of sampling (Wilson & Delahay 2001), or by vehicle to be able to make longer journeys in less time. Where multiple transects are used, they must be separated a sufficient distance to minimize the probability that the footprints of the same individual will be registered in more than one transects (Smallwood & Fitzhugh 1995).During monitoring can be also collected genetic samples (e.g., hairs, scars) from snow-tracks or day beds (Squires et al. 2004).

Fingerprint monitoring can also be carried out on mud or sand (Conover & Linder 2009, Barlow et al. 2008, Long 2008, Beltrán et al. 1991). The type of habitat adjacent to fingerprint transects affects the indices of traces of red fox, regardless of its density, due to differences in the number of times each individual crosses the transects in different habitats (Stanley & Bart 1991). In fact, substrate quality is also a factor that may influence index values (Smallwood & Fitzhugh 1995, Wilson & Delahay 2001).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: low (just index of abundance).

- Reliability: Requires experienced staff for recognition (Squires et al. 2004), this method might be biased by several factors, like experience and motivation of observers and weather conditions.
- Cost and effort required: low costs/efforts, easy, volunteers, well working system ("tradition")
- Spatial scale: local.
- Habitat: all, snow / sand need for predefined coefficients.
- Comparability: low among populations, if standardized, useful for comparison of the same population along time.

Practical guidelines:

- To avoid impacting the animal's behaviour, track sets should be backtracked 1st(Van Etten et al. 2007).
- Snow tracking also allows collection of scat and hair for DNA studies.

## 4. Scent-station

Objective: To calculate a relative abundance index by using the frequency of red fox visits to scent-stations.

Methodology: This technique has been used to detect changes in the population growth rate through time in carnivore species, an important parameter for population dynamics modelling and management (Travaini et al. 1996). The scent-station normally uses the line like the sampling unit, so that every transect must be spaced one km away from the others to minimize the probability of an animal visiting more than one line of stations in a night. Within each transect, the scent stations are arranged at a distance of 300-500 m from each other (Soyumert & Gürkan 2013, Travaini et al. 1996). In the centre of each station, a smelling bait is placed. The perfume stations are activated for 1-3 consecutive nights, per month or per season. It is preferable to apply this method in dry seasons as rain can make the scent-stations inactive (Soyumert & Gürkan 2013).

The stations are checked every day and the eventual footprints are noted (Travaini et al. 1996). The soil, which may possibly be altered by traces, is prepared again and the station was re-activated with bait (Soyumert & Gürkan 2013).

Nights are not independent sampling units because red foxes may visit the same or nearby stations on consecutive nights. Travaini et al. (1996) considered that "a paired sampling unit is defined as each 10 x 2 line of 10 scent stations run for 2 days. The daily total of visits of the red fox is used to calculate a relative abundance index as follows:

$$Relative\ abundance = \frac{Total\ red\ fox\ visits}{Total\ operative\ scent\ stations}\ x\ 1000$$

To collected footprints, it can also be used track plate foot (Gompper et al. 2006, Sargeant & Cypher 2003), a method in which aluminium plates are used to applied soot, animals pass the box first walk on the sooted surface. Soot is transferred by the feet to the slightly adhesive shelf-paper where it is deposited in the form of highly detailed tracks. Tracks left on the shelf-paper are often much clearer and more distinctive than those made on the sooted surface, often allowing one to distinguish between even closely related species.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance
- Accuracy and precision: low, it is only allowed to identify the species but not individuals, so it is only possible determine whether or not each station has been visited by each species of interest. Sargeant and Cypher (2003) show that repeated operation of stations can lead to habituation and reduce, rather than increase, precision of results.
- Reliability: low, it requires specific prior training of the personnel, the rates of visits to scent stations are affected in addition to the species density, due to various factors such as climatology, the season of the year, habitat characteristics and human activity (Sargeant et al. 1998). A disadvantage is its low sensitivity to small changes, the impossibility of compare different areas or habitats with each other, poor spatial and temporal resolution or low statistical power when evaluating changes.
- Cost and effort required: Low cost and effort, the main advantage of this method is its simplicity and speed of obtaining results.
- Spatial scale: Local
- Comparability: Comparable across time when sampling condition are repeatable, difficult to standardize comparisons among areas.

Practical Guidelines:

- In Southern Spain Travaini et al. (1996) used synthetic fermented egg (SFE), however it is also possible to use, for instance, chicken meat (Soyumert & Gürkan 2013), bobcat urine (or other animals), which have been successfully used is place (Beltrán et al. 1991).
- Requirements for success of this technique are: accurate track identification, careful preparation of the tracking substrate surface, favourable weather (no precipitation and lack of wind) and the need for stations to be conveniently located but not disturbed (Schauster et al. 2002).
- Roughton and Sweeny (1982), Smith et al. (1994) and Sargeant et al. (1998) should be revised before scent-station surveys are initiated (Schauster et al. 2002).
- Expert staff is required for fingerprint recognition.
- The visit rate to scent stations is affected also by the species density, due to various factors such as weather, the season of the year, habitat characteristics and human activity, the dry season is the one recommended for applying this method (Sargeant & Cypher 2003).

As some predators may avoid artificial materials (Gehring & Swihart 2003), to obtain proper footprints, scent-stations can be prepared naturally by cleaning up and sieving the natural soil (Soyumert & Gürkan 2013).

- Scent stations may be necessary to detect species rarely detected by scats.
- Track plates should be checked and rebaited every 2-3 days and left them on site for 11-15 days per locality.

**5. Den counts**

Objective: Count the number of breeding fox dens present in a territory to estimate the number of breeding animals and a relative of abundance of the population

Methodology: Counting breeding dens for estimating fox density is a widely tested method in various European countries (Storm et al. 1976, Lindstrom 1980, Harris 1981, Hewson 1986, Prigioni et al. 1991, Goszczyński et al. 2008, Wandeler et al. 2010, Keuling et al. 2011). Counts of breeding fox dens in late spring or summer depending on the study area (Lloyd 1980, Marks & Bloomfield 1999, Goszczyński et al. 2008) can be used to determine the absolute abundance of red foxes. Fox groups typically produce one litter of cubs annually so that counts of litters or breeding dens indicate the density of social groups (Sargeant et al. 1975, Insley 1977, Harris 1981, Hewson 1986, Lindstrom 1989, Goszczyński 1999, Marks & Bloomfield 1999, Heydon et al. 2000). To understand if a den is used for breeding, traces such as droppings of puppies, remains of fresh prey, smell, footprints and visual observations of foxes are used (Heydon et al. 2000). Assuming that each den corresponds to a pair of adult foxes and taking into account that each social group of foxes produces a litter per year, it is possible to make counts of breeding burrows that can indicate the density of the social groups and allow to estimate the minimum number of breeding adults (Uraguchi & Takahashi 1998). However, it is essential to take into consideration (Insley 1977) that in the red fox population there may be floating individuals, not breeders who are not integrated into any social group. In Wales, Lloyd (1980) estimated that 21% of adult females do not reproduce, therefore, the abundance estimates obtained from the dens count underestimate the real population by not taking into account non-breeding individuals, it would be better to confront this estimate with another method such as the batted (Goszczyński et al. 2008). To apply this method correctly therefore, it is necessary to determine correction factors for the occurrence of multiple litters in the same group and the loss of litters prior to emergence. In the absence of such factors, estimates of fox density may be unreliable (Baines et al. 1995). Sargeant et al. (1975) detected 84% of 270 fox families in their study area. Den location also provides a good basis for further work such as capturing and population management. This estimation method probably has as a main advantage the high level of accuracy obtained for the estimation of the reproductive population. The main disadvantage of applying it to estimating absolute density is that it is necessary to know (or assume) certain information such as the number of individuals per den. On the other hand, this method does not take into account the population of transient, non-reproductive individuals who are not part of a family group.

Evaluation:

- Appropriateness to estimate density or abundance: density and minimum number of breeding adults
- Accuracy and precision: low, error of underestimation
- Reliability: low, adaptable to local conditions, practicable, conductible. It is necessary to determine correction factors

- Cost and effort required: low costs and efforts, mainly manpower
- Spatial scale: local, adaptable to regional studies
- Habitat: all type, including cultivated areas
- Comparability: low among populations, if standardized, useful for comparison of the same population along time.
- Recommendations to improve comparability and accuracy:
    - Better to apply it in the period when there are puppies
    - Use other methods in comparison

Practical guidelines:

- It is already possible to count puppies when they are between the 4th and 6th week of age, because they start consuming solid food and they come out of the den, even if they are always dependent on the mother.
- Hunting can negatively influence the percentage of occupied dens (Lloyd 1981).

## 6. Hunting statistics

Objective: Estimates the population abundance with data obtained from hunting events for local (e. g. hunting grounds and management areas) and larger areas (Goszczyński et al. 2008).

Methodology: The number of foxes culled annually (hunting bags: e.g. (Hewson & Kolb 2010, Hewson 1984, Tapper 1992) or the number 'moved' or taken by hunts (Artois & Andral 1980, Gortázar et al. 1998, Nyenhuis 2000, Heydon et al. 2000) represent potential indices for monitoring changes in fox abundance. However, such techniques are heavily dependent on standardizing the amount of effort expended to obtain reliable and comparable data (see Mcdonald & Harris 1999). The cumulative number of foxes killed on a given area may not indicate fox density (typically when the number of foxes shot failed to reach an asymptote) due to the immigration of juveniles from neighbouring areas (Reynolds 1995).

The hunting statistics may be an acceptable indicator of the population trends of foxes to a regional or national scale, as long as the data has been recorded in a way consistent over the years and hunting pressure has remained constant. Records obtained for extended periods allow identification trends and relate the changes in the indices with specific events. However, it is not applicable to areas which are not hunted, and fox abundance obtained from collected data on these estates may be not representative of the rest of the region/country.

In summary, the problems with hunting bags include bias due to (1) different hunting traditions and hunting methods in each hunting area; (2) changes in hunting effort, quotas and hunter saturation, legal restrictions; (3) environmental conditions (e.g. weather, food availability and population density), and (4) variability due to non-hunted populations in urban and protected areas. Additionally, if the hunting bag is limited by a quota, it is no longer proportional to the local abundance and may be used as a target figure, particularly if it can affect the quota in the following year (Sadlier et al. 2004). For all this motivation hunting statistics can be a useful source of information, but its results must be interpreted with great caution.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance
- Accuracy and precision: low, highly variable
- Reliability: low to moderate reliability, depending on hunting legal restrictions, weather conditions, will and ability of hunters, red fox densities, report of hunting bags and dependent on knowledge of reproductive rate and sex ratio. The applicability of hunting statistics for monitoring fox abundance is greatly limited by differences in culling effort
- Cost and effort required: data more or less everywhere available, often long-term trends
- Spatial scale: local and large scale possible
- Comparability: It's necessary standardized red fox data collection model for hunting statistics

Practical Guidelines:

- See guidelines for drive counts during hunting
- Harmonised protocols for hunting data must be established to allow comparisons.

The Figure 7 illustrates some charasteristics of red fox and methods applied to estimate abundance.

The Table 1 shows a classification of different population density or relative abundance estimations based on desirable characteristics for red fox monitoring populations in local management units, practicability, and applicability.


**Figure 7. Red fox (*Vulpes vulpes*).** (a) Footprints; snow tracking has been used to calculate abundance indexes based on counts of tracks detected per unit of sampling effort (image: L. Klwak). (b) faeces easily visible because usually placed on high places with the aim of marking the territory. In addition to the abundance of species, the number of faeces found is determined not only by the abandonment of the species but also by the rate of defecation and by the rate of disappearance (images K. Petrovic and J.A. Blanco). (c-d) illustrate seasonal variations in fur, which varies from dark brown to beige on the upper parts, while the lower parts are white

(images: S. Baz, J. Horig and A. Grabowski). (e-f) camera trap images during night and daytime, respectively (image: R. Hohmann). (g-e) red fox dens (images: J. Hörig and K. Petrovic). Counting the number of breeding fox dens present in a territory is used to estimate the number of breeding animals and a relative of abundance of the population.

**Table 1**: Classification of different population density or relative abundance estimations based on desirable characteristics for red fox (*Vulpes vulpes*) monitoring populations in local management units, practicability, and applicability in epidemiological studies.

| Red fox (*Vulpes vulpes*) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linear transect | Linear transect: DS | Capture-Mark-recapture (CMR) | Telemetry | Camera trapping | Driven counts | Non-invasive genetic sampling or genetic capture-recapture | Pellet counts | Den counts | Track counts | Scent stations | Hunting bag statistics |
| Abundance/density | A/D | A/D | D | D | D | A/D | D | A/D | A/D | A/D | A | A/D |
| Temporal trend | annual/seasonal | annual/seasonal | annual/seasonal | annual | annual/seasonal/short | annual | seasonal | annual | seasonal | annual/seasonal | annual/seasonal | seasonal |
| Info on population structure | y | y | y | n | y | y | y | n | n | n | n | y |
| Season | year round | year round | year round | year round | year round | year round | year round | year round | seasonal | year round | year round | seasonal |
| Costs | moderate | moderate | moderate to expensive | very high | moderate | low | high | low | low | low | low | very low |
| Effort | moderate | moderate | high | very high | moderate | moderate | moderate | moderate | moderate | low | moderate | low |
| Ease of learning | easy r | moderate | difficult | difficult[1] | easy[3] | easy | easy[4] | easy r | easy r | easy | easy | easy |
| Accuracy + precision (High, Medium, Low) | MA+MP | HA+HP | HA+HP | HA+HP | HA+HP | MA+MP | HA+HP | MA+MP | LA+LP | LA+LP | MA+MP | HA+LP |
| Reliability | moderate | moderate to reliable | reliable | moderate | reliable | low | reliable | moderate | low | low | moderate | low |
| Useful at local scale | y | y | y | y | y | y | y | y | y | y | y | y |
| Useful at large scale | y | y | y | n | r | r | y | r | y | y | n | y |
| Useful at very low density | r | r | y | y | y | y | r | r | r | y | r | n |
| Useful at very high density | y | y | y | y | y | y | y | y | y | y | y | y |
| Suitable at all | y | y | y | y[2] | y | y | y | r | r | r | r | r |

1 - requires professional personnel trained with capturing and handling wild animals, 2 - suitable, but not recommended as the method used solely for monitoring, 3 - requires basic statistical skills to apply CMR models, 4 - requires the use of molecular techniques, but possible with use of commercial service, r - restricted.

## 4.2. Wolf (*Canis lupus*)

Wolves live in family groups (packs) that consist of the breeding pair, pups and a few subadults born in the previous years. Most of young wolves, however, leave the pack and try to find a mate to establish their own family pack and territory. The size of wolf territory ranges from 100-150 km$^2$ in southern Europe up to above 1000 km$^2$ in the northern latitudes (Jędrzejewski et al. 2007). In the family group reproduce usually only one wolf pair. The mating period occurs in February or at the beginning of March, while pups are born in April-May (Schmidt at al. 2008). At early summer the pack activity concentrates around breeding sites but from September-October new-born pups are able to follow the rest of pack members and survey entire territory (Theuerkauf et al. 2003). Wolves mark their territories with scent marking, that includes urine, feces and anal gland scents that are usually left on tree trunks, bushes or stones (Zub et al. 2003). The same places are also often marked by ground-scratching. In addition, wolf can inform other wolves about their presence in the area with howling (Okarma 2015).

The large territories, high mobility and low densities of wolves make the monitoring of wolf highly challenging. There have been many methods used to monitor the species, however there is no universal method that can be used throughout species range. Therefore, in each region or country monitoring is adjusted to the local environmental and weather conditions and financial capacity. At large spatial scales (country level) the combination of indirect methods are used to estimate the number of wolf packs and their composition (Kaczenski et al. 2013). The packs are differented based on the assumption that each family group is territorial and produce one litter per year, thus the presence of spatially separate litter of pups in summer provide information on location of different wolf family groups. Reproduction is confirmed with variety of methods such as den surveys, stimulated howling, genetic analyses of scats, camera trapping, direct observation of pups, and snow-tracking. The pack size, in turn, is estimated based on snow/sand tracking, camera trapping, direct observation, wolf-howling or genetic methods (Wabakken et al. 2001, Jędrzejewski et al. 2002, Lieberg et al. 2012, Passilongo et al. 2015, Reinhardt et al. 2015). The population size of wolves is calculated as a sum of individuals assessed in each recognized pack and individuals living out of family groups (assumed proportion of the population or determined based on snow tracking or DNA analyses; Wabakken et. al 2001). This can be also calculated by multiplying number of packs by average pack size and adding the number of individuals living out of family groups (Nowak & Mysłajek 2019). More accurate and precise methods of wolf density estimation utilize capture-recapture framework (camera-trap and genetic capture-recapture) or telemetry (Fuller et al. 2012; López-Bao et al. 2018; Mattioli et al. 2018). Although recommendable, they can be applied at local scales due to their complexity and costs.

**Direct methods**

**1. Camera-trap capture-recapture**

Objective: To calculate population density by identifying animals recorded during camera-trap surveys and estimating the proportion of the recaptured.

Methodology: Camera-traps are set at scent-marking sites used by wolves along forest roads, at road crossroads to maximize detection probability. Focal individuals (alfa) are recognized in videos registered by photo-traps based on their morphological (body size, fur colour, length, shape and carriage of tail and ears) and behavioural traits (scent-marking display; Galaverni et al. 2012; Mattioli et al. 2018). The recognition of the other pack member, crucial for estimation of the pack size and composition, are defined based on individual-specific traits or by the association with a known alfa pair. The frequency of marked individuals (alfa) observed in subsequent capture sessions is then used to estimate abundance. Spatially explicit capture-recapture models (SCR; Efford 2004, Borchers 2012) are used, both for closed populations (without births / deaths or emigration / immigration) and for open ones (which are advisable if no information is provided about population status). SCR models incorporate latent variable that represents location and number of individual's home ranges or their activity center that allows to estimate population density. Population density is defined as the local intensity of a spatial point process, taking into account heterogeneity in detection probability that is assumed to be a function of the distance between animal's activity center and detector. SCR model can be provided with observations which collection was repeated either in time or space, i.e. one site surveyed multiple times or multiple sites sampled once (Royle et al. 2013). The duration of recording sessions is adjusted to the recapture rate – the higher recapture rate, the shorter recording sessions (Mattioli et al. 2018).

This methodological approach was successfully applied in Italian wolf population, where individual wolf recognition was facilitated further by high individual phenotypical variability resulting from introgression of canine genes. Individual recognition was validated with genetic methods (noninvasive genetic sampling; Canu et al. 2017). The wolf population density estimation was based on the pack density and the mean pack size as a conversion factor. Population density (summer and late winter-spring) was obtained by fitting two types (Bayesian and likelihood-based) spatially-explicit capture-recapture (SCR) models to wolf pack capture histories under assumption of population closure (Mattioli et al. 2018).

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high, when there are sufficient skilled operators at work and the proportion of extra-pack wolves in population is low.
- Reliability: reliable, when assumption of population closure is met, and surveyed area has a high pack density.
- Cost and effort required: high costs, high effort.
- Spatial scale: local.
- Comparability: high, if sufficient experienced researchers are implied with special reference to ability to spot individual differences.

Practical guidelines:

- Study area should be large to encompass at least 10-12 wolf packs to have sufficient sample size of recaptures necessary to fulfil the assumption of population closure. The area can range from 50.000-60.000 ha for areas with a high pack density to 150.000-200.000 ha for less densely populated areas.

- It is helpful to run pilot study to collect preliminary videos for focal animal identification and to assess the effectiveness of camera locations and capture rate.

- The highest capture rate is reached when location of cameras corresponds to scent-marking sites of wolves.
- The sampling period should be adjusted to the rate of capture success. When the rate is high it can be limited event to one month (Mattioli et al. 2018).
- Individual recognition of wolves in photos or videos should be confirmed by different observers and can be validated with DNA analyses.
- It is recommended to discard ambiguous cases of focal animal classifications from the capture history as erroneously recognized packs (e.g. merged two adjacent packs) can remarkable influence density estimation (Mattioli et al. 2018).

## 2. Telemetry

Objective: To estimate density of wolf populations by estimating their home range size with radio or GPS tracking of animals and combining this information with pack size and the range of the surveyed population.

Methodology: Animals are captured with nets (Okarma & Jędrzejewski 1997), foot-hold traps Kuehn et al. 1986), sedated with dart gun (Arnemo et al. 2011) or captured with net gun (Barrett et al. 1982) and equipped with a collar with radio or GPS transmitter. Animals are tracked at least one year and based on the collected locations home range size is estimated. The most common methods are minimum convex polygon method (MCP; Worton 1989), kernel model (KDE; Blundell et al. 2001; Bowman 1985), autocorrelated kernel model (AKDE, Fleming et al. 2015), local convex hull method (LoCoH; Getz & Wilmers 2007); Brownian bridge movement models (BBMM; Horne et al. 2007). It is recommended to delineate territory boundaries of all territorial packs in the surveyed area. To reach this goal the home range analyses for at least one pack member is needed (Fuller & Snow 1988). Then the pack number is combined with the information on pack size collected with different methods (aircraft surveys, snow-tracking, camera-traps; Fuller et al. 1992). When telemetry is limited only to a few individuals representing only a fraction of all wolf packs occurring in the study area, the wolf abundance can be estimated by extrapolation of average home range size and mean pack number to the non-studied part of the study area. Density of wolves is calculated as total number of wolves in all defined or extrapolated packs divided by surveyed area (Burch et al. 2005).

Evaluation:

- Appropriateness to estimate density or abundance: density.

- Accuracy and precision: high (when territory boundaries of all wolf packs are delimited with telemetry and pack size estimated in the field); moderate to low (when extrapolation for non-studied area is made and no information on pack size was obtained).
- Reliability: High.
- Cost and effort required: Expensive, high effort is needed to catch animals. Hence, recommended mainly for scientific purposes.
- Spatial scale: local.
- Comparability: Comparable when a significant proportion of the population is marked, and the same home range estimators are used.

Practical guidelines:

- Animal handling during collaring procedure should be focus on minimizing animal suffering.
- Capturing should be spatially separated to increase probability of capturing animals from different packs.
- Animal trapping should be done outside breeding period (Arnemo et al. 2011).
- GPS collars are recommended over radio-transmitters as their application considerably limit effort and increase data quality that facilitates home range analyses and delineation of population area (Burch et al. 2005).
- When calculating home ranges special care should be taken when choosing proper home range estimator, especially for high-resolution GPS-tracking data that are often highly autocorrelated.

### Indirect methods

#### 1. Non-invasive genetic sampling (genetic capture-recapture, GCR)

Objective: Determine unique genotypes from non-invasive genetic samples of wolf scats and apply results from DNA analyses in capture-recapture model to estimate wolf population size.

Methodology: Capture-recapture method has been applied to estimate wolf population number in different European countries (e.g. Italy: Marucco et al. 2009, Luicchini et al. 2002, Spain: Echegeray & Vila 2012, France: Cubaynes et al. 2010). Wolf scats for genetic analyses are collected in the field during several sampling sessions. Personnel collect only fresh scats and avoid gathering samples from one location during the same tracking session. To minimize individual capture heterogeneity due to differences in marking intensity between sex, age and social status, surveyed transects should cover area in a way giving each individual the same chance to be sampled (Ebert et al. 2010). In case of closed CR models, it is advisable to adjust sampling period relative to the assumed lifespan or dispersal rate of wolves in the area in order to avoid violation of the closure assumption. Collected samples are genetically analysed to detect individual genotypes. Difference sources of possible errors are checked and found errors deleted. Individual genotypes are classified as capture or recapture and implemented into CR model. The population size and the level of uncertainty around obtained estimate can be assessed with different estimators: rarefaction curve-fitting (Kohn et al. 1999), $M_h$-Chao (Chao 1988), $M_h$-jackknife (Burnham & Overton 1979), capwire (Miller et al. 2005, Pennell et al. 2013). As each estimator is based on different set of assumptions and provide various level of estimation uncertainty, the choice of the proper model depends on the situation and the priorities of researcher.

Alternative approach, which deals with the problem of undefined effective sampling area in closed capture-recapture models and of heterogeneity in detection probability, is an application of the spatially explicit capture-recapture models (SCR; Efford 2004, Borchers 2012). Under SCR models, the ordinary capture-recapture models are extended by latent variable that represents location and number of individual's home ranges or their activity center that allows to estimate population density. Population density is defined as the local intensity of a spatial point process, taking into account heterogeneity in detection probability that is assumed to be a function of the distance between animal's activity center and detector. SCR model can be provided with observations that collection was repeated either in time or space, i.e. one site surveyed multiple times or multiple sites sampled once (Royle et al. 2013). SCR approach has been already successfully applied to monitor population abundance of wolves in USA (Roffler et al. 2019) and Spain (López-Bao et al. 2018) at population-level scale and is a recommended method over a nonspatial capture-recapture approach.

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high, method accuracy and precision increase with increasing field and lab effort; high accuracy and precision are reached provided correct sampling design and lab protocol that enables to meet all assumptions of the chosen CR estimator.
- Reliability: high but dependent on multiple factors: sample size (recapture rate), individual capture homogeneity, personnel experience, work intensity, lab protocol, the degree of meeting assumptions of the chosen CR estimator.
- Cost and effort required: High personnel effort, strongly dependent on human experience (e.g. scat collection design, lab protocols). Expensive method due to high costs of DNA analyses.
- Spatial scale: local due to great field and lab effort as well as substantial costs.
- Level of comparability: Difficult to assess as there have been no studies comparing results of this method with other methods aiming at estimating wolf abundance. Comparability between regions/countries possible provided that similar sampling design, lab protocols, and CR models are used.

Practical guidelines:

- Scat collection:

- The field effort during scat collection should be high and spatially uniform. It would be best to cover all inhabited area with the net of transects.

  - When adopting a closed CR models, the length of scat collection sessions should be as short as possible to meet population closure assumption. However, this condition can be difficult to achieve as collection of scats from all individuals in short timeframes can be often not feasible. Then, an open CR models are recommended (Marucco et al. 2009). The best time for collection sessions is winter, as wolves can be easier tracked and DNA contained in scats is better preserved.
  - Personnel should actively track individuals to collect scats from all individuals occurring in the surveyed area to minimize individual capture heterogeneity. It is not advisable to gather scats only along roads or trails as these are typical places of territory marking by dominant individuals, while subordinates can defecate in other parts of the territory (Marucco et al. 2009).
  - The sample size of collected scats should be representative for the size of surveyed population. Because of a variable lab success, Solberg et al. (2006) recommended to collect 2.5-3 times more samples than assumed number of individuals in the studied area.
  - It would be helpful to conduct pilot study to determine sample success rate in DNA analyses and work out best practices for collection and storing of scats.

- Lab analyses:

- Poor quality samples should be removed otherwise their quality should be taken into consideration in further analyses.
- It is advisable to adopt optimal lab protocol which allow to minimize genotyping errors and costs (e.g. selection of adequate molecular markers, screening for allelic dropouts, null alleles and human errors; Marucco et al. 2011).

- Capture-Recapture (CR) modelling:

- The fit of the applied CR model should be checked along with degree to which model assumptions are met (Marucco et al. 2011).
- When any of the model assumptions are violated or the model fit is poor it would be recommendable to take into consideration application of another CR estimator (Miller et al. 2005).
- If model assumptions are violated due to individual capture heterogeneity, the possible solution is to correct for its effects during modelling (Marucco et al. 2009) or applying spatially explicit capture-recapture models that take into account individual capture heterogeneity.

## 2. Snow-tracking

Objective: To calculate the index of relative wolf abundance by counting:(1) wolf tracks crossing line transects (2) packs (social units with confirmed reproduction) or to estimate the total population size by counting all individuals in the surveyed area.

Methodology: Snow-tracking can serve to get wolf abundance estimates within three different approaches: (1) transect counts, (2) pack counts (reproductive families), (3) total count. Transect counts assume counting of the tracks imprinted in the snow (animal crossings) along the transects of predefined length. To standardize the time of track accumulation, the tracking is performed one or two days after the snowfall that completely cover all old tracks or after pre-checking when all old tracks are marked or covered with snow (Lindén et al. 1996). The index of relative abundance of wolves is calculated as the number of crossing per day per 10km (Kojola et al. 2015).

To count the number of packs that reproduce in surveyed year the trackers follow the tracks of wolf groups and count the group members. Reproduction within territory is confirmed by comparing the number of individuals in packs from one winter to the next. An increase of at least two wolves is considered as the confirmation of summer reproduction. For newly established pairs, reproduction is confirmed when pack size increased to four individuals in the surveyed winter (Wabakken et al. 2001). The index of relative abundance of the social groups (packs) is calculated as the sum of packs with confirmed reproduction across surveyed area.

When using snow-tracking for estimation of the total population size (total count), the wolves are tracked the day after fresh snowfall, over whole surveyed area to discriminate all resident wolf packs and individuals (Jędrzejewski et al. 2002, Nowak & Mysłajek 2016). To separate all individuals and wolf groups trackers follow all found individual and group of tracks till the day-bed. In case of the group of tracks the number of pack members is recognized based on the number of individual tracks observed at places where group temporally separate. The distance between the tracks of neighbouring groups or individuals serves for differentiation of wolf packs. At the end the total population number of wolves is calculated as the sum of recognized pack members and solitary individuals.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance (transect and pack count) and density (total count).
- Accuracy and precision: medium accuracy and medium precision; highly dependent on proper study design, manpower involved, tracker experience and snow conditions.
- Reliability: moderate, more reliable at local scale; dependable on snow conditions.
- Costs and effort required: moderate costs and effort, in case of large-scale total counts high effort and costs connected with tracker employment and car renting.
- Spatial scale: from local to large spatial scales.
- Comparability: high for transect counts and moderate for packs counts and total counts as many assumptions should be met to get comparable estimates (e.g. same tracking conditions, tracker experience, road density).

Practical guidelines:

- Tracking sessions should be conducted in the period when wolf packs exhibit the highest cohesion and outside mating period (February) to minimize interference with mating behaviour (incorrect assignment of multiple tracks of mating wolf to family group).

- The snow cannot be too high, otherwise the tracks are difficult to recognize and differentiate from dog or lynx tracks.
- The timing of tracking is very important. There should elapse enough time between snowfall and tracking to allow animal to leave tracks on the surveyed routes.
- In case of pack and total counts the tracking routes should cover surveyed area as densely as possible.
- Tracker should pay high attention not to miss any of the crossing tracks and to properly count group members.
- It is recommended to repeat snow-tracking at least once to increase the probability that all individuals and groups were found.
- The steady decrease of snow cover in many parts of Europe (especially in Southern Europe) makes this method decreasingly applicable.

### 3. Den surveys

Objective: Ground surveys to count occupied wolf dens to estimate the index of relative abundance of the social groups (packs).

Methodology: Den survey should be done throughout surveyed area either shortly before or after reproduction. In spring freshly excavated or renovated den confirms its occupancy, while in autumn occupied dens are recognized by the sings left by temporal wolf presence (e.g. prey remnants, old scats; Schmidt et al. 2008, Reinhardt et al. 2015). Den occupancy is often confirmed with other methods such as simulated howling or photo-traps (Nowak & Mysłajek 2016). Once the number of occupied dens is known, the index of relative pack abundance is calculated by dividing the pack number by area of surveyed site.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: moderate.
- Reliability: moderate.
- Cost and effort required: low cost but requires high tracker experience and moderate effort.
- Spatial scale: local.
- Comparability: comparable between populations when the same effort is made.

Practical guidelines:

- Before searching for dens, it is useful to get information about places of increased wolf activity in breeding season (intensive marking with scats and scratching) that can indicate the possible location of occupied dens and about den occupancy from the previous years, when such information is available.

- Surveys should be concentrated in remote areas far from human activities with high vegetation and water availability as such areas are preferred for den location by wolves (Ausband et al. 2010).
- It is recommended to confirm den occupancy (reproduction) with other available methods (simulated howling, photo-traps).

## 4. Vocalisation – acoustic surveys

Objective: Acoustic surveys to confirm reproduction in wolf families and estimate the index of relative abundance of the social groups (packs).

Methodology: As resident wolves and their youngs are prone to respond to external vocal stimuli, elicited howling provides a tool to localize and confirm reproduction in wolf packs. This method consists in the acoustic stimulation produced by human simulation or playback of wolf howling records during sit-and-wait sessions from vantage points (Blanco et al. 1992, Llaneza et al. 2005). The elicited howling is done in summer and early autumn when pack spends most of its time at the den and rendezvous time where pups are reared (Harrington & Mech 1982, Fuller & Sampson 1988, Gazzola et al. 2002, Nowak et al. 2007). Howling session locations should be spread across whole surveyed area and they should be chosen based on landscape characteristics that helps locating putative rendezvous sites (remote areas with low human activities and permanent water availability). When available, the information on localization of rendezvous site or den from previous years is also used to localize vantage points. Howling sessions usually start at sunset and last for 2-5 hours (Llaneza et al. 2005, Jiménez et al. 2016).

Alternatively, to get more accurate and reliable index of breeding pack abundance howling surveys can be combined with sign surveys in rendezvous sites. Number of sites with breeding packs, then, is estimated with application of a multistate modelling approach based on Bayesian hierarchical-site-occupancy models (Jiménez et al. 2016).

Simulated howling provides also information on minimum number of pack members, as inspection of spectrograms and spectrum allows to count the number of voices in a wolf chorus (Passilongo et al. 2015), therefore minimum population number or density can be estimated.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance. Potentially, minimum population density when pack members are counted based on the chorus size (bioacoustic approach; Passilongo et al. 2015).
- Accuracy and precision: moderate, there are many factors that can interfere sound detectability and influence reply rate (i.e. land relief, weather conditions) (Crête & Messier 1987, Harrington & Mech 1982).
- Reliability: moderate.
- Cost and effort required: low costs, moderate effort of qualified staff, specialized recording equipment can increase the costs.
- Spatial scale: local.

- Comparability: high, when the same effort is made, and the staff represents similar qualifications.

Practical guidelines:

- Fieldwork should be avoided during windy or rainy nights (Llaneza et al. 2005).
- Recommended periods are summer and early autumn (Gazzola et al. 2002).
- The stimulated howling should be performed shortly after sunset when wolves start increasing their activity (Harrington & Mech 1982, Theuerkauf et al. 2003).
- Background noises should be avoided.
- It is recommended to emit three 6- to 7-s-long howls separated by 2- to 3-s-long breaks (Nowak et al. 2007).
- It is recommended to use directional microphone and digital recorder to record soundtracks and to analyse them with spectrometer as aural estimate is strongly biased with respect to spectral analyses and true number of pack members.

**5. Hunting statistics**

Objective: To estimate the abundance index based on the data on the hunting bags collected at the level of hunting ground or other management unit.

Methodology: This method can be applied in areas where wolves are regularly hunted. Hunters collect the information on the number of hunted wolves in hunting season. This method assumes the presence of positive correlation between the number of hunted wolves and their abundance. The comparison of hunting statistics over years allows for indication of population trends. Hunting quotas are a valid indicator of wolf abundance as long as the hunting quotas represent the same proportion of population over the years. However, hunting statistics are prone to be biased due to different hunting methods, effort, and environmental conditions among spatial units. In addition, hunting bags very often are not proportional to the abundance as the hunting quotas depend on political criteria in many regions (Blanco & Cortés 2012, Kaczenski et al. 2013).

Annual hunting bags can potentially serve for estimation of the minimum populations size or density. In areas, where wolves are hunted with hunting drives it is possible to estimate the proportion of the hunted to observed wolves during all registered hunting drives. Obtained proportion, then, is applied to the number of wolves shot in drive hunts during one year across entire surveyed area and the minimum population size or density is estimated (Blanco & Cortés 2012).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance (density potentially).
- Accuracy and precision: low, highly variable.
- Reliability: low reliability, depending on effort, hunting method, weather conditions, population densities, legal restrictions and political issues.
- Cost and effort required: low cost and effort.
- Spatial scale: from local to large scale.

- Comparability: low, difficult to standardize between different areas

The Figure 8 illustrates some charasteristics of wolf and methods applied to estimate abundance. The Figure 9 illustrates vocalisation – acoustic surveys, which are appropriate to estimate wolf relative abundance, potentially, minimum population density when pack members are counted based on the chorus size (Passilongo et al. 2015).

The Table 2 shows a classification of different population density or relative abundance estimations based on desirable characteristics for wolf monitoring populations in local management units, practicability, and applicability.


**Figure 8. Wolf (*Canis lupus*).** (a) faeces easily visible (image: G. Grygoruk). (b) Scent marking by wolf (image: M. Scandura). Determining unique genotypes from non-invasive wolf samples (mostly scats) and applying results from DNA analyses in capture-recapture model allows estimating wolf population size. (c) footprints (Image: R. Kryza), sometimes, it may be hard to differentiate tracks of the wolf and large domestic dogs (https://sidorovich.blog/2018/02/26/how-to-distinguish-tracks-of-wolves-and-dogs/). (d) Snow-tracking can serve to get wolf abundance estimates with different approaches (image: L. Mattioli). (e) equipment for howling surveys to confirm reproduction in wolf families and estimate an index of relative abundance (minimum pack size). (f) pack of wolves howling (image: S. Luccarini). (g) direct observation of wolf during

daytime (image: R. Mance). Wolves live in family groups (packs) that consist of the breeding pair, pups and a few subadults. (h) the skull identification requires morphological differentiation from dogs (image: P. Kaliszuk).

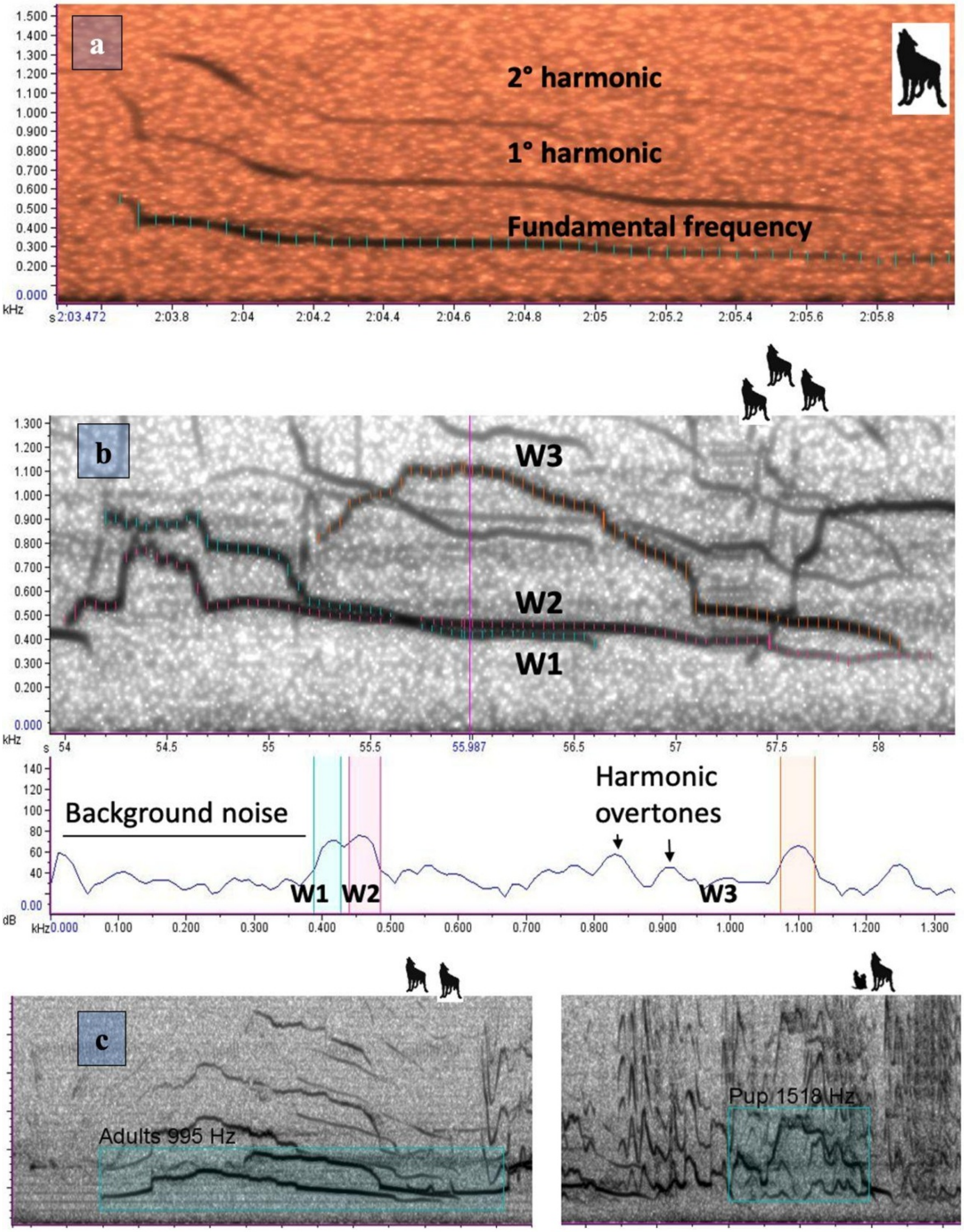

**Figure 9. Wolf (*Canis lupus*).** Vocalisation – acoustic surveys are appropriate to estimate wolf relative abundance, potentially, minimum population density when pack members are counted based on the chorus size (bioacoustic approach; Passilongo et al. 2015). This figure represents acoustic analysis of wolf's vocalizations. (a) a single wolf howl appears as a fundamental frequency (F0) and its harmonic overtones. (b) minimum pack size estimation: each different F0 at a given time corresponds to a different wolf. (c) discrimination between adults and offspring: vocalizations emitted by offspring, are recognizable until 6/7 months of age for their high frequencies and instability of the vocal structure (Harrington & Mech 1978; Harrington & Asa 2003) (images: M. Apollonio).

**Table 2**: Classification of different population density or relative abundance estimators based on desirable characteristics for wolf (*Canis lupus*) monitoring populations in local management units, practicability, and applicability in epidemiological studies.

| **Wolf (*Canis lupus*)** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Camera trap capture-recapture | Telemetry | Non-invasive genetic sampling or genetic capture-recapture | Snow-tracking | Den surveys | Vocalization | Hunting statistics |
| Abundance/density | D | D | D | A/D | A | A/D | A/D |
| Temporal trend | annual/seasonal | annual | seasonal | seasonal | seasonal | seasonal | seasonal |
| Info on population structure | y | y | y | n | n | n | y |
| Season | year-round | year-round | summer, winter | seasonal | seasonal | seasonal | seasonal |
| Costs | high | high | high | moderate | low | low | low |
| Effort | high | high | high | moderate | moderate | moderate | low |
| Ease of learning | difficut | difficult | difficult | easy | easy | easy | easy |
| Accuracy + precision | HA+HP | HA+HP | HA+HP | MA+MP | MA+MP | MA+MP | LA+LP |
| Reliability | high | high | high | moderate | moderate | moderate | low |
| Useful at local scale | y | y | y | y | y | y | y |
| Useful at large scale | n | n | r | y | r | n | y |
| Useful at very low density | r | y | r | y | y | y | y |
| Useful at very high density | y | y | y | y | y | y | y |
| Suitable at all | y | y | y | y | y | y | r |

## 4.3. Golden jackal (*Canis aureus*)

The golden jackal (*Canis aureus* L, 1758) is a medium-size carnivore. It is around 70 to 105 cm in length, 38 to 50 cm in height at the shoulder and weights 7 to 15 kg (Negi 2014). In Europe, after a century of fluctuation, the distribution of the species has been in expansion since the 1980s (Arnold et al. 2012). Signs of its presence have been recorded in Balkan peninsula, where the highest densities are reached (Stoyanov 2012), Italy, Austria, Slovenia, Czech republic, Denmark, Estonia, Latvia, Moldova, Romania, Hungary, Russia and Turkey, with presence also in France, Italy, Switzerland, Netherland, Germany, Poland (where recently the northernmost breeding has been detected, Kowalczyk et al. 2020), Lithuania and Belarus, with an estimated population size between 97,000 and 117,000 according to the IUCN red list (Hoffman et al. 2018). The factors affecting the jackal expansion across Europe are under discussion and several hypotheses have been proposed.

Due to its tolerance of dry conditions and its omnivorous diet, the golden jackal can adapt to a wide variety of habitats: in Europe, it prefers cultivated areas and wetlands in lower elevations and heterogeneous habitats with adequate cover for hiding and breeding (Šálek et al. 2014). Jackals are opportunistic and will venture into human habitation at night to feed on garbage or garbage dumps (Ćirović et al. 2016). This species manifests preference for human-dominated landscapes, with consequences (Jhala & Moehlman 2004). The golden jackal represents a major reservoir host of rabies in parts of Asia and Africa, with cases reported in Europe (Müller et al. 2015). Therefore, it is essential to better understand interactions between golden jackal and other species, including humans for management purposes.

Breeding period occurs during April-May (Szabó et al. 2007, Krofel 2008), with dens located in natural and man-made embankments (Jhala & Moehlman 2004). Packs are generally constituted of 2-4 individuals: the breeding pair, the fundamental unit, can be accompanied by the helpers (offspring from previous litter) and the cubs from the current litter. Single jackals usually hunt smaller prey, but whole pack predations have been recorded, especially for larger prays. Affiliative behaviours like greeting ceremonies, grooming, and group vocalisations are common in jackal social interactions (Golani & Keller 1975). Vocalisation consists of a complex howl repertoire beginning with 2-3 simple, low pitch howls and culminating in a high-pitched staccato of calls. Jackals are easily induced to howl and a single howl evokes responses from several jackals in the vicinity (Jhala & Moehlman 2004).

**Direct methods**

Direct methods involve counting animals themselves, using either live or dead specimens. Stratification of subsamples to different habitat types or land classes may increase the validity, usefulness, and precision of the surveys. They can also provide data about the structure of the population (e.g. Stoyanov 2012), but they can vary depending on the seasonality of the golden jackal. In 2016 an assessment was published by the GOJAGE (Golden Jackal Informal Study Group Europe), in which principal direct and indirect methods have been listed.

### 1. Linear transect (General)

Objective: By observing individuals / groups along transects, the objective is to estimate the local density or relative abundance.

Methodology: The transects can be carried out using a vehicle (Durant et al. 2010, 2011, Yirga et al. 2017) or, if distances allow it, on foot (Prerna et al. 2015; Singh et al. 2016), which is difficult to perform at spatial scales of several hundreds of $km^2$. Fixed point transects, where observation takes place from fixed points, are also an option. Golden jackals are mostly nocturnal animals, so transect surveys are usually performed after dusk (starting 1 hour after sunset), with the use of spotlights (spotlight surveys) (Debnath & Choudhury 2013, Yirga et al. 2017); moreover, the presence of the *tapetum lucidum*, which reflects the light if hit by it, may increase the probability to detect the animals in the dark.

Practical guidelines:

- An autumn census could be used to detect the increase in jackals due to recruitment and dispersal, while an early spring estimate would comprise largely breeding individuals.
- Golden jackals are mostly nocturnal animals, so transect surveys are usually performed after dusk.
- Within each season, spotlighting is conducted several nights in case of low density and/or low detectability.
- In the study design phase, it is important that a subsample of sites is visited multiple times to perform an analysis of detectability. These precautions can serve to avoid the effects of false absences generated by low detectability and allow obtaining more reliable estimates.
- A disadvantage of using spotlights is that its application depends largely on the availability of passable tracks or roads in vehicles. Therefore, it is not applicable in regions with limited vehicle access.
- In areas where animals are hunted, they may exhibit flight behaviours when perceiving the proximity of the vehicle, so the detection probability will be lower.

Transect methods include different techniques to detect animals and to estimate population density or indices of abundance:

*KAI* (Kilometric Abundance index)

Objective: By observing individual jackals along transects, it is possible to derive local density or relative abundance

Methodology: As stated, the transects can be carried out using a vehicle or on foot, preferably using spotlights. Date, time, GPS position, number of individuals (Durant et al. 2011) and, whenever possible, sex and age class (Prerna et al. 2015) are recorded for each observation. The KAI can be used to estimate abundance, and this can be obtained from the total number of specimens counted each night divided by the total number of kilometres travelled.

$$KAI = \frac{N°golden\ jackals}{N°\ nights \cdot N°\ km/night}\ or \frac{N°jackals\ (total)}{Nº\ km\ (total)}$$

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: moderate. Needs good visibility. Three samples per site will approximate the optimum to minimize the variance of the estimates of occupation

(MacKenzie & Royle 2005), and 2-3 samples per site will maximize the power to detect trends (Field et al. 2005b). However, high levels of occupancy and / or low detectability will tend to increase the number of repetitions required, confirming the importance of several replicas in each sampling.

- Reliability: moderate. Differences in seasonal behaviour can affect the results of studies performed in different times of the year. Also, some assumptions are required: (i) animals in the vicinity of the transect are detected; (ii) do not move before being detected; (iii) each individual is detected only once; (iv) the detection of each individual is independent of the other detections; (v) the influence of variables such as the observer, station or the weather.
- Cost and effort required: this method is not expensive, but it requires a moderate effort in terms of labour and operators, especially when it involves nigh time surveys.
- Spatial scale: local to large territories.
- Comparability: Debnath and Choudhury (2013) in their study showed that results in terms of detected individuals and density in linear transect were lower than in vocalisation surveys and higher than den surveys.

Practical guidelines:

- Consider the principles of sampling (summarized at https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1449) when calculating population parameters on large areas inhabited by golden jackal. It is necessary to select some proportion of surface in which density or relative abundance is estimated by different transects. The results of these estimates give rise to an average that will be extrapolated to the whole area of study. A correct study design means minimising bias during sampling and applying sufficient effort to produce precise reliable estimates of density or relative abundance. Jackal spatial distribution is a result of its spatial ecology, land use and distribution of resources, as well as other factors. We therefore need to have notions of the distribution of the population and habitat selection in the territory in order to make a good study design.
- Field operations developed during the same season
- Random placement of transects, at least repeated over years for comparisons purposes along time.
- Transects should be stratified by habitat type, avoiding roads and other singular features. All relevant environments (different habitat which similar use for jackal or characteristics may be grouped) within the study area (which may impact jackal distribution) must be considered for the sampling design.
- It should be tested whether stratification allows for similar sampling effort and bias in each habitat class. This is even more important when comparing different methods in a given area (no bias should occur due to different sampling effort in each habitat type by the different methodologies).
- Transects must be placed using a fine scale maps of the estate and should be stratified while also taking into account the description of the habitat composition.
- The sampling effort must be quantified per habitat type (e. g. as the proportion of transects across the different habitat types).
- The result of a given procedure when stratified design is not performed is biased estimations of relative abundance and density towards those habitats that are over-

represented in the sampling. This would thus cause low precision and incomparability with other values obtained from different study areas.

- The vehicle speed should be constant and slow enough to spot the animals, i.e. 20 km/h (20 km/h (Durant et al. 2011); at least one driver and one observer are required (Durant et al. 2011).
- Apart from spotlights, torches and night-vision binoculars are recommended (Yirga et al. 2017).
- In order to have reliable and accurate data, transects can be performed in different times of the year (in order to detect animals despite difference in seasonal activities) and repeated several times (Debnath & Choudhury 2013) or monthly (Durant et al. 2010).
- Other methods to survey the area with transects can be the parallel lines (Schaller et al. 1966; Durant et al. 2011) or frequency of sightings made at various locations during field surveys (Vijayan 1991), but they are considered as less robust methods (Singh et al. 2016).

*Fixed width transects*

<u>Objective:</u> By observing individuals / groups along transects and within a pre-defined detection band, the objective is to estimate the local density.

<u>Methodology:</u> The transects, usually by spotlighting after dusk, are used to estimate the densities by defining a band on the sides of the transect in which all the individuals present are assumed to be observed (Durant et al. 2011, Yirga et al. 2017). The width of this band is defined by the distance perpendicular from the travel line where visibility is close to 100%, which is usually around 200m (Durant et al. 2011), but it depends on technology provided, habitat and local behaviour of the species. Durant and colleagues (2001) adapted the distance-sampling density estimation to fixed width transect method, where data are not partitioned into clusters:

$$D\ (density) = \frac{N°golden\ jackals}{band\ width \cdot transect\ lenght \cdot probability\ of\ detectability}$$

with probability of detectability within a strip of half-width derived using detection functions from distance-sapling calculations (Buckland et al. 2001, 2004, Durant et al. 2011).

<u>Evaluation:</u>

- Appropriateness to estimate density or abundance: density and relative abundance.
- Accuracy and precision: high, depending on four assumptions: (i) groups of animals are distributed randomly with respect to transects, (ii) groups on the transect line are detected with certainty, (iii) groups are detected at their original location, (iv) measurements are exact (Durant et al. 2011).
- Reliability: Size bias (tendency to observe more large clusters at large distances) should be considered when doing the model estimations.
- Cost and effort required: as well as linear transect, distance sampling is not expensive in terms of devices, but is demanding in terms of operators work.
- Spatial scale: local.

- Comparability: Durant and colleagues work (2011) did identify distance sampling method to be adequate to detect long-term trends and abrupt changes in population size (see below). Distance based transects provide estimations of densities with greater accuracy and precision than fixed width transects (Durant et al. 2011).

Practical guidelines:

- Stratified sampling studies with habitat differentiation can be useful to avoid habitat bias (Durant et al. 2011).
- Consider the practical guidelines above regarding IKA, and track field designs.
- To detect the distance of the animal or group from the observer, either a laser rangefinder (Prerna et al. 2015) or eye estimations according to cut points (e.g. 0, 10, 50, 100, 150, 200, 300, 400, 500 and >500 m ) can be used (Durant et al. 2011), the larger distances being only applicable to open habitats.
- In order to obtain reliable estimates of density at least 30 observations are necessary.

## 2. Distance sampling on transects

Objective: By detecting individuals or groups along transects and recording group size and relative position to the observer, the objective is to estimate the density after modelling the probability of detection.

Methodology: This method is based on the concept that the probability of observing an animal decreases with increasing distance to the observer. The method is analogous to the linear transect survey, but in this case more information about each observation is required: date, time, cluster size, species, numbers, sex, age class, distance and bearing of the individual/group (Prerna et al. 2015). In order to obtain the detectability functions, the distance is perpendicular to the route line, whereas the bearing is the angle to the line of progression of the observer: the distance to the observer is therefore calculated by trigonometry.

To select the most appropriate model in terms of quality of fit and increased number of parameters, the Akaike information criterion (AIC) is used (Buckland et al. 2001, Durant et al. 2011, Prerna et al. 2015). The adjustment of detectability functions and density calculations can be performed through computer programs such as "DISTANCE" (Thomas et al. 2010, Durant et al. 2011, Prerna et al. 2015, Singh et al. 2016). With this model, the probability of detection and the average cluster size can be calculated (Durant et al. 2011). Exploratory analyses may be carried out (Buckland et al. 2001) to check for any evidence of evasive movement before detection and to truncate outlier observations, if necessary, for improving model fit (Prerna et al. 2015). Eventually, golden jackal density can be calculated as (Buckland et al. 2001, 2004, Durant et al. 2011):

$$D\ (density) = \frac{N°clusters \cdot expected\ cluster\ size}{2 \cdot trucation\ distance \cdot transect\ lenght \cdot probability\ of\ detectability}$$

Because those studies are all performed in different habitats from Europe, it is important to mark that vegetation can be a drawback on visibility and, especially for night surveys, open zones are preferably used. This can be a limitation in territories with forest cover and stratified studies are therefore recommended.

Evaluation:

- Appropriateness to estimate density or abundance: population density.
- Accuracy and precision: medium-high.
- Reliability: need for assumptions of different seasons not known, record the number of animals in the area (limited), which is the one that the observer can actually sample.
- Cost and effort required: The high effort required is compensated by low cost, as trained volunteers are often available (experienced hunters). Nonetheless, volunteers have to be motivated.
- Spatial scale: local.
- Comparability: A minimum number of transects must be sampled depending on the distribution of jackals in the study area, requires areas of good visibility and stratified design to be comparable among sites.

Practical guidelines for distance sampling:

- Consider the practical guidelines above regarding KAI and fixed width transects, and particularly, stratification of design, which is even more relevant for distance sampling to produce reliable and comparable results
- It is recommended to use a rangefinder to estimate the distance because the estimation of distances by direct observation is subject to errors, especially at night and they can also vary considerably between observers.
- Need of at least 50 sightings
- Cluster size is usually between 2 and 4 individuals. Real pack size is usually estimated by acoustic methods, differentiating the number of individuals answering to the call (Comazzi et al. 2016). In this case, it has to be kept into account that single individuals may not respond to the call, just breeding packs. Other methods would be direct sighting or sample correcting in the area where golden jackals live (which cannot determine the exact number of puppies).

## 3. Telemetry

Objective: By estimating the home range or territory size of single radio/GPS-collared jackals and combining those data with average pack dimension, the objective is to derive density estimates of the study population

Methodology: Animals are usually captured using soft leg-hold traps baited with appealing food (Admasu et al. 2004, Kapota et al. 2016, Charaspet et al. 2019), then a collar is put on. The main type of tracking is Global Position System (GPS). Recorded data via website should include date, time and GPS coordinates (Charaspet et al. 2019). Another option is to locate the specimens on foot or by car using a hand-held receiver at fixed times in the day (Admasu et al. 2004, Aiyadurai & Jhala 2006). Home range can be estimated using different methods and therefore density derived, sich as minimum Convex Polygon (MCP) method (Worton 1989, White & Garrott 1990, Aiyadurai & Jhala, 2006), adaptive (or fixed) kernel model (Worton 1989, Blundell et al. 2001). And others /e.g. the ellipse, Boulanger and White (1990), and the grid cells method (Siniff & Tester 1965, White & Garrott 1990). This is an area is constantly developing.

Marking with transmitters most of the population and tracking them for a period of at least one year is currently considered one of the most reliable method to determine the density of territorial carnivores, so it is often used as a reference to contrast results obtained by other methods (Schauster et al. 2002). However, this method indicates only the minimum density since it assumes that each home range is used exclusively by a single animal, which is never true. Determine the minimum area required for a daytime cover in order to hold a jackal family group is one of the goals prospected in the golden jackal action plan of WWF (Giannatos 2004).

<u>Evaluation:</u>

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: moderate. Because golden jackals can be highly social depending on availability and distribution of food resources, with around 20% of the population living in more than three individuals packs (Jhala & Moehlman 2004), critics about this method can be extended to golden jackal's case too (e.g., Mech 1973a, Fritts & Mech 1981, Fuller 1989, Gese et al. 1989).
- Reliability: High. Depending on sociality of the golden jackals in different areas, long term studies are very reliable for density estimations
- Cost and effort required: High. Telemetry is very labour intensive and costly; therefore, this method may only be used for scientific purposes.
- Spatial scale: local.
- Comparability: It is necessary to mark a significant proportion of the population this methodology can be used to complete data from camera-trapping (Charaspet et al. 2019) to calibrate less expensive methods (Gese 2001).

<u>Practical guidelines:</u>

- Field anaesthesiology must be performed in order to manage the animal (Admasu et al. 2004).
- Before wearing the collar, the animals should be sexed, aged (based on tooth wear and reproductive conditions) and weighted (Admasu et al. 2004, Aiyadurai & Jhala 2006). Both juvenile and adults can be collared.
- Applying colourful ear tags can help identify animals caught
- The selected period for trapping is mainly pre-breeding (Aiyadurai & Jhala 2006, Charaspet et al. 2019).
- Radio-collar can be integrated with a mortality sensor (Advance Telemetry System) (Kapota et al. 2016) and a VHF signal to help tracking the animal in the field with telemetry antennas (Charaspet et al. 2019).
- Data collection frequency can vary depending on available technology and study aims. To avoid autocorrelation between relocation one location per individual every 24 hours can be obtained (Rotem et al. 2008); other studies did record data on a weekly basis

(Kapota et al. 2016); Charaspet and colleagues (2019) set the collar to send a signal every 4 hours.

- To calculate pack size, refer to the practical guidelines of distance sampling

## 4. Abundance indexes based on camera trapping

*Abundance indexes based on camera trapping*

A relative abundance index (RAI) can be calculated as the mean value of the minimum number of recognizable individuals registered for 100 camera trap days in camera traps in the study area (Tsingarska et al. 2018), or as the number of photo captures per 100 trap nights (Kais et al. 2009, Jenks et al. 2011, Çoğal & Sözen 2020). Although standardized (which allows to compare different studies), this RAIs are not synonymous with an actual index of relative abundance because they are not been correlated with data on population size and do not qualify for statistical trend analysis in the same way as occupancy models (Jenks et al. 2011). The occurrence of the golden jackal is calculated as part of the test areas from their overall number within a study area with an established species presence (Tsingarska et al. 2018).

Evaluation:

- Appropriateness to estimate density or abundance: abundance
- Accuracy and precision: medium
- Reliability: medium
- Cost and effort required: affordable, moderate costs, need for CT, theft of cameras in some locations, medium effort, manpower required to analyse photo/video material, photos might be collected via online-photo-databases.
- Spatial scale: local, adaptable to regional studies.
- Comparability: Evaluate missing detections with your specific camera model and under your study circumstances. All assumptions should be stated in literature.

Practical guidelines:

- Field operations should be developed during the same season in order to provide comparable abundance estimations across sites and time.
- With photo identification systems many photos may be deleted due to image quality or individual's lack of identification, risking the loss of important information in population studies, and are likely to lead to erroneous conclusions in a number of behavioural studies.
- The use of lure in front of the camera trap was tested in several studies and can be useful. Cat or dog food or also fish and cod liver oil can be used and mixed to the soil or rubbed on trees. This also induces the animal to spend more time in front of the camera, with more probability to take a good picture (Georgiev et al. 2015, Pecorella & Lapini 2015, Tsingarska et al. 2018).
- Still needed studies on the efficacy of different lures.

## Other direct methods potentially useful

### 1. Capture-Mark-Recapture (CMR)

Objective: By identifying part of the animals and estimating the proportion of the recaptured during samplings, density can be estimated.

Methodology: Different methods included in CMR are reviewed in Buckland et al. (2000). Trapping and marking individuals are needed (Chandler & Royle 2013), or at least, the presence of recognisable identification signs (Karanth & Nichols 1998, Sarmento et al. 2009). The frequency of marked individuals observed in subsequent capture sessions is then used to estimate abundance. Spatially explicit capture-recapture models, together with generalized spatial mark re-sight, is a new methodology that permits estimation through individual identification of target species using camera-trap sampling (see below). There are different mathematical models to estimate the population size, both for closed populations (without births / deaths or emigration / immigration) and for open ones (which are advisable if no information is provided about population status) and there are computer programs specific for this (e.g. CAPTURE, Rextad & Burnham 1991).

Spatially explicit capture-recapture (SCR) models (or spatially explicit mark-resight, SEMR models) can be used for abundance and density estimation, frequently through individual identification of target species using camera-trap sampling, still not applied to this species to our known. Generalized spatial mark-resight (Gen-SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks (Gen-SMR is included in the section "Camera trapping without individual recognition". However, information of its application on jackals is still needed). For indirect CMR see "Genetic analyses of pellets (genetic CMR)". Research in other species suggests that the use of effective attractants at CT sites can significantly increase detection probabilities. To bait the traps, it is advisable to use fish liver oil (such as cod liver oil) as well as dry food for cat (Pecorella & Lapini 2014, Georgiev et al. 2015), which are proved to have similar attraction.

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high.
- Reliability: reliable but need for assumptions: (i) the population is closed (without immigration or emigration) and there are no births or deaths between captures; (ii) that all individuals have the same probability of being captured.
- Cost and effort required: moderate to expensive costs, high effort. It is necessary to mark a significant proportion of the population, which is very expensive, although accurate and precise. Due to its high effort and local conductibility, this method may only be used for scientific purposes, knowing that better methods exist.
- Spatial scale: local.
- Comparability: While the use of effective attractants can significantly increase detection probabilities, they may differently affect jackal detection probabilities in CMR studies by CT; and therefore, it is needed to assess the effectiveness of several attractants to evaluate their usefulness for non-invasive survey methods.

Practical guidelines:

- Adequate licensing for some procedures (capture) may be necessary in most countries.
- Recapturing is highly affected according to the first capture and experiences of individuals and it may increase the potential risks of injuries, also lethal, for the recaptured individuals.

## 2. Recently developed camera trap methods

*Random encounter model (REM)*

This method was developed and tested in several species including carnivores (Rowcliffe et al. 2008, Rovero & Marshall 2009, Rovero et al. 2010, Rowcliffe et al. 2011, Rovero et al. 2013, Rowcliffe et al. 2013). For more details, see the section of red fox.

*Random encounter rate and staying time (REST)*

REST is an extension of REM (Nakashima et al. 2018). The speed parameter is substituted by resting time in the camera view. It still has not been tested in golden jackal to our known.

*Point transect using camera traps (CT-DS)*

This method consists of an adaption of point transect methods (distance sampling) to camera traps (CT-DS) (Howe et al. 2017). It still has not been tested in golden jackal to our known.

*SCR (Spatial Capture-Recapture) for unmarked*

This method (Royle 2004, Chandler & Royle 2013, Forsyth et al. 2019, Jimenez et al. 2019) involves individual identification of target species, so that this is basically an extension of CMR avoids capturing individuals (Chandler & Royle 2013, Ramsey et al. 2015). Generalized spatial mark-resight (Gen-SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks. It still has not been tested in golden jackal to our known.

Practical guidelines:

- Since different CT methods are available, we will focus on those most practical, previously classified as of relative medium effort, and able to generate reliable data over a wide range of situations across Europe. Random encounter model (REM and its extensions, untested in jackal) does not require individual recognition, whereas Spatial mark-resight models (SMR) work recognizes only a part of individuals. We do not exclude the use of other CT methods, but we recommend those in terms of practicability as they provide an excellent balance between the required effort (and level of knowledge) and reliability.
- See practical guidelines in the red fox section.

- Individuals are often distinguishable by specific face mask, tail shape, reproductive status, urination, features like scars or missing parts (Ivanov et al. 2016, Tsingarska et al. 2018).
- See comments above about the use of lure.

**Indirect methods**

These methods allow to estimate relative abundance from indirect signs of golden jackals' presence. Absolute abundance is not provided, but a comparison between relative and absolute indices should be done in order to confirm a positive correlation. Also, as these methods are generally less expensive, prior comparison with a more expensive method is advisable as a calibration of the first one. They also should be applied consistently in order to make comparisons between areas, habitats or time (Gese 2001). The assessment of GOJAGE can be consulted about reliability of method depending on operators working on it (Hatlauf et al. 2016).

**1. Genetic sampling and non-invasive genetic sampling (genetic capture-recapture, GCR)**

Objective: By collecting golden jackals' samples, the objective is to identify individuals and obtain population estimates. It is also possible to make consideration abut population structure.

Methodology: This method can be performed either with golden jackals' presence signs collection, such as scats or hair, or directly from culled or road-killed individuals, such as blood, tissue (e.g. ears or muscle) or hair (Zachos et al. 2009, Cohen et al. 2013, Fabbri et al. 2013, Rutkowski et al. 2015, Yumnam et al. 2015) and blood sampling from captured animals (Yumnam et al. 2015). Once samples are taken, processing involves especially DNA microsatellite loci identification. Rutkowski and colleagues (2015) list 15 loci that were proved to have polymorphism in golden jackal (Fabbri et al. 2013) and that this number of polymorphic markers is proved efficient to detect genetic structure and describe genetic diversity within populations (Koskinen et al. 2004; Rutkowski et al. 2015). Amplification of hypervariable domain of the mitochondrial DNA (mtDNA) control-region can also be performed (Rutkowski et al. 2015). This way it is possible to identify individuals and thus obtain population estimates. Population size can be derived as the asymptote of the function between the number of different genotypes and the number of analysed samples. Alternatively, the mark-recapture model can be used (Kohn et al. 1999). Also, statistical analysis can be performed with software such as STRUCTRE, version 2.3.4 (Pritchard et al. 2000): this way, assertions can be made about population structure and evolution through the centuries.

Evaluation:

- Appropriateness to estimate density or abundance: density and individual presence.
- Accuracy and precision: high. Genetic analysis allows avoiding species misidentification when indirect sampling.
- Reliability: high.
- Cost and effort required: moderate.

- Spatial scale: local.
- Comparability: It is well remarked that genetic variability in golden jackals is higher in microsatellite loci and STRs than in mitochondrial DNA. When comes to acoustic surveys, Comazzi and colleagues (2016) state that scat genetic analysis may be useful to set a frame for the development of new non-invasive monitoring methods, which can also lead to individual censuses.

Practical guidelines:

- Scats are usually deposed in denning areas or intensively used trails (Jhala & Moehlman 2004), whereas hair can be collected along den, scat or track transects or surveyed areas.
- When samples are collected directly on individuals, specific sampling areas are identified and specimen number, sex, locality information, date and geographical coordinates are recorded (Rutkowski et al. 2015).
- See recommended guidelines for faeces and hair in red fox and wold sections.

## 2. Pellet count

Objective: By calculating/collecting the number of scats per unit of effort **(transects or plots)**, it is possible to derive an index of relative abundance.

Methodology: Although scat sampling is often used as a collateral method to confirm golden jackal's presence in the surveyed area (Lal et al. 2016), some examples of relative abundance estimations by scat sampling, which is very common for other canids (Gese 2001), are present also in this species. Road transects (scat deposition transects) are designed along main road or trails, depending on land conditions. To identify sampling spots, Jaeger and colleagues (2007) defined four random 1-ha plots in both random and fixed 1 km blocks along transects, with no difference in total number of collected scats within the two techniques.

*Pellet counts with cleaning approach*

This method involves that all the scats are cleared from the area, then the scat collection along the transect is repeated periodically, e.g. every two weeks (Gese 2001) or monthly (Jaeger et al. 2007). The rate at which scats are deposed should be standardised at scat/km/day and allows to estimate an index of relative abundance (Gese 2001). Especially when it comes to studies that sample the area all year long, differences in scat deposition rates can be relevant, but, due to different seasonal and food habits, rather than population density variation (Jaeger et al. 2007). Other variables to keep into consideration are the rate of defecation and the rate of disappearance. The defecation rate can be estimated with captive animals, with variations regarding sex, age, social status, type of food and different marking behaviours; the disappearance rate can be very variable depending on various factors of both climate type. See also the red fox section for pellet counts with cleaning.

To avoid scat misidentification problems and to estimate the number of individual animals, DNA techniques can be used too (Kohn et al. 1999, Gese 2001).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance and local density.
- Accuracy and precision: moderate. It can be improved with DNA techniques.
- Reliability: moderate. Requires experienced staff for recognition. Differences in the number of collected scats can be related to differences in food availability and seasonality (Jaeger et al. 2007). It is also to remark that smaller prey types have relatively greater surface area in relation to their volume and thus their consumption results in the production of relatively more predator scats when compared with the larger prey types (Singh et al. 2016).
- Cost and effort required: low cost/effort. However, operators should be trained to recognize golden jackal scats.
- Spatial scale: local.
- Comparability: This technique allows comparison of relative abundance between different times (trend analyses) at the same season, but comparisons among areas are constrained by many sources of variation.

Practical guidelines:

- For long-term monitoring, the transects should be conducted along the same routes at the same time of the year (Gese 2001)
- It is to keep into consideration that golden jackal's scent marking by urination and defecation is common around denning areas and on intensively used trails (Jhala & Moehlman 2004).

### 3. Track-counts along transects

Objective: By recording the number of tracks left by golden jackals in the sampling effort unit, the objective is to calculate an index of relative abundance.

Methodology: Track-count surveys are conducted by walking along transects (usually divided into shorter segments, which constitute the units of sampling); tracks left in sand or mud along riverbeds, dry washes, sandy fire breaks or roads are counted (Gese 2001). The collection of genetic samples can be useful to confirm the identity of the tracks.

Indices can be calculated as number of set of tracks per day, presence/absence of tracks or either length of interval between sets of footprints (Wilson & Delaha, 2001). The abundance index of the population can be calculated (Roženko & Volokh 2010):

$$Abundance\ index = \frac{N°tracks \cdot 1.57}{transect\ lenght \cdot average\ lenght\ of\ animal\ movement\ per\ day}$$

The average length of animal movement per day is the one calculated by Formozov (1932).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: Low. Precision can be improved by increasing sampling effort or the length of the transect. Much of the power of this estimator is dependent upon a high rate of encountering sign along the transects (Kendall et al. 1992, Gese 2001).
- Reliability: moderate, staff must be well trained to recognise golden jackal tracks.
- Cost and effort required: low.
- Spatial scale: local.
- Comparability: low among populations, if standardized, useful for comparison of the same population along time. Need to calculate reliable daily ranges (see REM).

Practical guidelines:

- The choice of habitat can have an impact on the results of the study. Although adapted to a variety of habitats, some studies (e.g. Roženko & Volokh 2010) have revealed that golden jackals mostly prefer wetlands, coastal sites and shallows. Important biotopes for the jackal are also maritime and leman meadows, characterized by high numbers of voles and mice. Generally, the jackals like investigating roads where they eat animals died under car wheels (Roženko & Volokh 2010).

#### 4. Den surveys

Objective: Ground or aerial surveys are done along transects or areas to count the number of golden jackal's dens to index relative abundance of social groups (packs).

Methodology: Even though this method is mainly used with other canids, especially red foxes, some studies have included den surveys to estimate relative abundance also with golden jackals (Banea et al. 2012, Debnath & Choudhury 2013). Those surveys are done during pre-breeding season (Debnath & Choudhury, 2013), when the population is stable, and no new individuals are there. The number of total dens, along transects or defined areas, is used to index relative abundance; however, ground surveys along transects can also be used to calculate the density of dens, if the perpendicular distance from the transect to the den is recorded (Gese 2001). An important distinction to mark is between active and non-active dens. The first ones can be identified by presence of hair, scat, pugmark and direct sighting by the local people. Those are as well useful tools to attribute a den at a golden jackal (differentiating it from the ones belonging to other canids). It is remarkable that, making comparison with howling studies, the absence of active dens in the surveyed areas does not mean the absence of golden jackals. Once the number of active dens is detected, density can be estimated knowing the average size of the social group. Difficulties in locate dens can be higher in close forest areas, dominated scrub, shrub and herb dominated areas (Debnath & Choudhury 2013). The main disadvantage of applying it to estimating absolute density is that it is necessary to know (or assume) certain information such as the number of individuals per den. On the other hand, this method does not take into account the population of transient, non-reproductive individuals who are not part of a family group.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance and minimum number of breeding adults. Potentially, density can be calculated.
- Accuracy and precision: low, error of underestimation. Being the golden jackal a social animal, this method is more adequate to estimate packs rather than individuals' number. Estimations of total population can be done based on pack dimension information (Gese, 2001).
- Reliability: low. Proof of the den being active and belonging to a golden jackal must be founded (Debnath & Choudhury 2013). It should be adapted to local conditions.
- Cost and effort required: low cost, but ground research is labour intense and may require many operators (Gese 2001).
- Spatial scale: local, adaptable to regional studies.
- Comparability: Compared studies with howling surveys have pointed out that the number of golden jackals is usually founded less (Banea et al. 2012, Debnath & Choudhury 2013).

Practical guidelines:

- Dens may have 1-3 openings and typically are about 2-3m long and 0.5-1.0m deep (Jhala & Moehlman 2004).
- In order to determine the relative abundance, it should be kept into account that females are generally monoestrus, so that one litter per year is given. The number of cubs is usually between 2 and 5 (Lapini et al. 2018).
- It is unknown which is the minimum size of the dense vegetation patch in order that a jackal family group feels safe to breed into. The habitat conditions and human use around the hiding patches probably influence its occupation. There are indications that the shape of the daytime cover areas is also important, as breeding jackals do not seem to prefer the narrow hedgerows along the edges of fields (Giannatos 2004).

## 5. Vocalisation - acoustic surveys

Objective: By broadcasting a group-yim jackal howl and detecting the animals' response, the objective is to identify the presence of territorial groups of jackals. Abundance or density of territorial jackal groups/individuals is therefore calculated by relating the identified groups with the maximum human hearing distance (Giannatos et al. 2005).

Methodology: This method is widely used for social carnivores that utilize long-range vocalizations to communicate (McCarley 1975, Harrington & Mech 1982, Creel & Creel 1996, Jaegeret al. 1996) and it is the most common and effective with golden jackal. The area of interest is covered by randomly placed calling stations at an average linear distance of 2 km, which is the average breeding female territory (Giannatos et al. 2005). From each calling station, recorded group-hip howls are performed; number (single/group) of responding jackals, number of groups and delay in response are recorded. A compass can be used to identify the azimuth of each howling group in order to avoid double counting (Heltai et al. 2013, Šálek et al. 2013). Geographical coordinates and altitude are recorded for each station.

To calculate densities the maximum human hearing distance on windless nights in an open terrain was determined to be of 1.8 to 2.0 km, from which effective area can be estimated (Giannatos et al. 2005, Krofel 2008). If some obstacles to the sound are spotted, one quarter of the area can be subtracted (Trbojević et al. 2018). It can be assumed that only territorial groups respond to the broadcast and each response corresponds to one group (Giannatos et al. 2005). To estimate the minimum number of jackals, only two members of the group are listed, to minimize the repetition of the individuals (Debnath & Choudhuri 2013). Alternatively, by recording the answer of golden jackals and processing it with spectrographic inspection programs, it is possible to determine the minimum number of vocalizers; the estimated number is usually lower than the one estimated during recording sessions (Comazzi et al. 2016).

This method is usually supported by the direct observation of the golden jackals with spotlights or night vision goggles. Spatial analysis may be performed by ArcGIS (Banea et al. 2012). Mills and colleagues (2001) did also propose a probability model for estimating the population size in a given habitat based on the response counts.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance. Potentially, density of jackal packs.
- Accuracy and precision: moderate, when a group is composed by more than two individuals, it is not possible to distinguish how many jackals are howling, plus there are different factors such as wind or wrong landscape, that can interfere with a plain detection of the sound (Giannatos et al. 2005, Šálek et al. 2013). Even so, the development of vocal differentiation study techniques can allow to recognise individuals (Comazzi et al. 2016).
- Reliability: moderate, single golden jackal's howls can be confounded with wolf's or dog's (Lapini et al. 2018).
- Cost and effort required: low cost, nightwork required (Krofel 2008).
- Spatial scale: local.
- Comparability: this method is usually accompanied by direct observation (Giannatos et al. 2005, Šálek et al. 2013) and was compared to direct surveys and den counting (Debnath & Choudhuri 2013). Indirect signs such as footprints, scats or pray remains can also be used in addition (Krofel 2008). Standardization and consistency are needed for reliable and comparable results for trend analyses (Gese 2001).

Practical guidelines:

- Fieldwork should be avoided during windy or rainy nights (Giannatos et al. 2005, Banea et al. 2012).
- Recommended periods are reproductive (February-March) and first common hunts (autumn) (Szabó 2016).
- Background noises should be avoided. Calling stations should be chosen based on topographic characteristics, in order to optimize sound transmission (Giannatos et al. 2005, Krofel 2008).
- Howls are performed for 30 seconds, followed by a pause of 5 minutes and repeated six times, so that the whole session lasts for around 30 minutes. The direction is changed every two or three howls (Giannatos et al. 2005). Banea and colleagues (2012)

recommend to always perform a least 5 howls, although the response rate may decrease with the number of emissions in a session (Comazzi et al. 2015, Szabó 2016).

- The acoustic survey is better to be performed from 1 hour after sunset to midnight (Giannatos et al. 2005) or 1 hour before sunrise (Krofel 2008), when jackals are more active, and especially during early breeding periods (Giannatos et al. 2005).

## 6. Hunting reports

Objective: By collecting hunting reports, both historical and recent, in golden jackal hunting zones, it is possible to calculate an index of abundance.

Methodology: Where governments pay hunters a bounty for predators it is common practice to record the location where the predator was killed (usually identified as hunting unit), and records are usually collated on an annual basis (Newsome et al. 2017). Bounty data have been used to derive indices of predator abundances, based on the notion that predator abundance generally correlates positively with the number of bounty returns (Newsome et al. 2017). No other complementary predator abundance data exist at the spatial scales of region or country. Data is collected directly by hunting associations regarding the hunting units selected, national game management database (Heltai et al. 2013) or competent agency. Another possible way is to directly interview hunting managers, but it requires checking the ability of golden jackal recognition, to avoid misidentification (Trbojević et al. 2018). The number of harvested jackals reported could be considered correct because of awards being paid for every shot animal (Stoyanov 2012). From this, an index of abundance can be derived by calculating the total number of killed specimens in each hunting zone (Krofel et al. 2017, Selanec et al. 2011). It is important to remark that this method often lacks in standardization and many elements can act as bias in the survey, such as the immigration of new individuals, differences in hunting conditions, seasonality. It is therefore more appropriate to utilize it for trends in population better than estimation of density index.

Evaluation:

- Appropriateness to estimate density or relative abundance: relative abundance.
- Accuracy and precision: low. There is usually a lack of scientific data about bias factors such as general state of environment, anthropogenic effects, predator effects and illegal hunting. Therefore, the obtained results should be interpreted only as trends in population numbers, without detailed discussion on the specific influence of any ecological factor (Markov 2012).
- Reliability: low to moderate, since different elements may interfere and be a source of bias: differences in hunting traditions and methods, changes in hunting effort, environmental conditions, presence of populations in non-hunting areas.
- Cost and effort required: low, data are more or less everywhere available. However, hunting managers' interviews may require a bigger labour effort.
- Spatial scale: regional (Krofel et al. 2017). Local and large scale are possible. The species is not always huntable across its distribution range in Europe.

- Comparability: although being a very cheap method, it has often been performed together with acoustic surveys (Banea et al. 2012, Heltai et al. 2013, Krofel et al. 2017).

## 7. Hunting data and age structure: Population reconstruction

Objective: To estimate population size by using harvest data and population age structure. By collecting hunting reports and skulls of shot jackals for one ot several years and estimating the age of every animal in the sample in golden jackal hunting zones, it is possible to estimate Population size/Hunting bag ratio by reconstructing population age structure (Stoyanov 2013).

Methodology: Age distribution data are some of the most popular data used for survival analysis, particularly for harvested species. Age distribution (and particularly age ratio) data also have applications in the indirect estimation of reproduction rates (Caughley 1977, Williams et al. 2002, Conroy & Carroll 2009). For some species, ages are relatively easy to determine with reasonable accuracy, and data can be available in large quantities. Where governments pay hunters a bounty for predators it is common practice to record the location where the predator was killed (usually identified as hunting unit), and records are usually collated on an annual basis (Newsome et al. 2017). Bounty data have been used to derive indices of predator abundances, based on the notion that predator abundance generally correlates positively with the number of bounty returns (Newsome et al. 2017). Data is collected directly by hunting associations regarding the hunting units selected, national game management database (Heltai et al. 2013) or competent agency. Along with hunting bag reports it is possible to collect skulls of big sample of shot animals. Age of harvested jackals is determined in consideration of upper incisive teeth wear (Lombaard 1971) or by counting the annual cementum layers in canines (Klevezal & Kleinenberg 1967). Both methods are reliable enough for the purposes of abundance estimation and provide accurate results, with precision up to one year for the second one (Harris et al. 1992, Rajchev 2002). However, counting the annual cementum layers in canines requires a lab, trained staff and is more elaborate. The skulls are assigned to three age groups: juveniles, subadults and adults. Juveniles are defined as individuals with fully developed second dentition but less than 10 months old, subadults as individuals older than 11 months, when they reach sexual maturity, but less than two years old, and adults as two years and older. It is possible to divide two years old from three years and older by upper incisive teeth wear (Lombaard 1971), although the method is not quite precise. In Bugarian golden jackal population only about 10 % of jackals from one cohort could reach the age of 5 years and more, and jackals above 3 years comprise only 6 % of the population (Stoyanov 2013). The proportion of juveniles in the population could be approximated by juveniles/adults ratio in the sample of collected skulls. The *juveniles/adults* ratio approximates birth rate excluding cubs mortality, i.e. it represents number of young jackals in population that survive to the autumn. If the population is stationary or have reached a stable age distribution (SAD) with known annual rate of increase ($\lambda$), the minimal population size could be estimated by the following equation (Stoyanov 2012, Stoyanov 2013):

$$\frac{Population\ size_{min}}{Harvest} = \frac{1}{1 + b - \lambda},$$

where: $\lambda$ - finite annual rate of increase, $b$ - ratio of juveniles to subadults and adults in the sample. The following assumptions are important: (i) the population is stationary or reached stable age distribution with relatively constant and known rate of increase ($\lambda$); (ii) - the sample is not biased, i.e. juveliles are not more vulnerable to hunting and hence overrepresented in the sample; (iii) - all hunting bags are reported; (iv) - constant hunting effort in the period of monitoring, if the method is applied for several years. Confidensce intervals are estimated by using bootstrap and resampling the age distribution of skulls.

If the natural mortality could be estimated as a part of the total mortality, a more precise estimation of population size is possible. For example, if the hunting mortality is 80 % of the total mortality, the ratio *Population size/Harvest* will be 1.25 times bigger than estimated. More precise aging of collected animals and collecting data for several successive years allow constructing the life table, based on the age distribution of shot jackals, following the methods described by Caughley (1977) for vertical life table and Udevitz & Ballachey (1998) for depositional life table. A more detailed description of the development of last method is presented by Skalski et al. (2005). Population reconstruction and estimation of life table vital rates allow more precise abundance estimation (Stoyanov 2013). Population reconstruction refers to the calculation of the abundance and age distribution of a cohort (group of animals all born in the same year) at some initial time. Reconstruction is usually based on "ages at death", the idea being that if we can enumerate all of these, we can work backwards to the original cohort size at age $x = 0$, using the fact that that animal that is age $x$ at time $t$ is age $x - 1$ at time $t - 1$ (McCullough et al. 1990). However, population reconstruction has serious drawbacks (Williams et al. 2002, Conroy & Carroll 2009) and is not recommended for estimation of vital rates. More direct analysis of actual sample data should be favoured, e.g. following cohorts of marked animals by using radio tags or gps transmitters.

Evaluation:

- Appropriateness to estimate density or abundance: absolute abundance or absolute density.
- Accuracy and precision: moderate, depending on four assumptions: (i) the population is stationary or reached stable age distribution with relatively constant and known rate of increase ($\lambda$); (ii) - the sample is not biased, i.e. juveliles are not more vulnerable to hunting and hence overrepresented in the sample; (iii) - all hunting bags are reported; (iv) - constant hunting effort in the period of monitoring, if the method is applied for several years (Stoyanov 2013). If the harvest is reported and governments pay bounties for collecting skulls, the sample size will be large enough to allow precise estimation. Collecting data on a big scale, i.e. on national level improves the accuracy and precision. The bias could be estimated if data is collected every year for a long period.
- Reliability: moderate, since different elements may interfere and be a source of bias: differences in hunting traditions and methods, changes in hunting effort, proportion of natural mortality, juveniles overrepresented in the sample, lack of correct hunting bag reports, environmental conditions.
- Cost and effort required: low, in countries with high density and where golden jackals are hunted, data is available in large quantities. However, collecting and aging of animals may require a bigger labour effort.
- Spatial scale: regional or national (Stoyanov 2013, Krofel et al. 2017). The species is not always huntable across its distribution range in Europe.
- Comparability: although being a very cheap method, it has often been performed together with acoustic surveys (Banea et al. 2012, Heltai et al. 2013, Krofel et al. 2017) and hunting reports.

Practical guidelines:

- The number of harvested jackals reported could be considered correct if awards are being paid for every shot animal (Stoyanov 2012). The government could provide bounties for reporting harvest or collecting skulls of hunted animals.
- The golden jackal population should be stationary or reached stable age distribution with relatively constant and known rate of increase ($\lambda$) (Stoyanov 2013).
- The finite rate of invrease ($\lambda$) could be estimated by using hunting bags ratio for several successive years. If hunting effort is comparable between years predator abundance generally correlates positively with the hunting bags (Newsome et al. 2017).
- One possible source of bias is that the sample of collected skulls could not be representative for the population. Often it is supposed that young animals are more vulnerable and their proportion in the sample obtained by hunting will exceed the real share they have in the population. If harvest sample is biased, then $\frac{n_0}{\sum_{x=1}^{w} n_x}$ should differ from $\frac{n_1}{\sum_{x=2}^{w} n_x}$ (where $n_x$ is the number of animals from age class $x$, and $w$ is the oldest age class). More juveniles and subadults would be included in the sample than those from other age groups (Stoyanov 2013).
- If different hunting methods are applied it is presumed lack of selection, because every method could be selective for different age groups. Moreover, there is no way to distinguish between different ages in the field and hunters do not have preferences as in trophy hunting of wild ungulates.
- Collecting of jackal skulls and aging of shot animals should be made by well-trained staff. The national database for collecting and storing information should be established and maintained.
- The age structure and age distribution will be more accurately estimated if the data is collected for large period, e.g. 5-10 years.
- Data collection is year-round activity, but should be more active during autumn and winter, when more jackals are hunted.

The Figure 10 illustrates some charasteristics of Golden jackal and methods applied to estimate abundance.

The table 3 shows a classification of different population density or relative abundance estimations based on desirable characteristics for Golden jackal monitoring populations in local management units, practicability, and applicability.


**Figure 10. Golden jackal (*Canis aureus*).** (a) camera trap image of a litter of three males and a female. (b) Chest displays in two golden jackals stimulated by cod liver oil used as olfactory attractant. (c) young female (9.7 kg) road killed on a highway near Farra d'Isonzo (Gorizia). (d) material used in bio-acoustic campaigns. (e) bio-acoustic survey performed along the river Tagliamento (Carnian Pre-Alps, Udine). Images: (a) A.L. Dreon, L. Lapini; (b) L. Lapini, S. Pecorella (c, d, e) L. Lapini.

**Table 3**: Classification of different population density or relative abundance estimations based on desirable characteristics for Golden jackal (*Canis aureus*) monitoring populations in local management units, practicability, and applicability in epidemiological studies.

| | **Golden jackal (*Canis aureus*)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linear transect | Linear transect: DS | CMR | Telemetry | Camera trapping | Non-invasive genetic sampling or genetic capture-recapture | Pellet counts | Track counts | Den counts | Vocal method | Hunting bag statistics | Hunting bag and age structure |
| Abundance/density | A/D | A/D | D | D | D | D | A/D | A | A /D | A | A/D | A/D |
| Temporal trend | annual/seasonal | annual/seasonal | annual/seasonal | annual | short | seasonal | annual | annual | seasonal | seasonal | seasonal | annual |
| Info on population structure | y | y | y | y | y | y | n | y | y | n | y | y |
| Season | year round | year round | year round | year round | year round | year round | year round | year round | seasonal | seasonal | seasonal | year round |
| Costs | moderate | moderate | moderate to expensive | very high | moderate | high | low | low | low | moderate | low | Low to moderate |
| Effort | moderate | moderate | high | very high | moderate | moderate | moderate | low | moderate | moderate | low | Low to moderate |
| Ease of learning | easy r | moderate | moderate | difficult[1] | easy[3] | Easy[3] | easy r | easy | easy r | easy | easy | difficult |
| Accuracy + precision | MA+MP | HA+HP | HA+HP | HA+HP | HA+HP | HA+HP | MA+MP | LA+LP | LA+LP | MA+MP | HA+LP | MA+MP |
| Reliability | moderate | moderate | reliable | reliable | reliable | reliable | moderate | moderate | low | moderate | reliable | moderate |
| Useful at local scale | y | y | y | y | y | y | y | y | y | y | y | y |
| Useful at large scale | y | y | n | n | r | r | r | y | r | n | y | y |
| Useful at very low density | r | r | y | y | y | r | r | y | r | y | n | n |
| Useful at very high density | y | y | y | y | y | y | y | y | y | y | y | y |
| Suitable at all | y | y | y | y[2] | y | y | r | y | r | y | y | y |

## 4.4. Brown bear (*Ursus arctos*)

Bears, as other large carnivores, are high on the food web and this greatly limits their densities. Individuals have large home ranges and move over those ranges extensively. Additionally, bears have developed shyness towards people and are hard to be seen, especially in the terrain that is most often rugged and densely vegetated. They are not territorial, which also limits the methods to estimate abundance and density. Obviously, all methods have some weaknesses and disadvantages, in addition to certain positive aspects. The best monitoring system for brown bear populations should therefore be a combination of methods that support each other. The only "stand-alone" method would be mark-recapture modelling based on genetic analyses of sufficient number of non-invasive samples (faeces or hair) collected in limited time and supported by significant logistic and financial resources. Recent developments in spatial capture-recapture models (SCR) allow to estimate the area to which the population estimates apply, therefore appropriate for open populations (Kendall et al. 2019). Mark-recapture methods provide the best and most robust statistical estimates of population size, but they require intensive field work and sometimes can be applied rather within a limited area. Nevertheless, data collected repeatedly in appropriate timescale are required for reliable results that could serve management and conservation decisions (see e.g. Linnell et al. 1998 for review).

**Direct methods**

**1. Counts of females with cubs of the year**

Objective: By counting the number of females with cubs of the year (FCOY) it is possible to obtain an index of relative abundance and to monitor population trends.

Methodology: FCOY are the most easily identifiable population segment. Litter sizes are mostly limited to 1-3, and cubs usually have recognizable marks. FCOY are more active at day, move less and have lower home-range overlap with other age-sex classes. Sightings of FCOY from the public are inadequate for population monitoring and underestimate population size (Solberg et al. 2006). However, when annual counts are designed according to local conditions, conducted systematically by trained observers every year, and follow rules, such as a distance-based criteria to distinguish unique FCOY, the method has been proven useful to estimate several demographic parameters (total number of cubs, total number of females with cubs, and the rate of population change $\lambda$; Harris et al. 2007, Schwartz et al. 2008). FCOY are judged to be unique based primarily on three criteria: 1) distance between sightings, 2) family group descriptions (number of cubs and marks), and 3) dates of sightings (Knight et al. 1995). The population trend derived from the Chao2 estimate of annual number of FCOY is similar to the population trend derived from vital rates of radio-collared individuals. In the case of increasing populations, the trend estimates will likely be biased low (Schwartz et al. 2008). This index can serve as indicative of population size.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance or abundance index.
- Accuracy and precision: high in small bear populations and/or low densities.

- Reliability: reliable with a proper sampling design, adapted to local conditions. FCOY sighting rates depend on sampling effort, sighting capability, food availability, habitat use, weather conditions.
- Costs and efforts required: relatively inexpensive and more practical and cost-effective than capture-mark-recapture methods. Requires trained observers (can be volunteers) and that sufficient sampling effort and coverage are ensured. It must be repeated periodically for population trend estimates.
- Spatial scale: local
- Comparability: between years. The trend in this segment of the population (FCOY) and its rate of change can be estimated from the annual estimates and can reflect rates of change in the population.

Practical guidelines

**Design: rules for field observations and estimation of demographic parameters**

- The sampling design should be adapted to local conditions.
- The ideal period to identify unique FCOY is the mating season (from early spring till the end of June). Depending on the area, most successful observation sessions should be conducted in late summer (e.g. Tosoni et al. 2017).
- Field observations should be conducted in several seasonal replicates of simultaneous sessions each year, involving the same or a similar number of trained observers. Observers scan a wide area from vantage points using binoculars and/or spotting scopes. Each simultaneous session comprises bouts of a few hours of observation, usually at dusk and dawn each day for a few consecutive days. These systematic simultaneous observations can be completed (i) by opportunistic observations by a team of observers repetitively scanning areas of high probability of FCOY sightings and verifying sighting reports by third parties, and (ii) by incidental sightings by trained observers during other activities.
- Family groups are distinct if seen simultaneously at different locations by ≥1 observer. In case of non-simultaneous sightings, family groups are distinct if they include individually recognizable bear(s) based on clearly detectable marks or tags. If not, distance-based criteria are developed *ad hoc* and FCOY are distinct if observed beyond the distance threshold at a time lag equal to the time elapsed between the sightings (Tosoni et al. 2017).
- Minimum distance criteria (for two observations conducted 30 days apart in early spring-June period) to consider distinct FCOY recommended in Europe are 13 km in the boreal forest and 15 and 7 km in southern and central Europe for released and native FCOY, respectively (Ordiz et al. 2007). In the Apennines, two sightings 30 days apart were considered to be of distinct family groups if they were > 11 km apart in spring and early summer, and >10 km apart in late summer (Tosoni et al. 2017). In the Cantabrian population, a conservative criterion of 14.5 km (the double distance than recommended by Ordiz et al. 2007) was used to assign whether two females with the same number of cubs were distinct (Palomero et al. 2007). In Yellowstone, an evaluation of the distance rule of 30 km (Knight et al. 1995) turned inaccurate and yielded that observations >15 km and <5 days apart most likely came from different FCOY (Schwartz et al. 2008).

- The observation program should be conducted in a systematic and comparable fashion year after year.
- The Chao2 estimator is recommended to estimate the total number of females with cubs present from the number observed. The estimate of the rate of change of FCOY can be a good measure of the rate of change (λ) of the entire population (Harris et al. 2007, Schwartz et al. 2008).

## 2. Counts at feeding sites

Objective: To estimate a relative abundance index that can be used to monitor population trends based on the data from bear counts at feeding sites

Methodology: Artificial feeding of game animals has been a common practice in many European countries (Council of Europe 2018), including feeding targeted to brown bear, which is hunted in half of the European countries inhabited by the species and in 7 out of the 11 populations. Wildlife feeding occurs year-round in many cases and mostly at established feeding sites. Currently, more than 80% (26 of 21) of the European countries where bears occur feed them intentionally or unintentionally. Being omnivores, brown bears are particularly affected by this practice as they often also use the food provided to ungulates and to other carnivores. The main reasons for bear feeding are bear hunting followed by viewing/photographing, and then for damage management and population monitoring. Corn and livestock (whole carcasses and slaughter remains) are the most common foods used to feed. Traditionally, in some countries where bears were hunted over bait, counting them at feeding sites has been the main and only used method of estimating population size and making decisions about the hunting quotas. The rules to count bears at feeding sites are the following:

- Fixed number of counting (feeding) stations, which is kept in every annual counting;
- Same feeding sites (over years);
- Same nights in each observation session (one in spring, one in autumn);
- Same bait (amount and type);
- All observations are noted in the form of raw data;
- The observers are requested to be in the hides or hunting towers (otherwise used for hunting or photographing) by the feeding sites from dusk till midnight on two preselected days (one in spring and one in fall) with full moon. They record each bear seen, including family groups, in a standardized form.

To continue using data from the counts at feeding sites, the traditional beliefs about the accuracy and the ultimate reach of the method are to be changed. It needs to be acknowledged that not all bears in the area can be seen and counted by this method. Along with other uncertainties illustrated in Figure 11, the visitation of bears to the feeding sites also depends on the productivity of natural bear food (e.g. beechnuts) in the given year (Figure 12).

Evaluation:

- Appropriateness to estimate density or abundance: usable for relative abundance or abundance index, and for trend.
- Accuracy and precision: the number of bears seen is precise but there is no accurate information on population density and size.
- Reliability: reliable with a proper standardised procedure but only for the relative abundance and trend.
- Costs and efforts required: inexpensive where it is already done as a routine practice. It must be repeated annually for population trend estimates.
- Spatial scale: can be at population level if organised properly.
- Comparability: Between multiple years. The trend reflects rates of change in the population.

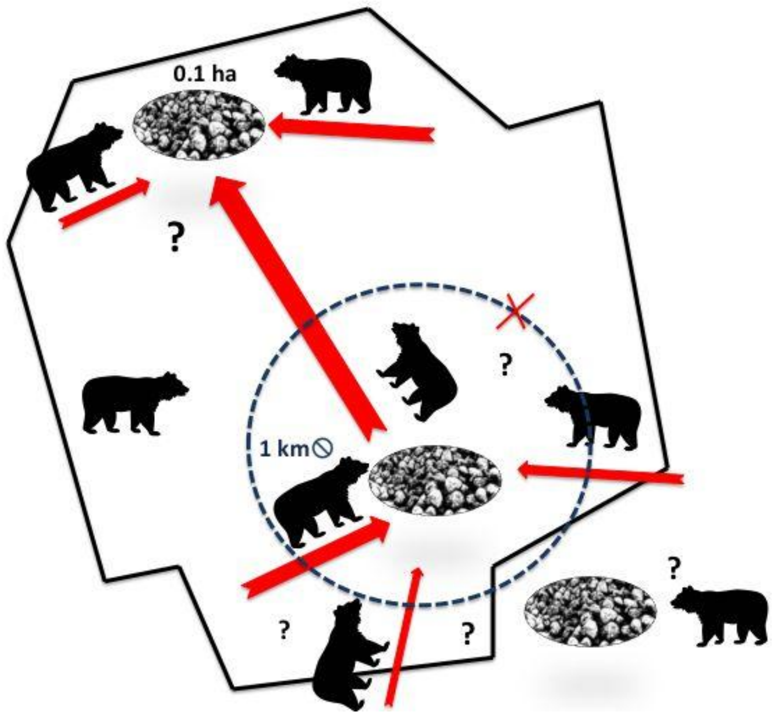


**Figure 11**. Hypothetical bear management unit with schematic feeding sites as plots of relatively small size (provisionally 0.1 ha) that attract bears from the surrounding area. The illustration emphasizes uncertainties related to counting bears at feeding sites. The distances from which bears do come to the feeding site are highly variable, including the chances of the same bear to be counted on more than one feeding site in the same day or entering the area from the neighbouring management unit, as well as not showing up at any feeding site at all.

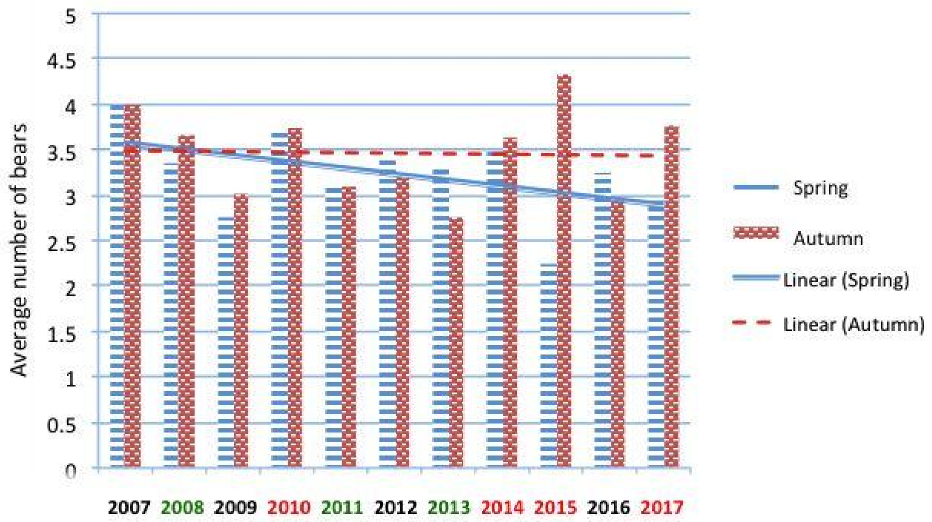


**Figure 12.** The example of the result of multiyear counting of bears at around 100 feeding sites in Croatia during springs (blue) and autumns (red). Good beechnut years (masting index ≥3) are indicated in green based on the annual masting index for Croatia (Ascoli et al. 2017). Figure adapted from Bišćan et al. (2019).

Practical guidelines:

- Acknowledging all the listed uncertainties, it must be understood that counting bears at feeding sites cannot provide usable data on population densities and can only indicate abundance.
- Where feeding bears is already an established routine, it should be used to count the bears in a systematic way. The advantage is that in some countries hunters have been doing that already for decades. It is also their duty according to specific hunting regulations and it does not require any additional training and cost (Huber et al. 2008a, 2008b).
- Additionally, counts at feeding sites can and should be used to monitor the trend and then to calibrate the data from the genetic counts in the years when genetic counts have not been performed. That means that counts at feeding sites are to be continued in all countries where they are already a routine, assuming the strict compliance of the listed "key rules".
- **Important considerations**: On the negative side is that in recent decades, the amount of food provided to wildlife, as well as the number of sites and length of the period where and when is provided, have considerably increased worldwide. These anthropogenic food subsidies can also potentially have important unintended negative consequences for species and ecosystems, such as alterations of social and trophic interactions, behaviour, activity and movement patterns, reproduction, spread of exotics, and pathogen transmission. Therefore, the rise of this practice is of increasing conservation concern and needs to be evaluated on a case by case basis (Council of Europe 2018). In Europe, artificial feeding practices are currently not always properly regulated. More than 60% of the areas where targeted bear feeding occurs have regulations regarding the type and

amount of food that can be provided and the periods during which it may be provided, although often such regulations are not properly implemented, and feeding is not controlled in practice. This is happening also in countries where bears are not the primary target of feeding (Council of Europe 2018). In any case no new supplemental feeding sites should be established regardless of the motivation, including the bear counts.

## 3. Capture-mark-recapture (CMR)

Objective: To estimate abundance by identifying a sample of individuals from the population and counting their proportion in the group of individuals identified over repeated sampling events. Density estimates can be derived afterwards.

Methodology: Identification of individual animals in their natural environment relies either on man-made marks (e.g. ear tags), variations in natural marks or genetic markers (Palsbøll 1999). Because morphological traits of brown bears rarely allow individual identification, direct capture-mark-recapture (CMR) methods involve live-capture and marking, and thus potential animal welfare risks related to chemical immobilization and physical handling (Arnemo et al. 2006). These ethical aspects need consideration and impose legal requirements on parties that conduct associated procedures, especially on endangered populations. Being high in cost, effort and risk, direct CMR methods should be applied only if benefitting from studies conducted for other scientific purposes, such as telemetry, rather than as a population monitoring tool *per se*. Depending on the capture method (culvert or Aldrich trap) and the use of bait (consumable food or a scent lure that provides no food reward), recapturing may be affected by either trap-happy or trap-shy behavioural response of experienced individuals (Kendall & McKelvey 2008).

From the variety of models and software available (e.g. MARK, CAPWIRE, RCapture) that requires different assumptions regarding i.a. demographic and geographic population closure and capture probability, the selection of the appropriate one(s) must be done considering the sampling scheme. Very important is that the number of marked and identified animals is proportionally high in relation to the total population size. This secures that the number of recaptures is high enough for the calculations and the margin of error is minimized, thus, the population estimates are more reliable. This is rather unrealistic with invasive techniques, but commonly used in non-invasive studies, e.g. with samples like scats and/or hairs collected for genetic tagging (see section "Non-invasive genetic sampling").

For the brown bear, CMR was conducted using aerial visual surveys of radio-collared and unmarked individuals (Miller et al. 1987, 1997), as well as of oestrus females, both collared and unmarked, consorting with collared adult males during the breeding season (Solberg et al. 2006). In the study by Swenson et al. (1994), aerial searches of females in oestrus were complemented by observations made from the ground. Another approach is to combine direct and indirect CMR methods. For instance, Bellemain and colleagues (2005) considered radio-collared bears as the captured group and the genetic faecal samples as the recaptured group.

Evaluation:

- Appropriateness to estimate density or abundance: abundance. It requires various ad hoc approaches to convert estimates of population size to estimates of density.
- Accuracy and precision: can be high if all the underlying model assumptions are met.
- Reliability: reliable but rarely used outside the scope of scientific investigations. It needs assumptions.
- Cost and effort required: very high.
- Spatial scale: local. Can be also applied at the population level but with big logistic and financial resources.
- Comparability: can be high for temporal analysis of the population (between years), but not for spatial (between populations).

Practical guidelines:

- Not recommended as a monitoring tool. Should be applied only if capture-recapture data derives from scientific investigations that raise broader research questions than population abundance estimation.

## 4. Camera trapping

Objective: To estimate local density and abundance from camera trap data using mark-recapture or -resight estimators. With and without individual recognition.

Methodology: Camera traps have become an important tool in wildlife monitoring and are commonly used in non-invasive studies, especially of rare and elusive species (Rovero et al. 2013). However, low precision and bias caused by individual variation in detection probability and small sample size may limit the efficacy of these methods to estimate population abundance parameters (Wilton et al. 2016, Murphy et al. 2019). Although there are examples of population estimators that account for variation in detection probability (e.g. Murphy et al. 2019), knowledge of specific ecological and behavioural differences within populations needs to be applied to improve sampling designs (Wilton et al. 2016). Certain demographic classes may be favoured with spacing of traps and extent of trap coverage (e.g. Sollmann et al. 2012), as well as temporal variation in detection introduced, dependent on the timing or duration of sampling (e.g. Wegan et al. 2012). Moreover, sampling low-density populations may yield insufficient sample size to correct during analysis for unequal detection. Thus, identifying possible sources of variation in capture probability in the field becomes crucial for improving efficacy of such sampling designs (Wilton et al. 2016).

Traditional photographic capture-recapture techniques face several limitations, one of which is that the species must be identifiable to the individual level, thus most precise and accurate for species with unique pelage patterns (Rich et al. 2014). To address the limitations of traditional capture-recapture, spatially explicit capture-recapture (SERC) models were developed, where the probability of being photographed is modelled as a function of the distance between traps and the animal homerange centre, which is unknown, but the spatial information from the traps where the individual was photographed is used in that respect (Royle et al. 2008). However, SERC models require individual recognition. Here, mark-resight models provide an alternative to spatial and non-spatial capture-recapture techniques when only a subset of individuals is recognizable by artificial (collars or ear tags) or natural marks (color patterns etc.). Those models take into account three classes of photographed animals: marked, unmarked and marked but not identifiable individually (McClintock et al. 2009, Rich et al. 2014). Additionally, for studies on species without unique patterns (and unmarked) a mark-resight framework has been proven useful for density and abundance estimates (Alonso et al. 2015). It has been used previously to collect an index of abundance for difficult to see species like bears (see e.g. Linnell et al. 1998). Nevertheless, population estimates of brown bears with camera traps increase their accuracy and precision in combination with other monitoring methods. As a sole method it is more commonly used for bear occupancy surveys (e.g. Fisher et al. 2016).

*Camera trapping without individual recognition*

Rowcliffe et al. (2008) developed a random encounter model without the need for individual recognition, that has been used to analyse the camera trapping data to estimate the population density of brown bears in Bulgaria in Central Balkan Mountains (Popova et al. 2018). This model is based on the rate of the contacts between the animals and the camera trap, taking into account the average daily distance travelled and the average number of individuals in a group, and the angle and radius as additional parameters in the formula. See red fox section for more details.

*Camera trapping with individual recognition*

Some bear species have natural marks that make individual identification possible and are used to estimate population parameters from trap camera images (Reyes et al. 2012, Van Horn et al. 2014). In the case of brown bears, cubs might have white markings on the neck that in some cases persist into adulthood and allow individual recognition. Recently, Clapham et al. (2018) have been testing high resolution images as a tool for individual recognition of brown bears by distinct facial features and have shown that brown bears can be identified to the individual level with a relatively high degree of accuracy (93.28 ± 4.9%). If successfully tested in the field, it presents a promising tool for future use in bear monitoring. With individual recognition that does not include only collared or otherwise marked individuals (e.g. ear-tags), the accuracy of local density and abundance estimates based on camera trapping would increase.

Evaluation:

- Appropriateness to estimate density or abundance: trends in density and in abundance
- Accuracy and precision: limited due to variation in detection probability and small sample size; increased when used in combination with other population monitoring methods.
- Reliability: mark-resight and recapture models without individual recognition have improved and are promising, therefore can be reliable for trends in local density and abundance.
- Cost and effort required: moderate costs in small populations or for local or seasonal densities in core areas, probability of equipment loss due to theft. Moderate effort, manpower required to analyse photo/video material, photos might be collected via online-photo-databases.
- Spatial scale: local, adaptable to regional studies; can cover the entire distribution range only in small and isolated populations.
- Comparability: local trends between seasons and years, if conducted following the same design.
- Insight on activity rhythm: daily, seasonal.

Practical guidelines:

- Recommended in combination with other population monitoring methods.
- Standardized trapping design is to be used with camera traps located to allow systematic sampling, e.g. one per 10-km x 10-km grid cells, at regular intervals.
- The choice of a specific site should aim at increased detection probability.
- Telemetry data should be used to define and correct the effective camera trap area.
- The camera setup should combine a series of consecutive pictures and video upon triggering. Proper setting of date and time, memory space on card, battery life, regular downloads, proper storage and labelling of photos are to be considered.
- Models that permit direct estimation of population density without assuming geographic closure are advantageous, and more appropriate over conventional approaches in bear population studies where recaptures at multiple locations are likely, and fairly accurate and precise density estimates are required (see e.g. Keiter et al. 2017).
- Caution is to be applied about the "edge effect", a bias that causes abundance overestimated relative to the size of the study area. Bears range widely and are available for trapping with only parts of their home ranges, depending on how study area covers the distribution of the population under study (see e.g. Obbard et al. 2010).
- Caution is to be applied for capture-mark-recapture estimators over the assumption that marks are not lost or overlooked, therefore estimator susceptibility to effects of bear movement, detection rates etc., and interaction of these factors (see e.g. Keiter et al. 2017).

**Indirect methods**

**1. Track counts**

Objective: To estimate local abundance (presence) and local demographic trends (presence of reproduction sites) based on systematically collected field signs data

Methodology: One of the widely used indirect methods of the monitoring of brown bear populations is counting signs on transects. The possible signs of the brown bear presence in the area are tracks (on snow, mud, sand), faeces, marking signs on tree trunks, presence of dens/ reproduction sites, signs of feeding behaviour (destroyed anthills, damaged fruit trees).

This method requires conducting systematic and repeated surveys along with given-length segments of forest roads or other set-up transects within the study area. The study area has to be divided into a set grid cells, for example: 5 km × 5 km, 3 km × 3 km or other, suitable for the research area or monitored area (as used for example in the study by Fernandez et al. 2012). Depending on the scale of the monitored area, within each randomly or systematically selected grid cell, the transects have to be traced, separated from each other by 1-2 km. Counting on transects should be performed systematically, every given period (for e.g. every two months, every spring/winter season). In most cases counting signs on transects is performed during winter (snow-tracking on the fresh snow), yet it can be also conducted during spring/summer when tracks on the mud are counted.

The bear presence signs should be marked with the GPS coordinates and then recorded in the databases.

When using sign surveys for estimating density, also biometric data on detected brown bear tracks have been used by means **of measurements of tracks for potential identification of individuals** (Popescu et al. 2017), like width and length of anterior and posterior foot track, yet identification of individuals by track measures is a highly unreliable method.

While **monitoring brown bear reproduction sites/dens,** the preferred season is spring after bears leave the den sites. Tracks noticed in the early spring should be investigated and back tracked to the den (if possible). The location should be marked with the GPS coordinates and then recorded in the databases. When finding bear reproduction sites or den, one can confirm the brown bear denning presence and provide data on denning habitat selection.

Brown bear population monitoring using field surveys like tracking, track or scats surveys (mixed with other, direct methods) are used in Romania (Cazacu et al. 2014, Popescu et al. 2017) and Bulgaria (Spassov et al. 2015). Researchers in Romania estimate brown bears abundance along transects using the number of unique tracks observed per survey period via N-mixture hierarchical models, which account for imperfect detection (Popescu et al. 2017).

Data on the bear signs could be also collected via various types of on-line or paper questionnaires, systematically (i.e. 2-3 times a year with the reference to winter and spring season) sent to the local administration institutions, responsible for forest and wildlife management (e.g. forestry district offices, natural/regional/national parks, directorates of natural/environmental protection, etc.). The questionnaire should be also based on the set grid, where respondents (foresters or wildlife managers) could mark the observations of brown bear signs, with detailed information on the location (GPS coordinates), date of observation, number of observed signs. All the data should be gathered in databases and used for mapping of brown bear presence in the monitored area. To evaluate collected data, one should set indices for "abundance" (presence) or "reproduction sites" with values defined as "favourable population status" (FV), "unfavourable/inadequate" (U1) and "unfavourable/bad" (U2), as defined for reporting under Article 17 of the Habitats Directive.

Surveys of field signs of brown bears might be useful as an additional, supporting method for assessing the abundance of species of a given population. Although people trained and experienced in the fieldwork may undeniably confirm the presence of brown bears in the area, yet those methods do not allow for reliable estimations of population density, population size or dynamics.

Evaluation:

- Appropriateness to estimate abundance: trends in abundance, more useful for presence surveys, registration of reproduction sites can be used for trends in the population dynamics.
- Accuracy and precision: high for monitoring the brown bear presence, limited when monitoring trends in abundance, trends in the population dynamics. It provides good proxies to compare with data gathered through direct methods.
- Reliability: not reliable for density and abundance estimates when used as a sole method; yet surveys of field signs conducted via on-line or paper questionnaires systematically

sent to the local administration institutions responsible for forest and wildlife management. May be a good method to estimate the brown bear population presence and distribution in the monitored area, as well as registering the presence of reproduction sites. One responsible institution should gather data in the database.
- Costs and efforts required: surveys of field signs realized in the field (tracking on transects) are costly and time-consuming and require trained personnel and appropriate logistics. Snow tracking or counting signs on transects depends on favourable weather conditions (fresh snow, stable weather conditions, etc.), the experience of the tracking team, thus the results might be biased. Surveys of field signs conducted via on-line or paper questionnaires systematically sent to the local administration institutions responsible for forest and wildlife management can be considered as a low-cost method, yet it requires a person maintaining the database and distributing questionnaires or information on the need to fill them.
- Spatial scale: local and national
- Comparability: presence, distribution, abundance, and presence of reproduction sites comparable between years and seasons.

Practical guidelines:

- What is the most important for monitoring the brown bear population by surveying field signs, is the design of the data gathering mode. This method requires conducting systematic and repeated surveys within the set-up area. The area should be predefined and divided into set grid cells (as proposed by Fernandez et al. 2012). Depending on the scale of the monitored area, within each/randomly/systematically selected grid cell, the transects should be traced.
- Survey of the brown bear signs of presence should be performed systematically, every given period (seasonally or yearly) on the set-up grid cell and transects.
- Tracking teams should be trained to recognize signs on the brown bear presence, like tracks (on snow, mud, sand), faeces, marking signs on tree trunks, presence of dens / reproduction sites, signs of feeding behaviour (destroyed anthills, damaged fruit trees).
- Gathering data on the brown bear presence via on-line or paper questionnaires should be conducted systematically (i.e. 2-3 times a year with the reference to winter and spring season, yearly) using a standardized set of questions or information required. The on-line questionnaire should be based on the same grid cells as used for the field studies. The observers should be asked to mark the detailed information on the location (GPS coordinates), date of observation, number of observed signs, type of observed signs and some personal data of the observer (in case of the need of the observation verification).
- All the data should be gathered in databases maintained preferably by one institution or one team and used for estimating/mapping of brown bear presence in the monitored area.

## 2. Hunting statistics and other mortality sources

Objective: To provide an index of relative abundance and trend based on hunting return rates.

Methodology: Dead animals are not members of the population anymore, but they can represent its size, structure and changes if used properly. Many hunted wildlife populations have been monitored only through hunting success for decades, even centuries. Hence, the state of hunted bear populations can be additionally monitored through hunting results.

Parameters to be systematically recorded are:

- number of bears hunted and the number that died from other causes,
- size of quota (i.e. the planned number of hunted bears),
- hunting effort involved.

Each record of a dead bear should contain information on:

- date and time,
- geographic coordinates,
- cause of death,
- sex,
- age,
- samples taken (including for DNA),
- any additional information (e.g. eventual post-mortem and results of laboratory analyses; Huber et al. 2008a).

When possible, the hunting data can be combined with the survey of sightings and of signs as was successfully done in Scandinavia (Kinberg et al. 2009). Hunting data could also be added to the mark-recapture method, for the latter, i.e. recapture phase. Previously marked animals could and should be recognized and reported when shot. However, usually only a few animals were marked for various purposes, like collaring, ear tagging or microchipping. In intensively hunted populations, tetracycline has been used as a biomarker due to its binding to the bones and teeth and being visible under ultraviolet light after the tissue of dead animal is obtained and tested. This method has been tested in both black and polar bears (see Linnell et al. 1998 for a review) and also other species of wildlife (Johnston et al. 2005), yet it has never been used in brown bears.

<u>Evaluation:</u>

- Appropriateness to estimate density or abundance: directly only relative abundance or abundance index and trend. The data on density can be obtained only if supplemented with data from other methods.
- Accuracy and precision: obtained numbers are very precise but do not give accurate results.
- Reliability: reliable when records are complete and systematically collected.
- Costs and efforts required: inexpensive as hunting and other mortality should be monitored anyhow.
- Spatial scale: depending on the area of data collection can be up to the population level.
- Comparability: along the series of years. Putting all the available data together one could reasonably explain what the reason for eventual change in the population trend was, as well as to provide the scientifically backed advice on possible management actions.

Practical guidelines:

- Total mortality tracked over the number of years, provides quite a complete picture of the state of population, including the health situation. For proper analyses numerous external information must be obtained:

  - Hunting effort - in the sense of number of days hunters being in the hides (in the case of hunting at feeding sites) or stalking in the field.
  - For traffic mortalities - in the sense of traffic intensity or eventual new traffic routes and regulations.
  - For other mortalities (including intervention removals) - eventual outbreaks of certain diseases or other environmental changes (like variations of annual production of natural food - beechnuts or other).

- When recorded over the longer period, and assuming that there is no additional unrecorded mortality (like illegal killings), the mere hunting mortality data can provide clear insight in the trend of the population, however, without telling about the actual number. It can prove satisfactory for the management purposes, i.e. for decisions about continuation of hunting under certain regime, unchanged or modified. For a more complete picture the total bear mortality should be known and considered.
- The recorded change in trend - total mortality in this case - expressed in the percentages, can then be used to calibrate the counts done by genetics; in the years between the two genetic counts of the whole population. This is the same as the use of counts of bears at the feeding sites.

### 3. Damage claims

Objective: To obtain a relative index of abundance using the number of claims for brown bear damage.

Methodology: In most European countries, damage caused by brown bears to private property is compensated, after being claimed and verified by damage inspectors (Bautista et al. 2017, 2019). The locations of these claimed damages can well complement other methods to monitor the species distribution range (permanent presence and sporadic occurrence, Chapron et al. 2014). However, the number of claimed damages has no relation with the number of bears in a given management unit (Bautista et al. 2017) and, thus, cannot provide an index of relative abundance or be indicative of population trends.

Evaluation:

- Appropriateness to estimate density or abundance: inappropriate. It only can complement data on the area of occurrence (e.g. Selva et al. 2011).
- ·Accuracy and precision: inaccurate.
- Reliability: the number of claimed damages depends on numerous factors other than the number of bears, such as the compensation and prevention programs, the existence of "problem" individuals responsible for most damage, the number and distribution of

livestock and beehives, the application of preventive measures, and the availability of natural foods. Large fluctuations among years in the number of claimed damages are observed.
- Costs and efforts required. Inexpensive, given that damage statistics are available in most European countries.
- Spatial scale: local and larger scales.
- Comparability: possible among areas and within the same area among years.

Practical guidelines: Not recommended for estimations of population abundance and density.

## 4. Non-invasive genetic sampling (genetic capture-recapture, GCR)

Objective: To estimate abundance and/or density based on identification of individuals via non-invasive genetic tagging.

Methodology: Genetic tagging allows unique identification of individuals by their DNA profile. The most common sources of DNA in the context of indirect sampling of wild animals are hair and faeces (Waits & Paetkau 2005). However, residual saliva left on carcasses has been also used to estimate the population size of brown bears (Wheat et al. 2016).Due to extensive methodological and analytical development that overcame limitations imposed by the low-quality and/or quantities of DNA obtained from non-invasive samples (Carroll et al. 2018), systematic and opportunistic collection of hair and faeces is of prime importance in genetic monitoring of brown bears.

Non-invasive hair sampling relies on the use of different hair collection devices. Although their effectiveness varies depending on the type (Berezowska-Cnota et al. 2017), those constructed of barbed wire are most popular (Stetz et al. 2010). Hairs can be passively left by bears during the course of their normal activities (e.g. on natural rubbing trees), whereas baited methods need to evoke a certain behavioural response from an individual to collect a hair sample (Kendall & KcKelvey 2008). Population estimation methods relying on collection of faecal samples should aim at collecting 2.5-3 times the number of samples as the "assumed" number of animals (Solberg et al. 2006).

Among a variety of modelling approaches available that use genetic data to estimate abundance (Carroll et al. 2018), spatial mark-recapture models SECR/SCR (Efford 2004, Royle & Young 2008) deserve special attention as they provide advantages over traditional non-spatial CMR due to spatial organization of individuals relative to sampling devices (Sun et al. 2014). Despite the high effort required for implementation of adequate sampling scheme, spatial models are increasingly used in bear research and management (e.g. Howe et al. 2016).

While the reliability of population estimates depends upon many factors, the sampling design is still considered to be the main source of bias. For the brown bear, combining multiple data sources and different sampling strategies improves detection probability, and thus the accuracy of the population size and density estimates (Boulanger et al. 2008, DeBarba et al. 2010). Nonetheless, the detector layout (extent, spacing, number, geometry) that adequately reflect population distributions and individual variation in space use is crucial for obtaining accurate and precise estimates of density and abundance (Wilton et al. 2014).

Evaluation:

- Appropriateness to estimate density or abundance: high if using spatial models (SCR/SECR).
- Accuracy and precision: high if the sampling design is proper and all the underlying model assumptions are met.
- Reliability: it depends upon many factors including study design, sampling methods, molecular techniques, population density, habitat, climate, and ultimately the resources available.
- Cost and effort required: high logistic and financial effort.
- Spatial scale: local or larger scales.
- Comparability: possible between areas if using density estimates, and within-area between years for both abundance and density estimates, however with some restrictions.

Practical guidelines:

- Spatial capture-recapture models (SECR/SCR) are clearly recommended for estimation of brown bear population abundance and density.
- The sampling design must consider the population closure assumption (i.e. no births/deaths or immigration/emigration occur during the sampling period), as well as ensure achieving an adequate and representative (large enough) number of samples. This is an important trade-off to be considered when deciding about the length and the timing of the sampling period.
- Even though consistent bear population size and density estimates can be obtained through spatial capture-recapture models regardless the type of molecular markers used (microsatellites vs. SNPs; López-Bao et al. 2020), microsatellite markers still seems to be the most common in brown bear research and monitoring.
- The sampling scheme should be adjusted to the local weather conditions due to the effects of temperature, sunlight and rainfall on amplification rates of non-invasive genetic samples (Lamb et al. 2016, Murphy et al. 2007, Piggott 2004, Stetz et al. 2015). Ideally, the weather should be low humid. Choose dry and shaded locations for hair traps to limit exposure of samples to the moisture and direct sun.As the success of non-invasive genetic studies involving hair-trapping strongly depends on the type of traps used, using the smola (beechwood tar) tree-traps together with natural rubbing trees is highly recommended for hair collection. For further methodological details regarding tree-traps, see Berezowska-Cnota et al. (2017).
- A quick field system of hair-sample quality classification can optimize selection of samples for genetic analysis if costs preclude processing all (Wirsing et al. 2020).
- When collecting faecal samples, the at-site selection of only fresh ones (less than 5 days old) can markedly increase the genotyping success. Proper labelling, preservation and storing till analyses is essential as well.
- While establishing a monitoring protocol, it is crucial to know how sampling techniques perform in a particular study area, and the financial and human resources required to obtain suitable data. Therefore, performing pilot or/and simulation studies may help to

optimize the sampling design and implicitly achieve the project goals (DeBarba et al. 2010, Solberg et al. 2006).

- Genetic monitoring should be performed on a relatively regular time basis if aiming at determining temporal trends in population demographic parameters. Due to high cost and effort, it is recommended to conduct it every six years, to coincide with reporting on conservation status of habitat and species (DG Environment 2017).

The Figure 13 illustrates some charasteristics of brown bears and methods applied to estimate abundance. The table 4 shows a classification of different population density or relative abundance estimations based on desirable characteristics for brown bear monitoring populations.


**Figure 13. Brown bear (*Ursus arctos*).** (a) Brown bear family groups at the feeding site in Croatia (image: T. Kovacevic). (b) Brown bear female with cubs of the year (at the feeding site) with disposed horse carcass (image: courtesy of Carpathian Brown Bear Project). (c) brown bear faecal sample collection. Proper labelling and preserving of each sample are crucial (image: A.

Sergiel). (d) brown bear tracks on the mud (Image: iMammalia and Nuria Selva). (e) brown bear hair sample on the tree trap (image: N. Selva). (f) hair trap baited with beechwood tar and (g) beehives destroyed by a bear in Poland (images: Nuria Selva and T. Berezowska-Cnota).

**Table 4**: Classification of different population density or relative abundance estimations based on desirable characteristics for Brown bear (*Ursus arctos*) monitoring populations in local management units, practicability, and applicability in epidemiological studies.
1=yes with restrictions, 2=depending on management rules, 3=depending on population size, habitat type (visibility), personnel available, 4=where already a routine, 5=should

| Brown bear (*Ursus arctos*) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Counts of FCOY | Counts at feeding sites | Capture-mark-recapture (direct) | Camera trapping | Survey of field signs | Hunting bags | Damage claims | Non-invasive genetic sampling or genetic capture-recapture |
| Abundance/density | A | A | A/D | A/D | A | A | n | A/D |
| Temporal trend | annual | annual | seasonal and annual | seasonal and annual | annual | over years | n | seasonal and annual |
| Info on population structure | y[1] | y | y | y | n | y[2] | n | y |
| Season | spring[3] | spring and fall | all year | all year | all year | hunting season(s) | all year | from spring to fall |
| Costs | low | low[4] | very high | moderate | moderate | low | low | high |
| Effort | medium | low[4] | very high | moderate | high | low[5] | low | high |
| Ease of learning | easy | easy | difficult | easy | moderate[6] | easy | easy | moderate |
| Accuracy + precision | H/M A + H/M P | H A + L P | H A + H P | M A + M P | L/M A + L/M P | L A + L P | low | H A + H P |
| Reliability | reliable[3] | unreliable | reliable[1] | reliable[1] | unreliable[7] | unreliable[8] | unreliable | reliable[1] |
| Useful at local scale | y | y | y | y | y | y | n | y |
| Useful at very low density | y | n | n | n | y[7] | n | n | n |
| Useful at very high density | y[9] | y | y | y | y[7] | y | n | y |
| Suitable at all | y | n[10,11] | y[12] | y | n[10] | n[10,11] | n[10] | y |

be obligatory routine, 6=depending on experience in field work, 7=useful only when combined with other methods, e.g. genetic, 8=but useful for mortality records, 9=maybe valid for monitoring and used as relative abundance for a given population, 10=only for trend, 11=only for relative abundance, 12=only if benefitting from studies conducted for other scientific purposes

## 4.5. European badger (*Meles meles*)

Estimating the size of badger populations at a local scale using direct observation is difficult because of their nocturnal and elusive lifestyle. Methods based on live-trapping can provide robust estimates of badger numbers, but are very labour-intensive and expensive. Widely used method for badger density estimation is sett survey. In high-density populations capture-recapture methods can be applied.

**Direct**

### 1. Badger sett survey

Objective: Estimation of number of main badger setts occupied in a monitored area and group size for density estimation.

The method is widely applied in high-density badger populations (Reid et al. 2011, Judge et al. 2013), but was also successfully used in medium- and low-density populations (Goszczyński & Skoczyńska 1996, Kowalczyk et al. 2003). This method was also used in reference areas for a larger scale (Keuling et al. 2011). It combines indirect and direct methods of survey.

Presence of badgers in an area is usually easy to detect because of the burrows, called setts, and also from the presence of field signs such as latrines, paths and foraging areas (Neal & Cheeseman 1996). In addition, because badgers live in social groups, and each group usually inhabits a single main sett, the number of social groups in an area can be estimated by counting the number of main setts (e.g. Cresswell et al. 1989, Wilson et al. 1997, Ostler & Roper 1998).

Badger setts are distinct structures, which exhibit various signs of activity. Each badger group occupy one main sett (used for reproduction, winter sleep and as a main shelter throughout the year), which can be distinguished by a variety of criteria such as number of entrances and size of spoil heaps (Kruuk 1978, Kowalczyk et al. 2003, Thornton 1988). While badger sett surveys are well suited to estimating the abundance of social groups, on their own they are limited in their suitability for estimating populations of individual animals (Wilson et al. 2001). This is because sett characteristics are a poor predictor of badger numbers (Lara-Romero et al. 2012), and group size can vary among the study areas (Wilson et al. 2003). Thus, the method includes identification of all main setts in the area and an estimation of average number of badgers (adult and cubs) occupying the setts.

Methodology: Depending on the size of the study area, a total area survey or stratified survey of randomly or regularly spaced on a grid sample plots, covering at least 10% of area, can be conducted. Survey should take into account habitat structure. Stratified survey allows to identify sorts of habitats that badgers select and avoid and calculate sett densities in areas with different habitat structure.

As badgers utilise setts for longer periods (decades or even hundreds of years) (Kowalczyk et al. 2000, Neal & Cheeseman 1996) information on main sett locations are usually known and can be collected from naturalists, forestry personnel, game wardens etc. A stratified survey is recommended for less known areas, particularly for initial survey, since it allow to obtain a good over-view of local badger population for considerably less effort than is required by a total sett survey. Usually one $km^2$ are surveyed as this is a convenient-sized sample units, since in many areas such areas can be surveyed by one person in a single day.

To increase accuracy of sett estimation, especially of initial surveys, a double-survey method (Magnusson et al. 1978) can be applied. It is based on two independent counts of setts by a single observers or group of observers. Sett number in the area is calculated with use of following formula:

$$N= ((S1 + B + 1)(S2 + B + 1)/(B + 1))-1$$

where: S1 - number of burrows found by the first observer only, S2 - number of burrows found by the second observer but not by the first, B - number of dens recorded by both.

Sett survey can be conducted year-round, however period from autumn to early spring, when ground vegetation is minimal, and setts are most easily detected is recommended. Setts are usually located in rough and wooded areas, so wood- and shrub patches need to be sampled using either a zig-zag transect system where patches are small and the number of surveyors limited, or a line of surveyors spaced out at regular intervals where patches are large and a greater number of surveyors is available. Badger trails (runs) radiating from the boundaries into the middle of fields can be followed if there was a possibility they would lead to a sett and around buildings were surveyed. Surveyors should especially inspect linear features, woodland edges and other rough terrain to identify badger runs and latrines. More attention was paid to higher excavated areas of diversified relief (top of hills, steep dope of hills, and walls of ravines), because such places are frequently chosen by badger for sett location.

All burrows found during the survey are classified on the basis of their characteristics and evidence of use (Thornton 1988). Main setts have a larger number of entrances (used and disused) with large spoil heaps. They look well used, i.e. the paths between entrances and to and from the sett are very obvious and well worn. There is only one main sett per social group. During direct observations or camera trapping at main setts, badgers are usually observed.

Group size estimation

Number of badgers inhabiting a sett can be estimated by several methods including:

- Direct observation
- Camera-traps or captures
- Individuals genotyping

Direct observations

Counting badgers at the sett may not be very accurate method for determining group size (e.g. Macdonald et al. 1998), but this refers to high-density populations rather than to low-density ones (comp. Goszczyński 1999). Direct observations at main setts should be conducted from May to July, when cubs are emerging from setts. They should start 1-2 hours before sunset and end when visibility drops due to dark, although night vision devices can be used. Observer should stay 20-40 meters from sett, viewing spots should be selected few days before watching (to not disturb badgers) and cover most of active entrances. As on some days some badgers rest in setts other than the main one, 2-5 surveys at each sett is recommended. The maximum number of adults and cubs seen is accepted as the group size.

Camera-trapping

Camera-traps are increasingly used to monitor wildlife (Wearn & Glover-Kapfer 2019). They were also successfully used to monitor badgers (Mori et al. 2015). Similarly to direct observations, camera trapping should be conducted from May to July, when cubs are emerging from setts. Camera traps need to be tied to trees about 5 m from active entrances to cover all used holes. Apparently, unused holes can be gently closed with branches as to prevent or detect their use during the trapping session. Badgers are not individually recognizable, the synchronization of the date and time of all cameras, together with the triggering interval set, helped to prevent double-counts.

Individuals genotyping

Size of social groups of badgers can be estimated by genotyping DNA extracted from remotely plucked hair with use of hair traps (usually barbed-wire) (Scheppers et al. 2007, Judge et al. 2017). The traps can be placed above well-used badger paths and sett entrances. It is also possible to use using baited barbed-wire enclosures (Frantz et al. 2004). Hair-trapping offers a method of censusing badgers that is relatively accurate and precise, comparatively non-invasive, potentially applicable in a variety of habitats and at different population densities, and not prohibitively expensive. For details of genetic analysis of collected hair see Carpenter et al. 2003, Frantz et al. 2004, Scheppers et al. 2007. See Table 5 for examples of group size estimations.

**Table 5:** Badger group size in different location in Europe (see also Acevedo et al. 2014).

| Locality | Group size | Source |
|---|---|---|
| Białowieża Forest, E Poland | 3.9 | Kowalczyk et al. 2003 |
| Rogów, Central Poland | 3.5 | Goszczyński & Skoczyńska 1996 |
| Saint-Blaise-Cressier-Thielle, Switzerland | 4.5 | Do Lin San et al. 2007 |
| Luxembourg | 5.5 | Scheppers et al. 2007 |
| Coto del Rey, Spain | 4.6 | Revilla & Palomares, 2002 |
| Cornwall, UK | 4.8 | Cheeseman et al. 1981 |
| Avon, UK | 5.7 | Cheeseman et al. 1981 |
| Aviemore, Scotland, UK | 5.7 | Kruuk & Parish 1987 |
| Woodchester Park, UK | 9.6 | Vicente et al. 2007 |

Evaluation:

- Appropriateness to estimate density or abundance: both.
- Accuracy and precision: method accuracy and precision increase with increasing field effort; accuracy higher at high density populations;
- Reliability: dependent on multiple factors: size of the study area, population density, knowledge of the area and earlier data on the occurrence of badgers, observer experience, work intensity;
- Cost and effort required: High personnel effort, strongly dependent on human experience. Relatively cheap at standard methods
- Spatial scale: local and large size area (stratified sampling).
- Level of comparability: the method is widely used in badger monitoring what gives opportunity of comparison between study areas or countries.

## 2. Capture-recapture

Objective: Determine number of individuals occupying setts or unique genotypes from non-invasive genetic sampling with use of capture-recapture models.

Methodology: Mark-recapture techniques are frequently used to estimate abundance of elusive or nocturnal animals in small-to-medium sized areas (Cormack 1972, Krebs, 1989). They have been also applied to estimate badger population number in different European countries (Tuyttens et al. 1999, Reid 2012). It is based on live-trapping of badgers and is recommended especially in high-density populations. Most often an adaptation of standard mark-recapture techniques called closed-subpopulation model (CSM) is used. It provides a flexible framework to increase the amount of data used for estimation of demographic parameters, by taking into account characteristics of the population and using a variety of additional non-trapping data about the occurrence of individually identifiable animals (such as from radiotelemetry, sightings, or road-traffic accidents) in addition to mark-recapture records. The model defines a subsection of the population that is known to be alive within the study area during a specific period, regardless of which animals were actually caught.

Population size is estimated from the proportion of animals in this closed subpopulation that were actually captured. Estimates of population size generated by CSM is likely to be more precise than those generated by standard techniques (for details see Tuyttens et al. 1999). Under the CSM, a simple estimator of population size at the time of the *i*th trapping occasion, $N_i$, is:

$$N_i = n_iT_i/t_i,$$

where $T_i$ - the number of badgers in the closed subsection of the population for which it was assumed that all badgers were alive and within the study area and, therefore, could have been trapped during trapping occasion *i* (number of badgers captured and marked); $t_i$ - the number of $T_i$ badgers recaptured; and $n_i$ - the total number of badgers trapped (marked and unmarked).

Badgers are caught in cage-traps placed near active setts following standard protocols (Cheeseman & Mallinson, 1980; Rogers et al. 1997). At first capture, anesthetized badgers are marked with a permanent and unique tattoo (Cheeseman & Harris 1982) or Passive Integrated Transponder (PIT) tags (Smyth & Nebel 2013). The modification of the method is CR based on genotyping individuals from hairs collected at setts (see Individuals genotyping). The number of badgers associated with each social group (i.e. with each main sett) is estimated by a mark-recapture analysis. Individual genotypes are classified as capture or recapture of hairs from individual badgers implemented into CR model. Hair traps are deployed, and hairs collected following a methodology developed for previous applications of this approach for estimating badger group size (Scheppers et al. 2007). Estimation of the number of individuals in each social group can be undertaken using the two intrinsic rates model and a "Capwire" a method designed for estimating the size of small populations from genetic mark-recapture data (Miller et al. 2005).

Evaluation:

- Appropriateness to estimate density or abundance: both.
- Accuracy and precision: method accuracy and precision increase with increasing field effort; In low-density population low trapability can occur.
- Reliability: dependent on multiple factors such as population density, trappability success sample size (recapture rate), personnel experience, work intensity.
- Cost and effort required: relatively low costs and effort to collect hair samples. Expensive method due to high costs of DNA analyses.
- Spatial scale: local due to field and lab effort as well as substantial costs.
- Level of comparability: There have been only no studies comparing results of this method with other methods aiming at estimating wolf abundance. Comparability between regions/countries possible provided that similar sampling design, lab protocols, and CR models are used.

The table 6 shows a classification of different population density or relative abundance estimations based on desirable characteristics for badger monitoring populations.

**Table 6**: Classification of different population density or relative abundance estimations based on desirable characteristics for badger (*Meles meles*) monitoring populations in local management units, practicability, and applicability.

| **Badger (*Meles meles*)** | | | | | |
|---|---|---|---|---|---|
| | Sett survey | Direct observation | Camera-traps | Individual genotyping | Capture-recapture |
| Abundance/density | D | D | D | D | D |
| Temporal trend | annual | seasonal | seasonal | seasonal | seasonal |
| Info on population structure | n | y | y | y | y |
| Season | year round | spring-summer | spring-summer | spring-autumn | spring-autumn |
| Costs | low | low | moderate | high | high |
| Effort | moderate | high | low | high | high |
| Ease of learning | easy | easy | easy | difficult | difficult |
| Accuracy + precision | MA+MP | MA+MP | MA+MP | HA+HP | MA+MP |

| | | | | | |
|---|---|---|---|---|---|
| Reliability | reliable | reliable | reliable | reliable | reliable |
| Useful at local scale | y | y | y | y | y |
| Useful at large scale | y | n | y | n | n |
| Useful at very low density | y | y | y | y | n |
| Useful at very high density | y | y | y | y | y |
| Suitable at all | y | y | y | y | y |

The Figure 14 illustrates some charasteristics of badgers and methods applied to estimate abundance.

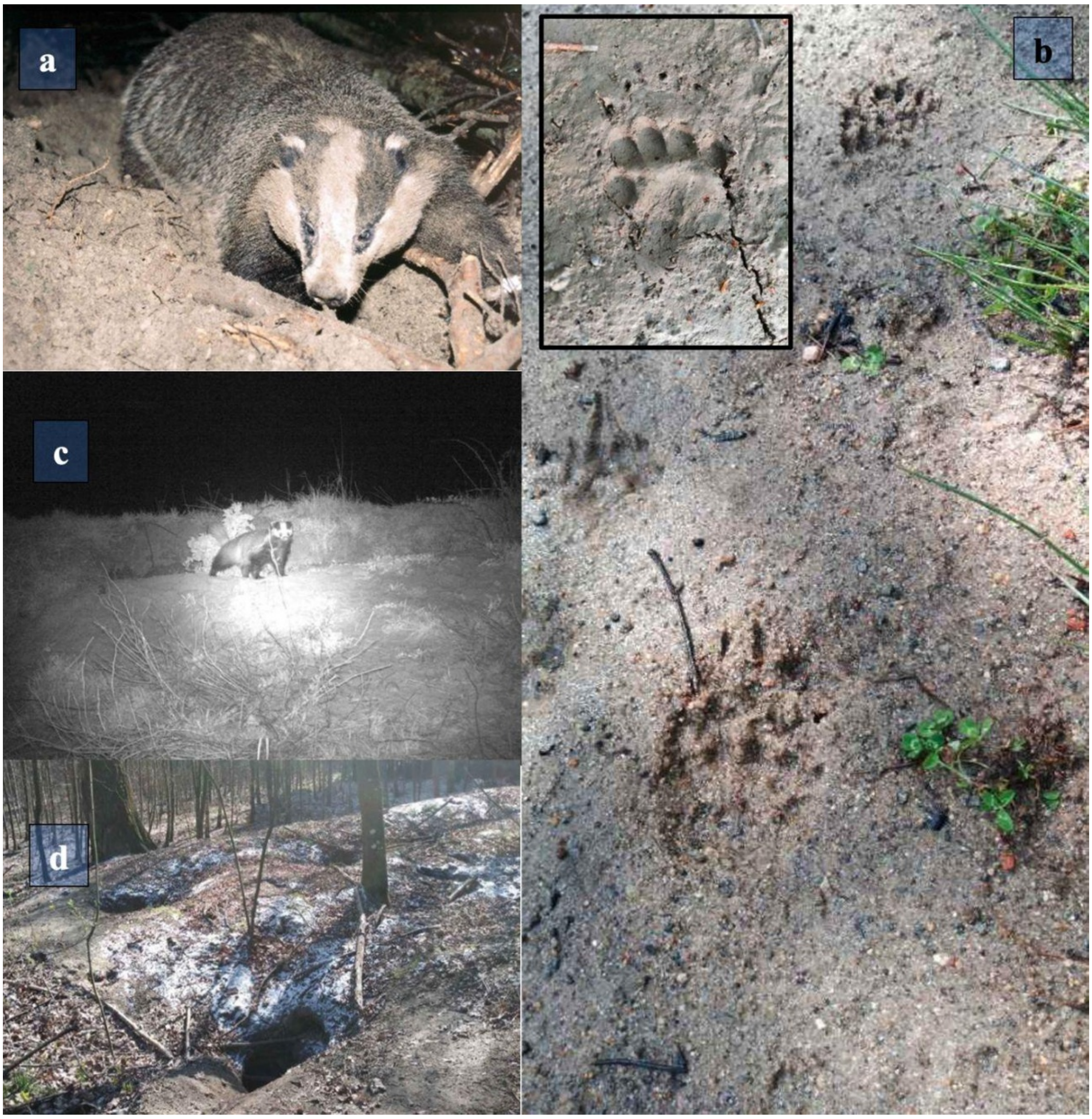


**Figure 14. Eurasian badger (*Meles meles*).** (a) badger characteristic striped face (Image: W. Jędrzejewski). In high-density populations capture-recapture methods can be applied. (b) Footprints (images: J. Ferreres and N. Fabijanic). (c) camera trap picture during night-time at the badger sett (image: A. Schnneider) and (d) badger sett (Image: M. Beblot). A widely used method for badger density estimation is sett survey. Badgers are normally caught in cage-traps placed near active setts for capture-recapture methods.

## 4.6. Eurasian Lynx (*Lynx lynx*)

The methods available and used to monitor the population of the Eurasian lynx (*Lynx lynx*) include a wide array of procedures that provide results with extremely different degrees of confidence, from guess-estimates based on hunter observations (Männil & Veeroja 2010), through detecting the lynx occurrence based on occasionally collected signs of its presence (Molinari et al. 2012) or non-invasive genetic individual identification (Davoli et al. 2012) up to the total counts based on the capture-mark-recapture approach (Breitenmoser-Würsten et al. 2007). There is no single method that can be used reliably to provide absolute population size with highest accuracy and precision. Even the most accurate method with use of radio- or GPS-marking live-captured individuals requires that it is supplemented with tracking data to obtain an estimate of unmarked portion of the population (Breitenmoser et al. 1993, Jędrzejewski et al. 1996, Breitenmoser-Würsten et al. 2007). Moreover, no single method can be equally feasibly applied in every population, since they all are based on the elements that have various limitations. Although the range of the Eurasian lynx is largely overlapping the temperate climatic zone characterised by snowy winters, using the snow-tracking based methods is often no longer possible due to global warming. On the other hand, the utility of the recently well accepted and widely used camera-trapping may sometimes be of limited use as it is based on the individual recognition, which is not always equally possible due to various share of individually distinguishable individuals in different populations (Thüler 2002, Kubala et al. 2020). Both direct and indirect methods provide tools for either estimating population densities, relative abundance or merely the occurrence of the species depending on the approach used. For instance, the snow-tracking is most often used to obtain the index of relative abundance when applied at line-transects Lohmus (2001) or can provide density estimates when total count is targeted with back-tracking approach (Jędrzejewski et al. 1996). On the other hand, some methods can only be used for detecting the species occurrence only. The recently developed method of environmental DNA (eDNA) metabarcoding already tested on both Eurasian (Harper et al. 2019) and Canada lynx (Franklin et al. 2019) despite of its sophisticated character based on the quantitative PCR of DNA samples collected from snow or water is not yet capable for identifying sex or individual (Franklin et al. 2019). Although this method would reduce the need of laborious long-distance back-tracking the animals to collect the samples of scats or hair for DNA identification, it is still limited to merely detecting the lynx presence.

There are following methods available, of which only major methods that are actually used for lynx monitoring are described in detail in this section. Aerial counts will not be evaluated here due to its limited use for lynx surveys conducted so far. The results from the aerial surveys can be rather used as a starting point for a more thorough, ground-based work and not as a single method (Schneider & Dettki 2017). Moreover, the method is not suitable in the forested areas where the majority of lynx population occur, and its costs are very high. Also, the method of estimating the population numbers by hunters that is used in Estonia (Männil & Veeroja 2010) will not be discussed due to its low reliability.

**Direct methods**

There are only two methods available for large carnivores that allow for locating and counting the individuals directly, which are reliable and logistically feasible. They include either live-capturing individual for marking with telemetry collars or photo-capturing by camera-traps.

## 1. Camera trapping (CT)

Objective: to obtain the estimate of the population size and density based on capture-mark-recapture approach.

Methodology: The CT has been already widely tested and used for the Eurasian lynx population density estimation in various parts of its range (Germany: Weingarth et al. 2012; Switzerland: PesentI & Zimmermann 2013; France: Blanc et al. 2013, Gimenez et al. 2019; Turkey: Avgan et al. 2014; Norway: Odden 2015; Slovenia: Fležar et al. 2019; Slovakia: Kubala et al. 2019). The method is based on the CMR approach, which assumes that individuals are uniquely identifiable (White & Garrott 1990). Capturing and recapturing rates of recognized individuals allow to calculate the detection probabilities of particular animals and estimation of density assuming that all individuals present in the population have a chance to be detected by camera traps. The Eurasian lynx is morphologically very variable with types and intensity of spots pattern differing within and between populations (Thüler 2002), which allows to recognize different individuals based on the unique spotting (but see below).

There are two main statistical approaches to the use of capture-recapture method for estimation of densities with the CT data. The CMR requires that the population is closed, which means that the number of animals is constant during the trapping session (no emigration, immigration, natality nor mortality). The population closure can be tested with the program CloseTest 3.0 developed by Stanley and Burnham (1999). However, to assure that the sampling area effectively captures the whole surveyed population one can choose between the non-spatial capture-recapture models and the spatially explicit capture-recapture (SECR) models (Blanc et al. 2013; Gimenez et al. 2019). The non-spatial models rely on the accuracy of designing the boundaries of the effective sampling area that can be defined by adding a buffer zone around the range covered with camera traps based on the estimation of mean maximum movement distance (MMDM) (Zimmermann et al. 2013). The MMDM can be estimated either by calculating the distances the individuals moved between camera-traps or from the daily movements data from the telemetry study available for the population being surveyed (Weingarth et al. 2012). With the non-spatial approach, it is advised to use ½ MMDM to determine the buffer width as it provides more reliable and less underestimated density estimation (Pesenti & Zimmermann 2013).

The spatially explicit approach (SECR) allows to directly estimate population density using information on capture histories and their spatial locations in the Bayesian modeling framework. An important difference from the non-spatial models is that each identified individual is assumed to have an independent activity center (or home range) and that the probability of its detection decreases with the distance from this center (Pesenti & Zimmermann 2013, Gimenez et al. 2019). The model requires information on capture details, timing of camera-trap deployments and potential location of the home-range (activity) centers. It is essential that the area of camera-trapping should be included within a larger buffer, which has to be large enough so that it contains all individuals that could have been exposed to the camera-survey (Zimmermann et al. 2013).

The SECR models seem to provide yet more reliable estimates than non-spatial models as there is no need to delimit the borders of the area surveyed, because the camera-trapping grid is included in a larger area allowing for animal movements beyond the trapping area (Pesenti & Zimmerann 2013, Royle et al. 2016). Moreover, it was shown that the density estimates by SECR models are less susceptible to the size of the area surveyed than the non-spatial models (Zimmermann et al. 2013). Therefore, SECR models are recommended as superior to the non-spatial models for estimation of the Eurasian lynx densities (Zimmermann et al. 2013; Blanc et al. 2013, Gimenez et al. 2019).

The studies with use of CT in these felids have been conducted so far in populations characterised with the greatest intra-population variability of the coat spottiness, so that recognition of individuals have been very feasible (Carpathians, Alps, Norway). However, the share of individuals with clear spots vary among populations and some of them consist of animals completely devoid of spots (Thüler 2002, Kubala et al. 2020). Although the unspotted lynx usually possesses a few spots on the inner side of the legs, which may be used for identification, the potential of determining the diagnostic signs is limited. Therefore, in populations where most or all individuals are unspotted the use of the CMR method is hardly applicable. In such cases the densities can be estimated with a point transect distance sampling (DS) with use of camera traps (Howe et al. 2017). This approach is based on the estimates of the distance between the animal and the camera, the view angle and the proportion of time animals are available for detection. Multiple observations of each animal provide data to calculate detection probability estimates. However, the DS, has never been used for the Eurasian lynx and requires validating.

<u>Evaluation</u>:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high accuracy and high precision.
- Reliability: very reliable, however it is highly dependent on the morphological differentiation of individuals, and good quality camera-traps allowing for clear pictures of the spots pattern.
- Costs and effort required: moderate costs, which include the purchase of camera traps, maintenance of the CT in the field, manpower for analysing the photo-material.
- Spatial scale: local, though the size of area surveyed is recommended to cover several hundred square kilometres to include sufficient sample size.
- Comparability: potentially very comparable among different sites though very much dependent on the camera-trap models used and the capture-recapture models (spatial or non-spatial and the type of MMDM estimates). The SCR and ½ MMDM models have been shown to give comparable results to the telemetry estimates (Pesenti & Zimmermann 2013).

<u>Practical guidelines</u>:

- The camera-trap sampling design should ensure that the area surveyed include no gaps that could contain an entire animal's home range. The grid suggested for the Eurasian lynx CT monitoring is 2.7x2.7 km (Zimmermann et al. 2013, Blanc et al. 2013, Gimenez et al. 2019).

- The surveyed area should include a minimum of 20 individuals and the capture probability should be greater than or equal to 0.1 to obtain reliable abundance estimates (Zimmermann et al. 2013). At least 20 recaptures are needed for a precise estimate using an SECR model (Efford et al. 2009). To achieve these conditions the sampling area should be large enough area to warrant capturing sufficient number of lynxes.
- Zimmermann et al. (2013) concluded that due to very low densities of lynx populations several measures should be taken into account to design a reliable CT survey. First, to increase the detection probability, the placement of CT should follow the landscape features that are most likely used by the lynx while traveling. Second, the minimum survey area should cover at least 760 $km^2$ to reach the minimum 20 individuals necessary for reliable density estimation. Third, lynx densities should be estimated with SECR models as they consider animal movements explicitly and are not biased by an estimation of the effective sampling area.

## 2. Telemetry

Objective: to estimate the total population size within a specified area and the population density.

Methodology: Telemetry is not specifically designed to monitor the abundance and density of wildlife, but when used in a complementary way with other methods, it provides the most accurate estimates of the population size approaching the true densities (Linnell et al. 1998, Balme et al. 2009). However, the method includes live-capturing animals that requires professional personnel trained with capturing and handling wild animals as well as official licensing for handling wildlife. Either VHF or GPS transmitters can be used to mark individuals. The population size and density can be estimated directly by summing up the number of collared individuals and observations of tracks that could not be attributed to the marked individuals (Breitenmoser et al. 1993, Jędrzejewski et al. 1996, Breitenmoser-Würsten et al. 2007). This approach is a simplification of the capture-mark-recapture (CMR) technique which uses a statistical procedure such as the Lincoln-Petersen estimate to derive the population size estimate (White & Garrot 1990). The animals marked with the telemetry devices constitute the known (captured) number of individuals in the population and the re-captured individuals are those collared individuals identified during snow-tracking (or camera-trapping) census. The CMR can also be applied with the telemetry data by calculating the probability of observing an individual in the population based on the rate of its locations within the study area as proposed by White and Shenk (2001).

Scientific data obtained from research using GPS or radio-telemetry are also providing important information for other monitoring methods. Data on home range size and daily movement distances make a baseline knowledge for the lynx family groups counts (Andrén et al. 2002, Linnell et al. 2007b, Gervasi et al. 2013) and can also be used to set a mean maximum distance moved (MMDM) for the non-spatial capture-recapture models of population density estimation in camera-trapping data analysis (Weingarth et al. 2012).

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high accuracy and high precision (but dependent on the statistical model used).

- Reliability: potentially most reliable method provided that a substantial portion of the population is marked simultaneously. However, due to high costs and efforts, it is practically exclusively used as a by-product of research focused on specific ecological questions.
- Costs and effort required: most expensive method, very high costs of the telemetry equipment plus additional costs and high effort of the field staff to capture animals.
- Spatial scale: local.
- Comparability: highly comparable among various population provided the same technical approaches are used. Abundant previous experience allows to follow the same technology, parameters and statistical methods to obtain and analyse data. As the CMR method the results are comparable to the camera trapping techniques.

Practical guidelines:

- method not specifically recommended if the sole purpose is to monitor the population size;
- to derive reliable population estimates, the majority of individuals in the population should be marked.

**Indirect methods**

**1. Snow-tracking**

Objective: to record the presence and/or the number of tracks to calculate an abundance index of tracks per unit of line transect or the area or to estimate the total count of lynx family groups or total population size.

Methodology: There are various methodological approaches to the snow-tracking performance and interpreting its results for evaluation of the Eurasian lynx abundance. They can be grouped into three main types: 1) transect count, 2) family group count and 3) total count. The transect counts are conducted on permanent line transects to obtain a track index per 1 km. It provides a relative abundance measures and can be used to monitor temporal trends of population changes. It is regularly used in Russia (Bragina et al. 2015), Estonia (Lohmus 2001) and Finland (Anonymous 2007), but also to some extent in Norway (Linnell et al. 2007a).

Snow-tracking data accumulated over a longer time can be converted to estimation of family groups based on the information on maximum distances moved and home range size derived from the telemetry studies (Andrén et al. 2002, Linnell et al. 2007b, Gervasi et al. 2013). It is assumed that tracks of several individuals travelling together, recorded in mid-winter (December-February), belong to females with kittens and not to mating individuals, because the offspring stays with their mothers till March when the mating begins. This way the observations of females collected during a reasonably short period can be separated to different families (Andrén et tal. 2002). The method is successfully applied for the lynx monitoring and management in Fennoscandia. However, due to high variability of the range of lynx movements across its distribution area (Herfindal et al. 2005) it is necessary that the movement parameters applied to distinguish different lynx families should be based on locally collected data from telemetry studies (Linnell et al. 2007b).

Total counts by snow tracking have been attempted by two methods. First method involves intensive searching for lynx tracks coordinated on large regions with use of numerous trackers during a single day after a fresh (1-2 days) snowfall (Andrén et tal. 2002; Jędrzejewski et al. 2002). The trackers attempt to discriminate between different individuals or groups by following each track until the day-bed. The second method is based on extrapolation of the number of family groups obtained from snow-tracking to the total population size using data on survival and reproduction from telemetry studies. Based on the proportion of family groups among all marked individuals an extrapolation factor can be established to convert the number of family groups to the total population counts (Andrén et tal. 2002).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance or density, depending on the approach used (transect or total count).
- Accuracy and precision: medium accuracy and medium precision; highly dependent on the availability of movement data in local populations and the engagement and motivation of local trackers.
- Reliability: dependent on good coordination of survey in large areas; more reliable at local scale.
- Costs and effort required: moderate costs, includes high number of trackers and costs of using cars.
- Spatial scale: possible at large spatial scales with high effort and good coordination.
- Comparability: transect counts are well comparable as they provide just a relative abundance; both family counts and total counts are potentially comparable provided that correct source data (distance rules, extrapolation factors) representative for local populations are used for population size estimation.

Practical guidelines:

- tracking sessions should be conducted between December - February to minimize interference with mating behaviour (incorrect assignment of tracks of mating lynx to family groups);
- when using distance rules to separate family groups one should account for variability of daily movements among different survey areas (Linnell et al. 2007b);
- extrapolation factors should be adjusted to the local conditions (Andrén et al. 2002);
- a considerable effort should be made to ensure that back- or forward-tracking to differentiate day-beds of different individuals/family groups is reliable;
- 2 - 3 sessions of snow-tracking should be performed for one winter to increase detectability of individuals.

## 2. Non-invasive genetic sampling

Objective: to identify genetically lynx individuals and determine the population size.

Methodology: The method requires obtaining the material being a source of DNA from wild living lynx. Two methods are available and have been already tested in the Eurasian lynx. The first is to collect the lynx hairs with use of hair-traps (Schmidt & Kowalczyk 2006) and the second involves collecting lynx scats (Hollerbach et al. 2018). Collecting hairs is possible due to the natural cheek rubbing behavior typical for felids, and several types of hair traps have already been proposed (Schmidt & Kowalczyk 2006, Heurich et al. 2012, Patko et al. 2016). The lynx should be attracted to the hair-trap with a specific lure which induces and strengthens rubbing behaviour. The beaver castoreum and catnip oil has been shown to work well in both captive conditions (Heurich et al. 2012) and in the wild (Schmidt & Kowalczyk 2006). Another source of DNA - scats can simply be searched and collected following the forest roads and trails in the area occupied by lynx, however, random search may be inefficient, and scats may not be correctly identified to the species. To facilitate searching for the lynx scats the use of dogs has been recently tested in the Bavarian Forest National Park, Germany, with optimistic results (Hollerbach et al. 2018). Both hair-traps and "detection dogs" have been shown similarly efficient in providing the PCR success rate of about 30% with regard to all collected material. However, if only the hair samples selected for genetic analyses (a sufficient hair number with roots) are considered, the success rate increased to 67% (Davoli et al. 2013). The total population size (Nc) based on the genotypes identified from hair (or scat) samples can be calculated with a software CAPWIRE (Miller et al. 2005) and the effective population size (Ne) can be obtained based on the linkage disequilibrium with software LDNE (Waples 2006).

Evaluation:

- Appropriateness to estimate density or abundance: total (and effective) population size, density.
- Accuracy and precision: high accuracy and precision.
- Reliability: high; however, it is recommended to be used as a supplementary method to other monitoring techniques, such as radio-tracking, camera-trapping or snow-tracking to reach highest reliability (Davoli et al. 2013).
- Costs and effort required: expensive, major costs include the genetic analyses; hair traps may be much cheaper for sample collection than detection dogs if costs of contracting dogs, wage for dog handlers are necessary to be considered.
- Spatial scale: local but could be extended to regional scale by shifting surveyed areas in consecutive years.
- Comparability: to assure comparability among areas, a standardized protocol should be used that includes both sampling and laboratory analyses (use of genetic markers). The method uses the CMR approach to estimate lynx density, so it is comparable to CT and telemetry (but see reliability).

Practical guidelines:

- most reliable if conducted simultaneously with other methods (telemetry, camera-trapping);
- hair traps should be placed along the forest roads and trails in conspicuous sites being potential scent-marking posts;
- hair-trapping is most effective during the pre-mating and mating season, when scent-marking intensifies;
- hair-trap type should be designed in a way allowing replacing the trap each time when hairs are deposited to avoid contamination with hairs of different individuals; thus, a piece of carpet with short nails is recommended as very practicable (Schmidt & Kowalczyk 2006);
- hairs should be dried immediately and can be stored dry for extended periods;
- maximum likelihood of each genotype should be calculated to exclude non-reliable genotypes with high accuracy;
- the "shadow effect" (different individuals showing the same multilocus genotype: Mills et al. 2000) should be minimized by increasing the number of loci;
- attention should be given to possibility of collecting mixed hair samples of different individuals at one trap, which may have produced false alleles in the genotypes. FA should be recognized and rejected.

### 3. Registration of animal signs

Objective: to obtain estimates of the population occurrence and relative abundance at larger areas.

Methodology: This method is mainly aimed at monitoring the occurrence and distribution of species on larger spatial scales, which is why it is doomed to lack of estimated detailed data on abundance. It is based on collecting reliable signs of lynx presence, such as tracks, scats, occasional photographs, foraging signs, dead individuals (e.g. road kills), which are accompanied by geographic location. It can be shown as a number of signs per unit area, as it is occasionally used in Latvia (Ozoliņš et al. 2017). A standard classification of the Eurasian lynx observation records has been developed by international lynx experts from the region of the Alps within the framework of the project "Status and Conservation of the Alpine Lynx Population" (SCALP) (Molinari et al. 2012). The records are classified into one of three categories according to their reliability: category C1 represents 'hard fact' data (e.g. dead lynx); category C2 includes confirmed data (e.g. tracks verified by an expert); category C3 comprises unconfirmed data (e.g. any kind of direct visual observation). The survey is based on an established network of experts trained to recognize and assess the different signs of lynx presence, who provide data annually into the SCALP database (Molinari et al. 2012). The SCALP categorization has been already adopted in many countries (e.g. Germany: Kaphegyi et al. 2006; Slovenia: Kos et al. 2012; Czech Republic: Kutal et al. 2013; France: Marboutain et al. 2006; Switzerland: Molinari et al. 2012).

The signs of lynx presence can also be registered with use od "citizen science" - collecting data on a voluntary basis from the public (Schneider & Dettki 2017). This can be realized via specifically designed webpages like in Sweden (http://www.skandobs.se) or a questionnaire survey in the public preceded by a media campaign (Balciauskas et al. 2017).

Evaluation:

- Appropriateness to estimate density or abundance: occurrence or relative abundance.

- Accuracy and precision: not applicable.
- Reliability: high in terms of the occurrence, though it depends on the category of reliability (C1, C2 or C3).
- Costs and effort required: low costs and effort, however some extra costs of manpower may be related to the database management.
- Spatial scale: large scale.
- Comparability: the information on species distribution is essential for effective conservation and management of a large carnivore. Therefore, the use of standard categorization of lynx observations throughout the entire European range of the species should ensure reliable monitoring of changes in distribution on a large spatial scale.

Practical guidelines:

- establish well-organized network of trained local assessors;
- consider a high probability of false-positive records in the C3 category of data, which may lead to overestimation of the occupied area; in the extreme case it may result in a dangerous overlooking the population decline;
- discard the least reliable records.

## 4. Hunting bag statistics

Objective: to estimate population size and population structure based on data on lynx harvested.

Methodology: This method is limited to countries were the lynx is a hunted species. However, it is not widely used for lynx population monitoring and management. The harvest data are basic for estimation of population size based on sex and age structure of hunted individuals in Latvia (Bagrade et al. 2016, Ozoliņš et al. 2017). The age structure of all harvested lynx is assumed to correspond with the age structure of sampled lynx and is estimated by dividing the number of samples in a specific age group with the sample proportion to the total number of harvested lynxes. Reconstruction of lynx population size using the age cohort analysis was found to match the estimates based on counting family groups in Norway (Nilsen et al. 2012).

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: the method can be accurate provided that all shot animals are available for necropsies and that the level of illegal hunting and other mortalities are accounted for.
- Reliability: highly reliable provided that all conditions for accuracy are filled.
- Costs and effort required: low costs and effort.
- Spatial scale: large scale.

- Comparability: as shown by the Norwegian test (Nilsen et al. 2012) the hunting harvest data can be well comparable with the main survey method based on family group count.

Practical guidelines:

- non-harvest mortality rates should be considered for reconstructing population number based on harvest data;
- all harvested lynx should be necropsied for sex, age, reproductive and physical condition, and samples collected for genetic, parasitological and other health-related investigations.

The Figure 15 illustrates some charasteristics of Eurasian lynx and methods applied to estimate abundance. The table 7 shows a classification of different population density or relative abundance estimations based on desirable characteristics for Eurasian lynx monitoring populations in local management units, practicability, and applicability.


**Figure 15. Eurasian lynx (*Lynx lynx*).** (a) hair traps should be placed along the forest roads and trails in conspicuous sites being potential scent-marking posts. Non-invasive genetic sampling allows identifying genetically lynx individuals and determine the population size. The method requires obtaining the material being a source of DNA from wild living lynx: hairs with use of hair-traps or lynx scats. (b) in lynx populations where most or all individuals are unspotted the use of the CMR method is hardly applicable (images: K. Schmidt). Capturing and recapturing rates of recognized individuals allow to calculate the detection probabilities of particular animals

and estimation of density assuming that all individuals present in the population have a chance to be detected by camera traps. (c) Although the range of the Eurasian lynx is largely overlapping the temperate climatic zone characterized by snowy winters, using the snow-tracking based methods is often no longer possible due to global warming. (d) the presence of the *tapetum lucidum*, which reflects the light if hit by it (e. g. camera rap), may increase the probability to detect the animals in the dark. Note the spotted pattern of the fur (c, d) compared to (b) (images c and d: Anil Soyumert & Alper Ertürk; project supported by Kastamonu University and Ministry of Agriculture and Forestry, Turkey).

**Table 7**: Classification of different population density or relative abundance estimations based on desirable characteristics for Eurasian Lynx.

| **Eurasian lynx (*Lynx lynx*)** | | | | | | |
|---|---|---|---|---|---|---|
| | Telemetry | Camera trapping | Snow-tracking | Non-invasive genetic sampling | Registration of animal signs | Hunting bag statistics |
| Abundance/density | D | D | A/D | D | A | A/D |
| Temporal trend | annual | short | seasonal | seasonal | seasonal | seasonal |
| Info on population structure | y | y | y | y | n | y |
| Season | year round | year round | winter | winter | year round | winter |
| Costs | very high | moderate | low | high | low | low |
| Effort | very high | moderate | low | moderate | low | low |
| Ease of learning | difficult[1] | easy[3] | easy | easy[4] | easy | easy |
| Accuracy + precision | HA+HP | HA+HP | MA+MP | HA+HP | na | HA+LP |
| Reliability | reliable | reliable | reliable | reliable | reliable[5] | reliable[6] |
| Useful at local scale | y | y | y | y | y | y |
| Useful at large scale | n | r | y | r | y | y |
| Useful at very low density | y | y | y | r | y | n |
| Useful at very high density | y | y | y | y | y | y |
| Suitable at all | y[2] | y | y | y | y | y |

1 - requires professional personnel trained with capturing and handling wild animals, 2 - suitable, but not recomended as the method used solely for monitoring, 3 - requires basic statistical skills to apply CMR models, 4 - requires the use of molecular techniques, but possible with use of commercial service, 5 - concerns the occurrence, 6 - highly reliable provided that all conditions for accuracy are filled, r - restricted, na - not applicable.

## 4.7. Iberian Lynx (*Lynx pardinus*)

The Iberian lynx (*Lynx pardinus*) is a wild cat species endemic to the Iberian Peninsula and listed as Endangered on the IUCN Red List (Rodríguez & Calzada 2015). By the beginning of the 21st century, the Iberian lynx was closely to extinction, as only about 100 individuals survived in two isolated subpopulations in South Spain (Andalusia). However, conservation measures were implemented since 2002 which included improving habitat, restocking of rabbits, translocating, re-introducing and monitoring Iberian lynxes; and by 2020, the population had notably increased.

The Iberian lynx is a medium size carnivore (males 7-15.9 kg, Females 9.2-10 kg (20-22 lb) with spotted fur, whose staple prey is the European Rabbit (*Oryctolagus cuniculus*) The habitat preferences include heterogeneous environments of open grassland mixed with dense shrubs and *Quercus* trees. Nevertheless, recent findings suggest that Iberian lynxes hold greater ecological plasticity than previously thought. Thus, habitats that were formerly considered as non-suitable, such as agricultural land, with low scrubland coverage could indeed permit lynx establishment if wild rabbit densities are adequate (Garrote et al. 2016, Gaston et al. 2016). This species is territorial, and the home ranges of adults are normally stable over years. The size of the territory varies depending on the abundance of its main prey, the wild rabbit, with the territories of the males being greater than those of the females (Female: 300-800 ha; male: 600-1200ha) The Iberian lynx plays the role of apex predator of the terrestrial vertebrate community in the Mediterranean ecosystem. The presence of the species n lynx affects the spatial distribution of other mesocarnivores as red fox (*Vulpes vulpes*), Egyptian mongoose (*Herpestes ichneumon*), beech marten (*Martes foina*), wildcat (*Felis sylvestris*), and common genet (*Genetta genetta*) (Garrote et al. 2019)

Unverifiable observations, a type of anecdotal occurrence data, or tracks and scats detection and species assignment based on morphology are often used to assess the ranges or abundances of carnivores (Al-Johany 2007; Din & Nawaz 2010). However, the use of such data has been widely criticized since misidentification is likely to occur (Garrote & Ayala 2015, Monterroso et al. 2013). In the late 1990s, non-invasive sampling began to be applied widely, as sampling of DNA-analysed scats and photo-trapping, that provide verifiable physical evidence of the species (Garrote et al. 2011, Garrote & Perez de Ayala 2015). Both methods were used to carry out the national survey of the Iberian Lynx at the beginning of the 21st century (Guzman et al. 2004), and which laid the backgrounds for the subsequent monitoring of the species. This is when the first estimation of the Iberian lynx population using capture - recapture analysis of camera-trapping data was made (Garrote et al. 2011). Likewise, scat surveys not only allowed establishing the distribution area of the species at the Iberian level, but also the utility of the scat count to estimate population densities (Garrote & Perez de Ayala 2015).

At that time the species reached its all-time minimum, when 100 individuals in just two isolated populations - in Andújar-Cardeña and Doñana - were known to exist (Guzmán et al. 2004) in a reduced occupied surface area (500 $km^2$; Guzman et al. 2004, Sarmento et al. 2009). Due to this situation and the contribution of various conservation programs, the monitoring of the species during these two decades has been very intensive, where most of the occupied lynx range surveyed annually by camera-trapping (Gil Sanchez et al. 2011). This long-term camera-trap monitoring program has allowed us to obtain not only the annual population size, but also to know the breeding females and their home ranges (Gil Sánchez et al. 2011), reproductive rates (Monterroso et al. 2016), as well as survival rates using CR analysis for open populations (Perez de Ayala 2017).

The area occupied by the species annually is established by combining the data obtained by camera trap, scat surveys and radio-tracking data (Gil Sánchez et al. 2011). Radio-tracking has been used to understand aspects of the spatial ecology and habitat selection of the species (Gil Sanchez et al. 2011, Gaston et al. 2016), mortality rates, as well as to provide information on the distribution of the species, but it has not been used in recent decades to estimate abundance or density. This is due to camera trap has allowed obtaining accurate estimates throughout the distribution range of the species, something impossible to obtain by radio-tracking However, radio tracking data can be used to support some aspects of the estimates by camera trapping (see below).

As can be deduced from the above, the main methodology for estimating the abundance and density of the Iberian lynx is camera trapping, so it will be the methodology that will be described here in detail. Likewise, the usefulness of scat counts will be described as a potential method for making these estimates.

**Direct methods**

**1. Camera trapping (CT)**

*Capture-recapture approach*

Objective: Estimation of population size and density using capture-recapture analysis of camera-trapping data.

Methodology: Traditionally, estimates of population abundances and densities using photo-trapping cameras have been performed with non-spatially explicit capture recapture (CR) models for closed populations. The objective of CR surveys is to estimate the number of individuals within a sample area. In general terms, this estimate is obtained by first estimating capture probability based on the "capture histories" of individuals that are caught at least once. All lynxes photographed can be individually identified through a comparison of distinguishing natural body marking (spots) (Garrote et al. 2011). The Iberian Lynx is the most heavily spotted member of the genus lynx (Wilson & Mitterneier 2009) and have three types of spots pattern: small, intermediate and thick spot. The proportion of each type of spot pattern varies in each population, but in all cases the spot pattern is clear and allows to recognize different individuals. The capture history consists in a string of ones and zeros indicating whether the individual was camera trapped (1) or not (0) during each trapping occasion. Statistical modelling of capture histories leads to an estimate of the unsampled fraction of the population and hence the total number. Once the abundance is calculated, the density is obtained by dividing that abundance by the effective sampling area (O'Brien 2011). The sampling areas is calculated adding a buffer to the area defined by the outer camera trap polygon. The most widely used method to establish this buffer is based on the average of the maximum distances moved by the individuals (MDMM; Wilson & Anderson 1985), obtained as the average of the maximum distances between cameras in which each animal in the study has been photographed. The measure applied as a buffer is ½ MDMM. The choice of this measure is based on the assumption that ½ MDMM is greater than or equal to the radius of the circle whose area is equal to the mean territory of the animal under study. Thus, animals whose centre of their (circular) territory is within of the buffer, they will probably be included in the sampled area (O'Brien 2011). In the case of the Iberian lynx, density estimates were compared with telemetry *versus* density estimates with CR and it was concluded that ½ MDMM is the one that best estimates the actual sampling area (Soria 2016). However, this is not the case for other felid species, such as jaguar or tiger, whose density estimates are less biased using the full MDMM (Maffei et al. 2011, Parmenter et al. 2003, Soisalo & Cavalcanti 2006).

It is also possible to use telemetry data to identify the size of the width buffer, where the buffer width is equal to the average of the home-range radius of the radio-collared lynxes. The home-range radius for each of the lynx is computed by assuming that the home range approximated a circle (Sharma et al. 2010).

Spatially explicit capture and recapture (SECR) models do not require calculation of the effective sampling area. These models estimate the density using information from the capture history in combination with the location of the individual captures, under either Bayesian or probabilistic analysis (Borchers & Efford 2008, Romairone et al. 2018). Recently, SECR models have been used to estimate Iberian lynx populations at different spatial scales (Garrote et al 2020, Jimemez et al 2019, Sarmento & Carrapato 2019, Soria 2016). SECR models are based on the assumptions that animals have randomly distributed circular action areas and that successive catches are independent (Noss et al. 2013). SECR uses two distinct sub-models within its workflow to compute densities (D). One sub-model simulates an animal's distribution from the capture history to give the individual's activity centre as an output, while the second simulates the capture process on the basis of the radial distance between the estimated centre of activity and the traps (Efford et al. 2009). Capture probability is a declining function of the distance between activity centres and camera traps and are analogous to distance sampling (Buckland et al. 2001). Input data is included in two files, one containing the name and geographical coordinates of the detectors (cameras) and another containing the capture histories (i.e. season, animal identification, the occasion and the detector), thus associating each capture of an individual with the respective coordinates of the camera station and the day of capture.

The SECR methodology does not require the calculation of the effective sampling area, the main source of variability when calculating the density (Foster & Harmsen 2011). But it also has disadvantages such as the assumption that the animals have independent centres of activity (somewhat unrealistic in the case of carnivores), the need to have multiple photo-trapping stations per medium territory and the high number of recaptures necessary to obtain an accurate estimate (Foster & Harmsen 2011).

When carrying out a capture-recapture study through camera-trap sampling, two main assumptions must be taken into account (Nichols 1998) that will determine the sampling design:

- **The population must be closed,** that is, there are no births, deaths, immigration or emigration of individuals during the sampling period (O'Brien 2011). Few wild populations are closed, so it is assumed that this assumption is met by limiting the duration of the study. In the case of the Iberian lynx, it has been verified that between 1-2 months, which was sufficiently short to meet closed population assumptions and time necessary to stabilise the cumulative curve (Garrote et al. 2011, Garrote et al. 2012). Anyway, the population closure must be statistically tested (Stanley & Burnham 1999).
- **All individuals have a probability of capture greater than zero,** that is, each individual must have the probability of being captured at least once. To avoid this, there must be at least one camera in an individual's area of action during sampling (Noss et al.

2013). This assumption limits the maximum distance between cameras, as there cannot be gaps large enough between them so that there is an area of action of an individual without sampling. A conservative strategy is to take the smallest occupied home range size known for any given age - sex Class documented for the species in the geographic region studied, to calculate the diameter of a circle with that area, with that diameter being the maximum distance that can exist between two photo trapping stations (Noss et al. 2013). In general, a minimum of up to 2 stations per average action area is recommended (Dillony 2007). However, in the case of intensive monitoring of the Iberian lynx, a density of 1 camera / 100ha has been applied, which implies more than 4 cameras per potential average home range (Garrote et al. 2011, 2012, Gil et al. 2011, Sarmento et al. 2017). This was a clear contribution to increase capture probability and to decrease the coefficient of variation (CV) of parameters estimates (Sarmento & Carrapato 2019).

Evaluation:

- Appropriateness to estimate density or abundance: density and abundance.
- Accuracy and precision: high accuracy and high precision.
- Reliability: Very reliable, as long as the fieldwork is carried out properly and the experimental design adheres to the method assumptions.
- Costs and effort required: Moderato to high cost. The initial costs of purchasing camera traps can be high. These costs are offset in long term monitoring programs. Photo processing work can be high depending on the spatial scale addressed.
- Spatial scale: Local to regional. Its efficiency has been proven applied to populations that occupy areas of several hundred square kilometres.
- Comparability: Very comparable as long as the fieldwork is carried out properly and the experimental design adheres to the method assumptions.

Practical guidelines:

- The use of attractants is recommended, as it has been proven that it increases the effectiveness, accuracy, and efficiency of capture-recapture abundance estimates in the Iberian lynx (Garrote et al. 2012).
- It is recommended to sample the entire study area simultaneously. However, this is not always logistically possible. In this case, it is recommended to divide the study area into blocks that will be sampled sequentially (Garrote et al. 2011).

*Random encounter model approach*

Objective: Estimation of population density by rescaling the trapping rate (y/t) to population density using the day range (DR, i.e. daily distance travelled by an individual), group size (i.e., the mean number of animals in a group), and camera-related parameters (radius and angle of camera detection).

Methodology: The random encounter model (Rowcliffe et al. 2008), a method for estimating animal density using camera traps without the need for individual recognition, has been developed over the past decade. Obviously, the precision and accuracy of the CR estimates in which it is possible to identify the individuals is higher, but recently the viability of this method to estimate densities of Iberian lynx has been verified and is therefore exposed here. An advantage of the method over CR is that the required photo processing work time is lower, since the identification of individuals is time-consuming. On a small scale, where the number of individuals is not very large, this work can be relatively fast, but the time dedicated to this work increases exponentially with the number of individuals detected.

This method is based on modelling random encounters between animals and cameras and takes into account variables affecting the trapping rate. To convert camera-trapping rates into lynx density must be used the equation:

$$gD = \frac{y \quad\quad \pi}{tvr(2 + \theta)}$$

in which y is the number of independent photographic events, t the total camera survey effort, V the average speed of animal movement, and r and θ the radius and angle of the camera trap detection zone, respectively.

A key assumption of this model is that cameras are placed randomly in relation to animal movements, requiring that cameras are not set only at sites thought to have high animal traffic. Therefore, REM method could not be applied to study design, commonly used in carnivores, as Iberian lynx, where cameras are placed on tracks to maximize capture probability. Nevertheless, Garrote et al. (2019) developed a correction factor (CF) for expected deviations from REM density estimates using data generated by conventional camera-trap design. The correction factor corrects for the differential use-rate between tracks and the rest of the area made by lynx:

$$\text{CF} = \frac{\text{road availability}}{\text{road use}} = \frac{\frac{\text{road surface}}{\text{total surface}}}{\frac{\text{on-road localizations}}{\text{total localizations}}}$$

To estimate the use of roads by lynx, is necessary use data from a GPS collar. This factor corrects the REM results and gives the corrected REM (REMc) result as:

$$REMc = REM \times CF$$

<u>Evaluation</u>:

- Appropriateness to estimate density or abundance: density
- Accuracy and precision: Medium accuracy and precision. Requires knowledge of basic ecology parameters of the species in the study area.
- Reliability: Medium or low reliability, since there is still only one study for the species. It is necessary to test it in different environmental conditions and study areas.

- Costs and effort required: Moderate to high cost. The initial costs of purchasing camera traps can be high. These costs are offset in long term monitoring programs.
- Spatial scale: Local to regional.
- Comparability: Unknown, since there is still only one study for the species. It is necessary to test it in different environmental conditions and study areas.

Practical guidelines: See red fox section

- Field operations developed during the same season in order for density estimations to be comparable across sites and time.
- To estimate the use of roads by Iberian lynx it is necessary the use of data from a GPS collar. VHF collars are not precise enough to identify the location of animals on the paths to be studied.
- Install the CTs and roads with similar characteristics (e.g. width) since there may be a difference in the use of different roads (e.g. dirty road *vs* path) by animals.

**Indirect methods**

**1. Scat (faecal pellet) counts**

Objective: Estimation of population size and density using analysis of scat counts data.

Methodology: Scat surveys have been described as suitable, cheap and quick methods of assessing detecting lynx presence (Guzmán et al. 2004, Gil Sánchez et al. 2011) and dispersion and/or colonization of new areas, and for generating data on other aspects of lynx ecology (Fernandez et al. 2006). Scat counts can be used as simple indices of abundance (nº scats / distance), but also, for the Iberian lynx, this index of abundance can be transformed in a measure of absolute density. Garrote & Ayala (2014) found a significant positive linear relationship between the scat number per kilometre and absolute density stablished by camera-tapping (nº Lynx/100ha = 0.065 + 1.056 nº Scat/km). This method was applied to estimate the lynx population in the Doñana area (Guzman et al. 2004). Scat censuses were carried out along 14-18-km itineraries in each 5x5-km grid square. The search effort was focused on trails, tracks and paths in each square (Sarmento et al. 2009). All lynx-like scats found during the censuses were collected for molecular analysis. The samples were collected with latex gloves to prevent genetic contamination and were placed in paper bags which in turn were placed in plastic pots filled with desiccant to prevent DNA degradation up to laboratory processing. Scats were analysed with molecular techniques that identify scats unambiguously to species level (Garrote & Ayala 2014). The abundance index expressed as 'scat / km' obtained for each grid square, was converted to lynx / 100ha using the relation indicated above. This density was then multiplied by the potential habitat area of each grid. Using this method, it was estimated that the number of lynxes in the study area was 33. The number of lynxes estimated by means of CR analysis with camera-trapping data was 26. This method can provide a reliable index of the spatial distribution of lynx density that is comparable to the results obtained by the more expensive camera traps. However, it is important to stress that the application of this methodological approach to Iberian lynx monitoring must be subject to certain criteria. Only scat counts carried out under conditions as much similar a possible, and in comparable annual periods will be useful for monitoring inter-annual and inter-areas changes since various variables can affect the scat detectability. Different behaviours of marking may vary throughout the year, just as the abundance of the species undergoes seasonal changes due to recruitment of young individuals after breeding (Garrote & Atyala 2015). Other seasonal changes such as the effects of the weather (Cavallini & Santini 1996, Andelt & Andelt 1998) and the presence of coprophagous beetles (Norris & Michalsky 2010), more abundant in spring and summer, may affect scat survival and detectability. The effect of all these environmental correlates on scat availability, which varies between years, seasons and areas, needs to be investigated before any extensive application of scat counts to lynx monitoring be considered.

Scat counts are an alternative for calculating Iberian lynx abundance in large areas where, due to logistical or cost constraints, it is too expensive to employ camera traps. However, the equation shown by Garrote and Ayala was established when the species was at minimum population, its distribution was very restricted, and the habitats were relatively similar. Currently the species has gone from being present in 2 populations in an area of 500 $km^2$, to living in 8 populations that occupy more than 3000 $km^2$, also expanding the range of habitats and original environmental conditions. Therefore, the effect of environmental correlates on scat availability, which varies between habitats, years and seasons, needs to be investigated before any extensive application of scat counts to lynx monitoring be considered.

The use of scats data for estimating carnivore populations through CR analysis is a method widely used in other species of carnivores. This method requires scat individual identification, technique already available for the species. However, this method has not been applied to the estimation of Iberian lynx populations until now. This is due to the fact that, up to now, it has been possible to develop an intensive monitoring with photo-trapping, that has managed to cover its distribution area very efficiently. However, given the viability shown with other species on large spatial scales, and the expansion the species is currently experiencing, it would be necessary to contrast this methodology for the Iberian lynx, to be applied in the future.

Evaluation:

- Appropriateness to estimate density or abundance: density and abundance
- Accuracy and precision: Medium accuracy and precision.
- Reliability: Medium reliability in original distribution areas, where the method was tested. Low reliability in new areas with different environmental and habitat conditions.
- Costs and effort required: Low to moderate. It depends on the spatial scale. Very low effort and cost on a local scale.
- Spatial scale: Local, regional or national. It has been possible to apply this method in the national survey of the species.
- Comparability: Comparable between areas with similar habitats. The effect of environmental correlates on scat availability, which varies between habitats, years and seasons, needs to be investigated before any extensive application of scat counts to lynx monitoring be considered. It is necessary to deepen on the study of the effect of covariates in the detection of scats.

Practical guidelines:

- Field surveys developed during the same season in order for density estimations to be comparable across sites and time.
- It is recommended to avoid carrying out surveys in rainy seasons in order to better preserve excreta before and after collection, thus minimizing the risk of degradation. A highly degraded scat can prevent conclusive results from genetic analysis.

The Figure 16 illustrates some charasteristics of Iberian lynx and methods applied to estimate abundance.

The table 8 shows a classification of different population density or relative abundance estimations based on desirable characteristics for Iberian lynx monitoring populations.

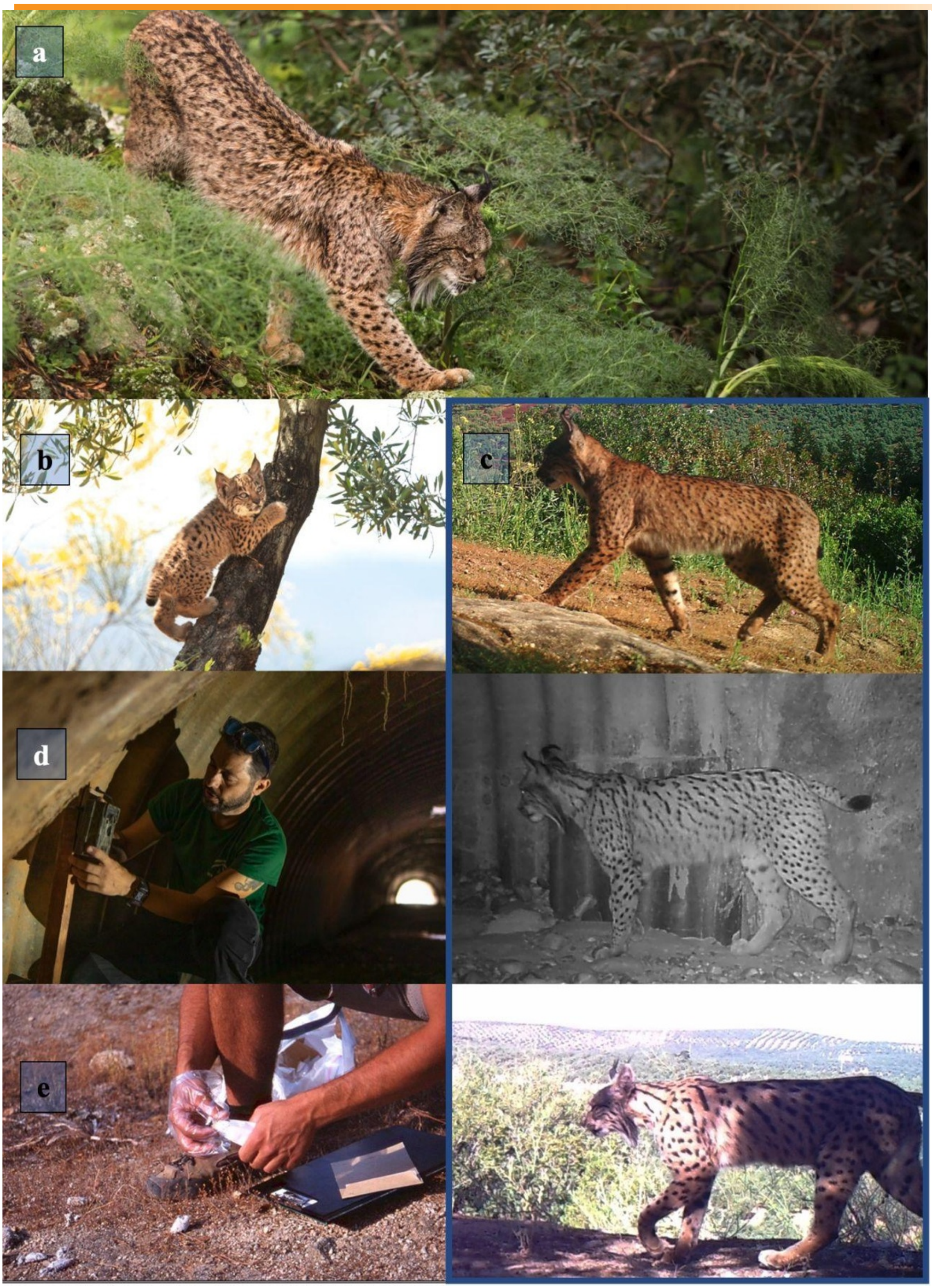


**Figure 16. Iberian lynx (*Lynx pardinus*).** (a) the Iberian lynx is a medium size carnivore with spotted fur (image: S. Marijuan). (b) Iberian lynx cub climbing an olive tree (image: S. Marijuan). Habitats that were formerly considered as non-suitable, such as agricultural land, with low scrubland coverage could indeed permit lynx establishment if wild rabbit densities are adequate. (c) The Iberian lynx is the most heavily

spotted member of the genus *Lynx* and have three types of spots pattern: small, intermediate and thick spot (images: G. Garrote). (d) the main methodology for estimating the abundance and density of the Iberian lynx is camera trapping (image: S. Marijuan & F. García). (e) Iberian lynx faecal sample collection. Proper labelling and preserving of each sample are crucial (image: G. Garrote).

**Table 8**: Classification of different population density or relative abundance estimations based on desirable characteristics for Iberian lynx (*Lynx pardinus*) monitoring populations in local management units, practicability, and applicability.

1 - requires basic statistical skills to apply CMR models, 2 - highly reliable provided that all conditions for accuracy are filled, r - restricted

| **Iberian lynx (*Lynx pardinus*)** | | | |
|---|---|---|---|
| | Camera trapping (CR) | Camera trapping (REM) | Scat counts |
| Abundance/density | A/D | D | D |
| Temporal trend | short/seasonal | short | seasonal |
| Info on population structure | y | n | n |
| Season | year round | year round | Sprint |
| Costs | moderate | moderate | low |
| Effort | moderate | moderate | low |
| Ease of learning | Easy[1] | easy | easy |
| Accuracy + precision | HA+HP | MA+MP | MA + MP |
| Reliability | reliable | reliable[2] | reliable[2] |
| Useful at local scale | y | y | y |
| Useful at large scale | r | r | y |
| Useful at very low density | y | | y |
| Useful at very high density | y | | y |
| Suitable at all | y | | y |

## 4.8. Northern raccoon (*Procyon lotor*)

The northern raccoon (*Procyon lotor*) is a mesopredator carnivore species, belonging to the family of *Procyonidae* (Timm et al. 2016). It is native of North and Central America, but in the 1920s, first alien populations were introduced and settled in Europe (Mori et al. 2015, Beltrán-Beck et al. 2012, Canova & Rossi 2009). Currently, it has been established (i.e. reproduction and population growth) in at least 20 European countries, including the three Caucasian republics; in 10 of them, expanding invasive populations occur (Salgado 2018). In addition, recent introductions through the pet trade have resulted in the establishment of new feral raccoon populations in Europe (e.g. Spain or Italy, Alda et al. 2013, Boscherini et al. 2019).

It is a medium-sized carnivore, with an unmistakable black facial mask. It has relatively short legs and a characteristics tail with clear alternating rings and dark (3-8 rings, rarely up to 10; Michler 2018). The weight varies between 4-10 kg for the adult, according to the geographical area and of the season and the length of the head and trunk of adult animals between 40 and 70 cm (Hausser & Schmid 1995, Michler 2018).

It is nocturnal and omnivorous and well adapted to various types of habitats (Mori et al. 2015), where it prefers the immediate surroundings of rivers or water courses, occupies woods and bushes, marshes with dense vegetation, dunes, coastal ponds and even mangroves in sub-tropical areas (Canova & Rossi 2009, Hohmann et al. 2000, Beasley et al. 2007). However, it also can be present in agricultural areas and urban environments where finds easily accessible remains of food and can reach very high local densities (Ikeda et al. 2004, Michler et al. 2004, Bartoszewicz et al. 2008; Timm et al. 2016; https://lifeasap.eu/index.php/it/component/content/article/11-ias/50-procione). Due to its high ecological plasticity the raccoon has had particular success in human settlement areas, which notably applicable in Central Europe where raccoon densities up to 100 individuals per 100 ha are reported (Michler & Hohman 2005).

During the winter they do not hibernate but reduce their activity and therefore avoid areas covered with snow (Lotze & Anderson 1979, Zeveloff 2002, Kamler & Gipson 2003, Beasley et al. 2007, Mori et al. 2015). The raccoon shows a high plasticity in the spatial structure and no pronounced territoriality, the size of the home range depends on the type of habitat and the abundance of food resources. Generally, in urban areas its home range is smaller than in the wooded and rural areas (Ikeda et al. 2004, Bartoszewicz et al. 2008) but high densities have sometimes been recorded (Ikeda et al. 2004). As indicative, in Germany the annual home range median of adult male home ranges over both seasons is approximately 600 ha and for adult females 300 ha (Hohmann et al. 2000, Michler 2018). In urban areas the raccoons roam smaller home range with an (average of 129 ha, the females' home range was significantly, 36 ha, than the males' home range average of 210 ha (Michler & Hohman 2005). In western Poland, averaged 100 ha in suburban areas (n = 5 individuals), 1000 ha in a wetland area, and 6000 ha for a single individual studied inhabiting woodlands (Bartoszewicz et al. 2008). Raccoons are one of the most opportunistic, omnivorous carnivores, being able to take advantage of almost every setting offering suitable food sources (Zeveloff 2002).

The raccoon has very adaptable social systems. Females show a characteristic fission-fusion social system (resource orientated) and males regularly form male coalitions with 2-5 individuals: mating occurs in February-March in North America, pregnancy lasts 63-65 days and the litter size is about 2-5 puppies (Gehrt 2003). There can be a second birth in autumn when the female was not pregnant in spring or when the puppies died. The births occur inside dens, that can be tree cavities, burrows of other mammals or rock outcroppings, used for shelter and raising young, also in winter, when there is low temperature. The male kits leave the mother home range in spring, females remaining close to their natal area (Aliev & Sanderson 1966, Lotze & Anderson 1979, Endres & Smith 1999, Bartoszewicz et al. 2008, Mori et al. 2015, Michler 2018, "Raccoons | Washington Department of Fish & Wildlife," n.d.).

In Europe the raccoon is considered as invasive species by the EU regulation (http://data.europa.eu/eli/reg/2014/1143/oj) on the prevention and management of the introduction and spread of invasive alien species. It is known to be host to a number of disease agents that could be transmitted to humans, domestic animals and other wildlife (Beltrán-Beck et al. 2012; Fischer et al. 2020), including *Baylisascaris procyonis*, *Toxoplasma gondii, Leptospires,* and *Salmonellae* (Compton et al. 2008, Michler 2020). Nevertheless, its overall epidemiological relevance in Central Europe is currently considered to be of minor importance (Michler & Michler 2012). As raccoon population growth in central Europe has increased at exponential rates since the 1990s, an efficient strategy for managing raccoon populations overlarge areas a long period is needed in Europe (Salgado 2018). Management of invasive species is a discipline that still struggles with biased approach and methodological limitations when it comes to assessing their real impacts (Bonanno 2016). In Central Europe an impairment of the population development of other species by the raccoon is currently not given (Lutz 1980, Winter et al. 2005, Kovarik 2010, Michler & Michler 2012, Michler 2017, Fischer et al. 2020).

**Direct methods**

Direct methods involve counting the animal itself, using either live or dead specimens. Stratification of subsamples to different habitat types or land classes may increase the validity, usefulness, and precision of the surveys. Study designs must account for the spatial distribution and aggregation of resources because they determine that of raccoons. Direct methods can also provide data about the structure of the population, but they can vary depending on seasonal factors such as social behaviour, food availability (and its distribution) or even just biases that can interfere with capture/spotting probability. Being in the centre of anti-rabies fight, this species was on the spot for the ORV (oral rabies vaccine) and thus baits and capture methods have been highly investigated. Also, to make the ORV program efficient, an accurate density study is usually required. Capture methods have also been developed in raccoon control as alien species. As detailed below, the best suggested approach to estimate the real population density of raccoons is probably the combination of trapping and marking, telemetry and camera traps. Individual recognition of raccoons is possible via camera trap pictures (Michler 2018).

**1. Linear transect (general)**

Objective: By observing individuals / groups along transects, the objective is to estimate the local density or relative abundance.

Methodology: The transects can be carried out using a vehicle or, if distances allow it, on foot, which is difficult to perform at spatial scales of several hundreds of $km^2$ (Blackwell et al. 2004). Fixed point transects, where observation takes place from fixed points, are also an option. Northern raccoons are mostly nocturnal animals, so transect surveys are usually performed after dusk (starting 1 hour after sunset), with the use of spotlights (spotlight surveys) (Hartman & Eastman 1999, Gehrt 2002); moreover, the presence of the *tapetum lucidum*, which reflects the light if hit by it, may increase the probability to detect the animals in the dark.

Detailed evaluation is provided below for each specific method.

Practical guidelines:

- An autumn census could be used to detect the increase in raccoons due to recruitment and dispersal, while an early spring estimate would comprise largely breeding individuals. Peak in raccoon population is usually around August (Blackwell et al. 2004).
- Within each season, spotlighting is conducted several nights in case of low density and/or low detectability.
- In the study design phase, it is important that a subsample of sites is visited multiple times to perform an analysis of detectability. These precautions can serve to avoid the effects of false absences generated by low detectability and allow obtaining more reliable estimates.
- A disadvantage of using spotlights is that its application depends largely on the availability of passable tracks or roads in vehicles. Therefore, it is not applicable in regions with limited vehicle access.
- In areas where animals are hunted, they may exhibit flight behaviours when perceiving the proximity of the vehicle, so the detection probability will be lower. On the other side, in some areas human presence can play an attractive role (Blackwell et al. 2004).
- Linear transects reveal themselves very useful in areas where capture (and related methods) are not possible (Ramey 2005)

Transect methods include different techniques to detect animals and to estimate population density or indices of abundance:

*KAI* (Kilometric Abundance Index)

Objective: By observing individual raccoons along transects, it is possible to derive local density or relative abundance.

Methodology: As stated, the transects can be carried out using a vehicle or on foot, preferably using spotlights. Date, time, GPS position, number of individuals and, whenever possible, sex and age class are recorded for each observation.

The KAI can be used to estimate abundance, and this can be obtained from the total number of specimens counted each night divided by the total number of kilometres travelled.

$$KAI = \frac{N°\, northern\, raccoons}{N°\, nights \cdot N°\, km/night}\ or\ \frac{N°\, northern\, raccoons\, (total)}{Nº\, km\, (total)}$$

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance.
- Accuracy and precision: moderate. Needs good visibility. Three samples per site will approximate the optimum to minimize the variance of the estimates of occupation (MacKenzie & Royle 2005), and 2-3 samples per site will maximize the power to detect trends (Field et al. 2005b). However, high levels of occupancy and / or low detectability will tend to increase the number of repetitions required, confirming the importance of several replicas in each sampling.
- Reliability: moderate. Differences in seasonal behaviour can affect the results of studies performed in different times of the year. Also, some assumptions are required: (i) animals in the vicinity of the transect are detected; (ii) do not move before being detected; (iii) each individual is detected only once; (iv) the detection of each individual is independent of the other detections; (v) the influence of variables such as the observer, station or the weather.
- Cost and effort required: this method is not expensive, but it requires a moderate effort in terms of labour and operators, especially when it involves nighttime surveys.
- Spatial scale: local to large territories.
- Comparability: in comparison to scat surveys, spotlight transect may give a lower density estimation. This is to be considered when coming to control measures implying spotlight shooting (Hartman & Eastman 1999). Moreover, spotlight surveys seem to not always give the same density estimations as capture-mark-recapture, even if trends have correlated variations along the year. Spotlight and road-kill surveys are useful techniques for monitoring changes in local populations of raccoons, but they must be used with caution when comparing indices among populations with different habitat characteristics or when evaluating low-density population (Gehrt 2002).

Practical guidelines:

- Consider the principles of sampling (summarized at https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1449) when calculating population parameters on large areas inhabited by northern raccoon. It is necessary to select some proportion of surface in which density or relative abundance is estimated by different transects. The results of these estimates give rise to an average that will be extrapolated to the whole area of study. A correct study design means minimising bias during sampling and applying sufficient effort to produce precise reliable estimates of density or relative abundance. Raccoon spatial distribution is a result of its spatial ecology, land use and distribution of resources, as well as other factors. We therefore need to have notions of the distribution of the population and habitat selection in the territory in order to make a good study design.
- Field operations developed during the same season

- Random placement of transects, at least repeated over years for comparisons purposes along time.
- Transects should be stratified by habitat type, avoiding roads and other singular features. All relevant environments (different habitat which similar use for raccoon or characteristics may be grouped) within the study area (which may impact raccoon distribution) must be considered for the sampling design.
- It should be tested whether stratification allows for similar sampling effort and bias in each habitat class. This is even more important when comparing different methods in a given area (no bias should occur due to different sampling effort in each habitat type by the different methodologies).
- Transects must be placed using a fine scale maps of the estate and should be stratified while also taking into account the description of the habitat composition.
- The sampling effort must be quantified per habitat type (e. g. as the proportion of transects across the different habitat types).
- The result of a given procedure when stratified design is not performed is biased estimations of relative abundance and density towards those habitats that are over-represented in the sampling. This would thus cause low precision and incomparability with other values obtained from different study areas.
- The vehicle speed should be constant and slow enough to spot the animals, i.e. 20 km/h; at least one driver and one observer are required.
- In order to have reliable and accurate data, transects can be performed in different times of the year (in order to detect animals despite difference in seasonal activities) and repeated several times or monthly.

*Linear transects in fixed width band*

Objective: By observing individuals / groups along transects and within a pre-defined detection band, the objective is to estimate the local density.

Methodology: The transects, usually by spotlighting after dusk, are used to estimate the densities by defining a band on the sides of the transect in which all the individuals present are assumed to be observed. The width of this band is defined by the distance perpendicular from the travel line where visibility is close to 100%, which is usually around 200m, but it depends on technology provided, habitat and local behaviour of the species. The density can be calculated as:

$$D\ (density) = \frac{N°northern\ raccoons}{band\ width \cdot transect\ lenght{\cdot}probability\ of\ detectability}$$

with probability of detectability within a strip of half-width derived using detection functions from distance-sapling calculations (Buckland et al. 2001, 2004). It still has not been tested in northern raccoon to our known.

Evaluation:

- Appropriateness to estimate density or abundance: density and relative abundance.
- Accuracy and precision: high, depending on four assumptions: (i) groups of animals are distributed randomly with respect to transects, (ii) groups on the transect line are detected with certainty, (iii) groups are detected at their original location, (iv) measurements are exact (Durant et al. 2011).
- Reliability: Size bias (tendency to observe more large clusters at large distances) should be considered when doing the model estimations.
- Cost and effort required: as well as linear transect, distance sampling is not expensive in terms of devices, but is demanding in terms of operators work.
- Spatial scale: local.
- Comparability: comparisons still require specific evaluations for each situation against other methods. Useful for multi-species monitoring.

Practical guidelines:

- Stratified sampling studies with habitat differentiation can be useful to avoid habitat bias.
- Consider the practical guidelines above regarding IKA, and track field designs.
- In order to obtain reliable estimates of density at least 30 observations are necessary.

## 2. Distance sampling on transects

Objective: By detecting individuals or groups along transects and recording group size and relative position to the observer, the objective is to estimate the density after modelling the probability of detection.

Methodology: This method is based on the concept that the probability of observing an animal decreases with increasing distance to the observer. The method is analogous to the linear transect survey, but in this case more information about each observation is required: number of individuals and perpendicular distance to the route have to be noted, as well as main vegetation type, to avoid visibility biases (Blackwell et al. 2004, Ramey 2005).

More details on the method can be seen in the section of red fox. This method can be useful when estimating the density of raccoon population in oral rabies vaccination programs. With another method, such as capture-mark-recapture, the parallel use of vaccine baits would introduce some biases (Blackwell et al. 2004).

Evaluation:

- Appropriateness to estimate density or abundance: population density.
- Accuracy and precision: medium-high.

- Reliability: need for assumptions: (i) all animals on the transect are detected; (ii) the distances are measured accurately and (iii) the animals are detected at their original location.
- Cost and effort required: The high effort required is compensated by low cost, as trained volunteers are often available (experienced hunters). Nonetheless, volunteers have to be motivated.
- Spatial scale: local.
- Comparability: A minimum number of transects must be sampled depending on the distribution of raccoons in the study area, requires areas of good visibility and stratified design to be comparable among sites. Distance sampling can provide lower density estimations than capture-mark-recapture.

Practical guidelines for distance sampling:

- Consider the practical guidelines above regarding IKA, and particularly, stratification of design, which is even more relevant for distance sampling to produce reliable and comparable results
- As potential food resources changes with season (e.g., ripening mast [*Quercus* spp.], corn [*Zea mays*]), the frequency in which animals are observed on or close to the survey route can decrease (Blackwell et al. 2004).
- It is recommended to use a rangefinder to estimate the distance because the estimation of distances by direct observation is subject to errors, especially at night and they can also vary considerably between observers (Blackwell et al. 2004).
- Infrared technology can help to detect the raccoons (Blackwell et al. 2004).
- Need of at least 50 sightings

## 3. Capture-Mark-Recapture (CMR)

Objective: By identifying part of the animals and estimating the proportion of the recaptured during samplings, density can be estimated.

Methodology: Different methods included in CMR are reviewed in Buckland et al. (2000). Trapping and marking individuals are needed (Chandler & Royle 2013), or at least, the presence of recognisable identification signs (Karanth & Nichols 1998, Sarmento et al. 2009). The frequency of marked individuals observed in subsequent capture sessions is then used to estimate abundance. There are different mathematical models to estimate the population size, both for closed populations (without births / deaths or emigration / immigration) and for open ones (which are advisable if no information is provided about population status), which can be defined either way or the other for example with radiotelemetry data (Ramey 2005), and there are computer programs specific for this (e.g. CAPTURE, Rextad & Burnham 1991). Capture and marking are often performed during rabies vaccination campaigns (Rosatte et al. 2007).

In studies of rare and elusive species, the number of marked individuals is usually small, leading to biases in estimations. Such species are often studied with radio and GPS telemetry. Where simultaneous resighting and telemetry data are available, both types of data can be combined in a spatial mark-resight model to obtain estimates of population density even if sample sizes are small (Sollmann et al. 2013a). Spatially explicit capture-recapture (SCR) models (or spatially explicit mark-resight, SEMR models) can be used for abundance and density estimation, frequently through individual identification of target species using camera-trap sampling. Generalized spatial mark-resight (Gen-SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks (Gen-SMR is included in the section "Camera trapping without individual recognition").

The use of classical capture-recapture studies often does not provide realistic results for raccoons. Reasons for this are (especially at higher population density levels) for instance a lack of territoriality and the associated possible aggregation of animals at resource hotspots. i.e. those of anthropogenic origin, artificial feeders, etc. This can be avoided by censusing when food is not provided and/or when racoon behaviour is less aggregated. However, such information can only be obtained through accompanying telemetric studies (Michler 2018).

Other types of CMR can be the genetic CMR with DNA identification in scats or radioisotope tagging: in this last one, Cadmium-115m is injected in capture animals, then scats are collected during a period of time such as 5 weeks (after previous scat cleaning of the area) and analysed for radioisotopes in them (Conner & Labisky 1985).

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: high. Coming to radioisotope tagging, two criteria are important to maximise accuracy and precision: (i) proper scat identification and (ii) maximisation of collected scat number (Conner & Labisky 1985).
- Reliability: low. Two assumptions are needed: (i) the population is closed (without immigration or emigration) and there are no births or deaths between captures; (ii) that all individuals have the same probability of being captured. In this case, the capture probability of raccoons varies depending on food resources (Michler 2018).
- Cost and effort required: moderate to expensive costs, high effort. It is necessary to mark a significant proportion of the population, which is very expensive, although accurate and precise. Due to its high effort and local conductibility, this method may only be used for scientific purposes, knowing that better methods exist.
- Spatial scale: local.
- Comparability: in a comparison study with RAI models (the index of abundance derived by dividing the number of unique individuals captured by the total study area), CMR provides with higher densities, suggesting an underestimation of RAI (Beasley et al. 2012).

Practical guidelines:

- Adequate licensing for some procedures (capture) are required.
- Some important factors can affect capture or recapture probability and should be kept into account:
    - Season: the highest probability in successful capture can be achieved during autumn or winter, when food availability is lower (although at this time general activity is less)
    - Temperature: low temperatures may inhibit the raccoons to move
    - Relation between trapping areas and home ranges
    - Males and juveniles (depending on the season) are more prone to capture and recapture, due to their social behaviour (Gehrt et al. 2018)
    - Drugs: apparently, recapture probability can be higher when animals are anesthetised with Telazol® than with Ketamine-acepromazine (Gehrt et al. 2018)
- Commercial attractive are available, such as Hard-Core 1 Raccoon Lure #1 (Wildlife Research Center, Ramsey, MN), however simpler lures can be used, especially fish (Disney et al. 2008, sardines, Michler B, pers. comm.) or other food (dry cat food) (Beasley et al. 2012, Beltrán-Beck et al. 2012)
- Genetic samples collection can be useful in case of loosing of eartag

## 4. Telemetry

Objective: Radio-tracking, or wildlife telemetry is the technique of determining information about an animal through the use of radio signals from or to a device carried by the animal. Marking with radio-emitters the individuals of a population, estimate the density assuming that each individual contributes to density with equal proportion to the proportion of their home range included within the catch area.

Methodology: The main type of radio-tracking in use today for northern raccoon is global Positioning System (GPS). Thanks to radio-tracking it is possible to identify the home range and their use by radio-followed animals (Gehrt & Frttzell 1997, Ellis 1964, Beasley et al. 2007, Sollmann et al. 2013, Rosatte et al. 2010). Density can be estimated indirectly from radio-tracking data based on the inverse relationship between the size of the home range and the population density. Marking with radio-emitters most of the population and tracking them for a period of at least year is currently considered one of the most reliable method to determine the density of carnivores; is often combined with other methods such as camera trapping studies (Sollmann et al. 2013) and it can be used as a reference to contrast results obtained by other methods.

However, this method indicates only the minimum density since it assumes that each home range is used exclusively by a single animal, which is never true. The density is estimated in the area where there is certainty of having marked most of the existing individuals, which is usually defined by the polygon that encompasses all catches ("catch area"). It is assumed that each individual contributes to density with equal proportion to the proportion of their area of champion included within the catch area. The density is calculated by adding the contributions of different individuals.

Evaluation:

- Appropriateness to estimate density or abundance: density.
- Accuracy and precision: medium
- Reliability: reliable but need for assumption.
- Cost and effort required: Expensive costs, high effort, difficult to catch animals. Due to its high effort and local conductibility, this method may only be used for scientific purposes.
- Spatial scale: local, can be applied in all habitats.
- Comparability: It is necessary to mark a significant proportion of the population to compare among populations.

Practical guidelines:

- Applying ear tags can help identify animals caught (Rosatte et al. 2010).
- Collar transmitters can be provided with reflective tapes (Oralite® Reflective Film 5500) so that the marked ones when illuminated at night from afar, animals could be quickly identified as transmitter animals (Michler 2018).
- The traps to capture animals is baited with sardines or cat food (Hohmann et al. 2000; (Rosatte et al. 2010, Kramer et. al. 1999, Michler 2018).
- Upon capture, raccoons are anesthetized, weighed, sexed, aged (see Grau et al. 1970), marked, and radio collared.
- For ease of checking and to prevent disturbance of the trap sites, radio transmitters is installed at the traps (Hohmann et al. 2000).
- To increase accuracy of the location estimate, eliminate any locations that is estimated with fewer than 3 satellites (Rosatte et al. 2010).
- Each seasonal data set is considered as unbiased by sample size if the size of the home range not increase over 20 added fixes (Hohmann et al. 2000).
- Knowledge of the ecology of raccoons should be used during planning for disease management (Rosatte et al. 2010).

## 5. Camera trapping

Objective: Converting trap rates into estimates of the size of a population (local density), with or without the need for individual identification and/or where animals are unmarked.

Methodology: Most density estimators used for population monitoring of carnivores are based on CMR data that are often based on the individual recognition, implying invasive costly marking techniques and individual tagging. Beyond CMR, a number of models to estimate the size of a population where animals are unmarked and/or without the need of individual identification has recently been published (Rowcliffe et al. 2008). These camera trapping methods without individual recognition are promising to estimate carnivore population density and could be useful to evaluate a possible use also for the raccoon.

*SCR for unmarked*

To estimate density or population size for explicit spatial regions, can be used Spatial capture-recapture (SCR) models (Beth Gardner et al. 2010, Royle 2004, Forsyth et al. 2019, Jimenez et al. 2019, Chandler & Royle 2013) through individual identification of target species using camera-trap sampling, as indicated in the section above. These are an extension of CMR without capturing for unmarked or unidentifiable individuals (Chandler & Royle 2013). This is achieved by modelling the number and positions of the individual centres of activity as well as distance-related heterogeneity in capture probability. Because the models are spatially explicit, a by-product of the estimation process is the likely locations of home-range centres within the sampled area. Generalized spatial mark-resight (Gen-SMR) is a recently developed SCR extension that allows for abundance estimation when only a subset of the population is recognizable by artificial or natural marks and incorporating telemetry data allows to inform model parameters related to movement and individual location (Sollmann et al. 2013, Beth Gardner et al.2010).

However, in many cases, it is not possible to read the marks in camera-trap pictures, even though individuals can be recognized as marked. A new extension of Gen-SMR has been applied to raccoon density estimation that allows for this type of incomplete identification, demonstrate good performance (Jimenez et al. 2019). This extension of Gen-SMR is broadly applicable as it addresses the common problem of incomplete identification of marked individuals during resighting surveys.

*CT in general*

Raccoons are particularly well suited to camera trapping, no showing any kind of timid reaction and sensitivity to flashlight. Moreover, they can easily be lured because of their curiosity (Michler et. al. 2008).

<u>Evaluation:</u>

- Appropriateness to estimate density or abundance: density
- Accuracy and precision: High (also in low densities), although do not always provide appropriate confidence intervals
- Reliability: quite reliable, different analysis-methods adaptable to local conditions, practicable, conductible (for several species at the same time), need for assumptions and previous information. For instance, the REM and spatial explicit methods, respectively, require prior knowledge of average speed and home-range size parameters of the studied population.
- Cost and effort required: those without the need of capture (non-invasive) are affordable, moderate costs, need for CT, theft of cameras in some locations, medium effort, manpower required to analyse photo/video material, photos might be collected via online-photo-databases.

- Spatial scale: local, adaptable to regional studies.
- Comparability: Evaluate missing detections (which, for instance, specially affect REST) with your specific camera model and under your study circumstances (camera did not release although animal was there, Amelin 2014, Fischer 2018). All assumptions should be stated in literature.

Practical guidelines:

- Since different CT methods are available, we will focus on those most practical, previously classified as of relative medium effort, and able to generate reliable data over a wide range of situations across Europe. Random encounter model (REM and its extensions, see below and red fox section) does not require individual recognition, whereas Spatial mark-resight models (SMR) work recognizes only a part of individuals. We do not exclude the use of other CT methods, but we recommend those in terms of practicability as they provide an excellent balance between the required effort (and level of knowledge) and reliability.
- For capture/recapture (i.e. CT not randomly placed), CTs have to be put in the relevant habitats and at the important landmarks. Detailed Information can be found in Michler (2018, 36-41 pp). Proper baiting is also very important for which cat food works very well.
- Field operations developed during the same season (optimally during summer, pre-hunting) in order for density estimations to be comparable across sites and time.
- Random placement of CTs (recording coordinates) to obtain a regular uniform distribution: a minimum 5 x 5 CTs (to guide the location using a buffer of 100 m around the nodes of the grid) at 1.0 Km x 1.0 Km or less for SMR, 1.5 Km x 1.5 Km for REM (see below and red fox section).
- Use a grid of CTs covering all the habitats of the study area, stratifying (at least one grid / 5000 ha) according to habitats, so that no of CTs in each habitat are finally proportional to habitat availability.
- Infrared CTs located on posts or vegetation 30 cm above the ground. Use sticks to reference the animal captured by the camera-trap in REM method as indicated in Keuling et al. (2018).
- Infrared CTs set 24h per day and to take up to three consecutive pictures at high sensitivity, selecting the minimum time lap between bursts (1 second if possible).
- The date and time of capture automatically stamped onto each image or recorded as metadata
- CTs checked weekly to change batteries and memory cards, as well as ensure proper functioning and baiting.
- Overall sampling period minimum of 1 month.
- Key CT settings have to be known angle of detection and effective range must be known to determine the surveyed surface.
- For REM (see below and red fox section), average daily travelled distance can be calculated from trapping records, which requires indicating distances in a blank picture,

signalized as follows to describe the speed of passes (after taking this blank picture, signals can be removed).

## Direct methods untested in raccoon in Europe

### 1. Camera trap

*Random encounter model (REM)*

This method was developed and tested in several species (Rowcliffe et al. 2008, Rovero & Marshall 2009, Rovero et al. 2010, Rowcliffe et al. 2011, Rovero et al. 2013, Rowcliffe et al. 2013) and some carnivores (Cusack et al. 2015, Anıl Soyumert 2020, Tosh & Twining 2017). More details are provided in the section of red fox

*Random encounter rate and staying time (REST)*

REST is an extension of REM (Nakashima et al. 2018, 2020). The speed parameter is not necessary, and it is substituted by resting time in the camera view. It still has not been tested in northern raccoon to our known.

*Point transect using camera traps (CT-DS)*

It consists of an adaption of point transect methods (distance sampling) to camera traps (CT-DS) (Howe et al. 2017). This approach has not yet been validated against other methods for carnivores. Similarly, it still has not been tested in northern raccoon to our known.

*N-Mixture models*

The number of observed individuals by any method rarely represents a reliable estimation of the number of individuals occurring in a given area. See red fox section.

## Indirect methods

Indirect methods allow to estimate relative abundance from signs of the animals (e.g. track, droppings, breeding refugia) that can be found in a wide variety of habitats, and a trained staff is needed for their recognition. Generally, are less expensive than direct methods and can be applied at local and large scale. Indirect methods are used to measure relative changes in animal density in the same region over time, and there are few evidences to indicate that they can be used to obtain quantifiable estimates of animal density.

### 1. Non-invasive genetic sampling (genetic capture-recapture, CGR)

Objective: Determining the effective population size by genetics (individuals or pellets). Several conceptually different types of $N_e$ can be distinguished, but the most commonly used is the number of individuals in a population who contribute offspring to the next generation (Ridley 2004). The estimates of $N_e$ tend to provide a lower number than an actual population size.

Methodology: Non-invasive genetic sampling (NGS) is a safe and cost-effective alternative to physical capture and can be used to obtain information on sex ratio and home range of individuals in a population. Multilocus genetic band-sharing data is used to study the spatial and genetic between individuals (Ratnayeke et al. 2002). By genetic analysis of polymorphic mini or microsatellites extracted from excrement it is also possible to identify individuals and thus obtain population estimates. This molecular technique can be used for faeces and hair from which DNA can be extracted (Kendall & Mckelvey, 2006). Specifically, faeces contain a small amount of DNA on their surface coming from its intestinal wall of the animal that produced them (Kohn & Wayne 1997). It's also possible to do a genetic analysis of pellets (genetic CMR) marking of faeces with radioactive isotopes (Conner & Labisky 1985). To obtained information on genetic diversity and population genetic structure of northern raccoon it is possible to use tissue samples from subjects legally hunted or killed on the street (Frantz et al. 2013, Kendall & Mckelvey 2006). Raccoons defecate in dedicated latrines (Harfenist et al. 1999), places like tall branches, fallen tree trunks and trunks that act as a bridge over a body of water formed typical structures that raccoons used as toilets (Michler 2018). Hair can be collected from den sites, or other areas frequented by the species of interest. To collect hair, you can use barbed wire, or glue or adhesives or brushes; the devices will be effective if hair is caught and hair samples containing useful DNA are obtained (Kendall & Mckelvey 2006, Belant & Wolford 2007, Long 2008). It is also possible to use an odorous attractant to attract animals to a sampling point where a hair collection device will be present (Kendall & Mckelvey 2006). Hair is identified by macro and microscopic examination, it's a technique with great potential because capture in not required, has a low cost and do no restrain or harm the animals (Foran et al. 1997).

Evaluation:

- Appropriateness to estimate density or abundance: density and individual presence
- Accuracy and precision: High
- Reliability: High, however determining the $N_e$ (local) by genetics is possible if appropriate sampling and corrections based on population dynamics parameters are applied

- Cost and effort required: high effort, expensive, need for assumptions, hardly applicable in low densities, still needs to be combine with other sampling techniques in most cases. This approach is time demanding and relatively expensive.
- Spatial scale: local
- Habitat: all, performs well in forest areas
- Recommendations to improve comparability and accuracy: these techniques have the molecular modelling part (modelling of marking and recapture has been already discussed before). The following is regarding the molecular side:
- Make robust designs for DNA sampling; avoiding biases in the collection of data associated with the behaviour of animals or habitat selection.

- Assesses the probability of capture locally to consider the necessary effort.
- Standardize and test laboratory protocols.
- Collect information on hunted (sampled) animals.
- Make a good selection of quality samples.
- Optimize the use of genetic markers (within and between laboratories).

Practical guidelines for faeces:

- For a program at regional level of study of populations of carnivores, genetic analysis methods are not recommended for the high cost, although it will decrease in the future.
- DNA is extracted from fecal sample by using kits like QIAamp DNA Stool Mini Kit (Qiagen). As positive controls, DNA can be extracted from muscle tissue (e.g. by a DNeasy Tissue Kit, Qiagen, Shimatani et al. 2008).
- Shimatani et al. (2008) designed new species-specific PCR primers for *N. procyonoides.*
- Wang (2011) developed 23 species specific primer pairs, based on mitochondrial DNA, to differentiate invasive raccoon dog and other carnivores and PCR reactions were optimized by using muscle samples.

Practical guidelines for hair:

- Because of greater performance, low cost, and ease of construction, Belant and Wolford (2007) recommend use of bucket snares over wood box snares.
- Wooden track stations were constructed as described by Zielinski and Kucera (1995).
- Belant and Wolford (2007) used photocopy toner on aluminium track plates placed in the bottom of the box to identify tracks of animals entering the snare.
- DNA degradation may be rapid in warm, wet environments and hair snares may become snow covered in the winter.
- DNA from hair is often less degraded than DNA extracted from scat, and generally provides more consistent results at far lower cost.
- Given that the use of a bait is based on a behavioural response from the animal it is essential to know biology and understand the behaviours to be successful in the sampling.
- Hair collection can survey large, remote areas and locate rare, cryptic animals. Also, it allows discrimination between closely related species, individuals, gender.
- The collection structure must permit or promote use by the animal.
- For most species, hair sampling is currently less effective for estimating population size than for estimating occupancy (Kendall & Mckelvey 2006) because hair collection methods may not be efficient enough to provide a capture-recapture sample size sufficient for meeting this objective. Moreover, extracted DNA may be of too low quality to reliably identify individuals.

## 2. Pellet count

Objective: By recording the number / frequency of northern raccoon droppings per unit of effort it is possible calculate local density or relative abundance.

Methodology: Pellet counts are frequently used to monitor wildlife species (Gese 2001). There are lots of methods based on counting the number of pellets or their frequency along transects or in plots. It allows comparisons of abundance relative to over time, or between different areas, although it requires trained personnel in the search and identification of excrement, and it can be used also in areas where den counts and spotlighting are not suitable. Raccoons prefer river shores and defecate in dedicated latrines. That is why this method is also called latrine transect (Gaston & Masselink 1997). As stated, the peak of raccoon population usually occurs in August, when the newborns leave their mother home range. In addition to the abundance of species, the number of faeces found is determined not only by the abandonment of the species but also by the rate of defecation and by the rate of disappearance. The defecation rate can be estimated in trials with captive animals, although variations may exist due to sex, age, social status, type of food and different behaviours of marking that may vary throughout the year. On the other hand, the disappearance rate can be very variable depending on various factors of both climate type (temperature, humidity, rain).

*Pellet counts with cleaning*

Objective: Starting from the estimate of the accumulation rate of excrements per unit of time and surface unit (Putman 1984) and relating them to the defecation rate per individual and unit of time, calculate local density or a relative abundance (Putman 1984).

Methodology: Unlike the pellet count without cleaning, all transects within the study area are initially crossed to remove all previously existing scat (cleaning). After 2-6 weeks walking the same transects a second time the number of accumulated droppings between the two visits is counted; if the land is flooded or if there is snow on the ground, the area shouldn't be included in any analyses (Gompper et al. 2006). It is estimated an accumulation rate of excrement per day and unit of surface and related to the daily defecation rate per individual. Several factors like the daily defecation rate can affect this estimation, but captive tests can help to get reference values and avoid biases. The number of pellets on a latrine does not lead to the number of individuals since raccoons usually defecate once a day (depending on the season), and there is quick eluviation in wet periods. The only way of attribute the pellets to individuals is genotyping (Michler 2020)

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance and local density
- Accuracy and precision: low (in most cases can just be used as an index of abundance)
- Reliability: low. Requires experienced staff for recognition. Parameters to estimate density (defecation and disappearance rates are values) must be locally calculated and cannot be taken from literature)

- Cost and effort required: low costs/efforts. For density, local parameters (defecation and disappearance rates are values) requires performing in situ experiment, which increase the effort required
- Spatial scale: local
- Comparability: To estimate density, calculate local defecation rate and dung persistence rate for that population during that season and for the year. More accurate relative abundance estimations can be derived from DNA recognition of scats (see section about genetic sampling), rather than using pellet counts along transects (Michler 2018)

Practical guidelines:

- Observers should be given a guide describing the morphological characteristics and odour of raccoon faeces in comparison with those of other species. They must record information like the transect route, the position of scats found on both visits and habitat information.
- Raccoons usually defecate once a day but the number of pellets on a latrine does not indicate the number of individuals.
- Quick eluviation of faeces in wet periods.
- The use of 2 observers is recommended to minimize the chance of missing or misidentifying scat along the route.
- Animals are motivated to deposit "new" scats when "old" ones are encountered.
- Extraction of DNA from faecal samples may enable comparisons to be made over small spatial scales.
- The use of non-random transects associated with linear features vs. random transects data may have possible bias although some studies suggest that non-random transect placement may not unduly affect density estimates (Walsh & White 1999) but may significantly reduce the logistical and cost implications of large-scale surveys.
- All habitat information can be analysed in GIS for measurement of areas and lengths, habitat diversity (e.g. Simpson's diversity index) to examine the effect of strata, survey protocols and culling and habitat-related variables on relative raccoon density.
- Raccoons latrines can be situated either on high trees or at their basis, fallen trunks, root piles or on eminences near river shores (Gaston & Masselink 1997, Michler 2020)
- A minimum of 30 sites may be required to obtain a consistent estimate of relative raccoon density.

### 3. Track counts on transects

Objective: By recording the number and frequency of tracks per unit of effort, it is possible to calculate an abundance index for a local population. Indices can be calculated as number of set of tracks per day, presence/absence of tracks or either length of interval between sets of footprints.

Methodology: Snow tracking have been used to calculate abundance indexes based on counts of tracks detected per unit of sampling effort. As raccoon activity during the year decreases in wintertime (when they tend to spend most of the time in dens) and they avoid snow sites, general tracking on dusty or muddy surfaces can be used instead (Gese 2001, Wilson & Delahay 2001) Indices can be calculated from the total number of sets of tracks per transect per day, or the presence or absence of footprints in each length of transect, or the length of interval between sets of footprints.

Track surveys are conducted by walking along the transects that usually are divided into shorter segments, such as kilometres, which constitute the units of sampling. Where multiple transects are used, they must be separated by a sufficient distance to minimize the probability that the footprints of the same individual will be registered in more than one transects.

The type of habitat adjacent to fingerprint transects affects the indices of raccoons, regardless of its density, due to differences in the number of times each individual crosses the transects in different habitats.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance
- Accuracy and precision: low (just index of abundance). A stratification with different kinds of habitats may be required.
- Reliability: Requires experienced staff for recognition, this method might be biased by several factors, like experience and motivation of observers and weather conditions.
- Cost and effort required: low costs/efforts, easy, volunteers, well working system ("tradition")
- Spatial scale: local
- Habitat: all, snow / sand need for predefined coefficients
- Comparability: low among populations, if standardized, useful for comparison of the same population along time. It has been pointed out that track stations can provide significantly higher visitation rate than scent stations or smoke plates (Burton 1999).

Practical guidelines:

- To avoid impacting the animal's behaviour, track sets should be backtracked 1st.
- This methodology also allows collection of scat and hair for DNA studies.
- The hind print is 8 to 10 cm long, much longer than wide, and the foreprint is shorter, about 7.5 inches long, and about as wide as long. The average distance between prints of a walking raccoon is 35 inches, with the left hindfoot almost beside the right forefoot. Five toes and claws are visible on all feet (Signorelli-Pappas 1994).
- Flour may be spread to reveal tracks from night activity (Signorelli-Pappas 1994).

## 4. Den counts

Raccoons change den sites every day with a high rate of reusage (Michler 2018: on average 47 different sites per raccoon/year with new sites every year). Without telemetry there is no reliable sign for usage (besides maybe scratch marks or hair on the trees (den sites on the ground cannot be detected at all without telemetry). Tree cavities can be very high (Michler 2020: medium 10 m up to 31 m) and can hardly be climbed in order to examine a cavity. Raccoons can be detected lying in branches and with the age of 4-6 weeks young raccoons can be detected climbing in the trees. Females raising their young are extremely careful and will not show up while watching a supposed den. Therefore, count the number of breeding dens present in a territory to estimate the number of breeding animals and a relative of abundance of the population is not appropriate for this species. Some studies of raccoon have included den surveys to estimate the survivorship of a northern raccoon (Gehrt & Fritzell 1999), the influence of age, sex, season and availability on den selection (Endres & Smith 1999) and the use of den sites (Nixon et al. 2001, Owen et al. 2015). However, we are not aware of scientific articles on the estimation of the density of the raccoon starting from the count of the dens.

## 5. Scent station

Objective: To calculate a relative abundance index by using the frequency of northern raccoon visits to scent-stations.

Methodology: This technique has been used to detect changes in the population growth rate through time in carnivore species, an important parameter for population dynamics modelling and management (Nottingham et al. 1989, Disney et al. 2008). The scent-station normally uses the line like the sampling unit, so that every transect must be spaced one km away from the others to minimize the probability of an animal visiting more than one line of stations in a night. Within each transect the scent stations are arranged at a distance of 300-500 m from each other (Nottingham et al. 1989, Smith et al. 1994). In the centre of each station, a smelling bait is placed. The perfume stations are activated for 1-3 consecutive nights, per month or per season. It is preferable to apply this method in dry seasons as rain can make the scent-stations inactive (Disney et al. 2008).

The stations are checked every day and the eventual footprints are noted (Gompper et al. 2006). The soil, which may possibly be altered by traces, is prepared again and the station was re-activated with bait. Nights are not independent sampling units because northern raccoon may visit the same or nearby stations on consecutive nights. The daily total of visits of the northern raccoon (number of visits / numbers of station-nights) is used to calculate a relative abundance index as follows (Smith et al. 1994, Disney et al. 2008):

$$Relative\ abundance = \frac{Total\ northern\ raccoon\ visits}{Total\ operative\ scent\ stations}\ x\ 1000$$

To collected raccoon footprints can also use track plate foot (Gompper et al. 2006, Ellison & Swanson 2016), a method in which aluminium plates are used to applied soot, animals pass the box first walk on the sooted surface. Soot is transferred by the feet to the slightly adhesive shelf-paper where it is deposited in the form of highly detailed tracks. Tracks left on the shelf-paper are often much clearer and more distinctive than those made on the sooted surface, often allowing one to distinguish between even closely related species.

Evaluation:

- Appropriateness to estimate density or abundance: relative abundance
- Accuracy and precision: low, it is only allowed to identify the species but not individuals, so it is only possible determine whether or not each station has been visited by each species of interest. Sargeant and Cypher (2003) in a research on red fox show that repeated operation of stations can lead to habituation and reduce, rather than increase, precision of results.
- Reliability: low, it requires specific prior training of the personnel, the rates of visits to scent stations are affected in addition to the species density, due to various factors such as climatology, the season of the year, habitat characteristics and human activity. A disadvantage is its low sensitivity to small changes, the impossibility of compare different areas or habitats with each other, poor spatial and temporal resolution or low statistical power when evaluating changes.
- Cost and effort required: Low cost and effort, the main advantage of this method is its simplicity and speed of obtaining results.
- Spatial scale: Local
- Comparability: Comparable across time when sampling condition are repeatable, difficult to standardize comparisons among areas.

Practical Guidelines:

- A cotton ball saturated with urine of bobcat (*Lynx rufus*) can be applied at scent station (Disney et al. 2008).
- Like lure can applied bobcat urine, cod liver oil, fatty acid scent (FAS), or an unscented cotton ball at scent stations but Nottingham et al. (1989) showed no preference among the 3 attractants; cotton ball probably is attractive as a visual anomaly.
- Shellfish oil replaced fatty-acid scent as an attractant to maximize the likelihood of visits (Smith et al. 1994)
- The transects are set also along a creek.
- Toe clipping can be used to differentiate individuals (Smith et al. 1994), but Disney et al. (2008) fears an impact on foraging behaviour.
- Each scent station also can be monitored with a camera trap set 10 cm above ground, 2-5 m from the scent tablet (Gompper et al. 2006).
- Requirements for success of this technique are accurate track identification, careful preparation of the tracking substrate surface, favourable weather (no precipitation and lack of wind) and the need for stations to be conveniently located but not disturbed (Disney et al. 2008)

- Roughton and Sweeny (1982), Smith et al. (1994) and Sargeant et al. (1998) should be consulted before scent-station surveys are initiated.
- Expert staff is required for fingerprint recognition.
- The visit rate to scent stations is affected also by the species density, due to various factors such as weather, the season of the year, habitat characteristics and human activity, the dry season is the one recommended for applying this method
- As some predators may avoid artificial materials (Gehring & Swihart 2003), to obtain proper footprints, scent-stations can be prepared naturally by cleaning up and sieving the natural soil (Soyumert & Gürkan 2013).
- Scent stations may be necessary to detect species rarely detected by scats.
- In track plates method checked and rebaited trackplates every 2-3 days and left them on site for 11-15 days per locality.
- 

## 6. Hunting bags statistics

Objective: Estimates the population abundance with data obtained from hunting events for local (e. g. hunting grounds and management areas) and larger areas.

Methodology: Raccoons can be culled for different reasons: sport hunting, pelt trapping, alien population control and they can be a victim of road kills as well, more or less frequently depending on density (García et al. 2012, 2013, Timm et al. 2016). Hunting bag data as indicator for raccoon relative abundance and represents a potential method for monitoring changes in their abundance (Fischer et al. 2016, Tomaschek, 2008). Culling numbers of the alien predator species raccoon dog and raccoon are increasing in Austria, Czech Republic, Slovakia and very dramatically in Germany; however, there are some countries, such as Switzerland, where raccoons are not hunted at all (Reimoser & Reimoser 2016). Some biases can be pointed out (Ranta et al. 2008). First of all, such techniques are heavily dependent on standardizing the amount of effort expended to obtain reliable and comparable data, because of different hunting traditions and methods. The cumulative number of raccoons killed on a given area may not indicate density also due to the immigration of individuals (such as juveniles) from neighbouring areas.

The statistics of hunting may be an acceptable indicator of the population trends to a regional or national scale, assuming that the data has been recorded in a way consistent over the years and hunting pressure has remained constant and that there have not been changes in hunting effort, quotas and hunters saturation or legal restriction. However, it is not applicable to non-hunting areas and hunting estates can be not representative of the rest of the region/country. Finally, environmental conditions (weather, food availability and population density) can interfere too. Despite all of this, several comparisons of census data and hunting bag statistics suggested largely similar conclusions from both data sources (Fischer et al. 2016)

Evaluation:

- Appropriateness to estimate density or abundance: density
- Accuracy and precision: low, highly variable

- Reliability: low to moderate reliability, depending on hunting legal restrictions, weather conditions, will and ability of hunters, animal densities, report of hunting bags and dependent on knowledge of reproductive rate and sex ratio. The applicability of hunting statistics for monitoring raccoon abundance is greatly limited by differences in culling effort.
- Cost and effort required: data more or less everywhere available, often long-term trends
- Spatial scale: local and large scale possible.
- Comparability: It is necessary standardized data collection model for hunting statistics.

Practical Guidelines:

- See guidelines for drive counts during hunting. It is to keep into account that availability of hunting bag data is strictly depending on hunting permissions for raccoons in different countries.
- Harmonised protocols for hunting data must be established to allow comparisons.
- Information can be taken both from raccoon hunting season and coon-dog training season, when dogs are trained to recognise and find racoons (Rogers & Rogers 2020).
- Hunting period for raccoon is usually autumn, starting month can vary depending on nations (Rogers & Rogers 2020).

The Figure 17 illustrates some charasteristics of Northern raccoon and methods applied to estimate abundance.

The table 9 shows a classification of different population density or relative abundance estimations based on desirable characteristics for Northern raccoon monitoring populations in local management units, practicability, and applicability.

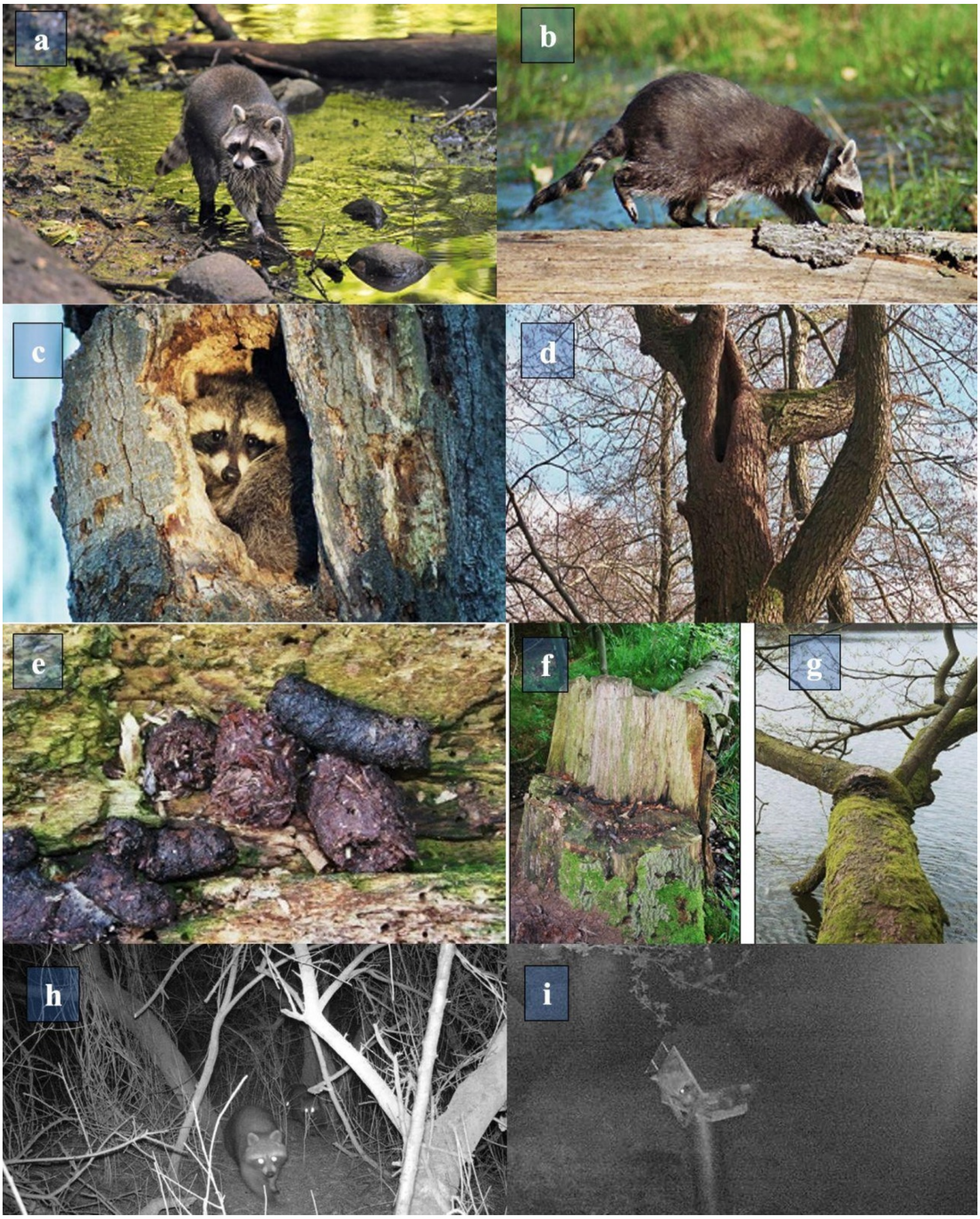


**Figure 17. Northern raccoon (*Procyon lotor*).** (a) Raccoon in riparian habitat (image: R. Vitt), with an unmistakable black facial mask. (b) raccoon bearing a GPS collar (image: F. Michler). Capture-Mark-Recapture (CMR) requires trapping and marking individuals. (c-d) The births occur inside dens, that can be tree cavities, burrows of other mammals or rock outcroppings. Tree cavities can be very high and can hardly be climbed in order to examine a cavity. Raccoons can be detected lying in branches and with the age of 4-6 weeks young raccoons can be detected climbing in the trees. Females raising their young are extremely careful and will not show up while watching a supposed den (images: F. Michler). (e-f) raccoons defecate in dedicated latrines, places like tall branches, fallen tree trunks and trunks that act as a bridge

over a body of water formed typical structures that raccoons used as toilets (images: F. Michler). (h) camera trap image of a raccoon group (Image: J. Hörig). (i) raccoon at bird feeding place in a garden (image: V. Reineke).

**Table 9**: Classification of different population density or relative abundance estimations based on desirable characteristics for Northern raccoon (*Procyon lotor*) monitoring populations in local management units, practicability, and applicability.

| | **Northern raccoon (*Procyon lotor*)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Linear transect | Linear transect: DS | CMR | Telemetry | Camera trapping | Non-invasive genetic sampling | Pellet counts | Track counts | Scent stations | Hunting statistics |
| Abundance/density | A/D | A/D | D | D | D | D | A/D | A/D | A | A/D |
| Temporal trend | annual/seasonal | annual/seasonal | annual/seasonal | annual | short | seasonal | annual | annual/seasonal | annual/seasonal | seasonal |
| Info on population structure | y | y | y | y | y | y | n | y | n | y |
| Season | year round | year round | year round | year round | year round | year round | year round | year round | year round | seasonal |
| Costs | moderate | moderate | moderate to expensive | very high | moderate | high | low | low | low | low |
| Effort | moderate | moderate | high | very high | moderate | moderate | moderate | low | moderate | low |
| Ease of learning | easy r | moderate | moderate | difficult[1] | easy[3] | easy[4] | easy r | easy | easy | easy |
| Accuracy + precision (High, Medium, Low) | MA+MP | MA+MP | HA+HP | HA+HP | HA+HP | HA+HP | MA+MP | LA+LP | MA+MP | LA+LP |
| Reliability | moderate | moderate | reliable | reliable | reliable | reliable | moderate | low | moderate | low |
| Useful at local scale | y | y | y | y | y | y | y | y | y | n |
| Useful at large scale | y | y | n | n | r | r | r | y | n | n |
| Useful at very low density | r | r | y | y | y | r | r | y | r | n |
| Useful at very high density | y | y | y | y | y | y | y | y | y | n |
| Suitable at all | y | y | y | y[2] | y | y | r | y | r | n |

1 - requires professional personnel trained with capturing and handling wild animals, 2 - suitable, but not recommended as the method used solely for monitoring, 3 - requires basic statistical skills to apply CMR models, 4 - requires the use of molecular techniques, but possible with use of commercial service, r - restricted.

## 4.9. Raccoon dog (*Nyctereutes procyonoides*)

Raccoon dog is an invasive species with increasing distribution range in Europe (Kauhala & Kowalczyk 2011), however limited number of studies were conducted on the species. Due to their nocturnal and elusive lifestyle, estimating the population densities using direct observation is difficult. Because raccoon dogs are relatively easily caught (Drygala et al. 2008, Kowalczyk et al. 2008) and usually live in higher densities than native medium-sized carnivores (such as red fox and badger), methods based on live-trapping such as mark-recapture can provide robust estimates of raccoon dog densities.

**Direct methods**

**1. Capture-recapture**

Objective: By identifying part of the animals and estimating the proportion of the recaptured, density can be estimated.

Methodology: Mark-recapture techniques are frequently used to estimate abundance of elusive or nocturnal animals in small-to-medium sized areas (Cormack 1972, Krebs 1989, Seber & Schofield 2019). They can be also applied to estimate raccoon dog population size. Raccoon dogs can be captured using wire-boxtraps and fish or meet bait (Kowalczyk et al. 2009, Drygala et al. 2010). Traps should be spaced within the areas to ensure adequate coverage of the entire study area. To increase trapping success traps should be set in wet habitats, preferred by raccoon dog, along rivers and streams, lake shores, however they use variety of habitats including agriculture. If possible, pre-baiting for few days is recommended. The traps should be opened and checked for 10 days. Raccoon dog are easy to handle and do not need to be immobilized (Drygala et al. 2008). Captured animals are sexed and fitted with numbered plastic ear-tags (Drygala et al. 2010) or Passive Integrated Transponder (PIT) tags (Smyth & Nebel 2013).

Most often an adaptation of standard mark-recapture techniques called closed-subpopulation model (CSM) is used. It provides a flexible framework to increase the amount of data used for estimation of demographic parameters, by taking into account characteristics of the population and using a variety of additional non-trapping data about the occurrence of individually identifiable animals (such as from radiotelemetry, sightings, or road-traffic accidents) in addition to mark-recapture records. The model defines a subsection of the population that is known to be alive within the study area during a specific period, regardless of which animals were actually caught.

Population size is estimated from the proportion of animals in this closed subpopulation that were actually captured. Estimates of population size generated by CSM is likely to be more precise than those generated by standard techniques (for details see Tuyttens et al. 1999). Under the CSM, a simple estimator of population size at the time of the th trapping occasion, $N_i$, is:

$$N_i = n_i T_i / t_i,$$

where $T_i$ - the number of raccoon dog in the closed subsection of the population for which it was assumed that all raccoon dogs were alive and within the study area and, therefore, could have been trapped during trapping occasion *i* (number of raccoon dogs captured and marked); $t_i$ - the number of $T_i$ raccoon dogs recaptured; and $n_i$ - the total number of raccoon dogs trapped (marked and unmarked).

Several capture-recapture software can be used for data analysis such as MARK (White & Burnham 1999) or others (see: http://www.capturerecapture.co.uk/software.html)

Evaluation:

- Appropriateness to estimate density or abundance: both.
- Accuracy and precision: method accuracy and precision increase with increasing field and lab effort; high accuracy and precision are reached provided correct sampling design and lab protocol that enables to meet all assumptions of the chosen CR estimator.
- Reliability: dependent on multiple factors: sample size (recapture rate), personnel experience, work intensity, lab protocol, the degree of meeting assumptions of the chosen CR estimator.
- Cost and effort required: relatively low costs and effort
- Spatial scale: local due to field effort.
- Level of comparability: There have been no studies comparing results of this method with other methods aiming at estimating raccoon dog abundance. Comparability between regions/countries possible provided that similar sampling design, lab protocols, and CR models are used.

## 2. Capture-recapture: combination of individual marking and camera-trapping

Estimating abundances and demographic parameters from camera traps has become prominent in wildlife. Recently developed spatial capture-recapture models for camera traps with marked animals allow estimation of their populations (O'Brien 2011). The method can be applied in medium- and high-density raccoon dog populations. It combines trapping and marking of animals (Popescu et al. 2014) with ear-tags or collars with small bands of infrared reflective tape. Thanks to this, marked animals can be recognized in the night. Animals are captured using wire-box traps and fish or meet bait (see above). Traps should be spaced within the areas to ensure adequate coverage of the entire study area. Each animal is marked with the collar or ear tag. Camera trap for photo-trapping session can be distributed regularly in a grid or in specific sites to increase visitation by raccoon dogs (baiting sites for other wildlife). Camera stations can be baited with meat, fish, fruits or corn.

On the first trapping occasion, $n_1$ animals are marked with collars. On the photo-trapping occasion, $n_2$ animals are photographed, of which $m$ animals were captured on the first trapping occasion and collared. The proportion of marked animals in the population after the first capture occasion is $n_1/N$. If all animals have equal capture probabilities, then the proportion of marked animals in the population should be the same as the proportion of marked animals in the second sample. If true,

$$n_1/N = m/n_2$$

which leads to estimator

$$N = n1n2/m$$

Several capture-recapture software can be used for data analysis such as MARK (White & Burnham 1999) or others (see: http://www.capturerecapture.co.uk/software.html)

Evaluation:

- Appropriateness to estimate density or abundance: both.
- Accuracy and precision: method accuracy and precision increase with increasing field effort; accuracy higher at high density populations;
- Reliability: dependent on multiple factors: size of the study area, population density, trapping effort;
- Cost and effort required: Relatively high personnel effort, it require to purchase some number of traps and camera-traps.
- Spatial scale: local
- Level of comparability: the method is novel and there have been no studies comparing results of this method with other methods aiming at estimating raccoon dog abundance. It was also not used for raccoon dogs so far. Comparability between regions/countries possible provided that similar sampling design, lab protocols, and CR models are used.

The Figure 18 illustrates some charasteristics of Raccoon dog. The table 10 shows a classification of different population density or relative abundance estimations based on desirable characteristics for Raccoon dog monitoring.


**Figure 18. Raccoon dog (*Nyctereutes procyonoides*).** (a) raccoon dogs. Despite its appearance and name, its closest relatives are true foxes and not the American raccoons (image: R. Kowalczyk). (b) Camera trap image of a raccoon dog at the same place northern raccoons were also photographed (see figure 7i, image: J. Hörig). Camera stations can be baited with meat, fish, fruits or corn. Unfortunately, no studies comparing results of recently developed camara trap methods with other methods aiming at estimating raccoon dog abundance are available. (c) Adult raccoon dog road killed in Poland (image: Andrzej Grabowski). Note variations in fur respect to (a).

**Table 10**: Classification of different population density or relative abundance estimations based on desirable characteristics for Raccoon dog (*Nyctereutes procyonoides*) monitoring populations in local management units, practicability, and applicability.

| Raccoon dog (*Nyctereutes procyonoides*) | | | |
|---|---|---|---|
| | Capture-recapture | Capture recapture: individual marking and photo trapping | Camera trapping |
| Abundance/density | A/D | A/D | D |
| Temporal trend | seasonal/annual | seasonal/annual | short |
| Info on population structure | y | y | y |
| Season | spring or autumn | spring or autumn | year round |
| Costs | medium | high | moderate |
| Effort | medium | medium | moderate |
| Ease of learning | medium | medium | easy |
| Accuracy + precision | MA+MP | MA+MP | HA+HP[1] |
| Reliability | reliable | reliable | reliable |
| Useful at local scale | y | y | y |
| Useful at very low density | n | n | r |
| Useful at very high density | y | y | y |
| Suitable at all | y | y | y |

[1]Keuling O (pers. Conv)

## 5. Summary tables

The tables 11 and 12 summarize the evaluation of different direct and indirect census-methods, respectively, based on desirable characteristics for all the carnivore species addressed in this report.

**Table 11**: Evaluation of different **direct density or relative abundance methods** based on desirable characteristics for all the carnivore species.

| **Direct methods** | | Linear transect | Linear transect: DS | Capture-mark-recapture (direct) | Telemetry | Camera trapping | Driven counts | Counts at feeding sites | Counts of FCOY | Badger sett survey |
|---|---|---|---|---|---|---|---|---|---|---|
| WOLF (*Canis lupus*) | Abundance/density | | | D | D | | | | | |
| | Cost/effort | | | H | H | | | | | |
| | Accuracy/ Precision | | | HA+HP | HA+HP | | | | | |
| GOLDEN JACKAL (*Canis aureus*) | Abundance/density | A/D | A/D | D | D | D | | | | |
| | Cost/effort | M | M | moderate to expensive | H | M | | | | |
| | Accuracy/ Precision | MA+MP | HA+HP | HA+HP | HA+HP | HA+HP | | | | |
| RED FOX (*Vulpes vulpes*) | Abundance/density | A/D | A/D | D | D | D | A/D | | | |
| | Cost/effort | M | M | moderate to expensive | H | M | low cost, moderate effort | | | |
| | Accuracy/ Precision | MA+MP | HA+HP | HA+HP | HA+HP | HA+HP | MA+MP | | | |
| BROWN BEAR (*Ursus arctos*) | Abundance/density | | | A/D | | A/D | | A | A | |
| | Cost/effort | | | very high | | M | | low[4] | low cost, medium effort | |
| | Accuracy/ Precision | | | HA+HP | | MA+MP | | HA+LP | H/MA + H/MP | |
| EURASIAN LYNX (*Lynx lynx*) | Abundance/density | | | | D | D | | | | |
| | Cost/effort | | | | H | M | | | | |
| | Accuracy/ Precision | | | | HA+HP | HA+HP | | | | |
| IBERIAN LYNX (*Lynx pardinus*) | Abundance/density | | | | | CR: A/D REM:D | | | | |
| | Cost/effort | | | | | M | | | | |
| | Accuracy/ Precision | | | | | CR: HA+HP REM: MA+MPP | | | | |
| EUROPEAN BADGER (*Meles meles*) | Abundance/density | | | A/D | | | | | | A/D |
| | Cost/effort | | | high cost, low effort | | | | | | low cost, high effort |
| | Accuracy/ Precision | | | HA + HP | | | | | | HA + HP |
| NORTHERN RACCOON (*Procyon lotor*) | Abundance/density | A/D | A/D | D | D | D | | | | |
| | Cost/effort | M | M | moderate to expensive | H | M | | | | |
| | Accuracy/ Precision | MA+MP | MA+MP | HA+HP | HA+HP | HA+HP | | | | |
| RACCOON DOG (*Nyctereutes procyonoides*) | Abundance/density | | | A/D | | D | | | | |
| | Cost/effort | | | Medium | | M | | | | |
| | Accuracy/Precision | | | MA + MP | | REM: HA+HP | | | | |

A = abundance, D = density, Cost/effort: H = high, M = moderate, L = low, Accuracy/precision: HA = high accuracy, MA = moderate accuracy, LA = low accuracy, HP = high precision, MP = moderate precision, LP = low precision. u = unknown

**Table 12**: Summary table of the evaluation of different **indirect density or relative abundance methods** based on desirable characteristics for all the carnivore species. No indirect methods has been tested for raccoon dog.

| **Indirect methods** | | Non-invasive genetic sampling | Den counts | Track counts | Scent stations | Vocal method | Damage claims | Hunting bag statistics | Hunting statistics and age structure |
|---|---|---|---|---|---|---|---|---|---|
| WOLF (*Canis lupus*) | Abundance/density | D | A | A/D | | A/D | | A/D | |
| | Cost/effort | H | low cost, moderate effort | moderate cost and effort | | low cost, moderate effort | | low cost, low effort | |
| | Accuracy/ Precision | HA + HP | MA+MP | MA+MP | | MA+MP | | LA+LP | |
| GOLDEN JACKAL (*Canis aureus*) | Abundance/density | D | A /D | A | | A | | | A/D |
| | Cost/effort | high cost, moderate effort | low cost, moderate effort | L | | M | | | moderate cost and effort |
| | Accuracy/ Precision | HA+HP | LA+LP | LA+LP | | MA+MP | | | MA+MP |
| RED FOX (*Vulpes vulpes*) | Abundance/density | D | A/D | A/D | A | | | A/D | |
| | Cost/effort | high cost, moderate effort | low cost, moderate effort | L | low cost, moderate effort | | | L | |
| | Accuracy/ Precision | HA+HP | LA+LP | LA+LP | MA+MP | | | HA+LP | |
| BROWN BEAR (*Ursus arctos*) | Abundance/density | A/D | | | | | no | A | |
| | Cost/effort | H | | | | | L | L | |
| | Accuracy/ Precision | HA+HP | | | | | LA+LP | LA+LP | |
| EURASIAN LYNX (*Lynx lynx*) | Abundance/density | D | | A/D | | | | A/D | |
| | Cost/effort | high cost, moderate effort | | L | | | | L | |
| | Accuracy/ Precision | HA+HP | | MA+MP | | | | HA+LP | |
| IBERIAN LYNX (*Lynx pardinus*) | Abundance/density | | | | | | | | |
| | Cost/effort | | | | | | | | |
| | Accuracy/ Precision | | | | | | | | |
| EUROPEAN BADGER (*Meles meles*) | Abundance/density | | | | | | | | |
| | Cost/effort | | | | | | | | |
| | Accuracy/ Precision | | | | | | | | |
| NORTHERN RACCOON (*Procyon lotor*) | Abundance/density | D | | A/D | A | | | A/D | |
| | Cost/effort | high cost, moderate effort | | L | low cost, moderate effort | | | L | |
| | Accuracy/ Precision | HA+HP | | LA+LP | MA+MP | | | LA+LP | |

A = abundance, D = density, Cost/effort: H = high, M = moderate, L = low, Accuracy/precision: HA = high accuracy, MA = moderate accuracy, LA = low accuracy, HP = high precision, MP = moderate precision, LP = low precision.

# 6. Statistical modelling

The objective of statistical modelling is to quantify the effect of a group of predictors on the distribution, density, or relative abundance of a species (wild carnivore population parameters in our case) usually at large spatial scale.

Predictive modelling is another way to predict population's relative abundance, and even density, with the aim to determine general spatial patterns of species, which is of great value for further studies on conservation, management and epidemiological analyses of risk factors. Modelling allows relating data on the species (presence/absence, abundance, fitness, etc.) with environmental variables in order to obtain an output that is related with the habitat suitability for the species (e.g. Honda & Kawauchi 2011). The model can be used to provide understanding and/or to predict the species' distribution across a landscape (Elith & Leathwick 2009). Model predictions should be validated with independent data since there are several factors modulating that relation. The distribution of different wild carnivore species has been obtained using spatially explicit modelling procedures based on distribution data (e.g., Baltensperger and Joly 2019; Melis et al. 2009; Pittiglio et al. 2018; Ross et al. 2019). Statistical models have been used to estimate population size and distribution of all mammals in Great Britain (Acevedo et al. 2014, Croft et al. 2017). Future attempts might explore combining modelling with alternative source data, such as camera trapping.

There are not simple equations (Figure 19) but complex spatial explicit models can determine abundance and/or predicted densities that are reliable at larger scales.

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_nX_n$$

where $b_0$ is the constante and $X_{1 \text{ to } n}$ are the predictors.

**Figure 19.** Illustration of the equation of spatial explicit models to determine relative abundance and/or predicted density of a wild carnivore species (y). Data on the species (occurrence, abundance) is related with explanatory variables (predictors), such as environmental variables that allow for the projecting of the predicted distribution/abundance of the species.

In this report, we only evaluated the methods that can be used as direct measurement tools, scientific monitoring, or for calibrating other data as basis for statistical modelling. Within the ENETWILD project, in a follow up report, statistical spatial modelling approaches for wild carnivores will be reviewed (but see ENETWILD Consortium 2018c for wild boar), proposed, and used to determine wild carnivore density in areas where there are only data of limited quality available. Hunting statistics can only directly contribute to this if collected at a very small scale (~1 $km^2$), but all methods that produce a density estimate can be used (for instance, sett surveys have been used for this respect in UK, Byrne et al. 2014).

# 7. Final remarks

In this report we wanted to make an assessment of the estimation methods applied to wild carnivore species of greatest interest for disease management but also conservation and socio-ecological relevance. Abundance estimation of wild carnivores is essential for their management and to provide baseline population information for epidemiological purposes (e.g. risk factor analyses of shared diseases). Therefore, there is need of harmonized application of methods in generate comparable population estimations and to monitor population changes.

Different approaches have been used to determine the abundance of wild carnivore species in Europe. They are elusive animals, many of them are nocturnal, live at low densities and are difficult to observe. Therefore, most methodological approaches attempt to increase the probability of detection. The methods available for estimating the population are not always completely reliable, or it is not sufficiently known and/or remain completely untested. In addition, comparisons between different areas, habitats, seasons and density levels are often complex to conduct. Altogether, this indicates that there is not universal best method for each species or for each environment, although SCR are nowadays widely considered as reference). The geographical, geomorphological, vegetation and land use conditions differ from area to area where each species responds with different distribution patterns and different population dynamics, which makes that an evaluation of the method that best fits our needs has to be done in each case. When planning an estimation of population abundance, it is essential to consider the final objectives. Much thought should be given to choosing a method appropriate to the characteristics of the study area (habitat, infrastructure, dirt roads, etc.), the estimated efforts and the expected results, the need for density value or just an index of relative abundance to monitor the trend in space and time, the necessary level of accuracy and precision; and design of the sample size to obtain a correct interpretation of the data. With repeated sampling over time, both relative indexes and absolute estimates of animal abundance can be used to monitor population trends (see tables 11 and 12). However, camera trap methods, especially those recently developed, are promising and are able to cover a wide range of species, habitats and densities with minimal adjustments. In conclusion, apart from the elaborated methods for estimating wild carnivore relative abundance and density, there is **need for compiling and validating abundance data at different spatial scales**. Developing a permanent network and a data platform to collect and share local density estimates, so as abundance in the EU, would allow to identify gaps in the data on wild carnivores and to focus on these areas for improving spatial model predictions. It is essential to involve the national and regional hunting administrations in data collection and sharing, as well as the hunter and conservationist associations.

**Density estimation at local scale and large spatial scale**

- Population density can be calculated with several methods such as capture-recapture (in a broad sense: CR, CMR by capture, camera trapping, genetics), telemetry, non-invasive genetics sampling or hunting statistics.
- In general, direct counts with high accuracy are very costly. However, camera traps and non-invasive genetic approaches are becoming more cost-efficient.
- Telemetry can be applied in all habitats and produce estimate with high level of accuracy and precision (only for territorial species), but the disadvantages are the high cost and effort to catch animals and applicability limited to a local scale only.
- Non-invasive genetics sampling produce estimate with high level of accuracy and precision, but is expensive and therefore difficult to apply on a large scale.
- The best suggested method to estimate the real population density in several species, unless until some recently developed CT methods are tested, is the combination of trapping, marking, telemetry and camera traps. CTs can allow individual recognition as well and

subsequent capture-recapture models. However, high effort is required to catch, mark and follow the animals.

- After evaluating the cost, effort, and accuracy, we concluded that the most (potentially) effective method is camera-trapping, which has a moderate/high cost and moderate effort, high accuracy and precision, also in low densities, and can be used in most condition.
- Capture- mark- recapture (CMR) produce estimate with high level of accuracy and precision but requires the capture and marking of the animals, greatly increasing the costs and sampling efforts. However, methods that use camera-trap and not require individual recognition have recently been developed and are a good compensation of cost, effort and accuracy.
- Spatial capture-recapture (SCR and its extensions) are in continuous development, and they also allow for the estimation of home-range centres within the sampled area.
- REM (and its extensions) and potentially distance sampling with CT, has been used for some carnivores and promising results has already been obtained to estimate population density without individual recognition. REM requires knowledge of average speed and home-range size parameters of the studied population, while REST does not. For both methods, all needed parameters can be calculated from camera trapping without need of marking or capturing individuals.
- In the context that CT are becoming more popular, and it useful for multispecies, a framework based on a stratified design of study sites and application of CT would be an excellent to monitor trend of densities and to be used as a benchmark and calibration for indirect methods and relative abundance.
- Distance sampling on transects is a cost effective alternative, specially at medium-high densities and where good detectability is good. It can be applied also to calculate relative abundance. The negative effect of vegetation on visibility can be a limitation in areas with a large proportion of the land with forest cover, where this methodology requires high efforts.
- In general, all methods require stratified design of sampling and analysis of data in relation to habitat are required to avoid sampling bias.

**Relative abundance estimates at local scale and large spatial scale**

- Relative abundance can be calculated with several methods that focus on finding signs of animal presence, which can also be used also to calculate local density. The latter often needs calculating local parameters which cannot be extrapolated to other populations or seasons.
- Indirect methods that rely on counts of the signs of the animals (e.g. droppings, breeding refugia) are less expensive than direct methods and can be applied to the range of habitats found in Europe. Sett and den surveys can be used at large spatial scales in different species.
- Despite the relatively low costs and ease of learning, these methods may have low reliability, accuracy and precision and may depend on the season and often are applied at local scale. However, at present, these indirect methods can only be used to measure relative changes in animal density in the same region over time, and there are few evidences to indicate that they can be used to compare between regions or to obtain quantifiable estimates of animal density. When possible, they should be applied with direct methods to confirmed data.
- Good results can be obtained by genetic capture-recapture, but genetic analyses of samples can be expensive.

- Linear transects are cost-effective method to calculate relative abundance because they can be applied at local and large scale, with high accuracy, moderate reliability and moderate cost and efforts. This approach includes several techniques for detecting carnivores and are adaptable according to the ecology of the animal and the climatic and environmental conditions. Particularly, KAI uses transect to calculate relative abundance. It is applicable at spatial scales of several hundred $km^2$ and useful for monitoring large regions after stratification of sampling for widely distributed species (e.g. red foxes, badgers). Random placement of transects, at least repeated over years for comparisons purposes along time, and it should be tested whether stratification allows for similar sampling effort and bias in each habitat class.
- Hunting bag statistics deliver large but inaccurate data amounts with low effort, and only for some, non-protected hunted species (e.g. red fox). Hunting data may be sufficient to provide information on large-scale density and relative abundance, long-term trends and in specific hunting areas. However, not all species are hunted, and methods are diverse. Hunting statistics are irregularly and not-completely collected, and in most cases, not possible to be compared. It is necessary to harmonize the hunting data collection frameworks to make them usable and comparable.
- Vocal methods in big canids have been so far some the most diffused way to perform census and relative abundance estimation, however, they have some important disadvantages: they can just be performed seasonally, only territorial groups can respond, and differentiating individuals requires sophisticated digital sound analysis techniques.

## 8. Glossary

**Accuracy of an estimate:** Difference between the real number of individuals in an area and the estimated number.

**Bias**: Any deviation from the reality that can occur when carrying out an estimate.

**Calibration**: comparison of abundance/density values derived from a given method under test with those of a calibration standard of known accuracy. The calibration standard method should be a method already validated under the specific conditions of the study.

**Catch (Hunting bag):** The number of individuals captured (hunted) in a given area and period of time.

**Catch per unit of effort:** Unit of relative abundance that can be used to measure the changes of hunted populations. It is obtained from the ratio between the number of individuals captured (hunted) and the capture effort made (for example, hunting days, number of hunts or hunters, etc.).

**Coefficient of variation (CV):** Accuracy measure of an estimate (the lower the more accurate). It is usually expressed as a percentage.

**Confidence interval:** These are the limit values that an estimate with a certain probability could have. Normally it is a 95% probability, which means that the probability that the real number is outside this range is only 5%.

**Culling**: the killing of animals during the process of management of the population for control or exploitation. Usually, the implication is killing for control.

**Detectability**: Probability of detecting the individuals that makes up the sample of a population.

**Direct method**: it is a method to estimate density or relative abundance that is based on the direct observation of animals, also with statistical calibration.

**Distance sampling method**: Density estimation method based on the decrease in the detectability of the animals as distance to observer increases. It calculates the detectability for a series of distance intervals with respect to observer.

**Drive count method**: Density estimation method based on the count of individuals displaced in the course of a beat in a area of known surface.

**Effort**: It is the intensity of the work done (hunting days, traps x day, etc.) to get a number of direct or indirect observations in order to calculate a measure of density or relative abundance.

**Estimation or estimate of a population**: It is an approximate calculation of the real number based on a statistical procedure and usually based on a representative sample.

**Extraction**: Number of individuals removed (hunted) from a population.

**Fixed quota of extraction**: Is that extraction that is done previously fixing the number of animals that are going to be hunted.

**Harvest/harvesting**: by whatever method (rifle, shotgun, bow, trap, ect.), the implication in the use of this word is sustainable exploitation of the population concerned.

**Home range**: The home range of an animal is the area where it spends its time; it is the region that encompasses all the resources the animal requires to survive and reproduce. One of the easiest and most widely used methods of estimating home ranges is the Minimum Convex Polygon (MCP). The concept is to construct the smallest possible convex polygon around the XY locations (point set). MCP has several downsides, however they are good for exploratory analysis and visualization. Usually, all points with a distance greater than a selected quantile are removed (95%= $MCP_{95}$).

**Hunting days**: number of days hunting activity has been performed in a given hunting ground and period of time.

**Hunting district:** administrative district in terms of organization of hunting activities. A hunting district usually includes several (a few or many) hunting grounds (public and/or private).

**Hunting ground:** continuum area subject to similar hunting management.

**Indirect method**: it is a method to estimate density or relative abundance that is based on the detection of presence signs, but not on living animal observations.

**Kilometric abundance index (KAI):** It is an index of relative abundance that expresses a certain count based on a unit (km) of the path travelled to perform the observation effort. It is calculated by dividing the number of observations (individuals, groups, pellets, etc.) by the number of kilometres travelled.

**Management**: the concept of "taking responsibility for" or influencing the dynamics of animal populations, and the activities associated with that.

**Management unit**: continuum area subject to similar wildlife (including hunting) management.

**Native/Introduced/Exotic:** Native is a population which is considered to have had continuous presence in a given area (=autochthonous). Introduced is a species which could occur in any geographic region but in practice is not native in that particular country and has been introduced within historic time by humans. Exotic is a species introduced by humans well outside its natural geographic distribution.

**Observation effort**: It is the equivalent of the capture effort applied to the observation of animals or their signs of presence. The effort of observation is usually measured in number of observations per kilometres travelled, days of work, people involved, etc.

**Population census:** It is a complete count, although it is practically impossible to count all the individuals that make up the population of a wild species. Colloquially, a census is usually called a census that is intended to count all individuals, but usually falls below the real number. To approximate the real number with greater security, population estimates are used.

**Population count**: It is the result of counting the individuals that compose the population in all the study area. Hardly, all individuals can be counted (see population census).

**Population density** (d) is a measurement of population size per area unit, i.e., population size divided by total land area. The absolute density usually is expressed in heads per 100 ha. Multiplying the population density by the studied surface, we obtain the population size. It can be calculated by different methods (either direct tor indirect).

**Population size or absolute abundance (N)**: It is the size of the population. It can be a known or estimated number, expressed in number of individuals. When related to area unit it gives the population density.

**Precision of an estimate**: Degree of statistical error that entails an estimate. It can be measured by the coefficient of variation (CV).

**Relative abundance or abundance index**: refers to the relative representation of a species in a particular ecosystem. Relative abundance can be calculated by different methods (either direct or indirect, summarized in Tables 1 and 2). The relative abundance reflects the temporal or spatial variations of the size (N) or density (d) of a population but does not directly estimate these parameters. Since relative abundance covariates with the population density, it is useful for monitoring animal populations over time, as well as for conducting large-scale studies on the factors that determine the abundance of species. Nonetheless, this relationship is not linear (Figure 1). Sometimes, due to financial, logistical, or time constraints, wild carnivore surveys can only deliver relative abundance such as those obtained from camera trap surveys, instead of total population size or density estimates.

**Repetitions**: Each time a given transect or observation station is repeated to counteract variations in specific conditions a given day (e.g., adverse weather), and its effect o detectability.

**Standard deviation**: It is a measure of variability of the estimate that allows calculating the probability that the estimated size of a population may vary. It is the square root of the variance. The standard deviation of a sample is represented by an *s*.

**Standard error**: It is a measure of variability of the estimate that allows us to compare two estimates. It combines the value of the standard deviation with the size of the sample. By increasing the number of samples, we can reduce the typical error. It is represented by the letters SE.

**Stratification**: Technique that allows improving the precision of the results of a estimate by dividing the samples into more homogeneous parts (strata, e. g. habitats).

**Transect**: An itinerary along which all the individuals or sings we can see are counted. It can be applied in the form of KAI (index of relative abundance) or by applying a distance sampling method to obtain an absolute density.

**Variable quota of extraction**: Is that extraction that is done previously fixing the percentage of the population that is going to be hunted.

**Variance:** Value that indicates the dispersion of the measurements with respect to a central value (the mean or in this case the estimate). It is the square of the standard deviation. The sample variance is represented by VAR or by s2.

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