BY ENETWILD

A PRACTICAL GUIDANCE ON ESTIMATION OF EUROPEAN WILD UNGULATE POPULATION DENSITY

A SELECTION OF RELIABLE PRACTICAL METHODS FOR HARMONIZED POPULATION MONITORING IN EUROPE

THE ENETWILD CONSORTIUM

ACEVEDO P
APOLLONIO M
BEVILACQUA C
BLANCO-AGUIAR JA
BRIVIO F
CASAER J

FERROGLIO E
GRIGNOLIO S
JANSEN P
ILLANAS S
KAVČIĆ K
K KEULING O

PALENCIA P
PLIS K
PODGÓRSKI T
ROWCLIFFE M
RUIZ C
SCANDURA M

SMITH G
SORIGUER R
ŠPREM N
VADA R
ZANET S
VICENTE J

National Institute on Wildlife Research (IREC), UCLM-CSIC-JCCM
This Research Institute is Spanish national reference on wildlife management, and it has as its foundational objectives to ensure the sustainability of hunting, contributing to the maintenance of biodiversity, and promoting its socioeconomic performance. It coordinates ENETWILD and MAMMALNET projects. https://www.irec.es/

Department of Veterinary Medicine, University of Sassari
The Dept. is an academic institution dealing with research and teaching activities in the field of animal science and animal health. Within the general frame of the one health concept, the Dept. carries out research in the fields of zoology, animal physiology, genetics, microbiology and parasitology, animal food products, both referred to domestic and wild species. https://www.veterinaria.uniss.it/en/department

Research Institute of Nature and Forest (INBO)
It is the independent research institute of the Flemish government that underpins and evaluates biodiversity policy and management by means of applied scientific research, data, and knowledge sharing. INBO provide evidence-based support for nature policy and its implementation. https://www.vlaanderen.be/inbo/en-gb/homepage/

Department of Veterinary Sciences, University of Torino
The Department´s mission is to provide excellence in teaching, learning and research in the field of Veterinary Sciences for the benefit of both animal and human life and health and the environment. Staff is involved in research projects related to epidemiology and pathogenesis of parasites and wildlife diseases of public health interest. https://veteren.campusnet.unito.it/do/home.pl

Department of Environmental Sciences, Wageningen University
Its staff coordinates the Vertebrate Program of ForestGEO (http://www.forestgeo.si.edu), the Tropical Ecology Assessment and Monitoring (TEAM) program (the Worlds largest camera-trapping program) for the Smithsonian (www.teamnetwork.org). Involved in eMammal (emammal.si.edu) and Agouti (agouti.eu), two platforms for collecting, processing, archiving, and sharing wildlife images and data. https://research.wur.nl/en/organisations/department-of-environmental-science

Department of Fisheries, Apiculture, Wildlife Management and special Zoology, University of Zagreb
Its research in the field of wildlife management focuses on the proper management and conservation of wildlife through the application of molecular markers, quantitative ecology, and telemetry for better study of biology and population ecology of wildlife. http://www.unizg.hr/

Institute for Terrestrial and Aquatic Wildlife Research (ITAW), University of Veterinary Medicine Hannover
In ITAW, veterinarians, biologists and other natural scientists work together on diverse topics to make possible to meet the great challenges of wildlife research. Within the discipline of wildlife biology, ITAW focuses on basic research, applied research and monitoring. https://www.tiho-hannover.de/itaw

Mammal Research Institute (MRI), Polish Academy of Sciences (PAS)
MRI is an independent scientific research unit of the PAS. Research has been shaping the development of theriology (mammalogy) in Poland since the founding of the Institute. Studies have theoretical character, but also find application in wildlife conservation and management of animal populations. https://ibs.bialowieza.pl/en/

The Zoological Society of London (ZSL)
The ZSL, a charity founded in 1826, is a world-renowned center of excellence for conservation science and applied conservation. ZSL´s mission is to promote and achieve the worldwide conservation of animals and their habitats. It crucially contributed to develop methods based on camera trapping to estimate reliable wildlife density. www.zsl.org

National Wildlife Management Centre (NWMC), Animal and Plant Health Agency (APHA)
NWMC provides high quality research to provide evidence-based support for wildlife policy and advise commercial parties on wildlife management and humaneness issues. With the largest concentration of vertebrate ecologists in the UK and state-of-the-art facilities, its staff are leaders in wildlife management. http://apha.defra.gov.uk/apha-scientific

A PRACTICAL GUIDANCE ON ESTIMATION OF EUROPEAN WILD UNGULATE POPULATION DENSITY

A practical guidance on estimation of European wild ungulate population density
A selection of reliable practical methods for harmonized population monitoring in Europe
Acevedo P[1], Apollonio M[2], Blanco-Aguiar JA[1], Bevilacqua C[1], Brivio F[2], Casaer J[3], Ferroglio E[4], Grignolio S[2], Jansen P[5], Illanas S[1], Kavčić K[6], Keuling O[7], Palencia P[1], Plis K[8], Podgórski T[8,9], Rowcliffe M[10], Ruiz C[1], Soriguer R[1], Šprem N[6], Smith G[11], Scandura M[2], Vada R[1,4], Zanet S[4], Vicente[1]

[1]National Institute on Wildlife Research (IREC), University of Castilla-La Mancha, Consejo Superior de Investigaciones Científicas and Junta de Comunidades de Castilla-La Mancha, Ciudad Real, Spain
[2]Department of Veterinary Medicine, University of Sassari, Italy
[3]Research Institute of Nature and Forest, INBO, Belgium
[4]Department of Veterinary Sciences, University of Torino, Italy
[5]Department of Environmental Sciences, Wageningen University, The Netherlands
[6]Department of Fisheries, Apiculture, Wildlife management and special Zoology. Faculty of Agriculture University of Zagreb, Croatia
[7]Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Hannover, Germany
[8]Mammal Research Institute, Polish Academy of Sciences, Białowieza, Poland
[9]Czech University of Life Sciences, Prague, Czech Republic
[10]Institute of Zoology, Zoological Society of London (ZSL), United Kingdom
[11]National Wildlife Management Centre, Animal and Plant Health Agency, Sand Hutton, York, United Kingdom

Image sources:
Joaquín Vicente (pag. 7 wild boar, 11, 34 wild boar, vultures, red deer, 27, 29 fallow deer, 31 drone commands, staff, drone, map, 32 wild boar, 42 deer pellets) - Luca Giordano (pag. 7, 9, 45 Apine Ibex, 10, 14, 27 Alpine chamois, 20 wild boar)- Tomasz Podgórski (pag. 11 Eur. bison) - Christian Gortázar (pag. 11, 23 red deer, fallow deer, 26 Iberian wild goat, 28 Alpine Ibex, 32 beaters, 66 moose, 68 Alpine chamois) - Carlos Ciudad (pag. 11 wild boar)- Moisés González (pag. 12 Barbary sheep) - Charles Smith-Jones - Francisco Carro (23 distance sampling from car, 25, 64 red deer thermal vision, 42 pellet counts, 44 car, 64 wild boar thermal vision) - Sonia Illanas (pag. 27 telescope, red deer)- Ewa Plasturga (pag. 29 Eur. bison) - Sykut Maciej (pag. 29 Eur. bison) - Mara Mulero (pag. 30 drone picture)- Lars Hillström (pag. 34 moose) - Oliver Keuling (pag. 37 camera trap attachment, camera trap field view top) – Patricia Barroso (pag. 66 hunter beating) Bledi Hoxha (pag. 38, wild boar) - Kresimir Kavčić (pag. 39 wild boar)- Uliana de Castro (pag. 39, 40 maps)- Ramón Soriguer (front Cover, pag. 13, 47, 64 red deer) - Marco Apollonio (pag. 10 roe deer) - David Ferrer (pag. 24 red deer spotlighted, and spotlighters, 37 camera trap setting, 42 line transect top), Javier Ferreres (pag. 31 beaters, 32 beaters) - Pablo Palencia (pag. 10 red fox, 34, 35 wild boar capture, 37 camera trap field veiw top, 39 red deer, fallow deer, 43 sampling deer, pictures Fig. A1) - German Garrote (pag. 44 sampling feces) - Massimo Scandura (pag. 44, DNA test) - Daniel Burón (pag. 58 red deer).

Graphic design
B I, Lemus Lara, TUINBIT

FOREWORD

The European Food Safety Authority (EFSA) funds ENETWILD project (www.enetwild.com) to collect comparable data on wildlife populations at European level, which is essential to conserve wildlife and manage wildlife-related conflicts, but the same data are also needed to analyse risks of spread of diseases shared between wildlife, livestock, and humans. In January 2018 ENETWILD project was publicly launched and organised discussion workshops for experts in the field of the ecology, management and epidemiology of wildlife, and three questions were addressed: (1) what kind of data is needed to develop wildlife abundance maps?; (2) how can estimates of abundance be harmonised between regions/countries?; and (3) how can the collection of distribution and abundance data be improved? The following step performed by ENETWILD consisted of developing the standards for data and metadata collection, and the appropriate collection of data, using a data model and supported by a data-sharing agreement. Therefore, important progress has been made in the field of spatial distribution modelling of wildlife at European level. Guides for reliable abundance estimation of wild mammals and detailed protocols on field methods have been published, which now we present in more practical and graphical format. With this guidance on study design, methods, and field protocols to estimate wild ungulate density, we aim reaching European wildlife professionals and support a network of population data providers (data and metadata for distribution, abundance, and density), in parallel to the continuous training activities offered by ENETWILD project.

Now, ENETWILD makes progress towards new targets: The European Observatory of Wildlife (EOW). It is conceived as a European network of "observation points" with common population estimation protocols and data collection standards to facilitate harmonization and interoperability, with the aims of:

• Providing sound, scientific guidance on methods and protocols for those involved in implementing wildlife monitoring, in close collaboration with European Institutions.

• Generating independent information on population abundance for those involved in developing, adopting, implementing, and evaluating environmental policy in Europe.

• Becoming a real observatory, able to provide trends on wildlife population change at European level based on a network of "observation points" monitored over time.

• Being a "laboratory" of population abundance estimation methods: continuous improvement of protocols, calibrations of methods, use of information technology tools (ITs), artificial intelligence (AI) and citizen science (e.g., MammalNet project, www.mammalnet.com)

To contribute to improve the European capacities for a common, harmonized, and reliable wildlife population monitoring in the context of the EOW, ENETWILD has produced this practical guidance. All together, we aim to plant the seed of a Pan-European network capable of providing reliable data on wildlife abundance on a long-term basis, and to overcome existing data gaps and workflow bottlenecks in the context of current European-wide frameworks for population monitoring of terrestrial mammals.

THE ENETWID CONSORTIUM

EXECUTIVE SUMMARY

Wild ungulates commonly have a dominant role in the development of European landscapes, but also are associated with conflicts with human activities, mainly agricultural and forestry ones. Thus, reliable and harmonized (comparable) estimates of population size (absolute abundance) and densities (population size per area unit) are needed for monitoring their population trends. This is essential to develop management and for further risk assessment. Population density is an absolute value and therefore more informative and comparable among populations, even when values are obtained from different methodologies. Abundance indices are relative, measured in different scales/units and therefore does not inform on population size (but on its variation over the time) and comparisons among population are not feasible in general.

Given the diversity of available methods and the geographical diversity of Europe, methodological harmonization is duly needed. The general aim of this guidance is to review the methods for estimating density in European wild ungulates. This guidance is based in previous comprehensive reviews carried out by the ENETWILD Consortium, which proposed general recommendations for practical implementation of methods to estimate wild ungulate density:

> • The sampling strategy should optimize accuracy while avoiding the bias of density estimations. No method will provide accurate, precise data if the design of the study (sampling strategy) is not representative. This is especially true for species with an aggregated pattern of spatial distribution and marked habitat selection.

> • To produce comparable data, density estimates rather than abundance indices should be used, if and where possible.

We present nine methods used in nineteen wild ruminant species and wild boar across Europe, paying special attention to most practical methods for further implementation in the field to calculate reliable density estimates, allowing further comparable results over their distribution ranges. This guidance provides recommendations to select the methods to estimate the density and its implementations for ungulate populations with the aim of increasing the output reliability (good accuracy and precision). The habitat type plays a key role in the selection of the best method to determine, and this is partially irrespective to species characteristics. Camera trapping (CT) is a method that can be conducted in different environmental conditions and at any time to collect robust data. We present some basic instructions for the practical use of camera traps (CTs) to estimate wild ungulate density, which should be adapted to local specificities. Since different CT methods are available, we focus on those most practical, classified as of relative medium effort, and able to generate reliable data over a wide range of situations across Europe: the Random Encounter

Model (REM) and Random Encounter and Staying Time (REST), and distance sampling with CTs (CT-DS), which do not require individual recognition. We present a field protocol which is compatible with these three methods. In open areas, where CT may require an excessive effort, we suggest using methods involving the direct detection of animals: vantage points, linear transects and subsequent application of distance sampling methodologies; block counts, which can also be performed during communal hunting activities, or random points.

The method should be used in a harmonized way: we provide detailed instructions for the application of most recommended methods, but specific protocols must be specifically adapted to local conditions. However, we concluded that every method on estimating reliable and comparable wild ungulate population density has some advantages and disadvantages depending on the habitat, the weather conditions and the benefit and do not discard their use if applied in a harmonized way.

TABLE OF CONTENTS

1. INTRODUCTION

1. INTRODUCTION

The wild ungulates are the largest terrestrial mammals occurring in Europe, where 19 species of ruminants and wild boar are present, including autochthonous and exotic ones (Apollonio et al. 2010). Some taxa require conservation strategy, coupled with management measures designed to safeguard dwindling population and encourage their recovery because they are rare and threatened of extinction. Still other populations are threatened due to a genetic introduction of exotic individuals or alien species. On the other hand, most European wild ungulates species are abundant, and they can represent a pest in local situations. As a matter of fact, in some areas the densities of ungulates overtake 30 - 45 heads per square kilometre. In Europe, apart from some islands and the area close to polar circle, there are only very limited regions where wild ungulates are not present. Consequently, wild ungulates commonly have a dominant role in the development of landscape affecting their structure, as well as composition richness and relative abundance of the vegetal species and the entire community (e.g., Putman 2004, Smit and Putman 2011, Marcon et al. 2019a). Whatever their role in the ecological dynamics of natural or semi-natural systems, in the last decades the impact of wild ungulate species caused conflicts with human activities, mainly agricultural ones, and forestry management (e.g. Ammer 1996, Putman & Moore 1998, Putman 2004). Moreover, an increase of concerns over the negative effects on habitat conservation are reported for European environments (Ammer 1996, Flowerdew & Ellwood 2001, Fuller 2001, Rae et al. 2014, Carpio et al. 2017).

The abundance of wild ungulates across Europe has favoured a dynamic situation where new pathogens emerge; disease agents boost their virulence and widen their host range taking advantage of the increase of the host population density and the behavioural patterns used by host in these ecological conditions. They may carry many unknown or newly discovered agents (Postel et al. 2016, Cagatay et al. 2018) of substantial economic importance (i.e., veterinary administration, human medicine, trading, food production etc., Höfle et al. 2004). Many pathogens can infect multiple hosts and thus may result in an outbreak of infectious diseases in wildlife as well as in livestock and humans (Ferroglio et al. 2011). The changes in livestock industry from a more intensive to more extensive farming management - i.e., with a lower human presence on the field - together with the increase of distribution and densities of wild ungulates caused a pivotal increase of the contact risks between wild ungulates and livestock (Laddomada et al. 1994, Gortazar et al. 2007).

The current approaches to the management of ungulates are different among European countries and they are constantly evolving (Apollonio et al. 2010). Partially, it is due to different species present in each country and, consequently to the relative conflicts experienced between wild ungulates species and human activities. In addition, there is a very high heterogeneity in the cultural approach to hunting management as well as in national traditions. This diversity of objectives and variety of traditions drove to marked differences in legislative frameworks often even between neighbour countries. In some cases, this led to different approaches in the methods used to estimate wild ungulate abundance, in recorded data and in the timing of estimations/hunting practise implementation (see a detailed overview in Apollonio et al. 2010).

Most current European wildlife pathogen surveillance schemes, including African Swine fever (ASF), lack integration with appropriate population monitoring (i.e., the denominator data). Integrated monitoring means combining population and disease monitoring (Cardoso et al. 2021). 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 wild ungulates across Europe for conducting efficient population management and to reduce the epidemic risks (Depner et al. 2017). The population density is a measurement of population size (absolute abundance) per area unit, but estimation of wildlife population density is a difficult task (see Glossary). Reportedly by recent scientific literature, most methods traditionally used by wildlife management are neither precise nor accurate enough to be considered as a gold standard.

Differences between density and abundance estimates are noticeable for wild ungulates across studies, which are not due only to variations in the density but to limitations in the quality of data, biases (or even absence) of sampling design, and the use of different analytical methods. Wild ungulate species are widely

distributed across Europe, and they use different habitat types (e.g. roe deer: Andersen et al. 1998, red deer: Clutton-Brock et al. 1982). Hence, many approaches have been used to assess population size to increase the detectability probability of each target species (Apollonio et al. 2010). As a result, comparisons among different areas are often complex to be conducted. However, density estimation rather than abundance, make possible comparisons when methods are applied in a harmonized way. Another consequence is that it is impossible to highlight the best density estimation method for each species or for each environment because the geographical, geomorphological, vegetational, and land use conditions create a plethora of situations where each species respond with different distribution patterns and diverse population dynamics. For these reasons, this document aims to give a practical overview of the methods used in different European countries to estimate density of wild ungulates

species, pointing out the drawbacks and the advantages of each one. In the last part of this document, we provide guidance to implement the more advantageous methodologies in the more common habitat types. We present some basic instructions for the practical use of camera traps (CTs) to estimate wild ungulate density, which should be adapted to local specificities. Since different CT methods are available, we focus on those most practical, classified as of relative medium effort, and able to generate reliable data over a wide range of situations across Europe: the Random Encounter Model (REM) and Random Encounter and Staying Time (REST), and distance sampling with CTs (CT-DS), which do not require individual recognition. However, this guide should be considered as an open document that will evolve as new practical, reliable approaches, methods and protocols are developed.

2. DATA AND
METHODOLOGIES

2. DATA AND METHODOLOGIES

The guidance is based on research and literature review performed on databases owned by ENETWILD. Additional knowledge on recent literature as well as experiences on the presented population density estimation methods came from own experiences and advice from experts within the ENETWILD consortium and from external ones. We present an evaluation of different methods according to the desirable characteristics for monitoring populations in local management units, practicability, applicability, and accuracy.

2.1 DEFINITION OF WILD UNGULATE POPULATION PARAMETERS

Generally, the plans to manage or to conserve ungulate populations require to know a reliable assessment of the demographic situation and, thus they consider the estimates of population density as the minimal information required (Williams et al. 2002, Morellet et al. 2011). Consequently, wildlife managers must choose between two main options when trying to assess the population dynamics: i) estimate the absolute population size/density; or ii) estimate a relative index of annual variation in population size/density (Morellet et al. 2011). Population density is an absolute value and therefore more informative and comparable among populations, even when values are obtained from different methodologies. Abundance indices are relative, measured in different scales/units and therefore does not inform on population size (but on its variation over the time) and comparisons among population are not feasible. In the last decades, researchers reported several pieces of evidence of bias or imprecision of the methods used by managers, highlighting as the assessment of these estimations is complicated. For instance, it is particularly difficult to estimate the size of the wild boar populations (number of wild boar), because it is an elusive species with a highly variable spatial distribution, most of the usual procedures for determining the size of wild ungulate populations are inapplicable.

Based on the above, this guidance focuses on wild ungulate density estimation, and not relative abundance indices. 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 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 a 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 variations of the size (N) or density (d) of a population but does not directly estimate these parameters. Since relative abundance increases 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 cannot be linear (Figure 1). Sometimes, due to financial, logistical, or time constraints, wild ungulate surveys can only deliver relative abundance, instead of total population size or density estimates.

Population density (d): it is a measurement of estimated population per area unit, i.e., number of individuals divided by total land area surveyed. The density usually is expressed in heads per 100 ha or km^2. It can be calculated by different methods (either direct or indirect, summarized in Table 1).
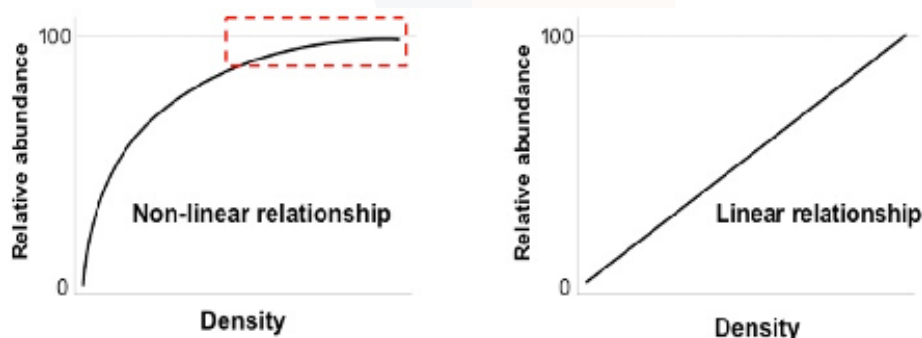
Figure 1. The best indices of relative abundance are those that have a linear relationship with the population density (right) in a given area, but often, these relationships lead to saturations for large abundance values (left). The Y-axes indicate the relative (expressed from 0 to 100) value of relative abundance.

To compare and make use of wild ungulate population estimates, it requires accuracy, precision, and reliability of such population estimates, which must be expressed in the same (comparable) units (density or relative abundance) or scale (e. g. absent, low, medium, high):

The accuracy of estimates of relative abundance or population 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 in this report considers (i) how trustable estimation is when repeated 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, often due to deficient sampling design. For example, when the survey staff is poorly trained, CT 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 of wild ungulates (Table 1). High accuracy of average values (Figure 2, left) allows for comparative purposes along time for a given population and for spatial comparisons among populations.
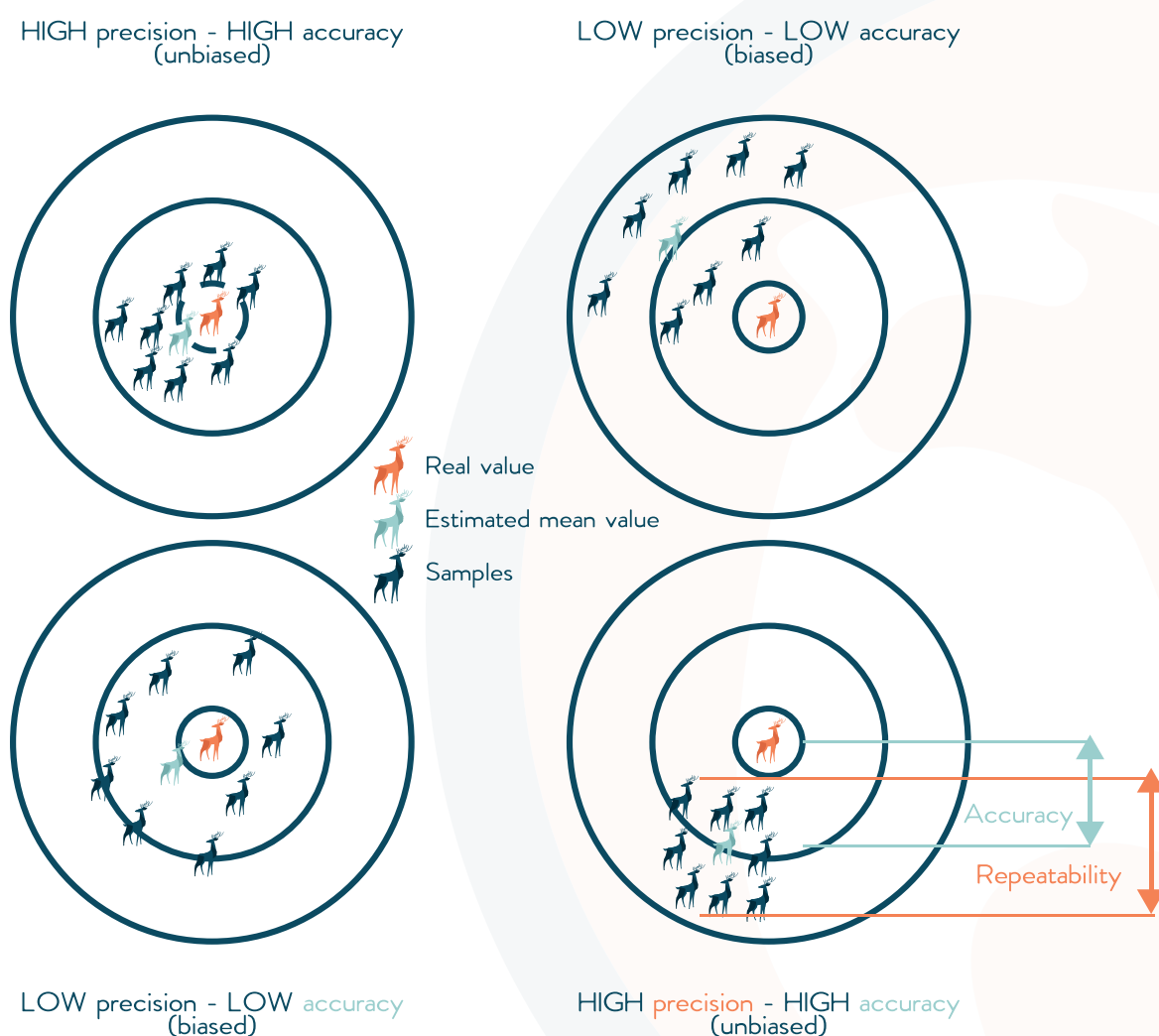

Figure 2. Accuracy, precision, and bias of population estimates. The first situation (top left) can be labelled as reliable.

Sampling is used when calculating population parameters on large areas. The design of the study and the sampling strategy are essential to correctly estimate precise unbiased (and therefore reliable) density estimate 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 as obtained for species with marked habitat selection patterns in heterogeneous landscapes. 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 is 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.

Spatial distribution of several ungulates is clamped and clustered because 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 density estimations. Therefore, we need to have notions of the distribution of the population in the territory to make a good study design.

UNIFORM OR REGULAR DISTRIBUTION

$$S^2 < M$$
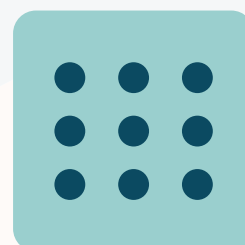
RANDOM DISTRIBUTION

$$S^2 = M$$
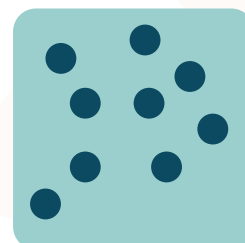
AGGREGATED DISTRIBUTION

$$S^2 > M$$
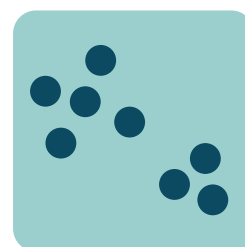
Figure 3 Patterns of distribution of species, which affect the final population size estimation, for which a correct study design is needed. The aggregated distribution represents a contagious spatial distribution, typical of many European wild ungulate species (S2=variance of data, M= mean value).

Since such species tend to be aggregated and not randomly distributed, transects, sampling plots, CT 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 ungulate 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 with a random origin (e.g. a CT every 1.5 km). 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 following randomly chosen sampling units. When possible, stratifying by (relative) abundance can increase the precision because equivalent encounter/trapping rate (e.g. CTs) would be achieved within strata. 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 CT sites must be placed using a fine scale map of the study area and can 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) when design is stratified.

• 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 estimations of 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.
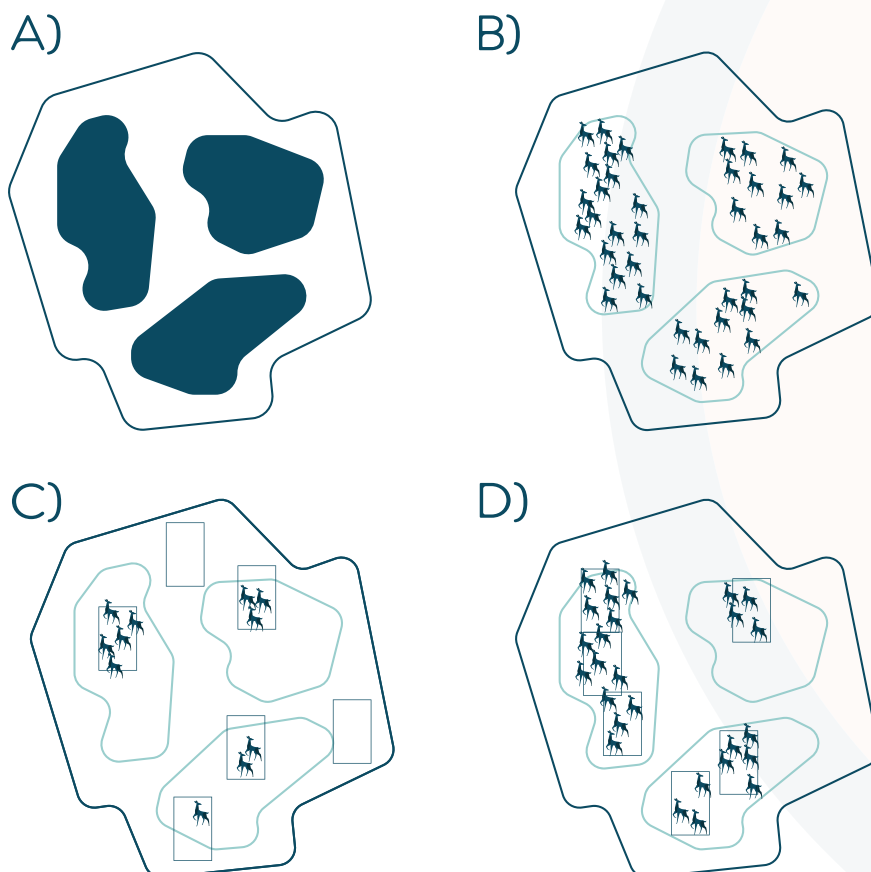
Figure 4. Area of habitat used by the species (A, green areas), animal distribution (B). A random sampling (C), a stratified sampling (D). In this case, a stratified design increases accuracy and precision of density estimation (and therefore total population) once relativized to the sampling area (stratum 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).
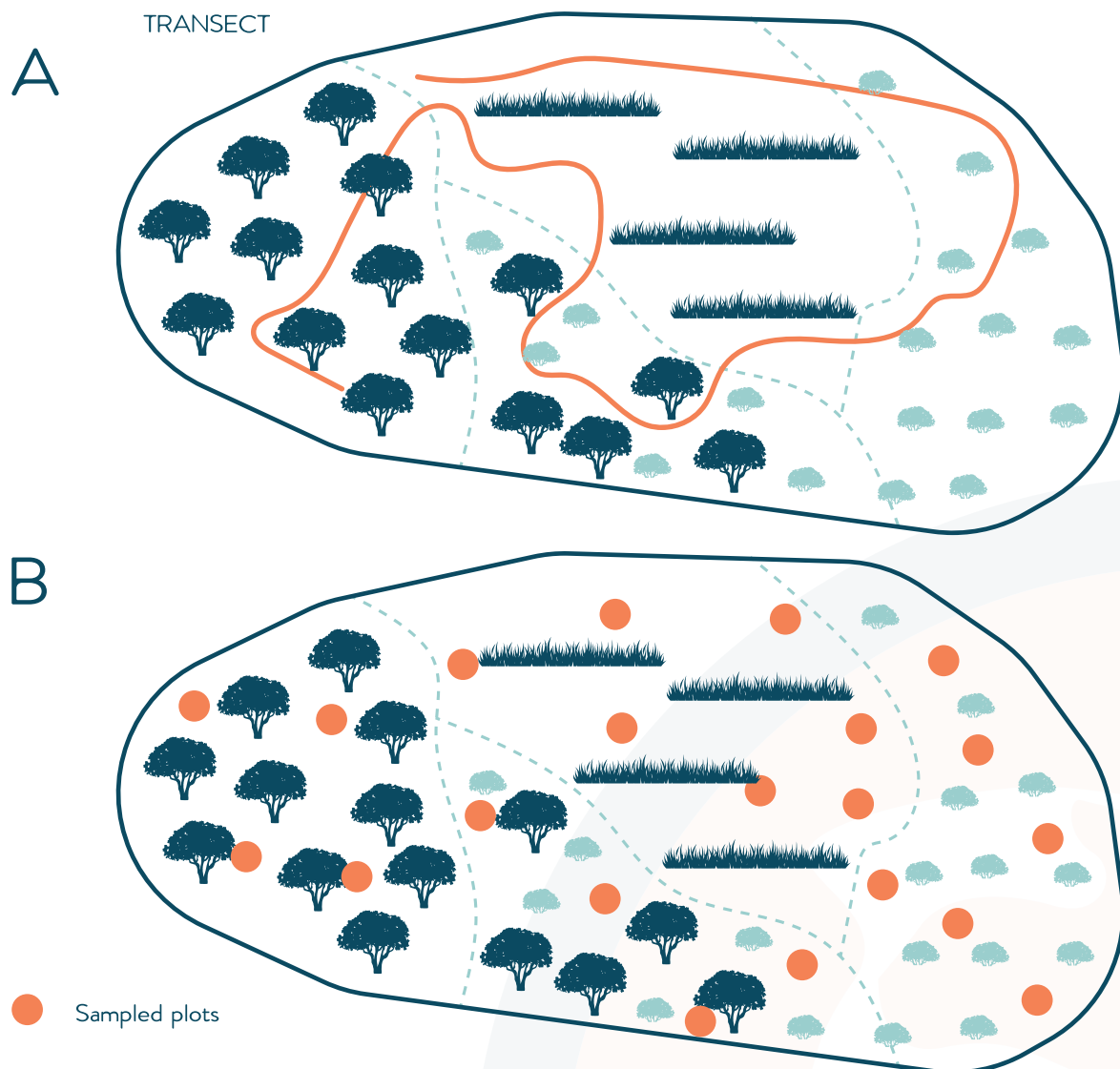
Figure 5. Schematic representation of the designs for a transect (A) or counts within plots (B) (e.g. from hides, beat areas or camera trap placements) considering an habitat stratified approach. As a mixture of both approaches, several short transects, independent and within each stratum can be preferable.

3. AVAILABLE METHODS FOR WILD UNGULATE ESTIMATION

3. AVAILABLE METHODS FOR WILD UNGULATE ESTIMATION

There are 13 native or historical introduced (Suidae: Sus scrofa, Cervidae: red deer *Cervus elaphus,* roe deer *Capreolus capreolus,* fallow deer *Dama dama,* moose *Alces alces*, reindeer *Rangifer tarandus,* Bovidae, Caprinae: Alpine ibex Capra ibex, Iberian wild goat C. *pyrenaica,* wild goat *C. aegagrus;* mouflon Ovis *gmeline musimon,* Northern chamois *Rupicapra rupricapra* and southern chamois *R. pyrenaica,* Bovinae: European bison *Bison bonasus)* and 7 introduced (white-tailed deer *Odocoileus virginianus,* sika deer Cervus *nippon,* Axis deer *Axis axis,* Chinese water deer *Hydropotes inermis,* muntjac *Muntiacus reevesi,* Musk ox *Ovibos moschatus,* Barbary sheep *Ammotragus lervia)* wild ungulate species roaming in Europe. Thus, we would assume, there is also the variability of methods needed to monitor the different species. However, in most cases, the applicability does rely more on the habitat than on the species itself (Table 1). As some species have specific habitat preferences, not all the methods can be performed with all the wild ungulates.

Most monitoring methods have restrictions in species with low densities or, aggregated distributions. Methods based on statistical models (e.g., distance sampling,

REM) perform well for solitary species like roe deer, whilst comprehensive direct counts (block, vantage point, aerial) perform quite well on group living species in open areas, like Alpine ungulates. Due to visibility obviously affecting the detectability, many methods are conductible in forest or bushland only with high effort and manpower (bad performance, bad practicability). The absence of a reflecting *tapetum lucidum* in the case of wild boar, makes nocturnal counts more difficult as compared to cervids. Many methods have been conducted in several European countries for scientific purposes, i.e. for knowing exact numbers in a study area, for testing the methods or to have a calibration for other methods (compare Morellet et al. 2011, Gräber et al. 2015).

Generally, authors proposed a classification of the density estimation methods based in two main groups (Figure 6): direct and indirect methods, i.e., according to if they are based or not on direct observation of animals.
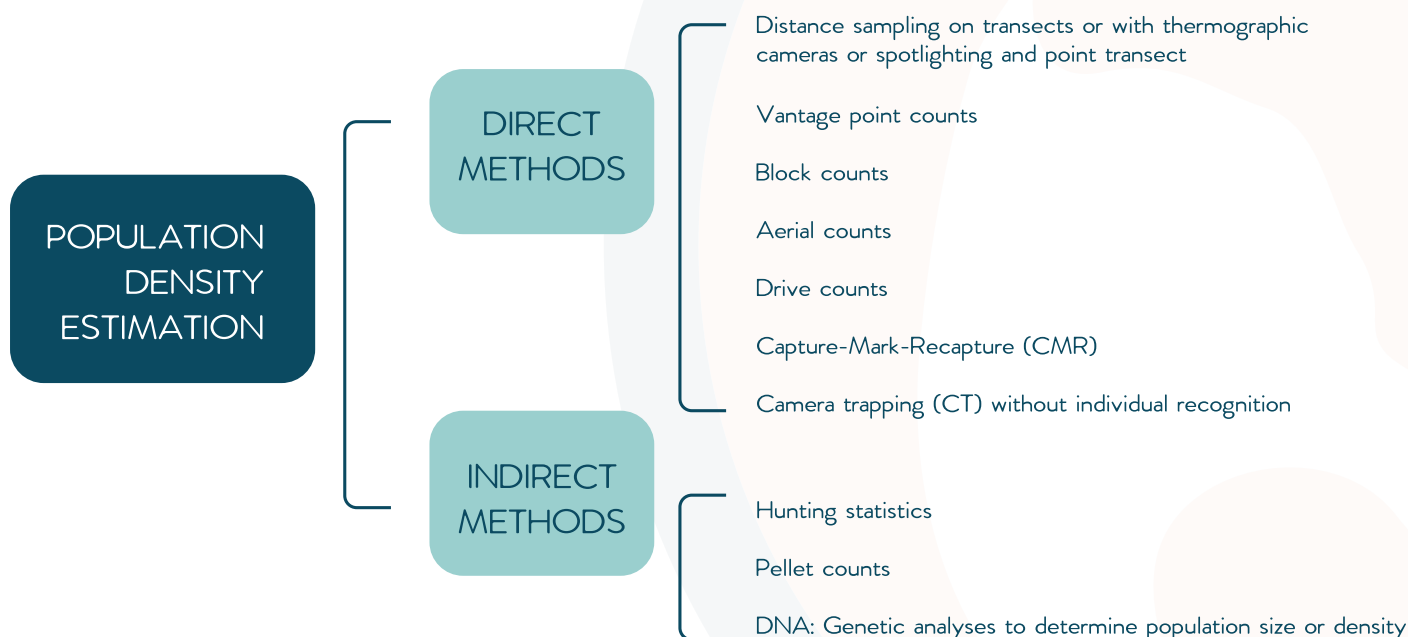
Figure 6. Classification of available methods for estimating wild ungulate population density. Direct methods: methods based on the direct observation of animals, Indirect methods: methods based on the detection of presence signs, see the text for more details.

DIRECT METHODS

3.1. DIRECT METHODS

Since these methods are based on the direct observation of animals, they depend on the daily and seasonality activity rhythm of the species and provide data on the structure (sex and age classes distribution) of the population.
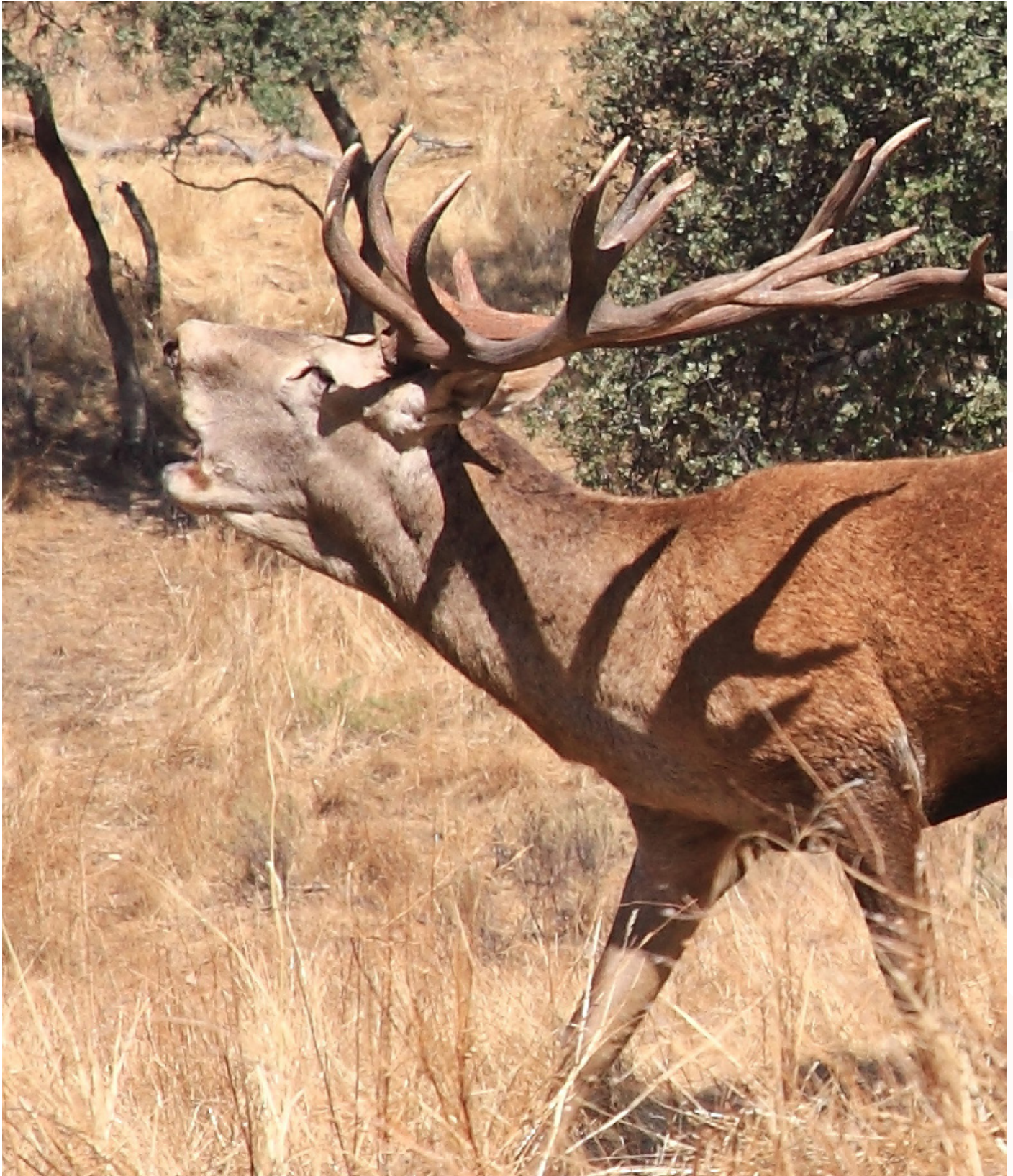
3.1.1. Distance sampling on transects with thermographic cameras or spotlighting, and point transect

Objective

By observing individuals / groups along transects and, if the distance sampling is used to analyse data (see below), recording the distances to which they have made such observations. The objective is to obtain a detectability function with which to estimate the local density of the wild ungulate population.

Measure estimated

Population density and population structure

Applicability

Potentially all ungulates with restrictions due to visibility and/or detectability of species (e.g., wild boar) or habitats (closed).

Methodology

Transect methods include different techniques to detect animals and to estimate population density. The method requires that the observer travelling along a transect records the perpendicular distances (or the sighting distance and sighting angle) of all animals visible from the transect. In other cases, i.e., line transects, there is no assumption that all animals are detected, but the final density estimation uses a detectability function calculated from the distribution of observation distances (i.e., perpendicular distance of the animals from the line of the transect) (Buckland et al. 1993, Buckland et al. 2001, Morellet et al. 2011).

Transects can be carried out on foot, but it may be demanding in terms of sampling effort, and it is difficult to apply at spatial scales of several hundred km² (typical scale of deer management units). Depending on the accessibility of the territory, alternatively, transects may be carried out by using a vehicle to monitor large areas (e.g., Pellerin et al. 2017 - See pictures in this section). The animals can be detected by using point transect also. In this case, the observations of individuals / groups take place from fixed points (e.g., from hide-outs or high seats) and, using distance sampling, by recording the distances from the observer to the animals.

Transect methods can be implemented during the day, in general during crepuscular hours, by using binoculars, or during the nightlight by using spotlights to detect the individuals (e.g., Garel et al. 2010, Corlatti et al. 2016). The presence of a reflecting *tapetum lucidum* increase the probability to detect individuals during nocturnal spotlight counts (by car). The absence of a reflecting *tapetum*

lucidum in wild boar makes nocturnal spotlight counts (by car) more difficult as compared to cervids. Spotlight counts are only useful for deer species but do not really work in wild boar (even as distance sampling) (Gräber et al. 2015). The problem of low visibility of wild boar during spotlight counts was solved successfully in Italy (Focardi et al. 2002, Franzetti et al. 2012) and in GB (Gill & Ferryman 2015) using distance sampling on transects with thermographic cameras (thermographic imaging TI; Buckland et al. 2004). The use of thermal imaging allows the detection of animals by detecting the long-wave energy radiated by warm-bodied. This technique enhances the probability of detecting animals before being perceived, thus reducing disturbance due to the observers (Gill et al. 1997; Ward et al. 2004; La Morgia et al. 2015). Even if the method works well when applying a high effort, in low densities and in habitats with dense understory (Gill et al. 1997, Franzetti et al. 2012, Focardi et al. 2013, Wäber and Dolman 2015), it can fail in other situations (e.g., Gill & Brandt 2010). Evaluation of this method can be seen in Table 1. The price of new-generation infrared cameras is now more attainable and present better performance, which can increase the cost-effectiveness and applicability of this method once it is fine-tuned.

Evaluation

· Pro: large areas observed with low disturbance, very accurate in high densities for local areas, low cost-effectiveness (currently no for thermographic camera).

· Con: need for assumptions using distance sampling, in study sites with low densities, dense understory, and high hunting pressure a very high sampling effort is needed, need for thermographic camera => expensive, mainly in winter.

· Accuracy: high.

· Habitat: open to mixed, less useful in pure forest.

Recommendations to improve comparability and accuracy

• Previous studies/knowledge are needed to prepare the study (transects) design, as well as basic biological information about the animal (activity rhythms).

• In the design of the study, attention should be paid to the fact that the population to be estimated does not have any type of correlation with the transect line in its distribution.

• Sampling design and data analysis must consider the type of habitat.

• The centre line of each transect must be clearly marked: the observer can determine their position at each moment.

• A minimum number of transects must be sampled depending on the distribution of the target species in the study area.

• Using distance sampling, all animals in the centre of the transect line are viewed with probability equal to one.

• The width of the transect should be the maximum possible. The points that are considered external must be eliminated during the analysis.

• Three basic measurements: perpendicular distance, observation distance and observation angle.

• All the measurements of angles and distances must be accurate.

• The measurements must be saved separately for each segment of the total length of the transect.

• The data collection must be carried out by competent, motivated, and trained staff.

• Point transects requires areas of good visibility

• At least 40 objects (individuals or groups), preferably 60-80 objects, should be recorded.

[2] See definition at section 2.1

3.1.2. Vantage point counts

Objective

By observing individuals / groups from a vantage point (e.g., pick of hill / mountain, high seat, random point) it is possible to estimate local density by using both total counts and counts in sample areas.

Measure estimated

Population density and population structure.

Applicability

Potentially most wild ruminant.

Methodology

In all cases, the assumption is that all animals present in an area are detected by the observers and that one individual can be observed only by one observer. The second part of the assumption can be partially relaxed if the observers take note of the direction and time of animals moving out from their view. After the data recording, the manager must exclude the potential double-counting to assess the density. A variant of such methodology is to attract the individuals of the target species by using artificial feed and/or salt licks (Morellet et al. 2011).

The main drawback of this method is that sampling error may be large and difficult to assess. For this reason, this method may be unfit to comparing the ruminant densities of different areas. By using random point method and distance sampling (see 3.1.1) to assess the covered area, the managers can also estimate the coefficient of variation (CV) and provide relative density, to our knowledge, there was just one attempt using this method for wild boar (Passon et al. 2012, Keuling et al. 2014).

Evaluation

· Pro: low costs in relation to the efforts.

· Con: risk of underestimation in dense habitats, and difficulty to delimit the area in which counted animals are referred to.

· Accuracy: potentially high in open areas, limited in dense environments.

· Habitat: open (esp. hilly and mountainous, marshes, artic), possible in mixed landscapes, less useful in large forest areas (high effort, only as distance sampling).

Christian Gortazar

3.1.3. Block counts

Objective

By observing individuals/groups present in a sector (block) moving within it.

Measure estimated

Population density and population structure.

Applicability

Ruminants, mostly in Alpine environment.

Methodology

The procedures to implement this method are like vantage point counts, but the observer moves within a sector to detect the greatest possible number of individuals, obviously avoiding double-counting. This method should be implemented only in open areas where operators can be sure avoiding double-counting. Indeed, it is often used to estimate wild ruminants in Alpine environment (e.g., Alpine chamois, Alpine ibex; Corlatti et al. 2015, Jacobson et al. 2004).

The implementation of block counts needs a relatively large number of operators to simultaneously collect data in neighbouring sectors. Often the operator must have the skills to use proper optical instruments and to recognize sex-age classes from afar. The wildlife managers should be aware that by using block counts they can obtain the minimum number of alive individuals in the sample area and not absolute population size. Like vantage point counts, also for block counts is hard to assess the sampling error, which can be large due to occasional conditions (e.g., unfavourable weather). However, for instance in chamois block counts yields a minimum population size underestimated in respect with the population size estimated by using mark-resight (Corlatti et al. 2015), even if the value estimated by using block counts falls in the range of confidence values range of the mark-resight R one.

Evaluation

· Pro: low costs in relation to the efforts.
· Con: risk of underestimation.
· Accuracy: medium to high: possible underestimation, accuracy potentially affected by environmental conditions, especially broken environments.
· Habitat: open (esp. hilly and mountainous), possible in mixed landscapes, not applicable in pure forest landscapes.

3.1.4. Aerial counts

Objective

By observing individuals / groups from aerial devices, it is possible to estimate local density following a total count or applying distance sampling methodology (recording the distances to which they have made such observations).

Measure estimated

Population density.

Applicability

Ungulates in relatively open areas.

Methodology

Aerial counting is not a single method. Aerial counting is used as a tool within other methods (like direct counts, strip and line transect, distance sampling, CMR). There are several possibilities conducting aerial counts: helicopters, gyrocopters, small planes, microlight aircrafts, drones, blimp. However, in many countries these are only allowed to fly by daylight.

For counting hoofed game, aerial counts may be used as direct counts or as distance sampling. A well working setup is using a microlight with thermographic imaging (TI, for detection) and parallel video (for distinguishing species) (Franke et al. 2012, Gräber et al. 2015). These are good methods for inaccessible areas, and they work well in swamps and reeds (Franke pers. comm.). In open areas, the noisy vehicles may cause the flight of animals resulting in underestimated and biased estimation of the population parameters.

This method may give a fast overview on population size and distribution (e.g., conducted with drones). However, the researcher opinions about precision and accuracy of aerial surveys are conflicting, likely due to the difficulty of obtaining the correct number of individuals in each area (e.g., Jachmann 2002, Ronnegard et al. 2008). Unmanned aircraft systems are promising alternatives in studies that require the estimation of spatial patterns of different species in large areas –like epidemiological ones- where traditional methods (e.g., line transects, drive counts, etc.) are not feasible due to logistical reasons (Barasona et al. 2014).

Especially on large areas in more open habitats it can be the only way to get reliable census data in a short time: e.g., seals on the shore, reindeer, and moose in northern Scandinavia).

Evaluation

· Pro: working quite well for wild ruminant in open area, larger areas might be achieved during one count. It is possible determining spatial distribution patterns (e.g., at the wildlife/livestock interface).

· Con: high costs, with colour cameras, and in many countries, only by daylight that results in low detectability crepuscular or nocturnal wild ruminant during daytime (hidden under trees), need of thermographic cameras, mainly in winter, weather and habitat structure dependent, evaluation comparatively costly. High expertise required (e.g., drones)

· Accuracy: limited – high, in relation to the environmental conditions.

· Habitat: very good for large open landscapes (Tundra, swamps, reed, moors), possible in mixed landscapes in winter, least accuracy in forest areas.

Recommendations to improve comparability and accuracy: Currently, that the use of thermographic and night vision cameras associated to aerial counts is still to be developed, there is the need to calibrate this technology for wild ungulates.
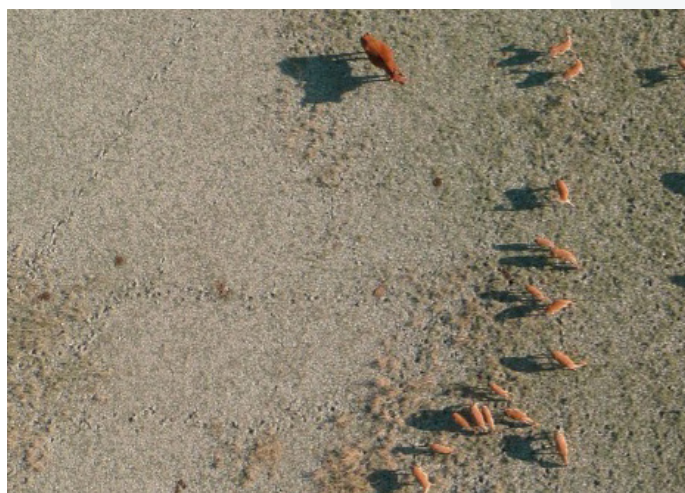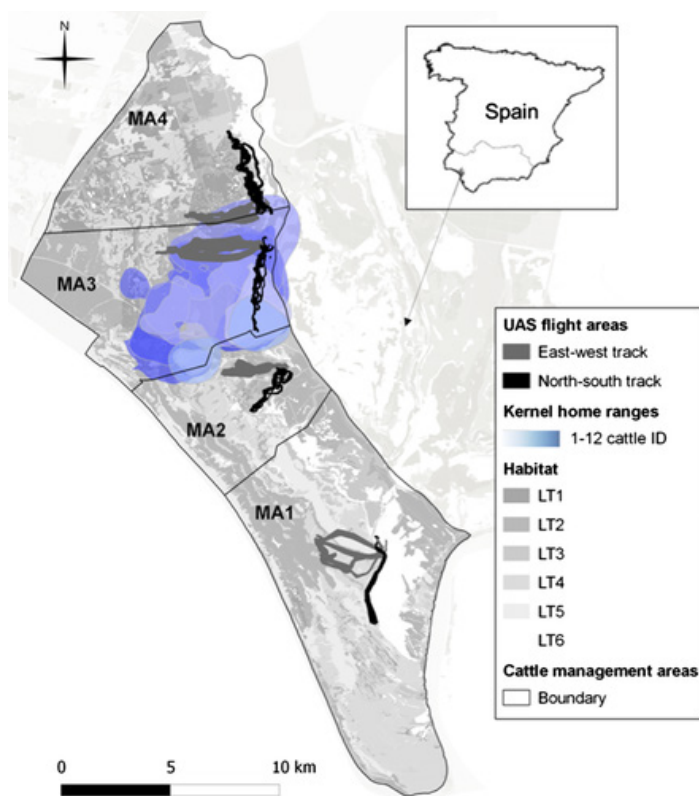
3.1.5. Drive counts

Objective

Move and count all the animals of a known surface area to estimate local density.

Measure estimated

Population density and, in some species, population structure.

Applicability

Potentially all ungulates.

Methodology

Drive counts are frequently used to estimate population densities in ungulates inhabiting forested areas (Dzieciolowski 1976, Borkowski et al. 2011). Drive counts may be conducted as drives with beaters (with or without dogs, ENETWILD consortium et al. 2019). All beaters and fixed position observers on the border of the drive do record all animals seen during the drives. This may also be conducted during drive hunts (usually driven with beaters and dogs), where the hunters in hides record all wild ungulates seen. Hunters can be used as experienced observers and therefore the hunting activity, if carried out by instructed and motivated personnel, can be a cost-effective alternative to monitor ungulates (Mysterud et al. 2007). The method is still widely used in several countries (e.g., Italy, Poland, Portugal, Switzerland) even if some authors recognised that it suffers of an increasing rate of underestimation with the increasing of population density (Maillard et al. 2010, Morellet et al. 2011). Drive counts by hunters is a method currently used to monitor wild boar population in Spain, the Czech Republic, and some parts of Poland (Plhal et al. 2010, Borkowski et al. 2011, Segura et al. 2014).

Dependent on several conditions, many wild ungulates may be missed. Thus, this method has a high effort with low reliability (but compare Borkowski et al. 2011) and it is very difficult to estimate the precision and accuracy. However, In Italy, the accuracy of the method has been tested using capture–mark–recapture techniques (radio-collared deer), and the average underestimate is estimated to be 20–25% of the actual population (Apollonio, pers. comm.).

Evaluation

· Pro: experienced volunteers, easy to conduct, also possible within regular hunting activities.
· Con: high personnel effort, dependent on experience and number of helpers (sometimes also dogs needed), dependent on population density and experience of animals, during hunt biased by willingness of hunters, mainly to be conducted during winter/hunting season. Need to assess double counts.
· Accuracy: highly dependent from context and experience of volunteers.
· Habitat: well applicable in forest and mixed landscapes, possible in open landscapes (but other methods perform better).

Christian Gortazar

Christian Gortazar

3.1.6. 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.

Measure estimated

Population density.

Applicability

All ungulates.

Methodology

CMR methods include a large family of methodologies reviewed in Buckland et al. (2000). This method needs trapping and marking, or at least, individually identifiable animals through CT (e.g., by means of antlers/horn shape, spotted fur) (e.g., CT based spatial Capture-Recapture, SCR) or other means (e.g. DNA analysis). 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.

CMR was conducted with recapturing for wild boar (Focardi et al. 2008), by hunting bags (Toïgo et al. 2008) or with re-sightings by CTs (marking or individually identifiable animals are needed; e.g., Sweitzer et al. 2000, Hanson et al. 2008, Hebeisen et al. 2008, Plhal et al. 2011; Morimando et al. 2016; Ünala and Çulhacı 2018).

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. 2014, Jiménez et al. 2017). For management purposes, traditional CMR is not suitable, as the release of animals instead of culling will be counterproductive to the management aims of population reduction.

Evaluation

· Pro: moderate to expensive costs, very precise and accurate if assumptions are respected, rarely used outside scientific investigations.
· Con: high effort, need for assumptions.
· Accuracy: : high.
· Habitat: all, depends on capturing possibilities, depending more on species then on habitats.

Recommendations to improve comparability and accuracy:
• It is necessary to mark/recognize a significant proportion of the population (not in SCR, Jiménez et al. 2017). Although it is very precise and accurate, it is expensive.

• For indirect CMR see "Genetic analyses of pellets (genetic CMR)". See also "Camera trapping without individual recognition (CT) – SCR".

3.1.7. Camera trapping (CT) without individual recognition

Beyond CMR, several models to estimate the size of a population where animals are unmarked has recently been published. Observation of animals with CTs was recently identified as a promising method to estimate a relative abundance (Rovero & Marshall 2009) and population density (which we will focus on) (Rowcliffe et al. 2008). Recently, promising results were obtained applying CT to estimate wild ungulate population density without the need for individual recognition (Marcon et al. 2019a, Pfeffer et al. 2018). Some different methods of analysing photo-data and calculating densities/abundances have been recently tested in several species. Assumptions for cam-trapping methods (deviation written below at specific method): random placement of cam-traps and/or probability of contacting any individual of the population, closed population, no influence of cam-trap on behaviour, cameras settings must be known (e.g., angle of detection, effective range). Evaluation of these methods can be seen in Table 1.

3.1.7.1.1. 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), including wild ungulates (Rovero & Marshall 2009, Zero et al. 2013, Pfeffer et al. 2018, Marcon et al. 2019b). This method has been successfully tested in wild boar (Eversmann 2014, Keuling et al. 2014, Massei et al. 2017, Palencia et al. 2021a) and other wild ungulates. This method rescales the trapping rate (y / t) to population density using the day range (DR, i.e., daily distance travelled by an individual), and camera-related parameters (radius and angle of camera detection).

$$D \text{ (density)} = \frac{y}{t} \cdot \frac{\pi}{v \cdot r \cdot (2+\alpha)}$$

Where ∝ is the angle and r the radius of detection of the cameras, v = DR, i.e. the daily range of displacement.

The model assumes the following:

• CTs can capture animals in any direction, and animal signals are detectable from any direction
• The model assumes that animals randomly move with respect to CTs. The animals are in a homogeneous environment and move in straight lines of random direction with velocity
• CTs can capture animals at a detection distancer and that if an animal moves within this detection zone, they are captured with a probability of one.

We recommend estimating all the needed parameters to apply the REM for each sampled population. Especially considering the lack of comparative studies that have tested these parameters in different species and environmental conditions.

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 (see our recommendations below). However, comparative studies to finely describe the distances travelled among different regions, habitats and seasons are required to evaluate the potential practicability of REM. A recent study has evidenced to classify behaviours observed with the cameras into two or more categories (feeding, i.e., exploiting resources; and moving between habitats; i.e. searching resources) in order to estimate reliable and more accurate DR estimates (Palencia et al. 2019; 2021b) for species that behave differently (moving or feeding) in front of the CTs. This approach has been successfully applied in a range of wild ungulates: e.g., red deer, fallow deer, roe deer, wild boar, chamois (e.g. Kavčić et al. 2021; Palencia et al. 2021a).

Considering a scenario of high variance in trapping rate, a practical limitation of REM is that a minimum of 60 CT placements should be sampled to obtain a CV lower than 20%. Density precision heavily depends on the variance in trapping rate. Increasing the sampling effort or to considering a stratified design could improve the overall variance.

3.1.7.1.2. Random encounter rate and staying time (REST)

REST is an extension of REM (Nakashima et al. 2018). The REST describes the relationship among population density, trapping rate and staying time (amount of time that detected animals remain within a specific area with probability of detection of 1 within the field of view of a CT) of animals in a predetermined detection zone. This allows a full likelihood approach and probably a good coverage of confidence limits (not available in REM). To estimate the detection zone, it is necessary to do a pilot study to estimate the area in which the probability of detect an individual of the target species (and with a specific cam-trap model) is 1 (see our recommendations below).

The REST have been proposed as more efficiency and feasibility than REM because DR it is not needed. Despite the REST has been published recently, it has been applied to some ungulate populations (Nakashima et al. 2020; Palencia et al. 2021a).

3.1.7.1.3. Point transects using camera traps (CT-DS)

Recently, Howe et al. (2017) adapted standard point transect distance sampling methods to CTs (CT-DS). This method can have great potential in low-density population because the continuous monitoring of the cameras allows to record more than one distance of detection for each detected animal enlarging the sampling size and optimizing the sampling effort. Distances from animal to camera must be measured, and this approach has been validated against other methods for ungulates (Palencia et al. 2021a).

Like REM, the CT-DS precision is usually low because of the high variance in encounter rate among cameras. Sampling more locations or stratifying the density estimates are useful to improve the precision.

3.1.7.1.4. CT without individual recognition in general

Objective:

Converting tap rates into estimates of the size of a population (local density), with or without, the need for individual identification.

Measure estimated:

Density.

Applicability:

All ungulates.

Methodology:

See protocol (Annex)

Evaluation

(REM, REST, CT-DS):

· Pro: (CT in general): different analysis-methods adaptable to local conditions (REM, REST and CT-DS recommended), medium effort, moderate costs, high precision and accuracy (also in low densities), adaptable to local and perhaps to regional studies (if the study design is stratified), all year, little delay, practicable, becoming more affordable, conductible (for several species at the same time), photos might be collected via online-photo-databases, all species in one method. Possibiility to incorporate artificial intelligence to image processing, which reduces workload.

· Con: assumptions needed for CT, theft of cameras in some locations, manpower required to analyse photo/video material, however automatic identification is progressing fast.

· Accuracy: high.

· Habitat: All. Performs well in forest areas, where other methods are not possible! Higher loss of cameras in open habitats.

Recommendations to improve comparability and accuracy:

• Further studies are required to define an adequate camera-trapping protocol to monitor different wild ungulate populations- for which precise data on movement parameters, activities, and other requirements in a range of different situations in the European context need to be gathered. Protocols for REM and REST already evaluated and available (see below).

• 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 (see Palencia et al. 2021b)

Example 1.
Camera delay is set to 0 sec taking three consecutive photos with each trigger (60° FOV, 30° L, 30° R)

First sequence – the same individual

Second sequence – the same individual

Third sequence – the same individual

…and fourth sequence – the individual behind
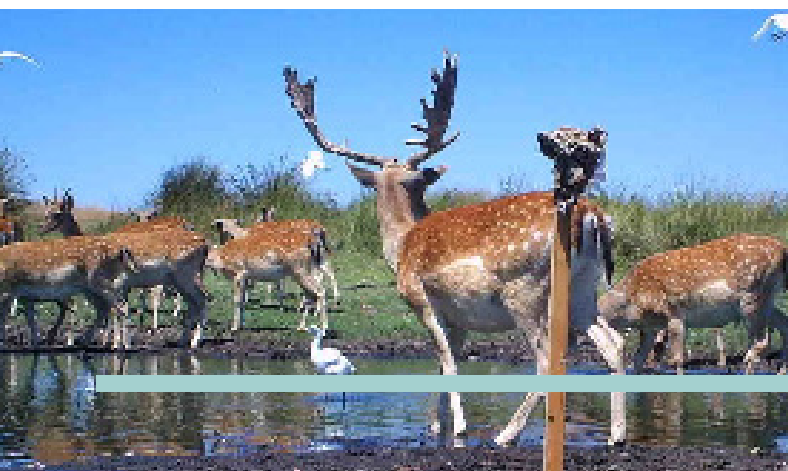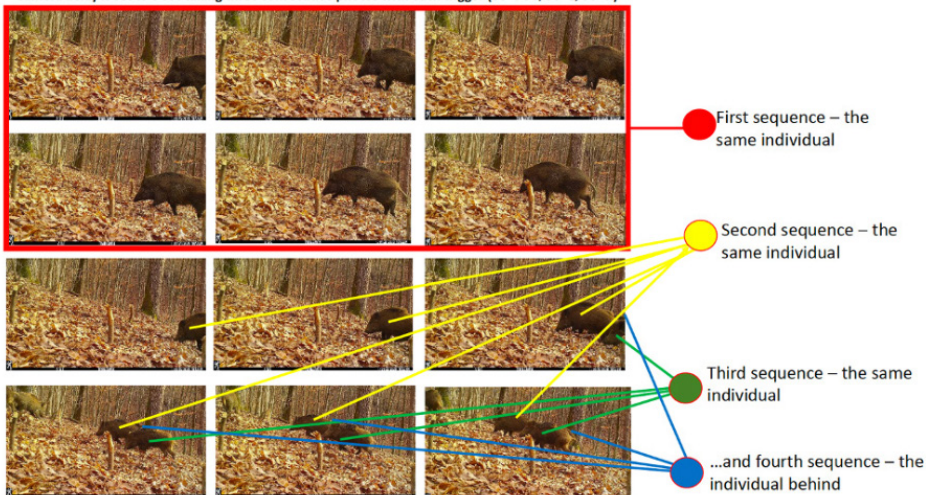
INDIRECT METHODS

3.2. INDIRECT METHODS

These methods are based on the detection of signs of presence, but not on living animals.

3.2.1 Pellet counts

Objective

Record the number / frequency of faecal pellet group per unit of effort to calculate local density.

Measure estimated

Local density.

Applicability

All ungulates.

Methodology

Pellet counts are frequently used to monitor wildlife species. In fact, there are lots of different approaches towards the estimation of ungulate abundance based on the counting of faecal pellet groups along transects or within plots. Two main groups of approach may be distinguished based on the cleaning or not of the investigate area before the counting of the faecal pellets (Putman, 1984). The faecal standing crop (FSC) is implemented by counting the number of accumulated pellet groups within randomly distributed sample quadrats, or along fixed transect lines without cleaning the study area. In this case, to estimate the population size, it needs to know the defecation rate (the increase of faecal pellet every day per each animal) and faecal decay rate (how many faecal groups disappeared).

Conversely, faecal accumulation rate (FAR) removes the source error intrinsic in the estimation of the decay rate. Initially the operators must clean the sample areas of all ungulate faeces and then re-examining the same areas after a fixed time to determine the number of pellet groups accumulated during this interval. The precision of the density estimation can be calculated by the dataset, whereas the accuracy of these different techniques has been estimated several times in several species obtaining variable conclusions. The precision of both FAR and FSC declines with declining pellet group density (Campbell et 2004).

One of the more important sources of variability is the defecation rates which are not uniform in space and in the time. Variations of defecation and decay rates, which can be very local, need to be assessed to make results comparable and able to be converted into estimates of local population density. This prevents this method to be used to estimate population density, but indices of abundance. A huge effort is required to locally estimate defecation and decay rate. Some approaches are used for wild boar (Massei et al. 1998, Ferretti et al. 2014, Plhal et al. 2014, Ferretti et al. 2016).

A proxy of the population aggregation can also be estimated from this method by statistically analysing the dispersion of faeces along the transects (Acevedo et al. 2007). Population abundance and aggregation are two key parameters for epidemiology. Therefore, this method is widely applied in epidemiological studies. Nonetheless, variations of defecation rate and dung persistence rate, which can be very local, need to be assessed to make results comparable and able to be converted into estimates of local wild boar population density. Evaluation of this method can be seen in Table 1.

Evaluation

· Appropriateness to estimate: Local density
· Pro: low costs/efforts.
· Con: defecation and decay rates should be known on a local basis and referred to a specific season for every study area.
· Accuracy: species, habitat and locally related.
· Habitat: all, performs well in forest and mixed landscapes.

Recommendations to improve comparability and accuracy:

• To estimate density, calculate local defecation rate and dung persistence rate for that population during season and for the year when count is to be performed.

• Design must be adapted to aggregation of faecal pellets distribution.

3.2.2 DNA: Genetic analyses to determine population size or density

Objective

Determines the effective population size by genetics (individuals or pellets).

Measure estimated

Effective population size (Ne).

Applicability

All ungulates.

Methodology

Several conceptually different types of Ne can be distinguished, but the most used is the number of individuals in a population who contribute offspring to the next generation (Ridley 2004). The estimates of Ne tend to provide a lower number than an actual population size.

Determining the Ne (local) by genetics is possible if appropriate sampling and corrections based on population dynamics parameters are applied. Nonetheless, this approach is time demanding and relatively expensive (Luikart et al. 2010).

Nowadays, this method is also applied associated to SCR methods.

Genotyping of faecal pellets has been used to assess population parameters of wild ungulates (e.g., Blåhed et al. 2019; Ebert et al. 2009, Ebert et al. 2010; Ebert et al. 2012a 2012b; Goode et al. 2014; Morden et al. 2011; Poole et al. 2011). Genetic analyses could also be done from hair traps, which has been used for instance in roe deer (Fickel et al. 2012), but they do not always work well (e.g., Ebert et al. 2010). Pellets can be collected and genetically analysed for individual genotyping, providing an indirect way to count and identify individuals in each population (Broquet et al. 2007).

Although costs for DNA analyses have been decreasing in the last years, analyses of multiple samples (as requested to apply mark-recapture approaches) are time-consuming and expensive, and therefore, this method not commonly used in ungulates.
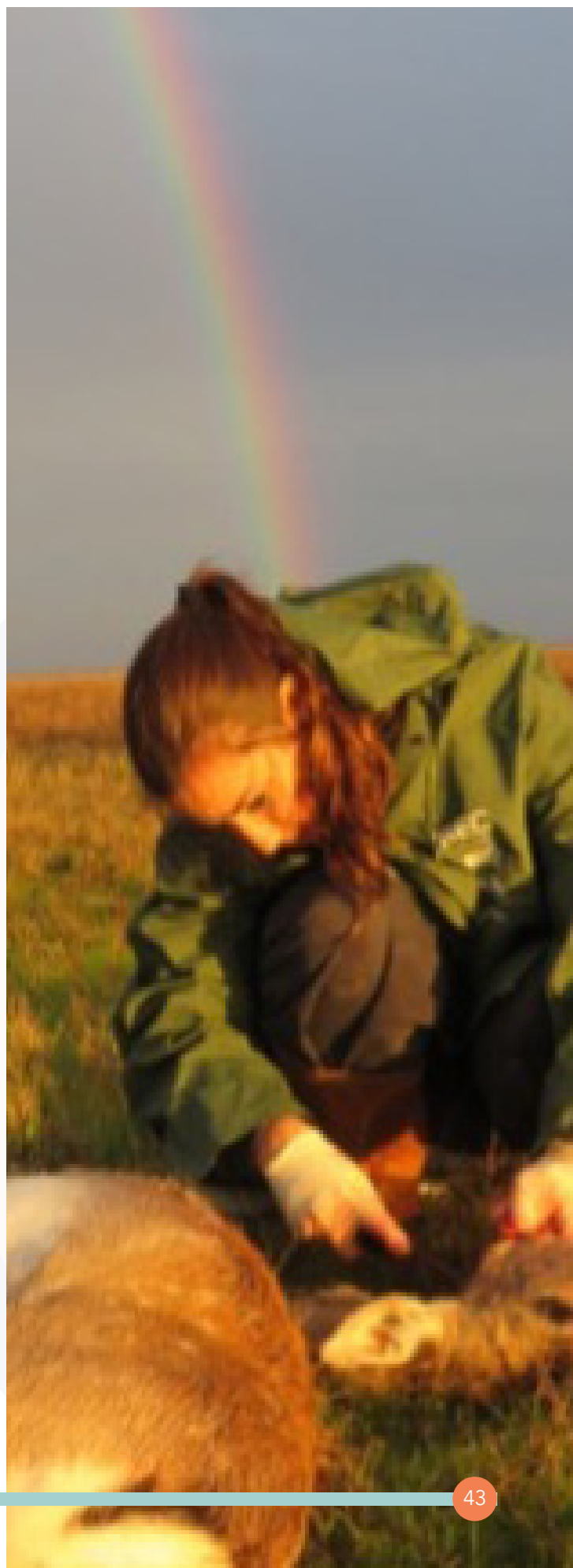
Evaluation

· Pro: high accuracy.
· Con: 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.
· Accuracy: high.
· 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) .
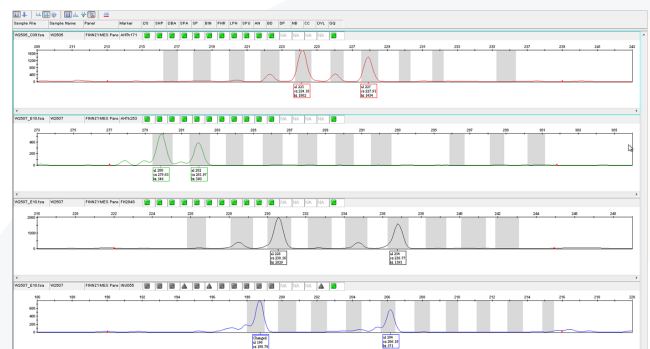
4. SUMMARY OF DESIRABLE CHARACTERISTICS OF METHODS TO ESTIMATE WILD UNGULATE DENSITY

4.SUMMARY OF DESIRABLE CHARACTERISTICS OF METHODS TO ESTIMATE WILD UNGULATE DENSITY

The Table 1 summarizes the classification of different methods to estimate of wild ruminants based on desirable characteristics for monitoring populations in local management units, practicability, and applicability in epidemiological aspects.

Table 1. Classification of different methods to estimate density of wild ungulate based on desirable characteristics for monitoring populations in local management units, practicability, and applicability in epidemiological aspects.

A=Abundance, D=density; y = yes, ~ = restricted, n = no, (…) = with restrictions, [~] = restricted possible, but…;
For accuracy + precisión 3=high accuracy or precision, 2=moderate accuracy or precision, 1=low accuracy or precision;

For reliability 3=Reliable and valid, 2=Reliable and valid for monitoring along time and space as relative abundance when precision or accuracy is low, 1= Not reliable, maybe valid for monitoring and used as relative abundance for a given population; e.g. 1/3 = Low accuracy and high precision.

Method	Animal	Density/ abundance	Habitat	Temporal trend	Info on population structure (Y/N)	Season	Costs	Effort	Ease of learning	Accuracy and precision	Reliability	Useful at local scale (mana-gament units)	Applicable at large scales	Useful at very low density	Useful at very high density
Drive counts	All ungulates	A (D)	forest/ bush	seasonal	n	winter	low	median	easy	2/3 3/1	1	y	~	n	y
Drive counts during hunt	All ungulates	A (D)	forest/ bush	seasonal	n	winter	low	median	easy	3/1	1	y	~	n	y
Distance sampling tracks	All ungulates	D	open, mountain, (forest)	seasonal	(y)	winter (with restrictions all year possible)	median	high	median	2/3	3	y	~	n	y
Distance sam-pling hides	All ungulates	D	open, mountain, (forest)	seasonal	(y)	all year	low	median	easy	3/3	3	y	~	n	y
Vantage point counts	Most wild ruminants	A (D)	open, mountain, (forest)	seasonal	(y)	all year	low	median	easy	2/2	3	y	~	n	y
Aerial counts	All ungulates	D	open, mountain	annual (seasonal)	(y)	winter	high	high	difficult	(2/3)	(3)	(y)	y	n	y
Camera trapping (REM, REST, CT-DS)	All ungulates	D	all	short	y	all year	median	median	easy	3/3	3	y	y	y	y
Block count	Wild ruminants	D	open, mountain	seasonal	(y)	winter (with restrictions all year possible)	median	median	easy	1/2	2	y	(y)	~	y
CMR	All ungulates	D	all	seasonal/ annual	(y)	~winter	median	high	median	3/3	3	y	~	y	~
DNA	All ungulates	D	all	seasonal/ annual	(y) only sex	winter (with restrictions all year possible)	high	high	difficult	3/3	3	(y)	(y)	y	y
Pellet counts	All ungulates	A (D)	all	seasonal	n	winter	low	low	easy	2/3	1	y	~	~	~

5. DISCUSSION AND
RECOMMENDATIONS

5. DISCUSSION AND RECOMMENDATIONS

In this practical guidance, we attempt to propose general guidelines which, starting from an environmental perspective and considering the necessary efforts to collect robust information, drive to select the best methodology. In fact, the amount, skills, motivation, and willingness of the operators play a pivotal role in determining the best method to be selected to obtain population data in the different environments. As new methods which require expertise arise (e.g., CT and subsequent statistical analysis), practical training of local staff (scientific and technical) has become a requirement, especially in areas where population data is not available (e.g., Eastern Europe).

The direct methods have the potential advantage to estimate local wild ungulates density and population structure (e.g., sex ratio, recruitment, distribution among age classes). Obviously, direct methods work well where the animal detectability is high, even if some direct methods (e.g., drive counts) can successfully be used also in areas with scarce visibility. After evaluating the pros, cons, and accuracy of these methods, we proposed three methods that may provide comparable local density data across the distribution range of wild ungulates:

• Distance sampling (with thermography for wild boar and forestall environments)

• Camera trap (REM, REST and DS-CT approach, other methods recently developed are promising)
• Drive counts

The three above mentioned methods have the potential to be used for validation among each other, with other direct or indirect methods, and they are characterised by good accuracy and reliability, moderate costs, are adaptable to local conditions (Table 1). In the case of drive counts performed during hunting activities, have the potential to be used also for regional (large scale) studies if proper hunting statistics collection is implemented (but this requires further calibration of the method).

Detailed information on the general protocols to be implemented for these three methods is provided in Annex 1.

· Camera traps (CTs), with a REM or REST approach, provide key advantages to count also elusive and nocturnal species. The biggest advantages of this method are its flexibility and the relatively low operative costs, but the high starting costs should be considered. Since different CT methods are available, we will focus on those most practical and tested, previously classified as characterized by relative medium effort, and able to generate reliable data over a wide range of situations across Europe.

   - REM or REST approach is a method which may be used in broad environmental conditions (almost everywhere) and for all ungulate species (REM and REST are well performing on all medium to large sized mammal species). Consequently, this method is usable across most of the distribution range of wild ungulates in Europe. When applied following a robust study design, it provides unbiased estimates of density, which are useful for spatio-temporal comparisons.

   - REM, and its extensions such as REST, does not require individual recognition, whereas Spatial mark-resight models (SMR) works with a part of population (individually or not) recognizable. We do not exclude the use of other 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. New CT methods without individual recognition are arising, and this guide will be continuously updated in the future

- Recently, REM and REST has demonstrated to be versatile enough to be successfully applied even in open habitats for gregarious species (e.g. Palencia et al. 2021a), although the loss of CTs in these habitats may be an issue. Considering the distribution of most ungulate species and populations across Europe, mainly associated to forestall habitats, the potential of CT to monitor population abundance is enormous, which should be complemented with methods performing well at affordable effort in open and alpine habitats for gregarious species (e.g., direct counts).

We present some basic instructions (Annex 1) for the practical use of CTs to estimate wild ungulate density, which should be adapted to local specificities.

· Drive counts performed during hunting activities, have the potential to be used also for regional (large scale) studies if proper hunting statistics collection is implemented. This requires further calibration of the method. The quality of the results obtained by using drive counts is recognised to be affected by some ecological factors, mainly the density of the target species. However, this method can be useful in dense forest (ENETWILD Consortium et al. 2019), particularly where it is already implemented, and a long-term dataset is available.

· Distance sampling is an accurate and established method for determining density of animal populations. Abundances on a local scale could even be estimated. Frequency and distance to observer (as well as group size) are used for calculating densities and abundances. This method is quite simple to conduct. However, some assumptions and requirements are needed and are described in Table 1 and Annex 1.

Density data obtained by these reliable methods has the potential to feed into the spatial modelling approach, but most important, they can be used to validate model outputs built on relative abundance or occurrence data.

The use of methods involving the direct detection of ruminants (vantage points, linear transects, block counts, random points) may provide accurate results in open and mountainous areas. These methods are affected by several environmental conditions (e.g., weather conditions) which, in turn, can affect animal behaviour and thus their detectability. For these reasons, we suggest repeating some sections of observation in the same areas using the same approach to have an estimation of the error.

Indirect methods providing density estimates (e.g. pellet counts) may be unbiased and even accurate for local populations where certain specific parameters are calculated (e.g., defecation rate for methods based on pellet counts) during a given timeframe (e.g., season). Therefore, validated available data may be usable as density. However, the required effort to calculate local specific parameters (which are not exportable to other locations/populations/seasons) is usually high, which makes the use of indirect unpractical for conducting them in large-scale areas. For that reason, we do not consider these as methods to promote the generation of new comparable data at broad scale, but if correctly used, they provide valid local densities.

· Hunting statistics should be standardized across Europe for comparisons (ENETWILD consortium 2018a, 2019). In so doing, recalculations (e.g., cohort analysis) would be applicable in ungulate species. Indeed, cohort analyses are possible as the reproduction is relatively stable over the years and can be used in the same way every year, unlike in wild boar, where reproduction is strongly fluctuating. Cohort analyses may be used for local management strategies for all species (if hunting bags are available), if calibrated with other methods. As commented before, not to forget is that drive counts can be performed during drive hunts (ENETWILD consortium 2018a). Nevertheless, for long term management and for monitoring purposes as well as for epidemiological models, where abundances on a large scale are needed, more reliable monitoring methods are necessary, at least to calibrate cohort analyses and hunting data as a basis for abundance models.

Based on ENETWILD initiative, we recommend developing a permanent network and a data platform to collect and share local density estimates (e.g., see https://wildlifeobservatory.org), which would enable to validate predictions for larger areas by modelling. It would allow to identify gaps in the data on wild ungulates and to focus on these areas for improving predictions. This platform must facilitate the reporting by wildlife policy makers and relevant stakeholders (hunting organizations), but also citizen science initiatives (e.g. www.mammalnet.com) and available open data.

To improve the estimates of wild ungulate population at large scale across Europe, we recommend:

- Standardising and harmonising data collection frameworks to validate and make them comparable across regions
- Involving the national and regional hunting administrations in data collection, so as the hunters associations.
- Calibrating local hunting statistics with potential to be compared across regions
- Collecting hunting effort and efficiency together with hunting bags in a representative set of wild ungulate populations .
- Evaluating the relationships between local densities and hunting statistics, how they vary and the factors affecting.
- To improve the modelling of wild ungulate distribution and abundance using reliable estimates of wild ungulate population at local and large scales by performing spatial modelling appropriate validation of the models.
- Exploring other data sources such as citizen science that may contribute to develop and improve current spatial models on the species distribution and relative abundance across Europe.

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/study populations 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): Precision measure of an estimate (the lower the more precise). 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: 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: Method to estimate density or relative abundance that is based on the direct observation of animals.

Distance sampling method: Density estimation method based on the decrease in the detectability of the animals as distance 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%= MCP95).

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

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.

GLOSSARY

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 or indirect, summarized in Table 1).

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 Table 1). 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 ruminant surveys can only deliver relative abundance such as those obtained from CT 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 S^2.

REFERENCES

Acevedo P, Vicente J, Höfle Ú, Cassinello J, Ruiz-Fons F, Gortazar C, 2007. Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. Epidemiol. Infect. 135, 519-527.

Andersen R, Duncan P, Linnell JDC (1998): The European roe deer: the biology of success. Scandinavian University Press, Oslo.

Amelin M (2014): Analyse von Auslösefehlern bei Wildkameras mittels Videoüberwachung und Reaktion von Wildtieren auf Infrarotblitze. BSc. Gottfried Wilhelm Leibniz Universität Hannover Hannover, 37 S.

Ammer C (1996): Impact of ungulates on structure and dynamics of natural regeneration of mixed mountain forests in the Bavarian Alps. Forestry Ecology and Management 88, 43-53.

Apollonio M, Andersen R, Putman R (2010): European Ungulates and Their Management in the 21st Century. Cambridge University Press, Cambridge, UK.

Baltensperger AP, Joly K (2019): Using seasonal landscape models to predict space use and migratory patterns of an arctic ungulate. Movement Ecology 7(1), 18 DOI: 10.1186/s40462-019-0162-8.

Barasona JA, Mulero-Pázmány M, Acevedo P, Negro JJ, Torres MJ, Gortázar C, Vicente J. (2014): Unmanned Aircraft Systems for Studying Spatial Abundance of Ungulates: Relevance to Spatial Epidemiology. PLoS ONE 9(12): e115608. https://doi.org/10.1371/journal.pone.0115608.

Blåhed IM, Ericsson G, Spong G (2019): Noninvasive population assessment of moose *(Alces alces)* by SNP genotyping of fecal pellets. Europ J Wild Res 65:96.

Boitani L, Trapanese P, Mattei L, 1995. Methods of population estimates of a hunted wild boar *(Sus scrofa L.)* population in Tuscany (Italy). IBEX J. Mt. Ecol. 3, 204-208.

Borkowski J., Palmer SCF, Borowski Z (2011): Drive counts as a method of estimating ungulate density in forests: mission impossible? Acta Theriol 56 (3) 239–253.

Broquet T, Ménard N, Petit E (2007): Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8 (1), 249-260. 10.1007/s10592-006-9146-5.

Buckland ST, Anderson DR, Burnham KP, Borchers DL, Thomas L (2004): Advanced Distance Sampling - Estimating abundance of biological populations. Oxford University Press, Oxford.

Buckland ST, Anderson DR, Burnham KP, Laake JL (1993): Distance Sampling: Estimating Abundance of Biological Populations. London: Chapman and Hall.

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001): Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press, Oxford.

Buckland ST, Goudie IBJ, Borchers DL (2000): Wildlife population assessment: past developments and future directions. Biometrics 56, 1–12.

Cagatay GN, Antos A, Meyer D, Maistrelli C, Keuling O, Becher P, Postel A (2018): Frequent infection of wild boar with atypical porcine pestivirus (APPV). Transbound Emerg Dis. 00:1–7. https://doi.org/10.1111/tbed.12854.

Campbell D, Swanson GM, Sales J (2004): Comparing the precision and cost- effectiveness of faecal pellet group count methods. Journal of Applied Ecology 41, 1185–1196.

Cardoso B, García-Bocanegra I, Acevedo P Cáceres G, Alves P C, Gortázar C. (2021): Stepping up from wildlife disease surveillance to integrated wildlife monitoring in Europe. Research in Veterinary Science. In press.

Carpio AJ, Guerrero-Casado J, Barasona JA, Tortosa FS (2017): Ecological Impacts of Wild Ungulate Overabundance on Mediterranean Basin Ecosystems. pp. 111-149 In Menendez A, Sands N (Eds.) Ungulates Evolution, Diversity and Ecology. Hauppauge, New York: Nova Science Publishers.

Chandler RB, Royle JA (2013): Spatially explicit models for inference about density in unmarked or partially marked populations. The Annals of Applied Statistics 7 (2), 936-954.

Chauvenet ALM, Gill RMA, Smith GC, Ward AI, Massei G (2017): Quantifying the bias in density estimated from distance sampling and camera trapping of unmarked individuals. Ecol. Modell. 350, 79-86.

Clutton-Brock TH, Guinness FE, Albon SD (1982) Red Deer: Behavior and Ecology of Two Sexes. University of Chicago Press, Chicago.

Corlatti L., Gugiatti A., Pedrotti L. (2016): Spring spotlight counts provide reliable indices to track changes in population size of mountain-dwelling red deer *Cervus elaphus*. Wildlife Biology, 22(6), 268-276.

Corlatti L., Fattorini L., Nelli L. (2015): The use of block counts, mark-resight and distance sampling to estimate population size of a mountain-dwelling ungulate. Popul Ecol 57:409–419.

Croft S, Chauvenet ALM, Smith GC (2017): A systematic approach to estimate the distribution and total abundance of British mammals. PLOS ONE 12 (6), e0176339. 10.1371/journal.pone.0176339.

Daniels E.J. (2006): Estimating red deer Cervus elaphus populations: an analysis of variation and cost-effectiveness of counting methods. Mammal Rev. 36 (3) 235–247.

Depner K, Gortazar C, Guberti V, Masiulis M, More S, Oļševskis E, Thulke H-H, Viltrop A, Woźniakowski G, Cortiñas Abrahantes J, Gogin A, Verdonck F, Dhollander S (2017): Epidemiological analyses of African swine fever in the Baltic States and Poland. EFSA Journal 15 (11), 59. doi: 10.2903/j.efsa.2017.5068.

Dzieciolowski R (1976): Estimating ungulate numbers in a forest by track counts. Acta Theriol. 21, 217-222.

Ebert C, Huckschlag D, Schulz HK, Hohmann U (2010): Can hair traps sample wild boar *(Sus scrofa)* randomly for the purpose of non-invasive population estimation? Eur. J. Wildl. Res. 56 (4), 583-590.

Ebert C, Knauer F, Spielberger B, Thiele B, Hohmann U (2012a): Estimating wild boar *Sus scrofa* population size using faecal DNA and capture-recapture modelling. Wildl. Biol. 18 (2), 142-152.

Ebert, C., Sandrini, J., Spielberger, B., Thiele, B., Hohmann, U. (2012b): Non-invasive genetic approaches for estimation of ungulate population size: A study on roe deer *(Capreolus capreolus)* based on faeces. Anim Biodiv and Cons 35(2), 267-275.

Elith J, Leathwick JR (2009): Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. Ann Rev Ecol Evol and System 40(1), 677-697.

ENETWILD Consortium, Keuling O, Sange M, Acevedo P, Podgorski T, Smith G, Scandura M, Apollonio M, Ferroglio E, Body G, Vicente J (2018a): Guidance on estimation of wild boar population abundance and density: methods, challenges, possibilities. EFSA supporting publication 2018:EN1449. 48 pp. doi:10.2903/sp.efsa.2018.EN-1449.

ENETWILD Consortium, J Vicente, R Plhal, J A Blanco-Aguiar, M Sange, T Podgórski, K Petrovic, M Scandura, A C Nabeiro, Gu Body, O Keuling, M Apollonio, E Ferroglio, S Zanet, F Brivio, G C Smith, S Croft, P Acevedo, RC Soriguer (2018b): Analysis of hunting statistics collection frameworks for wild boar across Europe and proposals for improving the harmonisation of data collection. EFSA supporting publication 2018:EN-1523. 33pp. doi:10.2903/sp.efsa.2018.EN-1523.

ENETWILD Consortium, Croft S, Smith GC, Acevedo P, Vicente J (2018c): Wild boar in focus: Review of existing models on spatial distribution and density of wild boar: Report containing the review of models, and proposal for next steps. EFSA supporting publication 2018:EN- 1490. 44 pp. doi:10.2903/sp.efsa.2018.EN-1490.

ENETWILD Consortium (2019): Harmonization of the use of hunting statistics for wild boar density estimation in different study areas. EFSA supporting publication 2019:EN-1706. 29 pp. doi:10.2903/sp.efsa.2019.EN-1706.

Ferretti F, Fattorini L, Sforzi A, Pisani C, 2016. The use of faeces counts to estimate relative densities of wild boar in a Mediterranean area. Population Ecology, 1-6. 10.1007/s10144-016-0536-3.

Ferretti F, Storer K, Coats J, Massei G, 2014. Temporal and spatial patterns of defecation in wild boar. The Wildlife Society Bullettin, n/a-n/a. 10.1002/wsb.494.

Ferroglio E, Gortázar C, Vincente J (2011): Wild Ungulate diseases and the risk for the livestock and public health. pp. 192-214 In Putman R, Apollonio M, Andersen R (Eds.) Ungulate Management in Europe: Problems and Practices. Cambridge University Press, Cambridge, UK.

Fickel J, Bubliy OA, Brand J, Mayer K, Heurich M (2012): Low genotyping error rates in non-invasively collected samples from roe deer of the Bavarian Forest National Park. Mamm Biol 77(1), 67-70.

Fischer A (2018): Wildkamera – Wildtier – Interaktion: Zuverlässigkeits- und Vergleichsanalyse von Kamerafallen. Rheinische Fridrich-Wilhelms-Universität Bonn.

Flowerdew JR, Ellwood SA (2001): Impacts of woodland deer on small mammal ecology. Forestry 74, 277–288.

Focardi S, Franzetti B, Ronchi F (2013): Nocturnal distance sampling of a Mediterranean population of fallow deer is consistent with population projections. Wildl Res 40 (6), 437-446.

Franke U, Goll B, Hohmann U, Heurich M (2012): Aerial ungulate surveys with a combination of infrared and high–resolution natural colour images. Anim Biodiv Conserv 35 (2), 285–293.

Franzetti B, Ronchi F, Marini F, Scacco M, Calmanti R, Calabrese A, Aragno P, Montanaro P, Focardi S (2012): Nocturnal line transect sampling of wild boar *(Sus scrofa)* in a Mediterranean forest: long-term comparison with capture–mark–resight population estimates. Eur J Wildl Res 58, 385–402.

Fuller RJ (2001): Responses of woodland birds to increasing numbers of deer: a review of evidence and mechanisms. Forestry 74, 289–298.

Garel M, Bonenfant C, Hamann J-L, Klein F, Gaillard J-M (2010): Are abundance indices derived from spotlight counts reliable to monitor red deer *Cervus elaphus* populations? Wildlife Biology 16: 77-84.

Gethöffer F, Sodeikat G, Pohlmeyer K (2007): Reproductive parameters of wild boar *(Sus scrofa)* in three different parts of Germany. Eur J Wildl Res 53, 287-297.

Gill R, Brandt G (2010): The use of thermal imaging to estimate densities of wild boar. 8th Symposium on Wild Boar and other suids York, 01.-04.09.2010. poster, 44.

Gill RMA, Thomas ML, Stocker DR (1997): The use of portable thermal imaging for estimating deer population density in forest habitats. J Appl Ecol 34:1273–1286.

Goode MJ, Beaver JT, Muller LI, Clark JD, Van Manen FT, Harper CA, Basinger PS (2014): Capture-recapture of white-tailed deer using DNA from fecal pellet groups. Wildl Bio 20 (5) 270-278.

Gortazar C, Ferroglio E, Höfle U, Frölich K, Vicente J (2007): Diseases shared between wildlife and livestock: a European perspective. Eur J Wildl Res 53, 241-256.

Gortazar C, Acevedo P, Ferroglio E (2015): APHAEA/EWDA Species Card: Eurasian wild boar, *Sus scrofa*. EFSA, https://www.aphaea.eu/cards/species/wildboar.

Gräber R, Ronnenberg K, Strauss E, Siebert U, Hohmann U, Sandrini J, Ebert C, Hettich U, Franke U (2015): Vergleichende Analyse verschiedener Methoden zur Erfassung von freilebenden Huftieren. Institut für Terrestrische und Aquatische Wildtierforschung - Stiftung Tierärztliche Hochschule Hannover, Hannover.

Hebeisen C, Fattebert J, Baubet E, Fischer C (2008): Estimating wild boar *(Sus scrofa)* abundance and density using capture–resights in Canton of Geneva, Switzerland. Eur. J. Wildl. Res. 54 (3), 391-401.

Höfle Ú, Vicente J, Fernández de Mera IG, Villanúa D, Acevedo P, Ruiz-Fons F, Gortázar C (2004): Health risks in game production: the wild boar. pp. 197-206. In: Fonseca C, Herrero J, Luis A, Soares AMVM (Hrsg.). Wild Boar Research 2002. A selection and edited papers from the "4th International Wild Boar Symposium", Lousa, Portugal, 19-22 September 2002. Vol. Galemys, 16 Special Issue.

Honda T, Kawauchi N (2011): Methods for constructing a wild boar relative-density map to resolve human-wild boar conflicts. Mammal Study 36 (2), 79-85.

Howe EJ, Buckland ST, Després-Einspenner M-L, Kühl HS (2017): Distance sampling with camera traps. Methods in Ecology and Evolution 8 (11), 1558-1565.

Imperio S, Ferrante M, Grignetti A, Santini G, Focardi S (2010): Investigating population dynamics in ungulates: Do hunting statistics make up a good index of population abundance? Wildl Biol 16 (2), 205-214.

Imperio S, Focardi S, Santini G, Provenzale A, 2012. Population dynamics in a guild of four Mediterranean ungulates: density-dependence, environmental effects and inter-specific interactions. Oikos 121 (10), 1613-1626.

Kavčić K, Palencia P. Apollonio M, Vicente J, Šprem N (2021). Random encounter model to estimate density of mountain-dwelling ungulate. European Journal of Wildlife Research (2021) 67:87.

Laddomada A, Patta C, Oggiano A, Caccia A, Ruiu A, Cossu P, Firinu A. (2002): Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African Swine fever. Veterinary Microbiology 145, 148-152.

La Morgia V, Calmanti R, Calabrese A, Focardi S (2015): Cost-effective nocturnal distance sampling for landscape monitoring of ungulate populations. Eur J Wildl Res 61:285–298.

Le Moullec M, Pedersen ÅØ, Yoccoz NG, Aanes R, Tufto J, Hansen BB (2017) Ungulate population monitoring in an open tundra landscape: distance sampling versus total counts. Wildlife Biology 2017: wlb.00299.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010): Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics 11, 355-373.

Jachmann H.(2002): Comparison of aerial counts with ground counts for large African herbivores. J Appl Ecol 39, 841–852.

Jacobson AR, Provenzale A, Von Hardenberg A, Bassano B, Fest A-Bianchet M (2004): Climate forcing and density dependence in a mountain ungulate population. Ecology 85(6), 1598–1610.

Jiménez J, Higuero R, Charre-Medellin JF, Acevedo P (2017): Spatial mark-resight models to estimate feral pig population density. Hystrix, the Italian Journal of Mammalogy 28 (2), 208-213.

Keever AC, McGowan CP, Ditchkoff SS, Acker PK, Grand JB, Newbolt CH (2017): Efficacy of N-mixture models for surveying and monitoring white-tailed deer populations. Mamm Res 62 (4), 413-422.

Keuling O, Baubet E, Duscher A, Ebert C, Fischer C, Monaco A, Podgórski T, Prevot C, Ronnenberg K, Sodeikat G, Stier N, Thurfjell H, 2013. Mortality rates of wild boar *Sus scrofa* L. in central Europe. European Journal of Wildlife Research 59 (6), 805-814.

Maillard D, Gaillard J-M, Hewison M, et al. (2010): Ungulates and their management in France. pp. 441–474 In M. Apollonio, R. Andersen and R. Putman (Eds.) European Ungulates and their Management in the 21st Century. Cambridge University Press, Cambridge, UK.

Malgras J, Maillard D (1996): Analyse spectrale et biologie des populations: analyse de l'activité de brame chez le cerf laphe (Cervus elaphus L.). Comptes Rendus de l'Académie des Sciences III: Science de la Vie 319, 921–929.

Mandujano S (2005): Track count calibration to estimate density of white-tailed deer *(Odocoileus virginianus)* in Mexican dry tropical forest. Southwestern Naturalist 50, 223–229.

Mansson J, Hauser CE, Andrén H, Possingham HP (2011): Survey method choice for wildlife management: the case of moose *Alces alces* in Sweden. Wildl. Biol. 17, 176-190.

Marcon A, Mladenoff DJ, Grignolio S, Apollonio M (2019a): Effects of forest management and roe deer impact on a mountain forest development in the Italian Apennines: A modelling approach using LANDIS-II. Plos One | https://doi.org/10.1371/journal.pone.0224788.

Marcon M., Battocchio D., Apollonio M., Grignolio S. (2019b): Assessing precision and requirements of three methods

to estimate roe deer density. Plos One | https://doi.org/10.1371/journal.pone.022234.

Massei G, Bacon P, Genov PV, 1998. Fallow Deer and Wild Boar Pellet Group Disappearance in a Mediterranean Area. Journal of Wildlife Management 62(3), 1086-1094.

Massei G, Kindberg J, Licoppe A, Gačić D, Šprem N, Kamler J, Baubet E, Hohmann U, Monaco A, Ozoliņš J, Cellina S, Podgórski T, Fonseca C, Markov N, Pokorny B, Rosell C, Náhlik A, 2015. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Management Science 71 (4), 492-500. 10.1002/ps.3965.

Melis C, Jedrzejewska B, Apollonio M, Barton KA, Jedrzejewski W, Linnell JDC, et al. (2009): Predation has a greater impact in less productive environments: variation in roe deer, *Capreolus capreolus,* population density across Europe. Global Ecol. Biogeogr. 18, 724–734.

Micu I, Náhlik A, Negus S, Mihalache I, Szabó I (2010): Ungulates and their management in Romania. pp. 319–337. In M. Apollonio, R. Andersen and R. Putman (Eds.) European Ungulates and their Management in the 21st Century. Cambridge University Press, Cambridge, UK.

Morden C-JC, Weladji RB, Ropstad E, Dahl E, Holand Ø (2011): Use of faecal pellet size to differentiate age classes in female Svalbard reindeer *Rangifer tarandus platyrhynchus.* Wildl Bio 17(4), 441-448.

Morellet N, Klein F, Solberg E, Andersen R (2011): The census and management of populations of ungulates in Europe. pp. 106-143. In: Putman R, Apollonio M, Andersen R (Eds.) Ungulate Management in Europe: Problems and Practices. Cambridge University Press, Cambridge, UK.

Morimando F, Focardi S, Andreev R, Capriotti S, Ahmed A, Lombardi S, Genov P (2016): A method for evaluating density of roe deer, *Capreolus capreolus* (Linnaeus, 1758), in a forested area in Bulgaria based on camera trapping and independent photo screening. Acta Zoologica Bulgarica 68 (3), 367-373.

Mysterud A, Tryjanowski P, Panek M, Pettorelli N, Stenseth NC (2007): Inter-specific synchrony of two contrasting ungulates: wild boar *(Sus scrofa)* and roe deer *(Capreolus capreolus).* Oecologia 151, 232-239.

Nakashima Y, Fukasawa K, Samejima H (2018): Estimating animal density without individual recognition using information derivable exclusively from camera traps. J. Appl. Ecol. 55 (2), 735-744. 10.1111/1365-2664.13059.

Nakashima, Y., Hongo, S., & Akomo-Okoue, E. F. (2020): Landscape-scale estimation of forest ungulate density and biomass using camera traps: Applying the REST model. Biol. Conserv. 241, 108381.

Palencia, P., Vicente, J., Barroso, P., Barasona, J. Á., Soriguer, R. C., & Acevedo, P. (2019): Estimating day range from camera‐trap data: the animals' behaviour as a key parameter. J. Zool, 309(3), 182-190.

Palencia P., Rowcliffe M., Vicente J., Acevedo P. (2021a): Assessing the camera trap methodologies used to estimate density of unmarked populations. Journal of Applied Ecology 58, 1583-1592.

Palencia, P., Vicente, J., Soriguer, R. C. & Acevedo, P. (2021b): Towards a best-practices guide for camera trapping: assessing differences among camera trap models and settings under field conditions. Journal of Zoology (in press).

Pellerin M, Bessière A, Maillard D, Capron G, Gaillard J-M, Michallet J, Bonenfant C (2017): Saving time and money by using diurnal vehicle counts to monitor roe deer abundance. Wildlife Biology 2017: wlb.00274.

Pfeffer (2016): Comparison of three different indirect methods to evaluate ungulate population densities. Master degree thesis in Biology at the Department of Wildlife, Fish, and Environmental Studies. Umeå (Schweden).

Pfeffer SE, Spitzer R, Andrew M, Allen AM, Hofmeester TR, Ericsson G, Widemo F, Singh NJ, Cromsigt JPGM (2018): Pictures or pellets? Comparing camera trapping and dung counts as methods for estimating population densities of ungulates. Remote Sensing in Ecology and Conservation 4(2), 173-183.

Pittiglio C, Khomenko S, Beltran-Alcrudo D (2018): Wild boar mapping using population-density statistics: From polygons to high resolution raster maps. PLoS ONE 13(5): e0193295. https://doi.org/10.1371/journal.pone.0193295.

Plhal R., Kamler J., Homolka M. (2014). Faecal pellet group counting as a promising method of wild boar population density estimation. Acta Theriologica, 1-9. 10.1007/s13364-014-0194-9.

Poole, KG., Reynolds, D.M., Mowat, G., Paetkau, D. (2011): Estimating mountain goat abundance using DNA from fecal pellets. Journal of Wildlife Management 75(6) 1527-1534.

Postel A, Hansmann F, Baechlein C, Fischer N, Alawi M, Grundhoff A, Derking S, Tenhündfeld J, Pfankuche VM, Herder V, Baumgärtner W, Wendt M, Becher P (2016): Presence of atypical porcine pestivirus (APPV) genomes in newborn piglets correlates with congenital tremor. Scientific Reports 6, 27735. 10.1038/srep27735.

Putman, RJ. (1984): Facts from faeces. Mammal Review 14, 79–97.

Putman RJ (2004): The deer Manager's Companion: A guide to Deer Management in the Wild and in the Parks. Shropshire, UK: Swan Hill Press.

Putman R, Apollonio M, Andersen R (2011): Ungulate Management in Europe: Problems and Practices. Cambridge University Press, Cambridge, UK.

Putman RJ, Moore NP (1998): Impact of deer in lowland Britain on agriculture, forestry and conservation habitats. Mamm. Rev. 28 141-164.

Rae LF, Whitaker DM, Warkentin IG (2014): Multiscale impacts of forest degradation through browsing by hyperabundant moose *(Alces alces)* on songbird assemblages. Diversity Distrib. 20, 382–395.

Reby D, Hewison AJM, Cargnelutti B, Angibault JM, Vincent JP (1998): Use of vocalizations to estimate population size of roe deer. Journal of Wildlife Management 62, 1342–1348.

Ridley M (2004): Evolution. Oxford University Press, U.S.A.

Ronnegard L., Sand H., Andrén H., Mansson J., Pehrson A. (2008): Evaluation of four methods used to estimate population density of moose *Alces alces* Wildlife Biology 14:3.

Ross, S., Al Jahdhami, M.H., Al Rawahi, H. (2019): Refining conservation strategies using distribution modelling: A case study of the Endangered Arabian tahr *Arabitragus jayakari.* ORYX 53(3) 532-541

Rovero F, Marshall AR (2009): Camera trapping photographic rate as an index of density in forest ungulates. J. Appl. Ecol. 46 (5), 1011-1017. 10.1111/j.1365-2664.2009.01705.x.

Rovero F, Tobler M, Sanderson J (2010): Camera trapping for inventory terrestrial vertebrates. In: Samyn Y, Vandenspiegel D, Degreef J (Eds.) Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring. Vol. 8(1). 100-129.

Rovero F, Zimmermann F, Berzi D, Meek P (2013): "Which camera trap type and how many do I need?" A review of camera features and study designs for a range of wildlife research applications. Hystrix, the Italian Journal of Mammalogy 24 (2).

Rowcliffe JM, Carbone C, Jansen PA, Kays R, Kranstauber B (2011): Quantifying the sensitivity of camera traps: an adapted distance sampling approach. Methods in Ecology and Evolution 2 (5), 464-476.

Rowcliffe JM, Field J, Turvey ST, Carbone C (2008): Estimating animal density using camera traps without the need for individual recognition. J. Appl. Ecol. 45 (4), 1228-1236.

Rowcliffe JM, Jansen PA, Kays R, Kranstauber B, Carbone C (2016): Wildlife speed cameras: measuring animal travel speed and day range using camera traps. Remote Sensing in Ecology and Conservation 2 (2), 84-94. 10.1002/rse2.17.

Rowcliffe JM, Kays R, Carbone C, Jansen PA (2013): Clarifying assumptions behind the estimation of animal density from camera trap rates. J. Wildl. Manage. 77 (5), 876-876. Doi 10.1002/Jwmg.533.

Royle JA (2004): N-Mixture Models for Estimating Population Size from Spatially Replicated Counts. Biometrics 60 (1), 108-115. 10.1111/j.0006-341X.2004.00142.x.

Royle JA, Chandler RB, Sollmann R, Gardner B (2014): Spatial Capture-Recapture. Academic Press, Boston, i S.

Ryser-Degiorgis M-P (2013): Wildlife health investigations: Needs, challenges and recommendations. BMC Vet Res 9:223.

Smart JCR, Ward AI, White PCL (2004) Monitoring woodland deer populations in the UK: an imprecise science. Mammal Rev. 34(1), 99–114.

Smit C, Putman RJ (2011): Large herbivores as 'environmental engineers' pp. 260-283 In: Putman R, Apollonio M, Andersen R (Eds.) Ungulate Management in Europe: Problems and Practices. Cambridge University Press, Cambridge, UK.

Stephens PA, Zaumyslova OY, Miquelle DG, Myslenkov AI, Hayward GD (2006): Estimating population density from indirect sign: track counts and the Formozov-Malyshev-Pereleshin formula. Animal Conservation 9, 339–348.

Silveira L, Jácomo ATA, and Diniz-Filho JAF (2003): Camera trap, line transect census and track surveys: a comparative evaluation. Biological Conservation 114, 351–355.

Telleria JL (1986): Manual para el censo de vertebrados terrestres. Raíces. Madrid.

Toïgo C, Servanty S, Gaillard JM, Brandt S, Baubet E (2008): Disentangling natural fom hunting mortality in an intensively hunted wild boar population. J. Wildl. Manage. 72 (7), 1532–1539.

Ueno M, Matsuishi T, Solberg EJ, Saithoh T (2009): Application of cohort analysis to harvest data of large terrestrial mammals. Mammal study 34, 65-76.

Ünala J., Çulhaci h. (2018): Investigation of fallow deer *(Cervus dama L.)* population densities by camera trap method in Antalya Düzlerçamı Eşenadası Breeding Station. Turkish Journal of Forestry 19(1): 57-62.

Vincent, J.-P., Hewison, A.J.M., Angibault, J.-M., Cargnelutti, B. (1996): Testing Density Estimators on a Fallow Deer Population of Known Size. The Journal of Wildlife Management, 60 (1) 18-28.

Vicente J, Segalés J, Höfle U, Balasch M, Plana-Durán J, Domingo M, Gortázar C, 2004. Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar *(Sus scrofa).* Veterinary Research 35 (2), 243-253.

Wäber K., Dolman P.M. (2015): Deer abundance estimation at landscape-scales in heterogeneous forests. Basic and Applied Ecology 16 (2015) 610–620.

Ward AI, White PCL, Critchley CH (2004): Roe deer *Capreolus capreolus* behaviour affects density estimates from distance sam- pling surveys. Mamm Rev 34:315–319.

Wawrzyniak, P., Jędrzejewski, W., Jędrzejewska, B. and Boro, T. (2010): Ungulates and their management in Poland. pp. 223–242. In M. Apollonio, R. Andersen and R. Putman (eds.) European Ungulates and their Management in the 21st Century. Cambridge, UK: Cambridge University Press

Williams BK, Nichols JD, Cobroy MJ (2002): Analysis and Management of Animals Populations. San Diego, CA: Academic Press.

Zero, V. H., Sundaresan, S. R., O'Brien, T. G., & Kinnaird, M. F. (2013). Monitoring an endangered savannah ungulate, Grevy's zebra *Equus grevyi*: choosing a method for estimating population densities. Oryx, 47(3), 410-419.

ANNEX 1.

1. INSTRUCTIONS FOR FIELD SURVEYS TO ESTIMATE POPULATION DENSITY OF MEDIUM SIZE MAMMALS AND UNGULATES USING CAMERA TRAPS

This card presents basic instructions to estimate the density of wild ungulates using camera traps (CTs). Since different methods are available, we will focus on a practical one that can generate reliable data in a wide range of situations (and species) throughout Europe. The random encounter (REM) model does not require individual recognition. However, it is necessary to collect certain information to determine the speed of movement (average daily movement range) of the wild ungulates. Therefore, it is necessary to place marks or stakes at a distance from the CTs that serves as a guide to subsequently mark the path followed by each animal, as indicated below. These instructions also apply to REST and CTs - Distance sampling methods. The field protocol (e.g., position of marks) will be modified in the near future once artificiall intelligence is tested to process images.

For comparison purposes, the work should be developed during late summer/autumn/early winter (optimally pre-hunting season), with the CTs placed a minimum of 60 days.

• CTs will be placed (registering the geographical coordinates) following a regular uniform distribution as a grid. A minimum of 45 camera placements during the study period are recommended. The separation between CTs will be approx. 1.5 km. The exact location can be within a diameter of less than 100m around the points of the grid, for instance, to avoid a road. If the number of CTs available is not enough to sample the 45 placements at the same time, the CTs should be moved during the experiment to cover the minimum of 45 locations. For instance, 15 CTs moved twice (every 3 weeks), which fit a study area of approximately 2500-3000 has. However, in case the study area is bigger, the distances between CTs can be larger that 1.5 km, and if possible, it is recommended placing more camera sites. Similarly, for smaller areas, CTs can be placed at 1 km grid.

• The grid must cover at least one patch beaten during the hunting season, if possible, more; or several grids for several patches. This is to compare density estimation based on CTs against hunting statistics (driven counts).

• Place stakes in 2.5m intervals (Figure A1). Connecting the stakes with signaling tape helps to better visualize distances (Fig A1-C). Finally, ensure that a photograph is taken from the CT where these stakes and the signaling tape are evident. Put natural marks (stones, branches…) before remove the stakes and other signals for later identification of the path of the animals photographed (Figure A1 B-D). Two of them can stay (5 and 10 m).

• The CT will be placed on poles or vegetation 40cm above the ground.

• The CT is configured with operation of 24 hours per day and to take up a burst of consecutive images (the maximum number possible), with the minimum waiting time (0 sec. if possible) between activations. Use medium sensitivity.

• The flash intensity should be set at medium (if possible) to avoid "overexposed photos".

• Check that the date and time are correctly set, and that they are printed automatically on each image.

• The CT should be reviewed at least in the half of the study period (minimum frequency once a month) to check its functioning and placement. Normally it will not be necessary to change the batteries and the memory cards, since the CTs are placed at random points and high wildlife activity is not expected.

• Choose a field of vision of the CT that is cleared of vegetation (it is not necessary to be totally clean, but that allows the detection of any wild ungulates that passes within the first 5 m), being better a north orientation.

• A form must be filled in (Form in Table 1), collecting the information of each CT during its placement, revision, and retrieval (see form below). All the information that is subsequently extracted must keep the traceability of the CT (mark the source camera of each memory card extracted and keep this nomenclature in the folders that are created on the computer to archive the images).

Figure A1. A) Scheme of the stick-structure (grey dots) used to reference the animal captured by the camera-trap (black point. XB indicates the position of the animals captured in the images B. Note these measurements are dependent on the camera characteristics. The dotted triangle is used for REST analysis and its size and distance to camera may vary according to the target species. B) Wild boar and red deer photo-captured. C) Photo of the structure installed in one photo-trapping sampling point. The camera should be oriented so that the well-centred stakes are displayed. D) Natural marks (stones) used as references after removing stakes.

Required material:

• CT adequately configured (see above), with proven batteries (alkaline) and compatible memory card. Check that the cards save the photos well, since sometimes they are not compatible with the camera model.
• Memory card of 8 GB minimum size recommended, 16 or 32 GB if the camera supports them.
• 50 cm stakes (or poles) and hammer to place them. 8 of them are required for the initial photograph of each study point. 2 of them will stay (5 and 10 m).
• Signalling tape.
• GPS for recording geographical coordinates.
• Single-use camps are very practical for fixing the cameras.
• Hoe for vegetation cleaning, only the strictly necessary within the first 5 meters.
• A form to record the information of each CT during its placement, revision, and retrieval (Table 1).

Key literature (for more details)

Howe EJ, Buckland ST, Després-Einspenner ML. Kühl HS (2017). Distance sampling with camera traps. Met Ecol Evol 8, 1558-1565.

Jiménez J, Higuero R, Charre-Medellin JF, Acevedo P (2017). Spatial mark-resight models to estimate feral pig population density. Hystrix, Ital J Mammal 28, 208-213.

Nakashima Y, Fukasawa K, Samejima H (2018). Estimating animal density without individual recognition using information derivable exclusively from camera traps. J Appl Ecol 55, 735-744.

Palencia P, Vicente J, Barroso P, Barasona JA, Soriguer RC, Acevedo P (2019). Estimating day range from camera-trap data: the animals' behaviour as a key parameter. J. Zool, 309, 182-190.

Palencia P, Fernández-López J, Vicente J, Acevedo P (2021). Innovations in movement and behavioural ecology from camera traps: day range as model parameter. Met Ecol Evol.

Palencia P, Rowcliffe JM, Vicente J, Acevedo P (2021). Assessing the camera trap methodologies used to estimate density of unmarked populations. J Appl Ecol 58, 1583-1592.

Rowcliffe JM, Carbone C (2008). Surveys using camera traps: are we looking to a brighter future? Anim Conserv 11, 185–186.

Rowcliffe JM, Jansen PA, Kays R, Kranstauber B, Carbone C (2016). Wildlife speed cameras: measuring animal travel speed and day range using camera traps. Remote Sensing Ecol Conserv 2, 84-94.

Royle JA, Chandler RB, Sollmann R, Gardner B (2014). Spatial Capture-Recapture. Academic Press, Boston.

http://wildlifeobservatory.org/wp-content/uploads/2022/01/Table.docx

N° of the study point	N° CT and memory card	Coordinate X	Coordinate Y	Date setting-up CT in the field	Time setting-up CT in the field	Picture of vision field with marks taken? (Y/N)	Date CT retrieval	Time CT retrieval	Observations: any eventuality, indicate if revision is made, the date of this, aspects of functioning of the CT, if it dropped down, if still correctly attached, any failure, change of memory or batteries, etc.
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									

Table 1 Annex 1. A form to record the information of each CT during its placement, revision, and retrieval.

2. DISTANCE SAMPLING FOR DENSITY ESTIMATION OF WILD UNGULATES AT LOCAL SCALE

Distance sampling is an accurate and established method for determining density of animal populations on a local scale. Frequency and distance to observer (as well as group size) are used for calculating densities and abundances. This method is quite simple to conduct. However, some assumptions and requirements are needed. The objective is to obtain a detectability function with which to estimate the local density of the wild ungulate population.

Distance sampling applied to transects requires that the observer travelling along a transect records the perpendicular distances (or the sighting distance and sighting angle) of all animals visible from the transect. The final density estimation uses a detectability function calculated from the distribution of observation distances (i.e., perpendicular distance of the animals from the line of the transect). Distance sampling to estimate density also provides detailed information about precision.

Design for Distance Sampling

- Field operations developed during the same season (optimally during summer, pre-hunting) in order density estimations to be comparable across sites and time.
- Transect methods can be implemented during the day, in general during crepuscular hours, by using binoculars, or during the nightlight by using spotlights to detect the individuals.
- The absence of a reflecting tapetum lucidum in wild boar makes nocturnal spotlight counts (by car) only useful for deer species.
- The problem of low visibility of wild boar during spotlight counts was solved successfully using distance sampling on transects with thermographic cameras.
- The use of thermal imaging allows the detection of animals by detecting the long-wave energy radiated by warm-bodied. The price of new-generation infrared cameras is now more attainable, which can increase the cost-effectiveness and applicability of this method once it is fine-tuned
- Even if the method works well when applying a high effort, in low densities and in habitats with dense understory, it can fail in other situations
- Random placement of transects
- Transects driven by car or walked by foot
- Use of a range finder for measuring distance to animal at first sight (Fig C: d)
- Use of angle measurement (α DISTANCE calculates distance (D) vertical to transect)
- Use software DISTANCE
- Need of at least 50 sightings
- Also random point sampling is a possible option: hunters might observe animals from hides

Key literature (for more details)

Acevedo P, Ruiz-Fons F, Vicente J, Reyes-García R, Alzaga V, Gortázar C (2008) Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. J Zool 276, 37-47.

Borchers DL, Buckland ST, Zucchini W (2002). Estimating Animal Abundance: Closed Populations. Statistics for Biology and Health. Springer Verlag, London.

Buckland ST, Anderson DR, Burnham KP, Borchers DL, Thomas L (2004). Advanced Distance Sampling - Estimating abundance of biological populations., Oxford University Press, Oxford.

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001). Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press.

Miller DL, Rexstad E, Burt L, Bravington MV, Hedley S (2015): Density Surface Modelling of Distance Sampling Data. CRAN.

Figure C. Scheme of how to count animals for calculating detection function and consequent abundances
d = distance to animal at first sighting
a = angle detection/transect
D = distance animal/transect
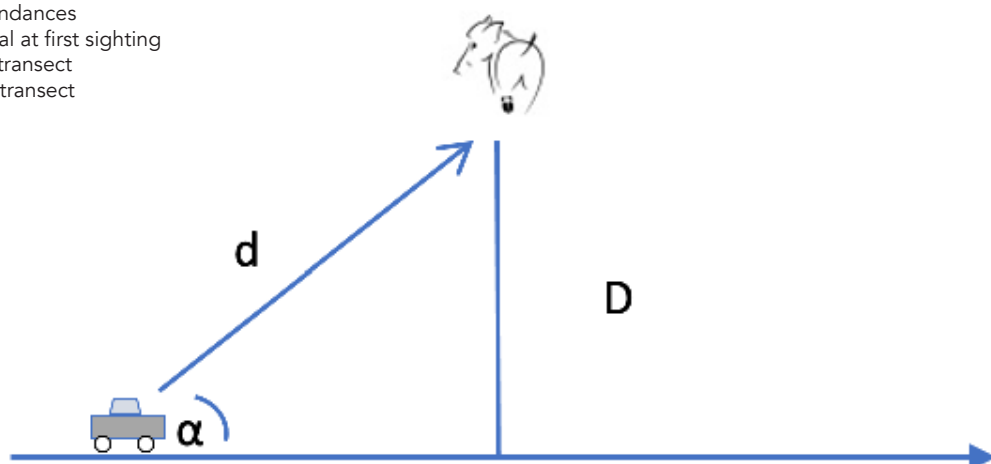
3. DRIVE COUNTS FOR DENSITY ESTIMATION OF WILD UNGULATE AT LOCAL SCALE

This section presents basic instructions to estimate the density of wild ungulates using drive counts. This method is frequently used to estimate population densities in ungulates inhabiting forested areas and may be conducted as drives with beater (with or without dogs). All beaters and special "counting persons" record all animals seen during the drives.

This method may also be conducted during drive hunts (usually driven with beaters and dogs), where the hunters in hides record all wild ungulates seen. Hunters can be used as experienced observers and therefore the hunting activity, if carried out by instructed and motivated personnel, can be a cost-effective alternative to monitor ungulates. Dependent on certain conditions, many wild ungulates may be missed. Thus, this method requires high effort and good organization, and it is very difficult to estimate the precision and accuracy.

Design for drive counts

Sample accurate hunting statistics for further calibration with drive counts
• Note hunting bags with reference to area (has)
• At least give all data needed for calculating hunting effort (number of hunters, beaters and dogs)
Drive counts (during hunt/without hunt)
• Drive counts can be used to estimate winter numbers of wild ungulates, such a wild boar (Figure A)
• The number of beaters should be almost equal to the number of observers (hunters) participating in the counts. The use of dogs may replace beaters.
• The observers should have sufficient experience to determine species, sex, and age (young/adult).
• Each observer records on an observation form the species and number of individuals of each group (a form is presented below), and if possible, also record the group composition leaving or entering the driven block on his right (or left) side.
• 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 to minimize the likelihood of double counting and inaccurate group sizes.

Key literature (for more details)

Acevedo P, Vicente J, Höfle U, Cassinello J, Ruiz-Fons F, Gortazar C (2007): Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. Epidemiol. Infect. 135, 519-527.
Boitani L, Trapanese P, Mattei L (1995): Methods of population estimates of a hunted wild boar (*Sus scrofa* L.) population in Tuscany (Italy). IBEX J. Mt. Ecol. 3, 204-208.
Borkowski J, Palmer SF, Borowski Z (2011): Drive counts as a method of estimating ungulate density in forests: mission impossible? Acta Theriol. 56 (3), 239-253.
ENETwild Consortium (2019): Harmonization of the use of hunting statistics for wild boar density estimation in different study areas. EFSA supporting publication 2019:EN-1706 29 pp. doi:10.2903/sp.efsa.2019.EN-1706 (https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2019.EN-1706).
Imperio S, Ferrante M, Grignetti A, Santini G, Focardi S (2010): Investigating population dynamics in ungulates: Do hunting statistics make up a good index of population abundance? Wildl. Biol. 16 (2), 205-214. 10.2981/08-051.
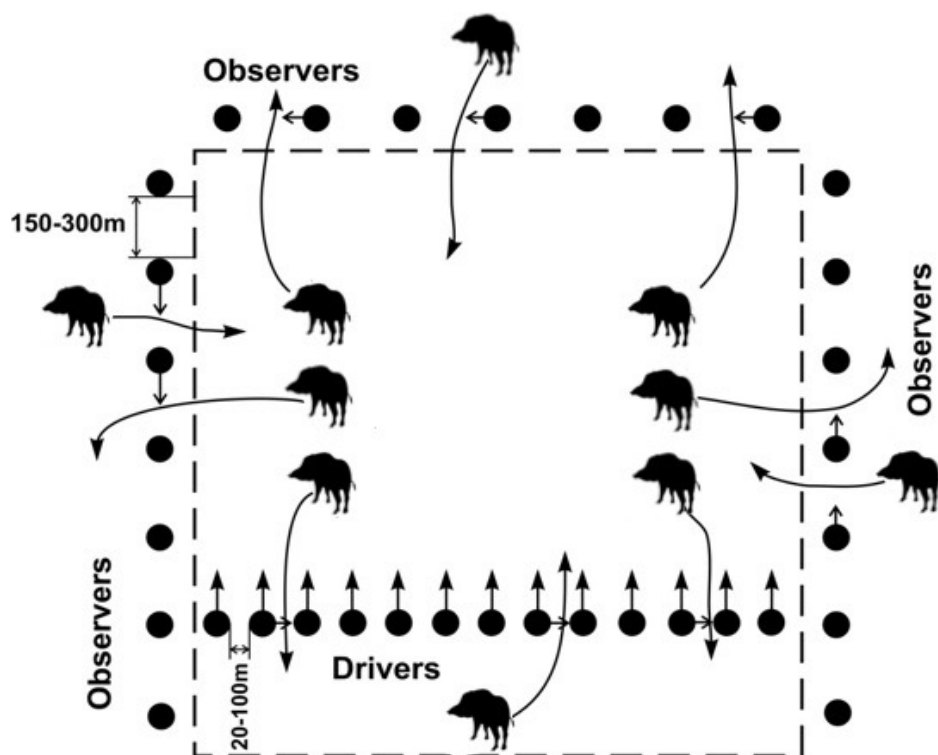
Figure A. Outline of a drive-count (Poland). In fact- all ungulates are counted during one drive-count event. Important note: driven-count blocks (here 1x1km) should be placed randomly over surveyed area and over at least 10% of its surface.

Table 2- Form to collect data during hunting drives (minimum data that should be collected).

http://wildlifeobservatory.org/wp-content/uploads/2022/01/Form.docx

FORM TO COLLECT DATA DURING HUNTING DRIVES (one drive one form)		
Name and position (organizer, ranger, etc.) of count coordinator: /		
E-mail:	Telephone:	
Date:	Municipality:	
Hunting ground ID:	Hunting ground name:	
Hunting drive (name of the patch covered and/or consecutive number within the season):		
Start time:	End time:	
Name and/or name of the stalking site:		
N° hunters (stalking sites):	N° beaters:	N° dogs:
Did you look for tracks before?		
Did you bait the hunted area?		
Beaten area (has):	Is there GIS file available? (yes/no):	
Total N° sighted wild boar (including those hunted):		
Total N° hunted wild boar:		
Total N° sighted red deer (including those hunted):		
Total N° hunted red deer:		
Total N° sighted _____ (indicate species, including those hunted):		
Total N° hunted _____ (indicate species):		
Total N° sighted _____ (indicate species, including those hunted):		
Total N° hunted _____ (indicate species):		
Total N° sighted _____ (indicate species, including those hunted):		
Total N° hunted _____ (indicate species):		

INSTRUCTIONS TO FILL THIS FORM

• Each stalked hunter must fill in this form for his position (fields above)
• Next, all data must be summarized in a single form by the co-ordinator of the drive count, who will fill in the form for the total count of the event. You should consider the possible double counting by neighbour hunting positions
• It is very important to fill in the form even if no piece has been seen or hunted, in this case in the corresponding boxes it will be set 0

Acknowledgements

A PRACTICAL GUIDANCE ON ESTIMATION OF EUROPEAN WILD UNGULATE POPULATION DENSITY

A SELECTION OF RELIABLE PRACTICAL METHODS FOR HARMONIZED POPULATION MONITORING IN EUROPE

Given the diversity of available methods and the geographical diversity of Europe, methodological harmonization is duly needed. The general aim of this guidance is to review the methods for estimating density in European wild ungulates. This guidance is based in previous comprehensive reviews carried out by the ENETWILD Consortium, which proposed general recommendations for practical implementation of methods to estimate wild ungulate density. We present 9 methods used in nineteen wild ruminant species and wild boar distributed across Europe, paying special attention to most practical methods for further implementation in the field to calculate reliable and accurate density estimates, allowing further comparable results over their distribution ranges. This guidance provides recommendations to select the methods to estimate the density and its implementations for ungulate populations with the aim of increasing the output quality (good accuracy and precision). 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. Every method on estimating reliable and comparable wild ungulate population density has some advantages and disadvantages depending on the habitat, the weather conditions and the benefit and do not discard their use if applied in a harmonized way. It arises from this guidance the need of developing a permanent network and a data platform to collect and share local density estimates, so as abundance in the EU.

Through THE ENETWILD CONSORTIUM (www.enetwild.com), the European Food Safety Authority (EFSA) aims to improve European capacities to monitor wildlife populations. First, developing standards for the collection and validation of wildlife data. Secondly, ENETWILD has created and promoted a data repository which initially focused on wild boar abundance and distribution data and is now moving to other groups of species (other ungulates and carnivores). Existing data were collated through a data sharing agreement with data owners and harmonized with the help of a specific data model. Therefore, important progresses have been made in the field of spatial distribution modelling of wildlife species at European level. Guidelines for reliable abundance estimation of wild mammals and detailed protocols on field methods have been published and now summarized here for wild ungulates in a practical format. A network of data providers (data and metadata on species distribution, abundance, and density) has been established and supported, including training on study design, methods, field protocols and easy-to-use data analysis tools to estimate local density of populations. All together these actions are planting the seed of a pan-European network (**The European Observatory of Wildlife**, https://wildlifeobservatory.org/), capable of providing reliable data on wildlife abundance on a long-term basis. This observatory aims to help overcome existing data gaps and workflow bottlenecks in the context of current European-wide frameworks for monitoring terrestrial mammal populations.