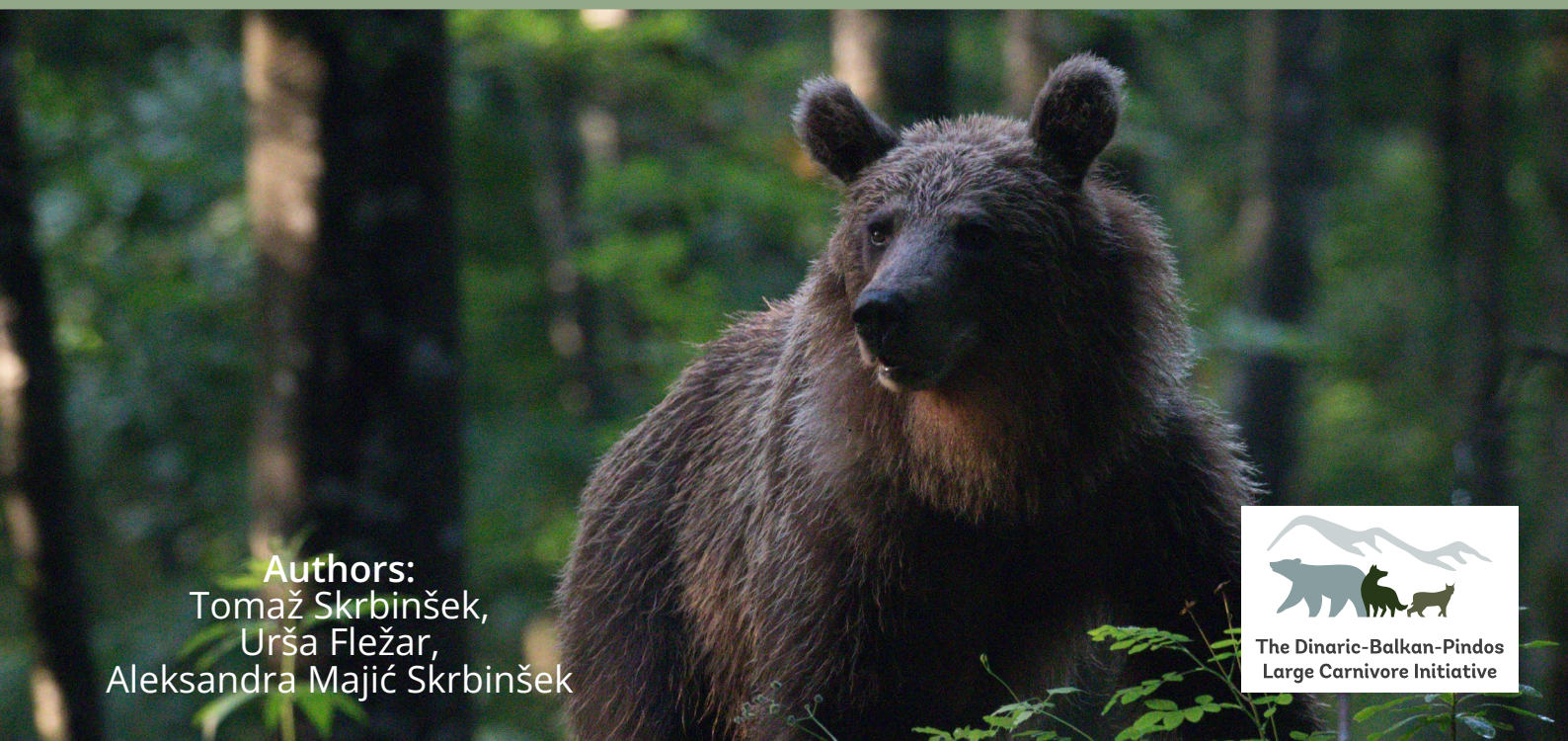
HANDBOOK FOR MONITORING OF LARGE CARNIVORES

IN THE DINARIC – BALKAN – PINDOS REGION



Authors:
Tomaž Skrbinšek,
Urša Fležar,
Aleksandra Majić Skrbinšek

The Dinaric-Balkan-Pindos Large Carnivore Initiative

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[1]DivjaLabs, Slovenia | [2] University of Ljubljana, Biotechnical Faculty, Slovenia |
[3] WWF Adria, Croatia

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Zagreb, 2024

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DiBaPi Platform: Transnational Cooperation for Large Carnivore Conservation in the Dinaric-Balkan-Pindos Region

1 DiBaPi Platform: Transnational Cooperation for Large Carnivore Conservation in the Dinaric-Balkan-Pindos Region

Large carnivores like bears, wolves, and lynx have vast territories that disregard political boundaries. Management decisions in one country can significantly impact populations in neighbouring areas. Consequently, collaboration and coordination among countries sharing these populations are critical for their long-term conservation. The Dinaric-Balkan-Pindos region is biodiversity hotspot and a home to numerous protected species and habitats including brown bears (*Ursus arctos*), wolves (*Canis lupus*), and Eurasian lynx (*Lynx lynx*), including the endangered Balkan subspecies (*Lynx lynx balcanicus*). To ensure their long-term survival and coexistence with people, transnational cooperation in management strategies is essential.

The Dinaric-Balkan-Pindos Large Carnivore Platform (DiBaPi Platform) serves as a crucial platform in the region for transnational exchange, bringing together authorities and stakeholders from Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Kosovo[1], Montenegro, North Macedonia, Greece, Serbia and Slovenia, to discuss and coordinate large carnivore management practices. It utilizes diverse formats for interaction, including platform meetings, thematic sessions on key management issues, and capacity-building events. First meeting of the platform was held on November 15th and 16th 2021 in Ljubljana, Slovenia.

The Platform membership is inclusive, encompassing representatives of:

- Managing authorities (ministries, departments, and agencies responsible for nature, agriculture, forestry, and hunting)
- Regional authorities
- Stakeholders (nature NGOs, hunters, farmers, and tourism operators)
- Scientists

The Dinaric-Balkan-Pindos Platform is governed by the Platform Secretariat which is providing organisation and technical support and background information for members interactions. The Platform is accompanied by scientific Advisory Board which is composed of experts from most participating countries.

1.1 Population monitoring working group

One notable achievement of the platform is the establishment of working groups focused on specific topics through in-depth technical discussions. The Population Monitoring Working Group exemplifies this approach. By addressing challenges and opportunities related to large carnivore monitoring, the working group's discussions directly informed the development of

[1] This designation is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence.

these practical guidelines tailored to the specific needs identified by DiBaPi countries.

Members of the monitoring working group also contributed to the development of the groups' vision for the future of population level monitoring of large carnivore populations in the region.

1.2 Vision for the future of population level monitoring of large carnivores in the region

"Our vision for the future of large carnivore population monitoring is a **transboundary approach** characterized by **standardized methodologies** and **collaborative efforts** across the entire range of these species in the Dinaric-Balkan-Pindos region. The establishment of the **Dinaric-Balkan-Pindos Platform** acts as a cornerstone, facilitating a dedicated task force responsible for **collecting, harmonizing, and exchanging monitoring data** within a **common database accessible to all partners**.

Population-level monitoring of large carnivores and their interactions within ecosystems and with humans is not only a scientific exercise but also a harmonized, coordinated effort supported by **secure funding**, **institutional commitment**, **stakeholder involvement**, and **international cooperation** defined within a **treaty among the participating countries**."

2

About monitoring of large carnivores

2 About monitoring of large carnivores

Monitoring large carnivore populations is not a simple task, and typically requires considerable effort and resources. However, **not monitoring is often not an option** because of the huge complexity of managing and conserving these species, diverse and highly motivated stakeholders, high public interest, and often considerable societal impacts that large carnivores have. **Monitoring provides the foundation on which all management decisions should be based**. As such, it is the key prerequisite for large carnivore management, and should be included in any large carnivore management strategy or plan.

The goal of this handbook is to provide practical guidance for anyone considering this daunting task in the Dinaric – Balkan - Pindos area. The main target audience are wildlife managers and other members of organizations that are involved in conservation and management of large carnivores. We tried to keep the handbook from becoming too technical, but provided further reading for people that wish to dive deeper into the topics covered here.

The term 'monitoring' has many definitions and usages. For the purpose of this book, we define monitoring as the cyclic process of gathering information about a a population parameter (such as the abundance or distribution of a species) to assess the state of the system and draw inferences about changes over time (Yoccoz et al. 2001). The key attribute of monitoring is a temporal component, meaning that it should be performed in biologically meaningful cycles to fulfil its mission. And it must be done in a consistent, methodologically sound manner to be effective.

Another important issue is scale. Over the last decades, there has been a shift in emphasis among conservation biologists from managing populations of threatened species at a single site to considering larger scale dynamics (Jones 2011). This has particular importance for large carnivores with their large homeranges and low population densities, and it is recognized that their management (including monitoring) should be done at the scale of populations (Linnell et al. 2008a). In the politically fragmented landscape of Europe this is often a difficult proposition and still seldom done in practice, but it's nevertheless something that should always be considered and strived towards implementing.

The issue that is most often the showstopper when ambitious monitoring initiatives are considered is the cost. Monitoring elusive species over large areas can be expensive, and often consumes large proportions of the budget spent on conservation, not always to the desired effect (Buxton et al. 2020). There will often be a trade-off between the cost of a monitoring method and the quality of the information it provides – if a low-cost method provides noisy data that does not allow trends to be detected robustly, it will be of little use for decision making and any resources invested will be wasted. On the other hand, an overly expensive and complex monitoring scheme may consume a lot of precious resources to produce data that exceeds what is required to achieve the management goals. There is wide spectrum between both extremes, and a thoughtful selection, implementation and validation of monitoring approaches can produce (relatively) cost effective monitoring programs that would be sustainable in the long term (Legg & Nagy 2006; Jones 2011; Buxton et al. 2020).

It is essential to understand the long-term costs and benefits of monitoring. A monitoring program may be costly to set up, and it will take some time for it to get established and optimized. The benefits of the data it generates may not be immediately evident, but the value of the data will increase over time as our understanding of the entire system improves and is translated into management.

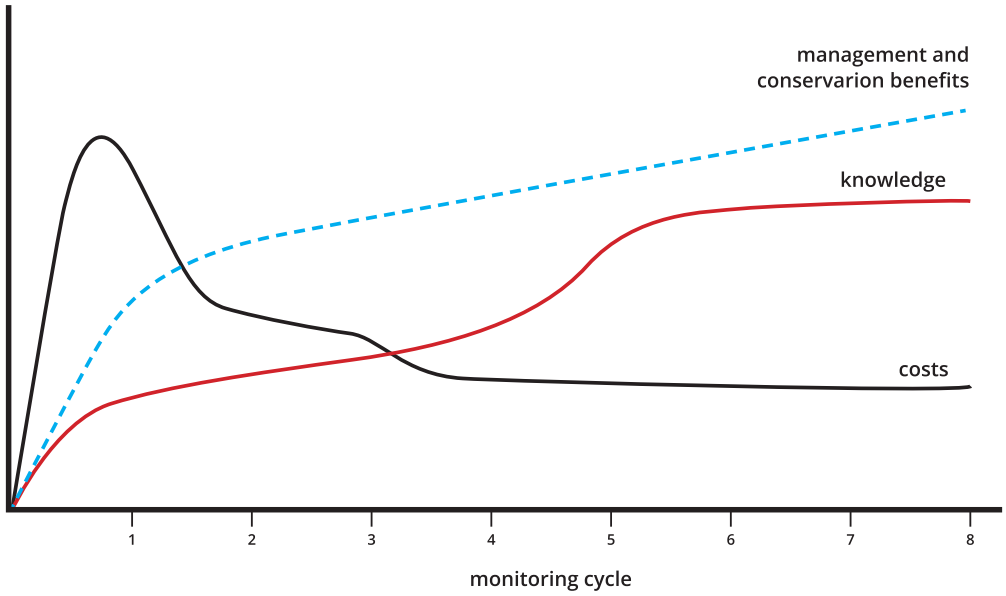

Figure 1: Establishment of a large carnivore monitoring program has a high up-front cost. While a comprehensive monitoring program will soon generate very important data for conservation and management (particularly if no systematic monitoring existed before), the real value is in long term accumulation of knowledge, and long-term conservation and management benefits as our understanding of the system improves. Costs should decrease as monitoring gets established and optimized, and initial costs will be offset by long-term gains (after (Dalton et al. 2024a).

It must also be understood that large carnivore monitoring is a long-term proposition that will exceed personal careers and should be able adapt to changes in methods, society, and environment. The only way for it to make sense is to ensure long-term continuity, so while an initiation of a monitoring program may be done through project funding, its continuation should receive systemic funding at a national level. For that to happen, a monitoring program should be founded in a national management plan for a large carnivore species and included in obligations of appropriate government bodies or governmental organizations.

At the time of writing of this handbook, there was still much to be desired in monitoring of large carnivores in the Dinaric –Balkan - Pindos area. We hope that this handbook will help change this in the future.

Concepts and definitions

3

3 Concepts and definitions

To be in sync with concepts and definitions in the contemporary literature, we use here the recent definitions provided in (Dalton et al. 2024b) and LCIE Monitoring Standards for Large Carnivores (Marucco et al. 2024), modified and adapted for the purpose of this book where appropriate.

Accuracy of estimate: Refers to the closeness of the estimate to the true population parameter we are interested in. It will be influenced by the sampling design and implementation, the amount of collected data and the choice of data analysis methods. See also: **bias, precision, estimate**.

Adaptive Management: A systematic approach to enhancing management policies and practices by learning from the outcomes of existing programs.

Area of Interest: Represents an area, habitat, environment, or ecosystem where monitoring occurs.

Bias: a systematic error or tendency to consistently overestimate or underestimate the true value of a parameter. Bias can occur because of a flawed sampling strategy (e.g. large spatial or temporal variations in sampling effort when estimating abundance or distribution), methodological problems in laboratory analysis (e.g. incorrect identifications of individuals from noninvasive genetic samples), or flawed data analysis (e.g. selection of an inappropriate statistical model or data analysis approach). Bias can be particularly problematic because it can skew the results in a consistent direction, leading to misleading conclusions. See also: **accuracy, precision, estimate**.

Conservation Goal: A specific target (trends, abundance, distribution, maximum inbreeding etc.) identified for achievement in conservation planning.

Conservation Obligation: Legally mandated conservation management actions, such as species monitoring or reporting to legislative bodies.

Conservation Outcome: The result of a management action aimed at conservation goals.

Estimate: for the purpose of this document, estimate is the best approximation of a parameter based on the obtained data. In estimates of animal abundance estimates consider detection probability and produce a measure of abundance with a level of precision. Various abundance estimators involve counting animals divided by an estimated probability of detection, such as Capture-Mark-Recapture or occupancy modelling.

Good Governance: Refers to principles such as legitimacy, transparency, accountability, inclusiveness, fairness, connectivity, and resilience, which facilitate positive management outcomes.

Home Range vs. Territory: Home ranges are confined areas where non-nomadic animals conduct daily activities, while territories are defended against conspecifics. Wolves and lynx exhibit high territorial behaviour, while bears do not.

Index: A minimum count of individuals or their signs constrained by the potential influence of changes in true abundance or the relationship between the index and true abundance.

Mature/Adult Individuals: Individuals capable of reproduction due to physiological and behavioural factors, particularly relevant for monitoring large carnivores.

Monitoring: The cyclic process of gathering information about a population parameter (such as the abundance or distribution of a species) to assess the state of the system and draw inferences about changes over time.

Monitoring Programme: An integral part of a conservation or management framework aimed at achieving conservation objectives through ongoing activities.

Monitoring Cycle: A single monitoring interval encompassing the entire routine from fieldwork to data analysis and archiving.

Objective of a Survey or Monitoring Program: Explicitly stated goals aimed at estimating the status and change over time of attributes such as distribution and abundance of large carnivore populations in Europe.

Precision: Refers to the agreement between measurements in multiple tests. Random error or imprecision is quantified by calculating the standard error of duplicate measurements.

Proxy Indicator: An indirect measure providing information about an indicator of interest.

Quality of Estimates and Indices: Estimates are considered higher quality than indices, but they are more expensive and require robust sampling designs. The quality of indices depends on their ability to reliably track changes in abundance across time, space, and management treatments, ensuring consistency in their relationship to true abundance.

4

Get it going! How to initiate and manage a large carnivore monitoring program

4 Get it going! How to initiate and manage a large carnivore monitoring program

In this section we leaned heavily on the "Framework for monitoring biodiversity in protected areas and other effective area-based conservation measures (OECMs)" (Dalton et al. 2023, 2024a). The publication provides an excellent framework for setting-up and running a monitoring program but has been written with many species and many different circumstances in mind. We used the concepts of that publication and adapted them to the specific requirements of monitoring of large carnivores in the Dinaric-Balkan-Pindos region.

While there are many parameters that can be monitored, we will focus in these guidelines on the ones identified by different stakeholders as the most important for the Dinaric-Balkan-Pindos region: **abundance and distribution for all three species, genetic conservation status in lynx, and hybridization with domestic dogs in wolves.**

Each large carnivore species has its own specific monitoring requirements and challenges, but there are several things that are in common. First, all large carnivores have **large spatial requirements**, which inherently means that monitoring will have to be large-scale to be meaningful. In the Dinaric-Balkan-Pindos setting this will usually mean that a monitoring program will as a minimum need to be considered at the level of the species distribution within a country. However, there will often be benefits if coordination or, even better, **collaboration between neighbouring countries** can be achieved. Monitoring should ideally be performed at the population level (Linnell et al. 2008a), but in the politically fragmented landscape of the Dinaric-Balkan-Pindos region this may be difficult to achieve in practice. Still, the minimum that the different countries should strive for is to coordinate the methods and effort, to make the results as compatible as possible, and to be able to draw conclusions about the monitored parameters at the population level.

Second, **large carnivores are flagship species** and usually receive considerable amount of public attention. The upside is that it is often possible to get direct help in monitoring through citizen science, and relatively easy to get media attention and attract "citizen scientist" collaborators. The perception of "too many" large carnivores easily develops based on frequent sightings of the same individual, isolated damage incidents, or, most concerning, attacks on humans. Social media's instant amplification and media's sensationalisation can fuel public anxieties, potentially leading to uninformed political decisions regarding population control. Conversely, advocacy groups may counter with claims of population vulnerability and endangered status. These conflicting narratives often dominate discussions surrounding large carnivore management. However, robust, documented population data can serve as a neutral foundation for informed decision-making. Establishing reliable population size and trend information can increase public acceptance of management strategies by alleviating uncertainty. The downside is that all results are usually heavily scrutinized, deficiencies criticized, any flaws exposed, and, if the worst comes to worst, results doubted or even contested. This means that any monitoring scheme requires full **transparency**, must be **scientifically sound**, and must be **communicated appropriately** to the public and the key interest groups. Directly involving the key interest groups in the monitoring can be of major

benefit in providing transparency and improving acceptance of the results.

All of the above means that **integrity** of the monitoring is absolutely key for a monitoring program to reach its goals. **The results of monitoring are only useful if decision makers, the key interest groups and the wider public believe them**. Large carnivores often cause considerable conflicts in any society (Rode et al., 2021) and are as a result often heavily politicized. If monitoring is perceived as biased, influenced by politics, scientifically poorly grounded or inadequate, any management decisions based on it will be perceived the same.

The framework proposed by (Dalton et al. 2023) consists of four phases: a) **preparatory** phase; b) **conceptual** phase; c) **implementation** phase with periodic interim evaluation guiding adaptive management; and d) periodic **re-evaluation** (Figure 1).

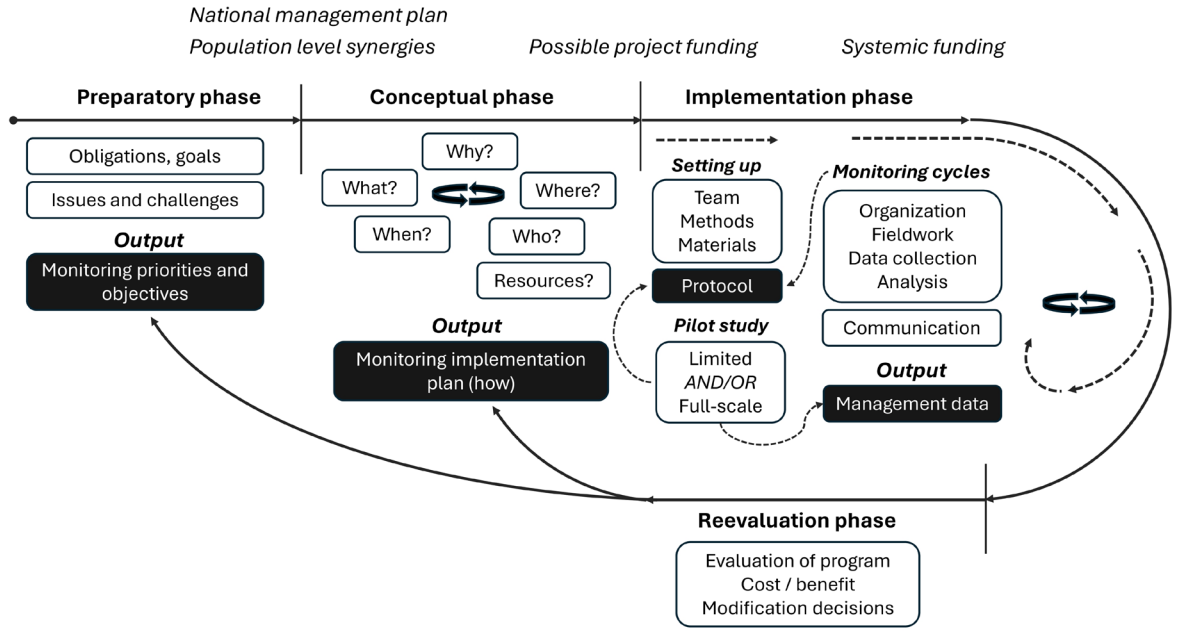

Figure 2: Four phases of framework for establishing a large carnivore monitoring programme (adapted from (Dalton et al. 2023)*. The preparatory phase provides an initial assessment of legal obligations, goals, issues and challenges, which leads to definition of the monitoring targets. In the conceptual phase the key interacting questions are addressed to develop a monitoring framework defined in an implementation plan that provides the basis for practical implementation. Implementation phase starts with initial setting up and development of the monitoring protocol, followed by a pilot study to test workflows and assumptions and modify the protocol. Monitoring is done in cycles that produce management data, and periodically re-evaluated. Monitoring should be grounded in a national management plan for the target large carnivore species and should look for population-level synergies with other countries sharing the same population. While the initial phases can be developed through project funding, the monitoring cycles should be systemically funded at the national level.*

An effective large carnivore monitoring program will correspond with the management goals and obligations and will be adjusted to natural and cultural specifics of an area. In the **preparatory phase**, a review of the management needs and obligations should be conducted, resulting in a monitoring statement of purpose. The **conceptual phase** addresses the basic key questions about the practical implementation of the monitoring program. The result is an implementation plan based on the understanding of the technical, methodological, and logistic considerations. A pilot study or a test run is conducted in the first cycle of the **implementation phase** to revise and optimize the implementation plan prior to the repeating

cycles of monitoring. Each monitoring cycle will produce data for management decisions. Detailed **re-evaluation of the program** occurs after a predetermined number of cycles to adjust the program to new methodological developments or changes in circumstances.

4.1 Preparatory phase: identifying the need, understanding the background, getting support

The main goal of the preparatory phase is to gather background materials and draft a **monitoring statement of purpose**, outlining and prioritizing the key objectives of the monitoring program.

Setting up a large carnivore monitoring program is a strategic decision-making process with long-term implications for conservation and management. **Sufficient time should be dedicated to the preparatory phase** to ensure the program's effective conceptualization and to identify management goals and challenges. These have ideally already been included in a pre-existing management plan, but if such a plan has not yet been produced, these topics will need to be thoroughly researched and identified. Background data and existing research about large carnivores in the area needs to be collected. Best practice examples from similar circumstances (neighbouring countries, similar landscape, similar conservation issues) should be collected and analysed. Decisions about the design of the actual monitoring should be informed by the information gathered in this phase.

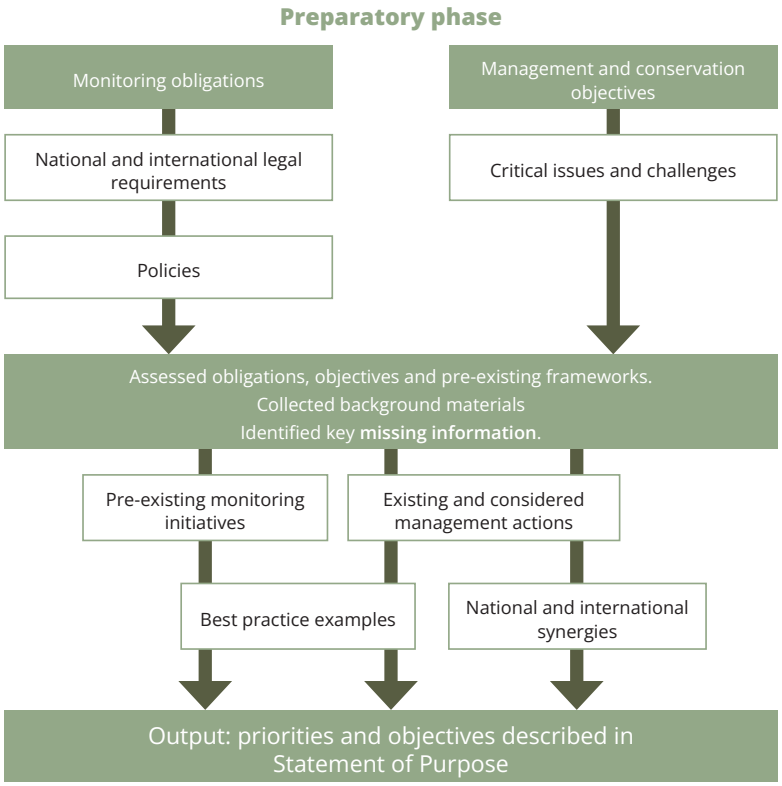

Figure 3: Preparatory phase. To develop an effective large carnivore monitoring program, we need to first obtain and document the management needs and objectives, national and international obligations, possible synergies with existing national and international monitoring programs, and specific challenges that the monitoring should address (adapted from (Dalton et al. 2023, 2024a).

4.1.1 International and national legal requirements

Because of their vulnerability and flagship species status, conservation of large carnivores is typically considered in national legislature, and international agreements and conventions. All European large carnivores are listed in the Bern Convention (Bern Convention Standing Committee 1979), the signatories of which include all countries in the Dinaric-Balkan-Pindos region. They are also listed in the Habitat's Directive (Council Directive 92/43/EEC) which is binding for all EU Countries, but which many non-EU countries are also implementing in their national legislature. Each country will typically have specific provisions for large carnivores in its national nature protection legislation, and frequently also in legislature concerning agriculture and natural resources utilization. This information will establish a binding basis for the monitoring program and will usually already be compiled in national management strategies for large carnivore species, if they exist.

4.1.2 Baseline assessment – what is already done and known

Even if there is no systematic monitoring in place, **there will usually be various country-level or site-level data about large carnivores available**. These data may be a result of research projects, a part of hunting or agricultural statistics, or collected sporadically or systematically by various institutions. They may include mortality records, harvest bags (if the species is legally hunted in the country), records of large carnivore damages, or similar data. Understanding completeness, availability, accessibility, and the methods behind such data can prove very important, as some of it may be systematically included in the monitoring program or help in framing it in the conceptual phase.

4.1.3 National and international synergies

Monitoring of large carnivores should be **ideally implemented at the population level** (Linnell et al. 2008a), which means that the larger picture should be considered when developing a national or an area monitoring program. Ideally, monitoring activities should be coordinated between neighbouring countries to obtain population level monitoring data. However, as a minimum, any existing monitoring program in other countries sharing the same large carnivore population should be examined and understood in this phase, and efforts should be made to coordinate new efforts with any pre-existing monitoring schemes. Examples would include using the same criteria when collecting occurrence data, using compatible genetic markers when collecting genetic data, using similar approaches when designing capture – mark – recapture abundance estimates etc.

4.1.4 Specifying conservation objectives, understanding challenges and opportunities

A monitoring program must be **designed based on desired conservation outcomes** and consider **specific challenges in a certain country or region**. Typically, all this should already

be included in a management plan if it exists. As a part of the preparatory phase, these desired conservation outcomes should be evaluated and clearly stated, as well as the monitoring data that would be required to achieve, verify, and maintain them.

The presence of large carnivores invariably carries social challenges through their impact on human society either directly through property damage and effects on people's livelihoods, or indirectly through (rational and irrational) fear for personal security. There can also be a considerable societal conflict between stakeholders with diverging positions regarding these species. On the other hand, large carnivore presence can also have positive impacts and provide opportunities through tourism and increased value of nature. A monitoring program should be designed in a manner that the data it generates helps in addressing the challenges and allows capitalizing on opportunities. Both aspects should be clearly understood in the preparatory phase.

4.1.5 Output: Statement of Purpose

A statement of purpose should briefly and clearly explain goals and objectives of monitoring. It should narrow down to specific indicators that would need to be monitored and explain the reasoning for why the data is needed, and how it addresses the management and conservation needs. This statement must align with the management plan's vision and objectives, if one exists, serving to communicate monitoring goals to stakeholders, local residents, and authorities. This statement will be the foundation for the conceptual phase, and the detailed plan will be built on.

4.2 Conceptual phase: clarifying objectives, defining means, estimating required resources

The conceptual phase of a large carnivore monitoring program is based on the overall goals and objectives recognized and stated in the Statement of Purpose as the result of the preparatory phase. The key in this phase is to provide concise answers to the key questions:

- **Why** do we want to do the monitoring?
- **What** will be monitored?
- **Where** will monitoring take place?
- **How** and with which methods should we monitor?
- **When** will monitoring be conducted?
- **Who** will be involved in the monitoring program? and
- What are the **required resources** to conduct monitoring?

The answers to these questions should interlink, and different scenarios and versions (technical variations, timing, cost frames…) can be considered and debated internally. Any existing national or international monitoring activities targeting the same large carnivore population that were identified in the preparatory phase should be debated and efforts should be made to meaningfully coordinate the developing monitoring program with them.

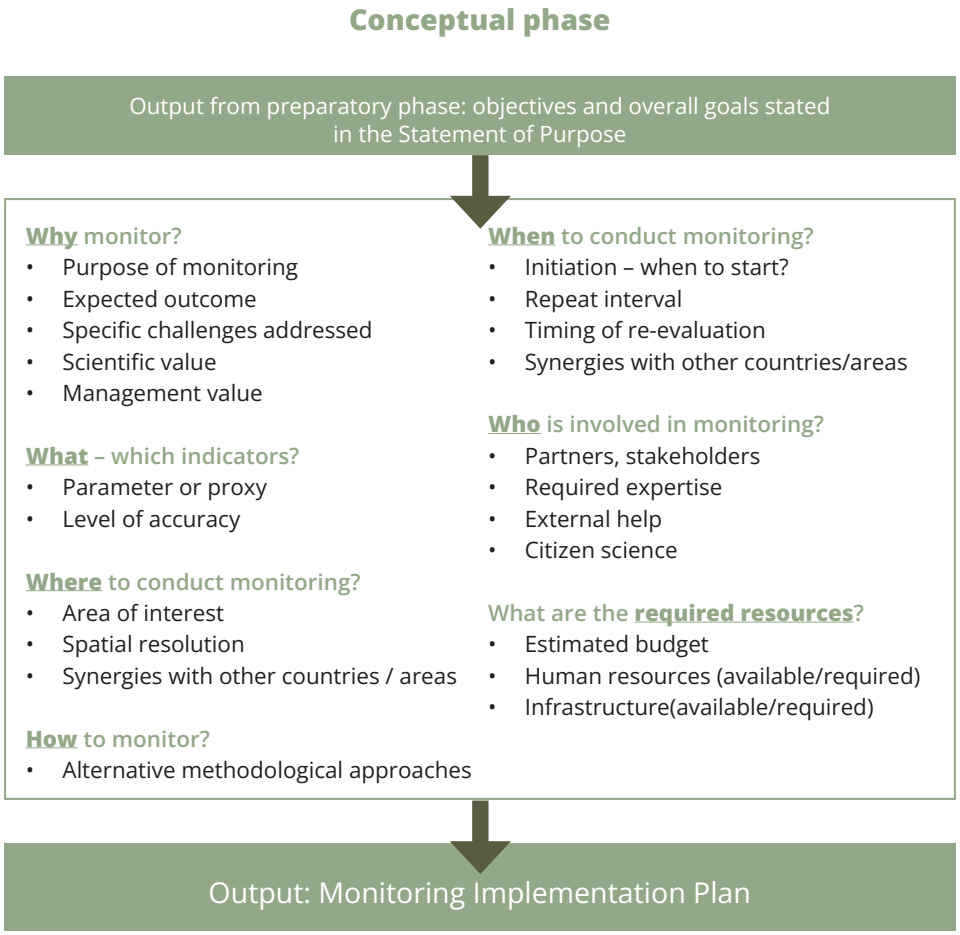

Figure 4: Conceptual phase. The conceptual phase builds on the statement of purpose from the preparatory phase. It considers management goals, particular needs and challenges, possible synergies, and available human and financial resources for the large carnivores monitoring program. This analysis determines the best scope for the program. Adapted after (Dalton et al. 2023, 2024a).

4.2.1 Additional help in conceptualizing a large carnivore monitoring program

There are additional tools (worksheets, guides, rating systems...) available in the annexes of the IUCN publication "A framework for monitoring biodiversity in protected areas and other effective area-based conservation measures" (Dalton et al. 2024a), which is freely available online. We follow the same logic in this handbook, and we encourage the use of these tools when working through the conceptual phase of a monitoring program development.

4.2.2 Why monitor?

The answer to this question will be based on the management and conservation targets, as well as the particular challenges that need to be addressed through management (e.g. conflicts with agriculture or hunting interests, concerns/fears of local residents etc.). It should **outline the use of the results** for management and conservation of the large carnivore species in the target area. The need to monitor is often also a legal obligation.

There are many different uses for monitoring results. One of the main purposes of a monitoring program is to provide evidence-based management and conservation (Sutherland et al. 2004) and help determine management effectiveness (Lindenmayer & Likens 2010). They guide adaptive management and can be used in outreach and educational purposes. They can also help resolve conflicts between stakeholders that are based on convictions about the status of large carnivore populations when hard data is lacking. An example of that would be abundance. In the absence of reliable data on the number of large carnivores living in an area, strong opinions can develop, leading to increased conflicts between various stakeholder groups and managers.

4.2.3 What to monitor?

The decision which of the population indicators or their proxies a monitoring program should target will be based on the answers to the "why" question and the available resources. The **indicators** are the parameters that are directly relevant for management and conservation goals and can be recorded and documented in a time series. If an indicator is too difficult or expensive to track, we can opt to track a **proxy** – a measurable quantity that can be used to infer the status of the indicator of interest. An example would be true population abundance as an indicator and counting of tracks or visual observations over an area, if done systematically, could be used as a proxy. The main challenge of using a proxy is to ensure that it accurately represents the status of the indicator we are actually interested in.

There are several indicators we typically wish to monitor in large carnivore populations, which will be covered in the "species focus" chapters that follow. Abundance (the number of animals) and distribution are often among of the most desired indicators for monitoring in all three large carnivore species. An important parameter for wolf is hybridization with domestic dogs, which is an important conservation threat for this species (Salvatori et al. 2020a). For lynx in the Dinaric-Balkan-Pindos region, genetic status, particularly inbreeding, is key, both for the reintroduced population in the northwest as well as for the remnant population of the Balkan lynx subspecies in southeast of the area. In the areas where large carnivore populations are expanding, monitoring of distribution is key to understanding the expansion process and providing timely management actions.

Apart from monitoring the biological characteristics of populations, an understanding of the interactions with humans is often needed for effective management. This includes a detailed record of anthropogenic mortality and any conflicts between large carnivores and humans (property damage including livestock damages, contact with humans including appearance of large carnivores in settlements, human injuries...). In some cases, it may also be beneficial to monitor the positive aspects of large carnivore presence – e.g. contribution to economy, direct and indirect, through large carnivore related tourism or hunting, if it is allowed.

The actual selection on what to monitor will critically depend on the importance of an indicator for management and conservation, and the resources required to establish appropriate monitoring. Both factors should be considered when setting priorities for a large carnivore monitoring program.

4.2.4 Where? The spatial context of monitoring

Developing an effective large carnivore monitoring program will require understanding the area of interest (where monitoring would take place), sampling approaches and statistical design of data collection.

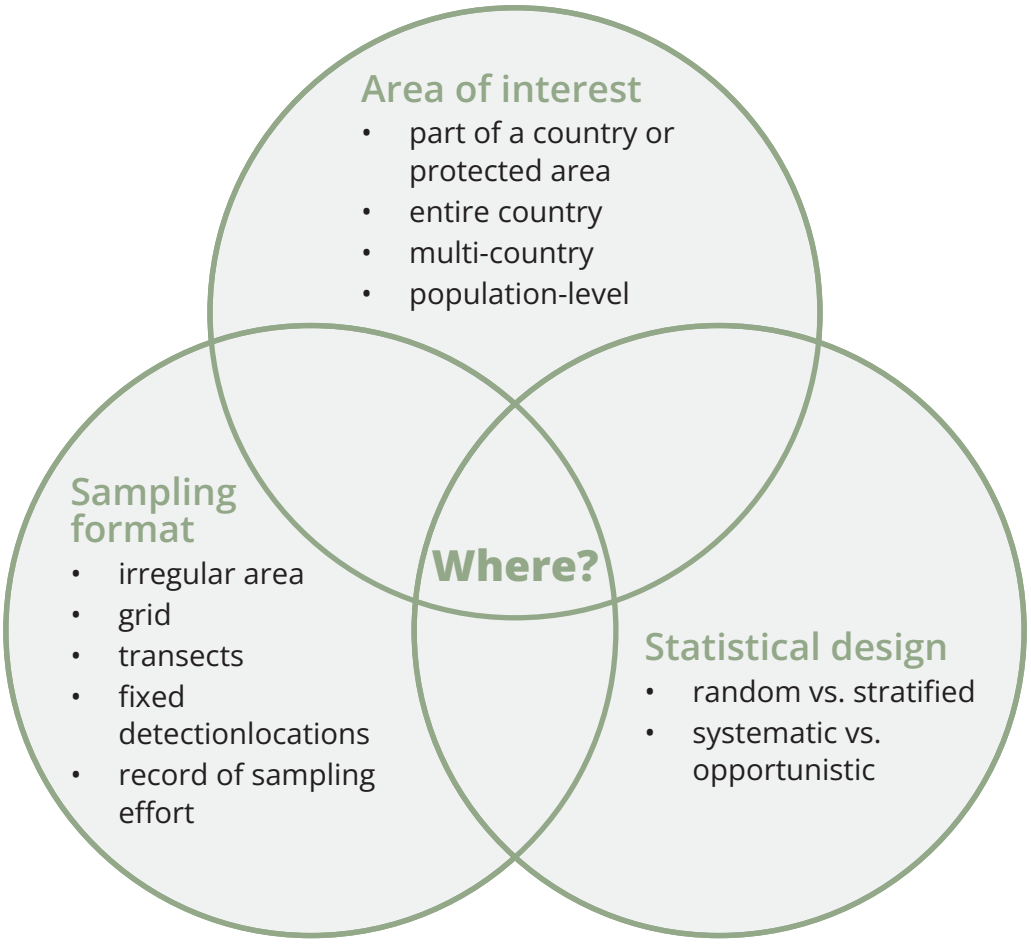

Figure 5: The spatial aspect of a large carnivore monitoring program for a certain indicator will depend on three critical considerations: the area of interest, the sampling format, and the statistical design. Different options can vary in resources needed and risk factors and should be debated in the conceptual phase. Adapted after (Dalton et al. 2023, 2024a).

First, we should define the **area of interest** – where will the monitoring take place. All large carnivore populations in the Dinaric-Balkan-Pindos region span more than one country, but their range in most cases doesn't cover entire areas of individual countries. For example, monitoring abundance would be ideally done at the level of entire populations or at least management units (parts of population that can be considered demographically independent, usually due to linear barriers or habitat discontinuities). However, in reality a monitoring program will often in the best-case scenario cover individual countries, or even smaller areas (protected areas, administrative units etc.).

The spatial context of **sampling approaches** will vary with regard to the indicator we wish to monitor, the methodology applied, and the resources we have available. For example, we can use random systematic genetic sampling over an entire area of interest to estimate population abundance of brown bears or wolves, or a spatially structured design to estimate lynx population density with photo traps and spatially explicit capture recapture (SCR). Other approaches and other indicators will of course each require a specific sampling format.

The third important consideration is the **statistical design** – how to obtain a sample that would as closely as possible satisfy the assumptions of the analytical method we wish to use to estimate an indicator. Sampling approaches can be either random or stratified and can be done opportunistically (e.g. with citizen scientist and with little constraints on where and when to sample) or systematically (defined spatial distribution, define sampling period(s), etc.).

All three considerations overlap to a large degree, and several solutions may be possible that provide similar end results for the indicator of interest but may carry different implementation risks and require different resources. These should be debated to find the optimal solution for a long-term monitoring program.

4.2.5 How? Outlining the methodological alternatives

There are often different methodological approaches available for monitoring a certain indicator. It is important to understand and debate the alternative approaches and their pros and cons. While the details of the practical application of the chosen methods should be worked out at the beginning of the implementation phase, a review of the available methodological approaches at this stage will help both in the identification of the team required to implement the monitoring (the "who" question), as well as help with realistic estimates of required resources.

4.2.6 When? Temporal dimensions of a monitoring program

We distinguish monitoring, which must have a temporal dimension, from a survey, which reflects a snapshot of population characteristics at a single point in time (Schwartz et al. 2007b). However, besides the realization that monitoring by definition produces time series data, there are key principles that should be considered: timing of program initiation, timing of the cyclic monitoring events (sessions), and the duration of the entire monitoring program.

The **timing for initiation of a monitoring program** for a large carnivore species in the Dinaric-Balkan-Pindos region will usually be "as soon as possible". Few countries have anything approaching robust monitoring programs in place even for the most important indicators, so timing of the monitoring initiation will typically not be an issue. That said, there may be circumstances that would require precise timing of monitoring initiation, particularly when some major changes in the habitat or populations is planned, when it would be important to obtain the "baseline status" before such a change happens and follow the effects after the change. Examples include important infrastructural developments (e.g. highway constructions) that impact large carnivore habitat or reintroduction – population

augmentation actions to improve conservation status. The latter is important for lynx, where for example a "baseline status" study was conducted in Slovenia and Croatia before population reinforcement in project LIFE LYNX (Skrbinšek et al. 2019a; Slijepčević et al. 2019).

Timing of the cyclic monitoring events (sessions) needs to consider phenology of the monitored species and indicator, both regarding the timing of each session, as well as timing between sessions. For example, if we are to monitor population abundance and dynamics, we must be careful that a monitoring session includes just a single reproduction cycle – from the time we're able to detect the yearly offspring in the sampling, and until before the time that the next generation of offspring becomes detectable. Failing to do that, we severely violate the population closure assumption that is key to most capture – mark – recapture methods useful for abundance estimates. Also, if we're sampling just a part of the population in bears, which are not territorial, and we have a pronounced "edge effect" where animals are migrating in and out of the population during sampling, we may wish to limit the duration of sampling as much as we can to "temporally close" the population (Skrbinšek et al. 2019c).

In a practical sense, some of the most important limiting factors that will influence the timing of monitoring sessions will be financial and human resources. In many cases it will be too expensive to monitor resource-demanding indicators (for example population size) every year, and a longer period may be selected. Duration between monitoring sessions will largely depend on practicality but must still be short enough to provide a solid enough foundation for management. Shorter periods between sessions will allow managers to react quickly to any changes but will be more resource intensive and costly.

Another important issue is the **duration of the monitoring program**. This may be defined in the national large carnivore management strategy or action plan, if such exist, otherwise it should be defined in the conceptual phase and will typically depend on availability and predictability of financial resources. As one of the main goals of monitoring is to detect trends, a monitoring program should continue for sufficient time as trends of an indicator can only be accurately observed after a sufficient number of monitoring cycles (White 2019).

We should strive for **long-term, consistent, and well-documented biodiversity monitoring programs** since the value of monitoring accumulates over time. Issues with large carnivores are most likely not going to "go away", so while monitoring programs will have to be modified and adjusted over time and may have a formally limited duration, they should in practice continue. This means that such programs (with modifications and iterations) should often last for decades, surpassing individual careers and institutional lifespans. Since turnover in staff and changes in institutions, laws, or finances are inevitable, it's **crucial to plan for continuity from the get-go**. Keeping **thorough records of monitoring protocols and objectives** is essential to maintain consistency and continuity despite these inevitable changes.

Finally, EU member states need to report to the European Commission on the conservation status of habitats and species protected under the Habitats Directive every six years, according to the Article 17 of the Directive, so this dynamic should be also taken into account when planning a monitoring programme.

4.2.7 Who? Identifying actors and stakeholders

Implementing a monitoring program in practice will require a core team that will lead and coordinate the monitoring activities, support staff, stakeholders that will be directly involved and stakeholders that need to be informed. The people and organizations involved in the monitoring, both directly and indirectly, will ultimately determine the success of the monitoring program.

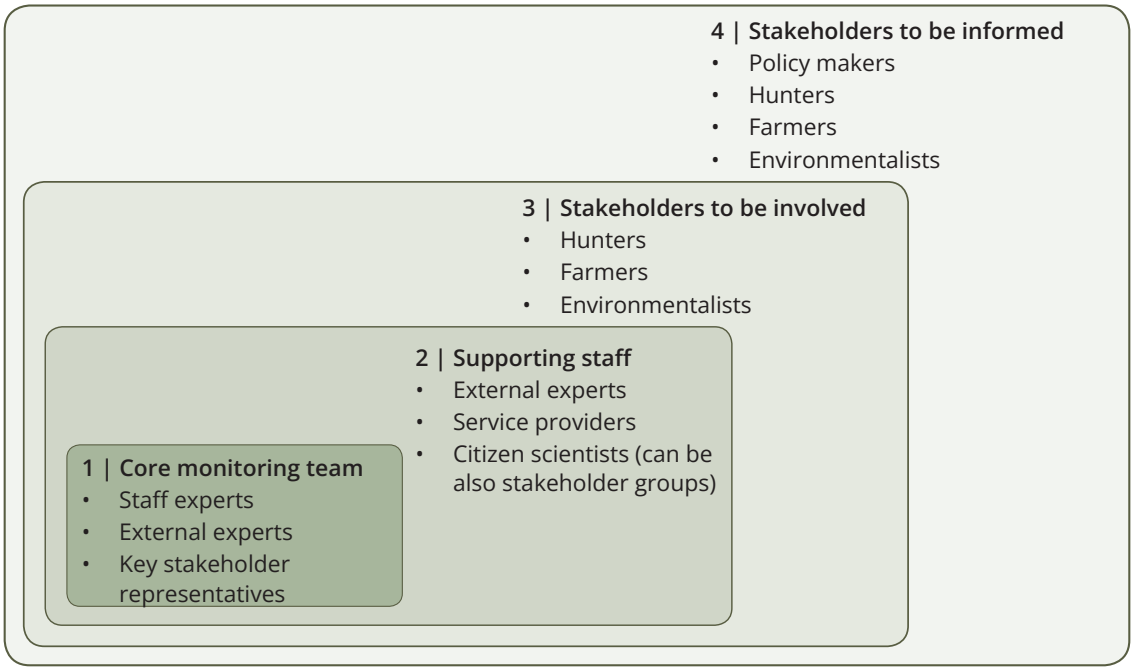

Figure 6: Potential participants in a large carnivore monitoring program. Adapted after (Dalton et al. 2024a).

The **core team** will guide the monitoring and make key decisions. It will typically be led by staff from the institution tasked with implementing the monitoring but may also involve experts from other institutions. The core team may also involve representatives from other stakeholders (e.g. hunters, farmers, environmentalists) that would get directly involved in the monitoring.

Supporting staff can be specialists providing particular expertise and may include scientists, GIS experts, IT experts, laboratory technicians or field crew leaders and intervention team members from different organizations (government institutions, universities, institutes, companies). It can also include representatives of different stakeholder groups or organizations that are can be included in the monitoring effort as citizen scientists (e.g. hunters, environmental NGOs, various outdoor enthusiasts like mountaineers or mountain bikers) or professionally (e.g. foresters, hunters, rangers).

Monitoring can benefit a lot if it is designed through active engagement of key actors and stakeholders, including governmental agencies, scientists, as well as the key interest groups. For the large carnivores the latter will often include hunters, but may also include farmers,

environmental NGOs, and other actors from the broader society. Hunters can be particularly valuable as they possess considerable traditional ecological knowledge, know their areas very well, and often spend a lot of time outdoors. Also, it is important to include the stakeholders sceptical about large carnivore management and conservation as such involvement can prevent conflicts and increase acceptance of results (Pocock et al. 2015). However, this shouldn't be done without thought, and managers of the monitoring program should acknowledge and address the underlying biodiversity conflicts (Young et al. 2013).

The main responsibilities of monitoring should be assigned to professionals and experts, but a lot can be accomplished through involvement of volunteers as "citizen scientists". While this requires a considerable amount of support measures (recruiting, training, motivating), it can result in a very strong support base for field activities that can more than offset the resources used in organizing these support measures. It also has additional benefits ranging from better visibility of the monitoring and education to better acceptance of monitoring results (Skrbinšek et al. 2019c)and possibly increased social acceptance of large carnivores by the key interest groups.

Intervention team members usually play a critical role in the systematic collection of certain types of data. Trained and obligated to report on their activities, they follow standardized protocols.

4.2.8 Identifying required resources.

The only way for a large carnivore monitoring program to succeed is if is supported by sufficient resources.

The first step is a realistic estimation of the **financial costs**. Some costs are obvious, but the others can be less obvious and hence missed in the conceptual phase, which can result in underfunded monitoring and seriously jeopardize the results. For example, it is quite obvious that financial resources would be needed for laboratory work if genetic monitoring is considered, but the effort required to organize field sampling is frequently underestimated, even if sampling is done by volunteers and/or organizations (e.g. hunters, foresters) that are operating in large carnivore habitat and willing to help with the sampling (personal observation by the author). It is crucial that the financial costs are thoroughly studied, debated, and understood, and that adequate financial support is ensured before any practical monitoring is attempted.

Another important consideration is the availability of **human resources**. While this can be often solved if sufficient financial resources are available, it may still prove to be a challenge to find trained personnel that is able to implement the monitoring activities in practice, particularly in the establishment phase of the monitoring program.

A consideration that should also not be ignored is the difference between the establishment phase and continuation in subsequent monitoring cycles. We can expect the establishment phase to generally require the greatest financial resources as the procedures get established and problems and mistakes in the implementation plan get kicked out (Dalton et al. 2024a).

This has been reported in complex ecological monitoring programs (Lindenmayer et al. 2012), and fits with the experience of the authors.

An **underfunded monitoring program has a high likelihood of failure**. If the estimation of the required resources in the conceptual phase indicates that the available resources are insufficient to achieve the desired monitoring objectives, an adaptation of monitoring objectives, indicators or methods will be required. It is also possible to look for other resources or synergies with other initiatives. Sometimes it is possible to obtain the funding for the initiation phase, which is the most resource-demanding, through specific funding instruments for nature conservation (e.g. EU LIFE program), EU cohesion funds (Interreg) or other national or international funding instruments, or even from private or public foundations. The initiation phase can also be done as a scientific project funded through national or international funding instruments for scientific research and development if the object of monitoring is of scientific importance and/or if the proposed monitoring applies novel, cutting-edge methods.

4.2.9 Output: Defined scope of the large carnivore monitoring program

Exploring and finding answers to the six key questions of 'why', 'what', 'where', 'when', 'who', and 'required resources' will allow us to frame the scope of the monitoring program. However, the implementation details that will allow the actual monitoring to proceed will need to be worked out in the implementation phase.

4.3 Implementation: from the first survey towards monitoring

The implementation phase is when the monitoring starts collecting data and providing the first results.

At the beginning **the infrastructure for monitoring needs to be set up**. The **core team** can be extended if there is expertise missing. A detailed study design should be elaborated, and a draft **monitoring protocol with a field manual** prepared which includes the sampling design and detailed description of methods both for data collection and analysis. **Tools and materials** needed for monitoring should be acquired. A strategy for involvement of **support staff** including volunteers should be prepared if such help is needed. Any **stakeholders** that should be directly involved in monitoring (e.g. hunters, forestry and/or nature protection services, NGOs...) should be brought onboard if that has not been done yet.

The next step is a **pilot study** where the monitoring protocol is applied in real-life. Depending on the methods, sampling design and objectives of monitoring this can be done either over a smaller area or immediately full-scale over the entire area of interest. A small-scale pilot study will typically be cheaper and allow for more alternative approaches to be tested, but it will take time and may not produce useful management data. A large-scale pilot study will be the first implementation cycle of the actual monitoring and may already produce the first useful data for management and conservation but will be inherently more expensive and

resource intensive. It will also have a higher chance of failure, which may jeopardize the entire long-term implementation of the monitoring program as an expensive failure may lead to retraction of financial resources. Higher financial and other resources should be planned for the pilot study than for regular monitoring cycles since mistakes and implementation problems should be expected at this stage. The ultimate decision on how to do the pilot study will ultimately need to be taken by the core team depending on circumstances, monitoring objectives and methodology.

The pilot study needs to be followed-up with a **thorough re-evaluation of the monitoring protocol**. In the pilot study, methodological and logistic deficiencies will become evident, mistakes will be made, and resources will be wasted. Results may turn out to be sub-optimal. Lessons learned in the pilot study should be studied, acknowledged, and solutions included in the updated monitoring protocol (manual).

After the pilot study and each monitoring cycle, results **should be communicated** to decision makers, stakeholders, and the general public. This shows how the monitoring contributes to large carnivore conservation and management, and provides the results needed for management and conservation to decision makers in an understandable form.

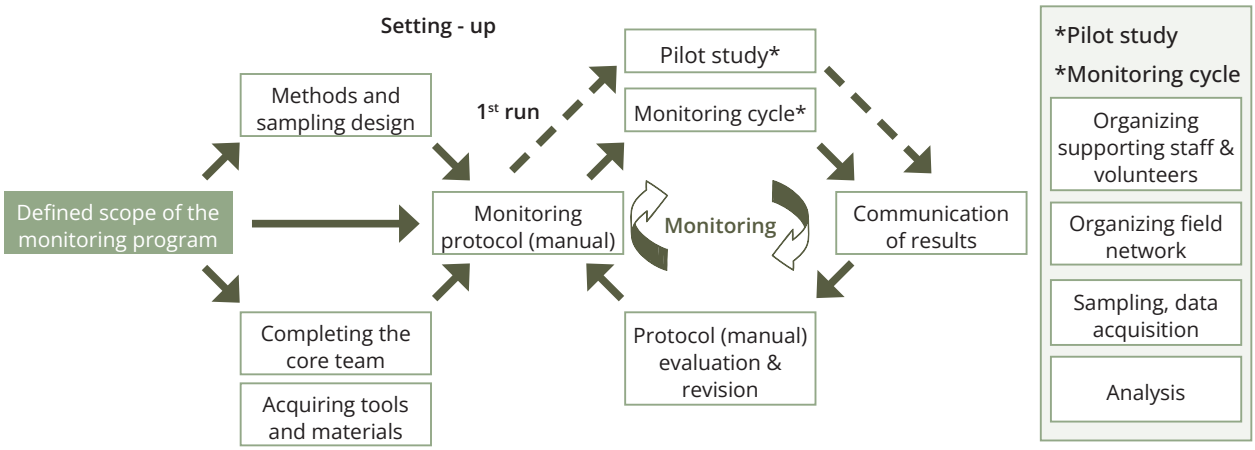

Figure 7: The implementation phase of a large carnivore monitoring program. Adapted after (Dalton et al. 2024a).

4.3.1 Detailing methods, elaborating sampling design and power analysis

For most indicators that we would wish to monitor, there is no universal methodology or sampling design that we could select "out of the box". Approaches that could be used would need to be tailored to the objectives of the monitoring program, but should also consider the available resources, target area characteristics, and characteristics of the large carnivore population we wish to monitor. The methodology section of this handbook provides descriptions of the most commonly used methods in monitoring of large carnivores, and the purpose is to provide managers with an overview of available methodologies. However, **specialist experts should be involved at this stage**, ideally with direct experience in the considered methodologies. Sampling design should be considered in collaboration with a statistician or analyst that understands the data analysis for the considered method, to ensure that the collected data will have appropriate statistical properties and yield acceptable results.

If possible, a **power analysis** should be done as a part of the sampling design. A power analysis is used to determine the probability of detecting an effect or difference if it truly exists in a given study design, or to understand how much field data needs to be collected to obtain the desired precision of an indicator estimate. In simple terms, it helps us assess the sample size needed to achieve a desired level of statistical power or estimate precision and should ideally be used to scale the field effort. The exact method to perform the power analysis will vary depending on the monitored indicator and the applied methodological approach.

If there are different approaches that are expected to provide similar results, the most cost-effective method should be prioritized. Still, care must be taken that the monitoring goals are not jeopardized in the desire to decrease costs.

4.3.2 Things will go wrong! Risk analysis and planning for contingencies

As Murphy's law states, **if things can go wrong, they will**. Particularly in the pilot study, the field protocols will not be optimized, equipment may malfunction, field personnel may not perform as expected, and the data collection may not proceed as planned. We should expect that, and plan for it! Pessimism at this point can prevent a lot of pain down the road!

A thoughtful review of the sampling design should be done to **identify risks (risk analysis)**:

a) **Check assumptions**, consider what would happen if they don't work out.
b) Try to identify possible **points of failure**. Debate "what if…" scenarios and try to find the ones that you think could possibly happen and would make your study produce poor results or even fail. Be pessimistic!
c) **Think of the worst-case scenarios** and work through solutions.
d) **Plan to monitor progress of the fieldwork in real time!** This can't be overstated. Monitoring activities are time-dependent, and for now it's impossible to go back in time to fix things. If something is not going as planned, you need to know about it! And you need to know about it when it's still time to fix it.

Use this to develop a **contingency plan**. The risk analysis and contingency plan should be an essential part of the monitoring protocol.

Box: Contingency planning for a survey of brown bear abundance in Slovenia and Croatia using non-invasive genetic sampling and capture-mark-recapture modelling.

The goal of the study was to estimate the number of brown bears in Slovenia and Croatia. The study was done within project LIFE DINALP BEAR (Skrbinšek et al. 2017).

Sampling of brown bear scat samples was planned between September and December 2015, and it included the entire bear range in both countries, approximately 20,000 km^2. We planned the sampling for autumn as the quality of samples and genotyping success rates were found to be higher (Skrbinšek 2020) and as bears tend to move less during that period because of hyperphagia. We also planned the sampling to be as short and intensive as possible to limit movement of bears in and out of the sampling area. A power analysis using mark-recapture simulations indicated that we should collect at least 3000 samples to have sufficient statistical power to obtain the desired precision of the estimate. We planned to include a large number of volunteers to have sufficient field resources. We focused on hunters as a motivated and knowledgeable stakeholder group, but also included foresters and forest service personnel, as well as some outdoor and conservation enthusiasts.

As we have done a similar study in Slovenia in 2007 (Skrbinšek et al. 2019c), we were confident that the laboratory analysis should not be a problem. However, the sample collection completely depended on the volunteers, and our main possible point of failure was too low volunteer support in the field, or even a boycott by critical stakeholders in some areas if the study was not presented correctly. As we planned to sample a very large area, hundreds of hunting clubs and many forestry districts would potentially be included in the sampling.

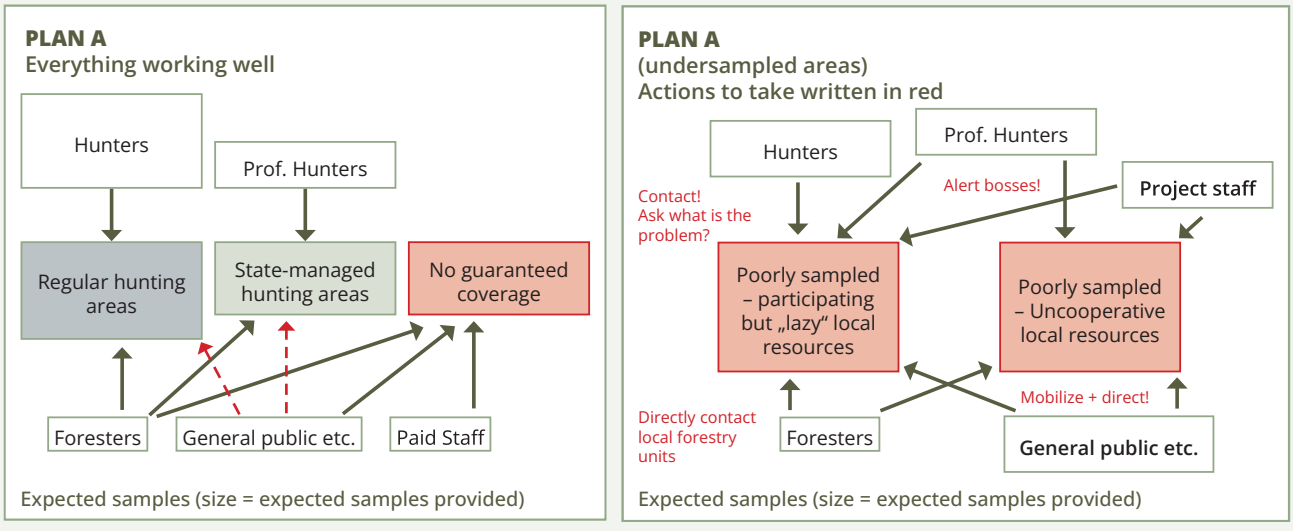

Figure 8: Actual slides shown to the project team at pre-sampling meetings. "Plan B" is contingency plan if insufficient sampling is detected in an area.

To minimize these risks, we planned and executed an information campaign which started six months before sampling. We published several articles in hunting magazines in both countries, talked to journalists from different media, organized dozens of workshops for hunters and forest service personnel, and spread the information through social media. We distributed nearly 20,000 sample collection tubes to the field before the start of the sampling.

Contingency plans for problems in local areas are presented in Figure 8. If sample collection was not going well in a local area, there would be attempts to motivate the local resources in increasing intensity. If this proved to be unsuccessful, the project team and a number of volunteers were on standby to "jump in" and directly sample the problematic areas. If the entire sampling would be going too slow, the contingency plan was to extend sample collection until early summer 2016 as the cubs of the year are still very small in spring and would likely not get sampled often.

The participants returned collected samples through regular post in pre-addressed, postage-paid envelopes. All received samples were entered into an online database on a nearly daily basis in both countries, and automatically mapped. Maps were regularly checked to monitor sampling success and see if sampling was progressing as expected.

In Slovenia, sampling mostly went without problems, and in the couple of areas where issues did occur, they were immediately detected and solved with a few simple phone calls. In Croatia there were some areas where we didn't get sufficient participation from local volunteers, and after some failed attempts to include and motivate local resources, these areas were sampled with project staff and volunteers from elsewhere.

We ended up collecting 4687 samples in three months over the entire 20,000 km^2 area, 56% more than the target. We received samples from 962 different people. The study was a complete success and it is being repeated in the same style in 2024.

4.3.3 Elaborating the monitoring protocol, preparing the field manual

A detailed monitoring protocol should be elaborated and described in a "field manual". The manual should describe how the monitoring is to be carried out in enough detail that a monitoring session (cycle) can be implemented in practice. It should be seen as a "living document" and updated as needed. The contents will vary with context, so here we'll present a brief possible outline:

a) **Goals and methods**. Goals of the monitoring and the methods that will be applied to reach these goals should be clearly stated.

b) **Sampling design**. The sampling design should be clear about the sampling area (where exactly will the sampling take place), timing of sampling (when the sampling starts and stops), who will do the sampling, how much data or samples should be collected (sampling target), and what is the expected dynamics of data collection. The last item will be important for monitoring of sampling progress.

c) **Description of pre-sampling activities**. A description of the activities that need to be done before a sampling cycle, with a timeline, should be included in the manual. This can include checking of the equipment, such as camera traps, for proper operation or preparation of material for genetic sampling but should also include communication with stakeholders. If volunteers or stakeholder groups are to be directly included in monitoring, a considerable amount of time and work should be planned to get them activated, motivated, and supplied with tools and materials to do their work.

d) **Sample/data collection and storage**. If samples or systematic data from camera traps are being collected, the manual should detail how and how often the samples or data will be collected from the field, where they will be stored, how will the accompanying data be recorded and stored, and who and how often will enter the data into the database. The typical problems are that samples remain with the field personnel until the end of the sampling or that they don't get recorded in the database regularly (impossible monitoring of sampling progress), or that they are inappropriately stored (lower analysis success). If just opportunistic data is being collected, it should be defined how these data are communicated, how and where they are stored, and who and how often will check the data quality as they arrive. Real-time backup options should be provided for all data.

e) **Monitoring of sampling progress**. The manual should detail how the sampling progress will be monitored. An expected trajectory of sample or data collection should be elaborated in the sampling design (how many data points or samples per time unit and spatial unit are expected). Actual collected samples or data should be checked against this expected trajectory during sampling or data collection. If any significant deviations are detected (not enough samples/data in an area or overall, oversampling in some areas etc.) this should be investigated, and a contingency plan activated if necessary.

f) **Risk analysis** and **contingency plans** should be elaborated (see above) and included in the manual.

g) **Laboratory and data analysis**. A description of laboratory analyses (e.g. which genetic markers are used if genetics is done and how they are analysed) or data processing (e.g. data quality protocol for observation data, method for lynx identification from photos) should be included. Data analysis and production of results (mark recapture modelling, preparation of distribution maps, etc.) should also be described.

h) **Communication**. Communication channels and workflows should be described. How, when and to whom the results need to be reported should be defined in the manual. It should also include (if needed with regard to the monitored indicator) a strategy for communicating results to the key stakeholders and the general public.

i) **Data quality and consistency**. Making sure of high data quality is of critical importance. Field forms (including labels on sampling containers) and datasheets should be carefully designed. Protocols for data entry into digital format should be elaborated, and appropriate databases prepared. Minimal standards for metadata

should be presented and explained in the manual. This includes who collected the sample or datapoint, exact location (GPS coordinates), date and time, and any other required method-specific or site-specific information, including field remarks and notes. It is also good practice to record who entered the data into the database. The collected data has to be relevant not to overburden the field personnel with needless extra work. The data that are found not to be usable in analysis should not be collected in the future cycles.

The **manual should be updated regularly**, particularly after the pilot study, but also after completion of each monitoring cycle if new lessons are learned or if circumstances change. While laboratory and data analysis methods descriptions may be more general or vague before the pilot study, after the pilot study (when there is direct experience) they should be described in detail.

4.3.4 Transboundary and population-level monitoring: looking for synergies

In the politically fragmented landscape of the Dinaric-Balkan-Pindos region, no single country hosts an entire population of a large carnivore species. As populations span the borders of several countries, **an effort should be made to do monitoring at the population level** (Linnell et al. 2008b), or at least to try to establish **transboundary cooperation** with neighbouring countries. The direct benefits of that are better and more relevant results and possible better utilization of resources. Indirect benefits, particularly forming of transboundary networks of experts, may be just as important and may help pave the way for eventual joint transboundary management of large carnivore populations.

4.3.5 Pilot study

There are two ways to do the pilot study: small scale on a limited, easily accessible study area, or full scale, which would basically already be the first cycle of the actual monitoring. There are numerous reasons why to go with a small-scale pilot study. First, things will go wrong, and it is almost certain that some mistakes and poor decisions were made when making the monitoring design. It is much cheaper to fail in a small pilot area than in a full-scale pilot. Second, the monitoring team can build up experience and optimize the field manual without burning too many resources. This can prove to be much more cost-efficient in the long run than the alternative. And, last but not least, an expensive failure in a full-scale pilot study can ultimately scare the decision makers away from a comprehensive monitoring program, deeming it too expensive or impossible to implement.

There are, however, advantages to going with the full-scale pilot study, the main being that it may already generate the real monitoring data which may be urgently needed. Another reason is that there may be political will and the related financial resources available for a full-scale study that may not be there in the future, and a successful proof-of-concept study could ensure support for a long-term monitoring program. Regardless of the reasoning, the decision to go with a full-scale pilot study should not be taken lightly. Considerably higher

resources should be planned than what would be estimated for ongoing monitoring cycles after it. A very thorough risk assessment should be done, and detailed contingency plans with sufficient financial and other resources to implement them if needed. Intensive involvement of experts from other countries that have already successfully used the same methodology in other areas is highly advisable as they could bring experience and increase the chances of the study's success. However, it should not be a-priori assumed that the workflows and approaches that worked in other areas would be directly applicable, and modifications to local circumstances should be made using the local knowledge.

4.3.6 Fieldwork

4.3.6.1 Preparing for fieldwork

Each monitoring cycle, including the pilot study, will need preparations before the field season starts. All these should be detailed in the monitoring manual. These will typically include obtaining and preparing the tools and materials, as well as assembling and training field personnel. If volunteers or stakeholders are expected to participate in the fieldwork, enough time and resources should be planned to recruit them, provide them with materials, tools, and written instructions for the fieldwork, and motivate them for participation. In large studies this can require many months and full engagement of several team members.

4.3.6.2 Running a field season

At the beginning of a field season, all participants should have everything they need to do the fieldwork and know exactly what they should be doing. Sampling and data collection should be monitored as closely as possible, according to the plan set out in the monitoring manual. Problems should be handled immediately, and preparations should be made to implement contingency plans if things aren't working as they should. The entire monitoring team should be fully engaged, as there will typically be a very small margin for mistakes. If sampling or data collection is done with volunteers, they should be motivated and engaged throughout the sampling season (phone calls, social media, emails etc.).

4.3.7 Data analysis and interpretation, reproducible research

Data analysis should be done by a competent expert with considerable knowledge of statistics and experience in similar analyses. Ideally, the same expert was already involved in the study design, and the data was collected in a manner and fits the assumptions of the analytical approaches that were already considered during the study design stage. However, as things can go wrong during the fieldwork, expertise is needed to evaluate if the data is appropriate for analysis with the considered methods, and data analysis methodology should be modified to fit the actual collected data if necessary.

We strongly encourage that the reproducible research paradigm (Gentleman & Temple Lang 2007) is followed during data analysis. The idea of reproducible research is to integrate the computations and code used in data analyses, methodological descriptions, simulations, and

so on with the documents that describe and rely on them. This integration allows readers to both verify and adapt the claims in the documents. Authors can check the entire analysis with other experts in the field, and easily reproduce the results in the future. The methodology used for the analysis can be consistently applied in future monitoring cycles.

There are excellent tools available for reproducible research in the main programming languages used for data analysis, R and Python. We would encourage use of tools that allow scripting and retain the full record of the analysis, including all analytical decisions and reasoning behind them, rather than use of point-and-click statistical programs or spreadsheet applications.

Interpretation is the process of understanding data and results in the context of the bigger picture. It should be done by the person (or persons) that did the analysis, in collaboration with local experts and members of the core monitoring team that were involved in implementation of the fieldwork. Any surprising findings should be scrutinized and re-checked for problems in data or analysis. As a rule of thumb, findings that strongly deviate from expectations are often caused by problems in data collection (poor statistical properties of data) or mistakes in data analysis and should be considered problematic until they are thoroughly re-checked and confirmed. Sometimes, however, the results may start making sense only after several monitoring cycles have been completed.

4.3.8 Data policy

Data is precious, and gains value through time. Scientific research relies heavily on data, and the ability to share and reuse it is crucial for advancing knowledge. In the context of large carnivore monitoring, time series data that is accessible across borders can provide crucial insights for long-term, transboundary management and conservation of these species. However, poor data management practices can hinder this progress. To address such challenges, the **FAIR data principles** were formulated (Wilkinson et al. 2016). FAIR stands for Findable, Accessible, Interoperable, and Reusable. These principles provide a framework for researchers to make their data more discoverable and usable by others.

Findability ensures data can be easily identified and located. This is achieved by using persistent identifiers (like DOIs) and rich metadata that describes the data content, creation process, and creators.

Accessibility guarantees that authorized users can access the data. This may involve depositing data in publicly accessible repositories or providing clear access procedures with minimal restrictions.

Interoperability allows data from different sources to be combined and compared. Standardized formats, vocabularies, and data models facilitate interoperability, enabling seamless integration with other datasets.

Reusability empowers users to understand and repurpose the data for new research questions. Detailed metadata, clear licensing information, and provenance (data history) are essential for reusability.

FAIR data sharing in large carnivore monitoring benefits the entire research, conservation, and management community. It fosters transparency, reproducibility, and collaboration. By following the FAIR principles, managers and researchers can maximize the value of the data monitoring produces and contribute to a richer knowledge base.

A monitoring program should have a **written, well formulated data management policy** that follows the FAIR principles.

4.3.9 Communicating results – providing the data for management, informing the public

The conclusions of the biodiversity monitoring program should support management decisions and reporting obligations (Dalton et al. 2024a). All methods and results should be completely transparent and easily available to increase trust in the monitoring. In large carnivores, where there are always strong and diverging attitudes of different stakeholders, and frequently a societal conflict, it is of particular importance that stakeholders believe the monitoring results.

The usual way to communicate results is through **reports** and **scientific publications**, but these may be too technical for communicating the results to stakeholders. For this purpose, it is helpful to also produce a brief "**layman report**" where the monitoring approach and results are explained in an approachable language for a wider public to understand. A **press conference** after each monitoring cycle may also attract media attention, and the layman report can be used as a hand-out for the journalists. A layman report can also be useful for decision makers since they may not have enough expert background and/or time to read through a very technical report but must understand the results for the decision-making process.

If the citizen science approach is used for sample or data collection, particular consideration should be given to providing the results as feedback for the people that helped. They should be the first to know the results or should be at least notified immediately when the results are made public.

4.4 Re-evaluation phase

Although a basic re-evaluation and optimization should be done after each monitoring cycle, a general re-evaluation of the entire monitoring program should be planned at pre-determined cycles. This allows to reflect on strengths and deficiencies and allows for inclusion of new technological advances and funding opportunities to be included in the monitoring, helping it become more efficient in the future.

As monitoring will often be included in the national management plan for a large carnivore species, it makes sense to plan for the re-evaluation of monitoring together with revisions of the management plans.

5

Focus on methods – monitoring how-to

5 Focus on methods – monitoring how-to

In this section introduce the most important methods currently used for monitoring different indicators of interest for conservation and management of large carnivores. The target audience is managers and decision makers considering a large carnivore monitoring program, and the goal is to provide an overview of the methods without delving deep into technical details, but to provide a working understanding required to make appropriate decisions.

5.1 Monitoring mortality

One essential indicator that needs to be monitored in all large carnivore populations is mortality (Boitani & Powell 2012). This should accomplish two immediate objectives: a thorough understanding of the direct and indirect reasons for the death of each individual, and the relative frequency and importance of each contributing cause of mortality to the population as a whole. But the data and samples collected from dead animals also have a much more long-term value, which may in the long run help understand how large carnivore populations evolve and respond to environmental pressures.

While systematic detection of mortality may be challenging, the minimum that should be done is that each detected mortality should be systematically recorded, examined, and samples collected. This provides essential data that can allow us to better understand the direct human impact on a large carnivore population (Boitani & Powell 2012; Benson et al. 2023), but it can also be important for detection of diseases that could threaten the carnivore population, domestic animals, or humans (Boitani & Powell 2012; Kelly et al. 2021).

We will briefly cover the key steps in recording mortality cases of large carnivores, but the reader is encouraged to read the more thorough treatment in Boitani & Powell (2012).

5.1.1 Establishing the reporting network

All detected mortality of large carnivores should be systematically reported to the monitoring team, which should ideally be included in national legislature. If the population is legally hunted, there must be an obligation of the hunting organization and the hunter to report the killed animal to the monitoring team and enable examination and sampling. All traffic mortality of large carnivores should also be reported to the monitoring team through respective services (police, veterinary authorities, railway authorities, highway authorities, hunting organizations etc.), as should any other detected mortality. The major task of the monitoring team is to facilitate putting such a system in place by helping create legal instruments that would oblige public agencies and other organizations (e.g. hunters) to report mortality and establish a reporting network that would allow each detected mortality to be recorded and examined. The usual way of doing this is drafting a large carnivore management plan that includes these provisions.

5.1.2 Field-data collection at mortality sites

While not strictly necessary if the cause of death is obvious (traffic mortality, legal killing), a thorough examination of a mortality site should be performed if the cause of death is unknow or if there are indications of foul play (illegal killing). If foul play is expected, the site shouldn't be disturbed, and police should be notified to start investigation. In other cases, veterinary authorities should be contacted and asked for advice on how to proceed.

5.1.3 Clinical necropsy

If indicated (unknown cause of death, indications of foul play), a clinical necropsy should be performed. This should be done by a trained veterinarian at an appropriate facility. Financial and other resources should be planned for such eventualities.

Clinical necropsies should also be considered for cases of mortality where the cause of death is known. This may enable early detection of diseases, and detection of signs of inbreeding depression, which is an important issue both for the Dinaric lynx population as well as for the Balkan lynx (Mueller et al. 2022).

Findings should be reported to the monitoring team and systematically recorded and archived.

5.1.4 Measurement and sampling

Biometric measurements of all dead animals should be systematically recorded. An outline protocol for standard body measurements is provided (for example) in Boitany & Powell (2012), but these standards should be agreed at the population level between neighbouring countries. Additionally, as a minimum, a tooth for age determination and a tissue sample for genetics should be taken from each animal and stored for analysis. Systematic sampling of hair and other tissues, as well as sampling of other material, should be considered for further analyses (e.g. stable isotopes, toxicological studies, parasitology...) in collaboration with experts in the respective fields. These data and samples may not always have immediate value, but their value will increase over time. Over decades they may end up providing invaluable insight into demography and evolution of large carnivore populations, including their responses to climate change and human pressure.

5.2 Monitoring population size (abundance)

The number of individuals in a population or area is often one of the key parameters we wish to monitor (Boitani & Powell 2012). This is even more true for large carnivores, as their abundance is often in the core of public debates that develop around these species and attitudes towards them (Dressel et al. 2014; Majić-Skrbinšek et al. 2016; Skrbinšek et al. 2019c). Unfortunately, it is also one of the more difficult and costly parameters to monitor with any precision.

That said, there is always a reported number of individuals for any population of a large carnivore species in various reports and management documents, even if no robust abundance surveys have ever been done for the population. Such reported numbers are

frequently produced by adding up guesses of experts and/or people with local knowledge and experience (most often hunters) and can sometimes be methodologically formalized regarding how these local guesses are obtained and added. It has been shown several times that accuracy of such "guesstimates" is low (e.g. (Skrbinšek et al., 2019) and that thus produced numbers are in the case of large carnivores typically overestimates. **"Estimates" of abundance based on expert opinions or questionnaires should not be a basis for a monitoring program** and will not be further considered in this document.

Other common traditional methods, such as counting at feeding sites or recording signs of presence, are not informative about population abundance (population size or density) but if done systematically can be informative about **relative abundance** (comparison with the data of previous year), thus potentially informing about long-term trends.

The most common is **counting bears at feeding sites**. In order for this method to be informative the approach has to be standardized on a long-term basis. It has to include:

· Consistent methodology: To ensure data comparability, a fixed number of counting stations is used year after year at the same feeding sites. Observations occur on pre-selected nights, ideally with a full moon. Additionally, the type and amount of bait remain constant across all counts. They can be done several times per year (for example once in spring and once in autumn).

· Data collection: All observations are recorded in a standardized format, including details on each bear seen (including family groups). Observers positioned in hides or existing hunting towers stationed near the feeding sites conduct the counts, typically from dusk until midnight.

· Acknowledging limitations: While this method provides valuable data for monitoring relative abundance and population trends, it's important to recognize its limitations. Not all bears in the area will visit the feeding sites, and visitation rates can be influenced by natural food availability.

Another established tradition is measuring bear footprints to "count" individual bears. This approach is highly unreliable (ENTEWILD, 2020). For more information on the values and limitations of the traditional methods we invite the reader to look up guidance prepared by the ENTEWILD consortium in 2020.

The need for robust and reliable estimates of wildlife abundance has driven the development of various statistical methods that allow robust and precise estimates (Pollock et al. 2002; Schwartz et al. 2006; Iijima 2020). We'll outline the most important current approaches applicable to large carnivores.

5.2.1 Estimating abundance through capture-mark-recapture modelling

The key principle for empirical estimations of population size in wildlife is marking animals, ideally with individually recognizable tags. While we can never ensure that each individual has been detected (marked), we can use repeated captures (recaptures) of already marked animals to account for imperfect detection and estimate the total population size (Otis et al., 1978). The key is that we can estimate the population size even if we don't capture or observe all animals. A simple explanation of the principle is provided in Figure 9. This simple model is called the

Lincoln – Petersen model (Lincoln 1930) and while it has very strong assumptions that are difficult to meet and is not used much in practice anymore, it illustrates the principle well.

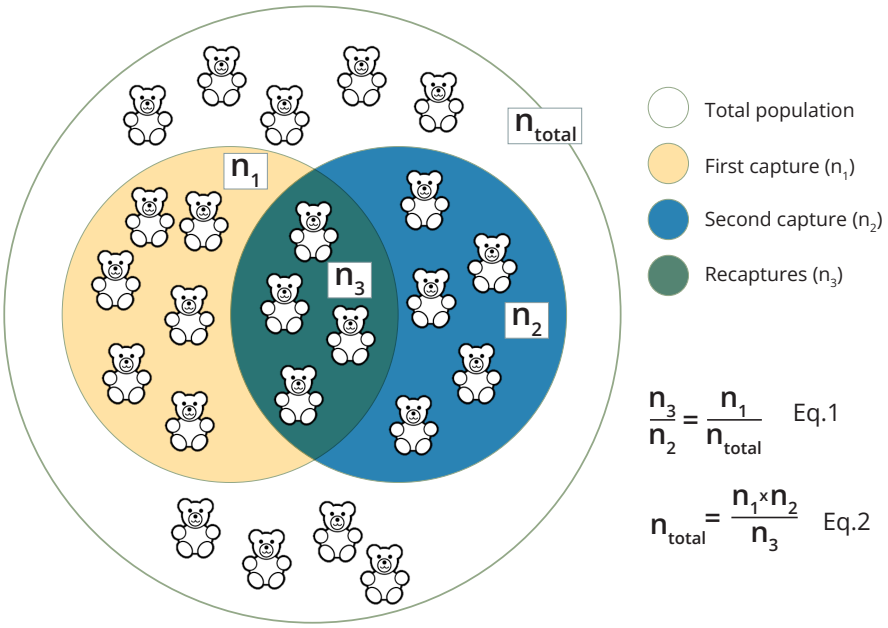

Figure 9: A simple explanation of the capture-mark-recapture principle. We capture, mark and release a proportion of the population (n_1) in the first capture (yellow). Some time passes, and we do the same in the second capture (blue), capturing and marking another part of the population (n_2). In the second capture, a proportion of animals (n_3) will already be marked from the second capture – these are recaptures. The proportion of animals marked in the first capture (n_1) within all animals in the population (n_{total}) should logically be the same as the proportion of marked animals (recaptures, n_3) in the second capture (n_2) (Eq. 1). We can use this to estimate the total number of animals in the population (Eq. 2), including the ones we never captured and marked.

To use CMR approaches in practice, we must be able to mark the animals (ideally individually), and to recognize these marks on recapture. In large carnivores, currently the only practical way to do CMR population size estimates is with non-invasive genetic sampling, or with camera traps for lynx, which can be reliably identified from photos via their coat pattern. Use of camera traps surveys for abundance estimates in bears or wolves is not feasible as individual animals cannot be reliably recognized from photographs. Approaches for estimating relative abundance indices using detection of unmarked individuals (not individually distinguishable) are being developed (see a recent review in (Gilbert et al. 2021)), but there are challenges that for now limit the usefulness of these approaches (Sollmann et al. 2013; Gilbert et al. 2021).

The concept of using genetics for marking animals is simple. Each animal has a unique genotype, which can be determined in laboratory analysis from a non-invasive genetic sample (scat, urine in snow, hair, saliva on prey...) or a tissue or blood sample when the animal is live captured or recovered dead. Methods for genotyping non-invasive and environmental samples are improving, and recently it became even possible to genotype tracks in snow (De Barba et al. 2024). When the first sample of an individual is found, this animal can be considered captured and marked. Whenever we find another sample of the same animal, we can identify the specific individual and we have a recapture. This allows us to track individuals in space and time and use the data in CMR analysis.

Use of capture – mark – recapture models has important assumptions that must be satisfied to obtain reliable results and which strongly affect the sampling design. There are textbooks written on the topic of CMR (e.g. (Amstrup et al. 2005; Cooch & White 2017), but we'll briefly cover some of the most important considerations for a CMR study of large carnivores.

5.2.1.1 Capture homogeneity – equal capture probability of all animals.

This is the most critical assumption estimates - all individuals must have the same probability of being captured. Its violation, **capture heterogeneity**, can severely bias abundance estimates. It is also one of the most difficult assumptions to satisfy, and the number one assumption that should be considered during sampling design. We will here consider CMR studies that use non-invasive genetic sampling. When using camera traps, or genetic sampling with hair traps for bears, it is better to use Spatial Capture Recapture (SCR). SCR can also be considered in other circumstances when we can record sampling effort in a spatial context – see chapter 5.2.2.

There are several sources of capture heterogeneity:

Spatially heterogeneous sampling. When a large area is sampled for genetic samples, sampling intensity may be different in different areas. The causes of this can be accessibility (more rugged terrain, less roads etc.), differences in numbers of motivation of field personnel or volunteers, or edge effect. The best solution is a careful sampling design that homogenizes the sampling effort as much as possible, and real-time monitoring of sampling progress that allows us to react when we detect under-sampled areas. If it is possible to record the field effort in a spatial manner, such data can possibly be analysed using SCR. However, in large studies utilizing volunteers it will often be impossible or impractical to record the sampling effort.

The edge effect appears when the border of a sampling area doesn't coincide with the edge of the sampled population or with an important landscape barrier that limits movement of individuals. The animals that have home ranges at the edge of the sampling area will spend some time inside and some outside the sampling area, which will modify their probability of being captured.

Behavioural response. If camera traps or hair traps are used to detect animals, this detection can be affected in subsequent detections by the experience of the first "capture" of the animal (if it is a positive or negative experience for the animal). The phenomenon is called a trap happy (higher probability of recapture) or trap shy (lower probability of recapture) behavioural response. This can be accounted for with appropriate modelling approaches, but steps should still be taken to avoid development of a behavioural response (using non-rewarding lures, changing of lures between sessions, not using lures for camera trapping etc.).

Misidentification of animals. This can be a serious problem in studies that utilize genetic samples as well as in studies based on camera trapping. If we incorrectly identify animals, we may severely bias the final results. Non-invasive genetic samples are very sensitive to genotyping error and contamination and require considerable expertise to genotype correctly. With explosive development of molecular genetics over the last decades there is an (over) abundance of genetic laboratories, but not many have experience in handling that type of material. There is a considerable body of literature providing strategies for correct genotyping of non-invasive samples (Taberlet et al. 1996, 1999; Broquet & Petit 2004; Mumma et al. 2015),

but the best strategy when using noninvasive genetic samples is to collaborate with a reputable laboratory with demonstratable experience in analysing non-invasive genetic samples.

Individual differences in detectability of animals regarding their sex, age class, social status etc. Sex of individuals has, for example, a great impact on detectability when hair traps are used to sample brown bears (Boulanger et al. 2004; Lamb et al. 2016). This can be accounted for in the models, but lower detectability of females with this method may render the female data too scarce for a reliable estimate. In many cases there may be individual capture heterogeneity that we can't describe.

There are CMR statistical models that relax the capture homogeneity assumption, and they should be applied when needed, but the monitoring team should make all efforts to **minimize capture heterogeneity as much as possible**. This can't be overstated. However, in most real-life situations, some degree of capture heterogeneity is unavoidable. The best solution for this is to **have a high capture probability** – in practice, this means having as intensive sampling as possible.

5.2.1.2 Population closure

Population is considered demographically closed when there is no immigration, no emigration, no births and no (undetected) deaths during a study. As these factors have a high impact on the capture – mark – recapture process, there are two types of CMR models, closed population models and open population models. While the latter are often closer to biological reality, their statistical power for estimating population size is typically lower. This means that in most cases when we want to estimate abundance, we will try to satisfy the population closure assumption as much as possible and use closed population models.

Spatial closure – if the edge of sampling area doesn't coincide with the edge of the studied population, we may have immigration into and emigration of the area occurring. The best way to handle that is to sample the entire population range, or, if this is not feasible, to sample as much of the population range as possible. For large carnivores this requires transboundary collaboration between countries sharing the same population, and population-level monitoring and management should be the goal for all European large carnivore populations (Linnell et al. 2008b). Spatial closure can also be facilitated by using a spatial barrier that limits movement of animals at the edge of the sampling area if this can be done in a meaningful way. A sampling season should be made as short and intensive as possible to minimize movement of animals in and out of the area. In brown bears, it helps if sampling is done in autumn as bears move less during their hyperphagia period (López-Alfaro et al. 2013), with the additional benefit of genotyping success being the highest during that period (Skrbinšek 2020).

Reproductive closure – in simple terms, sampling that has as a goal estimation of population size shouldn't include more than one reproductive cycle. While we can use CMR to estimate both population size and population dynamics through "robust design" models (Kendall et al. 1995), a single monitoring cycle shouldn't include more than one yearly reproduction. In studies that fail to collect enough data in a season, there may be a temptation to extend the sampling a bit and collect more data. This works until the new yearly litter starts being detected in sampling, which will be typically in summer for large carnivore species. After that, the detection of newly born animals will completely violate the closure assumption and may severely bias abundance estimates from such data.

5.2.1.3 A word of warning

Capture – mark – recapture, including the SCR approaches, provides a framework for reliable, statistically robust, evidence-based abundance estimates. These approaches are currently the only methods that allow direct estimates of abundance and are the "gold standard" for monitoring of this indicator. They can, however, be quite resource-intensive to implement in practice.

With this in mind, a word of warning: **capture – mark – recapture study that does not have enough recaptures (high enough intensity) will fail**. The only output from such a study will be the minimal number of animals (the number of actually detected individuals). As CMR studies tend to be expensive, a failure can jeopardize the entire monitoring program. A CMR study should be very carefully planned and executed, with sufficient resources to maximize the chance of success. If resources are insufficient, or if there are concerns that there may be problems in assuring high enough sampling intensity to obtain high recapture rates, the monitoring team should consider not implementing a CMR study or opt for a pilot study in a manageable area that would require less resources.

BOX: Steps for a successful implementation of a CMR study

- **Understand how CMR works**, particularly what are the critical assumptions.
- **Include a CMR specialist in your team** well ahead of your sampling.
- **If doing genetics, find a reliable laboratory before sampling and bring them aboard**. They will be able to provide advice regarding collection, storage, and transport of samples. It would be quite disappointing to have spent energy and resources for a successful field season just to have the study fail because the samples were stored inappropriately, and the genotyping success rate is low.
- Figure out how many captures you need to obtain the desired precision and use this to **scale the field effort**. If possible, do a power analysis. Program MARK (White & Burnham 1999) has a simulation module that can simulate a capture – mark – recapture process under different assumptions and provide you with an answer. Rule of thumb for studies that use non-invasive genetic samples is that you would need to collect **at least three times as many samples as you expect to have animals in the population you're sampling**. This ratio can be lower in large populations and high genotyping success rate but may need to be higher in small populations and if we expect the genotyping success rate to be low. The field effort can be decreased in routine monitoring cycles based on experience, but we'd strongly suggest planning for a very high field effort in the pilot study.
- Make a **precise study design** for field sampling. At least for the pilot study be pessimistic in scaling of the field effort – it's better to collect more data than to fail. You can optimize and economize in subsequent monitoring cycles when you gain experience.
- **Don't underestimate the costs and effort of fieldwork!** This can't be overstated. Even when working with volunteers, the organizational costs and effort are considerable. In

our experience, even when sampling is done by volunteers or pro-bono by personnel of participating organization (e.g. foresters, game wardens, rangers), the cost of organizing fieldwork can approach or exceed the cost of laboratory analyses. When sampling is done by paid personnel, sample collection costs may exceed the sample analysis costs by an order of magnitude.

- **Start preparing for a field season well ahead**, there will always be more things to do than you expect. Half a year would in many cases already be cutting it close. When the field season starts, everything and everyone must be ready to go!
- **Plan for contingencies**. Debate "what if" scenarios with your team, think of how to react. Be pessimistic! Write down your plans, discuss them with your team and be prepared to execute them if needed.
- **Monitor progress of sampling in real time.** Be ready to react if sampling is not going according to plan.

Fieldwork, from design to execution, is the most critical part of any CMR study of large carnivores. While laboratory and data analyses can often be repeated or improved, a field season can't be re-done or "fixed". If sampling intensity is too low, there will not be enough recaptures. **If there are no recaptures, there can be no CMR**, and the only thing you'd be able to tell after spending all the resources and time is the minimum number of animals.

5.2.2 Spatial Capture Recapture (SCR)

Capture mark recapture (CMR) models are allowing for estimation of abundance, which is often our parameter of interest (chapter 5.2.1). However, CMR models do not allow for an estimation of density, as they cannot accommodate any spatial aspect of the data collected, such as the size or shape of area surveyed, or the spatial characteristics of the studied species, such as their home range size. Any approximations or ad-hoc solutions used to account for these spatial aspects in order to infer about the density from a CMR-based abundance estimate can produce a strongly biased result (Pesenti & Zimmermann 2013; Royle et al. 2014a).

Spatially explicit capture recapture models (or simplified as spatial capture recapture models; SCR) are an extension of traditional capture recapture models. SCR was first described by (Efford 2004) and later comprehensively presented by (Royle et al. 2014a). SCR models account for the spatial organization of both individuals in a population and the observation mechanism (e.g. locations of traps). With such an approach, the sampling area is well defined, thus allowing estimation of **population density**, rather than only abundance. Density inherently relates to a unit of space, allowing for comparability of this demographic parameter with other study areas or study periods. Using density as a parameter for evaluating population status enables us to reliably track the changes over time regardless of potential change in sampling area, or effort, which is especially important for species of conservation concern (Kendall et al. 2019).

SCR is a method of choice for any surveys where we use fixed traps, e.g. camera traps, to obtain individual identities of a target species over a pre-determined spatial and temporal extent. It results in an estimation of density of a target species in a representative area of interest.

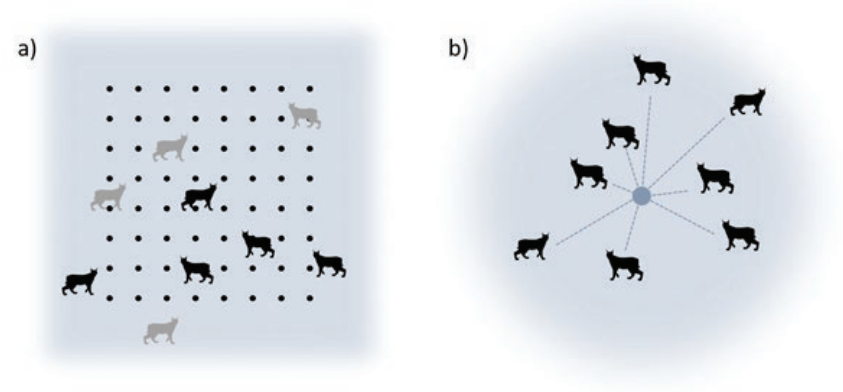

Figure 10: A schematic representation of sampling process for SCR modelling; adjusted from (Tourani 2022a)*. Figure a) shows how individuals are distributed across effective sampling area (outlined as blue box) and get (black lynxes) or do not get (grey lynxes) detected by traps (black dots) within the pre-determined trapping array. Figure b) shows the activity centre (blue dot) and a home range (outlined as blue circular area) of an individual animal, which was detected at different locations (black lynxes) within its home range.*

The most commonly deployed sampling techniques for SCR studies on large carnivores are hair trapping (e.g. for bears) and camera trapping (e.g. for Eurasian lynx) (Royle et al. 2014a). The approaches are both based on pre-determined grid of traps, which need to be repeatedly »capturing« the identity (DNA identity obtained from hair or visual identity obtained from photographs) of the individuals of the target species. The basic temporal unit of sampling is a trapping day (or night), i.e. a "sampling occasion". The resulting data form such approach are individual »capture histories«, i.e. when (at which occasion) each individual was captured, at each trap (see table 1 and 2, Figure 10). They represent the necessary input for any chosen SCR modelling framework (options described below).

Table 1: An example of a data frame with individual capture histories; each capture of an individual is associated with temporal (at which sampling occasion, e.g. camera trapping day, it was captured; "occasionID") and spatial (at which trap it was captured "trapID") information.

individualID	trapID*	occasionID
ind1	trap1	oc2
ind2	trap1	oc1
ind3	trap2	oc4
ind3	trap5	oc11
…	…	…

Table 2: An example of the data frame, defining the sampling effort; the extent of the trapping grid (X and Y coordinates of the traps in UTM coordinate system) and the operability (1 and 0 for yes and no, respectively) of each trap per sampling occasion.

trapID*	X	Y	oc1	oc2	oc3	…	oc120
trap1	61361	474906	1	1	1	…	1
trap2	59892	476811	1	1	0	…	0
trap3	57868	477658	1	0	0	…	1
…	…	…	…	…	…	…	…

5.2.2.1 Core assumptions of SCR models

Demographic closure

By assuming demographic closure, SCR model does not allow for any demographic processes taking place during the survey, i.e. no recruitment (births) and no mortality. In practice, that means that the survey should be planned in a way that we i) do not »miss« to detect the animals, e.g. during hibernation, denning or hunting season or ii) do not "count" the individuals from an age group which does not yet represent a functional part of a studied population, e.g. the juveniles (Iosif et al. 2022).

Geographic closure

The benefit of the SCR models is that the geographic closure assumption known from CMR models (Chapter 5.2.1) is relaxed. SCR explicitly account for the movement of animals within the sampling area, e.g. movement within the territories is detected by captures at different traps, while the location of the territory can be within or partly within the sampling area. However, there should be no immigration or emigration from the effective sampling area, i.e. the state-space (see section 5.2.2.2), as that would over- or underestimate the number of individuals actually living there. This is an especially important aspect in case of a translocation project being undertaken, where animals are artificially taken from or introduced to a population. In that case, we should limit the study period so it avoids the time of translocation activities.

Activity centres are randomly distributed

SCR models produce an estimate of density, which is essentially the distribution of individual activity centres within a species' habitat. An *activity centre* describes the area used by an individual during the sampling period, i.e. it is a proxy for a home range, or a territory (Royle et al. 2014a)(Royle et al. 2014b). The SCR model assumes that the distribution of activity centres is random and uniform within the habitat. However, if we suspect that might not be the case, we can account for it by adjusting the model structure, e.g. using landscape or habitat covariates to assess the spatial heterogeneity of the distribution of the individuals (the density) (Moqanaki et al. 2021).

Detection is a function of distance

This is an underlying paradigm of any SCR model; the probability of detecting an individual at a trap declines with distance from home range centre (Efford 2004) (Figure 11, see also section 5.2.2.2 below). If we had many detections of an individual at a trap, it means it was set up close to the activity centre. However, specific trap placement may violate these assumptions, e.g. setting some traps at territorial scent-making sites, which have highest detection rate at the edge of the true home range. In that case, we need to accommodate the specific set-up characteristics in our models by testing their potential effect on the detection probability (Fležar et al. 2023b).

Independence of encounters

SCR models assume that when an individual is detected by a trap, it's not influenced by the presence or actions of other individuals at that trap or by the individual's past encounters with other traps. In simpler terms, each individual's detection at a trap is considered independent

of other individuals' presence or capture at that same trap, as well as independent of the individual's previous detections at other traps. This assumption is crucial for designing SCR studies effectively. It means we need to carefully plan where we put the traps, taking into account the behavior of the species we are studying, ensuring they are spaced out appropriately, and determining the right length of time for sampling.

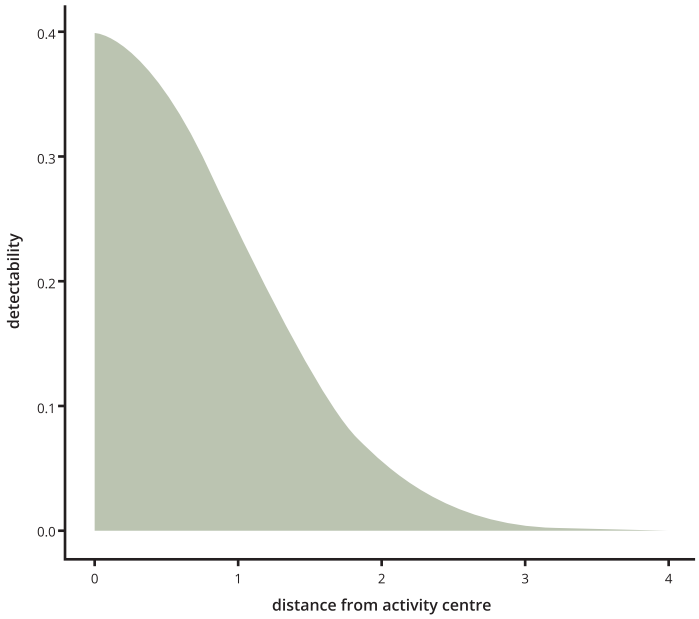

Figure 11: The half-normal function, describing the detectability of an individual animal; detectability is higher closer to the home range centre (distance = 0) and decreases by distance from the home range centre.

5.2.2.2 SCR MODEL STRUCTURE

One needs to be familiar with the SCR model structure in order to be able to design an SCR study (see section 5.2.2.3 below). SCR models have two main components:

1. The *spatial model of abundance*; estimating density (D) as the distribution of activity centres; may accommodate environmental covariates (Oberosler et al. 2021).
2. The *spatial model of detection*; relating capture rates to the distance between activity centre and a trap through two parameters:
    - baseline detection rate (*p0*); also a parameter of CR models; describes the probability of capturing an individual at a trap; may accommodate individual characteristics, such as sex (Sollmann et al. 2011), and trap characteristics, such as placement on/off road (Di Bitetti et al. 2014) or using a bait to lure the animals to the traps (Du Preez et al. 2014);
    - spatial scale parameter (σ): describes how rapidly the baseline detection rate drops with distance from the activity centre; may vary by individual characteristics, e.g. sex (Sollmann et al. 2011).

For a practical implementation of SCR models, it is mostly important to understand and pay attention to the possible variations of the three structural parameters (density, baseline

detection probability and spatial scale parameter). If they do not account for the specifics of the data collected, the entire SCR model might fail to produce unbiased estimates of density. For example, if we ignore the information of animal sex and do not accommodate it in our models, we can underestimate the population density (Efford & Mowat 2014).

Moreover, an important step of SCR modelling is defining a **"state space",** which represents the effective sampling area. State space is defined as the spatial region where all individuals, which could have been captured, reside (Royle et al. 2014a; Tourani 2022a). By default, it is notably larger than the sampling grid. It is outlined during the modelling process using the information from the data provided, mainly the distances between traps at which individuals were re-captured. Importantly, the state space should account for any potential restrictions in the landscape for the target species, i.e. the "non-habitat" areas should be cropped out (Figure 12). When the state space is known, it can be used to directly calculate the species abundance; by multiplying it with the density estimate (Blanc et al. 2013).

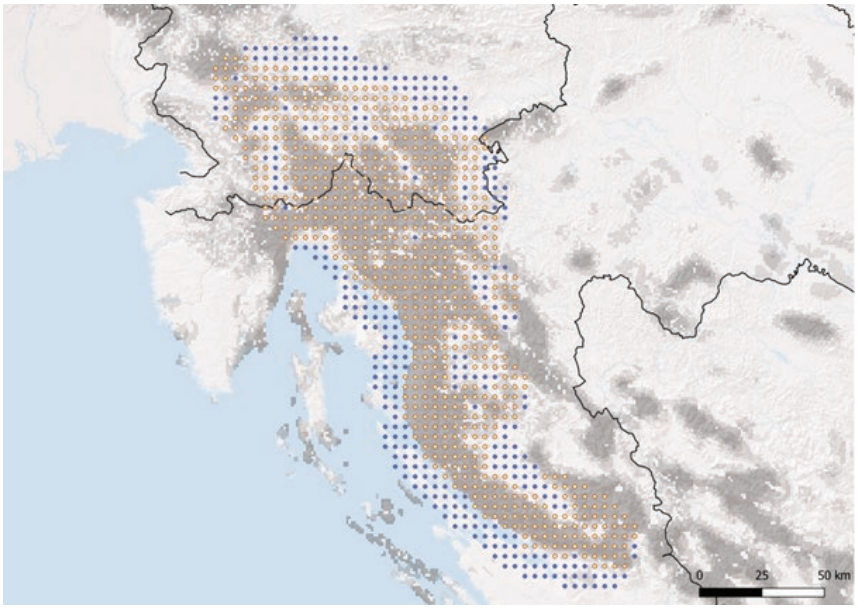

Figure 12: The adjusted state space; the yellow points represent the adjusted (cropped) state space, limited to habitat-only areas, while the blue ones represent the suggested state space before cropping. Black are the national borders (Slovenia, Croatia), blue is the Adriatic sea and the grey is the habitat suitability for the target species (Skrbinšek & Krofel 2008). Figure extracted from (Fležar et al. 2023b).

SCR modelling can be done in likelihood-based or Bayesian approaches. User friendly R packages have been developed in recent years to stimulate the use of SCR among practitioners, such as *oSCR* (Sutherland et al. 2019), *secr* (Efford 2019) or *nimbleSCR* (Bischof et al. 2020). No alternative software for SCR is available though, so knowledge of R is essential for anyone attempting SCR modelling.

5.2.23 Designing a SCR study

The main aim of designing an SCR study is to i) maximize the sample size, i.e. the number of captures of individuals, by maximizing the area sampled and ii) maximize the spatial captures of individuals, i.e. the number of captures of the same individual at different locations, by reducing the distance between adjacent traps (Dupont et al. 2021) (Figure 13). We should aim

at a minimum of two camera trapping sites per home range, while the size of the home range should be based on smallest (usually the female) home range information (Sun et al. 2014b). The quantity of data collected can be further improved by extending the period of sampling, provided we do not violate the demographic closure assumption.

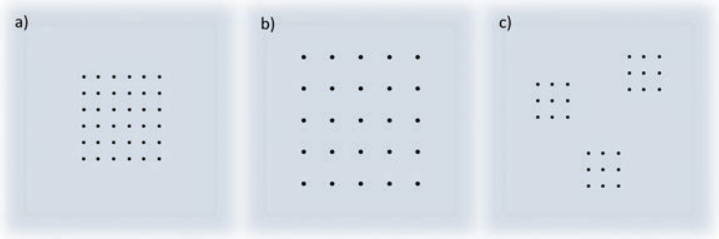

Figure 13: Three main design options; a) traps set up close together will detect few individuals but yield more spatial recaptures, b) traps spread out will capture many individuals, but yield little spatial recaptures, and c) clustered design will balance between the two options. For demonstrative purposes, these options are shown as regular grids however, it is possible to organize the camera trapping locations in a more irregular way in practice.

In practice, having many traps over a large area with small distance between adjacent traps is difficult to achieve, especially if the target species is a large carnivore. If we cannot find an acceptable trade-off, another option is mixing these two approaches in a "clustered design" as "holes" in the sampling grid are not an issue in SCR (Royle et al. 2014a). That also means that if there are some inaccessible areas in our study area, they should not be an issue for SCR model. The optimal distance between clusters of traps or the number of and distance between the adjacent traps depends on the goals of each individual study and there is no standard approach or recommendations on that.

Regardless of the approach, our datasets should involve at least 10 different individuals (Palmero, unpublished) and a minimum of 20 spatial recaptures (Efford 2004) for the SCR models to produce a meaningful output. Having this is mind, practitioners should have available sufficient funding and manpower to be able to plan and execute a survey producing data fit for SCR approach.

5.2.2.4 Limitations

SCR models represent a valuable tool in ecological research, offering a robust framework for modelling the distribution of individual activity centres within a species' habitat (Tourani 2022a). However, SCR is not the simplest ecological tool to comprehend, or implement. Familiarization with SCR requires a combination of having sufficient statistical knowledge and an understanding of ecological modelling. Thus, practitioners should aim for understanding these models so that they can balance between the requirements of SCR and the feasibility of the field design. In other words, if they wish to use SCR for density and abundance estimation, they need to understand how to collect suitable datasets.

On a technical note, SCR models are still under development to make better use of the data collected. For example, models, which allow for partial identities (Augustine et al. 2018) are highly relevant for species such as the Eurasian lynx where a substantial share of unspotted individuals may occur within a population (e.g. *Lynx lynx lynx*). However, such models are rarely used; the data from unidentifiable individuals are usually discarded (e.g. (Palmero et al. 2021)). Similarly, models acknowledging noncircular home ranges (Sutherland et al. 2015), are rarely used in practice. This calls for an integration of these accommodations into the existing tools, e.g. R packages for SCR.

BOX: The main steps for a successful implementation of a SCR survey.

- Clearly define the goal of your survey; what is your main expected result? *E.g. species population-level density vs the status of a species in a national park.*
- Design the field study which will allow you to reach your goal, accounting for the assumptions of the SCR approach and the available resources; i) define the size of the survey area, ii) define the time period that the traps need to operate to meet the demographic closure assumption, iii) decide about the best trap set up (standard/ clustered design) for your conditions, iv) define how many traps are needed based on the home range size of the target species, v) think about who will do the field work and who will be responsible for the data processing and analyses.
- Collect all the necessary data; the individual capture histories (make sure individuals are correctly identified) and the trap operability data (X and Y coordinates of traps and their daily performance; yes/no).
- Collect auxiliary data which could further inform SCR model parameters and potentially improve the density estimates (e.g. sex of individuals, type of camera trap setting).
- Make adjustments of the state space during the SCR modelling process, if needed.

5.3 SCALP criteria - ensuring reliable detection of signs of presence

Detecting signs of presence is essential to understand a species distribution. However, not all data have the same reliability, and not all data can be trusted. Non-critical treatment of all reports of large carnivore presence can lead to considerable errors and produce a skewed understanding of the actual distribution and status of a species.

To overcome this, criteria for data quality categorization have been developed within the framework of the SCALP initiative (Status and Conservation of the Alpine Lynx Population) to provide standardized criteria for interpretation of lynx monitoring data (Molinari-Jobin et al. 2012). These criteria became generally known as the SCALP criteria and are becoming increasingly used for other species and in other European countries (e.g. (Kaczensky et al. 2009; Reinhardt et al. 2015; Marucco et al. 2023a). They have also been included in the Monitoring standards for large carnivores in Europe (Marucco et al. 2024).

There are some key requirements to application of the SCALP criteria (Molinari-Jobin et al. 2012; Marucco et al. 2024):

- At least one experienced person must evaluate the field data, or the sign of presence needs to be documented in a manner that allows it to be evaluated by experts.
- An “experienced person” in this context means a person that has extensive field experience with the large carnivore species concerned.
- All observations must be checked for genuineness – the possibility of intentional deception must be ruled out.

The letter "C" stands for "category". The numbers 1, 2 and 3 don't indicate observer's qualifications, but are used to describe the level of validation for an observation.

C1: Hard evidence = Hard fact, i.e. evidence, that unambiguously confirms the presence of a target species (live capture, dead animal, genetic proof, photo, telemetric location).

C2: Confirmed observation = Indirect signs like tracks, scats, kills and dens confirmed by an experienced person as being caused by the target species. The experienced person can either confirm the signs in the field in the field or based on sufficient documentation provided by a third party.

C3: Unconfirmed observation = All observations that are not confirmed by an experienced person or observations which by their nature cannot be confirmed. This includes all sightings without photographic proof; all signs that are too old, unclear or incompletely documented; signs that, for other reasons, do not suffice to provide confirmation; and all signs that cannot be verified. Category C3 can be divided into the sub-categories "likely" and "unlikely".

False: false observations = observation for which a large carnivore can be ruled out as the cause.

Evaluation not possible = signs that cannot be evaluated because of lack of minimum information needed (e.g. reports of visual observations of tracks or kills).

Categorization of different signs of presence for individual large carnivore species according to the SCALP criteria is provided in Annex I.

5.4 Determining and mapping species distributions

Accurately determining the area of occurrence (AOO) for large carnivores is crucial for effective conservation strategies. AOO provides a baseline for understanding a species' distribution and potential threats (Boitani & Powell 2012; Madsen et al. 2020).

However, the problem of accurately determining an area of occurrence is not trivial (Boitani & Powell 2012). Large carnivores are elusive and live in low population densities, and their presence is not always easy to detect. This can lead to convenient "sampling designs" – interviews with local residents, reports of species presence, or, frequently, opportunistically collected data without a statistically sound sampling design (Anderson 2001). Such data are problematic – they provide presence-only information without estimates of error, they don't account for the areas where sampling was not conducted and are typically biased towards areas frequented by people.

The main problem are false absences. False absences occur when a species is considered absent from a site when some individuals are actually present but were not detected. This happens when probability of detecting a species in a certain area is < 1 (MacKenzie et al 2002).

There are two main approaches to determining the area of occurrence:

5.4.1 Minimum area of occurrence thorough detection of presence signs

The minimum area of occurrence are the grid cells where presence signs have been detected. The size of the grid can vary, but for large carnivores in Europe a standardize, pan-European 10 km × 10 km grid is standardly used (Kaczensky et al. 2012). Even if detection of signs is systematically organized, systematic and spatially well distributed, this should be considered an index, as the probability of detection is not taken into account and false absences are highly likely.

To enable a better understanding of spatial distribution, we can buffer the areas of occurrence of territorial animals (wolf packs/pairs, lynx family groups) with average territory size (Marucco et al. 2024).

5.4.2 Occupancy models – taking into account imperfect detection

Occupancy models allow for accurate estimation of species occurrence across a landscape, takin into account imperfect detection (Mackenzie et al. 2004; MacKenzie et al. 2006). Occupancy models are conceptually similar to capture – mark – recapture models, with the difference that the unit of interest is not an individual, but an area. Through repeated surveys each area (e.g. grid cell) obtains a detection history. The patterns of species detections and non-detection across the replicates provide the data needed to estimate occupancy, including its level of precision.

Occupancy models require appropriate sampling design (MacKenzie et al. 2006). We will not go into details in this handbook, but the readers are encouraged to read the appropriate literature and consult an expert if occupancy is considered to become a part of a monitoring program.

Occupancy models allow for a much more robust estimates of species area of occurrence, which is particularly valuable if the range of a large carnivore species is under expansion or contraction. If the area of occurrence is the key parameter that needs to be monitored, then the occupancy models are the appropriate tool.

5.5 Genetics as a monitoring tool

Over the last couple of decades, genetics became one of the most important tools in the management and research toolset for wildlife species (Schwartz et al. 2007a; Carroll et al. 2018). Genetic samples collected noninvasively (without any direct contact with the sampled individual) provide invaluable information that is impossible to obtain by any other manner while not disturbing the animal or coming into contact with it. On the other hand, tissue or blood samples from animals captured alive or recovered dead provide the same invaluable data, but can also be used for genomic studies that require higher quality and quantity of DNA.

We will here briefly review the current uses of genetics in large carnivore monitoring, the most common genetic markers currently used, their typical uses, and their respective advantages/ disadvantages. We will also list the main requirements for a genetic lab included in large

carnivore monitoring. In a more comprehensive section, we will provide detailed instructions for collecting genetic samples from large carnivores. These instructions have been taken from the field manual produced within the project LIFE LYNX (Skrbinšek 2017) and modified to include all large carnivores in the Dinaric-Balkan-Pindos area.

5.5.1 What can genetics be used for when monitoring large carnivores?

DNA provides a treasure-throve of information on all levels, from a single organism to the level of population and species. Besides being a "blueprint" for an organism and allowing individual recognition and, with genomic tools, an insight into characteristics of an individual, it also holds a record of demographic and evolutionary history of populations and species. As our tools to read this record are improving at an unprecedented pace, so does also the usefulness of genetics (and now also genomics) for monitoring of wildlife (Waits & Paetkau 2005; Allendorf et al. 2010; Carroll et al. 2018; Hohenlohe et al. 2021).

Our goal here is to provide a brief general overview of the current uses of genetics in large carnivore monitoring, to provide a general understanding to managers of monitoring programs. We would encourage the readers who are considering using these methods in practice to read a recent review since the methods are developing at a rapid pace, but in such cases, we would also highly recommend including a genetics expert in the monitoring team.

Monitoring abundance and distribution.

The number of animals in a population and the area they occupy are frequently some of the top priorities for monitoring, and in many cases molecular genetics paired with non-invasive genetic samples is the method of choice (Schwartz et al. 2007a; Carroll et al. 2018). Molecular markers allow us to individually identify animals from samples of the material they left in the environment – non-invasive genetic samples (also: minimally invasive samples). These detections of individuals can be used in capture-mark-recapture (Bellemain et al. 2005; Kendall et al. 2009; Caniglia et al. 2012) and spatial capture-recapture (López-Bao et al. 2018; Marucco et al. 2023b) models to estimate abundance, and in occupancy models for mapping distribution (Palomares et al. 2002; Long et al. 2011; Louvrier et al. 2018). These methods have also been used for surveying large carnivores in the Dinaric-Balkan-Pindosregion (e.g. Skrbinšek, Luštrik, et al. 2019; Karamanlidis et al. 2010).

Monitoring genetic diversity and inbreeding

Genetic diversity is of paramount importance, not just on an evolutionary but also on a conservation time scale (Allendorf et al. 2010; Moritz & Potter 2013). Some useful measures of genetic diversity (heterozygosity, allelic diversity) can be readily estimated with genetic markers standardly used for genetic monitoring, and it will be usually possible to estimate genetic diversity parameters in studies that use them. Going hand-in-hand with loss of genetic diversity is inbreeding, which can cause inbreeding depression and can be an important driver of extinction (Keller & Waller 2002; O'Grady et al. 2006; Hedrick & Garcia-Dorado 2016). Monitoring genetic diversity and inbreeding becomes particularly important in small populations where there is danger of genetic erosion (Hoban et al. 2013), and by monitoring

these parameters through time we can detect changes in genetic diversity that would warrant action (De Barba et al. 2010; Sindičić et al. 2013). In Dinaric-Balkan-Pindosregion the most critical species with these concerns that would need to be monitored is lynx, both the reintroduced Dinaric lynx population in the north, and even more the relict Balkan lynx population in the south (Sindičić et al. 2013; Mueller et al. 2022).

In recent years, large steps were made in measuring inbreeding in individuals and populations using genomic data (Hedrick & Garcia-Dorado 2016; Kardos et al. 2016; Mueller et al. 2022). As sequencing of whole genomes is rapidly becoming faster and cheaper, we can expect these genomic tools to become routinely used in monitoring of large carnivore populations.

Monitoring effective population size

Effective population size, or *Ne*, is one of the most important parameters in evolutionary and conservation biology (Frankham et al. 2002; Allendorf & Luikart 2009). We can (as a simplification) think of it as the number of animals breeding in a population corrected for variance in individual fitness. Not to be confused with the census population size (number of animals in a population), this elegant concept describes in a single index both a population's sensitivity to genetic stochasticity (loss of genetic diversity and inbreeding) and summarizes the key information about population's evolutionary potential and probability for long term survival.

While difficult to estimate reliably and often misunderstood in the past, the methodological and theoretical advances of the recent years made monitoring of this parameter in the wild feasible (Wang et al. 2016). Practical monitoring of this parameter in wildlife has also been demonstrated (Skrbinšek et al. 2012; Kamath et al. 2015). As the relationship between effective and census population size is increasingly better understood (Palstra & Ruzzante 2008; Palstra & Fraser 2012; Waples et al. 2013), monitoring of effective population size, which can be done relatively cost-effectively, could in the future also possibly be used as an index for census population size dynamics of wildlife populations, and allow early detection of population declines (Antao et al. 2011).

Monitoring geneflow and functional connectivity

The genetic picture of any population will change in time through genetic drift (Frankham et al. 2002; Allendorf & Luikart 2009). If the population is isolated from other populations of the same species, it will start to develop its unique "genetic signature". The higher the isolation and smaller the population's effective size, faster and stronger this differentiation will be. But besides this "coarse scale" population structuring, genetic differences will be visible also at a finer scale as the animals living closely together will be more related, and hence genetically more similar, than the animals living far apart. Any barriers to geneflow will also be reflected in the picture of the genetic landscape, both in space and time, and we can detect this with genetic markers and statistical analysis (e.g. (Jombart et al. 2008; Norman et al. 2017), a review in (Fenderson et al. 2020)). These principles have been applied to better understand population structuring and geneflow also in Dinaric-Balkan-Pindoslarge carnivores (Karamanlidis et al. 2017; Ericson et al. 2020; Šnjegota et al. 2021; Stronen et al. 2022b). In a monitoring context, these methods can be used to detect connectivity issues, and to monitor

effects of landscape changes (e.g. infrastructural development, landcover changes etc.) on functional connectivity (Luque et al. 2012).

5.5.2 Genetic markers used in monitoring of large carnivores

For the last two decades we have seen unprecedented developments in genetics and genomics that provided numerous new techniques and tools also for genetics studies and monitoring of wild populations (Allendorf 2017; Hohenlohe et al. 2021). Any genetic monitoring will require decisions regarding which markers to use. As the genetic toolbox is rapidly evolving, we would suggest that an expert is consulted when such decisions are made, but we provide here an overview to highlight some of the currently used markers, their usage, their advantages, and their drawbacks.

5.5.2.1 "Traditional" microsatellites genotyped using electrophoresis

Microsatellite markers, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are short DNA sequences consisting of tandemly repeated units of one to six base pairs in length (Tautz 1989). These repetitive sequences are dispersed throughout the genome and exhibit high levels of polymorphism due to variability in the number of repeats. Microsatellites are widely used in various genetic analyses, including population genetics, linkage mapping, forensic identification, and parentage analysis.

One key feature of microsatellites is their high degree of allelic variation, making them powerful tools for studying genetic diversity within and among populations. The variability in repeat number results in multiple alleles at a single locus, allowing for the discrimination of individuals based on their unique allelic composition. As the markers are biparentally inherited and very informative, they can be used for pedigree reconstructions.

Microsatellite markers are traditionally genotyped through polymerase chain reaction (PCR) amplification followed by fragment analysis, where the lengths of the amplified DNA fragments are determined by gel electrophoresis or capillary electrophoresis. Analysis of microsatellites using these approaches has been the "workhorse" approach in many conservation genetics studies and backbone of genetic monitoring of many species. They can be used for individual identification and hence for capture – mark – recapture (CMR) and spatial capture recapture (SCR) abundance estimates, for pedigree reconstruction, for analysis of population structure and geneflow, and for investigating genetic and demographic processes, also at fine scales and over recent time periods. They are very versatile, have a high information content (usually many alleles in a single locus), can be used in non-invasive genetic samples, and can be highly multiplexed (many markers analysed in a single analysis) to be cost effective.

However, microsatellites analysed in this manner also have some serious drawbacks and limitations (Pompanon et al. 2005; Putman et al. 2014). The main criticisms are technical, namely low throughput and automation, difficulty of scoring alleles, and difficulties in transferability of genotype data between platforms and laboratories. Because of this, microsatellites are falling out of favour with practitioners, but the problems are not limitations

of the microsatellites as markers, but rather a consequence of use of electrophoresis for their analysis (De Barba et al. 2017). New perspectives are opening with use of high-throughput sequencing for analysis of microsatellites which deals with practically all technical drawbacks mentioned above (De Barba et al. 2017; De Barba et al. 2024).

5.5.2.2 Next generation microsatellites – genotyping microsatellites using high-throughput DNA sequencing

While microsatellites analysed using the "classical" approach with electrophoresis are probably soon to become a thing of the past, a new method of their analysis using high-throughput sequencing (De Barba et al. 2017) is showing a lot of promise.

Instead of using electrophoresis, microsatellites are genotyped by sequencing on a high throughput sequencer and scored using a bioinformatic pipeline. This allows for a high level of automation and high throughput, and as a very large number of microsatellites can be multiplexed in a single PCR, a lot of data can be obtained in a cost-effective manner. Since alleles are determined by their DNA sequence, subjectivity is removed from scoring of alleles, and the data are future-proof and completely transferable between laboratories.

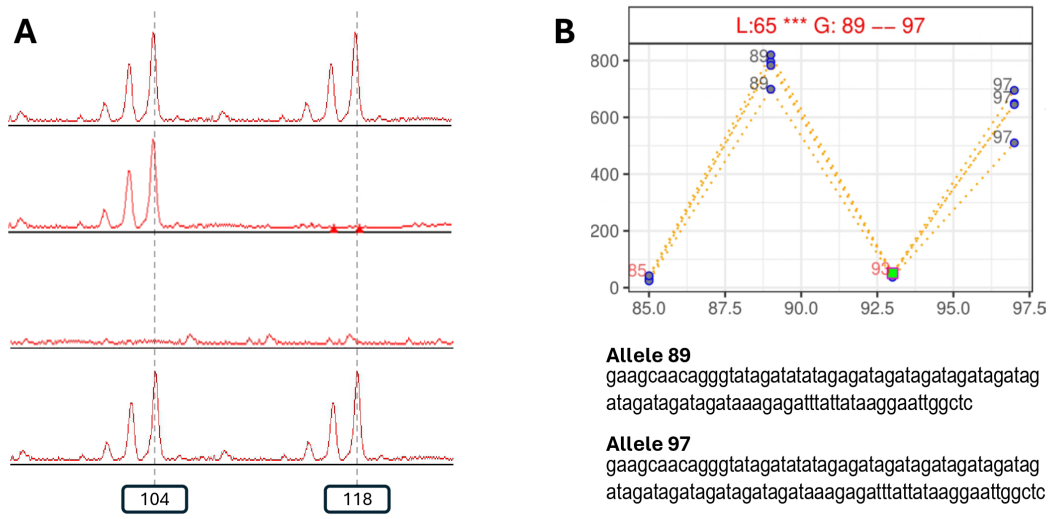

Figure 14: Microsatellite genotype (4 repeated PCRs) using capillary electrophoresis (A) and high-throughput sequencing (B). B, top: visualized genotype, locus UA65 (brown bear), x-axis = DNA fragment length, y-axis = number of reads. Each PCR is a separate line, red numbers are stutter peaks, black numbers are alleles. B, bottom: DNA sequences for the two detected alleles. In capillary electrophoresis, each PCR must be scored by a trained technician. The process is tedious, slow, and error prone. Allele names (104 and 118) are subjective, lab-specific and must be calibrated between laboratories to transfer data. In high-throughput sequencing allele scoring is automated through bioinformatics, but genotypes can be visualized for manual checking. Process is fast, efficient, and can be highly automated.

The first real-world experiences with these markers are very encouraging. They have been successfully used in large studies (Skrbinšek et al. 2017), and have been proven to be sensitive enough to enable individual recognition from environmental DNA from snow tracks (De Barba et al. 2024), which didn't seem possible with the previous approach. Genotyping success rates are higher than when using the previous approach (De Barba et al. 2024), in the case of wolf considerably so (85 % and 91 % genotyping success in two studies involving 149 and 169

samples, respectively (Skrbinšek et al. 2023)). They are since recently being routinely used for brown bear monitoring in Slovenia and Croatia (Skrbinšek et al. 2017) and for wolf monitoring in Slovenia, Croatia, and Switzerland (authors, personal observation; L. Fumagalli, personal communication), but their usage is likely to increase in the future.

5.5.2.3 Single Nucleotide Polymorphisms (SNPs)

SNPs (Single Nucleotide Polymorphisms) are variations in a single nucleotide occurring at a specific position in the genome, where each variation is present in a measurable fraction of the population (Allendorf et al. 2022). SNPs are abundant in the genome, with millions of SNPs distributed throughout mammalian genomes. Contrary to microsatellites, which can have many alleles, SNPs typically have just two alleles, so their information content per single marker is much lower than in the microsatellites. However, methods of their analyses allow simultaneous analysis of many SNPs, up to hundreds of thousands, which makes them very powerful markers for many questions.

SNPs used at the genomic level (analysed with genotyping arrays, RADseq, whole-genome sequencing) can be used for population genomics. This type of application for now requires high-quality DNA obtained directly from an animal (tissue, blood, buccal swab), but can be used, for example, for a very high-power monitoring of inbreeding (Hohenlohe et al. 2021). The genomic level data also allow us to link genotypes with phenotypes or environments (Wellenreuther et al. 2019), which facilitated development of rapidly advancing fields of population genomics and conservation genomics. Collectively, high throughput sequencing and rapid advancement of genomics is proving to be transformational for understanding the genetics and evolution of populations (Allendorf et al. 2022).

Over the recent decade, the methods became available that allow SNPs to be used also in non-invasive genetic samples (Von Thaden et al. 2017; von Thaden et al. 2020). These "reduced SNP panels" are optimized from large genomic datasets and can be used for genotyping low-quality samples with high reported success rates. SNPs in these panels are purpose-selected for their informativeness, for example for recognizing individuals or reconstructing pedigrees (Norman et al. 2013; von Thaden et al. 2020), understanding population structure and tracing gene flow (Stronen et al. 2022b), or purpose-designed to detect hybridization (Harmoinen et al. 2021).

There are some disadvantages of using SNPs in non-invasive samples. One is that using only SNPs, it is difficult to detect mixed (contaminated) samples. Mixed samples are a relatively common problem in non-invasive genetic samples (e.g. hairs from several animals collected from the same hair trap, fox marking a wolf scat...) and can be readily detected in microsatellite genotypes, but with SNPs, as they are mostly bi-allelic, this is lacking. If SNPs are used, for example, for individual identification and abundance estimates, this can cause us to "detect" non-existent animals and bias our abundance estimates. The other disadvantage is that the most common currently used method of analysis of the reduced SNP panels is with microfluidic arrays (Von Thaden et al. 2017), which is a less general-purpose and future-proof technology than DNA sequencing. However, SNPs can also be analysed by high-throughput sequencing, and the GT-seq method is already showing considerable promise in that regard (Campbell et al. 2015).

5.5.2.4 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a small, circular genome found within the mitochondria of cells, distinct from the nuclear DNA found in the cell nucleus. mtDNA is highly conserved within species but can vary significantly between species and is an important tool for species identification.

In the context of large carnivore monitoring, mtDNA is mostly used to identify the predator by analysing biological samples taken directly from the prey (saliva around bite wounds) or found in the vicinity (scat, hair), and to identify diet items using DNA metabarcoding analysis. As there are many more copies of mtDNA in a cell than there are of nuclear DNA, mtDNA is much easier to reliably analyse in poor and degraded samples and provides higher success rates. However, it doesn't provide resolution beyond species identification.

5.6 Recognizing wolf-dog hybrids and monitoring of hybridization

Through domestication, the domestic species have taken a completely different evolutionary trajectory from their wild counterparts, with artificial selection selecting for traits that can be maladaptive in the wild. When hybridization between such divergent species occurs, it can negatively impact the viability of the wild populations in many ways (Allendorf et al. 2001). Hybridization has been extensively documented in canids around the world, but its management has been generally neglected, including where it has been consistently reported (Salvatori et al., 2020).

In Europe, wolf-dog hybridization is increasingly reported from different parts of the continent (Salvatori et al., 2020). Introgression of dog genes into the wolf populations has the potential to represent a serious threat to genomic integrity of wolf populations (Hindrikson et al. 2017). Considering the rapid expansion of European wolf populations and increasing numbers of wolves all over the continent (Chapron et al. 2014), we argue that hybridization may be the most important conservation threat currently faced by wolves, and, if unchecked, can over time lead to extinction of the wolves as we know them through "hybridization vortex" (Allendorf et al. 2001; Rhymer & Simberloff 2003; Stronen et al. 2022a) and also possible loss of ecological function. As such, wolf-dog hybridization presents a complex societal, ecological, conservational, and technical issue (Stronen et al. 2022a). But if wolf-dog hybridization is not detected, no management actions can be taken, meaning that detection of wolf-dog hybridization should be an integral part of any wolf monitoring program.

5.6.1 Detecting wolf-dog hybrids with genetics

Although the first-generation (F1) and even later generations of wolf-dog hybrids may exhibit morphological traits atypical for wolves, which has been described also in Dinaric wolves (Kusak et al. 2018), morphology is not a reliable approach for determination of hybridization status of an animal (Lorenzini et al. 2014). The only reliable approaches are genetic or genomic, but they have limitations that we must be aware of.

5.6.2. Inclusion of hybridization detection in a population monitoring program

A wolf monitoring program should, as a minimum, systematically survey mortality cases and collect genetic samples from all detected wolf mortality (see chapter 5.1). These samples should be routinely analysed in a genetic laboratory for indication of wolf-dog hybridization regardless of morphological characteristics of the individual animals.

Field observations of animals with suspicious morphology should be followed-up with genetic sampling. Non-invasive genetic samples (see chapter 5.8) should be collected in the area of a pack with suspicious animals and analysed in a genetic lab for hybridization.

If genetic monitoring of wolves using non-invasive genetic sampling is being implemented, detection of hybrids should be included in the analytical workflow.

While the technical considerations of genetic identification of hybrids are beyond the scope of this handbook, we will provide a brief overview as we feel that understanding of these issues will allow the managers of monitoring programs to make better decisions.

To identify wolf-dog hybrids, we need to analyse nuclear DNA. Hybridization that can be detected in nature is usually between a female wolf and a male dog (but see Hindrikson et al. 2012). As mitochondrial DNA (mtDNA) is uniparentally inherited via the maternal line, this means that in most cases wolf-dog hybrids will have a wolf mtDNA haplotype.

A panel of genetic markers (microsatellites or single nucleotide polymorphisms) used for monitoring should be designed powerful enough to enable at least a reliable detection of F1 hybrids and detection of suspected hybrids up to the 2nd backcross generation. The power of a marker panel to distinguish hybrids of different generations can be tested through simulations (Godinho et al. 2011, 2015).

While detection of hybrids with standard genetic markers can be unambiguous in isolated populations (e.g. Godinho et al., 2011, 2015), it presents more of a challenge in presence of genetic structure (Hindrikson et al. 2017; Stronen et al. 2022a). Wolf populations in the Dinaric-Balkan-Pindos region exhibit high degree of genetic structuring (Šnjegota et al. 2021; Stronen et al. 2022b), and long-range dispersals that are common in wolves (Ražen et al. 2016) can make dispersers from distant population nuclei appearing almost anywhere (Ražen et al. 2016; Šnjegota et al. 2021). With standard genetic markers, such dispersers and their offspring can be accidentally misclassified as hybrids (Stronen et al. 2022a). For this reason, it is critical to include reference samples of all neighbouring populations in hybridization analysis. This will enable a more reliable identification of hybrids and will also allow detection of immigrants from other areas and their direct offspring, which is important for understanding how wolf (meta)populations function on large scale (Šnjegota et al. 2021; Stronen et al. 2022a).

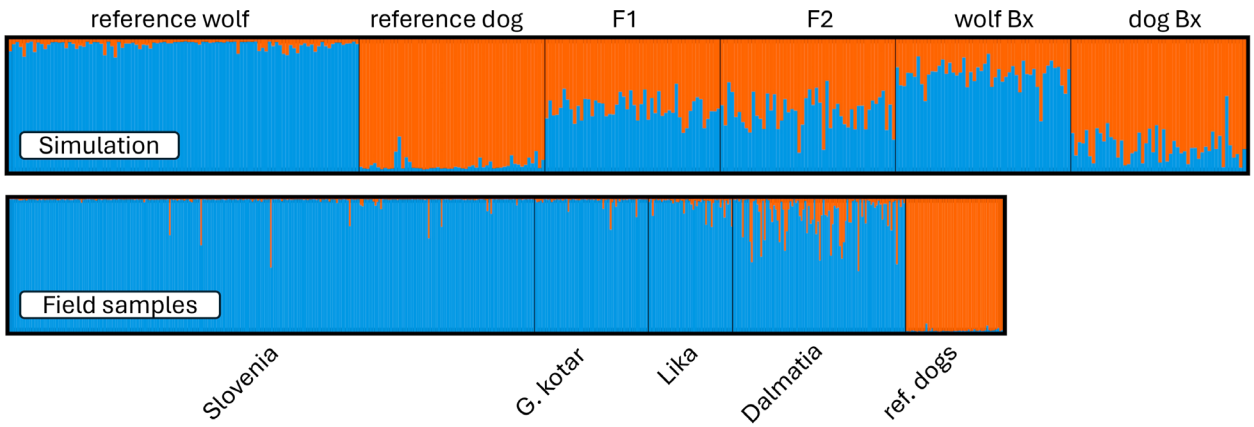

Figure 15: Detection of hybrids in NW Dinaric Mts. using microsatellite data and Bayesian structuring (Skrbinšek et al. 2019b)*. Each vertical column is an individual genotype, proportion of orange is the probability of assignment to the dog cluster (interpreted as proportion of dog ancestry), proportion of blue is the probability of assignment to the wolf cluster. Top: simulated hybridization using real reference genotypes of wolves and dogs to determine expected thresholds for different levels of hybridization; Bottom: field data from different areas and reference dogs. The individuals with a considerable proportion of dog ancestry (orange) in the field data are potential hybrids, which is particularly evident in Dalmatia region of Croatia (possible hybrid swarm). As the analysis was done on basis of microsatellite data, some of the detected hybrids could be immigrants from other populations. These samples were later genotyped also with the reduced SNP panel for hybrid detection and while most of the assignments held, some were indeed pure wolves.*

Recently, a reduced SNP panel has been developed for the specific purpose of identifying wolf-dog hybrids (Harmoinen et al. 2021). It provides a very high degree of differentiation between both taxa and seems to be unsensitive to population structuring in wolves. It can be used to reliably identify wolf-dog hybrids up to the 2nd generation backcrossing (1 in 8 ancestors). We would strongly suggest that the results obtained with standard markers are used to identify suspected hybrids, but that the final determination is confirmed using this specific marker panel. As usually not many samples will need to be processed in this manner, this can be done quite cost-effectively.

5.7 Citizen science – involving stakeholders and public in monitoring of large carnivores

Around the globe, thousands of research projects are engaging millions of people – many of whom are not trained as scientists – in collecting, categorizing, transcribing, or analysing scientific data (Bonney et al. 2014). Such projects obtain or manage scientific information at scales or resolutions unattainable by individual researchers or research teams, whether enrolling thousands of individuals collecting data across several continents, enlisting small armies of participants in categorizing vast quantities of online data, or organizing small groups of volunteers to tackle local problems. Over the last decades, the concept of citizen science is increasingly gaining recognition not only for its potential to make substantial contribution to scientific discovery, but also in its wide-ranging impacts on society (Bonney et al. 2009; Fraisl et al. 2022). Biodiversity monitoring is one of the fields where citizen science is making considerable contribution (Chandler et al. 2017; Fraisl et al. 2022), but there are several things one should consider when embarking on such endeavours.

As collection of data and samples for monitoring of large carnivores must be done at large scales and frequently requires a huge field effort, the use of citizen science is regularly practiced in many areas (e.g. (Skrbinšek et al. 2019c; Ražen et al. 2020; Marucco et al. 2023a). Considering the charismatic flagship species character of large carnivores, it is much easier to get people excited and motivated to volunteer their work for their monitoring and conservation that it would be for many (most) other species, and this "unfair advantage" is something that can and should be utilized in otherwise very resource demanding large carnivore monitoring programs. However, humans are complex, and there are no cookbook recipes on how to make a citizen science initiative work. We will try to provide some practical pointers on what to do, and what not to do, but at the end of the day it will require hard thinking and hard work to make it right. But if done correctly, the results will be more than worth the effort.

5.7.1 The key principles of working with volunteers: Respect, Recognition, Reward

One of the main premises of citizen science is something that may not come intuitively to managers and researchers running a monitoring program: **volunteers don't work for free**. They are just not paid money. They have their own lives, their own interests and problems, but for whatever reasons they've somehow decided to dedicate some of their time on earth to your project. This should be respected, recognized, and rewarded.

You should treat the volunteers participating in your project with **respect**. One would think that this should go without saying, but often researchers employ a small army of volunteers (think: students) for fieldwork just to forget about them the moment the data is collected. Managers or researchers involving stakeholders (a good example is hunters) are often not much different. This builds resentment and while it may work in the short term, it burns the bridges for any long-term collaboration and possibly turns people away from ever volunteering again. Volunteers participating in your project are your partners, and you should treat them as such.

All people like to be **recognized** for their work. Volunteers should be recognized for their participation, either individually in smaller projects, as stakeholder groups (if they are members of one), or as a group. This builds a sense of community and rewards the volunteers for the work they provided. Every communication of the results, or generally any public communication at any stage of monitoring, should be seen as an opportunity to recognize the participating volunteers.

The volunteers should also be **rewarded** for their contribution. This may be just recognition, a feeling of doing something worthwhile, or a feeling of belonging to a community. But this can only be achieved through frequent communication to build a sense of community, recognition when results are communicated to the public, and as detailed as possible feedback of the achievements and results. Also, any small token material reward will also be appreciated. A simple project t-shirt goes a very long way – it makes people happy and provides them with a sense of belonging, but also provides "marketing" for the monitoring and raises public awareness.

5.7.2 Don't underestimate the resources required to manage a large network of volunteers!

A frequent mistake in working with volunteers is to believe that there will be no associated costs. Organizing, for example, a large field sample collection effort with volunteers is a huge undertaking that will require considerable human and financial resources (Skrbinšek et al. 2017, 2019c). The monitoring team should be careful when estimating required resources since there will be costs that may not be immediately obvious, particularly in human resources. Organizing volunteers, delivering fieldwork materials, communication and troubleshooting during sampling, as well as communication after sampling will typically require several people employed full-time in any large-scale data or sample collection. Just managing social media when there are thousands of people participating is a full-time job.

To provide an example: a recent sampling of non-invasive genetic samples in Slovenia for brown bear abundance estimate (September – December 2023) was done with volunteers (mainly hunters, some nature enthusiasts) and Slovenia Forest Service personnel. Despite most of the participants volunteering their fieldwork, the costs of organizing and managing the sample collection consumed nearly ½ of the project budget, nearly as much as genotyping and data analysis.

5.7.3 Designing and testing field materials

If you wish for people to work for you, they need to take you seriously. **To be taken seriously, you must act seriously**. Nowhere is your professionalism (or lack of it) as evident as in the tools and materials you give to volunteers – you should make sure that they are thoughtfully designed, aesthetically pleasing, complete, and thoroughly tested before you give them out.

- Consider having your project's visual identity professionally designed. This will do wonders for the project's visibility, can be effectively used in many places (web page, t-shirts, publications…) and can be meaningfully recycled (with occasional facelifts) for many years.
- Make sure your tools and materials work. Are people collecting scat samples? Make sure that tubes don't leak! Are they recording snow tracks? Make sure they have a handbook to recognize them. Are they setting camera traps? Make sure that the cameras work, and that they know how to operate them. Thoroughly test everything, preferably with people outside of the monitoring core team.
- Provide detailed written instructions! If possible, have them professionally designed.
- If people are supposed to record data, make sure that the data forms are easy to understand and convenient to write into them. Make sure that fonts are not too small. Test this with several people outside of the monitoring core team. Think elderly relatives – a "grandma test" goes a long way for these things.
- If people are expected to take materials with them to the field, make sure they are easy to carry around.

- Prepare the materials well ahead of time as that procedure may take longer than expected. It will take some time to distribute the material to the volunteers. When field season starts, all volunteers should be fully equipped and ready to go!

Figure 16: Field materials for volunteers for genetic sampling of brown bears in Slovenia and Croatia, developed in project LIFE DINALP BEAR. Project visual identity was professionally designed. Collection flasks were thoroughly tested to be completely leak-proof. A nicely designed, illustrated instruction booklet was provided. Also provided were pre-addressed, postage-paid envelopes that simplified sending of collected samples for volunteers. The entire "sampling kit" was packaged into a neat individual box that could be handed out to a participant directly or sent to the participant by post.

5.7.4 Communication: building and maintaining a network of volunteers

If people don't know about your project, they can't participate. If they don't hear from you when the project is being implemented, they will forget about it. You should dedicate considerable resources to communication activities and make a communication strategy early on in the project. It is also not a bad idea to employ a communication expert at this stage if the project budget allows it.

Marketing & recruitment

To get people to participate, they must know about your project. In the first stage, the monitoring team should try to identify the target stakeholder groups or profiles in the general public that could be potentially involved. When this is done, you should think about the communication channels. Large carnivores are interesting and it's relatively easy to get the attention of the mainstream media, and they have the reach to a very large audience. Social media also provide important and useful communication channels, but they also require considerable work to get the message to a wide enough audience. The main advantage of social media is that it allows direct two-way communication with the audience, which is not the case with mainstream media.

The monitoring team should consider writing their own articles for different media outlets, not just contacting journalists. Editors need content and will often be happy to accept a (well written) contribution. This may offer the opportunity to the monitoring team to get a much more direct and concise message across.

Depending on local circumstances, it may make sense to target specific stakeholder groups. For large carnivore monitoring, this will frequently be the hunters as they have the benefit of considerable ecological knowledge and a good knowledge of their area. Additional benefit may be that by involving them directly in monitoring, they may be more likely to accept the monitoring results and support the management decisions (Skrbinšek et al. 2019c).

You should make it easy for people to sign-up for participation, through as many channels as possible – through Internet forms (can be communicated through social media), directly through their organizations (hunting clubs, NGOs, clubs for various outdoor activities...), by email... be creative. Make sure you obtain their **permission to contact them** once they sign up or you may get sued for unsolicited emails. Make sure that you handle the personal data in accordance with the national laws. The rules and regulations on safety of personal data are getting increasingly stricter, and missteps can result in severe penalties for the project.

Communication with volunteers before, during, and after the field season

Once volunteers are recruited, you should keep them involved. In cases when you need to provide some field materials or tools, you can organize a meeting where you can explain in-person what the project is about and distribute the materials. In large studies it may take many such meetings to kick-off a field season, but it is usually worth it.

If a stakeholder organization is involved, you should try using the internal communication channels of that organization to deliver your messages. For example, if a national hunting organization is involved, they will often publish (or be associated with) a national hunting magazine which hunters read. By regularly publishing in that magazine, you'd be directly addressing that entire stakeholder group.

You should maintain direct communication channels (email, social media) with volunteers and directly inform them prior to the field season about the work and plans, and during the field season about the progress.

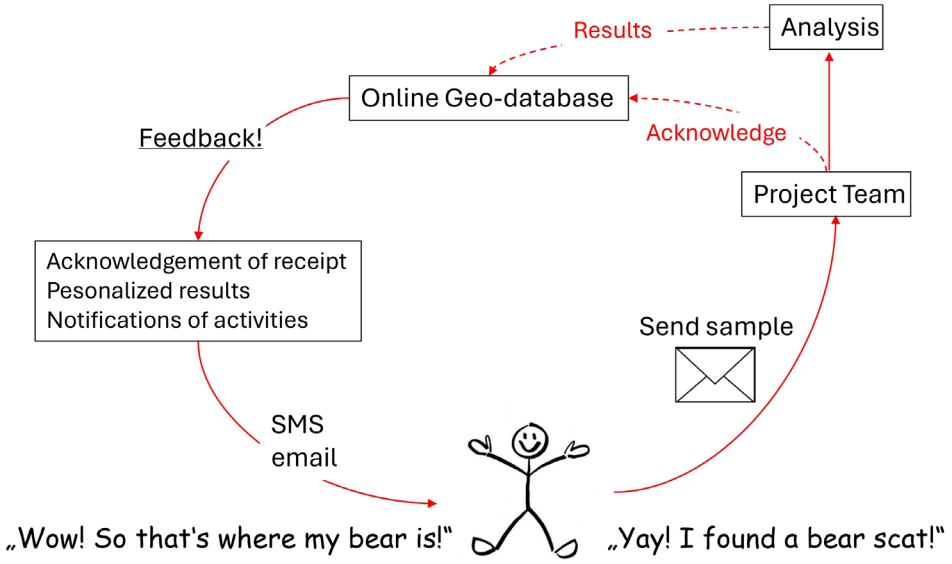

Figure 17: A slide from a steering group meeting of project LIFE DINALP BEAR describing the citizen science communication strategy for field sampling of brown bear non-invasive samples.

5.7.5 Make participation as simple as possible!

Remember: you need the volunteers to collect data or samples critical for your study. And you need them because the scale of this data or sample collection exceeds the capacity of the project team, possibly by quite a significant margin. Think about what you actually need from them and make it as simple as possible for them to provide that. This will maximize your chances of success.

Few pointers:

- Get tools and materials to the people, not the other way around. Devise an efficient system of getting materials needed for fieldwork to the people that have signed up for your study. You can distribute them at in-person meetings, send them by post, or think of some other way that will make sure that people get what they need with minimal effort. Don't make them come to you to pick up, for example, sampling flasks and instructions. Most of them won't show up.
- Minimize what they have to do to the bare essential minimum. Researchers thrive in collected data, and we want to know and record everything that's possible and a few things that aren't. But if you make a volunteer fill-out a three-page form for every sample they collect, they will think twice about participating. Keep it simple!
- Be careful about using technology! Mobile devices are wonderful, convenient, and everybody carries them around with them. However, it may be quite a hassle for a volunteer, who may not be very tech-savvy, to install a sophisticated app and learn how to use it just to collect two or three samples or data points in their entire "career". Paper and pencil have a very convenient user interface that we're all proficient with. If you do decide to use a sophisticated app for your volunteers to record data, make sure it doesn't do more harm than good. And provide alternatives.

- Organize convenient collection of samples and/or data. If the volunteers collect samples, organize local pick-up points. If possible, use the postal service to make it even easier. If they have to send data, try to minimize the data you require and make sending as convenient as possible.

Food for thought: while your world may revolve around your project, it is not so for your volunteers – they are in it just for the ride and the fun of it. Make sure you understand that, and act accordingly. You need them, they don't need you. Make participation easy and fun for them, and they'll get everything done, and enjoy doing that. If participation complicates their life, they'll turn tail immediately.

5.7.6 Provide feedback, acknowledge the work of volunteers!

Once the results are known, the volunteers should be the first to find out. Try to provide as much feedback as possible and keep the volunteers in the loop. If you have had organized meetings before the sampling season, consider organizing them again once you get the results. Remember, the volunteers are a part of the team – they should feel privileged and a part of the community.

The role of volunteers should be acknowledged in all media statements and articles, and in any project outputs, including reports. This is the only way to keep the sense of community and ensure participation in future studies.

5.7.7 Estimating lynx abundance and population density using SCR

Recently, SCR has become a standard for estimating lynx status in most of its range in Central Europe (Weingarth et al. 2015; Gimenez et al. 2019; Duľa et al. 2021; Palmero et al. 2021; Iosif et al. 2022; Fležar et al. 2023b). The means of data collection is via coordinated camera trapping, as the photos allow individual identification of lynxes based on their unique fur pattern. The placement of the camera traps on the field needs to be well planned so that we ensure the data collected would suffice the requirements of SCR (see chapter 5.2.2). The main steps of a successful SCR survey are outlined in the Box in chapter 5.2.2, while we give specific recommendations on the implementation of a SCR survey for assessing lynx status in the following sections.

Note that an SCR survey should be designed and implemented deterministically in time and space, meaning that photographic data, collected in an *opportunistic* manner, e.g. sent by hunters from their private camera traps, should not be considered for SCR modelling. However, such data is very useful to help decide where to implement the deterministic (systematic) camera trapping survey and what micro locations to use for a camera trap set up.

5.7.7.1 Planning the field design

The first and foremost thing is **defining the goal of the survey and the area of interest**. Are we interested in a population-level abundance, or density of lynx within a protected area? In any case, the area of interest needs to be big enough so that we can obtain estimates,

which are sensible for understanding the status of lynx there. At the same time, it should not be too big to prevent us sampling with the required intensity, as we would collect data too poor to have sufficient value for interpretation of lynx status. Population-level surveys, or surveys of areas over 10.000 km (Tourani 2022b), are difficult because they most probably involve international coordination, high costs and a lot of manpower, however they are not impossible (Fležar et al. 2023b). Thus, most of the existing surveys on lynx status limited to a representative area of interest, where camera trapping was feasible and still produced meaningful results, such as national parks (Palmero et al. 2021) or other area of interest (Iosif et al. 2022).

SCR models are quite robust to changes in trap array size, but **areas over 760 km²** produce reliable and precise estimates (Zimmermann et al. 2013; Palmero et al. 2021). We should place at least two cameras per female lynx home range (Sun et al. 2014a) and limit the camera set up to suitable lynx habitat. If no information on the size of lynx home ranges is available from our wider area of interest, we should use the information from the similar area. For example, the average female home range size in the Dinaric Mountains is just over 100 km² (Fležar et al. 2024), so any survey of lynx in the Dinaric Mountains can refer to that.

Furthermore, (Weingarth et al. 2015) have shown that an optimal camera trapping period for Eurasian lynx should **last for 80-120 days**, preferably in the **autumn – early winter** (September – December), which was confirmed also by (Fležar et al. 2019). By increasing the sampling period, we can increase the number of recaptures, which may further improve the precision of density estimates. However, in January, lynx dispersal begins, and the juveniles are getting more difficult to distinguish from adults on the photographs. Moreover, during the mating season (typically lasting from mid-February to mid-March) adult lynxes change their movement especially when on mating excursions, making them prone to capture at sites outside their normal home range and thus inflating the spatial scale parameter (see chapter 5.2.2 for details). Both aspects may induce bias in the camera trapping data so avoiding sampling in winter – spring period is recommended (but see (Gimenez et al. 2019)).

If we are sampling in an area for the first time, it is recommended to treat the first survey as a "pilot" survey in order to evaluate our approach (Fležar et al. 2019, 2023b). We can then adjust any spatial or temporal aspect of the survey in the next session, thus collect the necessary data for SCR modelling. Keep in mind that the dataset for SCR should involve capture histories of least 10 different individuals (Palmero, unpublished) and a minimum of 20 spatial recaptures (Efford 2004; Weingarth et al. 2013).

5.7.7.2 Choosing and preparing the camera traps

Literature on camera trapping is extensive, however the recommendations on the best model (Weingarth et al. 2013) are quickly outdated as the market is rapidly growing and the technical specifications of the cameras are improving. While Cuddeback (Cuddeback, Green Bay, Wisconsin) white flash camera traps have been used in most of the past European lynx surveys (Weingarth et al. 2015; Duľa et al. 2021; Palmero et al. 2021; Fležar et al. 2023b), they are difficult to purchase nowadays. A suggested alternative manufacturer is Reconyx, Inc., which produces a series of models for professional research. In fact, these cameras have been

shown to perform well even in the harshest conditions, e.g. snow leopard surveys in high altitude habitats (Suryawanshi et al. 2021). However, regardless of the choice, the important technical aspects, which need to be considered when choosing cameras for a lynx survey, are the following:

- Cameras with **white flash** (Xenon or LED) are a preferred model because they create **photographs of highest quality**, which is essential for lynx identification. They should be placed at locations, where you expect an animal to be passing by (e.g. a forest road). The intensity of flash should be adjusted to the specific location of the set up; the intensity should be higher when the distance of the camera and the area where we expect the animal to pass is larger. If the camera is set too close and the flash is too strong, the photos will be overexposed. Moreover, such cameras should not be used at scent marking sites not to disturb the lynx; **the lynx should not change its behaviour due to a camera trap**!
- Alternatively, we can use models with black **"no-glow" infra-red flash** producing high resolution images (>22MP) at sites where we expect the lynx to stop, e.g. scent marking sites.
- Use the same settings on all camera models to ensure comparable detectability between camera models; one to three photos per burst is recommended for movement trigger settings. Additionally, the **trigger speed** should be as fast as possible, e.g. 0.1 seconds, and **picture delay** as short as possible, e.g. < 1 second (Rovero et al. 2013). This setup facilitates the detection of consecutive animal movements, such as females with kittens, as they pass by.
- Using **time-lapse function** enables indubitable records about camera functioning; in addition to movement trigger settings, a time-lapse of 1 photo per 24 hours is recommended.
- Mark each camera with a unique ID to keep a good record of camera set up and their operability.
- Get familiar with the chosen model of camera through outdoor testing prior to the mounting on the field.
- Follow the recommendations on charging system and memory cards for chosen camera models, as they might influence camera performance and longevity.
- Account for the cost of housing, locks, SD cards and batteries, when planning the overall cost of camera traps.

5.7.7.3 Setting up camera traps on the field

Setting up cameras on the field is perhaps the most straightforward step of an SCR study, but there are nevertheless some principles we need to consider:

- Place cameras at the locations where you assume the highest chance of recording a lynx (Breitenmoser et al. 2006); at this point, **involving stakeholders** such as local hunters, might be essential (Fležar et al. 2023a).

- If cameras are set at different type of locations (Stergar & Slijepčević 2017), associate each camera ID with the characteristics of the set up location (e.g. road, off road, scent marking site, baited present or not); it is useful to create an online or conventional form about the camera trap set up and fill it for each camera set up. These **auxiliary data** need to be accounted for in the SCR model (see chapter 5.2.2).
- Choose the camera trap with **suitable illumination for the chosen location**; if cameras are set up at a location, where you expect the lynx to pass by (road/trail/...), one camera at each side of the road should be installed to record both left and right flank of the lynx (Rovero & Zimmermann 2016). Camera should be recording at a **height of 50 cm** which is approx. the height of an adult lynx. If placing cameras at many lynx scent marking locations, the number of cameras per location can be reduced to one, as full (left & right) identity of the lynx would be obtained at a marking site and individuals could be further distinguished only from one side of the body (Oberosler et al. 2021; Fležar et al. 2023b).
- Upon starting the camera, make sure the **date and time is correct and the settings are correctly defined.**
- Check cameras **at least every 2 weeks**; at each check-up, make sure the camera works properly and that the date and time is correct. If the camera seems to have failed temporarily, acknowledge that in the camera operability dataset. If you realize that the camera had a wrong date time defined, reconstruct the correct date and time; if it is not possible, discard the data.
- Keep track of the inconveniences, e.g. situations when camera failed or was stolen or destroyed.
- Unless you are following a clustered design with cluster displacement (e.g. moving clusters of cameras around your sampling area in a pre-determined matter), **do not move the cameras** during the survey!

5.7.7.4 Camera trapping data processing and lynx identification

Even if running a small-scale camera trapping survey, e.g. in a protected area, it is highly recommended to use **existing software** to store, manage and annotate all camera trapping data, e.g. Trapper (Bubnicki et al. 2016) or Camelot (Hendry & Mann 2017). An average lynx camera trapping survey will yield several thousand images where <1% of them may belong to lynx; the rest would be the non-target species, representing a valuable "by-catch" dataset (Mazzamuto et al. 2019; Hofmeester et al. 2021). Camera trapping software creates a structured workflow by importing, checking, sorting and annotating camera trap images, which reduces human error and create a straightforward data export (.csv file) which can be easily manipulated for further analyses. Recently, artificial intelligence solutions for camera trapping have been developed. For example, Wildlife Insights (https://www.wildlifeinsights.org/) offers managing and annotating the images on a public platform, while MegaDetector by Microsoft, Ltd. (Beery et al. 2019; Ahumada et al. 2020) can be integrated to aforementioned

platforms (e.g. Camelot) to classify people, vehicles and animals on the photographs.

To identify lynx, **multiple observers should be used** to avoid potential errors (Johansson et al. 2020). Observers should be conservative in identifying lynx; in case of poor-quality photos, or lack of clear fur pattern, they should rather skip the identification than erroneously determine the individual.

Each lynx should be named with **unique names or codes, which do not change over time** (unless an error is discovered. In case of implementing a cross-border survey identification should be harmonized between the neighbouring countries to avoid double counting of the lynxes with cross-border territories. I**nternational databases** can importantly assist this process (e.g. www.mbase.org). Artificial intelligence is making progress also for individual identification; tools such as Whiskerbook (www.whiskerbook.org) offer a promising solution for automatic identification of lynx in the future (Fležar et al. 2024).

Finally, published **guidelines for reporting** the results of a camera trapping survey, including using the correct terminology (Meek et al. 2014; Choo et al. 2020), should be acknowledged for any type of output document.

5.8 Collecting genetic samples for monitoring large carnivores

Genetics is an essential tool for monitoring large carnivores. But it all starts with samples.

A genetic sample needs to be collected correctly and appropriately stored. All required data need to be recorded, otherwise the sample is useless. Simple mistakes like collecting too much scat in a tube or waiting too long to analyse urine samples can significantly degrade genotyping success rates. This may decrease success of monitoring, or even jeopardize results. It makes a considerable difference if instead of having 80% of collected samples provide useful genotypes, this rate drops to only 30% just because the people in the field didn't get appropriate material or instructions.

Whenever you use genetics in monitoring, make sure you do the sample collection right. This is a key element that the entire downstream success (or failure) of monitoring will be hinged on.

5.8.1 Collecting scat samples

Scats can be considered "bread and butter" of European large carnivore studies that use non-invasive genetic sampling. They have many things going for them – they are cheap and easy to collect with minimum training, they can be efficiently stored and transported, and they usually have good statistical properties (little sex, spatial or temporal bias). As all non-invasive genetic samples, they require an experienced laboratory for analysis.

BOX: Checklist for collecting scat samples

1. **We recognize the species**
   … we make sure that the scat is really from our target species.
2. **We collect a sample**
   … from the surface of a scat, small amount (think "pea") or swab!
3. **We estimate the age of the scat**
   … fresher is better! Main guideline: smell!
4. **Write the data on the flask label**
   … a sample is useless if you don't know where and when it was collected!
5. **Mark the sample**
   … so it doesn't get picked-up again by someone else.
6. **Store and deliver the sample**
   … store in a cool and dark place, return for analysis as soon as possible.

5.8.1.1 Recognizing scats

Scats should be carefully evaluated before they are collected. **Sampling of non-target species**, if frequent, can increase the analysis costs (no useful data is obtained from such samples) and give a false impression of sampling success. While occasional non-target collections are unavoidable, effort should be made to provide enough training and instructions to the field personnel to make these as rare as possible. If many people are expected to participate in the sampling (large organizations, stakeholders, volunteers), a sampling manual with detailed instructions and photographs should be produced.

The easiest way to reliably recognize scats is if we ask ourselves about their **size, contents, shape and location**.

Lynx

Size: The typical diameter of the scat "sausage" in an adult lynx is around 2 – 3 cm, rarely more. Contents: Lynx scats will usually have some hair of its prey (either from ungulates or dormouse), but usually less than a typical wolf scat. It can have bone fragments. It may also have some plant material, usually individual blades of grass or leaves of trees, but this happens rarely. It shouldn't have pieces of anthropogenic foods. Shape: Lynx scat is usually in a single "sausage", often in several more or less connected segments and with blunt ends. *Location*: Lynx tend to hide/bury their scats (similar to domestic cats), but this is not always the rule, especially with older animals. Thus special attention should be given to heaps of snow along lynx tracks or heaps of leaves around kills sites and other locations frequently used

by lynx. If you find a scat on a visible spot (middle of a crossroads, exposed rock...) it usually means that it's NOT a lynx scat, but a different species (wolf, fox). Sometimes individual lynx repeatedly used same site for defecation, typically under rock shelters.

Possible mistakes: fox, wolf, dog, wildcat

Figure 18: Lynx scat. Notice blunt ends and the little "tuft" at one end, which is considered one of the best parts to collect for a genetic sample. (Illustration: Igor Pičulin; Photo: Franc Kljun)

Figure 19: Left: Buried lynx scat. Only a pile of leaves is visible. Right: Lynx scat taken from a snow pile, large (50 ml) sampling tube for size reference. We most often find lynx scats while snow tracking. (Photos: Miha Krofel)

Wolf

Size: diameter usually 3 cm, can vary from 2 cm in pups or smaller wolves and up to 4 cm in very large wolves. Contents: Wolf scats that we usually find have a lot of hair and usually some bones. We can sometimes find some plant material (grass leaves). Shape: They are usually in well-formed "sausages", and the ends are usually not blunt but more conical (different from lynx). An experienced person can also tell a wolf scat from that of a lynx or a bear by the characteristic smell. Wolves can also have very liquid scats with little hair (when they feed mainly on meat), but they will usually be found only in snow. Location: Wolves don't bury their scats and they will be often located on the forest roads and logging trails. As they also

use them to mark their territory, they are often left in a visible place (middle of the road, crossroads etc.). Sometimes there can be scratch marks on the ground close to the scat from the marking behaviour.

Possible mistakes: fox (very common error), dog, jackal, lynx, bear

Figure 20: WOLF scats. Notice that the ends are more "conical", not blunt like in lynx. There is often a lot of hair from the prey. When in doubt, still collect the sample and make a note. (Photos: Miha Krofel)

Bear

Bear scats are usually easy to recognize but considering their omnivorous diet and size differences between individuals, there can be mistakes. Size: Size of a scat can be very variable. In large bears they can almost get to the size of a cow dung, while they would be about the size of a dog scat in cubs in autumn. Contents: Contents can be very different regarding what the bear ate – grass, carrion, corn, fruits (very common in autumn), beechnuts, ants, wasps... It is typical that the plant foods are not finely ground as they are in ruminants, so we can see larger food fragments. Shape: A scat is usually in a single pile, there are no formed droppings (typical for wild boar). It can be fully formed or in large soft pile (depending on the diet).

Possible mistakes: wild boar, wolf, horse, donkey, red deer (summer scat).

Figure 20: Brown bear scats. Due to diverse diet and different body sizes of bears their scats can vary in appearance (Photos: Miha Krofel, Đuro Huber, Franc Kljun).

Frequent errors

Precise identification of large carnivore scats can be sometime problematic, and collections of scats of non-target species can occur.

Dog: Dog scats are problematic since they can be of various shapes and sizes and can be mistaken for any other large carnivore species. Dog scats typically don't contain hair. Sometimes we can see identifiable food items, e.g. dog food or other anthropogenic foodstuffs. If the scat is found while snow tracking, there will usually (but not always) be human snow tracks in vicinity. Dogs also don't bury their scats like lynx.

Wolf - bear: A wolf scat may be mistakenly collected as a bear scat, and vice versa. Bear scats are usually larger than wolf scat and even when the bear fed on carrion, it may contain food items that wouldn't be found in wolves (e.g. corn, fruits, wasps, mushrooms etc.). Wolf scats have also different shape than lynx scats (described above) and wolves don't bury their scats.

Fox: Fox scats often contain mammal hair (usually from small mammals), but frequently also contain seeds and other plant material, which is rarely found in lynx or wolf scats. Small mammal hair is typically short, but there may be long, thick vibrissae present that can be mistaken for large herbivore hair. Size is on average smaller, but can be similar to those of lynx scats, however fox never buries their scats and fox scats are often spirally twisted along the longitudinal axis, which does not occur in lynx scats. Fox scats will often be found on roads and various conspicuous sites (on large rocks, tree stumps etc.). Fox is the most common non-target collection in wolf studies.

Jackal: Jackal scats may be mistakenly collected as wolf scats. They are typically smaller than wolf scats, and, like fox, may have food items that would be unusual for wolves (small mammals, plant material, seeds...). If jackals are present in the study area, their scats are a relatively common non-target collection in wolf studies.

Wild cat: Wild cat scats are very similar shape to those of lynx and also wild cats often bury them. They mostly contain hair from small mammals (but keep in mind that also lynx scats frequently contain dormouse hair in Dinaric landscapes). Easiest way to distinguish them from lynx is size, as wild cat scats usually have diameter smaller than 2 cm.

Wild boar: Wild boar scats have similar structure to brown bear scats (coarsely ground plant material), and there is a large size overlap between scats of both species, so they can be mixed up. Wild boars often defecate while walking, and always standing up, so (contrary to bears) their scats are often found in several piles. They are also formed in characteristic irregularly shaped droppings, which we don't see in bear scats.

Some less frequent mistakes: There are scats of other species that may be mistakenly collected as bear scats: red deer summer scats (plant material is much more finely ground than in bears) and horse/donkey (typical shape and consistency of scats). These mistakes are rare, but can happen when sampling is done by untrained volunteers (citizen science initiatives).

5.8.1.2 Age estimate of scats

Estimate of scat's age is important since it gives us an idea of the expected DNA quality in the scat and allows us to plan the analysis. Estimating scat age is never precise, and the appearance of the scat will depend on weather conditions and content. However, we've noticed that most people can estimate how fresh a scat is using just common sense. This subjective estimate is an excellent predictor of genotyping success (Skrbinšek 2020) and as such of great help during analyses.

The goal should be to collect relatively fresh scats that **still have the characteristic smell** (unless they are frozen). Estimating age is more difficult in wolf and lynx than in bears since a fresh wolf or lynx scat may soon look dry and desiccated. **The best guide is your nose!** The rule of thumb is that **scats that subjectively seem more than five days old are not worth collecting** as they will most likely not provide useful results.

A few points to consider:

Fresh scat will appear fresh at first sight. It has a strong smell, looks moist and possibly slimy.

Older scat may still look fresh. The smell will be less intensive, but still characteristic. After 3-4 days the scat will not look slimy anymore. In dry weather in summer, especially in the sun, a scat may look older even after a single day, but it will still have a lot of smell, which indicates that it should be collected for genetics. It may have larvae of insects, particularly if the weather is warm.

Old scat loses most of the smell and is not slimy anymore. Such scats are usually dry but can be moist from a recent rain. They can have holes from insects that have already left after metamorphosis. Very old scats are without smell, dried-out and often light in colour. **It usually doesn't make sense to collect old samples that don't smell for genetics**.

Scat age is of less concern during winter – when a fresh scat freezes in the environment, genetic material in it will be conserved like in a freezer. Such scats are always worth collecting for genetics.

5.8.1.3 Collecting a scat sample

Scats can be collected in high-concertation ethanol (food grade, non-denatured) or the DETs buffer (DMSO – Tris – EDTA – NaCl). If a piece of scat is collected, it should be stored in a 8 ml up to 50 ml tube. The tubes should be **pre-filled with preservative** up to 3/4 or 4/5 of their volume before being put to the field to decrease the possibility of people collecting too much scat. It is **critical to test the tubes that are considered for sample collection** – there are not many things as demotivating for the field crew as spilled wolf or bear scats all over the gear in their backpacks. Our typical test is that a tube filled with ethanol must survive a heavy person jumping on it, and several hours in sun under a car windscreen in summer, without leakage. If it can't do that, don't use it.

Another option is to swab the scat with a swab. Ideal are "flocked" swabs (in effect small brushes) that have break-away tips. Instead of collecting a piece of scat, the scat is swabbed, and the tip broken off into an appropriately sized tube (2 ml) with preservative. We are using

the DETs buffer as preservative. Some caution may be warranted as this method is not yet widely used at the time of writing of this handbook, but we've been getting some very encouraging results with it.

A sample is collected from the **surface of the scat, if possible, from a part that is not in contact with the ground**. That part of the scat is the first to dry out, which conserves the DNA. If we see the end of the scat (the conical "tuft" or "tail" sticking out from one end) we should try collecting a sample from there.

If the sample was exposed to heavy rain, we try to take a sample from the least exposed part.

If there is **mucus** on the sample, we should try to collect it since it should contain a lot of target DNA.

We collect a **pea-sized** sample of the scat and put it in the flask with scat conservation liquid. Remember, **LESS IS MORE!** Make sure **not to collect too much sample** since in this case the DNA will not get conserved. The liquid in the flask should in no case spill over or even reach the edge of the flask, and there should be at least 4 times as much conservation liquid than there is scat.

Figure 21: Left: Collecting a scat sample (large (50 ml) tube for reference, shown with a wolf scat). Right: Small (8 ml) tube with a scat sample. We collect SMALL (PEA-SIZE) AMOUNT of sample – better less than more! (Illustrations: Tomaž Skrbinšek)

It's the easiest to collect the sample with wooden sticks which can be included in the sampling material and harmlessly discarded in the environment after sampling. If you don't have those, you can make a "tool" from a piece of wood or small tree branch. You can also use these sticks to mark the scat and let other people know that a sample has been collected. **You should use new "tools" for each scat to prevent cross-contamination of samples!**

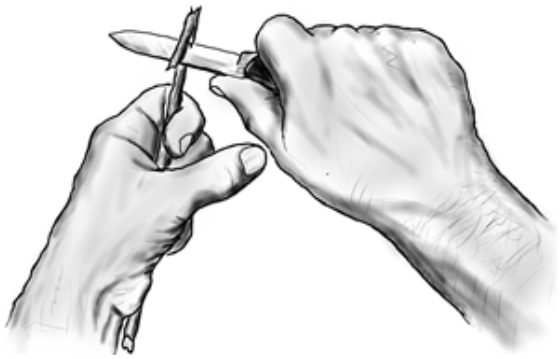

Figure 22: Making makeshift "tools" for scat collection (Illustration: Tomaž Skrbinšek).

If you collect a sample with a swab, make sure to make the swab dirty, but don't use it as a spoon to fill the sampling tube. Break-off the swab tip into the tube and seal it securely.

Figure 23: Collecting a scat sample with a swab. Scat is swabbed until dirty, and the tip broken off in the sampling tube with preservative. Data is recorded on a label on a resealable bag, and the sample tube sealed in the bag. (Photos: Gregor Simčič, Tomaž Skrbinšek).

5.8.1.4 Record the data for a sample

The data about the sample should ideally be recorded on the label of the sampling tube. If you're using a small (8 ml) tube or 2 ml tubes for swab tips, put label on a small resalable bag and distribute the tubes in such bags. The same data form can be used for both scat and urine samples, and different data are filled-in as appropriate.

These would be the **typical instructions for field personnel** regarding the data that needs to be recorded:

Species – in some areas, different species are being sampled in parallel.

Date of sample collection.

Name of the person that collected the scat which will enable you to get feedback about samples.

Location where the sample was found. If you have a GPS, write down the coordinates. If not, record the local name of the place where the sample was found. Please also note the wider area/region so that we can later place the sample on a map as precisely as possible. If you can't determine the precise location, still collect the sample and write down its approximate location.

Age of the scat – circle the number of how old you think the scat is.

Snow tracking data – if the sample was collected during snow tracking, please fill-in also the snow tracking data (track size, number of animals in the group).

Note – write down anything you consider useful noting with the scat, also if you have some particular questions.

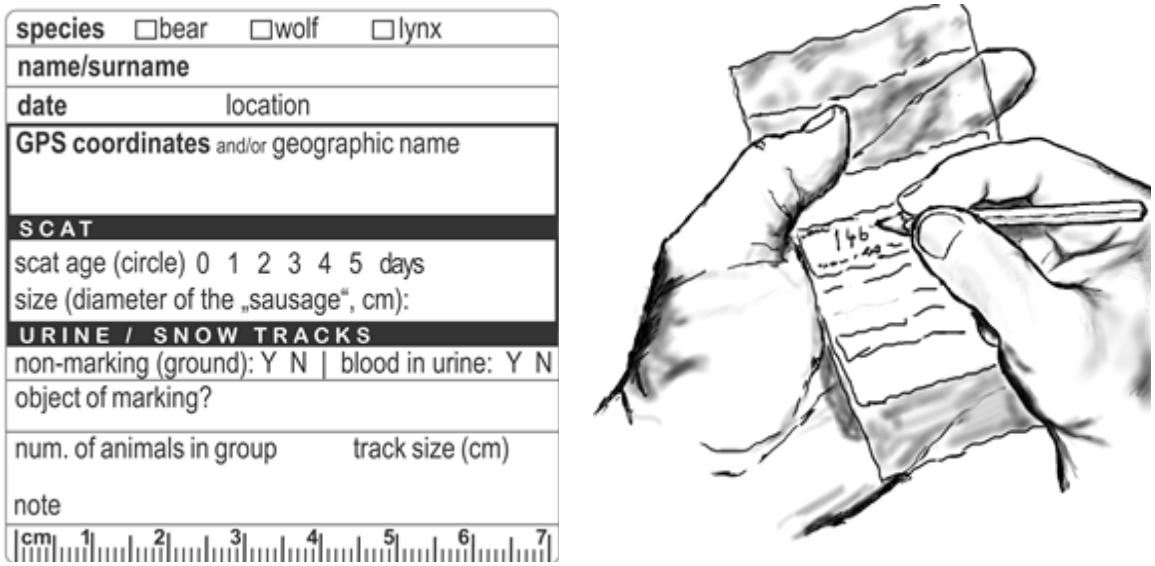

Figure 24: An example of a data-entry label for urine and scat samples, writing data on the bag label for small (8 ml) sampling tube or a 2 ml tube for swab tips. Large tubes have labels directly on the tube (Illustration: Tomaž Skrbinšek).

5.8.1.5 Mark the scat

After collection you must **mark or remove the scat**, so that it doesn't get sampled twice. The best is if you can cover it with a large rock or put some branches over it. If it's on a trail, you can also remove it using a rock or a branch.

The mark/removal must be permanent and clear!

Marked samples should not be collected again.

5.8.1.6 Store the scat sample and send it for analysis

After collection, keep the sample in a cool and dark place. Don't leave it in a car on the sun!

The sample should be **sent to be analysed** as soon as practical. While scat samples, once stored in the conservation medium, are more robust than other samples, this should still be done sooner rather than later. Specific details on how to do that will vary between countries and should be determined by the monitoring team.

While scat samples will be ok if stored for a short time at room temperature, keep them in a freezer at -20 °C for longer storage. Freeze – thaw cycles should be avoided as much as possible.

5.8.2 Collecting urine samples in the snow

Urine samples are also a viable material of DNA for genetic analyses. Since lynx and wolf use urine to mark their territory, such samples can often be abundant if a residential wolf pack or lynx is snow tracked.

Collecting a urine sample is just as simple as collecting a scat sample. We're usually using larger (50 ml) flasks which make urine collection easier, but the small (8 ml) flasks can also be used for this purpose to the same effect.

CHECKLIST FOR URINE COLLECTION

1. **We recognize a lynx or wolf tracks**

   ...we make sure that we're tracking our target species.

2. **We collect a sample**

   ...as much of the "yellow snow" as possible!

3. **We record the data**
   ...a sample is useless without the data and will not be analysed.

4. **Store and deliver the sample**
   ...store in a cool and dark place, return for analysis as soon as possible.

5.8.2.1 Are the tracks really from a lynx or a wolf?

While lynx tracks are relatively easy to recognize in ideal conditions, this becomes more difficult when snow conditions are less than ideal. Same goes for wolf tracks, which can also be mixed up with tracks of domestic dogs. Some pointers to consider:

Is the size and the shape of the tracks correct? Are tracks in-line?

A lynx footprint is round, with diameter between 7 and 9 cm in an adult lynx (forepaws are larger than back paws). Typical wolf track is elongated, bout 11.5 cm × 9 cm. However, one should be careful – as snow melts, footprints get larger and rounder, so a hare or fox track can easily be mistaken for a lynx or even a wolf. The main rule is always that several footprints should be observed if at all possible before making a decision.

Lynx tracks are usually in a straight line. Lynx are also very agile, meaning that they will often go on fallen trees, through dense vegetation, over rocky outcrops and make large vertical and horizontal jumps. Observing this type of behaviour in the tracked animal almost always indicates a lynx.

Figure 25: Lynx tracks. See the round shape and absence of claw marks (although not always!). Diameter is 7 - 9 cm, with the front paw larger than the hind paw. (Photo: Franc Kljun)

Wolf tracks may be sometimes tricky to distinguish from tracks of domestic dogs. There are some pointers we can consider:

- Size. Only few dog breeds have paws longer than 10 cm, so this is the first indicator. Some care must be taken since snow tracks get larger as snow is melting. We should always check several tracks and consider the smallest. Also, if there are tracks of several animals, tracks of a wolf pack should be of similar size. As a contrast, if there are several dogs walking together, there is a high possibility that some will have smaller tracks.
- Footprints of humans. This usually means that somebody took their dog for a walk.
- Behaviour. Wolf tracks are usually in a straight line, while dog tracks often zig-zag all over the place.

Did you see a single footprint, or are you able to follow the animal and observe many footprints? The most common mistakes are done when a single footprint is observed. With sufficient imagination and appropriate field conditions (melting, falling snow from trees...) a single footprint of almost any appropriately sized animal may be misidentified as a lynx or wolf footprint. If you've observed several footprints in a row and they all look like lynx or wolf, you may be reasonably sure you're tracking a lynx or a wolf.

Can you see claw marks in the track? Like most cats, lynx retract their claws when they walk and you usually don't see claw marks in a lynx track. In other words, if you're seeing claw marks you're probably not tracking a lynx. A notable exception to this may be when lynx walk on frozen snow or in steep terrain and can occasionally use claws as "crampons". Such claw marks are narrow and sharp, quite different than blunt claws we can observe in dogs, wolves, or foxes. Also keep in mind that claw prints may not necessarily be visible in canids in certain snow conditions.

5.8.2.2 Collecting a urine sample

First check if **more than one animal urinated in the same spot**. While this is not common with several lynx, it may happen in wolves. It also often happens that a fox will mark the same spot as a lynx or a wolf. Collecting such mixed samples should be avoided.

We try to collect as much as possible of the "yellow snow" in the flask. We can carefully remove some of the snow around the yellow spot with urine to make collection easier and get to the deeper parts of the urine stream. When a vertical object is marked, check also snow on the ground below the object, as urine often drips from the marked object. When finished, close the flask well to avoid leakage.

Large (50 ml) or small (8 ml) flasks can be used interchangeably, depending how easy/difficult a specific sample is to collect.

When **following a lynx track**, especially for males and during the mating season (February-March), we can find many urine samples of the same animal. Since not all samples will be successfully genotyped, it makes sense to collect several samples. The typical procedure is to collect the **first two urine samples** of the same animal that are fund in separate tubes. When **additional urine samples** are found while tracking along the same snow track from the same animal, they should be collected (as many as practical) in **a single large (50 ml) tube** (several samples in the same tube). If such "bulk" samples are collected, we should be very careful that **the samples are really from the same animal**. If there is any doubt, stop collecting new samples in the same flask.

In wolves we don't collect bulk samples, but we can nevertheless find samples of the same animal that is marking when following tracks of a wolf pack. This should be noted on the collected samples so that a decision can be made when they are considered for analysis whether they should all be analysed, or just some of them to save resources.

If you find a **non-marking urination** (on the floor) when tracking a lynx family, collect it as a single (not bulk) sample since it may belong to a kitten. Also be sure to collect non-marking urination in wolves as this may be from a non-reproductive pack member that may not mark.

5.8.2.3 Recording data for a urine sample

Most of the data is the same as with the scat sample, with a few exceptions.

The number of animals and track size is also recorded.

Marking/non marking behaviour – was urination on the ground (non-marking) or on an object (marking)? If marking, what was marked (e.g. small tree, forest house etc.).

Blood in urine – can be sometimes visible after mating in lynx, or in female wolves when they are in heat. Collection of such samples should be prioritized.

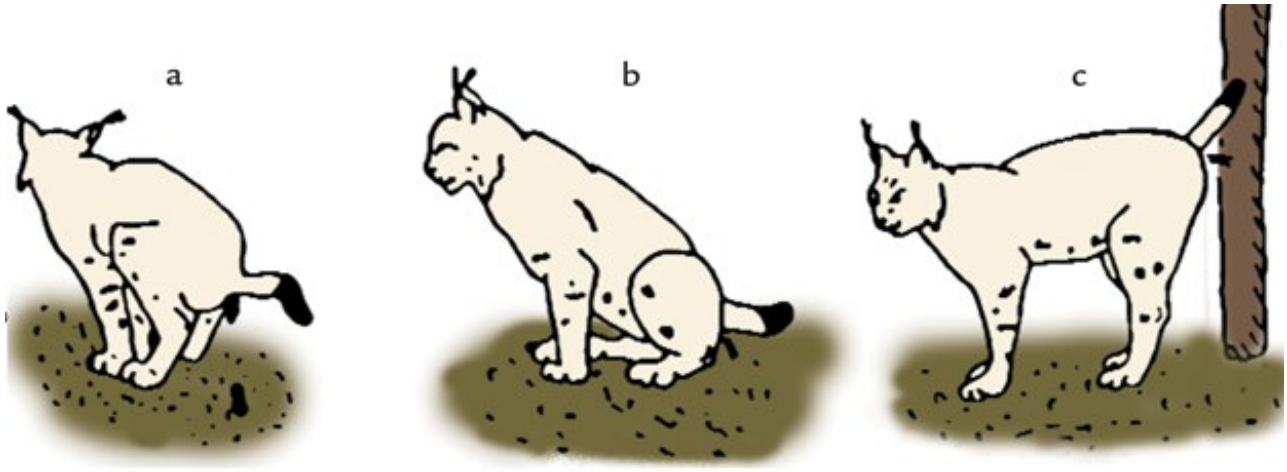

Figure 26: Defecation (a), urination (b) and marking (c). While urination will typically be on the floor and larger quantity of urine, marking is often a few drops sprayed against a vertical object. (Illustration after Hucht-Ciorga 1988).

5.8.2.4 Store and deliver the urine sample

After collection, keep the sample in a cool and dark place. Don't leave it in a car on the sun!

The sample should be **sent to be analysed** as soon as possible. **This is especially important for urine samples** since they degrade faster than scat samples. Specific details on how to do that will vary between countries and should be determined by the monitoring team.

While the urine can be stored at -20 °C for some time, stability of such samples is not assured (Cannas et al. 2009; Ng et al. 2018). The monitoring team should deliver these samples to the lab as soon as possible, preferably cooled, and the lab should process them at the earliest convenience. We don't recommend storing urine samples for long periods. Freeze – thaw cycles should be avoided as much as possible.

5.8.3 Collecting Hair Samples

Hair follicles provide a good source of DNA and can be used to sample bears or lynx. Hair samples can be collected systematically (using hair traps) or opportunistically when lynx or bear leave hair on various objects and in daybeds. Here we deal with opportunistic samples, but the same principles apply to systematically set hair-traps.

Like other non-invasive genetic samples, DNA in hair samples will degrade in environment and the probability that the analysis will be successful drops with time. However, different to scat samples they don't store that well and should be transferred to a laboratory for analysis as soon as possible.

CHECKLIST

1. We look for hair samples

...check entire hair trap, if you use them, each tuft of hair is its own sample. Opportunistic samples can be found at some places lynx or bears mark and where they are resting, but we need to look for them.

2. We collect a hair sample

...in a paper envelope, which we put in a sealed container / bag with desiccant.

3. Write the data

... a sample is useless if we don't know where and when it was collected!

4. Store and deliver the sample

... store in a dark place, return for analysis as soon as possible.

5.8.3.1 Finding opportunistic hair samples

Opportunistic hair samples of lynx will typically be found while snow tracking, meaning that we will already be quite sure that the hair is from a lynx. However, there are a few pointers where these can be found.

Look around a marking spot – lynx often rub against objects before they mark with urine, leaving some hair behind. They may mark the object they rub against, but it's not necessary. When you find marking with urine, make sure to look around for lynx hair. Hair will get stuck in wood, on a broken branch, even on a rock – look for objects that would appeal to a cat to rub against. But you will miss them unless you specifically look for them. If you are not sure whether hair is from lynx, check for the characteristic smell of felid urine that often accompanies hair at rubbing sites.

Look at the places where lynx rest – when snow tracking, you may find places where lynx lie down to rest. This will often be sheltered places with a good view – e.g. under a fell-down tree, under a rocky outcrop, edge of rock cliff etc. Carefully check such places for hair, usually several hair can be found.

Lynx have regular marking places where you may find hair samples also when there is no snow. Once you know terrain well, you will know places that lynx regularly visit. Interestingly, the same places seem to attract different lynx over many years. Once you know such places you may check them also outside of tracking season. A good idea is also to put a hair trap at such a place. However, samples found outside of a snow tracking session are of unknown age, which makes them less likely candidates for a successful genotyping since they may already be old and DNA in them may be degraded.

Recognize lynx hair – it's not always easy to recognize lynx hair, and lynx have different types of hair. Lynx hair is usually of light colour, relatively thin (compared, for example, with bear) and often with black tip. Guard hairs of the topcoat can be quite long and thicker, while the under coat hairs are shorter and very fine. Both should be collected if possible.

Opportunistic bear hair can be found on rub trees. However, we don't know how long the hair has been there (if it is still viable for analysis), and we can't be sure it's from a single bear. Bear hair will typically be collected in systematic studies utilizing hair traps. These are common in North America (e.g. (Kendall et al. 2008, 2009; Lamb et al. 2016), but are relatively rare in Europe (but see (Karamanlidis et al. 2010).

5.8.3.2 Collect a hair sample

Hair is collected in a **paper envelope**, which is then **stored** in a plastic flask or a gas impermeable zipper bag **with desiccant** (silica gel). Always use desiccant with humidity indicator that changes colour when it gets moist. Desiccant is critical since it dries-out the sample and conserves the DNA, but it must be in a sealed environment. Envelope, on the other hand, allows the sample to dry and protects it. If required, you can put several envelopes with samples in the same zipper bag / flask with desiccant.

WARNING. When exposed to air, desiccant will absorb moisture and become ineffective. Sampling kits for hair should ideally be vacuum-sealed and if the seal is not broken, they should keep indefinitely. However, once a kit is opened you should use it within a couple of days or throw it away if for whatever reason you can't collect a sample.

The part of the hair that has the DNA is the **follicle**, a bulbous end of a pulled-out hair. Not all hairs you may find will have follicles and they're often difficult to observe without a magnifying lens, so it makes sense to collect **as much hair as possible**. Also, make sure to **collect entire hair** – cut-off hair is useless. Also make sure to **also collect the thin and short undercoat hair**. Remember, the hair itself is not very important so its length doesn't matter, it's the follicle that has the DNA.

If there are **several places** with lynx or bear hair on the same site, collect **hair from each** of these places in a **different envelope**.

Ideally the hairs are collected by tweezers, which are burned with a flame (e.g. a pocket lighter) before each collection. However, since cross contamination is less of a problem with such samples and human DNA is not a problem, you can use your fingers to pluck the hairs and put them in an envelope if there is no other option.

Make sure you **seal the flask or bag with desiccant/sample envelope well**. Otherwise, moisture from the air will damage the sample.

5.8.3.3 Record the data

Much of the recorded data is the same as with other sample types, with some exceptions.

Hair age – how old do you think the hair is. There is no objective way to tell this directly from the hair, but often we can estimate (e.g. if found while snow tracking, you can estimate how old the track is with regard to last snowfall, other weather conditions, shape of tracks…). In many cases this will be a guess. You may also note the time interval since you last checked that marking site or hair trap for hair (which provides the maximum age).

Hair collected from – note if the hair was collected from a hair trap or something else. If other, note what type of object (e.g. dead tree, feeding place, forest cabin…).

Figure 28: Example d*ata collection label for a hair sample.*

5.8.3.4 Store and send the sample

First, always make sure that the envelopes with samples are well sealed in the supplied plastic bag or flask.

The sample should be **sent to be analysed** as soon as possible. **This is especially important for hair samples** since they degrade faster than scat samples. Specific details on how to do that will vary between countries and should be provided by the monitoring team.

For long term storage, hair samples should be stored in paper envelopes, in a gas-impermeable zipper bag with silica at -20 °C. Several envelopes can be stored in the same zipper bag. Optionally, add a humidity indicator card. Always use desiccant with humidity indicator. We don't recommend storing hair samples for long periods. Freeze – thaw cycles should be avoided as much as possible.

5.8.3.5 Saliva samples from prey

When we find a fresh lynx or wolf kill, we can try taking a saliva sample around bite wounds. The tricky part is contamination with scavenger DNA, but this is less of an issue with lynx since contamination with DNA of the most frequent scavenger, the fox, is not a problem in the analysis.

CHECKLIST

1. Find a good place to swab

...killing wound (neck), most recently eaten part of carcass.

2. Collect saliva samples

... collect 2-3 swabs at each prey, from different places. Seal well!

3. Write the data

... a sample is useless if we don't know where and when it was collected!

4. Store and send the sample

... store in a dark place, return for analysis as soon as possible.

5.8.3.6 Recognize the predator signs on the prey

Lynx usually kill larger prey (roe deer, red deer calf, chamois) by biting the neck from below. Occasionally they would kill the prey by biting the neck from above, severing the spinal cord. Wolves kill the prey biting the neck from below, but the wounds are usually much larger.

In lynx prey you can often see 4-8 deep wounds in the larynx area (neck, below). The wounds are typically clean, neat holes, without large lacerations that are typical in wolf kills. Sometimes no holes will be visible from the outside in a lynx kill. However, have in mind that this picture may be disturbed by scavengers. Fox, for example, will often chew through the neck and take the head away.

Lynx and wolves also differ in a manner how they consume the prey. Lynx eat muscle tissues, typically starting at upper thigh or shoulder. They don't consume the intestines, but usually (unless they catch another prey or the prey is taken away by a scavenger) return to eat for several days until all edible parts are consumed. Wolves, on the other hand, will also open and consume parts of intestines and internal organs. Lynx in most cases also cover their prey with snow, leaves or grass, which wolves don't do.


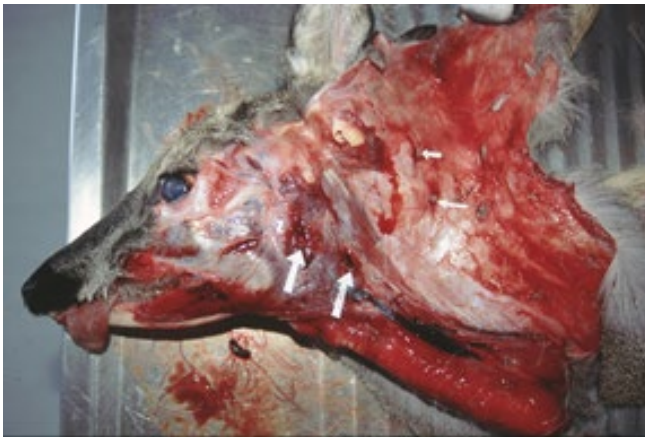

Figure 29: Killing wounds of a lynx, in nature (above) and dissected (below). Wounds (shown by arrows) made by teeth are usually neat holes with little laceration. A saliva sample would typically be taken by rubbing a forensic swab around and between the wounds shown in the left photo (Photos: Miha Krofel).


Figure 30: Lynx prey. Lynx starts eating muscle tissue, usually starting from butt or shoulder (left, Photo: Hubert Potočnik). If undisturbed, it will often consume all edible parts (right, Photo: Miha Krofel).

5.8.3.7 Find a good place to swab.

When the predator bites its prey, it leaves saliva which contains its DNA. So, wherever you find bite marks, you can try wiping the area around with a swab to pick-up the DNA. As always – the fresher, the better.

Since genotyping success from such samples is not good, particular with nuclear DNA if we also want to individually identify the predator, **always collect 2 or 3 samples** from different places on the carcass. One sample should always be from the **kill wound** (the neck). Predators often lick the neck after the kill so if the kill is fresh, there is a good chance to collect useful DNA.

The rest should be collected **at the place where the predator has most recently eaten**. Lynx return to feed on a prey for several days, so you should try to estimate which part of the carcass was most recently eaten and collect samples there. In wolves this is less of an issue as a wolf pack will consume a prey much faster.

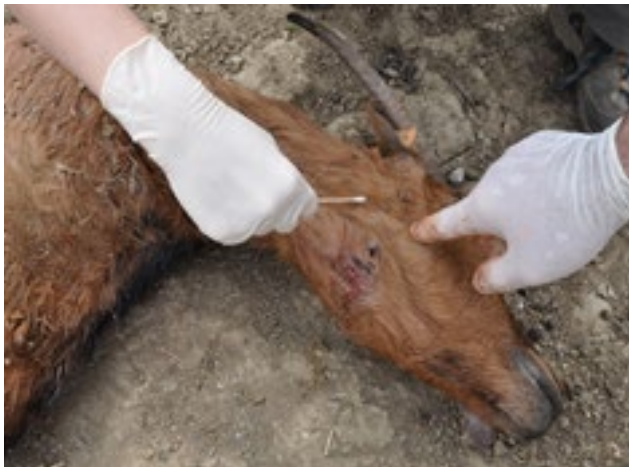


Figure 31: Collecting a saliva sample. We vigorously swab the area around and between bite wounds (left). When the carcass is already mostly eaten, we try to find the places where we think it's the highest possibility that the lynx ate last (right; Photo: Tomaž Skrbinšek).

5.8.3.8 Collect saliva samples

The best material we found for this are special **forensic swabs** which have desiccant integrated in the storage tube and are provided in sealed bags that prevent ingress of moisture. **Open the bag with the swab immediately before use** – don't open the swabs if you're not planning to use them. If you happen to unseal the swab and not collect the sample for whatever reason, this swab should be used in a couple of days or thrown away.

Once you identified the area you think has the predator saliva, **rub the tip of the swab over the entire area**. Don't swab too large areas – about the size of a human palm should be the maximum. Collect a single area with the same swab – e.g. one swab for the neck, two at two places where the predators have most recently eaten.

Some blood is unavoidable but avoid swabbing in areas **with a lot of blood** since your swab will quickly get soaked and won't collect any new material.

After collection, **return the swab to the original tube** it came in and **seal well**. If possible, seal the connection between plug (swab handle) and tube with the electrician's tape or other sealing material (e.g. parafilm). An airtight sample will remain conserved longer.

5.8.3.9 Record the data

This is similar to data collected for other types of samples, with a few exceptions.

Prey species, % consumed, prey description: Describe the prey... species, estimated age (young/old), sex & physical condition if possible. Also estimate how much prey has already been consumed.

Single predator/family: Does this look like it was a single lynx, a mother with young? A wolf pack? If you think there have been more animals, try to estimate how many (usually possible only in snow).

Sampled from: In a fresh kill, it is particularly important is to take a sample from the killing wound (neck) if possible. Note from which part of the prey the sample was taken.

Carcass decomposition: The state of decomposition gives some indication of the chance of obtaining a successful genotype.

species ☐ bear ☐ wolf ☐ lynx	**date:**
name/surname:	**location:**
GPS coordinates and/or geographic name:	
prey species:	**% consumed:**
prey description:	
single predator ☐ family ☐ if family, how many?	
sampled from: neck☐ rump☐ shoulder☐ other:	
carcass decomposition (circle): 0 1 2 3 4 5 (0 - fresh; 3 - bloated t.; 5 - liquification of tissue, mumification...) **notes:**	

Figure 32: Example sampling label, predator saliva on prey.

5.8.3.10 Store and send the sample

Double check if the swab tubes are well sealed. The sample should be **sent to be analysed** as soon as possible. Specific details on how to do that will vary between countries and should be provided by the monitoring team.

For long term storage, swabs should be stored in their original containers, in a gas-impermeable zipper bag with silica at -20 °C. Several swab containers can be stored in the same zipper bag. Optionally, add a humidity indicator card. We don't recommend storing saliva samples for long periods. Freeze – thaw cycles should be avoided as much as possible.

5.8.4 Tissue and blood samples, saliva samples taken from live captures

These types of samples provide high quality and quantity DNA and can be used for genomic studies. This makes them extremely valuable. While they are usually more reliable and easier to analyse than non-invasive samples, the same care should be taken when they are collected. They should also be carefully collected in appropriate preservation media and stored correctly until analysis.

Tissue samples

Tissue samples will usually be taken from dead animals. Soft tissue samples should be collected in ethanol (food grade or analysis grade). Do not use formaldehyde as it destroys the DNA in the sample. Make sure that **the volume of ethanol is at least 5 times as much as the volume of the sample** – if there is too much sample in comparison to the amount of ethanol, the sample will not get fully conserved and will continue to degrade. Not much of material needs to be taken for analysis – typically, the amount of tissue required for a DNA extraction

for genetic analyses is about the size of a grain of rice, so a walnut sized piece of tissue can support a large number of analyses. Still, as these samples may be taken for posterity and be utilized in research decades down the road, do take enough sample so that it's not used-up in a single analysis, if possible.

Samples of bones and teeth can be stored with desiccant (silica) in gas-impermeable zipper bags, but make sure that the silica is fresh, that there is enough of it, and that the container with the sample is thoroughly sealed. It is good practice to change silica a week or so after collection so that the sample completely dries out, and to store them with a humidity indicator card (commercially available) for long-term storage.

Which tissue to take will depend on the goal of the study and the status of the carcass. While internal organs (e.g. liver) have high DNA content, they degrade fast, and we don't recommend their collection for the purpose of genetic or genomic studies unless the carcass is completely fresh. Skeletal muscle is good choice in most carcasses except for severely decomposed, mummified, or skeletonized ones. In heavily decomposed carcasses it is better to take hard tissue (bone, tooth). Samples from such carcasses can be problematic, so collection of several samples of different material is advisable.

While correctly preserved tissue samples can in principle be stored at room temperature in a dark room, we would advise against that, particularly for valuable samples. In a belt-and-suspenders type of approach, we would suggest that the samples are stored in a freezer at -20 °C. For samples stored with desiccant, they should be left for a couple of days with fresh desiccant at room temperature to completely dry out before being put in the freezer. Freeze – thaw cycles should be avoided as much as possible.

Samples should be occasionally checked, and ethanol replaced if some of it evaporated. Humidity indicator cards or the colour of silica with indicator should be occasionally checked in dry-stored samples, and silica replaced if needed.

Blood samples

Blood samples are usually routinely obtained at live captures of animals. They provide an excellent source of DNA but must be stored appropriately.

Whole blood with anticoagulant (EDTA) should be immediately refrigerated, and frozen as soon as possible at -20 °C. This, however, should not be considered as long-term storage – for that, whole blood needs to be stored at -80 °C or in liquid nitrogen.

A much simpler solution is storing blood samples as Dried Blood Spots (DBS) on a filter paper (Grüner et al. 2015). There are different DBS cards available commercially, and they are very simple to use:

- Transfer anti-coagulated (EDTA) whole venous blood on the filter cards as soon as possible, maximum within 24 hours. You can fill several cards with the same blood sample to allow for more analyses.

- Dry the blood spots – put them on a clean paper towel in a dry environment and let them dry for at least 4 hours, preferably overnight, at room temperature. When dry, they should have uniformly dark brownish colour and no red areas are visible anymore.
- Store DBS cards in gas-impermeable zipper bags with desiccant (silica) to protect from moisture. Optionally, add a humidity indicator card.
- Keep bags with samples in freezer at -20°C or lower. Transport them on dry ice for long transports.

Buccal (saliva) samples directly collected from animals

Sometimes taking blood samples is not routinely done or desirable at live captures of animals (for example when sampling young animals in a den). In such cases, buccal samples can be taken directly from the mouth of these animals.

- The same forensic swabs as described for saliva collection from prey can be used for collection.
- Inside of cheeks of the animal should be swabbed with the swab. The swab should then be stored in the same tube it came in, which contains desiccant, and sealed. If possible, take several samples.
- For long term storage, swabs should be stored in a gas-impermeable zipper bag with silica at -20 °C. Optionally, add a humidity indicator card.

Direct saliva and buccal samples are quite stable when dried (Anthonappa et al. 2013), even when stored at the ambient temperature, but they should still be stored appropriately as soon as possible.

6

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6 References

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7

Annex I: LCIE Monitoring Standards for Large Carnivores

7 Annex I: LCIE Monitoring Standards for Large Carnivores

This part is directly copied from "Monitoring standards for large carnivores in Europe", a white paper recently prepared by the Large Carnivore Initiative for Europe (Marucco et al. 2024). This is copied in this place for convenience and for completeness of these guidelines. However, the text here is from an advanced draft - we encourage the readers to check the final version of the referenced document when using these standards in practice.

7.1 Wolf (*Canis lupus*)

7.1.1 Wolf signs, and their C categorization

C1	C2	C3
Captured or rescued alive animals	Tracks with typical trend/pattern, followed for at least 100 m in snow	Tracks If followed < 100 m or single footprints
Dead animals	Scats or hairs with expert check, or If collected along a C2 snow track *	Scats not confirmed by an expert and not associated with snow tracks
Radio-collar localization	Urine If collected along a C2 snow track*	Urine, hairs not associated with C2 tracks and without DNA evidence
High quality video and photos	Predation signs with typical bites and/or consumption and / or combined with other C2 data	Heavily eaten kills, livestock depredations not combined with other C2 data
Whatever DNA evidence confirms the biological sample (e.g. scats, hairs, urine, saliva)	Howl with wolf pups' presence, checked by an expert	Single howls
Indirectly certified presence signs from DNA evidence (e.g. tracks on snow)*		Sightings Not supported by photos and videos
		Poor quality videos and pictures

*if you find scat / hair / urine along a C2-tracks, than track + sign count as one C2-event, not as two C2 signs;
If the scat / urine / hair is genetically analysed and becomes C1, than track + sign count as one C1 event

7.1.2 Wolf – agreement on definitions

Wolf presence type in the area	
Single resident wolf	Single wolf living in an area for at least 6 months.
Pair	Male and female wolves travelling together but not (yet) having reproduced
Pack	A group of more than two wolves living together in a territory.
Reproductive pack	Consists of at least one mature wolf with confirmed reproduction.
Age class of wolves:	
Mature/Adult (reproducing)	≥ 24 months
Pup	Wolf in its first year of life. The transition from pup to yearling is considered on 1st May.
Yearling	Wolf in its second year of life
Monitoring year	1st May – 30th April
wolf-dog Hybrid*	wolf-dog hybrid documented with genetic analysis

* If hybrids are detected they should be excluded from the wolf population size estimate/index but should be reported separately. If hybrids are known to be present but not excluded from the counts (because precise data of their proportion in the population are not available), then this should be declared and reported as well.

7.1.3 Comparison of available methods for wolf monitoring

Method	Possible applications in Surveying or Monitoring	Required investment of resources	SCALP category of data obtained
Opportunistic sightings (not documented)	Where to concentrate monitoring efforts	Low	Not attributable
Questionnaires	Where to concentrate monitoring efforts	Low	Not attributable
Records on livestock damages	Where to concentrate monitoring efforts and conflict mitigation. If C1 (because saliva samples confirm wolf presence), then used for distribution / abundance and their trends.	Medium	C1, C2, C3
Signs collection by travelling transects or opportunistic, snow tracking	Distribution, indications of population trend, reproduction	Medium	C1, C2, C3
Recovery of dead wolves	Distribution, mortality, health	Low	C1
Camera trapping	Distribution, abundance, population trend, demography, dispersal, reproduction	Medium to high	C1, C3
Genetic analysis on biological samples	Distribution, abundance, population trend, dispersal, reproduction	High	C1
Telemetry	Abundance, dispersal, health and conflicts	Very high	C1

7.1.4 Parameters, recommended methods, and data needed to estimate area of occurrence and population size for wolves in Europe

Measure	Field Methods	Data needed
Area of occurrence in a cell	Presence sign survey, camera trapping	One C1 hard fact, or three independent C2 signs, or ten not independent C2 signs of the same type, per 10 x 10 km cell and year
Number of packs Number of pairs Number of individual territorial wolves	Presence sign survey, snow tracking, genetic analysis, howling survey, camera trapping, video / photo documentation	Occurrence of a pack, a pair or a territorial individual must be confirmed via C1 or C2 data
Population size	Capture-recapture methods or other modelling framework that accounts for imperfect detection	Individual identification from genetic analysis
Pack size	Presence sign survey, snow tracking, genetic analysis, howling survey, camera trapping, video/photo documentation	The minimum figure for pack size must be confirmed via C1 or C2 data
Reproduction Presence	(focus on pup tracks), snow tracking, genetic analysis, howling survey, video documentation of rendezvous sites, camera trapping	Successful reproduction has to be confirmed via C1 or two C2 data
Distinguishing between adjacent territories	Genetic analyses, camera traps, telemetry	Distinction possible by: genetic identification OR simultaneous proof of reproduction OR reproductions confirmed in May – July (August?) at least X* km apart OR telemetry data from radio collared wolves belonging to one of the adjacent territories OR individuals are clearly identifiable (e.g. by camera trap pictures / videos)

*This distance is population specific, e.g. for CEP population a distance of 10km is used (Reinhardt et al. 2015).

7.2 Brown bear (*Ursus arctos*)

7.2.1 Bear signs and their C categorization

C1	C2	C3
Captured or rescued alive animals	Tracks on snow / sand / mud with typical trend/pattern, assessed by an expert (expert on site or photo)	Tracks on snow / sand / mud
Dead animals	Scats with expert check	Single footprint
Radio-collar localization	Hairs collected e.g. on a rubbing tree plus expert check	Urine, hairs not associated with DNA evidence
High quality video and photos	Urine If collected along a C2 snow track	Heavily eaten kills, livestock depredations not combined with other C2 data
Whatever DNA evidence confirms the biological sample (i.e. scats, hairs, urine, saliva). Bear scat is quite distinct even without genetic confirmation.	Predation signs if combined with other C2 data and expert check	Inappropriate documentation provided by third party
Indirectly certified presence signs from DNA evidence (e.g. tracks on snow). Bear footprints are quite distinct even without genetic confirmation.	Medium quality video and photos with expert assessment	Sightings Not supported by photos and videos

7.2.2 Bear - agreement on definitions

Bear presence type in the area:	
	Single animal confirmed.
	Family group of a mother with confirmed number of accompanied cubs
	Several bears of different sizes (ages and sexes)
Age class of bear: by sightings	Cub – accompanied with the mother (or alone)
	Yearling – accompanied with the mother (or alone)
	Adult female – can be confirmed only if with cubs
	Adult male – confirmed by size (200+ kg)
Monitoring year	Fall AND spring

7.2.3 Comparison of available methods for bear monitoring

Method	Monitoring/research questions to be answered	Required investment of resources	SCALP category of data obtained
Reports from hunters	*Trend, abundance, reproduction*	*medium*	*C1, C2*
Questionnaires	Where to concentrate monitoring efforts	Low	Not attributable
Collection of chance observations	Distribution, indications of population trend, reproduction, health and conflicts	Low	C1, C2, C3
Snow tracking	Distribution, population trend, reproduction	Medium	C2
Camera trapping	Distribution, abundance, population trend, demography, dispersal, reproduction	Medium to high	C1
Genetic analysis	Distribution, abundance, population trend, dispersal, health, reproduction	High	C1
Telemetry	Dispersal, health and conflicts	Very high	C1

7.2.4 Parameters, recommended methods and data needed to estimate area of occurrence and population size for bear in Europe

Parameter	Methods	Data needed
Area of occurrence	Presence sign survey, camera trapping	One C1 hard fact, or two C2 signs per 10 x 10 km cell and year
Population size	Genetic analyses, counts at feeding sites,	Minimum estimate based on C1 or C2 data
Reproduction	Tracks, foto/video, sighting	Reproduction has to be confirmed via C1 or C2 data

7.3 Eurasian lynx (*Lynx lynx*)

7.3.1 Lynx signs, and their C categorization

C1	C2	C3
Captured animals	Tracks on snow documented and assessed by an expert	Tracks on snow not assessed by an expert
Video and photo (if in doubt =>discard)	Prey remains with typical signs documented and assessed by an expert	Single footprint
Dead animals		Prey remains not assessed by an expert
Whatever DNA evidence confirms the biological sample (i.e. scats, hairs, urine, saliva)		Presumed lynx scat not genetically analysed
Hair from lynx, identification based on hair structure		Vocalisations
Radio-collar localization		Sightings not supported by photos and videos

7.3.2 Lynx - agreement on definitions

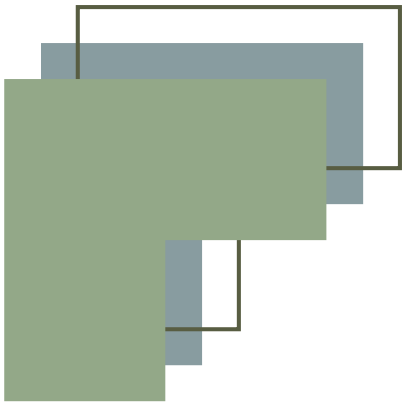
Lynx presence type in the area:	
Lynx presence	Confirmed presence of a lynx
Family group	Confirmed reproduction
Age class of lynx:	
Kitten	Until the age of 1 year
Independent	Age 1 and older, cut off date is 30. April
Monitoring year	1st May – 30th April; In Scandinavia 1st June – 31st May (Mattisson et al. 2022)

7.3.3 Comparison of available methods for lynx monitoring

Method	Monitoring/research questions to be answered	Required investment of resources	SCALP category of data obtained
Reports from hunters	Where to concentrate monitoring efforts	Low	Not attributable
Questionnaires	Where to concentrate monitoring efforts	Low	Not attributable
Collection of chance observations	Distribution, indications of population trend, reproduction, health and conflicts	Low	C1, C2, C3
Snow tracking	Distribution, population trend, reproduction	Medium	C2
Camera trapping	Distribution, abundance, population trend, demography, dispersal, reproduction	Medium to high	C1
Genetic analysis	Distribution, abundance, population trend, dispersal, health, reproduction	High	C1
Telemetry	Abundance, dispersal, health and conflicts	Very high	C1

7.3.4 Parameters, recommended methods and data needed to estimate area of occurrence and population size for lynx in Europe

Parameter	Field Methods	Data needed
Area of occurrence	Camera trapping, presence sign survey	One C1 or C2 sign per 10 x 10 km cell and year
Population size	Opportunistic camera trapping to determine minimum size. For larger populations, systematic camera trapping for statistical capture-recapture estimation in reference areas.	Minimum estimate based on C1 data, individual identification
Reproduction	Camera trapping, snow tracking, direct observation	Reproduction has to be confirmed via C1, C2 or C3 data, but needs to be distinguished



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