Guidelines for Long-Term Monitoring of European Bats

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1 Preface

This document provides guidance on methods for the surveillance and monitoring of European bat species and constitutes the most updated guidelines after the EUROBATS Publication Series No. 5, *"Guidelines for Surveillance and Monitoring of European Bats"*, in 2010. The guidelines are designed to accommodate regional variation and differing capacities, needs, and monitoring priorities across Europe.

Since 2010 the growing need to design a coordinated, pan-European bat monitoring programme that centralizes data and harmonizes methodologies across countries has become notorious. At present, monitoring efforts are often fragmented, with varying protocols, metrics, and levels of coverage that limit the comparability and scalability of results. Establishing a shared framework would enable the consolidation of datasets, facilitate the exchange of expertise and best practices, and improve overall data quality. Such coordination would also reduce duplication of effort and help ensure that monitoring is both efficient and scientifically robust.

The first, and to date only, concerted effort to estimate bat population trends across Europe was conducted by Van der Meij et al. (2015), more than a decade ago. However, this analysis was limited to cave-dwelling species monitored at hibernation sites, and therefore excluded a large proportion of European bat diversity and seasonal activity patterns. Since then, substantial advances in monitoring methodologies, particularly the rapid development of passive acoustic techniques and associated analytical tools, have greatly expanded the scope and feasibility of large-scale assessments. These technological improvements now make it possible to integrate data from a much broader range of species, habitats, and countries. We can move beyond taxonomically and spatially restricted assessments and towards more comprehensive, pan-European estimates of bat population trends.

A unified programme would make it possible to calculate consistent, large-scale trends in bat populations that transcend national borders, reflecting the ecological reality of such mobile species. By fostering collaboration among researchers, institutions, and policymakers, this approach would support more coherent conservation strategies and strengthen the evidence base for decision-making at regional and international levels. In an increasingly interconnected world, a pan-European perspective is essential to effectively assess and respond to the pressures facing bat populations. This works aims to contribute to this purpose by providing guidelines to harmonise, implement, share and archive protocols in the highest level possible.

Guidelines’ structure

These guidelines have been designed as a complementary tool to the online dataset *“Bat monitoring programmes and protocols for European bats”*, published by EUROBATS and accessible via the European Zenodo Repository (López-Baucells et al. 2025).

The publication is structured into three main sections. The first section links specific long-term monitoring schemes to bat species occurring in Europe, enabling practitioners to identify and select the most appropriate protocols for monitoring particular bat populations (Chapter 3). The second section provides practical guidance and recommendations for the implementation of monitoring schemes, including aspects such as survey periodicity, timing, and other related considerations (Chapter 4). The final section presents detailed information on the specific techniques required for each monitoring approach, with the aim of facilitating and improving their application in the field (Chapter 5).

The book also includes a dedicated section on data storage and management, recognising that this is an increasing concern among bat researchers and conservation practitioners, particularly in light of recent technological advances that enable the collection of large volumes of data (Chapter 6). To support data analysis and practical implementation, the publication also provides guidance and examples illustrating how long-term monitoring data can be analysed to assess and project bat population trends as well as to use them as indicators to multiple ecological drivers (Chapter 7).

Species accounts and distribution maps included in the previous edition have been omitted, as these data are highly dynamic and subject to frequent change, and because robust and authoritative alternative sources such as the IUCN Redlist already provide this information.

It should be noted that these guidelines will require periodic revision and updating as new methods are developed and adopted. Updated versions will be made available in the PDF edition of the guidelines on the EUROBATS website. These guidelines have been developed by the EUROBATS Intersessional Working Group on “Monitoring and Indicators”, with additional contributions from the advisors in each chapter or section (Fig. 1).

Fig. 1 Participants of the EUROBATS Advisory Committee meeting in Bonn , 2025.

2 Introduction

Bats are a diverse and ecologically significant component of European biodiversity. They are widely distributed across the continent and occupy a broad range of natural, semi-natural and human-modified landscapes, exploiting complex networks of roosts above and below ground. Many species exhibit complex life histories, including seasonal migration, long-distance movements between roosts and foraging areas, and reliance on heterogeneous habitat mosaics. Through their specialised feeding ecology and sensitivity to environmental change, bats perform essential ecosystem functions and serve as valuable indicators of ecosystem health and biodiversity status (Russo & Jones 2015, Tuneu-Corral et al. 2023).

European landscapes have long been shaped by intensive and varied human influences. Since the publication of earlier EUROBATS monitoring guidelines (Battersby 2010), the environmental context in Europe has changed substantially. Accelerated land-use change, agricultural intensification, urban expansion, forest management shifts, infrastructure development, renewable energy installations, pollution, and climate change are reshaping landscapes at unprecedented scales (Van Vliet et al. 2015; Plieninger et al. 2016). These drivers affect bats through alterations in roost availability, habitat structure, prey dynamics, phenology, and migration routes (Festa et al 2023). Intensifying threats, including collision mortality, light pollution, and disease (Browning et al. 2021), further highlight the need for coordinated, robust monitoring frameworks.

All European bat species are protected under national legislation and international agreements, reflecting both their conservation importance and their vulnerability. Despite this protection, many species remain in unfavourable conservation status in parts of their range. Reliable information on changes in distribution, abundance and population dynamics over time is therefore essential. Robust and coordinated surveillance and monitoring programmes are required at local, national and European levels to provide this information (e.g. Fig. 2).

Fig. 2 *Plecotus austriacus* in medieval benedictine monastery, an important cultural heritage site in Catalonia, where bat monitoring is needed to monitor its population.

Within conservation practice, monitoring and surveillance represent complementary approaches. Monitoring refers to standardised and repeated data collection designed to detect long-term trends in populations, distribution or ecological processes. Surveillance focuses on targeted efforts to detect specific species, emerging threats or changes in conservation status, often providing early warning signals that can trigger management responses. Together, these approaches form the foundation of adaptive conservation strategies for highly mobile and ecologically sensitive taxa such as bats.

Well-designed monitoring and surveillance programmes support conservation by:

- Detecting changes in distribution, range and abundance, and generating reliable long-term population trends.
- Informing national and international policy and the setting of conservation priorities.
- Assessing the effectiveness of habitat management, species protection measures and mitigation actions.
- Supporting public engagement, education and capacity building through the involvement of volunteer networks and citizen science initiatives.

In many European countries, volunteer-based monitoring schemes constitute a cornerstone of bat conservation (e.g. Barlow et al. 2015, Kerbiriou et al. 2015, Torre et al. 2021, Clarke et al. 2024, López-Bosch et al. 2024). These initiatives expand spatial coverage, strengthen long-term datasets, build technical expertise and foster public understanding of bat ecology and conservation challenges.

Fig. 3 Educational activities conducted to enhance volunteer-based monitoring programmes, providing capacity training and general information to the potential participants.

Conservation policy context and international obligations

The need for coordinated monitoring and surveillance of bats is recognised within several international conventions and legislative frameworks.

Under the Convention on the Conservation of Migratory Species of Wild Animals (CMS, Bonn Convention), all European bat species are listed on Appendix II, recognising that migratory species can only be effectively conserved through international cooperation across their entire range. The Agreement on the Conservation of Populations of European Bats (EUROBATS), established under Article IV of CMS, provides the principal framework for collaboration among Range States. Monitoring, research, education and coordinated action form the central pillars of the Agreement.

The Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) obliges Parties to protect breeding and resting sites of strictly protected species, including almost all European bat species. The Convention on Biological Diversity (CBD) requires Parties, under Article 7, to monitor components of biodiversity, with particular attention to threatened and indicator species.

Within the European Union, Directive 92/43/EEC on the Conservation of Natural and Semi-natural Habitats and of Wild Fauna and Flora (the Habitats Directive) requires Member States to maintain or restore species at favourable conservation status. Article 11 obliges Member States to undertake surveillance of the conservation status of species and habitats, and Article 17 requires periodic reporting based on robust monitoring data. Monitoring data also contribute to European Red List assessments and broader biodiversity indicators (Russo et al 2025).

These international and regional obligations underline the importance of harmonised methodologies, transparent analytical frameworks, data sharing and collaboration among Range States to ensure that monitoring results are comparable across regions and contribute to continental-scale conservation assessments.

Monitoring and surveillance at a European scale

Many bat species undertake seasonal movements between maternity, mating and hibernation sites, frequently crossing national boundaries (Fleming et al 2003). Effective conservation therefore requires coordinated monitoring at a continental scale to understand population dynamics, migration patterns and large-scale environmental drivers.

Pan-European monitoring initiatives aim to promote consistent data collection and standardised methodologies, enabling comparison of trends between regions and aggregation of national datasets into European-scale indicators. Harmonised approaches facilitate the identification of species or populations requiring targeted conservation action and support timely responses to emerging threats.

Standardisation also enables integration of datasets collected by researchers, government agencies, conservation organisations and volunteer networks. Collaborative frameworks under EUROBATS provide a platform for developing best-practice methodologies, sharing knowledge and promoting coordinated responses to conservation challenges across Europe.

Advances in monitoring approaches

Technological advances have significantly expanded the tools available for bat monitoring and surveillance. Passive acoustic monitoring, automated call classification, radiotelemetry and GPS tracking, thermal and infrared imaging, fibre-optic inspection, genetic monitoring and remote sensing technologies now allow bat populations to be studied at unprecedented spatial and temporal scales (Hristov et al. 2008, O'Mara et al. 2014, Hurme et al. 2025; Meramo et al. 2025, Roemer et al. 2025). These methods improve detection of species presence, behavioural patterns, migration routes and responses to environmental change (Fig. 3).

While technological innovation offers new opportunities, consistency of methodology, careful sampling design and clear data interpretation remain essential to ensure comparability across programmes and over time. Long-term monitoring requires stable protocols, repeatable sampling frameworks, secure data and metadata management and transparent statistical approaches capable of detecting genuine trends.

This manual provides guidance on the design and implementation of scientifically robust, standardised and adaptable monitoring and surveillance programmes for European bats. By strengthening methodological rigour, coordination and long-term continuity, EUROBATS Parties and partners can ensure that monitoring across Europe provides the reliable evidence base necessary to safeguard bat populations in a rapidly changing environment.

Fig. 4 Infrared video recording coupled with a passive ultrasound recorder set up in front of a cave entrance to census a migratory bat colony.

3 Selecting monitoring protocols per species

3.1 First steps to design a monitoring programme

In order to start developing a monitoring programme, it is crucial to understand what is being measured and to interpret the results with care. To begin assessing bat population trends, it is first necessary to determine which species are present in a given area (species occurrence). Then, observed changes in bat species abundance provide the baseline for estimating population trends over time.

- **Species occurrence.** Assessing changes in populations over time can be challenging when little information is available on the occurrence of a particular species. In such cases, determining the species' distribution across specific areas becomes a priority. The selected methods should aim to maximise the probability of detecting and correctly identifying the species (Limpens & Kapteyn 1991, Limpens & Roschen 1996, Limpens & Roschen 2002, Flaquer et al. 2007). Species distribution is typically presented in maps indicating where the species has been recorded over a defined time period (e.g. 5- or 10-year intervals), and species distribution modelling is commonly used to define potential distribution areas based on the available information.

- **Species abundance.** To assess bat population trends, the collection of abundance data is essential. However, estimating total population sizes is almost always impractical or impossible. In most cases, only a subset of the population can be sampled, and population trends must be inferred through extrapolation from these data. Consequently, different abundance metrics are typically used to model and interpret population trends. Depending on the monitoring technique employed (e.g. underground roost censuses versus acoustic surveys), either absolute abundance or relative abundance indices may be obtained, and results must be interpreted with caution. Abundance assessments may involve conducting a full census of all individuals and repeating the survey at regular intervals; however, such approaches are often time-consuming and costly, and therefore not always feasible.

There are many factors and concepts to consider when designing long-term surveillance and / or monitoring programmes. The terms "surveillance" and "monitoring" have been used somewhat interchangeably in the past, but in fact a distinction can be drawn between the two activities and this is quite important when considering the level of information required.

- **Surveillance**, in the context of measuring populations, consists of repeated and standardised observations of abundance over time, using methods that enable changes in numbers to be detected (Hellawell 1991). Surveillance is a means of assessing what is happening to populations of a particular species over time.

- **Monitoring** requires that targets are set, management recommendations made and carried out, the effectiveness of the management assessed and changes made to improve the process. Monitoring therefore involves surveillance, not only of the species in question but, so far as possible, also of the other factors likely to affect populations of that species.

Surveillance schemes needs to consider a broad variety of decisions in order to be designed appropriately to provide robust results and population trends:

- **Surveys frequency**: Surveillance schemes should collect data at regular intervals, ideally annually, in accordance with the annual cycle of bats, although less frequent monitoring may be appropriate for certain species. To distinguish real population trends from natural year-to-year fluctuations, surveillance should be conducted over the long term (exceeding 10 years), which necessitates sustained commitment and investment. Survey frequency for each protocol is indicated in 4 Designing long-term monitoring protocols.

- **Standardization**: Any long-term monitoring programme must ensure that the applied methods are standardised, so that they are repeatable across sites and over successive years. Only through such standardisation can comparisons remain valid and meaningful over extended periods of time (Walsh et al. 2001, Walsh et al. 2003).

- **Area coverage, stratification and sample size**: The spatial extent over which population trends are to be assessed strongly influences both the effort required and the selection of survey sites. As a general guideline, ~40 sites per species per region are considered sufficient to characterise bat population trends, although this number varies depending on the overall target area. Randomised site selection approaches are strongly recommended to ensure unbiased and representative sampling.

- **Species coverage**: Calculating population trends for multiple species is always recommended. However, if specific species must be prioritised due to resource or logistical constraints, the following factors should be considered: conservation priority at regional, national, or European levels, and the ease with which the species can be effectively surveyed.

- **Pilot phase**: It is extremely important to test the monitoring scheme during a pilot phase in order to ensure reliability of data collection, repeatability of the surveys and the ability to deliver the required level of information.

- **Biases**: Bats are difficult to count, and even using the best sampling methods, there will be uncertainties inherent in population estimates and trends. Repeatable counts do not have to be accurate in the sense that the population estimate is close to the actual population. If the counts are consistently done, the changes in bat population counts can still be measured accurately. The ability to count bats with the same detectability each year remains an essential attribute of a successful bat population monitoring scheme. The effects of small sources of bias are often over-emphasised in comparison with a lack of precision (Toms et al. 1999). For this reason, it is important to measure or justifiably estimate the magnitude of bias. Biases are described in 5 Techniques and methodologies for each particular technique.

- **Data collection, entry and storage**: Managing surveillance data is probably one of the most difficult and time consuming aspects of running a surveillance scheme. It is essential to have a database of survey results that can be easily accessed and analysed, potentially an online system that is dynamic and consistently updated, and as open as possible in terms of data sharing policy (e.g. www.batmonitoring.org). Data should be stored in a format that is accessible and can be maintained in perpetuity and made available to as wide an audience as possible. Long-term (i.e. over decades) organisational, financial, data archiving and data supply structures should be put in place. In particular procedures should exist to safeguard the foregoing irrespective of changes in personnel. More specific information about data storage and management is included in 6 Managing data: storing, sharing and archiving.

- **Database requirement and management**: The database should include appropriate metadata for each sighting (time, surveyor, locality, species, observations, methodology, biases, environmental conditions.) and should be easily comparable at pan-European level. In order to ensure comparability one can use a predefined database open for all European countries (e.g. www.batmonitoring.org) or ensure that metadata and collected data are being stored using standardized methods. More specific information about data storage and management is included in 6 Managing data: storing, sharing and archiving.

- **Validation and quality control**: There may be some concerns over the accuracy of raw data provided; a process of data validation should be put in place when entering the data electronically, so that the accuracy can be checked. Generally, surveillance data can be collected by relatively inexperienced surveyors, including volunteers, because the data collection process can be fairly simple. However, it is important to have some way of verifying the data they provide. It is also important that schemes include some form of training and feedback of results to surveyors.

- **Data analysis**: Many factors can influence the appearance of trends (apart from true changes in population size) and the magnitude of their effect should be estimated and methods for reducing their influence put into place to reduce the possibility of data misinterpretation. Specific recommendations for long-term population trends analyses are included in 7 Indicators and Population trends.

- **Surveyors.** In current monitoring programmes, it is essential to combine professional surveyors with recruited volunteers to collect data. A large network of volunteers enables coverage of numerous sites, achieving a level of spatial and temporal monitoring that would be prohibitively expensive using only professional surveyors. Volunteers frequently possess valuable local knowledge of the area. However, reliance on volunteers can introduce higher levels of uncertainty due to varying levels of expertise. These challenges should be mitigated through training programmes. Unfortunately, many European countries lack a tradition of engaging volunteers in the collection of natural history data, resulting in limited pools of volunteer surveyors. Consequently, considerable effort is required to recruit and retain volunteers.

- **Health and safety issues**: Fieldwork on bats is always accompanied by special risks for the surveyor, and all people and institutions being involved in a bat monitoring programme should take the utmost care to minimise these risks (work at night, complex caves, high trees inspections or ladders usage in the field, for instance). For this reason, survey work has to be planned carefully and all appropriate measures for safety of the surveyors should be taken very seriously.

3.2 Selecting the protocol for each species

Selecting the most appropriate methodology to monitor bat populations is a critical step in the design and implementation of local, regional, or national monitoring programmes. However, this decision is rarely straightforward, as it depends on multiple factors, including co-occurring species, regional context, population densities, and geographical variation in bat behaviour and distribution.

In the following table, we present the most commonly implemented monitoring protocols and indicate those that are universally applicable, as well as those that may be suitable under specific regional conditions, for monitoring European bat species. The table was developed through a series of round-table discussions held during the EUROBATS Annual Advisory Committee meetings, in collaboration with members of the Intersessional Working Group on Monitoring and Indicators.

Table 1. Most appropriate long-term monitoring protocols for each European bat species. The table indicates protocols considered suitable for all regions (Y) and those applicable only under specific regional conditions (site-specific). The suitability of any given protocol should always be verified through consultation with local experts.

Species	**Over- and underground roosts**				**Bat foraging Habitat**		**Bat boxes**
	Maternity	Hibernation	Migration	Swarming	Acoustics (PAM or transect)	Waterway surveys	
Barbastella barbastellus	Site-specific	Site-specific		Yes	Yes		
Barbastella caspica		Yes		Yes	Yes		
Eptesicus anatolicus							
Eptesicus isabellinus							
Eptesicus nilssonii		Yes		Yes	Site-specific		
Eptesicus ognevi					Site-specific		
Eptesicus serotinus	Yes	Yes			Site-specific		
Hypsugo savii	Yes	Yes			Yes		
Miniopterus pallidus	Yes	Yes	Yes				
Miniopterus schreibersii	Yes	Yes	Yes				
Myotis alcathoe				Yes			
Myotis bechsteinii				Yes			
Myotis blythii	Yes	Yes	Yes				
Myotis brandtii	Site-specific	Site-specific		Yes			
Myotis capaccinii	Yes	Yes	Site-specific		Site-specific	Site-specific	
Myotis crypticus				Yes			
Myotis dasycneme	Yes	Yes			Site-specific	Site-specific	
Myotis daubentonii	Yes	Yes			Site-specific	Site-specific	
Myotis davidii				Yes			

Myotis emarginatus	Yes	Yes	Yes	Yes			
Myotis escalerai	Yes			Yes			
Myotis hoveli							
Myotis myotis	Yes	Yes	Yes				
Myotis mystacinus	Site-specific	Site-specific		Yes			
Myotis nattereri	Site-specific	Site-specific		Yes			
Myotis punicus	Yes	Yes	Yes		Yes		
Myotis schaubi	Site-specific	Site-specific		Yes			
Myotis tschuliensis	Site-specific	Site-specific		Yes			
Nyctalus azoreum					Yes		Yes
Nyctalus lasiopterus	Yes				Site-specific		Yes
Nyctalus leisleri	Yes				Site-specific		Yes
Nyctalus noctula	Yes	Site-specific			Site-specific		Yes
Otonycteris hemprichii	Yes				Yes		
Pipistrellus hanaki					Yes		
Pipistrellus kuhlii	Yes				Site-specific		Yes
Pipistrellus maderensis	Yes				Site-specific		
Pipistrellus nathusii	Yes				Site-specific		Yes
Pipistrellus pipistrellus	Yes				Yes		Yes
Pipistrellus pygmaeus	Yes				Site-specific		Yes
Plecotus auritus	Yes	Yes					
Plecotus austriacus	Yes	Yes					
Plecotus gaisleri	Yes	Yes	Yes		Yes		
Plecotus kolombatovici	Yes						
Plecotus macrobullaris	Yes						
Plecotus sardus	Yes						
Plecotus teneriffae	Yes						
Rhinolophus blasii	Yes	Yes	Yes		Yes		
Rhinolophus euryale	Yes	Yes	Yes		Site-specific		
Rhinolophus ferrumequinum	Yes	Yes	Yes		Yes		
Rhinolophus hipposideros	Yes	Yes	Yes		Site-specific		
Rhinolophus mehelyi	Yes	Yes	Yes		Site-specific		
Rousettus aegyptiacus	Yes				Site-specific		

Tadarida teniotis	Site-specific				Site-specific		
Taphozous nudiventris	Yes				Yes		
Vespertilio murinus	Yes				Site-specific		

4 Designing long-term monitoring protocols

4.1 Introduction

If robust long-term population trends are to be obtained, the surveillance of bat species and their roosts can be conducted using a wide variety of methodologies and monitoring protocols (Fig. 5). The methodological choice depends on the target species, the type of data to be obtained, the available human and financial resources, and the region to be surveyed. These methods generally involve repeated counts and visits to the same sampling sites, at least annually, in order to detect temporal trends. It is therefore of paramount importance to adhere to a strict code of practice to ensure that surveillance activities do not adversely affect the bat populations under study.

It is also essential to maximise the standardisation of protocols across Europe to enable the establishment of population trends at a continental scale. At the same time, this standardisation must take into account the varying climatic regions across Europe, which can directly influence the phenology of bat populations, even within the same species, as well as different bat behaviours that can be found in different regions. In order to cover these differences, the Advisory Committee has grouped experts from all the Biogeographical regions of Europe, and the text has been revised taking into account all these expertises.

The protocols presented below address different aspects of the annual life cycle of European bat species and can be implemented in a standardised manner. They are arranged from the most frequently used protocols (e.g. underground roost monitoring) to the least commonly used or emerging methodologies (e.g. swarming surveys). For each protocol, general recommendations are specified, sometimes for the four principal European climatic regions: the Mediterranean, the Central European temperate, the Boreal and the Macaronesian regions (Fig. 6 & 7). The specific methodologies and techniques involved in each recommended protocol are described in greater detail in 5 Techniques and methodologies.

Fig. 5 Traditional census using speleology during the hibernation period.

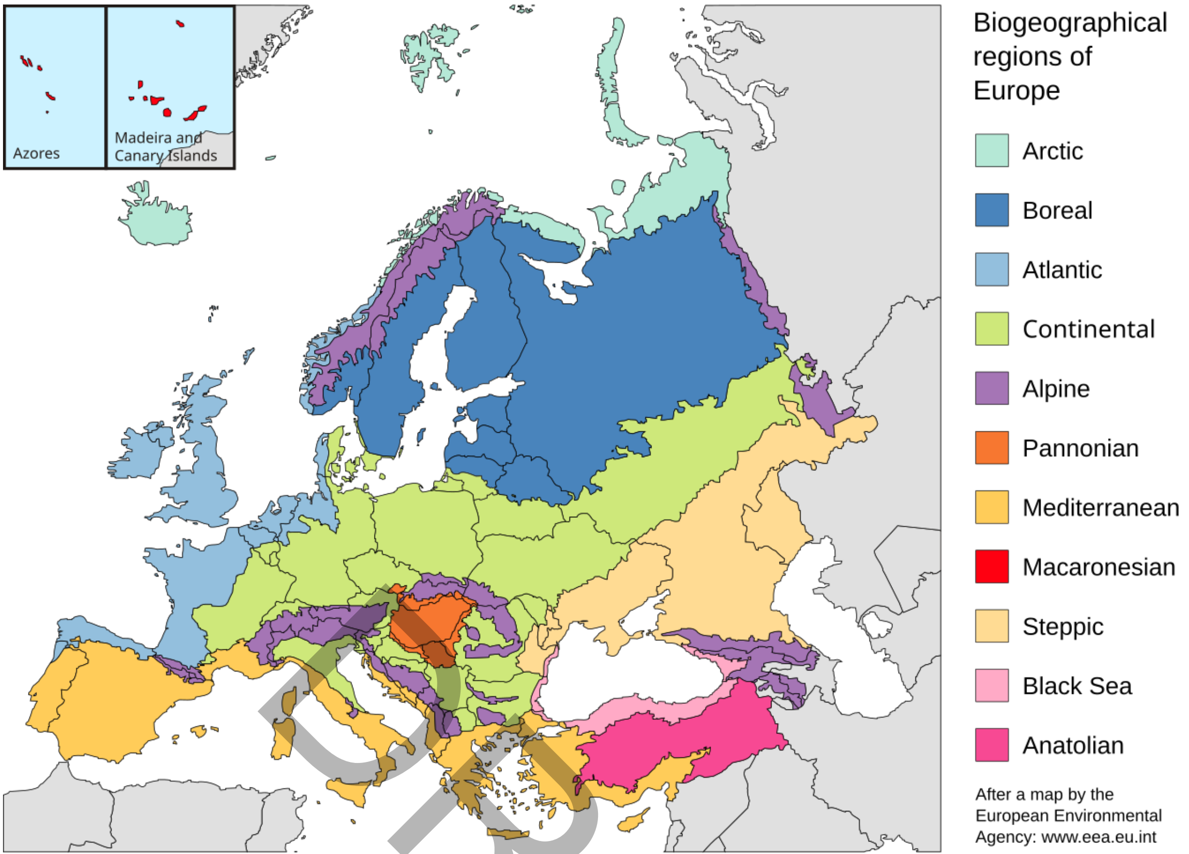

Fig. 6 Biogeographical regions of Europe. We grouped them as: Boreal (Arctic, Boreal and Alpine), Central European (Atlantic, Continental and Pannonian), Mediterranean (Mediterranean, Steppic, Black Sea and Anatolian) and Macaronesian regions. Source: “Europe's environment: the third assessment. Environmental assessment report No 10. Luxembourg: Office for Official Publications of the European Communities, p. 231.”

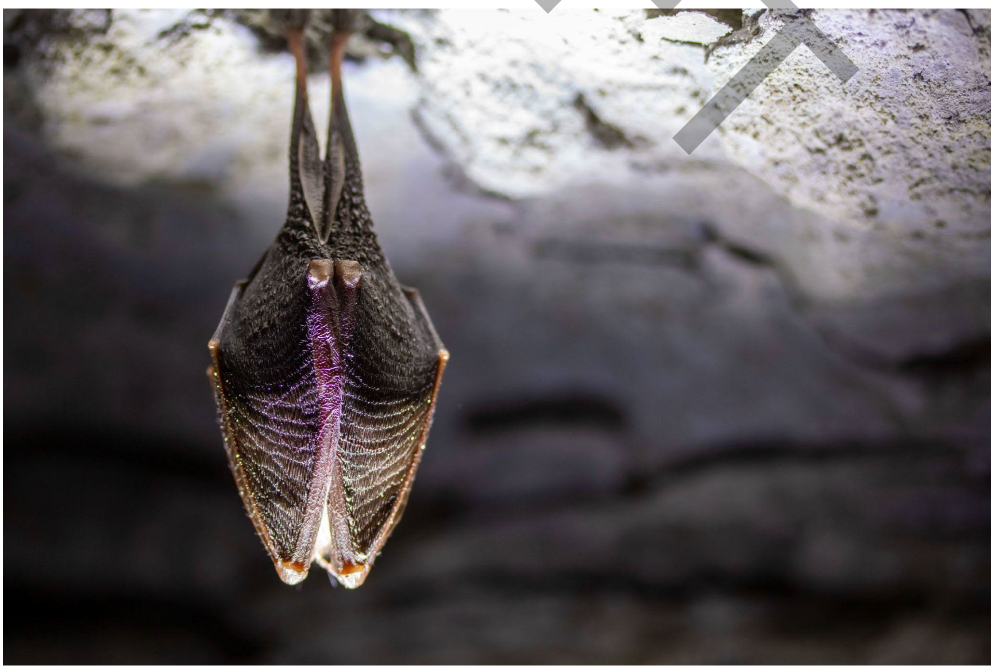

Fig. 7 Hibernation periods are probably those that are more affected by changes in climate along a north-south latitude gradient across Europe.

4.2 Under- and over-ground roost monitoring

Monitoring of cave-dwelling bat colonies is one of the most common monitoring protocols conducted in Europe. Depending on the species, bats can form colonies that vary from a few individuals to thousands of them. While the use of a specific roost can be very season-specific, bats are known to show strong philopatry in the use of a roost in a specific time of the year, thus making bat roost counts a trustful measurement of the state of bat populations, at least for species that form these numerous aggregations (3 Selecting the protocol per species). Because of this specific behaviour, these protocols are the ones that can potentially be more intrusive for bat colonies and it is of utmost importance to keep disturbance to a minimum, and professional advice and accompaniment are always recommended.

The Bat Monitoring Programme (www.batmonitoring.org) is the only platform currently available designed for professionals and volunteers to upload data from under- and overground roosts inspections across all European countries following a standardized protocol, and ensuring the use of FAIR data principles.

Sampling size

In countries or regions where the target species is widespread, a representative sample of roost sites should be monitored on a regular basis. Roosts should be selected to capture variability in colony size (number of individuals), geographic distribution, and surrounding land-use types. Stratified random sampling, with strata defined according to factors such as roost size and/or habitat characteristics, provides one of the most statistically robust approaches and allows for more reliable inference of population trends. Where the species is rare or highly localised, it may be feasible and preferable to monitor all known roosts.

The selection of sampling units should also consider the long-term accessibility and stability of roosts, as consistent monitoring of the same sites over time is essential for detecting population trends. Sites that are subject to frequent disturbance, loss, or changes in accessibility may introduce bias or reduce data continuity. Therefore, maintaining a balance between representativeness and practical feasibility is critical when designing monitoring schemes.

In addition, the spatial and temporal coverage of the sampling design should be sufficient to capture potential regional differences in population dynamics. Monitoring efforts should aim to include roosts across the full ecological range of the species within the study area, while ensuring that survey frequency aligns with key periods in the species' annual cycle (e.g. maternity, hibernation). Where resources allow, periodic reassessment of the sampling framework is recommended to incorporate newly discovered roosts or to adjust for changes in population distribution, thereby maintaining the relevance and robustness of the monitoring programme.

Statistical evidence suggests that it is always better to increase the number of sampled roosts than to increase the number of counts at each roost.

General methods for roost monitoring

Roost typologies are highly diverse, ranging from natural underground formations and mines to road infrastructures, abandoned buildings, and even inhabited private houses. The methods presented here are adapted to these different roost types, as certain approaches are more suitable for specific situations. Ultimately, the methodology and protocol selected should be tailored to the characteristics of each roost.

Given the sensitivity of bat colonies, non-invasive methods should always be prioritised. Whenever possible, surveyors should avoid entering the roost. In general bats are highly sensitive to human disturbance, and intrusion into roosting sites may lead to abandonment by adults, with potentially severe consequences for both the colony and the species.

For newly discovered roosts, however, it may be necessary to capture and handle individuals for accurate species identification. In such cases, the sampling methodology that minimises disturbance should be carefully selected. If visual identification upon entry is sufficient, this approach should be preferred. In cases involving cryptic species, such as those within the *Myotis nattereri* complex or whiskered bats, trapping sessions may be required to determine species presence (5.11 Trapping bats). These activities should be limited to initial assessments; trapping devices must be placed outside the roost (e.g. at entrances) and should not be considered part of routine monitoring protocols for most daytime roosts.

Emergence counts (using near infrared or thermal recordings coupled with acoustic recorders) should be the preferred monitoring method whenever feasible and are particularly well suited for underground roosts such as caves, mines, or tunnels with a single entrance. However, this method is only applicable when the entrance used by the colony is clearly identified and can be effectively monitored.

- For multispecific colonies, individuals should ideally be recorded using near infrared or thermal video systems for counting, combined with passive acoustic detectors for species identification (5.4 Thermal imaging and 5.5 Near-Infrared recording). When colony sizes are relatively small, counts may also be conducted manually using handheld ultrasound detectors during emergence. These methods require experienced or well-trained surveyors, as they can be challenging for inexperienced observers. It is also important to consider the substantial time required for post-processing, including synchronisation and analysis of audio and video recordings.
- In monospecific colonies, counts can be conducted through direct observation of bats emerging from the roost, using soft, low-intensity red lighting and a handheld detector. However, this process can also be further automated using infrared light barriers (5.6 Infrared light barriers). These can be either customizable or commercial The use of handheld bat detectors or night-vision devices can improve detection accuracy.

When emergence counts are not feasible, such as in roosts with multiple entrances, visual roost inspections (internally) may be necessary to count them through direct photography (5.3 Photography counts, Fig. 8). For example in buildings such as churches or castles that have very large attic spaces, allowing surveyors to go in and survey without disturbing the bats. Internal censuses usually allow the identification of several species (those more visible), and allow carrying out several censuses per day. In these cases, disturbance must be minimised at all times. The number of surveyors should be kept to the minimum required, noise levels should be strictly limited, and artificial lighting should be used only when necessary. Additionally, appropriate safety measures must be followed, including the use of helmets, headlamps, safety equipment, and, where applicable, certified speleological training.

The following general precautions should be followed during internal inspections:

- Survey groups should remain as small and quiet as possible, speaking only when necessary.
- The duration of the survey should be minimised.
- Waking or capturing bats inside a roost is strongly discouraged under any circumstances for monitoring purposes.

When selecting a method for internal counts, surveyors must evaluate which approach will result in the least disturbance. Direct visual counts may be less intrusive but can require longer presence within the roost. Photographic methods can reduce survey time but may involve the use of flash or artificial lighting, which can disturb bats.

Fig. 8 Typical photographic count of a *Rhinolophus* species.

Emerging technologies

As an alternative, emerging technologies such as LiDAR (5.8 LIDAR) offer promising solutions for counting large colonies with reduced disturbance, but most of them are still under development. Genetic analyses of bat droppings is now an emerging technique that is being developed as a new potential method to enhance bat population monitoring and has occasionally been used to identify individuals and populations sizes, structure and composition (5.12 Genetic monitoring).

Roost phenology

The use and dynamics of a bat roost can vary considerably throughout the year, largely depending on the species and the phenological stage during which the site is occupied. For newly discovered roosts, it is recommended to conduct an initial year-round monitoring programme to determine the periods of occupancy. This can be achieved, for example, through monthly censuses over 12 consecutive months. Such an assessment is essential to evaluate the relative importance of the roost at local, regional, national, and international scales.

To adequately account for seasonal variation in roost use, this section is further divided into the following subsections: maternity (summer), hibernation (winter), equinoctial migration (spring and autumn), and swarming (autumn).

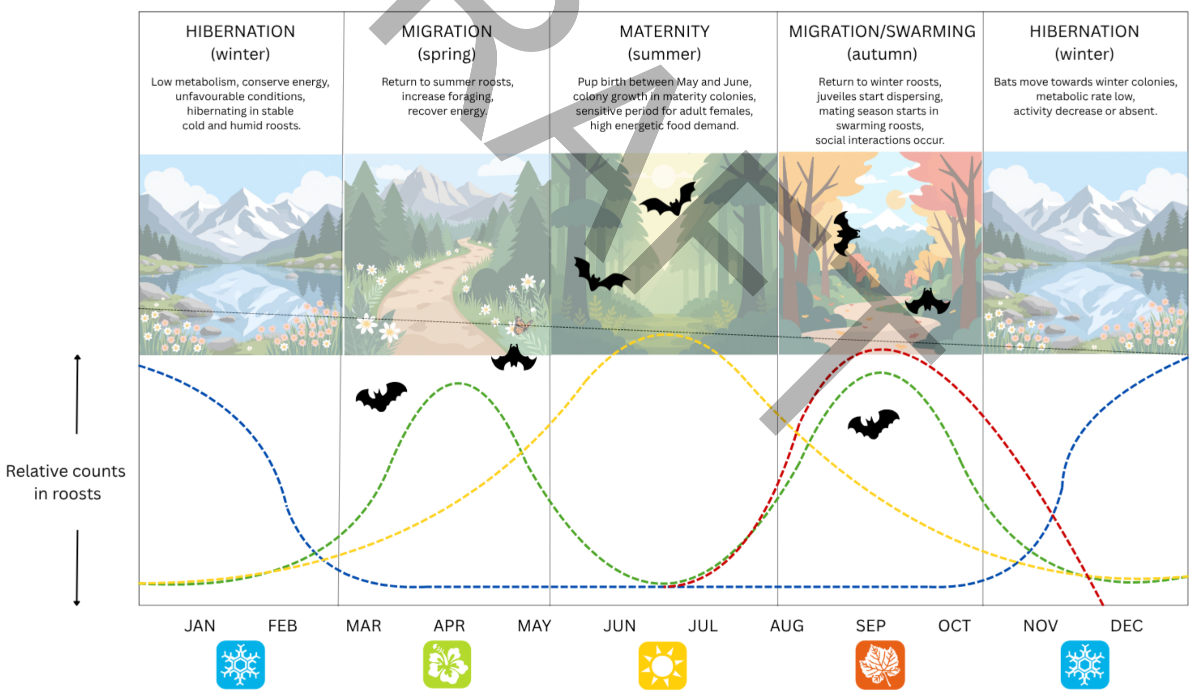

Fig. 9 Annual cycle of European bats: Conceptual phenological curves.

4.2.1 Maternity and summer colonies

Counting bats in their breeding colonies is a widely-used and well-established method for monitoring the status of certain bat populations within a given area. Surveys of maternity roosts can also provide valuable information on annual recruitment, particularly when juveniles can be distinguished from adults or when repeated counts are conducted at different stages of the breeding season (e.g. before and after the emergence of juveniles).

Long-term monitoring of these roosts is of great conservation importance as it can provide very precise information on the regional, local or continental population status of several species and the tendency of these species during the maternity period, a critical phase for species conservation and resilience.

Furthermore, during the summer period, some species segregate into sex-specific colonies, with females/males forming large separate colonies. Monitoring these colonies can potentially provide additional information about the demographics of a bat species' population. Therefore, whenever feasible, it is recommended that all relevant maternity and summer roosts within the target region are included in monitoring programmes.

Methods

The methods recommended for monitoring breeding roosts largely follow those previsouly described in the *General methods for roost monitoring* section. Preference should be given to non-invasive approaches, particularly emergence counts using 5.5 Near-infrared recording or 5.4 Thermal-imaging imaging combined with ultrasonic acoustic recording. These methods are generally favoured over internal visual inspections or photographic counts due to their lower potential for disturbance.

In cases where 5.7 Visual roost inspections are the only feasible option (usually when colonies are very large, or where bats roost in mixed species groups and identification with a bat detector on emergence is very difficult), efforts should be made to distinguish between adults and juveniles whenever possible, in order to maximise the information obtained from each survey.

The timing of censuses depends on the method employed. Visual inspections should be conducted during daylight hours, when bats are present inside the roost, preferably during the mornings. Emergence counts should begin at sunset and continue for at least two hours, or until no bats are observed emerging from or re-entering the roost for a continuous period of at least 10 minutes.

It should be noted that breeding females with dependent juveniles may undertake multiple foraging trips in rapid succession, repeatedly exiting and re-entering the roost. This behaviour can complicate emergence counts, particularly in large colonies. In such cases, video recordings should be carefully reviewed, preferably at reduced playback speed, to identify and exclude re-entering individuals from the final count.

<u>Sampling period and periodicity</u>

Surveys should be conducted during the breeding season of the target species. Local knowledge of the specific roost is often required to determine key phenological stages, such as when females (or males in non-breeding summer colonies) are fully established, the timing of parturition, the onset of juvenile flight, and the eventual abandonment of the roost. Although this period typically extends from late April to August, it can vary significantly depending on the climatic region in which the roost is located.

Maternity roost monitoring should include at least one census conducted after adult females have settled but before juveniles become volant, thereby avoiding their inclusion in the count. This provides a reliable estimate of the number of reproductive females associated with the site.

Recommended approximate survey periods vary across the four principal European climatic regions:

- Mediterranean: 15th May - 30th June
- Temperate: 1st June - 15th July
- Boreal: 15th June - 15th July
- Macaronesian: ???

As previously stated, the exact date should be chosen according to the specific phenology of the roost. Ideally, a second census should be conducted when juveniles are already volant, but before the colony leaves the roost. This will provide relevant information about the yearly recruitment of the colony. The dates should be around one month after the first census, usually between July and August, depending on the region.

Fig. 8 *Myotis myotis* colony which needs to be counted through photography.

4.2.2 Hibernation colonies

Hibernation is one of the most critical periods in the life cycle of European bats. The success of pre-hibernation foraging during autumn determines the amount of fat reserves available for winter, which in turn strongly influences survival throughout the hibernation period. Hibernation colonies in Europe can also represent some of the largest aggregations of wild mammals on the continent, with certain sites in Eastern Europe hosting tens of thousands of individuals within a single roost.

Hibernation roost counts are of paramount importance, not only because of the large number of individuals that may be present, but also due to the extreme sensitivity of bats during this period. Disturbance during hibernation can cause bats to arouse prematurely, leading to rapid depletion of critical fat reserves and, in severe cases, mortality. Repeated disturbances have cumulative effects, further increasing the risk to individuals and colonies. Therefore, it is essential to minimise disturbance to the greatest extent possible (Stapelfeldt et al. 2020). The protection of winter roosts is of high conservation priority and should be considered a key component of any bat conservation programme at local, regional, and continental scales.

Methods

Unlike the rest of the year, during the winter months bats are largely inactive and do not emerge from their roosts to feed. Therefore, the only reliable method for monitoring hibernation roosts is to conduct internal surveys and visually count hibernating individuals. These surveys should always be carried out under the guidance and supervision of experienced professionals, and with extreme caution to minimise disturbance. See the recommendations in the *General methods for bat roost monitoring* section.

As stated in the *General methods for bat roost monitoring* section, the method used to survey hibernation roosts should strike a balance between maximising accuracy and minimising disturbance to the colony. If the colony consists of a relatively small number of individuals dispersed across the ceiling (as in *Rhinolophus hipposideros* colonies), direct visual inspection is generally the most appropriate method (5.7 Visual inspection). However, some winter colonies can comprise thousands of individuals forming dense clusters, making direct counts impractical. In such cases, high-resolution photographic methods are recommended, allowing images to be analysed later (5.3 Photographic counts). Photographs should be of sufficient quality to enable accurate counting of individuals and, where possible, species identification.

In structurally complex underground roosts, where numerous crevices and inaccessible sections are present, it is advisable to use tools that facilitate inspection of these areas. While a small extendable hand mirror may be sufficient in many cases, the use of fibre-optic (endoscopic) cameras (see 5.9 Optic-fiber camera inspection) is becoming increasingly common, as they are relatively inexpensive, can be connected to mobile devices, and allow high-quality images to be captured for subsequent counting and species identification.

Detailed field notes should be recorded for each site, including the areas surveyed and the locations where bats were observed. This information is valuable for ensuring consistency in future monitoring efforts. Any expansion of survey coverage to previously unmonitored sections should be clearly documented and, where possible, analysed separately. Data should also be recorded separately for each site or sub-site, particularly in cases where sections may be periodically inaccessible due to flooding or other environmental conditions, which could otherwise introduce bias into long-term monitoring datasets.

<u>Sampling period and periodicity</u>

Winter roost surveys should be conducted during the coldest months of the year, with the aim of selecting periods when the roost hosts the highest number of individuals and species. Prior local knowledge of roost occupancy patterns may be required, particularly for newly identified hibernation sites. The extent to which bats occupy hibernation roosts is strongly influenced by local climatic conditions; in southern Europe, bats may remain active for much of the winter. Consequently, this method is generally more reliable in the northern parts of species' ranges, where bats remain in hibernation sites for longer and more stable periods.

Weather conditions can also influence the detectability of certain species, particularly those tolerant of low temperatures. For example, studies in Germany have shown a strong relationship between ambient temperatures prior to surveys and the number of hibernating individuals of species such as *Barbastella barbastellus* and *Myotis nattereri*, with lower counts recorded during milder conditions (5–10 °C) and higher counts when temperatures are close to or below 0 °C (e.g. Meschede & Rudolph 2004). Therefore, surveys should preferably be conducted during colder periods within the winter season to maximise count reliability.

It is recommended to minimise the number of visits to each roost in order to reduce disturbance. Ideally, a single census should be conducted during the coldest period of the year, typically between mid- and late January (approximately 15–30 January). From both a conservation and statistical perspective, it is generally preferable to increase the number of monitored sites rather than the frequency of visits to individual roosts. However, it is essential that a consistent survey effort is maintained across years, with counts conducted within the same time window to ensure comparability.

Nevertheless, it should be noted that species composition and abundance within hibernation roosts may vary throughout the winter season. In such cases, multiple censuses may be justified. The hibernation period can extend from November to March, depending on geographic location. Where repeated surveys are necessary, it is recommended to conduct a maximum of three counts per season, each separated by at least two weeks, to balance data quality with minimisation of disturbance. The recommended timing of these surveys, depending on the climatic region, is as follows:

- Mediterranean: 15th-30th December; 15th-30th January, and 15th-30th February
- Temperate: 1st-15th December, 15th-30th January, and 15th-30th February
- Boreal: 15th-30th November, 15th January-30th January, and 1st-15th March
- Macaronesian: ???

As previously stated, a single count is recommended if the colony is stable throughout the season, which would correspond to the second date in the previous indications. Yet, if the maximum occupancy of the roost differs from the proposed dates, the census' dates should be modified accordingly.

4.2.3 Migration colonies

While less frequently surveyed than maternity or hibernation colonies, migration roosts are of significant conservation importance, particularly for long-distance migratory bat species. Species such as *Miniopterus schreibersii* and *Myotis capaccinii* may use the same equinoctial roosts each year when moving between summer and winter sites, with thousands of individuals potentially aggregating for short periods. Additionally, migratory routes often include multiple stopover roosts, which may differ between spring and autumn migrations, further increasing the complexity of monitoring these sites.

The monitoring of migration roosts is important not only for ensuring their protection when colonies are present, but also because it can provide valuable insights into habitat use outside the most critical periods of the annual cycle, long-term phenological shifts (e.g. in response to climate or land-use change), and the structure and dynamics of migratory routes. This latter aspect is of particular conservation relevance, as it informs environmental risk assessments for infrastructures such as wind farms and power lines, which may pose significant threats to migratory bat species.

Methods

The methods recommended for monitoring migration roosts are those described in the *General methods for roost monitoring* section, with a clear preference for non-invasive approaches such as emergence counts (see 5.4 Thermal-imaging, 5.5 Near-Infrared recording, and 5.6 Infrared light barriers). In the context of migration, bats may exhibit increased levels of activity, sometimes even during daylight hours, which makes internal visual inspections (5.7 Visual roost inspection) both less reliable and more likely to disturb the colony.

Sampling period and periodicity

The short duration of use of migration roosts, together with interannual variation in their timing, makes them particularly challenging to monitor. These roosts should be surveyed during both migration periods, namely spring and autumn. It is essential that monitoring coincides with the peak occupancy of the target species, which can vary considerably depending on the roost's location relative to breeding and hibernation areas.

When surveying a migration roost for the first time, repeated censuses should be conducted throughout the entire migration period, from initial occupation through peak abundance to eventual abandonment (Fig. 9). For this initial assessment, it is recommended to carry out surveys using non-invasive methods at approximately 15-day intervals. If only internal visual inspections are feasible, these should be limited to a maximum of one visit per month to determine the presence or absence of bats, although, as noted previously, internal inspections are strongly discouraged for this type of roost. Depending on the climatic region, the recommended survey periods are as follows:

- Mediterranean: 15th February - 15th May and 15th August - 30th November
- Temperate: 15th March - 15th May and 1st August - 15th November
- Boreal: 15th April - 1st June and 1st August - 31st October.
- Macaronesian: ???

This initial assessment should allow the identification of the peak occupancy period of the roost. If such an intensive sampling effort cannot be maintained over multiple years, it is recommended to conduct at least three censuses during the previously-identified peak period. However, it is strongly advisable to repeat the full 15-day interval monitoring protocol every few years in order to detect potential shifts in the timing of arrival or peak occupancy, which might otherwise go unnoticed.

Such temporal changes are particularly important to consider, as shifts in peak occupancy could be misinterpreted as population declines if surveys are restricted to previously defined peak periods. Regular reassessment of roost use phenology is therefore essential to ensure accurate interpretation of long-term monitoring data.

Fig. 9 *Rhinolophus euryale* in a migration cave surveyed annually to describe the phenological curve of the species.

4.2.4 Swarming site monitoring

During late summer and early autumn, some species congregate at the entrance of specific cavities in large numbers and display a circling flight behaviour coupled with social vocalisations, a behaviour known as bat swarming (Fenton et al. 1969). Although widely reported, the role of this behaviour remains poorly understood, and various hypotheses have been proposed about its function. Swarming in bats is thought to serve multiple functions, including identifying suitable hibernation sites and orienting juveniles to potential hibernacula (Gottfried 2009; van Schaik et al. 2015). However, mating has been suggested to be the main purpose of swarming, as shown by the presence of bats from various locations converging at these sites to mate, thus promoting higher genetic diversification and gene flow between otherwise isolated populations (Bogdanowicz et al. 2012; Gottfried 2009; Kerth et al. 2003; Rivers et al. 2006).

Although the definition of swarming species is still debated, European species commonly reported to engage in swarming behaviour are non-migratory and forest-dwelling, including multiple *Myotis* species, *Barbastella barbastellus, Eptesicus nilssonii*, and *Plecotus auritus* (Furmankiewicz et al. 2013). Most of these do not use these swarming cavities for roosting, apparently leaving them empty during the day. Most importantly, a significant proportion of these species are threatened or considered vulnerable across several regions in Europe (Russo et al. 2023). Swarming sites attract very large populations of bats (thousands) from large catch ment areas (~ 100 km radius or more), with many hundreds visiting a site each night at the peak of the season (Fig. 10).

Surveillance of swarming sites can therefore provide a useful index of the status of a number of species over a very large area. Swarming populations are dominated by males (60-90%), but it is not known if this is a real sex bias or an artifact of differences in behaviour that make them easier to catch (Kerth et al. 2003, Rivers et al. 2006).

<u>Methods</u>

Although there are no experiences in monitoring bats in swarming sites that provide robust population trends, several methods can be considered to systematically survey these bats: 5.2.1. Remote acoustic stations and 5.11 Trapping bats (in order to get relative abundance measures for each swarming species. Automatic recording at underground roosts entrances can make species identification difficult because bats often change their typical sounds inside or close to roosts.

The numbers and positions of harp traps, mistnets and detectors should be identical each year. Traps and nets should not prevent bats from entering or leaving the site, to minimise disturbance. In these particular cases, length of the survey sessions would be about 8 hours after sunset, as bat activity usually peaks at 3-5 hours after sunset and decreases gradually along the night. It is not recommended to carry out trapping sessions on consecutive nights.

Sampling period and periodicity

Similarly to the migration roosts, peak activity in swarming sites can become quite unpredictable during the whole season, particularly because of the weather and interannual phenological variation (species composition usually substantially varies along the season); therefore, making repetitive surveys necessary to cover the days with the highest activity. Swarming activity peaks vary between species and depending on latitude. In southern Germany it starts with *Eptesicus nilssonii* in mid-July, followed by *Myotis brandtii* and *Barbastella barbastellus* at the end of July. In Spain and the UK, swarming activity peaks between mid-August and mid- September.

- Mediterranean: August 15th - October 15th.
- Temperate: ???
- Boreal: ???
- Macaronesian: ???

Fig. 10 During late summer and early autumn, some species congregate at the entrance of specific cavities in large numbers and display a circling flight behaviour coupled with social vocalisations, a behaviour known as bat swarming. Underground roosts with relatively large entrances and medium high elevations, surrounded by a high proportion of forest habitats, seems to be the common criteria to define swarming sites in southern Europe

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4.3 Bat foraging habitats monitoring

Monitoring bats in their foraging habitats has advanced considerably in recent decades, largely due to the rise of passive acoustic monitoring. Surveying bat populations in these areas has proven particularly effective for species that are rarely detected in underground or aboveground roosts, as well as for very common and abundant species whose numerous roosts are difficult to monitor comprehensively. Foraging grounds are often consistently used by many species and can therefore provide robust, standardized data on bat populations within their natural habitat. However, this monitoring approach is only suitable for a subset of European species (see 3 Selecting monitoring protocols per species, Table 1).

Sampling size

To obtain high-quality information and compile robust datasets for monitoring these species, surveys should include the full range of habitats they occupy, covering different altitudes and geographic regions. The number of sampling sites should be defined at the appropriate landscape scale, taking into account the specific objectives of the monitoring program, environmental variability, and local logistical capacity. Specific geographical units are selected for survey site selection (e.g. 10 x 10 km square grid). Within each area one or more main sites (known from atlases or inventories) should be selected to have the highest possible bat species richness for the region

Methods

While mist-netting and harp-trapping remain valid methods (5.11 Trapping bats, Fig. 11), capturing bats in foraging areas in Europe is generally inefficient and requires substantial effort and logistical support. In contrast, the use of automated recorders and acoustics transects enables the collection of extensive datasets and estimates of relative abundance through a comparatively simple approach (5.2 Acoustic surveys). Trapping sessions should always be standardized in duration, ideally lasting five hours after sunset, and trap/detectors should always be placed at the same places to avoid problems of imperfect detectability and biases due to the habitat's structural characteristics such as clutter.

Sampling period and periodicity

One of the main advantages of using bat detectors is that they can be deployed year-round, allowing the detection of bat activity even when it is relatively low during colder periods such as autumn and winter. For this reason, it is recommended to conduct acoustic surveys in each season, tailored to specific objectives.

In passive acoustic monitoring, a full night is considered the minimum sampling unit for assessing bat populations. While one night per season and locality may be sufficient when a large number of sites are included, conducting sessions of at least five full nights is strongly recommended to improve data reliability. For acoustic transects, a minimum of two nights per season is highly encouraged. Specifically in summer, sampling sessions should ideally align with the pre-parturition period to avoid the presence of volant juveniles from the current year. The annual survey date, start time, and starting location should remain consistent across years, and repeat surveys should be conducted under comparable environmental conditions.

In general, it is statistically preferable to sample more areas (use more replicates) than to sample areas more intensively (repeated measures at fewer sites). The following dates are suggested but should be adjusted to each biogeographical region to acknowledge local climatic variation:

- Winter: November - February
- Spring: March - Early May
- Summer: Late May - July
- Autumn: Late August - October

Fig. 11 Mistnetting in a Mediterranean river, with the aim to capture and identify trawling bats while foraging.

4.4 Trawling bats monitoring and waterway surveys

Trawling bats, species that forage by skimming over water surfaces to capture prey (i.e. *M. daubentonii, M. capacinii and M. dasycneme*) are closely associated with aquatic ecosystems and therefore require monitoring approaches specifically adapted to waterways. Surveys targeting these species typically focus on rivers, streams, lakes, and reservoirs, where linear and open habitats facilitate both bat activity and standardized sampling. Visual monitoring aided with acoustic detectors along waterways has become a key method in several countries (i.e. Ireland, Slovenia, Spain, UK, López-Bosch et al. 2023), as it allows for the efficient detection of these species and measuring their relative abundance or activity levels over extended distances and across multiple habitat types. Waterway surveys provide valuable insights into species presence and habitat use, ultimately contributing to the calculation of these species' populations trends.

Ireland and UK have the longest datasets of waterway surveys, and the most extended network of sampling points, with very robust *Myotis daubentonii* population trends. In parallel, a more recent initiative, namely the Bat Monitoring Programme (www.batmonitoring.org) is the only platform currently available designed for professionals and volunteers capable to register and upload data from waterway surveys across all European countries following a standardized protocol, and ensuring the use of FAIR data principles.

<u>Sampling size</u>

These surveys should be carried out in localities where trawling species do not co-occur, minimizing the risk of misidentification. Sampling localities should cover most of the range of the species in the study area, both in extension and habitat type, but also in altitude, as these species' abundance has been shown to vary with altitude (Russo 2009). The minimum number of localities required to calculate population trends depend on the overall size of the study area and the number of workers and volunteers that contribute to the surveys. This monitoring method is very well suited to citizen science projects, as it can be implemented by volunteers, potentially increasing the potential sample size.

<u>Methods</u>

Waterway surveys for trawling bats are designed to capture activity along rivers and streams, where these species concentrate their foraging activity. The most widely used approach is the visual quantification aided with acoustic monitoring across transects (5.2.3 Walked/car/bicycle/boat bat detectors transects). These transects count with a network of observation stations placed at strategic fixed points (ranging from four to 10) distributed along a 1 km transect, where trawling bats are manually counted. To count them, bat detectors and lights are typically positioned to maximize coverage of the water surface while minimizing obstruction from vegetation, perpendicularly to the river stream. Surveys are usually carried out under suitable weather conditions, avoiding strong wind, heavy rain, or low temperatures, during 40 minutes in total, starting approximately one hour after sunset.

Sampling period and periodicity

Waterway surveys are commonly carried out during August, although in specific areas they have also been conducted in July or June (previously to the juvenile recruitment) (López-Baucells et al. 2025). Two replicates are advised per locality, in order to capture differences due to habitat and phenological/seasonal variability, but always with a sensible time between the two censuses (i.e. a week between samplings).

Fig. 12 Waterway survey with volunteers, participating in a citizen science project in Catalonia © Pietat Pizarro.

4.5 Bat box monitoring

Although bat boxes have been widely used for surveillance and for multiple researches (e.g. Stapelfeldt et al. 2022), bat bot monitoring has rarely been used to assess bat populations or estimate population trends (e.g. Dondini et al. 2025). This is likely due to biases associated with box use among different species and sexes, as well as variation in the availability of natural roosts within a given habitat. However, several initiatives across Europe have adopted this approach, particularly targeting species that are otherwise difficult to monitor, such as *Pipistrellus nathusii* and *Nyctalus leisleri* (because of their migratory nature) or *Myotis bechsteinii, M. daubentonii, M. nattereri, Plecotus auritus* (because they are strict forest-dwelling bats, and their roosts are highly difficult to locate and monitor). Bat boxes are especially useful in artificial (mainly non-native coniferous) forests where natural roosts are lacking or rare. In such forests real population trends can be measured (e.g. Schmidt 2000, Blohm 2003, Heise & Blohm 2003; see also overview in Meschede & Heller 2000).

There are also concerns that installing large numbers of bat boxes in semi-natural woodlands could disrupt existing bat communities (Lisón et al. 2024). Therefore, the impacts of introducing bat boxes into these environments should be carefully evaluated prior to installation, ensuring, for example, a long-term monitoring scheme, or taking into consideration other positive side-effects such as educational outputs and activities that aid general bat protection within the area.

The Bat Monitoring Programme (www.batmonitoring.org) is the only platform currently available designed for professionals and volunteers to upload data from bat box checks across all European countries following a standardized protocol, and ensuring the use of FAIR data principles.

Sampling size

There is no specific agreement on the number of bat boxes needed for monitoring purposes. It will ultimately depend on the study area, the variability of habitats present in the area, the number of bat boxes that are occupied, etc. Some initiatives recommend to group boxes in stations and use them as they provide several different microhabitats conditions. Groups of at least three boxes are encouraged.

Methods

Although bat boxes can be checked during the day at any time, it is recommended to check them in the morning as the animals tend to be less active. Whenever possible, in order to minimize disturbance, bat boxes should only be checked using an endoscope camera as this is far less invasive than opening them (5.9 Optic-fiber camera inspection). Handling animals should be justified and conducted only for specific purposes.

Caution should be taken to avoid bat mortality due to overheating events (Flaquer et al. 2015, Ruegger et al. 2016, Crawford et al. 2022), especially in these countries or areas where temperatures regularly exceed 40ºC during the summer days (e.g. coastal sunny areas in the south of Europe; Fig. 13). Also, the typology of bat boxes, colouration and design should be accurately assessed and adjusted to the microhabitat weather condition and target species (Martin-BIdeguren et al. 2019).

Sampling period and periodicity

Bat boxes should be checked at least once per season (winter, spring, summer, autumn), though in many countries several revisions per season are also conducted. Sampling period of the bat boxes must be adjusted to local climate to ensure that boxes checked in the summer provides high-quality data about maternity colonies, in spring and autumn, high-quality data about migratory species and reproductive harems in the migratory species, and in winter, high-quality data about hibernating bats.

- Winter: December - February
- Spring: March - April
- Summer: May - August
- Autumn: September - November

Fig. 13 Bat box with a maternity colony of *Pipistrellus pygmaeus* in the Ebro Delta.

5 Techniques and methodologies

5. 1 Introduction

Monitoring bats in the European region poses several serious challenges to researchers and conservationists, due to nocturnal and elusive habits of most species, the inaccessibility of certain roosts, in deep caves, high cliffs or small crevices in rocks and mature trees and the relatively small population density. However, recent advances in technology have improved the suitability and feasibility of bat monitoring protocols and programmes in many aspects. Technological advances in bioacoustics, thermal and infrared video-recording and genetics, for instance, have fostered the creation of several new monitoring schemes across Europe and have allowed the establishment of wide-spread networks of volunteers monitoring bats within citizen science programmes in many countries, and the detection of elusive species that were practically impossible to monitor in the past.

The monitoring methods listed here are based on those agreed at the Second Meeting of Parties of the EUROBATS Agreement and outlined in Resolution 2.2 (Doc.EUROBATS. MOP2.5.AnnexBfin Resolution No. 2), but include other new methods that have been developed in recent years. It is important to remember that this section covers the general specificities of each method and describes good practice recommendations, examples of their application and specific settings to apply them accurately in bat monitoring protocols. It is recognised that methods may need to be amended to take account of regional variations and in the light of new information. However, the specific monitoring protocols are explained in detail in Chapter 4, and the potential target species for each protocol in Chapter 3.

A monitoring scheme should always aim to obtain high quality data using a repeatable protocol with standardized methods and techniques, which will be appropriate for robust forms of data analyses across regions and researchers. At the same time, the methods used should be as simple as possible, allowing surveyors with minimum skills and training to participate, thus increasing the possibility of obtaining a sufficiently large sample over the long. They should also be selected to minimise as much disturbance as possible to the bats, because of the long-term, repeated nature of the activity. term. Therefore, taking into consideration the abovementioned recommendations, selecting the appropriate technique turns out to be essential to ensure a good quality monitoring programme to endure over time.

5.2 Acoustic surveys

5.2.1 Remote Acoustic Stations

Method description

The first bat detector was designed in the early 20th century by George Pierce. Since then, the number of models, the technology behind them and their capacities have dramatically increased. New acoustic sensors designed for passive bat sensing now enable the collection of vast quantities of acoustic data suitable for bat monitoring. Amongst the range of different bat detectors that can be found in the market, the most appropriate to be used in remote acoustic stations are passive automatic recorders that can be programmed to record for long periods. These detectors are usually deployed and left in the field to gather data about bat presence/absence (for distribution and inventory projects) and bat activity levels (for population monitoring, activity patterns and behavioural studies). They can be used either for cave-dwelling bats monitoring or for free-flying bats within foraging habitats.

For acoustic monitoring, a wide range of bat detectors have been designed and developed over the last decades worldwide. Bat detectors are generally designed to record ultrasound acoustic waves (some, up to 500 kHz in frequency) automatically. There are three main types of bat recorders; (1) heterodyne, (2) frequency division and (3) full-spectrum recorders. Heterodyne recorders mix the bat call with a signal produced by the detector to produce an audible click; then, the identification of the species is made from the pattern of clicks. The frequency division recorders make bat calls audible by dividing the frequency of the call by a determined value (usually 10) (Jones et al. 2013, Browning 2017). These recorders usually have low memory and battery power requirements, making them suitable for long-term monitoring. However, the call information content is rather reduced, which turns the species identification challenging. Finally, full-spectrum recorders record directly at high sampling rates, retaining the full amplitude and frequency of the calls recorded. Due to the higher resolution of their recordings, which facilitates subsequent species identification, full-spectrum recorders are among the most widespread and widely used detectors in the Western Palearctic. Therefore, whenever possible, this type of recorder will be used to ensure the acquisition of as much high-quality data as possible (Browning et al., 2017).

If this surveying method is selected, it becomes important to bear in mind the placement of the detectors. On the one hand, the diversity of habitat use in bats must be considered; different bat species use different habitats to forage, drink, commute, and roost, so in a heterogeneous landscape, detectors should be placed in the different habitats in order to maximize the species registered (Loeb et al., 2015). On the other hand, the selection of the specific site/setting where the detector is deployed is also highly relevant, as it can strongly affect the quality and quantity of bat detections. Therefore, placement in the right orientation and at a sufficient distance from the clutter is crucial to avoid echoes and their effects on the sound quality. In this respect, a distinction must be made between unidirectional and omnidirectional microphones; while unidirectional microphones record in one direction, they must be oriented at a minimum of 3 to 5 meters from the clutter and at a minimum height of 1,4 meters from the ground. Omnidirectional microphones, on the other hand, require a greater distance from the clutter, as well as being placed higher to reduce noise and echoes from the ground (Loeb et al., 2015).

Although for monitoring purposes it is highly recommended to survey in all seasons, at least it should be conducted in the summer (preferably before the juveniles start flying, to count only adult males and females). Surveys must be always conducted when the weather is optimal (no rain or strong wind) and preferably on the same period every year (Loeb et al., 2015).

Necessary materials

- Passive acoustic recorders
- SD cards
- Batteries
- Tripod or support pole (If necessary, otherwise, it can be placed in trees or vegetation)
- Acoustic analyses software
- Acoustic identification guide and/or reference call library

Recommendations

- Ensure the chosen recorder can record ultrasounds (up to 250 or 400 kHz). It will not be a problem if a full-spectrum recorder is chosen.
- Ensure audio is stored as a ".wav" or another lossless format, to ensure valuable acoustic information is not lost.
- Recording ultrasounds for a long period requires a large amount of memory storage. It is recommended to choose a model that can hold multiple SD cards.
- Placing the detectors within the sampling habitat always pointing towards a semi-open space.
- Detectors should be set on poles or trees, and breast height and pointed horizontally.
- In the case of non-waterproof microphones (e.g., AudioMoth), these should be protected using waterproof membranes that have been previously tested to minimise sound attenuation.
- Although it has previously been suggested that recording during the first four hours should be enough to detect the peak of activity, it is advisable to record during the whole night, from 30' before the sunset to 30' after the sunrise.
- If possible, recording up to five nights per site per season, although one night would be the minimum amount of effort to be comparable if resources are limited.
- For long-term monitoring schemes, acoustic sampling should be conducted in all seasons (winter, spring, summer, and autumn).
- Using the unit of a "Bat pass" is strongly recommended to quantify relative abundance. A bat pass is considered as the sequence of a minimum of two echolocation pulses within a 5-second acoustic recording as in Millon et al. 2015.
- Sound recordings must always be stored and made available. Subsequent control is sometimes useful for verification when dealing with species records in new areas and in difficult identification cases.
- For species classification it is recommended using a first rough classification with automatic algorithms followed by a posterior visual validation (especially for those species that are threatened, data deficient, or that are particularly sensitive to misidentifications, e.g., Plecotus). Relying entirely on automatic classification should be avoided in order to avoid misidentifications.

Advantages

- Acoustic remote stations allow the collection of data for a longer period of time, as well as within a much more widespread network of sampling points than would be possible otherwise.
- Repeated surveys over the years, in the same selected areas and using a standardised method will reveal changes in rare species occurrence and distribution (those that are difficult to monitor using other techniques) and give the earliest indications of disappearance as well as expansion to new sites.

Limitations

- It is not possible to identify individuals (sex and age) and get absolute abundances of the species, nor is it possible to determine the reproductive status of the populations (e.g., lactating or mating).
- Comparisons between species should be avoided due to different species detectability.
- There is the possibility of malfunctioning of one or several detectors while they are deployed in the field, compromising the quality of data collection and prolonged data time-series.
- Analysing the acoustic data is time-consuming and demanding compared to other types of bat sampling techniques.
- The continuous improvement on technology, bat detectors designs and models, algorithms for automatic classification as well as software to store and visualise bat sound files hampers the standardisation of acoustic analyses over time.
- While data collection is rather objective and systematic, the identification of bat calls greatly depends on the researcher's expertise. Bat detector researchers need high quality training to attain the capability necessary to identify the bat pulses in the recordings and analyse bat sounds.
- The price of several detectors varies substantially, hindering their use by certain research groups in projects with low-funding or economical support.
- Clutter, temperature, humidity, and other environmental characteristics might affect the sensitivity of the microphones.
- It is difficult to extrapolate activity measure to population densities, as a station may record multiple passes from an individual throughout the night.

Data analyses

Once the audio data are collected from the field, it is time to process these files and classify it by identifying the bat calls. There are several open-source and paid acoustic software thare commonly used to visualize and analyse bat calls using spectrograms. The spectrogram consists in x-axis representing time and y-axis representing frequency, while amplitude is represented by colour intensity. With this visualization we can identify and classify the bat calls into species or *phonic groups* (groups of species that cannot be distinguished). This can be done manually or by automated systems; the manual method is labour-intensive and may be biased due to the specific researcher, while automated systems are never entirely reliable. For this reason, semi-automated classification is the most common, with automatic classification of signals followed by manual checking of the processed data (Browning et al., 2017, López-Baucells et al. 2019).

The acoustic data, measured as “number of bat passes per unit of time” (Fenton 1988 and 2001, Walsh & Harris 1996), and understood as a measure of activity may be extrapolated to relative abundance, and therefore population size (Browning et al., 2017). This type of extrapolation may be challenging, and several considerations may be taken into account. For example, it is necessary to contemplate the differences in detectability between species (quieter species are more challenging to detect), as well as environmental factors that may influence the detectability capacity of the recorders, and which, in addition, may vary between the surveyed areas (Darras et al. 2016; Browning et al. 2017). Therefore, statistical models that incorporate detectability or a call rate per specie can provide a reliable measure of population densities. For population trends, acoustic data over several years are needed (Browning et al., 2017).

Case studies

In Europe, a great example of a remote passive acoustic monitoring would be the Bat monitoring Programme (www.batmonitoring.org) carried out from the Biodiversity and Bioindicators research group (BiBio) based at the Natural Sciences Museum of Granollers (Spain), or the Vigie-Chiro project in France (https://www.vigienature.fr/fr/chauves-souris). The first monitoring programme includes a specific protocol to monitor bat populations acoustically using permanent remote acoustic stations deployed throughout Catalonia. This protocol is named Chirohabitats and its main objective is to survey bat populations in their foraging habitats. Two main approaches are carried out in this protocol; on the one hand, the summer approach samples the permanent stations during seven nights in summer, while the extended approach samples only one night for each season (Torre et al., 2021a). With this acoustic data accumulated annually, populations trends for the different bat species or phonic groups can be drawn.

The French programme, led by the research team in the Natural History Museum of Paris, uses a very similar dataset collection protocol and covers the vast area of France with the collaboration of a broad network of volunteers. With the collected data, species distribution modelling and population trends are published annually in their website.

In the Americas, the North American Bat Monitoring Program (NABat: www.nabatmonitoring.org) also consists of several protocols, one of which also relies on remote acoustic stations. This is a well-coordinated continent-wide program that provides data to promote and inform conservation decision-making, all by informing about bat long-term population trends and densities (Loeb et al., 2015).

5.2.2 Walked/car/bicycle/boat bat detector transects

Method description

Line-transect surveys require the observer to follow a pre-determined path of known length (walking, driving, cycling or navigating). This method consists of conducting transects along roadways and waterways using hand-held bat detectors, assessing the presence and/or activity by counting “bat passes”. The transects can be done continuously by moving or using listening points that generally do not include prolonged stops at a given point.

While bat monitoring may be difficult because bat populations exhibit high spatial and temporal variations in their activity levels, mobile acoustic methods may be a great solution to cope with this variability. Using this method, one can acquire high-quality distributional data for common species, specifically to detect distributional changes in common species with good sensitivity. These variations in bat activities are due to bats adjusting their foraging areas in order to unpredictable or unknown variables, such as weather, water availability or insect densities (Whitby et al., 2014). For this reason, mobile methods can cover larger areas at the landscape level, delivering high-quality distributional data and reflecting these spatial and temporal variations.

Sites are always surveyed under optimal weather conditions, during “prime time”, i.e. the first four hours after sunset, preferably starting 30 minutes after sunset. The different habitats and localities within the site are visited several times during this period to cover differences between species in their time of emergence, commuting and foraging.

In terms of driven transects, squares of 30x30 km are usually established along the territory to be surveyed, each with a determined number of transects of approximately 2 km. Each transect can be defined as an independent sampling unit. The route is driven annually (preferably twice) at a maximum of 25 km/h speed. The more transects are driven, the more it will increase the survey sensitivity (Roche et al., 2011).

In terms of walked transects, each line-transect should be 1.5 to 2 km long with surveyors taking about 8 minutes to walk each 100 m section (preferably in different habitats). Cycling and boat transects should be dimensioned according to local and specific characteristics.

Bat vocalisations are subsequently analysed using bat sound analysis software. Any detector system could be used, but once a system has been selected, the same system should be used on each survey to avoid biases due to the detector's capabilities. The sampling transect must be representative of the habitats present in the landscape.

Necessary materials

- Handheld bat detector (same model for each survey).
- For driven transects, vehicle (car, boat, or bicycle).
- Caution visual signals to call the attention of other drivers or inhabitants and make yourself visible.
- Bat sound analysis software.

Recommendations

- The annual survey date should coincide each year, at the same time and the same starting point, so that data from all years are consistent and comparable.
- Environmental conditions should be optimal for bat sampling (no rain, no strong winds).
- It is recommended that the surveillance period should coincide with the pre-parturition period, in order to avoid the sounds of volant juveniles.
- It is important to be careful when extrapolating the results from the road or waterway to the wider countryside because data from the transects may be biased.

Advantages

- The main advantage is that a few surveyors are needed to deliver a large volume of data. Based on power analysis, a single surveyor could collect sufficient data with ten nights of fieldwork to provide a statistically defensible surveillance project (Roche et al. 2005).
- This method can cover large landscape areas, delivering distributional data and reflecting spatial and temporal variations in bat activity.
- Roads are easy to follow and normally well-identified on maps.
- If the landscape to be surveyed is well divided and the transects well-disposed it is a relatively easy activity to be done.

Limitations

- This method is generally restricted to open and edge species that found roadsides and waterways as an optimal foraging habitat.
- An overestimated abundance for some species may be collected due to the tendency of some species to be attracted by streetlights on roadsides.
- For some species visual clues about behaviour and appearance are also needed, sometimes with a strong handheld lamp (Ahlén & Baagøe 1999).
- For these reasons, the main limitation is that the sampling may be biased due to a non-correspondence between the roadside and waterway habitat and the countryside habitat.
- The occurrence of rare species is often underestimated or missed altogether, and samples of rarer species will often be insufficient for statistical analyses.

Data analyses

Acoustic data analyses are carried out in the same way as for the Remote Acoustic Stations monitoring (Chapter 5.2.1).

Nevertheless, there are some considerations that must be taken into account in this specific case. As it has been said above, the main problem of this method is the data to be biassed, due to a lack of correspondence between the roadside areas with the wider countryside habitats. There is potential for streetlights to attract certain species and give an over-estimate of species abundance along roads in relation to actual abundance in the wider countryside. There is also potential for roads to be developed at a different rate and in a different way to the general countryside, introducing other biases in data collection. For these reasons, extreme caution must be taken when extrapolating the data provided by the roadways or waterways transects, because wrong conclusions can be drawn. If such extrapolation is required, then a roadside habitat assessment must be done. It is important therefore to note bat encounter rates at streetlights and any changes in type and number of lights over time, and any road development activity. For example, preliminary results from vehicle-based surveys in the UK suggest that pipistrelle bats are more likely to be encountered along roads with boundary features than featureless roads (see also Verboom 1998).

Case studies

In Europe, one of the greatest examples using this method is the car-based monitoring programme carried out in Ireland since 2003 (Roche et al. 2011). The monitoring is done by volunteers twice annually and has served to reveal new data about the Ireland bats' distributions (Roche et al., 2011). At the same time, they were capable of detecting the number of years of surveying required to achieve 90% power to detect Amber Alert (25% decline in 25 years) and Red Alert (50% decline over 25 years) for each species. Similarly, Russ et al. (2003) studied the seasonal patterns in activity and habitat use by *Pipistrellus pipistrellus* and *Nyctalus leisleri*, using roadway transects.

In the USA, Whitby et al. (2014), also implemented a mobile acoustic monitoring protocol and they quantified species richness and abundance along car and boat transects while testing which method was most effective. The efficiency was also compared to a stationary acoustic detectors protocol. They detected no differences in species density, but the stationary method accumulated species more quickly. Additionally, compared to the boat transects, higher diversity indices and species density were observed in the car-based method, concluding it was the most effective mobile acoustic method.

5.3 Photography counts

Method description

Photography counts are based on documenting bat colonies with various devices capable of capturing photos, and carrying out an exact or estimated manual count later and off site, using a PC and various photo editing software. With the evolution of digital cameras, or even the photo component of mobile phones, a good quality (i.e. useful, countable) photo is achievable virtually by anyone. Obtaining a countable photo usually depends on an adequate lightsource, which varies depending on the height where the colony is located inside the roost. On site, colonies to be monitored should be photographed in their entirety, either on one photo that encompasses the whole colony, or on multiple, partially overlapping photos, that can be assembled later with adequate software. Separate clusters can be photographed individually. Clusters or colonies should fill the frame as much as possible, without cutting off marginal sections of the colony.
In large cave colonies numbering tens of thousands of individuals, the number of bats can be estimated by determining the surface occupied by the colony (in m^2, see details in Battersby 2010 for *Miniopterus schreibersii*). However, these extrapolations may be done with extreme caution, since variable cluster densities may produce less consistent estimations than direct manual counting (O'Shea & Bogan, 2003; Meretsky et al., 2010, Loeb et al., 2015)

Necessary materials

- Single lens reflex (SLR) digital camera (minimum resolution of eight megapixels) with flash (Loeb et al., 2015; Meretsky et al., 2010).
- Zoom lens (for high ceilings).
- Tripod (useful in low light, or when photographing multiple clusters)
- Or high-quality photo camera of a mobile phone
- Personal Protective Equipment (PPE) for entering in caves.
- Torches and headlamps.
- Laser pointer, if a reference point is needed.
- Measured reference stick.
- Software for photo editing and analysis (ex. ImageJ, Adobe Photoshop,).

Recommendations

- Make preparations (checking camera, fixing speedlight, preparing tripod, etc.) before reaching the colony. In this way you will spend the least amount of time (i.e. disturbance) under the colony. This is especially the case of nursery colonies, which can (partially or fully) fly off after a few seconds of disturbance.
- Take photos, whenever possible, perpendicular to the overall layout of the colony, in order to have as many bats in focus, as possible.
- Photographic counts of nursery colonies should take place in the first part of the day, when bats are less active.
- The use of handheld laser pointers is recommended in order to facilitate focusing through the camera's autofocus. This may avoid the need to illuminate the cluster by a headlamp, and reduce disturbing the bats (Meretsky et al., 2010).

- After taking a picture, do a quick check of the obtained photo, to assess its quality for PC counting
- It is recommended to fill the cluster with the photograph. If it is not possible, several pictures of the cluster should be taken. In this case, it is important to ensure a minimum of overlapping among pictures to cover all the cluster (Loeb et al., 2015).
- 2 people are needed to take pictures with the stick of known length; one holding the stick next to the colony, and the other taking the picture (for large colonies).

<u>Advantages</u>

- Reduces direct disturbance of colonies
- Reduces the time spent inside a roost
- Requires less light than visual counts (only for focusing the camera)
- Yields exact counts on all visible bats, and minimizes estimation error
- Low-invasive method
- Monitors and documents at the same time
- If the species forming the colony is known from previous monitoring, photos can be taken even in bad circumstances (e.g. barely enough to count bats), and subsequent count will be possible.
- Relatively easy to implement (but site dependent)
- Can be applied in various environments, caves, buildings, etc.
- Can be applied in all major seasons of the bat life cycle: for nursery colonies, hibernation colonies, transitory colonies, male colonies etc.

<u>Limitations</u>

- Nursery colonies fly off quickly
- Can require powerful light / flashlight (but for a short time), in order to obtain an adequately lit / focused photo
- The count of colonies of tens of thousands of bats (ex. *M. schreibersii, P. pipistrellus*) based on photos can still be challenging
- Depending on the structure of the roost, an adequate photo, that truly represents the colony in question, can be hard to take, so proper training is encouraged.
- Not suitable for some species, ex. crevice dwelling species
- In the case of many small clusters of bats, photography counts may not save a significant amount of time, and hence, may not reduce disturbance adequately.
- If the colony is very large, the time spent on post processing images and counting should be appropriately considered.

<u>Data analyses</u>

Before counting photos on our PC, brightness, contrast and other parameters of the photo can be adjusted in adequate processing software, to ensure the best visibility of bats and the colony. Photos of small / medium colonies of a few hundred, 1000-2000 bats may be counted with simple software, manually counting bats with coloured dots placed on individuals relatively quickly (ex. MS Paint, Adobe Photoshop). Complex images, or photos containing several thousand bats may require special software. Geographic Information Systems (GIS) software can be used to digitise individuals, where noses can be a good individual identifier.

Once the individuals are marked, it can speed up considerably the arduous task of counting (Meretsky et al., 2010).

In the last decades the development of several AI tools has aided in the automation of these counts, which could be helpful when dealing with large amounts of data. CNN deep learning models have been developed in recent years that could be used to count images containing large numbers of animals and even identify different species and age groups (Wang et al. 2025). These can open the doors to automated photography counts, for example, using camera-trapping settings and devices, obtaining much precise counts and monitoring for some large-aggregating species.

<u>Case studies</u>

In the context of the United States Fish and Wildlife Service (USFWS) survey protocol for the endangered species *Myotis sodalis*, and the posterior recovery plan (USFWS, 2007), Meretsky et al. (2010) compared the different techniques comprised on it to survey and count bats at hibernaculum. They compared the photography method with 4 different visual-estimation approaches, observing that estimation techniques varied from 76% to 142% of counts from the photography-count approach, and demonstrating the photographic technique as the most reliable.

More advanced visual counts of roosting bats can involve LIDAR (Light Detection and Ranging) and pattern recognition (ex. Azmy et al. 2012, Shazali et al. 2017).

5.4 Thermal-imaging

Method description

Human eyes are sensitive to a region of the electromagnetic spectrum between about 400 to 750 nm wavelengths; the longer wavelengths are called infrared. The infrared red region is usually divided into near (wavelength between 700 to 1500 nm) and far (or thermal – 1500 to 1 million nm) infrared. Thermal infrared imaging (or infrared thermography) relies on the infrared spectrum of light (roughly 9,000 - 14,000 nm) which accounts for the heat emitted by objects (Kunz & Parsons 2009). This technology should not be confused with Infrared recording, which uses the near-infrared light emitted by an external light source and expresses images into the visible spectrum (see chapter 4.5).

A thermal infrared camera, which converts electromagnetic waves to electric signals for visual display, is usually coupled to a computer for recording images (called thermograms. These are analysed by vision algorithms distinguishing warm regions of bats (typically the body) from the vegetation, clouds and other objects of the background. Software compares each pixel's value with a range of recently measured values of pixels representing sky and vegetation and removes them from further analysis. Then, filters are used to detect image regions that include the warmest parts of bats (or other flying animals), eliminating pixels according to thermal threshold values.

This technology is used for a wide array of tasks, including medical, veterinary, security and engineering processes and research. The recording of wildlife based on the heat they emit is extremely useful when it occurs in low-light conditions or in absolute darkness, as it permits the observer to capture events, behaviours or to simply count animals in a way that would be almost impossible without this technology. Each passing year, new models of thermal-imaging cameras become available in the market, making this method more and more open and accessible to the general public.

Advantages

- Several types of bat monitoring benefit from the use of thermal infrared imaging as this method is completely non-invasive and is effective in complete darkness and also in lighted areas as recorded wavelengths are far from the visible spectrum, Stoecklé & Hénoux 2014). Bat monitoring using natural light sources can be difficult as the visibility is often far from ideal, e.g. for roost emergence surveys when visibility varies with clouds and moon conditions, or assessing the activity of trawling bats in an aquatic environment.
- Thermal infrared imaging is used to survey bat colonies emerging from natural roosts including tree holes and rock crevices or buildings, providing the number of issues is limited (Sabol & Hudson 1995, Betke *et al.* 2008, Gillam et al. 2010).
- Censusing colonies ranging from a few hundred to several million individuals is achievable (Kunz & Parsons 2009). Estimates were shown to be more reliable and repeatable than methods used previously (Hristov *et al.* 2010).
- Monitoring bats inside roosts during the day depends on the species, the number of individuals, the size of the cavity and the season (temperature of hibernating bats is often close to the substrate's, but arousals are particularly obvious). Temperature of

roosting places and airflows can be detected for explaining bat choice (Stoecklé & Hénoux 2014) and daily torpor in some cases (Hristov *et al.* 2008).
- It seems to be a potentially competitive new tool for sampling all flying bats without bias, as it benefits from thermal infrared imaging by a larger field of view than automatic ultrasound recording and can also detect non-echolocating bat species and whispering bat species at longer distance (Darras et al. 2021b).
- Thermal infrared imaging was also proved to be very promising for identifying the behaviour of bats in their roost and in foraging areas, flight paths including road crossing, and mainly in wind farms (Claireau *et al.* 2021, Correia *et al.* 2013, Cryan *et al.* 2014, 2021, Gilmour *et al.* 2021, Hristov *et al.* 2008, Huzzen *et al.* 2020).
- Moreover, this method can identify the number of foraging individuals in an area when acoustic surveys provide a level of activity (of one or several individuals) (Stahlschmidt *et al.* 2012).

<u>Limitations</u>

- Thermal imaging cameras are much more expensive than their visible-spectrum counterparts, and higher-end models are often export-restricted due to the military uses for this technology. The smaller temperature differences it detects (down to 0.03°C) and the higher number of pixels it uses (40x40 up to 1280x1024), the more expensive the camera is.
- This method is very effective for monitoring monospecific colonies or habitat use by an assemblage of bat species (Betke *et al.* 2008, Yang *et al.* 2013). However some bat species exhibit hot spots along the flanks that can help to identify them from species without such hot spots (Reichard *et al.* 2010). It is very convenient to couple it with acoustic detection (it is possible to synchronise recordings) (Gillam *et al.* 2010, Huzzen *et al.* 2020).
- As with most other methods counting bats with thermal infrared cameras can be challenging, owing in part to irregular topography at the site, adverse weather conditions (rain or wind), limited field of view, variability in the pathways of emerging bats (Kunz & Parsons 2009, Ahlberg et al. 2025).
- A detection limitation occurs as approaching animals can be clearly seen at distances up to 25 m, due to an infrared radiation signature generated by the horizon (Huzzen *et al.* 2020). Censuses of large emerging colonies may suffer mainly from parallax velocity and size, and from overall velocity (Frank *et al.* 2013).
- Yet, environmental temperature can play an important role on the resulting data, as it may differ between locations and can change survey results. Nevertheless, thermal imaging has increased in popularity among bat researchers as it provides a standardised and replicable method for different types of surveys even if it requires large recording storage and some amount of technological and/or informatic knowledge (e.g. Frank *et al.* 2003, Correia *et al.* 2013, Darras *et al.* 2021a).

<u>Recommendations</u>

- Spectral range: Long wave (LW/LWIR) category higher than Medium wave (MW/MWIR), which are usually more expensive.
- Temperature range: from -40 to +120℃. Enough to display background temperature.

- Video recording capacity. Always, if possible, to be able to store the data as radiometric data files, which store in each pixel the information regarding the original recording conditions and can be optimised later. If not possible, it should store video data as .AVI or .MOV files.
- The position of the thermal infrared camera must be carefully adapted to the environment and to the aim of the survey.
- It is very convenient to couple it with acoustic detection (it is possible to synchronise recordings) (Gillam et al. 2010, Huzzen et al. 2020).

<u>Data analyses</u>

To count emerging bats from roosts, and to identify all the species in the video, one should play back and analyse the thermal video and the audio file simultaneously. Both files can be synchronised using the shared audiovisual signal (e.g. clapping hands). Counts are carried usually out manually with the use of clickers and manual counters (Ammerman et al., 2009). However, there is the possibility to use recently developed computer vision softwares that incorporate automatic counting (Ganow et al., 2015, Corcoran et al. 2021, Corcoran et al. 2025) but with the inconvenience of still not being able to separate different species in mixed-species roosts. By checking the flight path and the echolocation calls of the recorded bats the identification of the species becomes easier and substantially more reliable than using videos alone. By using this method on roost emergencies, scoring both the numbers of emerging and entering bats, it is possible to determine a net number of bats each minute (Ammerman et al., 2009). Unfortunately, there is no available software to playback a video and an ultrasound spectrogram synchronously yet.

<u>Case studies</u>

Thermal infrared imaging of bats has been developed in the United States; however some studies have been conducted in Europe, apart from the use of this method for preparing a movie for the Life+ Chiro Med in France.

Stahlschmidt et al. (2012) evaluated the relation between recorded bat activity levels and the number of individuals when estimating the role of foraging habitats for bats. At retention ponds they observed a significant positive correlation between bat activity and the maximum number of bats observed by means of a thermal infrared imaging camera. This indicated that the ponds were not only foraging sites for a single or a few individuals, but that they did attract more bat individuals. Gilmour *et al.* (2021) also used thermal infrared cameras to reconstruct trajectories of several foraging bat species (identified by simultaneous acoustic recordings) and calculate trajectory variables including flight speed, flight height and tortuosity value. So, Flight paths were recorded by Claireau *et al.* (2011) to identify the best installation of overpasses aimed to increase habitat connectivity and reduce bat collision risk.

Foraging and/or crossing behaviour of bats in wind farms should be more investigated in the future as it was initiated by Rico & Lagrange (2018). Like Cryan *et al.* (2014, 2021) or Huzzen *et al.* (2020) in the United Stated, they were able to analyse the interactions of bats with wind towers and, in this study, identify the causes of fatalities.

Voortman & Bakker (2020) described phenology and spatial and temporal variation in maternity roost site usage by common pipistrelles (*Pipistrellus pipistrellus*). They noticed that colonies roosted in the same building throughout the maternity period (April-July), and that individuals frequently switched to several roost openings within each building. They also accurately recorded the emergence pattern (phenology, timing and clustering).

5.5 Near-Infrared recording

Method description

Similarly to thermal imaging (see chapter 4.4), near-infrared (NIR) imaging also uses the infrared part of the electromagnetic spectrum but, instead of recording medium to long wavelength radiation, it only records wavelengths between 700 to 1,500 nm. This technology works similar to the human eye, as it does not rely on the natural emission of the electromagnetic waves from objects or the environment, but it requires an external light source to emit NIR light that can be detected and recorded by the camera. This system provides images more similar to the ones obtained under natural light conditions, allowing better visualisation of the environment and easier identification of the target species. Additionally, while most animals are sensitive to visible light, they do not perceive IR light, so NIR recording has the advantage of not interfering with the behaviour of recorded species (Fure et al. 2006, Russo et al. 2017).

The use of NIR recording has increased in the last decades in monitoring and surveillance projects for different animal species. Apart from the widely-used camera traps, many different studies have been conducted using NIR recording to register different aspects of bat behaviour: from foraging patterns to social interactions between individuals or swarming behaviours (Rattclife & Dawson, 2001). NIR recording can also be used in waterway surveys as a non-invasive method to assess trawling bat behaviour without the potential disturbance of an external visible light source (Russo et al. 2017, Polak et al. 2011). Also, in relatively large bat boxes, NIR has also been proved to be a suitable method to count their colonies (Schoner et al. 2010). More recently, NIR recording is also commonly used in biodiversity impact assessments as a complementary tool with thermal imaging to monitor bat activity around wind turbines (Cryan et al. 2014).

Yet, the main use of NIR for bat workers and researchers regarding bat monitoring is the regular colonies census, mostly in underground roosts, both due to the lack of light and their inaccessibility. NIR recording can be used both in internal roost surveys using night vision scopes or goggles (Sabol et al. 1995) to detect bat clusters and count individuals in the dark, and during the emergence of the colony through the use of video recordings (Revilla et al. 2019). In the last decades roost emergence counts using NIR recordings has been increasing in popularity due to several factors: it is a non-invasive sampling method, it provides highly accurate counts, the increased autonomy of different battery types allows to monitor underground roosts through long periods of time, and the affordability of newly-available camera models makes this method ideal for citizen science programmes (Torre et al. 2021).

Necessary materials

- NIR video camera (sensitive to NIR light) with or without internal NIR light lamp.
    - Resolution: minimum of HD (720x1280 px)
    - Recording frequency: minimum of 25 frames/second.
    - IR lamps power: 12W.
    - It is advisable that the camera can record for a minimum of three hours continuously.
    - Preferred output videos should be saved as commonly used files (e.g. .avi, .mp4, .mov.).
- External NIR lamps (one or more depending on roost entrance size).
- If needed, external batteries to prolong battery life.
- Power cables to connect the camera and lamps to the external batteries.
- Tripods
- Hard drives for data storage
- Optional: Ultrasound detectors (preferably full spectrum recorders) that are capable of recording continuously.

<u>Recommendations</u>

Due to the bat sensitivity towards anthropic disturbance inside their roosts, it is always recommended to use NIR recordings during the emergence, outside the roost entrance. The use of NIR cameras inside the roosts is only recommended during hibernation surveys (Chapter 4.7).

Whether video recordings are used to count emerging bats from underground roosts:

- The camera should be pointing at the roost entrance, but not inside, in order to avoid recording animals flying in circles.
- Counts should not be made in bad weather conditions. Bad weather conditions include low temperature, rain or strong winds.
- The roost entrances should not be illuminated with white light (Downs et al. 2003).
- The camera should not be placed too far from the roost entrance, as its internal NIR light will probably not reach the emerging bats.
- The camera frame must cover the totality of the roost entrance, without leaving blind corners where bats could pass unnoticed.
- The external focus must be pointed to the emerging animals from the front, but special caution must be taken to avoid confusing shadows in the background surfaces, which could result in overestimated counts.
- It is highly recommended to save the video files for posterior cross-validation.
- It is highly recommended to combine NIR video and acoustic recordings using ultrasound recorders in order to avoid species misidentification.
- If an ultrasound recorder is used, it must be placed just at the emerging point (coinciding with the video camera frame), to ensure we are recording the emerging bats more intensively than the rest of the flying individuals (Revilla et al. 2019).
- In order to accurately synchronise the video and the audio recordings an audiovisual signal must be provided in front of the camera (e.g. clapping hands).
- Another method in cases of very large colonies (> 5,000 ind.) where thousands of bats emerge within a few minutes can be to count or assess, respectively, emerging bats over a period of one minute and to repeat this one-minute-count every five minutes. The number of bats for the five minutes- period then can be calculated by extrapolation

of the numbers of the oneminute- counts, taking the middle of two consecutive one-minute-counts per minute (Rudolph et al. 2005).

Whether video recordings are used to monitor and count trawling bats in waterways surveys:

- The camera should be placed perpendicularly to the river.
- The NIR light beam should be strictly aligned with the water surface for an optimal visualisation of trawling bats.
- In wide rivers, the NIR lamp should point towards an environmental object in the background to provide a visual reference clue.
- Acoustic detectors can be used simultaneously to aid species identification and counts.

<u>Advantages</u>

- This method allows accurate monitoring of bat populations causing the minimum disturbance to the animals avoiding the use of visible light in low-light conditions (Kunz et al., 2011).
- Compared to thermal imaging, NIR recordings provide higher accuracy in image details and might allow the identification of some species, the spatial relationships between flying targets and background objects, and the gathering of additional behavioural details (Cryan et al. 2014).
- Recorded videos allow for a posterior revision of any survey conducted and, as for emergence roost counts, it also allows to slow-down the image for a more accurate counting of the individuals.
- In combination with acoustic recorders and/or thermal imaging, emergence counts with NIR cameras can be used to monitor multispecific bat colonies (Darras et al. 2021).
- The required equipment is relatively affordable compared to thermal recording cameras (Elliot et al. 2006).

<u>Limitations</u>

- Although some authors are working on automatic software to improve emergence counts with NIR video recordings, there is currently no automatic algorithm that is user-friendly, economic and widely available capable of automatically counting individuals from infrared recordings accurately (Wu et al. 2014).
- For large colonies (several thousands of individuals) NIR recordings may be insufficient to provide accurate estimates for bat populations, as would happen with direct observation using visible light sources (see chapter 2.4 for surveying large colonies).
- A NIR camera can only be used to record one roost exit so, roosts with multiple entrances may need multiple recording sets to be properly surveyed.
- The physical characteristics of the roost entrance may not allow the use of NIR recording as the camera frame may not cover the whole entrance.

Data analyses

In order to count either emerging bats from roosts or trawling bats in waterway surveys, and whether there are several concurrent species, it is imperative to scan both the NIR video recording and the audio file simultaneously. Both files can be synchronised using the shared audiovisual signal (e.g. clapping hands). Counts are carried out manually with the use of clickers and manual counters (especially if several species occur at similar numbers). There is the possibility to use recently developed computer vision software that incorporates automatic counting (Ganow et al., 2015, Corcoran et al. 2021, Corcoran et al. 2025) but still with the inconvenience of not being able to separate different species in mixed-species roosts.

By checking the flight path and the echolocation calls of the recorded bats the identification of the species becomes easier and substantially more reliable than using videos alone. Unfortunately, there is no available software to playback a NIR video and an ultrasound spectrogram synchronously yet.

Case studies

Multiple studies have used NIR cameras to count and monitor bat populations in Europe, especially colonies found in underground roosts. For instance, Piksa et al. (2011) used NIR recordings (along with acoustics and mist-netting) to monitor the swarming behaviour of bats in three different caves in the Carpathian mountains, allowing them to unveil and describe the swarming periods for the different bat species that gathered in those locations. Also, automatic NIR monitors allowed the monitoring of the emergence and swarming behaviour of tree-dwelling maternity colonies of *Nyctalus leisleri* in Germany (Nado et al. 2015). And NIR recording has also been used in caves hosting small migratory colonies of *Miniopterus schreibersii* in north-eastern Iberia (Revilla et al. 2019).

Outside Europe, by using NIR recording, Diamond et al. (2014) evaluated the effects of gating on *Corynorhinus townsendii* flight behaviour at maternity colonies and found that gates mostly affected subadults during the initial-volancy periods. Elliot et al. (2006, 2011) used both infrared and thermal video recordings simultaneously to monitor *Myotis grisescens* populations in 14 caves from the USA. Using NIR recording allowed Shöner et al. (2010) to assess the improvement of bat box occupancy using bat lures to attract forest dwelling bat species. In a study conducted in Sumatra (Darras et al. 2022) a multimethod approach allowed the visual identification of flying bats thanks to NIR video recordings (combined with acoustics, and thermal imaging to automatically count individuals). Finally, IR recordings have also been used to test the efficiency of bat deterrents in wind farms in the USA (Szewczak, et al. 2007).

5.6 Infrared light barriers

Method description

Infrared light barriers are used to count bats entering and leaving a roost (hibernation sites, maternity roosts in houses, bat boxes, etc.). The installation requires electricity and an automatic counting system connected to an infrared grid. The infrared grid is installed at the entrance of a roost to detect any bat coming in and flying out. The light barrier consists of two parallel sensor bands. These sensors broadcast a double row of beams, which are interrupted when a bat passes through the barrier. The sequence of interruptions allows to inform the direction of the bat if it was entering or emerging. Total numbers of bats in the roost are calculated based on the difference between incoming and outgoing flights (Matthäus et al., 2022). Using the system over longer periods, seasonal (phenological) patterns are visible (Krivek et al., 2023). Additionally, a photo camera can be attached to take pictures from entering bats automatically, enabling species-specific analyses.

Necessary materials

- Light barrier
- Control unit (Matthäus et al., 2022)
- Batteries (V? a Matthaus 2022 12 V) or a permanent supply
- Software to analyse data
- Camera or camera trap (if required)

Recommendations

- The technology requires some Know-How to produce reliable data or to combine with a camera system to work as a camera trap. Involve someone who is familiar with the technique and already has experience (or professional service).
- Check the system regularly (e.g. once per month) to check technical problems.
- The infrared light barrier can be triggered to a camera trap that may facilitate the individuals identifications (Krivek et al., 2022).

Advantages

- Automated counting
- Long recording periods with relatively little time investment
- Non-invasive method
- In sites where bats are hidden it can get more accurate numbers compared to visual inspection counts
- Can be used at sites where humans cannot enter
- Can be remotely connected to a computer via internet

Limitations

- It is difficult to use in roosts with several entrances (each requires one light barrier).
- This method may have incorrect counts when not installed and maintained appropriately.

- It is not possible to distinguish species (except an additional camera is installed).
- The material required for these methods may be relatively expensive.

Fig. 2: Lightbarrier system with photo trap (ChiroTEC, Lohra, Germany) maintained with car batteries in a former military bunker in the Nossentiner/Schwinzer Heide (Germany). Upper right photo: greater mouse-eared bat (Myotis myotis) entering the photo trap © M. Fritze, AG Kerth).

Data analyses

This system provides a very straight forward count of bats for each roost. Total numbers of bats in the roost are calculated based on the difference between incoming and outgoing flights. Reentries must be subtracted from the total sequentially in order to avoid underestimates when several animals come back at the end of the emergence.

Case studies

In Germany, a monitoring programme of the greater mouse-eared bat (*Myotis myotis)* is carried out since 2002. The nationwide protocol consists of monitoring roosts, which are the sampling unit. Several methods have been used for this purpose, one of which is the use of light barriers. In this respect, Matthäus et al. (2022) evaluated different techniques used in the protocol to test which of these could be the most effective, proving the light barriers to be the most optimum for the greater mouse-eared monitoring.

In a similar way, Krivek et al. (2022) used infrared light barriers triggered to white flash camera traps in order to prove its efficiency and disturbance for bat monitoring. They proved this method as a minimally invasive and highly reliable, since the camera trap permitted a better and more reliable identification of the individuals detected by the infrared light barrier. One year later, Krivek (2023) used the same approach to study the annual phenologic curve in an specific roost.

5.7 Visual roost inspection

Method description

Roost counts can be a good tool for monitoring and estimating population densities. Is one of the oldest and more used techniques to estimate abundance of bats (Allyson et al., 2001; Loeb et al., 2015), although several considerations must be taken into account. First of all, it is important to note that the different bats species display different roosting preferences (Mitchell-Jones & McLeish, 2004), so the suitability of this method will depend on the target species. Therefore, this method is optimum for those species that roost in buildings and caves (for tree-dwelling species see 4.9) but is necessary to distinguish between those species that roost hanging freely in the open, and those that roost mainly in crevices. Those species such of the *Rhinolophus* genus may be the easiest to count, while many of the vespertilionid family tend to roost in crevices and cracks, doing its counting more difficult and required of equipment such as endoscopes (see 4.9) or mirrors (Collins, 2016; Hundt, 2012; Mitchell-Jones & McLeish, 2004).

Visual roost inspection requires entering or accessing a roost (hibernation site or maternity colony) in person for counting individual bats, without touching. Underground hibernation and maternity sites (e.g. cellars, mines, caves) are usually multi-species sites. Bats are counted with the help of a torch as little as possible and carried out with as few people as possible to avoid disturbances. In maternity roosts (e.g. roof trusses, bat boxes, cellars, mines, caves), females and juveniles of a certain species can often be distinguished during the lactation phase based on fur colour and size.

The sample unit is the roost, and although it is recommended to monitor it once per season, this should be done at least in winter or summer, depending on the specific use of the roost. For hibernation sites monitoring, surveying should be done during the coldest winter months, which is generally January and February (Allyson et al., 2001). In summer, maternity colonies should be surveyed during the two final weeks of pregnancy, avoiding in this way counting the juveniles and thus obtaining relatively stable colony numbers (Loeb et al., 2015). It is crucial to minimise disturbance during the lactation phase. These timing specifications will vary among species and geographic regions.

In both hibernation sites and maternity colonies, surveying by entering the roost is an intrusive method. In winter, hibernating bats may be disrupted by the presence of surveyors, due to increased levels of noise, light and heat, while in summer they may disrupt heavily pregnant females or dependent juveniles (Loeb et al., 2015). For this reason, hibernaculum and maternity surveys should not be conducted more frequently than once every season, and when possible, other methods such as emergence counts (see 4.4, 4.5 and 4.6) or photography counts (see 4.3) will be preferred. Hibernacula and maternity counts can be replaced using a light barrier system (see 2.6), whenever the local conditions allow.

According to Loeb et al. (2015), this type of method is appropriate when (1) acoustic monitoring is insufficient to detect a particular species in the area, (2) exit counts cannot be conducted effectively, or (3) species identification is unreliable either from acoustics or emergence counts.

Necessary materials

- Torch
- Headtorch
- Bat’s identification guide
- Binoculars (recommended)
- Camera (recommended, see 4.3)
- Mirrors for crevices and cracks.
- Endoscope for crevices and cracks (recommended, see 4.9)
- Personal Protective Equipment (PPE) (e.g. helmet, gloves, etc.)
- Elevation and baseline drawings of the building or structure.

Recommendations

- Use a standard protocol/scheme that is used by your colleagues.
- Multiple observer methods are recommended to conduct single internal surveys (Loeb et al., 2015)
- The use of red lights or night vision may reduce disturbance (Loeb et al., 2015).
- Remotely operated cameras may be a good way to obtain internal counts without disturbance (Loeb et al., 2015).
- Maternity roosts itis recommended to access sites in the morning when temperatures are low and bats are mostly inactive.
- It is recommended to stay inside the minimum time possible, not making excess noise or stay near bats longer than necessary to identify them (Mitchell-Jones & McLeish, 2004)
- Additionally, the surveys should be carried out with as few people as possible (in relation to the size of the site) to avoid noise and human heat (which can influence the microclimate especially in small hibernation sites, resulting in disturbance).
- Do not shine bright lights on bats for longer than necessary to identify them (Mitchell-Jones & McLeish, 2004).
- All surveyors must be able to identify bat species without handling them (Loeb et al., 2015).
- For large bat clusters it is recommended to measure the dimensions of the cluster and establish counting subsets. When a density measure is estimated for a subset, hall cluster density is then extrapolated (Loeb et al., 2015).
- It is recommended to access sites in the morning when temperatures are low and bats are mostly inactive.
- Droppings located underneath timbers and crevices are good indication of bat presence.

Advantages

- Easy and wide-spread method
- Low-cost
- Allows estimates about the health status of bats (e.g. white-nose disease in hibernacula).
- Allows to assess presence and abundance of whispering bats, that cannot be readily detected with acoustics (Loeb et al., 2015).
- Allows inspection of the roost conditions (manipulations, disturbances, structural changes).

Limitations

- Invasive, can cause disturbance.
- Counting events may not cover actual seasonal (phenological) abundances.
- Usually, statistical challenges when analysing many datasets by many observers over many years.
- Internal counts have some advantages from a research perspective but are generally useless for large-scale monitoring (Allyson et al., 2001).
- Counts may be not representative of the real abundance since many bat species select roosting in cracks and crevices, hidden from view (Allyson et al., 2001).
- Estimating population abundance may be difficult since bats move readily within and among roosts in both winter and summer (Loeb et al., 2015).

Data analyses

In small bat clusters (typically <60 bats), direct counts can be used, but for larger groups, it is common to use estimation approaches. The visual-estimation techniques need the measure of the clusters areas, by using rulers or measuring tapes. Some estimations can use a standard number of bats per area, determined previously depending on the target species, or consider different densities per cluster depending on the specific cluster configuration. To estimate the density depending on the cluster, it can be used the row by column method, which uses linear counts of bats in perpendicular directions in a previously measured cluster portion. Once the density is determined, it is multiplied and extrapolated to the entire cluster (Meretsky et al., 2010).

There are many factors that can cause roost counts to be biased. Increasing species abundance, roost structure irregularities, and variability in cluster density and dispersion may be the most known factors. For this reason, a model is needed to convert raw count data into a density estimate. An assumption to be made is that counts are always less than the total number of the colony, and that double counting may inflate the total number of bats.

Roost counts may be analysed using log-linear models (Loeb et al., 2015).

Case studies

In Europe, the UK's National Bat Monitoring Programme established by the Bat Conservation Trust in 1996 (www.bats.org.uk) comprises hibernation surveys that are conducted in caves, mines, and other underground structures such as cellars. This long-term integrated programme works with data collected by volunteers throughout the UK. The hibernation surveys are conducted through internal visual roost inspections and carried out by two daytime visits, one in January and one in February. It has been conducted on a national scale since 1997, providing in this way relative abundances and population trends (Barlow et al., 2015). Similarly, the Bat monitoring Programme (www.batmonitoring.org) also has a protocol, called ChiroRoosts, that consists in a long-term monitoring of under- and over-ground roosts, and that has obtained information on all species present in the area (Torre et al., 2021). This protocol is specially conducted in caves, complex mines, and buildings at least one time per each season of the year. Finally, the North American Bat Monitoring Programme (NABat) also includes internal roost counts protocol, which is conducted in maternity colonies (summer) or in hibernating roosts (winter) (Loeb et al., 2015).

5.8 LIDAR

Method description

Few studies have shown the potential of Light Detection and Ranging (LIDAR) systems to monitor cave-dwelling bats. The use of this system is well known in geology, seismology, and physics, but in recent years its use has been applied to other subjects such as in forestry and ecology studies. This dispositive broadcasts several laser beams to the studied area and allows it to map its 3D structure by the returning intensity. The laser generates millions of high-resolution points merged into a point cloud representing the studied surface. Each point cloud contains the spatial coordinates x, y and z and the reflectance values. Is by this way that individual bats can be distinguished from the cave surface and other objects; because bats possess a lower reflectance value than cave's surface due to the characteristics of its fur (Azmy et al., 2012). Therefore, the count of individuals is automatically by using a 3D point cloud processing software, which can distinguish between bat individuals and cave surface through the laser return intensity (Shazali et al., 2017).

Necessary materials

- LIDAR dispositive
- 3D point cloud processing software
- Headtorches and torches for cave surveying
- Personal Protective Equipment (PPE) for entering caves.

Recommendations

- Total darkness condition is the ideal, because the scan's quality is proportionally inverse to illumination (Azmy et al., 2012).
- Preliminary surveys are recommended in order to establish the points where the scanner will be deployed, as it requires a good line of sight (Azmy et al., 2012).

Advantages

- Non-invasive method.
- In addition to counting individuals also allows characterising the roost features, in several contexts: phreatic, vadose, bell chamber, open gallery, etc (Azmy et al., 2012).
- In the study of bats it can be a potential tool, since it allows to model the 3D structure of the cave, providing important bat microhabitat information.
- Provides information on the specific distribution of bats within the roost.
- Less intensive fieldwork compared to other methods.
- Counts seems to be fairly accurate.

Limitations

- It is limited to those species that hang freely in the roost.
- LIDAR dispositive are expensive.
- May be dependent of another techniques, such as captures or acoustics, to identify the species.

- On high-density clusters the returning laser pulse may interpret the cluster as a unique object (Azmy et al., 2012).
- In the case of co-roosting species, the identification and counting may be especially challenging. In mixed-species roosts, differentiating specie may require of human interpretation (Azmy et al., 2012).

Case studies

There are few studies demonstrating the possibilities of this method for counting and surveying bats, although there are no specific examples of its use for monitoring purposes. Even so, the few examples using it for counting cave-dwelling bats could be extrapolated for monitoring programmes. For example, Azmy et al. (2012) demonstrated its potential using it in the Gua Kelawar cave in Malaysia where they could count the individuals and even identify its species by modelling some characteristics of the species by classifying and filtering each 3D captured shape. Similarly, Shazali et al. (2017) used this system to assess bat roosts by determining the roost size and number of individuals in a cave also placed in Malaysia. They identified the different 9 species present, which were generally roosting in different locations within the cave. By this way they could map the roost sites according to their respective species, thus allowing to count the individuals of each species depending on the determined location. Even so, for some species that were found roosting mixed, they were only able to extract the number of the mixed group.

5.9 Optic-fiber camera inspection

Method description

This simple method consists of the introduction of an endoscope into potential or known roosts, including tree cavities, rock and building crevices and bat boxes. Most endoscopes consist of a close-focus camera-tipped probe with adjustable LED lighting. The most professional ones have a built-in screen, but there are also simpler options where the image can be viewed via wireless on a smartphone or tablet. Different lengths are available.
By carefully inserting the endoscope into the cavity, one should look inward, downward, and finally upwards, not forgetting to look at both sides of the cavity. It is important at this point to be very careful in case animals are found inside the cavity. It is possible to observe both individuals and their presence evidence (faeces) using this technique (Coronado et al., 2018). This method, if well implemented, causes minimal disturbance because it allows for inspection and photographing of anything found and to check and identify it being away from the roost (Andrews L, 2013).

Necessary materials

- Fiber-optic endoscope.
- Ladder: if needed, recommended for surveying trees, bat boxes and buildings.
- Mechanical Elevating Working Platform: if needed and if possible, recommended for surveying bat boxes and buildings.
- Arboreal climbing equipment: if needed, recommended for surveying trees.
- Optional: Telescopic pole. Can be useful for surveying bat boxes and higher holes, as the endoscope can be attached to the pole and surveying can be done from the ground (Myczko et al., 2017).

Recommendations

- The brightness of the endoscope should be kept to a minimum to ensure minimal disruption.
- Do not apply force to get the endoscope into the roost, it may harm the animals inside.
- The use of this technique should only be done when other less invasive techniques are not possible, such as the use of torches or mirrors.
- It is important to take extreme care during the winter and summer periods, when the disturbing could be especially damaging for the bats (Method Statement for the Appropriate Use of Endoscopes by Arborists, 2015).
- Characterisation of the surveyed roosts is highly recommended; measuring the entrance, the internal dimensions (height, width, and depth) and the internal conditions may be useful to carry out analyses (Coronado et al., 2018; Tillon & Aulagnier, 2014)

Advantages

- This method allows the monitoring of specific roosts with minimum disturbance to the animals, as the photographs allow for later review (Andrews L, 2013).
- It allows surveying roosts all the year, enabling monitoring throughout the four seasons of the year and the complete cycle of life of bats (Collins, 2016).

- Due to bats can roost in inaccessible cracks and crevices, leaving no evidence, this technique allows to find non-obvious roosts (Collins, 2016).
- Material is relatively cheap.

Limitations

- Monitoring holes in trees, due to their nature, is particularly difficult (Guixé et al., 2018).
- The use of endoscopes requires specific training (Collins, 2016).

Case studies

This well-known technique has been used in several studies. In Europe, for example, Tillon & Aulagnier (2014) used endoscopes in a temperate lowland forest to study the occupancy of roosts by bats, and to characterise them and identify the most relevant features for their use. Similarly, Otto et al. (2016) used this technique in tree holes to see if there were differences in roost characteristics depending on the species of bat. Outside Europe, Civjan et al. (2017) used this technique together with others to assess the use of bridges in New England as bats' roosts. Finally, endoscopes and smartphone-enabled technology allowed Blackburn & Unger (2019) to test this technique as a non-invasive surveying tool for identification and monitoring of bats.

5.10 Marking bats

Method description

Individual marking of bats can provide valuable data and information concerning population dynamics, movements and relationships between roosts or populations as well as seasonal migration. Mark-recapture long-term data can be analysed to obtain demographic parameters as well as survival and reproduction rates which can be more informative compared to population trends obtained by count data. Multiple techniques are known for individual bat marking. We can distinguish between short-duration marks (SDM) and long-duration marks (LDM), but only LDM are suitable for long-term bat monitoring protocols (Mitchell-Jones & McLeish, 2004). The most used techniques are the permanent tagging transponders and bandings, but necklaces can also be used in some situations for long-term monitoring. All methods require decent knowledge and training as well as licensing/permits concerning animal ethics.

Regarding the RFID (Radio Frequency Identification) transponder tagging, also known as PIT (Passive Integrated Transponder) tagging, it is a commonly used technique as it represents permanent and efficient markings. Transponders are subcutaneously implanted using a syringe or a needle (Smyth, B.Nebel, 2013), and it is for this reason that it is necessary a certain expertise as well as a license for its use (Rigby et al., 2012). The PIT tag is a small (about 8-32 mm long), pill-shaped cylinder of glass that contains a radio transponder with a unique code. The transponder does not actively transmit a signal, so it does not need a battery; it responds to a signal emitted from a scanner. The scanner or reader sends out an electromagnetic charge, and when a transponder is in its range, the transponder transmits its identification code (Rigby et al., 2012; Roussel et al., 2000). The scanner may be an automated antenna system attached to a roost or a handled device, for individuals that have been captured (Smyth, B.Nebel, 2013). It is important to consider that the detection range is usually between 7-45 cm. And the distance at which a transponder can be read depends on the tag size and the antenna design. For its implantation the hindlegs of the individual may be retracted caudally, creating a flatter surface where the transponder will be inserted. On the scapular region, by gently pulling up the skin of the bat's back, the needle is inserted longitudinally. Then the needle is slightly angled downward but carefully avoiding contact with the underlying body. The needle is introduced 10 to 15 mm and the trigger is squeezed, leaving the tag under the skin. Once the tag is implanted, it is necessary to find the transponder by using the fingers, and if necessary, massage it away from the insertion point. All materials must be sterilised before implantation, and if necessary anaesthesia can be used during the process (Rigby et al., 2012).

Bat banding is also a well-known implemented monitoring method for bats that consists of the use of aluminium bat bands, which are placed on the forearm, and are marked with a unique number (Steffens et al., 2007). Bat banding has a long tradition, especially in Europe. Using special bat bands (forearm clamp), bats are marked with individual numbers which can be read by recaptures. Although it was the first technique implemented for long-term monitoring, its popularity has been decreasing in the last years, thus some studies demonstrated that it can be harmful to the animals by causing wing membrane injuries (Baker et al. 2001, Lobato et al. 2023). The most common problems include in-grown bands, chewed bands that make it difficult the identification and infected forearms. Multiple factors that may cause injuries have been described, such as the use of wrong bands (not all types are appropriate for certain species), or bad placements (Ellison, 2008) (See also best practice guidance on ringing and catching bats has been produced by the EUROBATS Advisory Committee, EUROBATS Resolution 4.6: Guidelines for the Issue of Permits for the Capture and Study of Captured Wild Bats, adopted in 2003; Hutterer et al. 2005).

Recapture is necessary for the identification of the marked individuals, which is a problem, since refind rate is therefore usually less than 1%. Even so, a continuous follow-up over many years can achieve a refind rate of 30% (Steffens et al., 2007). Exist some specific features of the bands that may be useful, such as different colours for individuals classes or a sex specific marking, which make it possible to work from a distance, and make recapture not necessary (Steffens et al., 2007). Bands can be made of metal or plastic, and nowadays' designs have improved to minimise possible injuries to the animals. There are two sizes of bands, 2.9 mm and 4.2 mm, and each size maybe used depending on the species (see Mitchell-Jones & McLeish (2004) to review species' specifications). Correct fitting procedure is important to avoid harm to the individual; the band should be fitted as loosely as possible, so that it can slide along the forearm, but it should be closed tightly enough to avoid trapping the finger bones when the band is closed. If this method is still chosen it is required to be carried out by licensed bat experts (Mitchell-Jones & McLeish, 2004), licences of which vary by country.

A less used method is the use of necklaces. The necklace or collar is threated through a numbered aluminium ring that makes the individual identifiable. There are two necklaces' designs, a ball chain type and a plastic lighter type (Gannon M. R, 1993). Plastic necklaces seem to be easier to fit than the ball chain type. Plastic ones are constructed from adjustable cable ties, and threated through a medical grade tubing that avoids movement and irritation to the individual (Gannon, M. R, 1993; Gannon & Willing, 1999). The length of the necklace will vary with the bat species, as it is determined through the neck circumference. Once we have determined the right size of the necklace, it is dropped over the head of the bat and the remaining plastic is cut away (Gannon & Willing, 1999).

<u>Necessary materials</u>

Mist-nets, harp-traps and all material related to trapping bats (see chapter 4.11) is necessary to capture individuals and proceed with the marking methods.

- Transponder tagging
    - Transponder
    - Scanner (handled or automated system)
    - Syringe or needle
    - Anaesthesia (if necessary)
    - Specific license
- Banding
    - Rings (metal or plastic)
    - Pliers to remove rings
    - Specific license
- Necklaces
    - Aluminium rings with identification number
    - Necklaces (plastic cable tie or clasp ball chains)

- Medical grade tubing to cover the collar
- Scissors
- Specific license

Recommendations

- Transponders
    - Before insertion check the transponder to be in working with a scanner.
    - Once inserted, massage it with the fingers to situate it away from the insertion point.
- Banding
    - Be sure to have expertise in using this method, thus it is key to avoid harming the animals.
- Necklaces
    - Plastics designs are preferable to ball chains because are much lighter and easier to fit, due ball chains require excessive handling of the bat by two persons.
    - Small sizes of ball chains are difficult to obtain. Therefore, this method is preferable for animals weighing more than 15 grams.
    - Because it is a procedure that requires a lot of manipulation, it is preferable to do it between two people (Gannon M. R, 1993).

Advantages

- Transponders
    - The lack of a battery means an individual can be detected almost over the course of its entire life. Tags can remain operational for decades.
    - PIT transponders weigh much less than those that emit signal with a battery.
    - Seems to be not harmful for the individuals because it has not effect on its behaviour and physiology.
    - Allows monitoring population movements, demographic patterns, and survival rates.
    - Lifelong ID.
    - Its identification is highly reliable.
    - No recapture necessary when automated readers are used.
    - Individual data.
- Banding
    - Lifelong ID.
    - Bandings are easily to spot and recognize in the field.
    - Knowledge about migration patterns, especially long-distance.
    - Depending on band's type used, characteristics of individual may be recognised with no recapture, such as sex or individual classes.
- Necklaces
    - Individual data
    - Lifelong ID
    - Are less probably to harm the animals
    - Are less probably to be chewed by the animals

<u>Limitations</u>

- Transponders
  - When there are several tags in the magnetic charge field, they all speak at the same time, resulting in only the loudest tag being heard. This is an important consideration to take in account, especially in the case of automated scanners placed in roosts, where multiple tags will be read at the emergence time. A tag monitoring system should be designed to limit this effect.
  - Invasive method.
  - Special training required.
  - Decent statistical knowledge required for meaningful data analysis
- Banding
  - Invasive method.
  - Special training required.
  - Decent statistical knowledge required for meaningful data analysis.
  - Incorrect placement can harm the animals.
  - In most of the cases recapture is required.
- Necklaces
  - Invasive method
  - Special training required, plus an excessive handling of the individuals.
  - Decent statistical knowledge required for meaningful data analysis.
  - Incorrect placement can harm the animals.
  - Recapture is required.

<u>Data analyses</u>

On the one hand, refinds allow to understand the spatial behaviour of bats, by knowing which species migrate, where and when do they do it and the maximum distances they can reach. Translocation may be differentiated among:

- Seasonal migration between summer and winter roosts
- Migration to swarming and/or matting roosts
- Dispersal migrations of juveniles
- Daily nocturnal flights between roosts and foraging locations.

Seasonal migration between summer and winter roosts is the easier phenomenon to monitor by recording data over the years. Swarming and matting translocation, as well as dispersal migration and translocation between roosts and hunting areas require a special experimental design, in order to distinguish it from seasonal migrations between summer and winter roosts. To develop the data analyses of seasonal migrations, should be considered that if within a season the same individual has been recaptured at the same place various times, the animal is only counted once for this season, and if it was counted in other several locations, the more distant location will be the one to analyse. Summer and winter roosts periods must be established, and it can differ among species. Roost fidelity may be analysed by comparing the locations where the individuals were found in the same season (summer and winter) in consecutive years. Representation of translocation can be summarized in general maps for each specie (Steffens et al., 2007).

On the other hand, refinds also allow to calculate rates about the biology of the species, as well as population trends. The most commonly used method for analyse mark-recapture data is the Cormack-Jolly-Seber (CJS) model (Sedgeley, 2012). Principal interesting rates that can be calculated are survival rate (lx), mortality rate (qx), age specific mortality rate (qy), the life expectancy (ex), the mortality expectation rate, among others. Age distribution, as well as the growth rate of bat populations and roost communities can also be measured. Growth rate can be calculated through the mortality and the birth rate. Nevertheless, just in a few cases there are robust data to compute it (Steffens et al., 2007).

<u>Case studies</u>

Long-duration marks can provide valuable data about survival rates, species' phenology, population movements, demographic patterns, etc. For example, by using PIT transponders, Meier et al. (2022) collected data of 1100 individuals over seven years, and could analyse the differences and similarities between the hibernation particularities from *Myotis daubentonii* and *Myotis nattereri,* two sympatric species in Germany. In the use of ringing as a marking methodology for long-term monitoring a great example is the Bat Marking Centre from Dresden, in east Germany, which for more than 50 years has been marking individuals and improving the ringing technique, resulting in information on survival rates, growth rates of bat populations, site fidelity and roost interrelations, among others (Steffens et al., 2007). Regarding the necklace's technique, Gannon & Willing (1999) implemented and improved it on bats from the Luquillo Forest in Puerto Rico, where a long-term monitoring protocol were established by the use of this method, which allowed to provide data about populations trends and the effects of disturbances on it.

5.11 Trapping bats (mist-netting and harp-trapping)

<u>Method description</u>

Invasive approaches that involve capturing and handling bats, such as the use of bat lures, harp traps, and mist nets, are generally not recommended for routine surveillance, due to the potential for significant disturbance and the substantial effort required to obtain high-quality datasets. However, these methods remain highly valuable in specific contexts, including preliminary research prior to establishing monitoring schemes, periodic assessments of bat abundance, and situations where species identification needs to be confirmed through the capture of individuals. They may also be appropriate when primary surveillance methods, such as bat detector or roost counts, are not feasible or effective, and no suitable alternatives are available.

The method consists of capturing free-flying bats with mist-nets or harp-traps at bat roosts, swarming sites or during the bat commuting among the foraging and the roost area (Collins, 2016; Mitchell-Jones & McLeish, 2004). Bat lures can also be used to increase captures efficiency (Collins, 2016). The set-up of the harp-traps and/or mist-nets may follow the principle of bats' commuting, whereby bats move by using ways, paths, and waterways. To increase catch efficiency it is recommended to set-up the traps in places where the vegetation or other structures force the animals to go through the restricted space where the trap is located. Collins (2016) recommends the following locations:

- Woodland rides and edges with overhanging tree branches;
- Streams/river corridors and bridges;
- Low-hanging branches from large isolated trees;
- Gaps in treelines/hedgerow
- Next to water features such as lakes/rivers, especially adjacent to riparian woodland;
- Tunnel, cave and mine entrances and passages; or barn doors.

A daytime inspection should be done to locate the potential places where the traps will be set-up. The installation may be done before the sunset, and all the traps should be functional at sunset time. Nets should be checked in 15-minute intervals, while harps may be checked every 30-45 minutes (Collins, 2016).

Harp-traps consist in a frame with two or three vertical nylon lines. Sizes may vary among 1x1m, 1,5x1,5m and 2x2m. Its reduced trapping area, compared to a net, makes them particularly useful in narrow paths. When the bat passes through the lines, it slide-down and land into a collecting bag. It is important the lines to be fully tensioned to avoid harming the individuals. The animals should be extracted from the collecting bag (Mitchell-Jones & McLeish, 2004).

Mist-nets range from 2 to 18 meters of length, and consist of a fine nylon or terylene netting, and it is held in tension between two poles. The height of the net is divided into different sections, each one of which has a loose pocket where the animals are retained. The mesh size is usually 13" or 12", being preferable the smallest. The extraction is harder and requires more expertise compared to the harp method (Collins, 2016; Mitchell-Jones & McLeish, 2004). To extract the animal from the net, first step is to open the loose pocket, clear the feet and tail and then hold firmly the body of the animal. Then, free the less-tangled wing and finally the head and the other wing (Mitchell-Jones & McLeish, 2004).

When a bat is caught, it must be extracted from the mist-net or harp-trap as soon as possible. Then, necessary information such as species, sex, age class and breeding status (pregnant/lactating/post-lactating/non-breeding) is taken. If necessary, other morphological measurements such as weight and forearm length, or genetic samples can be taken. Normally it is sufficient to release them from the head height, but if we denote the animal is not going to fly, we can find a suitable tree and allow the animal to climb to a height where it is comfortable to launch (Collins, 2016).

Trapping is usually taken between May and October when bats are heavily active. The perfect timing for swarming purposes is between August and October, whereas to confirm the presence of breeding bats it should be between May and August. Even so, it should be avoided in the summertime when bats are heavily pregnant or lactating, unless such data are necessary. The trapping should begin at the sunset hour, and the nightly effort should be approximately five hours. The number of harp-traps and mist-nets will vary depending on the surveyed area. Weather is an important aspect to take in account, whereby surveys under wet and windy conditions will be less effective. It should be avoided during prolongated rainy days and when temperatures are below 8 ºC. These considerations are both to ensure maximum the effectiveness of the surveys and not to affect the animals' welfare (Collins, 2016).

The suitability of this technique for monitoring purposes will vary depending on how and where it takes place. On the one hand, trapping far from roosts, in commuting and foraging areas, could be used to estimate relative abundance and population size, although the firmness of these data would be very low (Sedgeley, 2012). Consequently, trapping far from roost for monitoring purposes would be recommended only as a support on acoustic monitoring, to confirm cryptic acoustic species and/or breeding status in the monitored area. On the other hand, it is highly recommended when carried out at roost sites, because combined with mark-recapture techniques (5.10 Marking bats), it is a useful tool to monitor survival, productivity, and population size rates. In this case, capture and recapture sessions must be standardised over the years (Sedgeley, 2012b, 2012a).

<u>Necessary materials</u>

- Specific license from the competent authority (Collins, 2016)
- Mist-nets
- Poles
- Harp-traps
- Bat-lures (if required)
- Head-torch (leaving hands free to work comfortably)
- Holding bags (With closure-strings and seams on the outside)
- Gloves (to handle bats)
- Dust mask (to handle bats)
- Rabies vaccination (to handle bats)
- Vernier caliper (to measure the forearm)
- Scale (precision?)
- Bat species identification guide

Recommendations

- No catching should be carried out during the expected time of parturition.
- Catching bats around breeding colonies when non-flying young are present should only be carried out if there is no other way to identify the species reliably using less invasive methods (e.g. daytime surveys, ultrasound detection). If catching bats is necessary then the number of individuals caught should be kept to a minimum.
- When removing bats from the net, check first if the netting is caught in the bat's teeth, and if so, it should be removed very carefully (this particularly concerns small species). Disentanglement should proceed as quickly as possible. The animal should be held softly with one hand and the other hand used to take the bat out the same way it entered the net (e.g. Kunz 1988, Barlow 1999).
- When the bat is disentangled it should be placed in a fabric bag to minimise stress for the animal.
- The time the individual is captured must be kept to the minimum necessary – ideally not exceeding ten minutes. Visibly pregnant and nursing females should be released immediately after removal from the net. If many bats are captured at the same time then additional catches should be stopped until all animals have been disentangled.
- In the event that a large number of bats are caught at the same time, preferably individuals should be placed in separate bags. Individuals of *Rhinolophus* spp. should always be in separate bags. Pregnant and nursing females should be dealt with immediately.
- After examination, bats should be released immediately. If a bat does not fly from the hand it should be placed on a tree trunk or a branch. Captured bats may lower their body temperature for energetic purposes and then need some time to heat up before departure. For this reason bats must not be thrown in the air for release.
- It is recommended to use harp-traps in the places where larger number of individuals will be catch, due is easier for them to escape from mist-nets and harp-traps are safer for the animals (Collins, 2016).
- Harps should be used at roost entrances, where large number of individuals is expected due to the emergence phenomenon (Kunz & Brock, 1975).
- It is recommended to complement trapping with bat acoustic surveys, to provide a more balanced data (Collins, 2016).
- If using bat-lures, it is necessary to ensure the acoustic recorders do not record the calls emitted by the lures (Collins, 2016).
- The use of a bat detector is recommended to monitor any bat activity when checking nets (Collins, 2016).
- Bat handling and identification skills need to be regularly practised (Collins, 2016).
- If not necessary survey between mid-June and mid-July should be avoided, due females may be heavily pregnant (Mitchell-Jones & McLeish, 2004)
- Bats are capable of detecting and avoiding both harp traps and mist nets, but careful positioning and the element of surprise allows both to be used with considerable success.
- Capture success declines rapidly if the bats are given time to learn the positions of nets and traps, so it is best to move them every night if catching is to be carried out on consecutive nights

Advantages

- The main advantage is the confidence in identifying bat species. Some species, such as those in the genus Myotis, are acoustically cryptic, so trapping allows us to confirm the species recorded with considerable confidence.
- Quiet echolocating species are usually under-recorded, so the trapping technique allow us to confirm the presence of these species, such as those in the genus Plecotus.
- Trapping allows us to confirm the sex, age class and breeding status of the individuals.
- In conjunction with marking methods can be used to estimate survival rates, productivity, build population models, etc.
- Mist-nets:
- Are cheaper and lightweight compared to harp-traps.
- Cover larger areas than harp-traps.
- Harp-traps:
- Less harmful for the animals
- Cover narrow paths

Limitations

- Invasive method
- Mis-netting can cause bats to entangle and damage during extraction.
- Capture rates may not be representative of the relative abundances.
- Is a very labour-intensive method
- Trapping and handling bats require high experience and skills
- Catching generally requires a license.
- More probability to harm the individual than with harps.
- More detectable by bats
- Require constant monitor to ensure individuals do not escape or harm themselves
- Harp-traps are more expensive and heavier than mis-nets.
- Harp-traps have smaller covered area
- The fine mist nets designed for catching bats are usually successful, but their efficiency declines rapidly under even moderately windy conditions.

Case studies

Year 2007, the Bat Conservation Trust in the UK established a Bechstein's bat monitoring programme (Miller, 2011). The monitoring was developed until 2011, and the principal aim was to get baseline data about the distribution of this rare mammal species. With this 4-years monitoring protocol, it was also expected to understand the habitat requirements of the species, as well as to provide the areas of the country where monitoring protocol for the species could be developed in the near future. The protocol was displayed by different bat groups in the country and consisted of trapping bats using harp traps and bat lures. This case is a good example of a monitoring protocol for those species that are difficult to detect using ultrasonic detectors, due to their acoustic cryptic condition.

Thomas & Davison (2022), demonstrated the potential of this method in conjunction with others in an example of an integrated monitoring protocol. The study was carried out for three years, between 2015 and 2017, and it was focused on the swarming behaviour of a multiple-species community of the genus Myotis. A part of the trapping method was also used passive acoustic and video monitoring. Trapping was an essential part of the monitoring protocol to measure sex ratios, and reproductive status and to confirm the *Myotis* species.

5.12 Genetic monitoring

5.12.1 Genetics for species identification

Method description

Genetic tools can be used to confirm the identification of bat species and characterise the whole bat community. Over the last few decades, the application of genetic tools helped resolve taxonomic uncertainties and greatly boosted the number of recognised bat species in Europe and elsewhere (e.g., Ibáñez *et al.* 2006; Mayer *et al.* 2007; Clare *et al.* 2011). As cryptic species (morphologically identical but genetically distinct species) are common in the order Chiroptera, genetic tools are often needed to support species identification. For instance, the common pipistrelle was considered as a single, widespread and morphologically uniform species across most of Europe for more than a century. When bat workers realized that local common pipistrelles could produce echolocation calls ranging from 42 to over 55 kHz, it was unclear whether this was due to individual variation or to another source of polymorphism. DNA showed that each of those two phonic types were genetically distinct and segregated into separate colonies, demonstrating the existence of two cryptic species (Barratt *et al.* 1997).

While tissue samples (wing biopsies) are traditionally used as the source material for genetic species identification in bats, less invasive samples (Puechmaille *et al.* 2007) also offer a good source of DNA for confirming species identity. For instance, genetic tools are used to confirm bat identity from guano pellets collected under nursery colonies or during roost surveys when bats are absent, inaccessible or cannot be trapped to confirm species identification. Current most widely used genetic method to monitor bat populations are based on a single (or a few) versatile markers, which can be obtained from non-invasive samples (Walker *et al.* 2016; Mota *et al.* 2021).

Whether the genetic material originates from tissue, saliva, hair or faecal sample collected directly from a caught bat or from guano samples collected from the environment, DNA is extracted from each sample and a standardised region of the genome (a barcode) is amplified with bat specific or generic mammalian primers, using Polymerase Chain Reaction (PCR). The PCR products (amplicons) are sequenced to obtain a sequence of DNA (barcode) that can be matched with available reference libraries for species identification (Hebert *et al.* 2003; Ruedi *et al.* 2023). The length of the DNA segment amplified varies between ca. 120 and 700 bp, depending on the quality of the source material (Haarsma *et al.* 2016).

Environmental DNA (eDNA), the extraction and identification of DNA from environmental samples (e.g. soil, water, air, faeces, sediments) has recently emerged as an important tool for surveying and monitoring biodiversity. Advances in sequencing technologies and bioinformatics made it possible to obtain genetic information from the environment without the need to sample the target species directly (Bohmann *et al.* 2014). DNA metabarcoding approaches use standardised DNA regions (e.g. mitochondrial DNA Cytochrome oxidase-I, COI, or 16S rRNA) and high throughput sequencing to simultaneously identify multiple species (or strains) from a single environmental sample (Ruppert *et al.* 2019).

Genetic confirmation of species identity is essential for monitoring changes in species composition over time, in particular for species that cannot be easily distinguished morphologically or based on their echolocation calls. Metabarcoding approaches can be applied to monitor changes in bat species composition in an area over time and track range expansion of species into new areas without the need to trap and sample them directly.

Necessary materials

For species ID based on the DNA barcoding or metabarcoding approaches, any source of genetic material obtained from tissue, saliva, faecal matter (guano), soil and air, is potentially useful for PCR amplification (see Hoban *et al.* 2022 for sources of DNA for genetic analysis and monitoring). However, the longer the amplified target, the better-quality DNA is needed to get reliable amplifications. For instance, universal COI primers (Ivanova *et al.* 2007; Hassanin *et al.* 2012) are versatile for most bats, but as they target a fragment of 650 to 750 bp, the amplification process should be based on relatively fresh biological material. Mini-barcodes of less than 200 bp (Walker *et al.* 2016; Kocher *et al.* 2017) can also be useful for species ID and could be amplified theoretically from much more degraded DNA such as historical or environmental DNA but they are also much more sensitive to exogen contaminations (Rishan *et al.* 2023).

- Any source of bat DNA (guano, tissue, soil, air).
- 1.5 ml tubes containing either 70-99% ethanol, a lysis buffer (e.g., DESS, DNAShield), or silica beads, and pencil or permanent marker to label the tubes.
- Paper, notebook or electronic spreadsheet to record metadata (sample ID, location, coordinates, date, sex, reproductive conditions, biometric measurements etc…).
- Access to a relatively simple laboratory to perform DNA extraction, PCR amplification and gel electrophoresis. Amplified products are then sent to a commercial laboratory for Sanger sequencing (barcoding) or high throughput sequencing (metabarcoding).
- A library (database) with reference sequences from well-identified samples. Commonly used online reference libraries are NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the Barcode of Life Database (BOLD, https://boldsystems.org/).

Recommendations

- Avoid contamination when collecting the samples (especially when collecting eDNA) by sterilising surfaces and equipment and wearing disposable gloves and face masks. This is also crucial for preventing the transmission of pathogens between individual bats and between humans and bats.
- Store tubes with biopsy samples in a freezer to minimise DNA degradation.
- Choose popular markers (COI and Cytochrome b) to have extensive comparative reference material with good geographical coverage.
- Include also nuclear markers to identify hybridising or introgressed species (see below).

Advantages

- Standard protocols and ease of process.
- Versatile markers are available for all bats.

- Sequence reference database available for most species on open access platforms.
- Commercial facilities offer affordable species identification services from guano.
- eDNA samples can be processed through metabarcoding approaches to get additional information on other taxa present in the area, bat diet or bat microbiome.

Limitations

- Some specie pairs (Eptesicus serotinus vs nilssonii; Eptesicus serotinus vs isabellinus, Myotis myotis vs blythii; M. mystacinus vs davidii; M. nattereri vs crypticus) cannot be differentiated by their DNA barcode because of mtDNA gene introgression. Nuclear markers should be used instead.
- Publicly available repositories of DNA sequences are plagued with mislabelling or identification errors. To minimise these errors, examine all matching sequences within >98% similarity to detect possible inconsistencies.

Data analyses

- Curate reliable reference databases for correct identification of bat species.
- Metabarcoding approaches require complex bioinformatics processing of the High Throughput Sequencing reads to group them into Operational Taxonomic Units (OTUs), Amplicon Sequence Variant (ASV) or Barcode Index Numbers (BINs) and remove potential contamination and sequencing errors.
- Community composition matrices, alpha and beta diversity measures, network analyses.

Case studies

Initial genetic surveys of European bats identified unexpected divergence within few species (e.g., Ibáñez *et al.* 2006; Mayer *et al.* 2007), some of which eventually proved the existence of cryptic species (e.g., Salicini *et al.* 2011), while others only reflected the persistence of ancient intraspecific lineages within otherwise panmictic populations (Andriollo *et al.* 2015). The combined use of mitochondrial DNA (i.e., transmitted clonally by females only) and nuclear DNA markers (i.e., transmitted with recombination by both parents) was essential to understand the origins of such unexpected molecular differences.

To further illustrate to usefulness and pitfalls of the DNA barcoding approach to identify mammal species, Ruedi *et al.* (2023) surveyed the genetic variation of 102 Swiss mammals (including 32 bat species) with three mtDNA markers (COI, Cytochrome b and 16S rDNA). Most bat species could be easily distinguished with these popular markers, exceptions being introgressed species pairs (*Eptesicus serotinus vs E. nilssonii*; *Myotis myotis vs M. blythii;* and *M. nattereri vs M.crypticus*). However, a more worrying result was that several sequences submitted to the GenBank or BOLD repositories for identification resulted in wrong or ambiguous species identifications. This uncertainty was not due to any biological reasons, but to misidentified or mislabelled sequences present in these public databases. For instance, the full mitochondrial genome of an "*Eptesicus serotinus*" proved to be a misidentified *Nyctalus noctula* (GenBank #MT584130), which implied wrong species ID (at 100% match) for all mtDNA markers. This study stressed the importance of building a carefully curated reference database to minimise such experimental errors for reliable species identification throughout the DNA barcoding approach.

5.12.2 Genetic populations composition and demography

Method description

Within-species genetic variation (genetic composition) is a fundamental component of biodiversity that underpins the long-term persistence of populations because it determines their capacity to adapt to environmental change (Hoban *et al.* 2022). The field of population genetics to monitor wild animals has been expanding rapidly over the past few decades. It allows us to estimate crucial information about population structure, population status and environmental adaptations, and how they change over time in non-model species. The genetic markers used in population genetic studies have evolved over the years, from allozymes and mtDNA, which relied on a small number of markers, to approaches that examine many markers at the same time, microsatellites (e.g., Gürün *et al.* 2019) and more recently single nucleotide polymorphisms (SNPs; Allendorf 2017). High throughput sequencing techniques, developed in the past two decades, enable the generation of thousands of molecular markers simultaneously, opening a new era of population genomics and offering unprecedented levels of resolution in terms of accuracy (Garg *et al.* 2023; Foley *et al.* 2024). However, even if such progress now provides access to remote genetic data, not all kinds of genetic information can be obtained in practical terms for long-term monitoring programmes.

If genetic markers are used to simply monitor the presence or absence of a species in each place, methods and analytical tools to reach this aim are cheap and generally straightforward (Clare *et al.*, 2011, Ruedi *et al.*, 2023). For more advanced studies, if the aim is to get useful information about the demographic history, levels of inbreeding or of connectivity among populations (Lehnen *et al.* 2021; Razgour *et al.* 2024), then laboratory settings and analyses (bioinformatics and genomic data analysis) are more labour-intensive and might become prohibitive for routine monitoring programmes. This is because of the thousands of molecular markers (SNPs) needed to reach statistical significance and costs of sequencing. Reduced representation genome sequencing approaches, such as RAD-sequencing (Restriction site-Associated DNA sequencing) or genotyping-by-sequencing, allow the sequencing of a large number of samples for reduced costs, and therefore have been applied extensively in molecular ecology studies to assess population structure, hybridization, demographic history and adaptive variation (Josić *et al.* 2024). Alternatively, genomic tools can be used to develop a small panel of markers specific for identifying either population structure, hybridization or population size, which can be genotyped at lower costs across a large number of individuals over time (Hohenlohe *et al.* 2021). However, with dropping sequencing costs, whole genome sequencing is becoming more affordable for long-term monitoring studies. Yet, more traditional genetic markers, like microsatellites, are still commonly used for wildlife monitoring (Allendorf, 2017).

DNA barcoding of non-invasive samples (e.g., from guano) may be used beyond confirming species identification to delineate Evolutionarily Significant Units (ESUs) or Management Units (MUs) that are very important tools for managing wild populations or recovery programmes, but because they are based on maternally-inherited markers, they cannot be used for more elaborate inferences on parameters of population size or connectivity. In this case, there is a need for better source of DNA (e.g., wing biopsy sample), and for specifically-designed nuclear DNA markers (microsatellites) or whole or reduced representation genome sequencing to generate large SNP datasets. Whole genome sequencing (or good genome coverage from reduced representation sequencing) is needed for inference about adaptive variation.

Visual counts or more traditional mark-recapture methods are perceived as more cost-effective and accessible for long-term monitoring programs than genetic monitoring. However, when based on non-invasive sampling (collection of hair, skin or faeces without even observing the animal), genetic data may be cheaper and easier to gather, especially for elusive species (e.g., Carroll *et al.* 2018; Ferreira *et al.* 2018).

Genetic monitoring programs for bats should focus on the four essential biodiversity variables proposed by Hoban *et al.* (2021) for monitoring and understanding changes in the genetic composition of populations: 1) genetic diversity; 2) genetic differentiation; 3) inbreeding; and 4) effective population size (N_e). As all these variables can be calculated using different genetic markers (e.g., whole genome sequencing data, SNPs, DNA sequences, microsatellites), they can be calculated from existing datasets thus reducing costs and disturbance to populations.

Necessary materials

- Source of DNA: tissue (wing biopsy), faecal matter, saliva, hair
- Sterile biopsy punches (3 mm) to collect good-quality DNA samples or buccal swabs (for genetics, but not genomics, work).
- Sterile 1.5 ml tubes containing either absolute ethanol or a lysis buffer (e.g., DNAShield or RNAlater) and pencil or permanent marker to label the tubes.
- Paper, notebook or electronic spreadsheet to record metadata (sample ID, location, coordinates, date, sex, reproductive conditions, biometric measurements etc…).
- For genomic monitoring, extracted DNA is usually sent to a commercial laboratory for library building and high throughput sequencing.
- Access to a high performance computing cluster for bioinformatics of high throughput sequencing data (some genomic facilities and companies offer bioinformatics services).

Recommendations

- Careful consideration of sampling design to ensure a sufficient number of samples from each population, number of populations (or individual locations), representation of environmental conditions, number of markers and sequencing depth. These parameters vary depending on the type of genetic/genomic markers used and the variables to be monitored.

Advantages

- Provide information on the conservation status of populations, in terms of genetic diversity, inbreeding and effective population size.
- Can be used to estimate historic changes in population size before monitoring has begun.
- Tracking how populations are adapting to environmental change or management actions.
- Track movement across large spatial and temporal scales through information on gene flow and population structure.

- Availability of new genomic resources for bats through the Bat1K initiative (https://bat1k.com/).

Limitations

- Generating genomic data can be time consuming and expensive.
- Advanced bioinformatics and statistical knowledge required for meaningful data analyses.
- Capture is often required to get good quality individual DNA samples.

Data analyses

- Bioinformatics to process raw sequencing data for genomic datasets or genotype scoring for microsatellite markers.
- Population structure, gene flow or genetic differentiation between populations, genetic diversity, inbreeding and other population genetic statistics. There are several R packages with good vignettes for population genetics and genomics data analysis (e.g., Paradis et al. 2017; Gain & François 2021).
- Demographic history analysis to identify historic changes in population size either based on a single sample or longitudinal sampling.

Case studies

The Greater and Lesser mouse-eared bats (*M. myotis* and *M. blythii*, respectively) are two sibling species which are morphologically very similar but differ markedly in their ecology. The former is relatively common throughout most of Europe and classified as Least Concern, while the latter is more meridional and listed as Near Threatened, e.g., in French Red Lists. Because both species may form mixed colonies, population size of each species is impossible to assess by visual counting. Afonso *et al.* (2017) used non-invasive methods to sample a mixed colony in the French Alps and genotyped fresh guano pellets with a panel of 11 microsatellite loci. This array of nuclear markers enabled them to discriminate every individual present and estimate effective population size and genetic diversity of each species separately. In addition, individual genotypes were also used to estimate levels of gene introgression (7.9%) in this mixed colony. Interestingly, none of those introgressed individuals were first generation hybrids (F1), suggesting that hybridization was rare among these sibling species.

Genomic tools can improve the precision of estimates of effective population size and reconstructions of demographic history (Hohenlohe *et al.* 2021). Razgour *et al*. (2024) generated a reduced representation genomic dataset (using the ddRAD-sequencing approach) for the Barbastelle bat, *Barbastella barbastellus*, to identify historic population changes and their drivers. They identified a severe population decline by around 99% over the past 300 and 500 years for the northern and southern British populations, respectively, which may be linked to the loss of large oak trees and native woodlands due to shipbuilding during the early colonial period. These estimates of the extent of population size declines helped place the conservation status of the barbastelle into a historic context and inform targets for species recovery. Razgour *et al.* (2024) further used the genomic dataset to identify the effect of anthropogenic land-use change on the conservation status of the barbastelle. They found that genetic diversity increases with woodland cover around the colony home range, while genetic connectivity between colonies is impeded by artificial lights and facilitated by rivers and broadleaf woodland cover.

Genomic tools can also be used to identify genes or genomic regions involved in adaptation to the environment, and therefore provide information on adaptive variation and the capacity of the population to evolve in response to environmental change (Hohenlohe *et al.* 2021). Razgour *et al.* (2019) used reduced representation genomic datasets for two cryptic Mediterranean bat species, *Myotis escalerai* and *M. crypticus*, to identify adaptations to key climatic conditions affecting bat survival and reproductive success, maximum summer temperatures and summer rainfall. They then used this information to generate genomic-informed species distribution models to project changes in range suitability for individuals adapted to hot and dry versus cold and wet conditions under future climate change. This has resulted in improved predictions of the vulnerability of these two bat species to climate change and has highlighted the importance of forest connectivity for their ability to shift their ranges to track changing climatic conditions.

6 Managing data: storing, sharing and archiving

6.1 FAIR principles

FAIR stands forFindable, Accessible, Interoperable and Reusable. These principles, if applied, will make it possible to reuse the data collected in a given study for a new purpose. The best case scenario is that the data is easy to find, access, interoperate and reuse, even if the author of the data is not available anymore.

Although many studies do not have the ambition to be published in scientific journals, it is worth applying FAIR principles to the data management of any study. This applies to any work on bats that is carried out systematically and repeatedly by either volunteers or professional bat workers, and also applies to impact assessment studies. A regional or national register of impact assessment studies is important, for instance, to make sure that other people know that bat data was collected at a given place and at a given time. Authors of the data and their contact information should be made public, as well as a description of the dataset so that the data can be requested. If possible, the raw data should be made public. Even if the data is confidential at the time, an embargo could be used as a tool to make the data public years later. Some countries require that biodiversity data collected in the frame of impact assessment studies are made public, which is the best case scenario (e.g. article L 411 1 A of the French environmental code). However, to be efficient, this requirement should make sure that the raw data and the metadata are published in standard reusable formats.

Good reasons for using FAIR principles include the transparency of the work so that readers of a report can correctly interpret the results and check the data if needed. Another particularly good reason is that data collected for a local objective (e.g. the inventory of bats of a Natura 2000 area or the environmental impact assessment of a project) could contribute years later to meta-analysis at the regional, national or European scale. In this case, the original data and any information about the methods (date, time, location, type of material and settings…) might be of the highest importance. As it is impossible to predict how technology will develop in the future, the raw data (e.g. sounds, videos, pictures or biological samples) collected in a study should always be archived on the long term to be re-analysed if necessary. Affordable solutions exist to preserve this precious material, including depositing the data in a collective database (e.g. Movebank for tracking data). Invasive studies implying bat capture or roost visits have an even higher ethical responsibility to make the data available for scientific purposes, as they have a potential impact on bats.

More information about how to apply FAIR principles can be found at www.go-fair.org.

6.2 Data Management Plan (DMP): anticipating the life cycle of data

Bat experts and researchers are no longer only relying on paper notebooks for their data. With the advancement of the technology, they are now handling complex datasets and are often involved in regional, national or international projects. This complexity in the management of data can be facilitated with good practices. A Data Management Plan (DMP) is a document describing how the data is managed, from the start to several years after the end of the project. It will help the data manager to achieve the FAIR principles. It can be used internally only or be made public. A DMP is designed to help data managers anticipate the life cycle of their data (e.g. *How much storage space will I need? Who owns the data and who needs to be acknowledged when results will be published? Where will the data be archived after the project is finished? Who will be authorised to download it?*). A DMP can be created for a particular study, or to describe how data of a certain type are generally managed in the structure. It can serve as a reference document for a structure in which several bat experts work together and need a solution to properly organise the structure's data . The best practice is to write a DMP before the start of data collection and update it every time it is needed, but DMP can also be written after data collection has started. It must be noted that a DMP has to be adapted to the needs of the data manager, so it can be very simple or more complex.

<u>General information</u>

In this section, the DMP describes the timing of the project, who is responsible for the project coordination (including contact information), for the data management, and who are the partners. The expected costs of data management can also be included here. Legal issues can be anticipated and described, in particular concerning personal information (emails, names and addresses) or intellectual property rights. If ethical approval or authorisations are needed to collect data, they can also be included.

<u>Collection and metadata</u>

This section describes who collects the data, who checks the quality of the data, and what kind of data and metadata are involved. The structure of the dataset should be described. For instance: a dataset is composed of *study sites* studied at different *dates* by *experts* who will collect information about the *number of bats* recorded for each *species*. If codes are used, they should be explained here. It can be convenient to mention which metadata are collected automatically and which manually. Data can be of a sensitive nature (e.g. roost location): it is possible to explain the strategy to keep this data confidential. This section should be thought of carefully to prepare future collaborative work. The structure of the dataset should, as much as possible, follow the regional, national or international practices. For instance, if standard tables are used on a regular basis to make a national synthesis of roost counts, then identical column names and value types (number, characters or factors) should be used to facilitate future data analysis.

Storage

Here, the total expected size of the dataset and the size of the data collected each year are described, as well as the type of storage where it is saved (e.g. SD cards, paper worksheets, hard drives or servers). If the format of the data changes along the management process (e.g. from sound files to tables), the different formats should be described. The backup of the database must be described: it provides a solution in case the original database is destroyed (e.g. fire, flood or human error).

Analysis

This section describes the different steps necessary to implement the FAIR principles. The tools required for the analysis should also be described, such as the different softwares used. A dataset can have different versions and grow continuously. It is important to describe the version control naming and procedure. A scenario of data usage can be described. A diagram can accompany the descriptions (e.g. Figure 3).

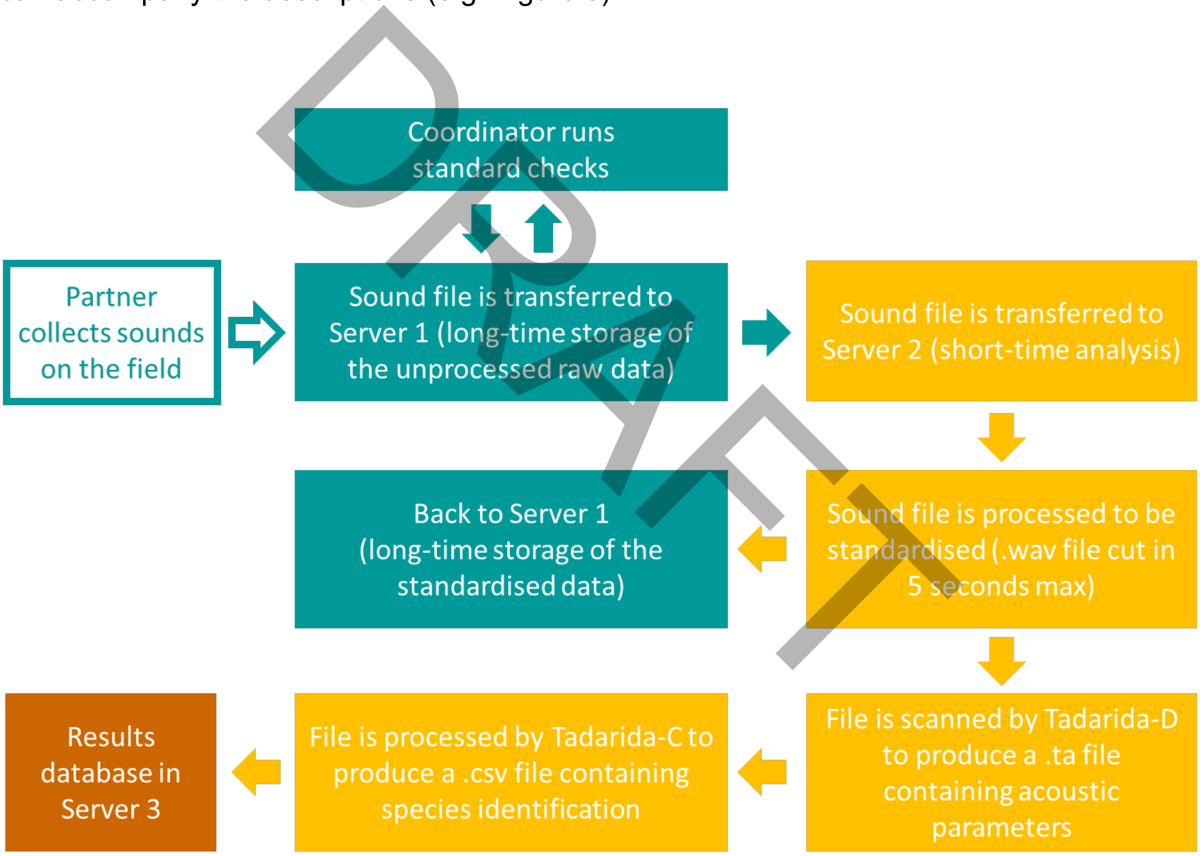

Figure 3: Example diagram of the analysis and storage workflow in the project Bat migration routes in Europe. Acoustic recordings are collected by multiple partners to feed a collective database. The data moves through three different storage locations during the process.

Sharing and interoperability

Here whether the dataset is destined to be shared or not is described. In both cases, the way to access the data should be described, as well as the kind of software required to use it. It is recommended to design solutions to share the raw data so that it can be re-analysed if needed. If the processed data is shared, the format should be readable across platforms (e.g. linux, windows or mac) as open standards like comma-separated-values.

Archiving

Archiving is the adequate term used for the long-time storage of the data. Archives are generally not accessed on a regular basis. The format and the support in which data are archived should be described.

More information about how to create a DMP can be found online.

6.3 Types of storage, sharing and archiving facilities for digital data

Data noted on paper should be digitalised, and digital data should be backed up as soon as possible to prevent the loss of data. A backup in a different building or even town than the original data is a stronger guarantee of data preservation.

Direct-attached storage (DAS)

These storage facilities are directly accessible to the user (e.g. SD card, USB key or hard drives). They can be used for temporary analyses, for storage and archive, to share data on an ad-hoc basis, but they are not suitable for datasets superior to a few terabytes. Using many hard drives to store datasets can hinder the implementation of the DMP:, for instance it will discourage updates in the organisation of the database, or result in a loss of time when managing data stored on different disks.

On-site server

A server is a computer that can be accessed by multiple users through a network. On-site servers are usually installed when several employees need access to a storage and archive facility on-site. It is possible to increase the storage capacity of an on-site server over time if needed by buying additional disks, and backup of the whole database is facilitated. On-site servers can be accessed with USB/ethernet connections from inside the building or from outside with an internet connection protected by a VPN. On-site servers are convenient to handle big datasets, however the associated costs of the facility must be accounted for.

Commercial online server or "Cloud"

A cloud is a storage space on a server hosted by a third-party and it is accessed with an internet connection. It is usually necessary to pay a subscription to obtain space, but many offer a few Gigabytes for free. Commercial clouds sometimes offer bigger space for free for non-profit organisations in their grant programs. Cloud solutions are better suited for sharing data on an ad-hoc basis.

Non-commercial third-party server (servers of other organisations or citizen science programs)

Hosting a dataset on a non-commercial third-party server usually means that there is a mutually beneficial agreement between the third party and the author of the data. For instance, NGOs and museums can develop online platforms to centralise biodiversity data in order to support research and conservation projects. It is convenient for users to store/archive their data on such facilities to make sure that their data has a second life. However it is often required to follow a particular protocol to upload data. Some platforms even offer the automatic and free analysis of the data.

These platforms have the aim to produce citizen science thanks to the contribution of users anywhere in Europe:

- **Bat Monitoring Programme** (Natural Sciences Museum of Granollers) (https://www.batmonitoring.org/) for:
    - Passive acoustic monitoring following specific settings
    - Underground and overground roost monitoring
    - Waterway surveys
    - Bat box monitoring
    - Capture/trapping monitoring
- **Batlas** (Universität Greifswald) for the monitoring of population trends with roost data (http://www.batlas.info/).
- **Bat migration routes in Europe** (French museum of natural history) for passive acoustic monitoring (https://bat-migration-europe.netlify.app/).
- **Noctule count** (Batlife Europe) for the visual monitoring of common noctules (https://www.batlife-europe.info/projects/noctule-count/).
- **DarkCideS** for underground roost monitoring (https://darkcides.wordpress.com/)

Non-commercial online server with DOI

When using the data for scientific purposes some dataset needs to be referenced. In these cases, non-commercial online servers can host the dataset and provide a Digital Object Identifier (DOI). Movebank is a platform hosting animal tracking data. Zenodo, Dryad or FigShare host all kinds of data but they generally need to be less than a few dozen gigabytes. These platforms generally also offer to provide a versioning of the dataset so that when it is updated a new version can be referenced.

Practical recommendations for digital data

Table 2. Long-term solutions to archive and share different types of raw data on the long-term. Processed data (e.g. sounds analysed and resulting in a data table) can also be archived and shared but are of a lower priority to achieve FAIR principles. Commercial cloud solutions are not recommended because they rely on a subscription that could represent an obstacle in the long term. NGOs or museums centralising data from different partners are generally considered to be a safe place to archive data and share it on the long term. They usually don't rely on long-term online solutions for data sharing because they make extractions of their database on demand (e.g. capture data, roost counts). Short-term or middle-term storage and sharing solutions are not included and are left to the appreciation of the data manager.

Method	Data obtained	Important metadata associated	Recommended archive facility (after the analysis)	Recommended long-term storing and sharing facility
Acoustic recording	Sound	Date, start and end time of session, time, coordinates, protocol: stationary / transect, recorder model, microphone model, calibration status of microphone, microphone height, settings of the recorder (at least sample rate, trigger level, filters), author name(s) and contact	• External hard drive with backup • On-site server with backup • Non-commercial online server	• For small datasets: non-commercial online server with DOI • If full-night passive recording: citizen science programs are not designed yet for the facilitated sharing of the raw data because of the size of the datasets. But they can process the data to contribute to new research projects.
Photography	Image	Date, time, coordinates, camera model, lens specificities, settings of the camera, author name(s) and contact	• External hard drive with backup • On-site server with backup • Non-commercial online server	• For small datasets: non-commercial online server with DOI
Filming	Video	Date, time, coordinates, technique used: video / IR / thermal, camera model, lens specificities, settings of the camera, author name(s) and contact	• External hard drive with backup • On-site server with backup • Non-commercial online server	• For small datasets: non-commercial online server with DOI If full-emergence: citizen science programs are not designed yet to store it because of the size of the datasets. But they can process the data.
Radar	Image	Date, start and end time of session, coordinates, model of the radar, settings of the radar, time of the images, author name(s) and contact	• External hard drive with backup • On-site server with backup • Non-commercial online server	• For small datasets: non-commercial online server with DOI

Roost count	Table	Date, coordinates, method used (in the roost, at roost emergence, or passive count with an IR-barrier), type of roost (tree, house, bat box, cave,...), species name, number of individuals, sex, age, author name(s) and contact	• Computer's hard drive with backup • On-site server with backup • Non-commercial online server	• It is recommended to centralise the data at regional/national organisations for bat conservation • This kind of sensitive data is generally not shared publicly • The Bat Monitoring Programme (www.batmonitoring.org) can store and manage this kind of data.
Capture	Table	Date, start and end time of session, coordinates, type and number of nets, time of capture, species name, sex, age, morphological measurements, author name(s) and contact, license for handling bats	• Computer's hard drive with backup • On-site server with backup • Non-commercial online server	• It is recommended to centralise the data at regional/national organisations for bat conservation • Non-commercial online server with DOI • The Bat Monitoring Programme (www.batmonitoring.org) can store and manage this kind of data.
Tracking (VHF, GPS, SigFox…)	Table	Date, start and end time of session, model of the logger, settings of the logger, coordinates and time of the fixes, species name, sex, age, author name(s) and contact, license for handling bats	• Computer's hard drive with backup • On-site server with backup • Non-commercial online server	• Non-commercial online server with DOI (e.g. Movebank)
Marking (ring, pit-tag)	Table	Date, type of marking device (ring, pit-tag), model of the tag, unique number of the tag, registry for the tag, species name, sex, age, author name(s) and contact, license for handling bats	• Computer's hard drive with backup • On-site server with backup • Non-commercial online server	• It is recommended to centralise the data at regional/national organisations for bat conservation • Non-commercial online server with DOI • The Bat Monitoring Programme (www.batmonitoring.org) can store and manage this kind of data.
Genetic	Table	Date, start and end time of session, coordinates, time of capture, species name, sex, age, morphological measurements, type of genetic analysis (mtdna, edna,...) author name(s) and contact, license for handling bats	• Computer's hard drive with backup • On-site server with backup • Non-commercial online server	• Non-commercial online server with DOI

7 Indicators and Population trends

7.1 Introduction

Long-term monitoring schemes are a top priority to assess population trends and detect changes associated with environmental or landscape transformations. The continuous technological advances and the increasing urgency of filling the knowledge gap on the bat population status are encouraging the establishment of standardized long-term monitoring schemes in a growing number of European countries. And fortunately, the vast amount of data already collected nowadays through the multiple methodologies, makes it possible to describe population trends.

Here we present a summary of the different statistical methods that have been used for population trend analysis. Trends are typically studied using either individual count data (if available), activity indices such as bat passes per unit of time, or specific indicator metrics, which usually do not follow a normal error distribution. For this reason, all the proposed statistical models are based on generalised models. The chosen model depends on the type of data, the amount of missing values, the length of the time series, and the desired result, amongst several other factors. Although no particular model has been established as the standard for describing population trends, TRIM has recently been gaining importance due to the possibilities it offers and the results it provides.

It is important to highlight that although many models do not generally require a minimum sample size to be computed, a reasonably long time series is necessary to obtain reliable and robust trends. In specific cases, data collected over five years may be sufficient, but ideally a minimum of ten years would be recommended. In general, data collected using standardized methods over several years, without gaps, across different locations and applying varied methodologies for each species will provide accurate bat monitoring reports.

In terms of indicators, although bats have long been recognized as valuable bioindicators of habitat health (Jones et al. 2009), their practical application as bioindicators using user-friendly metrics or indices remains rare in the literature (but see López-Baucells et al. 2017, Tuneu-Corral et al. 2020, López-Bosch et al. 2023), probably due to the lack of standardized measures and indices (Stahlschmidt et al. 2012).

Therefore, there is a clear need to develop, standardize, and implement robust bat-based indicator indices that translate complex ecological data into accessible, user-friendly metrics. Such tools would facilitate the effective integration of bat responses into environmental assessment, management, and legislative frameworks, ultimately strengthening evidence-based policy and conservation law.

7.2 Ecological Indices

Bats play an indisputable role as bioindicators, a fact which is widely recognised (Jones et al. 2009). Consequently, standardised ecological indices are necessary for examining the underlying factors contributing to fluctuations in these trends. For this reason, Tuneu-Corral et al. (2020) introduced novel community indices based on their ecological preferences:

- **Bat Community Thermal Index (bCTI)** was developed to assess changes in bat communities in response to temperature fluctuations, particularly in the context of escalating climate change. This index has been adapted from Devictor et al. (2012), who initially introduced it for birds and butterflies.
- **Bat Community Precipitation Index (bCPI)** shares a similar framework to the previous one, focusing on alterations in precipitation patterns such as prolonged arid periods or extreme storms, which are progressively gaining prevalence.
- **Bat Community Openness Index (bCOI)** was designed to assess habitat preference along a gradient ranging from open to forested areas. This index provides insights into land-cover structure alterations, such as instances of deforestation or reforestation. Herrando et al. (2016) established this index for birds and butterflies.
- **Bat Community Specialization Index (bCSI)** enables the evaluation of niche complexity by considering the presence of specialist and generalist species. The predominance of generalist species within bat communities indicates disturbed ecosystems with limited available niches, whereas the presence of specialist species represents more stable ecosystems characterised by a higher degree of niche specialization. This index is derived from Julliard et al. (2006) work, who originally formulated the index for European bird assemblages.

Calculating the Community Indices always involves a previous computation of the corresponding species-specific indices. Each Species Index is determined for each bat species, and it represents the average index (either of temperature (STI), precipitation (SPI), openness (SOI) or specialization (SSI)) within the specific range of each bat species. Once the Species Index is determined for each species, the Community Index for each sampling point is calculated by summing the Species Index of all species/sonotypes present at that location and weighting them based on their bat activity detected. All these ecological indices can be used to assess temporal trends, in a manner comparable to standard analyses of bat population data.

For a comprehensive understanding of the calculation methodology for each specific community and species index, we recommend referring to the study conducted by Tuneu-Corral et al. (2020).

7.3 Bat roost priority index (BRP)

The Bat Roost Priority Index (BRP) is a multicriteria conservation tool designed to prioritise bat roosts based on both ecological value and vulnerability. As described by López-Bosch et al. (2025), it integrates biotic data, such as species richness, abundance, and the presence of threatened or conservation-relevant species, with information on roost-specific pressures and threats. The index translates these components into a single, standardised score that reflects the overall conservation priority of each roost, enabling consistent comparison across sites and regions.

A key feature of the BRP is its foundation in structured, reproducible criteria that capture both community composition and site vulnerability. By combining measures of biological importance with indicators of disturbance or risk (e.g., human access, structural instability, or land-use pressures), the index provides a more holistic assessment than approaches based solely on bat counts or species presence. This framework improves upon previous prioritisation methods by offering a transparent and scalable way to evaluate roost significance across heterogeneous landscapes. Importantly, the BRPi is designed to support conservation planning and decision-making. It enables the classification of roosts into ordered categories of conservation priority, helping practitioners identify key sites for protection, monitoring, or mitigation actions.

The index is embedded within a broader bat monitoring platform (i.e. www.batmonitoring.org), is fully functional and ready to be used, as it can incorporate any community-generated data from all European countries, using open, FAIR datasets (Chapter 6. Managing data:storing, sharing and archiving). Its dynamic nature also facilitates continuous updating and refinement as new information becomes available.

Overall, the Bat Roost Priority Index represents a practical and adaptable tool for standardising roost assessments at large spatial scales such as Europe. Its multicriteria structure makes it particularly suitable for harmonising conservation priorities across administrative boundaries, supporting collaborative management, and informing evidence-based conservation policies for bat populations across regions and countries.

For a comprehensive understanding of the calculation methodology for this index, we recommend referring to the study conducted by López-Bosch et al. (2025).

7.4 Generalised Linear Models (GLM)

<u>Description</u>

Generalized Linear Model (GLM) is an extension of the Linear Models that allows working with broader types of response variables (count data, binary responses, or proportions), non-normal error distributions (Poisson and Binomial) and non-constant variance (Faraway 2010).

The main type of data used to assess population trends is counts, which usually do not present symmetric distributions. Counts may present Poisson error distribution where the increase in variance is equal to the mean. But in many cases, the counts of individuals or the bat passes display overdispersion, meaning the variance changes faster than the mean. In that case, negative binomial error distribution may be more appropriate to assume (Walsh et al. 2002, Faraway 2010).

GLM models with significant p-values (<0.05) confirm population trends, with positive tendencies when the estimate is >0 or negative when the estimate is <0. Estimate values near 0 and non-significant p-value indicate stable or inconclusive trends.

<u>Type of data</u>

GLMs can be used with the following response variables:

- Count data (Individual counts).
- Activity indices (Bat passes per unit of time, proportions).
- Binary response (Presence-Absence).
- Zero-inflated count data.

As predictive variables:

- Temporal variables (years, months, days, etc.). The response variable years can be included in the model in its linear and quadratic form to differentiate between exponential and logistic population growth (Zuur et al. 2009).
- Geographically data that may affect counts should be added as a covariable in the analysis (Walsh et al. 2002).
- Other explanatory variables can be used, such as meteorological, habitat or survey data.

<u>Advantages</u>

- The GLM is a statistical approach relatively easy to interpret and understand how predictors affect the response variable.
- Non-normally distributed variables can be used.
- Missing values in datasets are allowed. Monitoring datasets usually hamper gaps, especially if surveys are done by volunteers. Although the reason for the missing values has to be considered when discussing, the GLM approach allows to work with incomplete datasets (Ter Braak et al. 1994).

<u>Limitations</u>

- GLMs assume that the observations must be independent of each other. But data with temporal or spatial correlation won't comply with the independence assumption (Roche et al. 2011). A solution to this limitation is to use bootstrapping to produce confidence limits that are unaffected by temporal correlation (Fewster et al. 2000, Roche et al. 2011). Another option is to conduct a Generalised Linear Mixed Model (GLMM; see section 7.4).
- Trend tendencies do not always follow the log linear assumption, especially when long sequences of counts over time are studied. Whenever this occurs, a generalized additive model (GAM) might be more appropriate, or the application of both approaches GLM and GAM.

<u>Case studies</u>

An example of using GLMs to study bat population trends over the years is given by Warren et al. (2002). From 1993 to 1997, *Rhinolophus hipposideros* roosts were monitored across Wales. Initially, the volunteers counted the emerging individuals four times per roost each year, but finally only two counts were done. In total 79 roosts were surveyed, but there were missing data within and between years for most of them. The sampling period was between mid-May and mid-June, before giving birth. The GLMs were conducted with Poisson errors and roost count data as response variable. As explanatory variables they used site, date, and year but they also tested other variables such as weather conditions, time of first emergence, whether a tally counter was used or not, etc. A power analysis was conducted to assess the probability that the monitoring study would detect a trend over 5 years. As a population trend result, they showed a graphic with the maximum count observed at each site within each year, with no significant evidence that roost sizes of *R. hipposideros* increased or decreased over time.

Recently, Hernández-Brito et al. (2022) has also used Generalized Linear Models to study population trends on two parakeet species from 2013 to 2021. Using data from population census and citizen science platforms they conducted negative binomial GLMs with the number of each parakeet species as response variable and years as explanatory variable. The response variable years was included in the model in its linear and quadratic form to differentiate between exponential and logistic population growth. They also checked for temporal autocorrelation in census data using the Durbin–Watson test. The population trend results presented an exponential growth rate for the rose-ringed parakeet, while the monk parakeet population fitted a logistic growth.

7.5 Generalised Linear Mixed Models (GLMM)

<u>Description</u>

Generalised Linear Mixed Model (GLMM) is a combination of two statistical approaches. From one side, the GLM that allows non-normal error distributions. From the other side, an extension of the Linear Model, the Linear Mixed Model (LMM) which allows for the possible non-independence of observations (Bolker et al. 2009, Zuur et al. 2009).

A common situation when assessing population trends is to perform observational studies that measure the same community or ecosystem through the time, which does not meet the independence assumption of the GLMs. To handle this, incorporating random effects stands out as the best option. Thus, we must include the explanatory variables that we want to test (year, temperature, etc.) as fixed factors, and the variables that define dependence relations between the observations as random factors.

As the GLMs, the GLMMs allow working with non-normal error distributions. The main type of data used to assess population trends is counts, which usually do not present symmetric distributions. Counts may present Poisson error distribution where the increase in variance is equal to the mean. But in many cases, the counts of individuals or the bat passes display overdispersion, meaning the variance changes faster than the mean. In that case, negative binomial error distribution may be more appropriate to assume (Walsh et al. 2002, Faraway 2010).

GLMM models with significant p-values (<0.05) confirm population trends, with positive tendencies when the estimate is >0 or negative when the estimate is <0. Estimate values near 0 and non-significant p-value indicate stable or inconclusive trends.

<u>Type of data</u>

GLMMs can be used with the following response variables:

- Count data (Individual counts).
- Activity indices (Bat passes per unit of time, proportions).
- Binary response (Presence-Absence).
- Zero-inflated count data.

As predictive variables (Fixed factors):

- Temporal variables (years, months, days, etc.). The response variable years can be included in the model in its linear and quadratic form to differentiate between exponential and logistic population growth (Zuur et al. 2009).
- Geographically data that may affect counts should be added as a covariable in the analysis (Walsh et al. 2002).
- Other explanatory variables can be used, such as meteorological, habitat or survey data.

As random factors:

- Any variable that defines dependence relations between the observations (locations, times, etc.).

Advantages

- Incorporate random effects to account for correlated errors, as data with temporal or spatial correlation won't comply with the independence assumption of the GLMs (Bolker et al. 2009, Zuur et al. 2009, Roche et al. 2011).
- Relatively easy to interpret and understand how predictors affect the response variable.
- Non-normally distributed variables can be used.
- Missing values in datasets are allowed. Monitoring datasets usually hamper gaps, especially if surveys are done by volunteers. Although the reason for the missing values has to be considered when discussing, the GLM approach allows to work with incomplete datasets (Ter Braak et al. 1994).

Limitations

- Trend tendencies do not always follow the log linear assumption, especially when long sequences of counts over time are studied. Whenever this occurs, a generalised additive model (GAM) might be more appropriate, or the application of both approaches GLMM and GAM.

Case studies

Printz et al. (2021) used GLMMs to study *Nyctalus noctula* population trends from bat boxes in Germany. From 2009 to 2019, a total of 131 bat boxes, placed in 14 sampling sites, were monitored between April and early May. To assess the population trend, they conducted a negative binomial GLMM, including the bat box ID as a random effect to account for dependence among surveys of the same bat box during the year. In addition, they conducted another two GLMMs, one to test the effects of biotic and abiotic parameters, using sampling site and year as random effects. And another one to test the effect of habitat type and dead wood, while bat box ID and year were set as random factors. As a population trend result, they detect a significant decline of noctule bats over the 11 years (p-value < 0.001).

Another example of using GLMMs to assess population trends is given by Hernando et al. (2022) who studied alpine bird communities in the Iberian Peninsula. The surveys were carried out in a total of 10 random grids 0.2 km square placed along an elevational gradient. From 2008 to 2017, the sampling was conducted during the nesting period, with two sampling times per year the first three years, and one sampling time per year the last two. Poisson GLMMs were done to evaluate the trends of each bird species during the five years considering the elevational gradient. Bird abundance was fitted as response variable, while year, sampling date and elevation were fitted as explanatory variables, and the grid ID as random factor. In total, trends of six bird species were assessed, the two alpine specialist species had a negative trend in their abundance at the highest elevations, while stable and positive at lower elevations. The two mountain specialists presented a stable trend. While the two generalist species showed positive trends, more pronounced at lower elevations. Nevertheless, they also tested the effect of weather conditions in those bird populations by using a structural equation modelling approach.

7.6 Generalised Additive Models (GAM)

Description

Generalised Additive Model (GAM) is an extension of the GLMs, which rather than assuming a linear population trajectory, allows modelling a non-linear trend (Zuur et al. 2009). As a generalised model, it works with a broad variety of response variables (count data, binary responses, or proportions), non-normal error distributions (Poisson and Binomial) and non-constant variance (Faraway 2010).

To allow for non-linear relationships between the response and the explanatory variables, GAMs use smoothing curves. The degrees of freedom need to be specified to control how smooth the curve will be. These range from 1 (simple linear trend, like a GLM) to one less than the number of years ('saturated model', a sum of lines between each pair of consecutive years) (Zuur et al. 2009). Fewster et al. (2000) suggested a value 0.3 times the number of years used to produce trend curves. Nevertheless, it is always good to plot GAMs with different degrees of freedom to set the final value according to the data and objectives of the project. Only continuous co-variables can be smoothed, never categorical.

The main type of data used to assess population trends is counts, which usually do not present symmetric distributions. Counts may present Poisson error distribution where the increase in variance is equal to the mean. But in many cases, the counts of individuals or the bat passes (when activity indices is used) display overdispersion, meaning the variance changes faster than the mean. In that case, negative binomial error distribution may be more appropriate to assume (Walsh et al. 2002, Faraway 2010). Monitoring based on static detections, such as remote acoustic stations or waterway surveys, often accounts for multiple encounters of the same bat. To deal with this issue some authors prefer to work with presence-absence data for which a binomial distribution is required (Aughney et al. 2018).

The additive models test whether there is an effect of the predictive variable to the response variable. But unlike other models like GLM, it is not possible to interpret the magnitude of the population trend by the estimate sign, as they do not follow a linear trend. Graphs must therefore be represented graphically to explain the results.

Type of data

GLMs can be used with the following response variables:

- Count data (Individual counts).
- Activity indices (Bat passes per unit of time, proportions).
- Binary response (Presence-Absence).
- Zero-inflated count data.

As predictive variables:

- Temporal variables (years, months, days, etc.). The response variable years can be included in the model in its linear and quadratic form to differentiate between exponential and logistic population growth (Zuur et al. 2009).

- Geographically data that may affect counts should be added as a covariable in the analysis (Walsh et al. 2002).
- Other explanatory variables can be used, such as meteorological, habitat or survey data.

<u>Advantages</u>

- Non-linear functions allowed. GAMs can have greater predictive power when temporal and spatial effects are tested (Potts et al. 2018).
- The smoothed curves are robust against random variation between years (Battersby 2010, Fewster et al. 2000). Nevertheless, annual fluctuations and extreme outliers have more influence at the beginning and end of the time series. Therefore, it is suggested that the estimated trend for the earlier and later years remain provisional, represented with a dotted line, for example.
- Non-normally distributed variables can be used.
- Missing values in datasets are allowed. Monitoring datasets usually hamper gaps, especially if surveys are done by volunteers. Although the reason for the missing values has to be considered when discussing, the GAM approach allows to work with incomplete datasets (Ter Braak et al. 1994).

<u>Limitations</u>

- As previously explained, GAMs are not able to give a general trend on their own. To deal with this, different approaches has been used assuming a lineal trend: some authors complement the GAM with a GLM (Roche et al. 2011); others follow Fewster et al. (2000) method, who got the percentage of change by measuring the difference between the later year and the earlier year, divided by the earlier year; and others detect significant differences when the confidence intervals between the later and the earlier year (generated from the bootstrap) do not overlap (Barlow et al. 2015).
- GAMs assume that the observations must be independent of each other. But data with temporal or spatial correlation won't comply with the independence assumption (Roche et al. 2011). A solution to this limitation is to use bootstrapping to produce confidence limits that are unaffected by temporal correlation (Fewster et al. 2000, Roche et al. 2011). Another option is to include a random factor with Mixed Models.

<u>Case studies</u>

The reference study of bat population trends using GAMs is given by the National Bat Monitoring Programme in Great Britain. For many years they have followed the same protocol adapted from Fewster et al. (2000). Barlow et al. (2015) published the citizen science data collected from 1997 to 2012 in 3272 sites, for 10 bat species or species groups with four standardised methodologies. Poisson GAMs were conducted with degrees of freedom set as 0.3 times the number of years of survey data, and bootstrapping was used to produce confidence limits. Trends were significant when the confidence intervals from the baseline year and the last one did not overlap. As a response variable they used population index using the year 1999 as baseline (index = 100). For roost counts and hibernation surveys, relative abundance was used to produce the indices. Nevertheless, to avoid the associated problem of over-dispersion for bat activity, binomial models were used to analyse the trends from field and waterway surveys (proportion of point counts or transect sections in each survey where the species was observed). GLMMs were also used to detect variables that might influence the population trends. In consequence, GAMs from field surveys were done with microphone type and sensitivity range as covariates. The analysis revealed significant trend increase for 6 species from at least one method, although sometimes the trend directions from different surveys did not agree. One species (*P. pygmaeus*) showed a significant decrease from the roost counts but it was stable from the field survey. For the rest of the species there were no changes.

GAM models have been used in different bat population analysis, like the car-based monitoring programme carried out in Ireland (Roche et al. 2011). In that case, they used data from 7 years recorded from 15 transects per 30 km^2 square, a total of 28 squares. A Poisson GLM was conducted per each bat species (3 in total) with bat activity as response variable and years (from 2003 to 2009) as explanatory variable, although they also tested meteorological data. To account for the independence assumption, they used bootstrapping at the site level to produce confidence limits with no temporal correlation. GLMs were together analysed with GAMs for estimating smoothed trends. Using also 0.3 times the number of years to define the degrees of freedom. A power analysis was also conducted to assess the ability of the survey to detect Red or Amber Alert declines. Although the results must be interpreted carefully, they presented GLM and GAM graphics showing initially an increase, but a decrease after 2007 for common pipistrelle and the Leisler's bat. While the soprano pipistrelle presented a quite stable or a slightly increasing trend. From the power analysis they observed that red alert declines can be detected within 8 to 12 years for the three species if the survey is done in 25 squares twice a year.

7.7 TRends & Indices for Monitoring data (TRIM)

Description

TRends and Indices for Monitoring data (TRIM, Pannekoek et al. 2005) is a statistical method specifically proposed for the analysis of species populations over time. The method uses monitoring data to assess annual indices and trends, but also changes between years and the impact of covariates on these trends.

TRIM is based on a generalised linear model with Poisson error distribution, designed to correct possible overdispersion and serial correlation, and to account for missing data. When using TRIM several models can be implemented to include overdispersion and/or serial correlation (Van Strien et al. 2004, Pannekoek et al. 2005). The method can be computed through the TRIM software (Pannekoek et al. 2005) or the package “Rtrim” v 2.1.1 within R software (Bogaart et al. 2020).

As a result of the TRIM method, a population index is calculated based on count data, and represents the proportion of change between years and its associated standard error, calculated from the species abundances. The first monitoring year is considered the baseline year and assigned a value (often, index = 100), the next years are calculated from this referent value. The slope of the generated function is the trend of the population, and TRIM categorises the trends depending on the slope and its confidence interval (Soldaat et al. 2007, Van der Meij et al. 2015).

- Strong increase: significant increase > 5% per year (slope ± 1.96 * SE > 1.05).
- Moderate increase: significant increase < 5% per year (1 < slope - 1.96 * SE < 1.05).
- Stable: no significant increase or decrease < 5% per year (slope - 1.96 * SE > 0.95 and slope + 1.96 * SE < 1.05).
- Uncertain: no significant increase or decrease > 5% per year (all others).
- Moderate decrease: significant decrease < 5% per year (0.95 < slope + 1.96 * SE < 1.00).
- Strong decrease: significant decrease > 5% per year (slope ± 1.96 * SE < 0.95).

Type of data

TRIM can be implemented with the following response variable:

- Count data.

As predictive variables:

- Temporal variables (years).
- Other explanatory variables that could influence (covariates), such as meteorological conditions, habitat or survey date.

Advantages

- TRIM is a user-friendly statistical approach, already developed to easily process the monitoring data from a wide range of taxa and interpret the results.
- Standardised framework, the output results are comparable and can be combined between trends from other studies of even different taxa, habitat types, biogeographical regions, etc.
- It allows large monitoring datasets. TRIM is well-suited for long-term monitoring within several sites, as it allows up to 2000 sites, 80 years and 10 covariates (Van Strien et al. 2004).
- Missing values in datasets are allowed. Monitoring datasets usually hamper gaps, especially if surveys are done by volunteers. TRIM estimates the missing counts from a specific site from changes in other sites, to complete the data set with the predicted counts (Pannekoek et al. 2005).
- TRIM allows the use of weights to mitigate the potential biases of oversampling and undersampling. This is a common problem in monitoring programs as some areas are more sampled than others, leading to some regions being overrepresented while others underrepresented (Pannekoek et al. 2005, Van der Meij et al. 2015).
- It takes overdispersion into account. TRIM is based on a Poisson distribution, where the increase in variance is equal to the mean. But in many cases, the counts of individuals display overdispersion, meaning the variance changes faster than the mean. To deal with this, TRIM uses statistical procedures for estimation and testing (Pannekoek et al. 2005).
- It takes serial correlation into account. When monitoring, serial correlation is common as counts from a specific year will depend on the counts from the year before. Thus, TRIM uses statistical procedures for estimation and testing (Pannekoek et al. 2005).
- Effects of different covariates on the trends and indices can be included, to investigate the potential drivers of the population changes.
- There is a standard method to categorise the trends as strong increase, moderate increase, stable, uncertain, moderate decrease and strong decrease.

<u>Limitations</u>

- TRIM does not accept replicates, only allows for one survey per year per site. In many monitoring programs, multiple surveys are conducted at the same location to monitor throughout the whole year. But TRIM restricts the use of the complete dataset to a single count.

<u>Case studies</u>

A notable case study of the TRIM application can be found in the study conducted by Machado et al. (2017). The work aimed to establish the population trends of several bat species and evaluate the effectiveness of conservation activities conducted to protect their roosts. A total of 34 roosts (14 unprotected, 20 protected by fences) were monitored between May and July 1997 to 2014 in the Eastern Peninsula. Once a year the census was done at sunset using an infrared camera and an acoustic detector placed at the entrance of the roosts. The authors used TRIM program to analyse the population trends of the whole period (1997-2014) and the recent years (2003-2014), with a log-linear Poisson regression to estimate the missing values. In that case, they did not calculate the population index, they represented the trend just with the number of individual counts during the emergence. As population trend result, *Rhinolophus euryale* showed a moderate decrease in both periods. *R. ferrumequinum*, *Myotis myotis/blythii* and *Miniopterus schreibersii* presented uncertain trends for the whole period but were stable for the last few years. *R. hipposideros* was stable for the full period but during the last years presented a moderate decrease. Overall *R. mehelyi* presented a moderate decrease trend, but for the recent years the trend is uncertain. *Myotis capaccinii* and *M. escalerai* presented uncertain trends for both periods studied. *M. emarginatus* presented an uncertain trend but showed a strong decrease for the recent years. The study also detected positive trends in the protected roosts and most of the unprotected roosts showed negative trends.

With monitoring data across nine European countries, Van der Meij et al. (2015) used TRIM to assess a pan-European bat indicator, such as Van Strien et al. (2001) developed for birds. To do so, the authors compiled available data of 16 bat species and two cryptic groups from underground hibernacula for 19 years. The steps followed to develop the pan-European indicator were, first, to calculate the trends of all the species at national scale. Second, compute the regional trends combining the national trends for bio-geographical regions. Finally, combine the regional trends into a pan-European trend generating a final global bat indicator from the geometric means of the European trends of single species. All missing values were imputed by TRIM, and weighting factors were used to reflect the differences in population sizes between countries. The results for individual species at national scale showed an increase in many species for more than one country. At regional/biogeographic scale almost all species presented an increased trend in the Atlantic region, and none were declining, while in the Continental region six species were increasing and one (*Plecotus austriacus*) showed a decline trend. When all trends were combined at European level, nine species presented a significant increase and only *P. austriacus* had a moderate decline. Finally, the European bat indicator combined the indices for all 16 species from 1993 to 2011 showing a positive trend for bats at hibernation roosts.

8. Bat Monitoring Programmes in Europe

All national and regional monitoring schemes currently being carried out and implemented across Europe are available in a related online dataset, *"Bat monitoring programmes and protocols for European bats"*, published by EUROBATS and accessible via the European Zenodo Repository (López-Baucells et al. 2025). This dataset compiles and summarises all available information (including general settings, schedules and other particular specificities) on bat monitoring protocols and programmes being implemented across the EUROBATS Parties and Range States in order to make this information publicly accessible and dynamically updatable.

Beyond its descriptive value, this dataset represents a key tool for harmonizing monitoring approaches across Europe. It facilitates the comparison and alignment of protocols, particularly among neighbouring countries that share ecological regions and bat populations, and provides a foundation for standardization where monitoring efforts are currently limited or absent. In doing so, it supports greater coordination, knowledge exchange, and the development of consistent, large-scale assessments of bat population trends across borders.

9 References

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