Evaluation of different monitoring methods at maternity roosts of greater mouse-eared bats (*Myotis myotis*)

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Abstract

In recent years, biodiversity has declined faster than ever before in human history. Appropriate monitoring methods are needed to detect the decline of populations in time to be able to take conservation measures. Information on population dynamics can only be obtained by conducting standardised monitoring programmes, and the quality of the data depends on the survey method used. The present study aims to provide scientifically sound recommendations for the selection of a suitable survey method for emerging flights at greater mouse-eared bat (*Myotis myotis*) maternity roosts. For this purpose, three survey methods, namely infrared video recordings, counting by light barriers and visual counting of emerging bats, were used simultaneously for 4–5 nights at three different maternity roosts in 2019 and comparatively evaluated. Besides the quality of the counting data, the requirements and limitations of the different methods were compared and discussed. The results of this study showed that the number of emerging flights detected with all three survey methods were closely correlated, regardless of location, number of emerging flights per night (between 300 and 800) and season. Furthermore, it was shown that the presence of the observer and infrared video recordings had no significant influence on the emergence behaviour of the bats recorded by light barriers concerning the time of emergence, the duration of activity and the number of counts. As the three methods differed with regard to additional parameters, such as the need for technical equipment or qualified personnel, the time required, the costs and the error-proneness individual settings and requirements should be taken into account when deciding about the method used. However, for the continuous monitoring of greater mouse-eared bats at their maternity roosts, which is part of the monitoring requirements under the European Union Habitats Directive, the use of light barriers proved to be the method of choice, in particular as it allows to promptly detect changes in the bats' activity as a basis for conservation measures.

Keywords Light barrier · FFH-directive · Infrared video recording · Monitoring

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Introduction

Biodiversity has declined faster in recent years than ever before in human history. These massive species extinction has reached global proportions and can be attributed to factors such as habitat destruction, overexploitation and degradation, land-use change, invasive species and climate change (Barboza and Franz 2016; Kehoe et al. 2020; BMU 2018; IPBES 2019). To counteract this dramatic decline, national strategies for the conservation and sustainable use of biodiversity have been developed and translated into legislation. In 1992, the European Union (EU) adopted the EU Habitats Directive to conserve biodiversity (Directive 92/43/EEC). The Directive aims to safeguard and protect wild species of flora, fauna, their habitats as well as the connectivity of these habitats in the EU. With the adoption of the Habitats Directive, the EU Member States have committed themselves to monitor the conservation status of the habitat types and species covered by the Directive. This results in the need for Europe-wide monitoring (BfN 2017). The species affected by the Directive are highly endangered species. To protect these species, the Directive aims to designate protected areas on the one hand and to protect individuals of these species and their habitats on the other hand. A fundamental prerequisite for achieving this goal is to improve our knowledge about the status and development of biodiversity. To create basic information experts of species are needed to collect data on the status of species and habitats as part of standardised monitoring programmes as well as on potential stressors and conservation measures, over extended periods of time and by using standardised methods (BMU 2018; Hausmann et al. 2020; Beresford et al. 2020). Such monitoring studies can document species declines at an early stage (IPBES 2019) and thus provide a basis for implementing conservation measures (Gellermann 2001). The insect decline identified by a long-term study and which is very likely caused by human activities, might have taken a different course if corresponding monitoring studies had been conducted earlier and on a large geographical scale (Hallmann et al. 2017; Settele 2019).

The knowledge of strengths and weaknesses of relevant survey methods and the ability to assess the suitability of each method for the particular monitoring effort and setting are necessary to select the most appropriate method and subsequently evaluate the results (Schneider and Hammer 2007). To make the results of monitoring studies comparable the same study design and survey method has to be applied.

From the mid-1950s onwards, several European bat species experienced massive population declines, leading to local extinctions in the mid-1970s and, in the case of horseshoe bats, large-scale extinction (Dietz and Kiefer 2014). This period of decline can be attributed to changes in bat habitats, the use of pesticides, the destruction or disturbance of roosts and other factors (Dietz and Kiefer 2014). However, recent studies show that European hibernation populations appear to be stabilising and recovering after a period of sharp declines in the twentieth century (Van der Meij et al. 2015). To support this positive, international trend, it is essential to maintain bat conservation measures.

One of the species protected by the EU Habitats Directive, which must therefore be recorded through regular population monitoring, is the greater mouse-eared bat (*Myotis myotis*). Expert committees have developed recommendations for minimum requirements for the species and reference lists to record causes of endangerment and to identify needs and develop conservation and development measures to ensure the comparability of the data collected and the assessment of the respective conservation status (LUBW 2021b). The greater mouse-eared bat has a secondary synanthropic distribution and is classified as a species inhabiting buildings. It is therefore particularly at risk from

building renovations, habitat fragmentation due to road construction and thus increased collision risk, and as an insectivorous species also from insect decline and the accumulation of toxins in the environment along the trophic gradient (Dietz and Kiefer 2014). Germany has a special responsibility for this species (Meschede 2012), as a significant part of the greater mouse-eared bats' range and the main part of its total population are located in Germany.

The greater mouse-eared bat is easy to observe and well-suited for monitoring studies in Germany due to its fidelity to roosting sites and its preference for buildings as maternity roosts (Biedermann et al. 2003). Against this background, the first ideas for nationwide monitoring of greater mouse-eared bats were developed by a committee of experts as early as 1996. In 2002, a first standard programme for the monitoring of greater mouse-eared bat and the technical criteria to be applied were agreed upon. The test phase was to last two years and was carried out by volunteer observers. This first monitoring programme foresaw that colonies would be counted twice per activity period. The first count was to be carried out between mid-May and mid-June to record the number of adults. The second count was to take place between the end of June and mid-July, or 14 days after the birth of the young, to determine the proportion of offspring. Various methods have been used in the past to collect this data: emerging flight counts, where the person is outside the roost and observes the exit opening directly, counts in the roost (on the sleeping berth) are carried out during the day when the bats are present, for video recordings (infrared camera) the flight of the bats is filmed with the help of infrared spotlights and cameras and then counted thereafter on the screen, for the photo documentation (counting based on photos) photos are taken during the day of the hanging groups of bats in the roosts and which are then counted on the screen. For the count with the help of light barriers, these are mounted in the hatch opening, whereby each entry or exit of the bats is counted by interrupting the light barrier beams (Biedermann and Geiger 2005; Biedermann et al. 2003; Meschede 2012; Meyer and Biedermann 2003).

The data obtained with these methods are used for the nationwide monitoring of the greater mouse-eared bat in accordance with the EU Habitats Directive. With the framework of this monitoring, random surveys of the species are carried out in the Continental biogeographical region (cf. EU Habitats Directive). A full population survey of the greater mouse-eared bats is carried out exclusively in the Atlantic biogeographical region (Sachtel-eben et al. 2010). The sampling unit for monitoring under the Habitats Directive is the maternity roost. To estimate the population size of the species, three surveys per reporting year are currently required every 6 years, regardless of the method used. The count should include both adult females and, where possible, juveniles (BfN 2017; Sachtel-eben et al. 2010). The number of juveniles can so far only be determined by nightly counts within roosting sites, a method associated with the heavy disturbance of the maternity colonies. Emerging flight observations provide only approximate numbers of juveniles as these estimates are based on observed activity changes (Meschede 2012).

In Germany, the federal states are responsible for monitoring surveys. This leads to heterogeneity in practical implementation, which can be seen, for example, in the fact that the assessment of the conservation status of species is based on expert estimates instead of standardised counts, in contrast to the national standard. Also, resource and methodological limitations in the number of samples are accepted in individual countries, which leads to heterogeneity between the respective country-specific monitoring programmes (LAU 2021; LfULG 2021; LUBW 2021a). The results are therefore of varying quality and not comparable.

The present study aims to compare the data quality (consistency in the number of counted emerging flights) of the methods currently used for bat monitoring and, in particular, for the monitoring of greater mouse-eared bats. These methods included infrared video recordings, counting by light barriers and visual counting of emerging bats. In addition, we wanted to assess the disturbance to bats caused by the presence of a person during visual counts and infrared video recording. This study therefore recorded activity at three maternity roosts in different seasons using the three different monitoring methods simultaneously. It is generally believed that the video counts provide the most accurate results (Revilla-Martín et al. 2020). Therefore, counts obtained by video recording were used as a reference in the present study (Revilla-Martín et al. 2020). The methods used differ in terms of time required, technical equipment needed and applicability in relation to the conditions prevailing at the roosts studied. Therefore, another aim of this study was to evaluate the advantages and disadvantages of the different methods and to give recommendations for the choice of a suitable recording method under the given conditions. We hypothesized that the number of counts recorded by the light barrier should correlate highly with the numbers recorded by video recordings, while we assumed that the visual counts would have a higher variability of counts, especially in larger colonies. Furthermore, we assumed that the presence of a person during the visual count and the infrared video recording would influence the timing of emergence flights, their duration and the level of flight activity due to human disturbance (Ancillotto et al. 2019).

Methods

The greater mouse-eared bat (*Myotis myotis*)

The greater mouse-eared bat is the largest *Myotis* bat species in Europe (body mass 26–45 g; Braun and Dieterlen 2003). The usually very large maternity roosts in Central Europe with up to 1000 (rarely up to 5000) breeding females (Dietz and Kiefer 2014) are often located in undisturbed, draught-free, medium-sized to large roof spaces of old buildings (e. g. churches, historic buildings etc.) (BfN 2020). Similar to the original natural cave roosts, these roof spaces are relatively unstructured, but contain crevices into which the animals can retreat (Vogel 1988). Both building-dwelling and cave-dwelling greater mouse-eared bats are threatened by disturbance from renovations or cave tourists. The greater mouse-eared bat shows high fidelity to its maternity roost (Dietz et al. 2016; Simon and Boye 2004). Females use these roosts to give birth and raise their young. As a rule, they outnumber the males, although single males can also be found in individual, separate hiding places (Braun and Dieterlen 2003).

Greater mouse-eared bats usually give birth to only one young per year. The young are usually born between the end of May and the beginning of July, and from the beginning of August the maternity colonies gradually disperse (Braun and Dieterlen 2003). The mating season begins in early August at the mating sites. From October onwards, the animals retreat to their winter roosts. Hibernation lasts from September/October to March/April and hibernacula are usually located within caves, adits, bunkers and also rock crevices (Braun and Dieterlen 2003; Dietz and Kiefer 2014).

The greater mouse-eared bat usually leaves its day roost only in late twilight, about 30 min after astronomical sunset. Observations have shown that the rate of emerging flights

at a maternity roost rises rapidly at the beginning, peaks and then drops again. Approximately one to three hours before sunrise bats return to the roost, with a much longer period of entry activity due to individual return compared to exit activity (Braun and Dieterlen 2003). Even lactating females return to the maternity roost during the night to feed their young (Braun and Dieterlen 2003). In unfavourable weather conditions, bats may not return to the maternity roost in the morning, but spend the day in tree cavities outside the roost (Petersen et al. 2004).

Roost sites

The studies of the survey methods were carried out on three maternity roosts of the greater mouse-eared bat in Ettenheim, Schweighausen and Seglohe (Table 1). Ettenheim is located in the western part of Baden-Württemberg in the Rhine plain. Schweighausen is located 15 km east of Ettenheim in the Schutter valley in Upper Black Forest. Seglohe is located in the west of Bavaria, close to the border with Baden-Württemberg on the northern edge of the Nördlinger Ries. The maternity colonies are located in an old industrial cellar in Ettenheim, in an attic of a vicarage in Schweighausen and also in an attic of the local church in Seglohe. The exit opening of the roost in Seglohe is very small, so that the bats usually fly out individually. The exit in Ettenheim is somewhat larger than in Seglohe, so several bats can fly in and out at the same time. However, the bats here have to fly a tight curve directly after flying out to reach the free airspace. The roost in Schweighausen has two exit openings on opposite sides of the roof. The investigations here were limited to the exit that shows the greater activity.

Survey methods

Given the endangerment status and negative population trends of several bat species and their status as strictly protected species (Directive 92/43/EEC), the selection of appropriate survey methods for monitoring studies must focus on the least invasive methods. The application of this principle led to the selection of the three survey methods used, as all three allow monitoring to be carried out without entering the roost when the animals are present, thus avoiding potential disturbance.

The bats were recorded synchronously using three methods when they left their roost in the evening. The methods were (1) infrared video recordings, (2) counting by light barriers and (3) visual counting of emerging bats. A prerequisite for the application of all three

Table 1 Overview of the parameters of the individual roost sites (easting, northing, altitude, natural landscape unit, size of the exit opening)

Site parameter	Ettenheim	Schweighausen	Seglohe
Easting	7° 49' 06.60" E	7° 57' 53.1" E	10° 29' 21.87" E
Northing	48° 15' 16" N	48° 13' 08.45" N	48° 59' 39.85" N
Altitude a.s.l	177 m	415 m	468 m
Natural landscape unit (Meynen et al. 1953–62)	Central Upper Rhine Plain (Lahr-Emmendingen foothills)	Black Forest (central Black Forest)	Franconian Keuper-Lias Plains (foreland of the southern Franconian Jura)
Size of the exit opening	Approx. 70 × 25 cm	Approx. 60 cm Ø	Approx. 40 × 40 cm

methods is knowledge of the roosts' exit opening. Neither for the infrared video recordings nor the visual count of the emerging bats do the surveyors have to enter the maternity roosts. The light barriers are installed before the roosts are colonized by the bats in spring, so that there is no disturbance of the animals. Since some bat roosts have already been monitored by light barrier surveys for more than 15 years and the animals have not left these roosts [e. g. Kalkberg Cave Bad Segeberg (Kugelschafter and Lüdgers 1993)], no adverse effects on the bats are to be expected from the light barriers (Elliott et al. 2005).

The surveys were conducted between April and August 2019 during the maternity period. The evening departure of the bats from the roost was recorded, as this takes place in a relatively short time window. In contrast, the morning return flight or a possible nocturnal return to the maternity roost is more protracted and sporadic, making it difficult to accurately record the number of entries. If no further bats had left the roost for at least ten minutes after the evening emerging flight event, counts were suspended. No data are available for a visual count of emerging bats on 24 April 2019 at the roost in Ettenheim, as no emerging observation was planned at that time. Another data gap exists on 17 June 2019, as the light barrier in Seglohe failed that evening due to dirty diodes.

Infrared video recordings (IVR)

To capture the evening emergence of the bats with IVR, infrared emitters and an infrared video camera were used, as bats cannot see infrared light. The emitters were built by ChiroTEC and included two HSDL 4220 IR emitters with 30° diodes and one HSDL 4230 IR emitter with 17° diodes (880 nm, 8 watts with diffusing lens at close range). A Canon XA20 camera was used for IVR. Both the infrared video camera and the emitters were battery-powered.

For the surveys at the roosts, the infrared video camera and the IR emitters were aimed at a larger area (image width of approx. 4–10 m) around the exit opening from a distance of approximately 5–15 m. A larger image section facilitates later counting from the infrared video images. IVR generates comparatively large amounts of data and therefore requires sufficient storage capacity. Approximately 4 GB storage capacity is required to record an entire exit event. The counting of the evening emerging flights of the greater mouse-eared bats from each roost was always carried out by the same person working on a screen in a laboratory. For validation purposes, randomly selected infrared video segments were counted by a second person and the number of counts was compared. The infrared videos were played at half speed or even slower if necessary, and the emerging flights of individual bats were counted over the entire length of the recording. For the period covered by the IVR during the evening emerging flights of the bats, all emerging and entering animals were recorded and counted.

Light barriers (LB)

LB is a permanent, fixed detection technique that is used during the entire activity phase of the bats. They are installed so that they cover the entire exit opening and thus all animals can get through. A control unit known as 'tricorder' supplies power to the sensors (Tricorder 9008E by ChiroTEC), with the power source being either 12 V car batteries or a permanent power supply, depending on the infrastructure available at the roost. The tricorder also evaluates the interruptions signalled by the LB. The data are analysed with the help of a computer and the software ChiroSoft (Kugelschafter 1998).

LB technology is based on two anti-parallel rows of single beams that are connected to form a double row of beams. When a bat passes through, these beams are interrupted. The sequence of interruptions provides information about the bat's direction of flight. In order for a bat's flight to be registered as an entry or an exit, the bat must cover both light-beam curtains at the same time when it passes and the order of the interruptions must be correct (Kugelschafter 2004).

Visual counting of emerging bats (VCE)

VCE was conducted with binocular night vision goggles (Safran Vectronix Big25) when required. Depending on the visibility of the exit opening, the activity of the bats and the light conditions during the emerging event, it was also possible to carry out the counts without night vision goggles. The emerging bats were recorded with mechanical counters. On each of the survey nights, the counts were carried out by the same person (LM), with a second person carrying out parallel sample counts for validation.

To be able to compare the methods and their results, each of the three maternity colonies was surveyed on five nights with all three methods simultaneously (Suppl. Table 1). The five survey nights in the maternity colonies were distributed over the activity phase of the bats between March and October. Thus, survey results are available for a total of fifteen nights, during which emerging events of the bats were recorded synchronously using three different methods. In order to be able to record changes in the activity of a maternity roost, it is postulated that at least two survey sessions should be carried out per year to enable comparisons between years (Meschede 2012).

To investigate the disturbance level of the VCE and the IVR on emergence behaviour and bat activity, counting by LB at the three maternity colonies continued throughout the 2019 activity period (Suppl. Table 1).

Statistical analyses

For the comparison of the survey results obtained with the three methods described above, only the registered evening emerging flights were considered. Potential evening return entries were not considered.

We used R 4.0.1 (R Development Core Team 2020) and the package lme4 (Bates et al. 2014) to conduct linear mixed effects analyses. To obtain p-values from the mixed effects models, we used the package lmerTest (Kuznetsova et al. 2015), which uses the Satterthwaite approximation to determine degrees of freedom. The Shapiro–Wilk test was used to check the normal distribution of the residuals of the models, and homogeneity of variance was ensured by visual inspection of the residuals plotted against fitted values. In the case of multiple comparisons, a Tukey's post-hoc test was used (R package "multcomp"; Hothorn et al. 2008). The significance level was set at $p=0.05$.

To analyse the potential influence of the survey method used on the number of counts, we ran a linear mixed effects model where the number of counts as the dependent variable and the survey method used was the factor (3 levels: IVR, LB and VCE), including the survey night nested in the colony as random effects.

Regression analyses were also conducted to assess possible quantitative relationships between the number of counts obtained with IVR as the dependent variable and the counts obtained with LB or VCE as the explanatory variable. We assumed that number

of counts detected by the different methods should be linearly correlated. The emerging events captured by IVR were chosen as the benchmark for the other two methods, as it was assumed that these counts would be the most accurate and that the data analysis could be reproduced as often as desired and that the results would be verifiable, as the playback could be slowed down and it would be possible to view individual sequences repeatedly (Revilla-Martín et al. 2020).

To assess the methods IVR or VCE are associated with disturbance for the bats, we compared the number of emerging flights and according to Ancillotto et al. (2019) also emergence timing and the duration of emergence flights between the observation night and the respective nights before and after using the light barrier data as reference. We used a paired T-test if data were normally distributed and a paired U-test if not. The onset of the emerging flights was based on the emergence time of the first bat on each evening, and the duration was determined using the elapsed time between the first and the last bat leaving the roost on each night. The last flight was defined as the flight after which no other bat left the roost for 10 min. However, there were nights when the end of the emergence could not be clearly defined because the bats regularly flew in and out of the roost (e. g. nights with rain). In these cases, the emergence flight was defined as the last emergence flight after which no further bats flew out for the longest time.

Additional parameters

As a decision-making aid for the selection of a suitable survey method, the methods used were compared not only based on the quality of the survey data, but also taking other parameters into account, e. g. to show the method-specific cost–benefit ratio. The parameters used were assigned to different categories (definitions see below), while the personnel time was calculated in hours for three survey rounds per year.

For this purpose, the following variables were considered:

Availability of technical equipment

Yes: The technical equipment is commercially available without its own contribution.

Partly: Not all components required for the specific application are commercially available; minor own contribution is required.

No: The majority of the required technical components are not commercially available; major own contribution is required.

Personnel time

The three classification levels for personnel time are based on three rounds of surveys per year and the number of hours required in each case. For this purpose, personnel time was categorized into three classes: high, medium and low time expenditure.

Costs

The three classification levels for costs are based on the total costs for equipment and personnel. For this purpose, costs have been categorized into three classes: high, average and low cost.

Requirement of sufficiently qualified personnel

A low, medium or high level of biological/bat-specific or technical expertise is required to apply the survey method.

Identification of changes in activity

Changes not detectable: The use of snapshots does not allow identification of changes in activity at the maternity roost.

Changes only partially detectable: The use of a very high number of snapshots may allow the identification of changes in activity at the maternity roost.

Immediately detectable changes: The use of long-term recordings using automated methods allow the identification of changes in activity at the maternity roost.

Error-proneness

The qualitative assessment of error-proneness was carried out based on many years of experience in the application of the individual survey methods and the evaluation of existing literature.

The parameter ‘error-proneness’ is represented by a combination of the following sub-parameters: Dependence on weather conditions, dependence on the visibility of the exit opening, technical susceptibility to error, human counting error and reproducibility.

Due to weather conditions When evaluating and classifying the parameter “dependence on weather conditions”, a distinction was made for the three different methods used as to whether the application of the respective method is dependent on the prevailing weather conditions or not. In the case of precipitation or low ambient temperature, the activity of the bats may be lower, so that no statements can be made about abundance. By using continuous data collection short term changes in activity due to adverse weather conditions can be identified and data omitted. In addition, in unfavourable weather conditions, visibility may be limited and restrict the application of the methods carried out.

Due to the visibility of the exit opening For the evaluation of the parameter “dependence on the visibility of the exit opening” and the classification of the methods concerning this parameter, a distinction was made whether a person has to be present near the exit opening and be able to see it or not, so that the emerging flights of the bats can be recorded.

Technical susceptibility to errors Since it was not possible to quantitatively measure the technical error susceptibility of the processes used, empirical values were used for this purpose. In particular, the complexity of the technology and the system were taken into account.

Human counting errors For the assessment of the parameter human counting error, the possibility or probability of human errors in the counting of the resulting flights was assessed for the respective recording methods. A distinction was made as to whether the possibility of human counting errors exists or not. Additionally, it was considered whether the probability of human counting errors can be reduced or avoided by technical aspects, such as slow or repeated playback of video recordings.

Reproducibility of data Here a distinction was whether the reproducibility of the data of a recording method is possible, conditionally possible or not possible. However, it must be taken into account that the recording of the flight of the bats of one night is not reproducible, but only the evaluation of the recordings.

The primary reason for this approach is the fact that the reasons why errors can occur in the application of the respective recording method are different. For example, there may be a higher error-proneness in the case of a maternity roost due to the lack of visibility of the exit opening, while the reproducibility of the data is of decisive importance in another survey. In the following, therefore, only an overview of the possible sources of error of the individual survey methods will be given in order to take them into account when choosing the appropriate method.

Results

General data characteristics

The counts obtained with all three survey methods varied between roosts and survey evenings (Suppl. Table 2; Fig. 1). The highest activity and thus the highest number of individuals were recorded at the Seglohe roost with over 700 emerging flights per night, the lowest activity and number of emerging flights per night were recorded in Ettenheim with 311 flights.

The number of emerging flights recorded at the roosts also fluctuated during the year. While comparatively high numbers were counted in July, the records show fewer emerging flights towards the end of the maternity period in August (Fig. 1).

Influence of survey method

The number of emerging flights recorded per night at a specific roost and night did not vary between the different survey methods (Tukey Post Hoc Test: $p > 0.13$ for all comparisons, $n = 43$ nights).

The number of emerging flights recorded by IVR correlated closely with the number of flights recorded with the other two methods (Figs. 2 and 3). Significant deviations from the linear functions were not evident for any of the three roosts.

Linear regression analyses revealed highly significant correlations between the number of emerging flights recorded with the IVR and the LB or VCE (Fig. 2; Table 2), with more than 99% of the variance explained by the models. The number of emerging flights was not significantly influenced by the survey month or by the roost site (Tukey Post Hoc Test: $p > 0.15$ for all comparisons: months, study site).

Fig. 1 Number of emerging flights per night at each roost for the three survey methods (IVR, LB, VCE) on the 5 survey days during the 2019 active phase of the greater mouse-eared bat (*M. myotis*) in summer habitat and during the swarming period

Disturbance level due to the presence of a person and the infrared video recording

The number of emerging flights recorded with the LB during the observation nights did not differ from the numbers recorded in the nights before and after (Fig. 3, comparisons: before–during; paired U-test: $p > 0.28$; during–after: paired T-Test: $p > 0.9$, $n = 13$ observation nights).

The onset and duration of the emerging flights recorded with the LB during the observation nights also did not differ from those recorded in the nights before and after (onset of emerging flights: paired T-Test: $p > 0.4$ for all comparisons (duration of emergence: paired U-Test: $p > 0.3$ for all comparisons).

Fig. 2 Number of emerging flights per night (No. of counts) at the three different roosts on the respective survey nights. The LB data (left) and VCE data (right) are plotted against the IVR data ($n = 14$)

Fig. 3 Number of emerging flights before, during and after the observation night

Table 2 Results of the linear model explaining the variation between the different methods during the months and at the maternity roosts. Reference level of factor: IVR counts

	Estimate	SD	t value	p	Significance
Intercept	18.38	26.77	0.69	0.5	ns
Light barrier	0.95	0.06	15.83	<0.001	***
Intercept	25.18	11.57	2.18	0.06	ns
Visual counting of emerging bats	0.95	0.03	33.61	<0.001	***

*** $p < 0.001$

Table 3 Overview of results for additional parameters

Parameter	Infrared video recording	Light barrier	Visual counting of emerging bats
Availability of technical equipment	Partly	No	Yes
Personnel time (approx. hours required for 3 survey rounds per year)	20 h	20 h	5 h (excluding travel time)
Approx. costs (in €)	1800	7350 respectively 8050	7000
Requirement of sufficiently qualified personnel	Low	High	Medium
Identification of changes in activity	Only partially detectable	Immediately detectable	Only partially detectable
<i>Error-proneness</i>			
Dependence on weather conditions	Yes	No	Yes
Dependence on visibility of the exit opening	Yes	No	Yes
Technical susceptibility to errors	Medium	High	Low
Human counting errors	Possible/low	Not possible/low	Possible/high
Reproducibility of data	Yes	Partly	No

Evaluation of additional parameters

An overview of the results for the additional parameters can be found in Table 3.

Availability of technical equipment

Regarding VCE, it should be noted that suitable night vision goggles are commercially available, so there are no technical limitations (hence the rating ‘without own contribution’).

For IVR and analysis, commercially available cameras, power supplies and multimedia software can be used. In order to meet the specific requirements of the infrared emitters, it is recommended to build emitters oneself to avoid the qualitative limitations of commercially available products (low own contribution).

While the LBs are made of commercially available parts, self-build is required for their construction and assembly. Car batteries can be used as a power supply. However, the LB, the tricorder and the software required for data retrieval can only be purchased as individual components (high own contribution).

Personnel time

Personnel time includes not only the survey time, but also the time needed to evaluate the survey results.

Among the survey methods considered here, the personnel time required for the VCE is by far the lowest (excluding the travel time to the roost), as only the time for the resulting on-site count needs to be taken into account; no further evaluation is required (low time expenditure).

In order to operate a LB, it must first be individually installed at the exit opening to be counted and then regularly maintained. If no power supply is available on site, the batteries must be replaced at least every three months. If remote access to the LB is set up, this must also function reliably and be maintained. In addition, the time required to evaluate the LB data must be taken into account (average time expenditure).

In the case of IVR, the survey time on site is the same as for VCE. The subsequent evaluation of the recordings to determine the bat emerging flights is quite elaborate and time-consuming. The total time required depends on the duration of the recording, the orderly emergence of the bats from the roost and the experience of the surveyor and is estimated to be at least 4–5 h per survey night, excluding travel time.

Costs

In terms of costs, VCE only requires the purchase of night vision goggles (approx. €7000).

Recording the IVR requires the purchase of infrared emitters, a car battery as a power source and a video camera. Total costs of approx. €1800 can be expected for this equipment. Freeware can be used as software (low cost).

Counting by LB requires the purchase of the device itself, a tricorder, a power manager and the necessary software at a total cost of approx. €7350. If remote access is also to be set up, this costs an additional €700, bringing the total cost to €8050 (high costs).

Requirement of sufficiently qualified personnel

Another factor is the required technical qualification of the person performing the survey using the method in question. For example, performing IVR of emerging events requires only a low level of technical expertise and technical knowledge. Similarly, counting emerging flights in the video recordings requires only a certain level of practice, but no specific technical knowledge (low requirements).

For a VCE, observers should be particularly familiar with the bat behaviour to ensure valid recording of occurrence and identification of bats (medium requirements).

However, counting by LB requires both knowledge of bat biology and behaviour (flight behaviour and morphology, knowledge of diurnal activity of the animals) and a deeper technical understanding to analyse the data. This method has proven to be the most demanding in terms of staff qualifications (high requirements).

Identification of changes in activity

The parameter for assessing the usefulness of each survey method is the data set that can be generated. IVR and VCE can only provide snapshots of a survey night, as each survey episode requires the presence of an observer. Possible changes at the roost, such as a sharp decrease or increase in bat activity, cannot usually be recorded because the intervals between survey episodes are too long (only partially detectable).

In contrast, counting with LB involves continuous recording over several months. Due to the automation of the procedure, the observer does not need to be present during data collection. Changes at the roosting site can be easily recognised in the data records of the individual survey nights. If remote access to the LB is set up, changes can be noticed immediately and necessary measures can be taken (immediately detectable).

Error-proneness

Dependence on weather conditions: While surveys based on VCE and IVR are weather dependent (yes), continuous recording by LB allows the targeted selection of a specific emerging event during favourable weather conditions to assess bat activity (no).

Dependence on the visibility of the exit opening: While with VCE and IVR the exit opening must be visible (yes), with counts using LB the exit opening does not have to be visible from the outside (no).

Technical susceptibility to errors: The technical error-proneness of this method is classified as low due to the low technical equipment for VCE. Although various technical devices are used for IVR, they are only moderately prone to errors. In contrast, the technical susceptibility to errors of LB counting is classified as comparatively high, which is due, among other things, to the complexity of the sensor technology.

Human counting errors: As the recording of bat emerging flights with VCE is based exclusively on the observer's count and thus depends heavily on the observer's performance, the possibility of human errors in counting emerging flights is given. Especially in difficult counting conditions (high activity at the roost, poor visibility conditions, etc.), the probability of possible human counting errors is also quite high (possible/high). In contrast, the other two methods, IVR and LB, are much less prone to human counting errors. For example, in LB recording, human counting errors cannot occur in the first place, as the recording is exclusively technical and counting by humans is not necessary (not possible/

low). When recording emerging flights using IVR, there is a possibility of human counting errors when evaluating the video recordings, but this can be significantly reduced or avoided through technical aspects. For example, the video recordings can be slowed down significantly and played back as often as desired, so that the probability of counting errors by humans is significantly reduced and validation of the evaluation becomes possible (possible/low).

Reproducibility of data: While the VCE does not allow the survey to be repeated (no), the LB data can be evaluated (partly) and the emerging flights recorded by infrared video can be counted several times and are least prone to error due to this validation possibility (yes).

Discussion

Comparison of the methods based on the data collected

In order to observe trends in population dynamics, standardised monitoring studies are necessary to generate representative and meaningful data (Dietz and Simon 2013). The use of a constant study design is key to the comparability of results and thus to the identification of population declines (Dietz 2017). The choice of survey method is an important component of the study design. This study aims to provide scientifically justified recommendations for the choice of survey method to record emerging flights at greater mouse-eared bat maternity roosts.

The statistical analyses of the results of the three survey methods studied have shown that they provide comparable results and that the counts collected with LB and VCE differ only very slightly from those collected with IVR, which was chosen as benchmark for the two others, as it assumed that these counts would be the most accurate (Revilla-Martín et al. 2020). Thus, in terms of data quality, there are no significant differences between the three methods studied. Moreover, the close correlation between the records of the different methods was not influenced by the month or by the roost and colony size studied. The results are independent of colony size and it can be assumed that the methods used can be applied to maternity roosts within the size range of the colonies studied (300 to 800 emerging flights per night). As the maternity roosts of the greater mouse-eared bat in Central Europe usually consist of 50 to 1000, rarely up to 5000 females, the sizes of the colonies investigated in this study seem to be representative of this species. Similarly, we assume that the methods can be applied to smaller maternity roosts without additional restrictions. For roosts that host significantly more animals than the observed maternity roosts, further investigations on the applicability of the above-mentioned survey methods are necessary. The present comparison of methods deals exclusively with greater mouse-eared bat roosts. Therefore, the question arises to what extent the present study is transferred to other roost types or other species. The three survey methods used seem to be suitable for maternity colonies with different roost conditions. Thus different sizes of exit openings or different abundance indices are not decisive parameters for the choice of a particular survey method. In this respect, it is assumed that the comparison is equally applicable to other roost types with similar characteristics as the studied maternity roosts. For example, the conditions for recording methods are often similar for winter roosts, so the comparison should also be applicable there. The methods investigated can also be applied to box roosts, as light

barriers for bat boxes have already been developed. However, the situation is different for tree roosts, where further research is needed.

Concerning the transferability of the study to other bat species, it is also assumed that this is given. However, it should be noted that the methods used do not allow for the differentiation of the individual species. Thus, although the overall activity at the roosts used by different bat species can be recorded with the methods mentioned, a species-specific recording is not possible. This requires the use of other methods, such as the recording of entries and exits with the help of light barrier-controlled photo camera systems. As another method for conducting monitoring studies on bat roosts, an acoustic recording method has recently been developed by Revilla-Martín et al. (2020), which also aims to develop a non-invasive monitoring procedure for bats in caves with as little disturbance to the animals as possible. To this end, visual observations and infrared video recordings will be made at the beginning of the study, which will make it possible to determine a site-dependent regression. This regression is then used to infer bat abundance from the acoustic data. With this method, the number of emerging bats can be determined acoustically based on their calls. In this way, long-term monitoring data can be generated from acoustic recordings with comparably little effort. In addition, the acoustic method, like the light-barrier surveys, is a non-invasive long-term observation that goes beyond snapshots. The acoustic surveys were conducted at cave sites where a visual count does not provide reliable results. In addition, light barriers are already installed at cave entrances to record bat activity, so a direct comparison of the two methods would be possible in the context of further studies. To apply the acoustic method in a roost, it is first necessary to record the site conditions and collect comparative data using video recordings or visual observation in order to correct the counts for site-specific conditions. Light barrier monitoring, on the other hand, only requires the installation of the technical equipment so that data can be generated immediately afterwards. Both methods require occasional visits to the quarters to back up data, change batteries and check the equipment. With the light barrier, the data backup and battery change are not necessary under certain conditions, for example if a power connection is available. The main advantage of the acoustic method is that, at least to a limited extent, it is possible to distinguish species based on species-specific calls. However, compared to light barrier monitoring, weather conditions can affect the performance of the detectors and echo problems can occur within the cave. Most importantly, the fact that the acoustic method cannot distinguish between arrivals and departures can quickly lead to misinterpretations of roost abundance. For example, during light barrier monitoring, it was observed that the bats flew in and out several times during light rain, while in favourable weather conditions there were concentrated flights without multiple entries and exits. Thus, with the acoustic method, significantly higher activity would be detected on rainy nights.

Although Ancillotto et al. (2019) observed responses of a bat to human disturbance, the survey of the assessment of disturbance levels by observer presence at roosts during the present study revealed no significant differences between LB data for nights when the observer was on site to conduct IVR and VCE and the previous and subsequent nights when the observer was absent. Neither the number of emerging flights nor the onset or duration of the emerging flights seems to have been affected by the observer's presence and the infrared video recording. It should be noted that Ancillotto et al. (2019) investigated the influence of additional persons besides the observer, whereas in the present study only the disturbance caused by the presence of the observer and the infrared video recording was determined by LB counting.

The last recording night at the individual roosts was carried out during the swarming season of the bats from mid-August to October. During this time, there is an increased

activity of the bats in front of the roosts. During the swarming period, individual bats already move to potential winter roosts, so that abundance at the maternity roosts decreases (Fölling et al. 2013). This is also evident in the data collected here, especially for Seglohe (23.08.2019). The transition from the swarming season to hibernation can also be seen in the pattern of data from all three methods used.

In summary, all three methods are suitable in terms of data quality and the presence of the observer and the IVR did not seem to lead to a change in the behaviour of the bats during and after the observational night.

Comparison of methods based on additional parameters

LB and IVR are currently still rarely used in monitoring studies. This is because the relevant method standards and regulatory requirements continue to specify VCE as the survey method (Albrecht and Selzer 2015; Hessen Mobil 2017). This method is the oldest and best known of the methods used and can also be used by volunteers with little effort due to its low technical requirements.

Infrared video recording

Monitoring of emerging events using IVR is recommended when a high level of public interest is expected and therefore there may be doubts about the results, or when the observer does not have extensive bat-specific experience. Apart from infrared emitters, this survey method relies exclusively on commercially available equipment, and the requirements in terms of personnel time are average (Limpens and Roschen 2002). Similarly, both the costs of this method and the surveyor are comparatively low (Elliott et al. 2005). Another decisive factor is that the screen-based counting of emerging flights can be repeated as often as desired and thus, in case of doubt, additional recounts by third parties are possible for quality assurance purposes.

Surveys with IVR can be prone to error due to technical defects or human error. When installing the infrared emitters and the video camera, care must be taken to ensure that the infrared emitters cause as little shadowing as possible by the emerging bats, as shadows make the subsequent counting of the emerging flights more difficult. The accuracy of the results of the subsequent on-screen count from the video images depends on the exact technical installation of the recording equipment on site and the attention and concentration of the observer. Shadows cast and bats appearing at the same time can lead to erroneous counts (Elliott et al. 2005). With the help of technical aids, such as the possibility to slow down the playback of the video recordings as well as to replay the videos as often as necessary, human counting error can be significantly reduced or almost completely eliminated. The possibility of validating the count of emerging flights is thus given.

Provided that the exit opening can be captured by the infrared video camera and that the weather conditions are suitable for recording, this survey method is particularly recommended when doubts about the survey results are to be expected and it is sufficient to generate snapshots instead of long-term data series. This is all the more true as the results of the present study show that the use of the IVR has no influence on the bat behaviour and thus on the data quality.

Visual counting of emerging bats

For surveys where personnel time is a limiting factor, the use of VCE for greater mouse-eared bat maternity roosts is recommended. This method only requires travel time, which is variable and therefore not considered in this study, and actual recording time, without the need for extensive post-data analysis. The availability of technical equipment is also not necessarily a challenge for the observer, as night vision goggles are commercially available. It should be taken into account that although the availability of night vision equipment is not a limiting factor for this recording method, the one-time acquisition costs of approx. €7000 can be an exclusion criterion in individual cases. However, the price development of night vision devices in recent years gives hope that devices of suitable quality will become more and more affordable in the coming years. The application of this method does not require any special technical knowledge, but does presuppose knowledge about bats and their behaviour, biology and ecology. It should also be taken into account that VCE are only snapshots (Bürger et al. 2018). In addition, weather conditions should be suitable for the task and the exit opening must be visible.

Bat flight surveys using VCE are conducted without digital support and are the most dependent on the expertise of the observer of the three methods considered. Depending on the visibility of the exit opening, the light conditions during the emerging event, the bats' emergence behaviour as well as the experience of the observer, the results of the emerging count can deviate considerably from the actual emerging flights (Betke et al. 2008). The more orderly the emergence of the bats from the roost and the better the visibility of the exit opening, the more likely it is that all emerging flights will be counted (LBM 2011). If many bats emerge within a short period or if they continue to swarm near the roost, there may be a greater number of return flights. Such situations make counting much more difficult and can lead to multiple counts being made or emerging bats being overlooked, resulting in human counting errors. With increasing darkness, it becomes more difficult to visually detect the bats and thus to conduct a reliable count (LBM 2011). Night vision goggles were therefore used as a supporting measure. In the present study, night vision goggles were generally used in the last third of the nocturnal survey period. Mechanical counters were used to record the individuals, producing a tally sheet after the count was completed. For quality assurance, counts were conducted synchronously by two observers at random.

VCE are the right choice of method if the observer has sufficient experience with bat behaviour and only a small time budget is available. This is all the more true as the results of the present study show that the presence of the observer and the IVR do not seem to influence bat activity during and even after the observation night.

Light barrier

The recording of evening emerging flights of greater mouse-eared bats from their maternity roosts through LB is recommended especially when questions are involved that require long-term observations or are intended to detect changes in the population of a roost in a timely manner (Berková and Zukal 2006). A prerequisite for surveys with LB is sufficient funding (Limpens and Roschen 2002). Other prerequisites are sufficient technical understanding and knowledge of bats to ensure correct evaluation of the data obtained, as well as correct installation and maintenance of the LB. For counts using this method, sound technical expertise and knowledge about bats are indispensable, not least because of the large number of possible technical sources of error.

Possible sources of error with the LB method are power failures and soiling of light-emitting diodes. Such soiling can occur when bats fly near the sensors. Dirty sensors can no longer register entries or emerging flights. Since the LB are not only installed for single survey nights at roosts, but also continuous monitoring surveys over several months, remote access to the LBs has proven useful (Kugelschafter 2015). Remote access enables a daily function check of the devices, so that immediate repair is possible in case of a malfunction. If outliers are detected, the continuous monitoring allows the data to be compared with the previous or following night and correlated with weather data. Other potential sources of error in the LB data can occur when several bats emerge from the roost at the same time. In this case, only one entry or exit can be recorded. The same applies if the distance between two individuals is too small (e. g. in tandem flights), as the temporal distance between two bats must be greater than 0.25 s for them to be recorded as single individuals (Kugelschafter 2015). In the present case, the data showed that this did not influence the results for the surveyed roosts. Another factor to consider is the triggering of LB by birds rather than bats. Daytime recordings are usually due to birds, and in exceptional cases birds staying in the roosts overnight may trigger LB events. This problem can be easily remedied by defining suitable time windows.

Taking into account the possible sources of technical error, the use of LB is particularly justified for long-term studies, such as monitoring studies (Dietz 2017). It enables weather-independent, automated observations, but is associated with relatively high costs and requires well-trained personnel. Automated long-term observations in combination with remote access make it possible to detect possible population-related behavioural changes in the maternity colonies, such as activity during the night while the lactation period, in a timely manner and to take measures if necessary. Behavioural changes cannot be detected with the other two survey methods.

Overall assessment and conclusions

The comparison of methods presented above is to support volunteer and professional observers, scientists and competent authorities in choosing a suitable and reliable recording method. As the comparison has shown that there are no significant differences in data quality between the three methods used and that the presence of the observer on site or the installation of infrared lamps do not influence the results in terms of disturbing the emergence of the greater mouse-eared bat from the roost, the choice of an appropriate survey method can be made on a case-by-case basis depending on the context, the possible sources of errors and objectives of the particular study.

Censuses by LB are recommended for the planned nationwide permanent monitoring of greater mouse-eared bats, as envisaged in the context of monitoring under the EU Habitats Directive. Long-term recording with this method allows possible changes in the maternity roost population to be detected promptly and appropriate measures to be taken. In certain situations, it may be useful to compare sample counts by LBs with other methods, e.g. for validation purposes. Taking into account the above-mentioned aspects of the new method of Revilla-Martín et al. (2020) and the light barrier, it could therefore be examined whether long-term monitoring is possible by combining the two methods of acoustic recording and the light barrier if more than one species occurs in each case. In this way, the advantages of both methods could be used and a method for recording the abundance at bat roosts could be developed. The distinction between

arrivals and departures could be determined using the light barriers and the distinction between species or species complexes could be determined using the calls of the acoustic methods. In this way, long-term monitoring could also be carried out at roosts used by several species.

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