
**Improving bat monitoring and conservation through
infrared light barriers, camera traps and deep learning**

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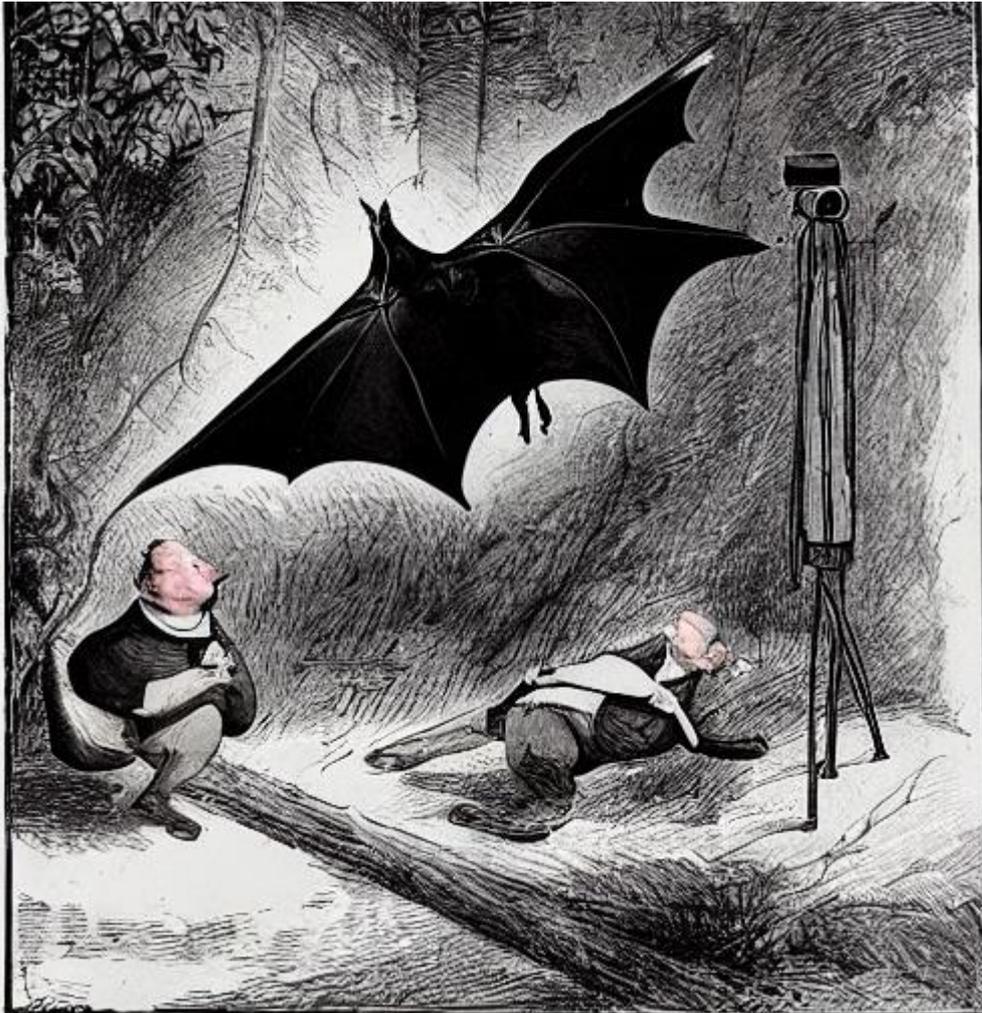
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How a neural network imagined my fieldwork in the beginning of my PhD:
monitoring bats with camera traps.

- image generated using DeepAI 2019 -

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Abstract

Amid the current global biodiversity crisis, being able to accurately monitor the changing state of biodiversity is essential for successful conservation actions and policy. Despite the pressing need for reliable and cost-effective monitoring methods, collecting such data remains extremely difficult for elusive species, such as temperate zone bats. Although bats are important indicators of environmental changes, monitoring bat populations is challenging because they are nocturnal, volant, small, and highly sensitive to human activities and disturbance. Thus far, population trends of temperate zone bats have been mainly based on visual surveys, including winter hibernation counts at underground sites. However, as bats may not always be roosting in visible locations within the hibernacula, it is currently unknown how these estimates relate to actual population sizes.

Infrared light barriers combined with camera traps are a novel method to monitor bats at underground sites. When installed at the entrance of hibernacula, infrared light barriers have the potential to estimate site-level population sizes more accurately than visual surveys, by counting all bats flying in and out of the site. Moreover, camera traps, consisting of a digital camera and white flash, can be used for species-level identification. However, for this new method to be applicable as a large-scale bat monitoring technique, it is important to characterize it with regard to three main criteria: is the method minimally invasive, is it accurate, and is it scalable in terms of spatial and temporal resolution? Therefore, the purpose of this thesis was to investigate the invasiveness and accuracy of this novel bat monitoring method, and to develop standardized and automated data analysis pipelines, both for the light barrier and camera trap data, to support the deployment of this method at scale.

In Publication I, we used light barrier data, infrared video recordings and acoustic data from an experimental field study to investigate whether the white flash of the camera trap has any measurable short- or long-term effect on bat activity and behavior. The flash of the camera trap was turned on and off every week at each site, which allowed us to compare the activity and behavior of bats between flash-on and flash-off nights. We found that despite the high sensitivity of bats to disturbance, they did not change their nightly activity patterns, flight direction, echolocation behavior, or long-term site use in response to the white flash of the camera trap. Based on these results, we concluded that camera traps using a white flash are a minimally invasive method for monitoring bat populations at hibernacula, providing high quality images that allows species-level identification.

In Publication II, we used infrared video surveillance to quantify the accuracy of infrared light barriers, and we described a standardized methodology to estimate population sizes and trends of hibernating bat assemblages using light barrier data. We showed that light barrier accuracy varies based on the model and location of the installation relative to the entrance, with the best combination achieving nearly perfect accuracy over the spring emergence phase. When compared to light barrier-based estimates, we found that visual counts markedly underestimated population sizes, recovering less than 10% of the bats at the most complex hibernacula. Moreover, light barrier-based population trends showed regional patterns of

growth and decline that were not detectable using the visual count data. Overall, we established that the light barrier data can be used to estimate the population size and trends of hibernating bat assemblages with unprecedented accuracy and in a standardized way.

In Publication III, we described a deep learning-based tool, *BatNet*, that can accurately and efficiently identify bat species from camera trap images. The baseline model was trained to identify 13 European bat species or species complexes using camera trap images collected at 32 hibernation sites (i.e., trained sites). We showed that the baseline model performance was very high across all 13 bat species on trained sites, as well as on untrained sites when the camera angle and distance from the entrance were comparable to the training images. At untrained sites with more atypical camera placements, we demonstrated the ability to retrain the baseline model and achieve an accuracy comparable to the trained sites. Additionally, we showed that the model can learn to identify a new species, while maintaining high classification accuracy for all original species. Finally, we established that *BatNet* can be used to accurately describe ecological metrics from camera trap images (i.e., species diversity, relative abundance, and species-specific phenology) that are relevant for bat conservation.

We conclude that infrared light barriers and camera traps offer a minimally invasive and accurate method to monitor site-level bat population trends and species-specific phenological estimates at underground sites. Such remote data collection approaches are particularly relevant for monitoring large, complex hibernation sites, where traditional visual surveys are not feasible or account only for a small fraction of the actual population. Combining this automated monitoring method with a deep learning-based species identification tool, *BatNet*, allows us quickly and accurately analyze millions of camera trap images resulting from large-scale, long-term camera trap studies. As a result, we can gain unprecedented insights into the behavior and population dynamics of these enigmatic species, drastically improving our ability to support data-driven bat conservation.

1. Introduction

1.1. Biodiversity monitoring and conservation

Biodiversity, the variety of life on this planet, underpins every aspect of our lives, yet it is currently declining at an unprecedented rate. The current global rate of biodiversity loss is extraordinary in human history: today, species go extinct tens to hundreds of times faster than they did on average in the past ten million years, and currently up to one million species are threatened with extinction (IPBES, 2019). Although the world's human population represents only 0.01% of all living things, humanity has already caused incredible damages on the planet. For example, we have cut down half of the 6 trillion trees that existed on the planet before human civilization, destroyed two third of the world's tropical rainforests, and caused the extinction of 83% of all wild mammals (Bar-On et al., 2018).

The main drivers of this biodiversity loss are changes in land and sea use, direct exploitation of organisms, pollution, invasive alien species, and the most-well known, climate change (IPBES, 2019). Rising global temperatures and more frequent extreme weather conditions are already affecting nature from the level of ecosystems to genetics (Scheffers et al., 2016), as well as directly affecting human populations (Steel et al., 2022). Perhaps as a result of this impact on human well-being, numerous international legislative agreements have been passed to mitigate the impact of climate change, and the majority of the general public now also considers climate change a global emergency (UNDP, 2021). In contrast, public awareness of the consequences and urgency of biodiversity loss are often lacking. This contributes to the relatively low political priority given to biodiversity issues, despite them being one of the greatest threats to the long-term viability of the human species (Díaz et al., 2006).

Although now there is a general consensus that we are entering the sixth mass extinction event (Ceballos et al., 2015), quantifying the exact extent of biodiversity loss remains difficult. Determining the spatial and temporal scales of biodiversity loss is essential to understanding its consequences, and developing strategies to mitigate its impact (Magurran, 2021). Since ecosystems consist of carefully balanced, complex interactions between species, the extinction of one species is likely to have far-reaching consequences (Sodhi et al., 2009). This was poignantly illustrated at the COP15 (UN Biodiversity Conference, Montreal, 2022) using the metaphor of a *Jenga* tower for an ecosystem, suggesting that we can take out pieces for a while and it remains standing, but we do not fully understand which combination of blocks that get removed from the tower will destabilize it, leading to its collapse. What is certain that with every species that becomes extinct, biodiversity dwindles, and with it the basis of life on Earth as well. In effort to halt or even reverse this biodiversity loss, several global targets have been set for the next decade, such as protecting and sustainably managing 30% of the Earth's land and marine environments (CBD, 2022). However, to achieve effective conservation and sustainable management of these protected areas, the first essential step is to be able to accurately monitor biodiversity and make informed predictions about how and why this diversity changes over space and time (Lindenmayer et al., 2012).

Accurately monitoring biodiversity provides a reliable baseline to track and understand long-term shifts in biological diversity. However, for most species, such analyses can focus only on recent changes, as data are limited for the past decades or if older data are available, then they are less accurate (Mihoub et al., 2017). Because the baselines are often recent and arbitrary, it is important to be cautious when interpreting stable or even increasing population trends as a sign of conservation success. For example, European bat populations showed a massive decline during the second half of the 20th century, followed by partial recovery of some species in the past 30 years, as a result of conservation legislation and habitat management (Van der Meij et al., 2015). However, the current bat populations are still likely to be smaller compared to the pre-decline figures, making them less robust to the considerable anthropogenic changes that continue to affect them. Moreover, in cases when we lack long-term monitoring data and knowledge about the past condition of a species, we are more likely to perceive such trends as normal, a term called ‘baseline shifting’ (Soga & Gaston, 2018). This shifting baseline syndrome increases the society’s tolerance for environmental degradation and species extinction, but it also changes the expectation of people as to what is a desirable state of nature, and thus, the species and habitats that are worth protecting (Papworth et al., 2009).

*“A shifting baseline has distorted our perception of all life on Earth. We have forgotten that once there were temperate forests that would take days to traverse, herds of bison that would take four hours to pass, and flocks of birds so vast and dense that they darkened the skies. Those things were normal only a few lifetimes ago. Not anymore. We have become accustomed to an impoverished planet. We have replaced the wild with the tame. We regard the Earth as **our** planet, run by humankind for humankind. There is little left for the rest of the living world. The truly wild world - that non-human world - has gone. We have overrun the Earth.”*

*(A Life on Our Planet:
My Witness Statement and a Vision for the Future
by David Attenborough)*

Obviously, we cannot go back in time to improve species monitoring and establish baselines that represent the population sizes before extensive anthropogenic impacts. What can be done, is to initiate the collection of accurate and consistent long-term monitoring data that can inform both the current and future generations about the changing state of biodiversity (Jones et al., 2020). Since biodiversity monitoring is a very broad term that encompasses many different approaches, it is essential that before starting the data collection, we clearly define *why* and *what* we want to monitor (Jones et al., 2013), and *how* the data should be collected and processed to support conservation actions and management decisions.

The rationale for starting a monitoring program (the “*why*”) is very often the simple idea that gaining more information about any biological system is always beneficial (Yoccoz et al., 2001). However, to ensure the success of monitoring programs, it is important to define explicit objectives. The monitoring objective can either be to contribute scientific knowledge about the behavior and dynamics of the monitored system, or to evaluate the effectiveness of conservation actions and make more-informed management decisions in the future (Yoccoz et al., 2001). In both cases, a set of *a priori* hypotheses are developed about how the monitored system will respond to environmental changes or management actions, and the resulting predictions are compared to the patterns observed from the collected data (McComb et al., 2010). This thesis focused on investigating the use of a novel monitoring method, and less on addressing these explicit objectives. However, in the future, the data collected with such a method can be used to evaluate both the impact of environmental changes and conservation actions on the monitored populations.

Next, we have to specify the level at which we want to document the changing state of biodiversity and the metrics we want to quantify (the “*what*”). Monitoring can range from ecosystems and communities to species and populations, or even to an individual level (Noss, 1990). In this thesis, biodiversity was monitored primarily at the community- (i.e., mixed species assemblages) and species-level. Indeed, most monitoring programs focus on species-level changes and prioritize the monitoring of species that are rare, endangered, or have high economic, social, cultural, educational, and aesthetic values (Gascon et al., 2015; McComb et al., 2010; Tribot et al., 2018). After choosing the monitored system, the next step is to define the characteristics of the system to be monitored. In this thesis, we focused on several ecological metrics that can be derived from a novel monitoring technique and have a direct relevance to understanding the biology of the monitored species or can be used to evaluate management actions that might affect them. For one, we established methods for detecting changes in the species composition of communities, which can contribute to better understanding the species-specific impact of management actions or to identifying species-specific threats. Moreover, we monitored population-level parameters, such as population sizes and trends, which enables us to determine the conservation status of species and evaluate how they are responding to human-induced environmental changes (Elzinga et al., 2001). Due to its spatial component, such monitoring data can also contribute to identifying sites and habitats of high conservation value that should be protected (Chape et al., 2005), and to measure the effectiveness of these protected areas. This evaluation is part of an important feedback loop to implement more informed, data-driven conservation policy and management strategies. Additionally, we monitored species-level phenological changes that have also important implications for conservation itself. For example, the phenology of hibernating mammals, such as temperate zone bats, can be directly linked to their fitness and survival (Iler et al., 2021). However, the timing of phenological events is heavily influenced by climatic cues, thus, climate change is expected to have consequences on the persistence of these species (Wells et al., 2022).

Finally, we have to establish how can we collect monitoring data that is required for obtaining relevant ecological metrics (the “how”). First, the chosen method should be minimally invasive, so that it does not negatively affect the target species or changes its behavior. Beyond the impact on the individual level, if the method causes disturbance, it may alter the metric that it is trying to observe, and the results cannot be used for inference about the broader population. Second, the collected data should be accurate enough that it can be used to evaluate the *a priori* hypotheses without the true patterns being masked by inaccurate data collection. Third, the spatial and temporal scale of monitoring should be sufficient to achieve the predefined objectives. Due to the rapid loss of biodiversity, there is a growing need for assessing the status of wildlife at large spatial and temporal scales (Schmeller et al., 2017), but this is often associated with high costs. Therefore, when developing monitoring programs, we should aim for using methods that allow cost- and time-efficient sampling, but also offer accurate assessment of changes in biodiversity at scale. Recent advancements in automated monitoring methods are important in this context, as they have the potential to increase the spatial and temporal scale, the accuracy, and the information content of the assessments of biodiversity changes (Kitzes & Schricker, 2019; Petrou et al., 2015). Moreover, remote data collection is particularly useful to overcome the challenges related to monitoring rare or cryptic taxa, such as bats, that are otherwise hard to accurately and efficiently monitor due to their elusive behavior (Rebelo & Jones, 2010). In this thesis, we focused on evaluating a novel, automated bat monitoring method and its potential to be a minimally invasive, accurate and scalable approach that can contribute to data-driven conservation.

1.2. Bat monitoring

Bat species around the globe provide ecosystem services, such as insect suppression, plant pollination and seed dispersal, which are essential for general ecosystem health and human well-being (Kunz et al., 2011). However, due to their sensitivity to changing environmental conditions, most bat species face a myriad of threats, such as habitat loss, emerging infectious diseases or global warming coupled with increasingly unpredictable climatic conditions (Frick et al., 2020). Since bats are long-lived animals with a slow rate of reproduction (Barclay et al., 2004), when environmental and anthropogenic changes cause population declines, bat populations will recover only slowly (Fleischer et al., 2017). Consequently, accurate and long-term monitoring of bat populations is fundamental for tracking the impact of environmental changes on their populations, and consequently, for biodiversity conservation.

Despite the bats’ key ecological role and the current biodiversity crisis, over one third of bat species assessed by IUCN (International Union for Conservation of Nature) are considered threatened or data deficient, and more than half of the assessed bat species have unknown or decreasing population trends (Frick et al., 2020). This means that currently almost a thousand bat species around the globe would require improvement to their population monitoring data or immediate conservation actions (IUCN, 2022).

Monitoring bats is a challenging task, particularly in the temperate zone, where most species are small, elusive, show nocturnal activity and are sensitive to the disturbance associated with many monitoring methods. Nearly all temperate zone bat species follow a similar annual life cycle (Schober & Grimmberger, 1989), which provides opportunities for monitoring them. For those species that use underground sites for hibernation, the annual life cycle consists of three main phases: *summer*, when female bats typically form species-specific maternity colonies and males roost separately either on their own or in small bachelor groups; *autumn*, when both females and males of multiple species gather at swarming sites for mating; and *winter*, when both sexes of multiple species hibernate together at underground sites (Dietz et al., 2009). Therefore, monitoring at underground sites is suitable for observing both the autumn mating and winter hibernation phases (Figure 1).



Figure 1. The annual life cycle of temperate zone bats that use underground sites for hibernation. The green portion of the ring indicates the phases when bats are present at the hibernacula. Also note that while the species and sexes (brown and black icons) are segregated in summer, they form mixed assemblages at the underground sites in autumn and winter.

Traditionally, many bat species have been monitored primarily by visual surveys at their summer maternity roosts. This can be done either by counting the adult and juvenile bats that are visible inside the roost – a method often associated with heavy disturbance; or by counting bats emerging from the roost for their nightly foraging trips, performed by surveyors outside of the roost causing minimal disturbance (Battersby, 2008). While monitoring summer maternity colonies is an essential component for understanding bat population dynamics, it also comes with some challenges. First, counts at maternity colonies are limited to female and juvenile bats, since the adult males are segregated during this time of the year. Second, this method requires prior information about the location of the maternity roosts, which is not always readily available for many populations. For example, for species that frequently switch roosts, it is also important to quantify the home range of their maternity colonies to ensure that the same colony is monitored between years. Moreover, for understanding the

population dynamics of species with fission-fusion dynamics, it is also crucial to consider that very often only an unknown subset of the population will be counted on any given survey. Finally, visual counts in and outside of large maternity roosts are both highly susceptible to observer bias. The accuracy and reproducibility of such counts can be increased by using automated counting methods. For example, emergence counts can be performed post-hoc from infrared and thermal videos that were recorded at the exit of the roosts. Furthermore, automated animal tracking methods can be applied to the video data (e.g., Bentley et al., 2022), providing more standardized count results with less human effort. However, due to high power and data storage requirements of the video recording devices, such approaches remain unfeasible for widespread or long-term bat monitoring.

In contrast to summer, the autumn and winter phase of the annual life cycle of most temperate zone bat species take places at the same underground sites. Although monitoring such underground sites is not suitable for tracking colony-level dynamics or approximating the reproductive output of colonies, these underground sites offer an opportunity to efficiently track regional population dynamics. This is because during autumn and winter the same underground sites are used by both sexes of multiple species, and by individuals from different maternity colonies (Dekeukeleire et al., 2016). The most common bat monitoring methods at such underground sites are swarming captures and acoustic surveys in autumn, and visual surveys during the winter hibernation period.

During autumn, temperate zone bat species assemble at underground sites for swarming, which is a behavior that can serve several purposes. First, it supports a promiscuous mating system and facilitates gene flow between the otherwise isolated summer maternity colonies (Furmankiewicz & Altringham, 2007; Kerth et al., 2003). Second, it provides opportunities for social information transfer regarding the location of the underground site (Humphrey & Cope, 1976), particularly to juveniles (Stumpf et al., 2017). Third, it supports the assessment of the suitability of an underground site as hibernaculum (Fenton, 1969; van Schaik et al., 2015). During autumn swarming, bats exhibit high flight activity and circling behavior at the entrance of the site (Parsons et al., 2003), which provides opportunities to capture bats using mist-nets and harp-traps. Although capturing and handling bats allows reliable species and sex identification, this method might expose the captured bats to considerable stress (Battersby, 2008). To minimize the potential stress caused to bats, the temporal and spatial resolution of swarming capture data is often limited. As result, this coarse data cannot be used to directly estimate population sizes and trends or to efficiently monitor species-level phenology.

As an alternative to swarming captures, acoustic detectors can also be used at swarming sites to non-invasively survey bat activity. Another advantage of acoustic surveys is the possibility to record species that would be difficult to observe with other traditional methods, such as captures (O'Farrell & Gannon, 1999). Moreover, passive acoustic detectors can be installed at swarming sites to record large volumes of bat activity data without the presence of a surveyor (Froidevaux et al., 2014). To speed up the data analysis, several automated classifiers of echolocation calls have been developed (Rydell et al., 2017). However, reliably distinguishing calls to species level is not always possible due to intraspecific call variation, interspecific overlap in the call features (Russo et al., 2018), and overlap of concurrently calling

individuals (Bergmann et al., 2022). Another limitation of the acoustic activity data is that it cannot be directly translated into population size estimates, and even comparing the relative volume of activity between sites is likely challenging due to site- and year-specific levels of swarming activity.

During winter, many temperate zone bat species share the same underground sites for hibernation. In this period, bats alternate between prolonged bouts of torpor (i.e., reduced body temperature and metabolic rate) and short arousals with a return to normal body temperature (Ruf & Geiser, 2015). This behavior constitutes a trade-off between energy conservation during unfavorable environmental conditions and the physiological and ecological costs of lowered metabolic rate (Humphries et al., 2003). Given this energetic constraint, it is important to consider that hibernating bats are sensitive to non-tactile stimuli and might arouse due to changes in microclimatic conditions, disturbance from other bats or humans. While hibernating bat assemblages are most commonly monitored by visual counts, the presence of observers may evoke arousal in hibernating bats, as a result to exposure to light, noise, air currents and heat (Davis, 1970; Pflitsch & Piasecki, 2003; Speakman et al., 1991; Thomas, 1995). Such arousals may result in high metabolic activity and loss of body mass (Johnson et al., 1998; Speakman et al., 1991; Thomas, 1995), which may also lower overwinter survival rates (Johnson et al., 1998). Although the use of modern technology (e.g., LED lamps instead of halogen or kerosine lamps) and following best practice guidelines has likely reduced the impact of visual surveys in recent decades (Stapelfeldt et al., 2020). Nevertheless, to limit the impact of human disturbance on hibernating bats, winter counts are usually limited to one occasion per year. Because they are relatively easy to perform, winter hibernation counts are the most widely used and oldest monitoring method used to track population dynamics of temperate zone bats (Van der Meij et al., 2015). However, it has been long acknowledged that there is a discrepancy between the visually observed and the actual population size of bat assemblages in hibernacula, as a result of bats hiding in deep cracks and crevices (Battersby, 2008).

In addition to these monitoring options (i.e., swarming captures, acoustic surveys, winter counts), the installation of infrared light barriers and camera traps at the entrance of hibernacula represents a promising, novel bat monitoring method. Light barriers can count all bats that enter or leave the site without being affected by the visibility of bats. Consequently, they are expected to provide more accurate population size and trend estimates than visual counts, but the discrepancy between the two methods has not been quantified before. Additionally, camera traps can be connected to the light barriers to gather species-level information even for rare or crevice-dwelling species that may remain hidden during visual surveys. The resulting camera trap data can be used to describe activity patterns and hibernation phenology of different bat species, and ultimately, to monitor species-level population trends. However, to gather images with sufficient quality that allows reliable species identification, camera traps must include a white flash. The use of white flash might cause disturbance to temperate zone bats that are generally considered sensitive to artificial light (Voigt et al., 2018), but the potential effects of the white flash on bats have not been investigated. Moreover, manual bat species identification from camera trap images is a time-

consuming and monotonous task that requires extensive experience with the subtle morphological differences between species. Given that a medium-size site with around 600 hibernating bats may yield up to 30,000 camera trap images every year, manually analyzing images from large-scale monitoring projects appears unrealistic. To overcome this bottleneck, deep learning-based species identification could offer a more efficient solution to process the resulting huge volume of images, and thus, ensure that the temporal and spatial resolution of camera trap-based monitoring can be vastly scaled up. Although such solutions exist for many other mammals (e.g., Norouzzadeh et al., 2018; Tabak et al., 2019), thus far no tools have been developed for automated identification of bat species from camera trap images. The aim of this thesis was to fill these knowledge gaps related to the invasiveness, accuracy, and scalability of infrared light barriers and camera traps used for bat monitoring.

1.3. Data collection

In this thesis, several types of techniques have been applied to monitor bats, and to quantify the accuracy and impact of the monitoring methods on the bats. In addition to traditional visual count at hibernacula, we used infrared light barriers, custom-built camera traps, self-built infrared video cameras and full-spectrum acoustic loggers at the entrance of hibernacula (Figure 2, for details see [Box 1](#)).

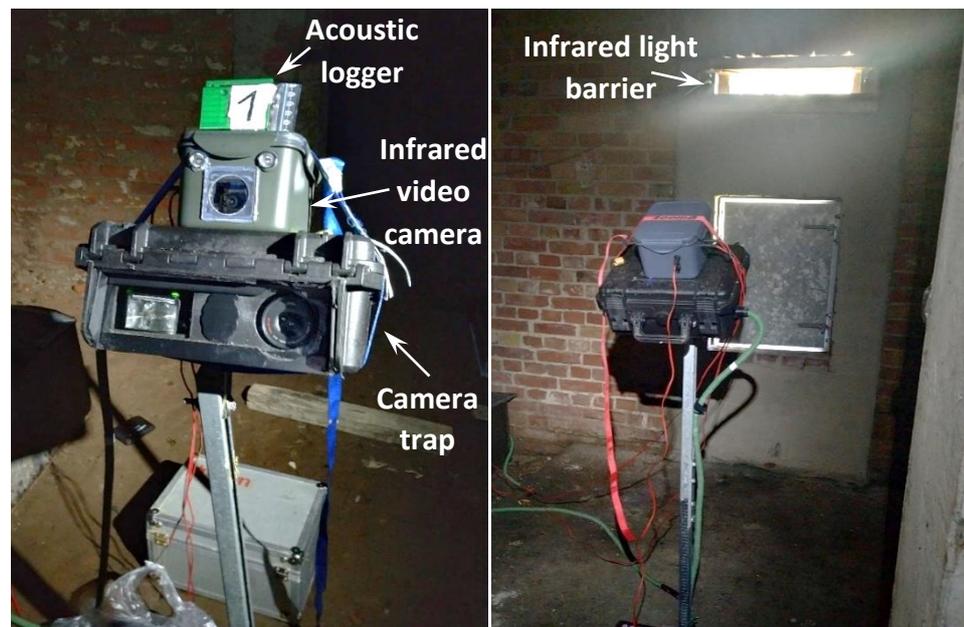


Figure 2. An automated bat monitoring system consisting of a custom-built camera trap, an infrared video camera and an acoustic logger, facing the entrance of a hibernaculum, where an infrared light barrier was installed.

Box 1. How does the automated bat monitoring technology used here work?**Infrared light barrier**

Infrared light barriers consist of a sensor array of infrared LEDs and corresponding receivers that create two parallel sets of infrared light beams, called ‘curtains’. These systems cycle through each transmitter-receiver pair at a frequency of 1kHz (i.e., 60 times a second) within each curtain. The curtain is considered blocked when any single receiver does not register the photoelectric signal from the LED during a cycle. The direction of the passes through the light barrier can be distinguished based on the order that the curtains are blocked (Figure 3). In addition to recording passes, the light barrier can save a log of all individual curtain triggers, which enables the identification of times when a curtain is blocked for an extended period of time (e.g., by a leaf or spiderweb in the entrance).

In this study, we used three light barrier models produced by ChiroTEC (Lohra, Germany). The models differ in the height of the monitored opening (Liba-4: 9.7 cm; Liba-16: 35.5 cm; Liba-16k: 20.1 cm), the number of sensors per curtain (Liba-4: 4 sensors; Liba-16 and Liba-16k: 16 sensors), and thus, the sensor density (Liba-4 and Liba-16: 2.2 cm between sensors, Liba-16k: 1.3 cm). These light barriers can either be powered directly via a power outlet or using car batteries. Using one 12V 100 Ah battery, a light barrier can continuously run for approximately six weeks. However, using a custom-made power manager tool, the light barrier can be connected simultaneously to four batteries, which will be depleted one after another in sequence.

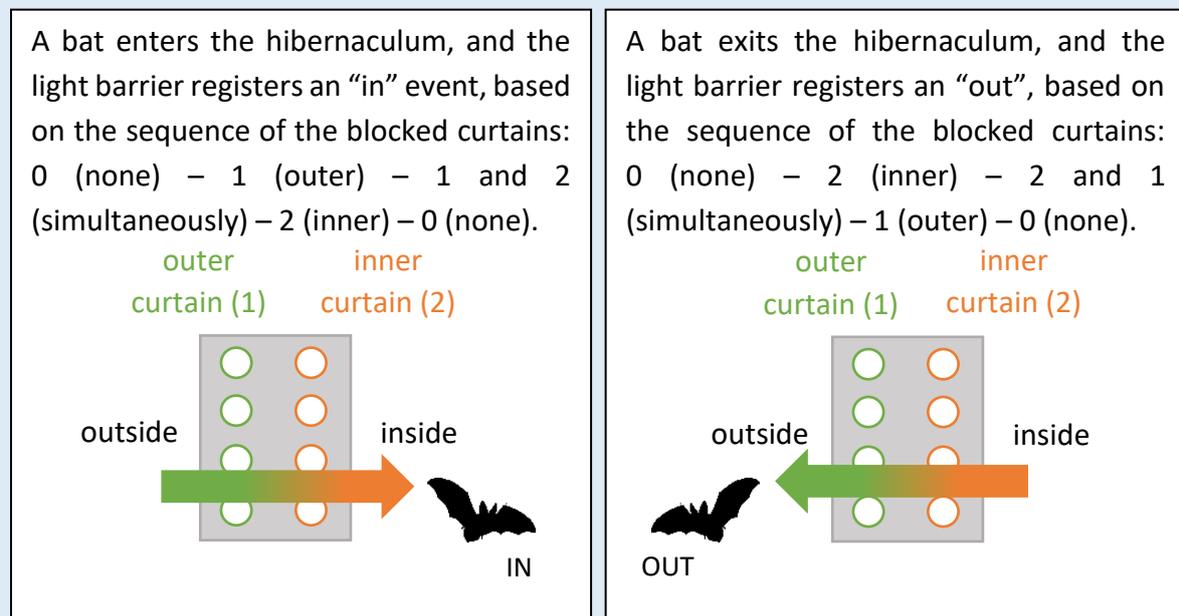


Figure 3. The logic behind how the infrared light barrier distinguishes the directionality of the registered bat passes (i.e., entry vs exit).

Box 1. (cont.)**Camera trap**

We used custom-built camera traps produced by ChiroTEC (Lohra, Germany; Figure 4A). These camera traps consist of a Panasonic Lumix G5 digital mirrorless camera and a Metz 58 AF-2 digital white flash with standardized settings (aperture 5.6, power 1/16, zoom 70). The camera trap is connected to the light barrier, and it is triggered on each entry or exit registered by the light barrier, depending on the settings. Such camera trap can be powered for up to four weeks using six car batteries (12V, 100Ah; four for camera and two for flash).

Infrared video camera

We used self-built infrared video cameras that consist of a Raspberry Pi 3, an 8 MP camera, and an 850 nm infrared illuminator (Figure 4B). They were mounted on top of the camera traps and recorded continuously between sunset and sunrise, with 800x600 resolution and 25 frames per second. The Raspberry Pi received a signal from the light barrier whenever it registered a pass, enabling the automatic extraction of short video clips around every registered pass. These video cameras could record for one week using two car batteries (12V, 100Ah).

Acoustic logger

We used AudioMoth acoustic loggers that were encased in a 3D printed protective casing (Figure 4C). The recorders were mounted directly on top of the infrared video cameras, with the microphone facing the entrance of the hibernaculum. They recorded with a sample rate of 192 kHz, on the medium gain setting. The recorders are originally powered by four AA batteries, but these were modified with a holder for four additional batteries. This allowed continuous recording every day between sunset and sunrise for one week.

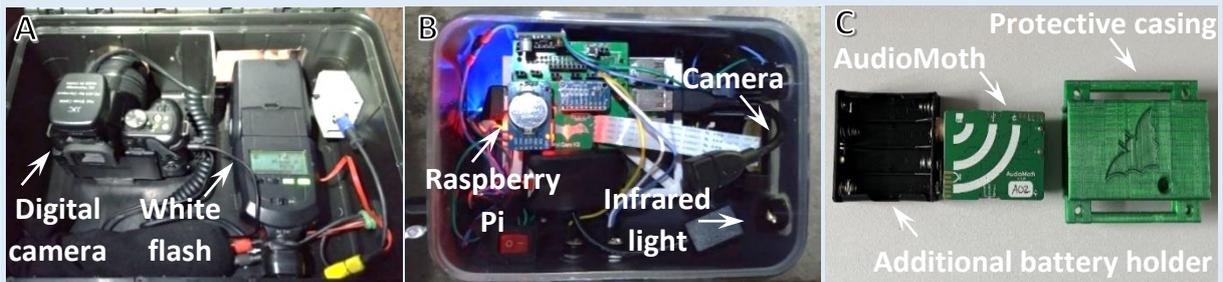


Figure 4. Overview of the automated bat monitoring techniques used in this study: **A)** a custom-built camera trap, consisting of a digital camera and a white flash, that is connected to an infrared light barrier; **B)** a self-built infrared video camera, consisting of a Raspberry Pi 3, an 8 MP camera, and an 850 nm infrared illuminator; **C)** an AudioMoth acoustic logger, modified to be powered with eight AA batteries, and covered with a 3D-printed protective casing.

Infrared light barriers enable us to constantly monitor bat activity at hibernacula throughout the entire year. Based on this data, we can distinguish five activity phases at underground sites that are not used by maternity colonies in summer (Figure 5): *mid-summer* with low activity and no substantial entry or exit; *autumn swarming phase*, with generally high activity and swarming behavior (i.e., flying in and out of the site and circling at the entrance of the site), gradually transitioning into a period of net entry into the site; *winter hibernation* with very low activity and little to no net exit or entry; *spring emergence*, with generally lower flight activity than in autumn, characterized by net exit from the site; and *early summer activity* with relatively low activity without significant net entry or exit.

Across all these activity phases, the light barrier registers every bat flying through the entrance. For each recorded pass, a light barrier event is saved that contains the direction of the pass (i.e., in or out) and a time stamp. Using these data, we can describe activity patterns (i.e., sum of all events, irrespective if it was an in or out), throughout the year, across seasons, or even on a nightly or hourly basis. Since we can distinguish the direction of the passes, we can assign a positive value (+1) to the entries (Figure 5, grey bars on the positive y-axis), and a negative value (-1) to the exits (Figure 5, grey bars on the negative y-axis). Consequently, we can calculate a net value for each night that represents how many bats entered or left the site. When the nightly net value is positive, more bats entered the site than left on that given night (Figure 5, light purple bars on the positive y-axis), and vice versa, when the nightly net is negative, more bats emerged from the site than entered it (Figure 5, dark purple bars on the negative y-axis). To estimate population sizes, we can either calculate the number of bats that entered the hibernaculum in autumn by adding up the nightly net values during the autumn swarming and hibernation entry phase; or calculate the number of bats that emerged from the hibernacula in spring by adding up the nightly net values during the spring emergence phase. However, precisely delineating these phases can be challenging due to the variation between species, sites, and years (Box 2).

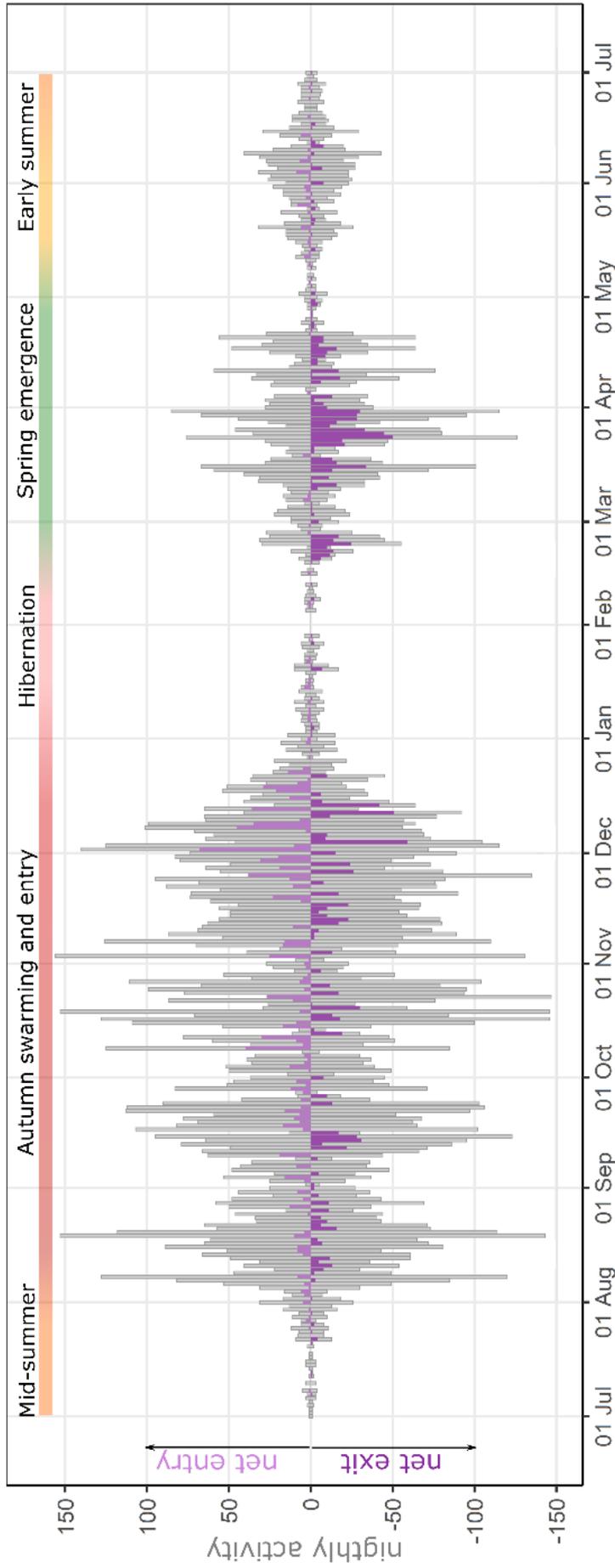


Figure 5. Annual bat activity at underground sites can be divided into five broad, partially overlapping phases (colored bar on top). The number of passes registered by the light barrier each night is indicated with gray bars (entries on the positive y-axis, exits on the negative y-axis). The net sum of all entries and exits per night is represented by the purple bars (net entry: light purple bars on positive y-axis; net emergence: dark purple bars on negative y-axis). Figure modified from Krivek et al., 2023.

Box 2. Delineating and selecting the most suitable period for estimating bat population sizes when using light barrier data.

To decide whether the autumn entry or spring emergence data are better to use for estimating population sizes, we must consider that the accuracy of the light barrier may vary with the number of registered passes (i.e., more passes in a short time increases the chance of passes being missed by the light barrier) and with the behavior of bats (i.e., bats swarming at the entrance are more likely to trigger false registrations). Consequently, the autumn entry data is expected to have lower accuracy compared to spring emergence data, for the following reasons. First, the highest activity peak of bats is recorded in autumn, which is several folds higher than in spring. Moreover, the autumn swarming phase cannot be reliably separated from the hibernation entry phase in the light barrier data (see Figure 5). This further inflates the number of registered passes and false triggers by swarming bats that circle on the inside of the hibernaculum without leaving, leading to higher error rate. In contrast, the spring emergence phase is characterized by low overall number of passes and less swarming activity at the entrance, and the emergence phase can also be relatively well separated from the early summer activity. However, none of these activity phases can be delineated with fixed dates, because their precise timing may differ between species, sexes, age classes, sites, and years (e.g., Meier et al., 2022). Therefore, the start and end dates of the emergence phase are usually determined visually by observing the activity patterns, which may introduce considerable bias to the final estimates.

In some cases, the emergence phase is followed by a short, low-activity period that separates it from the start of the early summer activity (e.g., Eldena 2017, Figure 6A). As a result, visually delineating the emergence and the early summer activity is relatively easy. Moreover, due to the low number of passes between the two phases, any differences in the human-defined time windows for emergence would have only a minor effect on the ultimate population size estimate. In other cases, when the emergence and early summer phases overlap without any clear gap (e.g., Eldena 2020, Figure 6B), visually separating the two phases becomes challenging. In such cases, the large variance in human-defined time windows for emergence is expected to have a significant effect on the population size estimates, due to the relatively high number of passes between the two phases.

Box 2. (cont.)

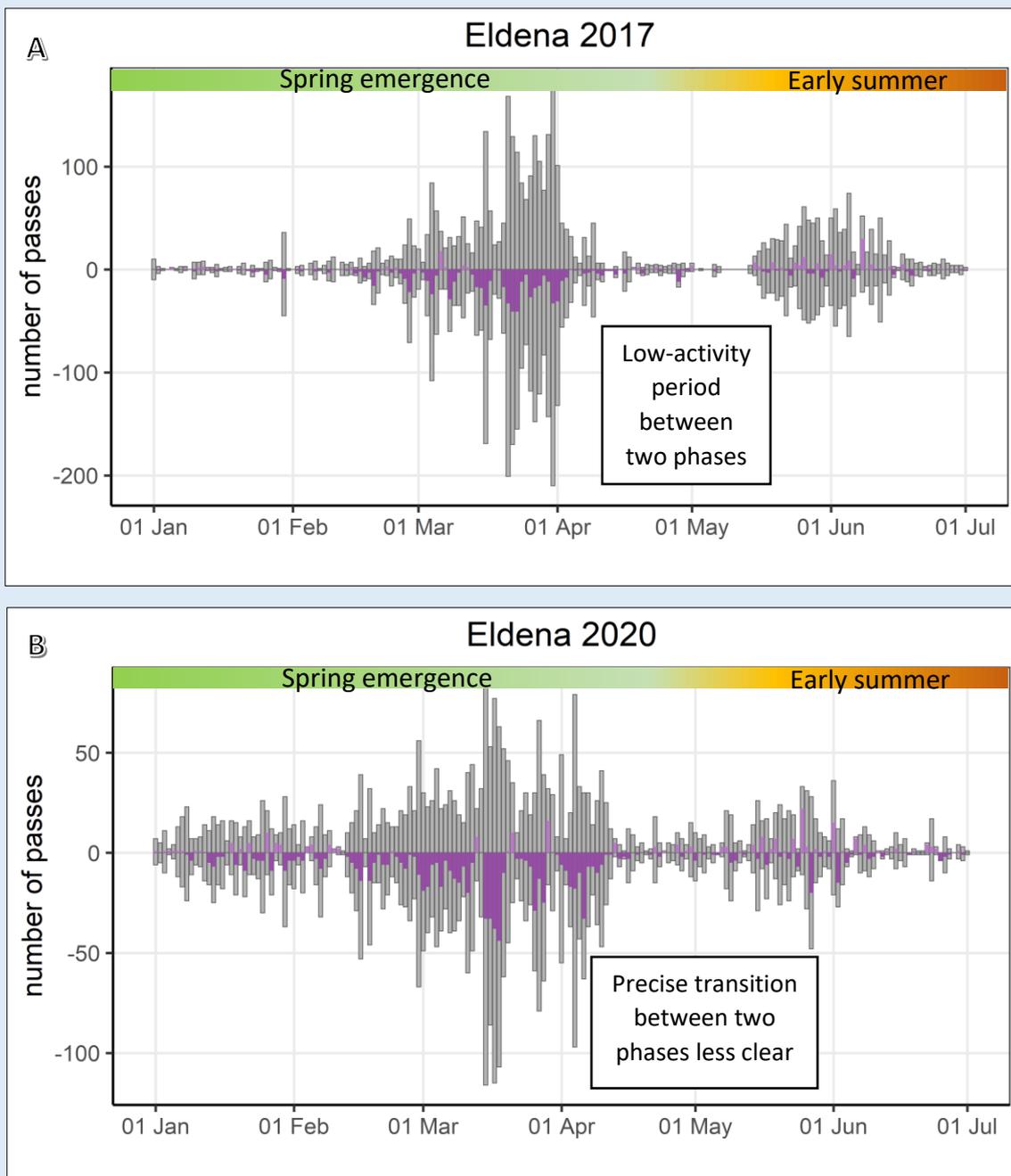


Figure 6. Light barrier data showing the spring emergence and early summer activity phases, collected in Eldena in **A)** 2017 and **B)** 2020. The number of passes registered each night are indicated with gray bars (entries on the positive y-axis, exits on the negative y-axis). The nightly net sum of all entries and exits is represented by the purple bars (net entry: light purple bars on positive y-axis; net emergence: dark purple bars on negative y-axis).

1.4. Study sites and species

For this study, we collected or were given access to bat monitoring data from 12 hibernation sites across Germany (Table 1), with most of the sites being located in Mecklenburg-Western Pomerania in northeast Germany. The type of collected data varied between sites, including data from infrared light barriers, camera traps, infrared video cameras, acoustic recorders, and visual surveys. Moreover, a curated collection of camera trap images from 33 other hibernacula was additionally used to develop an automated species identification tool. The monitored sites were mostly man-made structures, such as old cellars, bunkers, wells, and abandoned mines, but we also used light barrier data from one of the largest and most important natural bat hibernacula in northern Europe, the Kalkberg Cave (Kugelschafter et al., 2014). We specifically selected these hibernation sites to meet the following criteria: sites with one or two narrow entrances, where the dimensions of the existing opening did not have to be markedly reduced to fit an infrared light barrier (currently the maximum dimensions that can be covered with a light barrier is 35x300 cm). Conveniently, the entrances of many hibernation sites in Germany have already been reduced in size to prevent human access and disturbance (for examples see Figure 7). Therefore, installing light barriers at the entrance of these sites required only minimal modification.

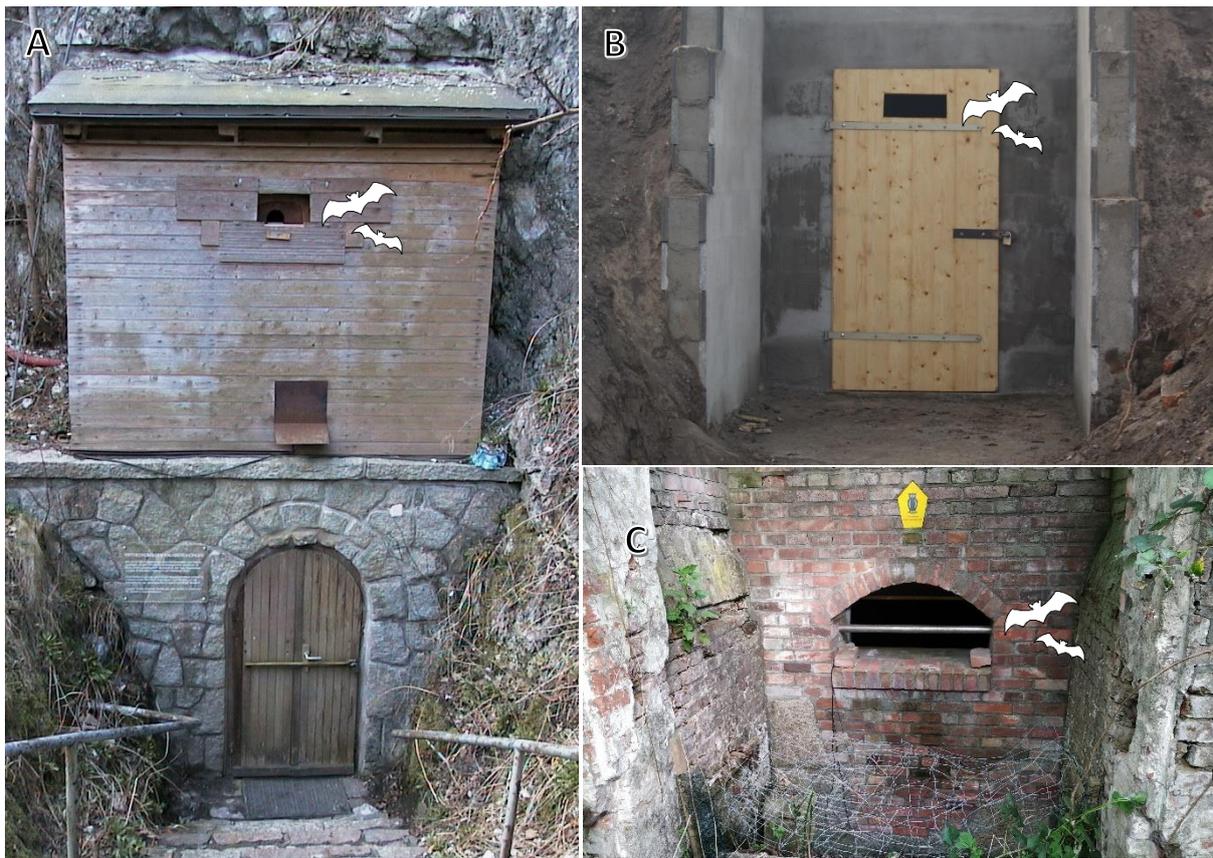


Figure 7. Entrances of the different types of hibernacula used by bats: **A)** a natural cave (Kalkberg Cave, photo: Karl Kugelschafter), **B)** a purpose-built hibernaculum in Demmin, and **C)** an old beer cellar in Eldena. The bat icons indicate the openings used by bats, where the infrared light barriers were installed.

Table 1. Overview of the bat hibernation sites monitored in this study, including their location within Germany, roost type, and the type of monitoring data collected at each site.

Site	Federal state*	Roost type	Light barrier (model, position)	Camera trap	Winter count	Video camera + acoustic recorder
Anklam	MV	cellar	Liba-4, within opening	✓	✓	✓
Demmin	MV	purpose-built hibernaculum	Liba-16k, within opening	✓	✓	✓
Friedland	MV	cellar	Liba-16, back of opening	✓	✓	✓
Peenemünde	MV	bunker	Liba-4, back of opening	✓	✓	✓
Eldena	MV	cellar	main: Liba-16, back of opening; Liba-16k, within opening	✓	✓	✓
			side: Liba-4, within opening			
Comthurey	MV	bunker	Liba-4, back of opening		✓	
Putbus	MV	cellar	Liba-4, within opening		✓	
Strasburg	MV	cellar	Liba-4, within opening	✓	✓	
Trollenhagen	MV	bunker	main: Liba-16, within opening		✓	
			side: Liba-16k, within opening			
Kalkberghöhle	SH	natural cave	main: Liba-16k, within opening		✓	
			side: Liba-16k, within opening			
Baumberge 1	NRW	well	Liba-16, within opening			
Baumberge 2	NRW	well	Liba-16, within opening			
33 other hibernacula	across Germany	cellars, bunkers, and mines	Liba-4, Liba-16 or Liba-16k	✓		

* German federal state abbreviations: MV - Mecklenburg-Western Pomerania, NRW - North Rhine-Westphalia, SH - Schleswig-Holstein.

Depending on the entrance dimensions, we selected the most suitable light barrier model: Liba-4 for smaller, Liba-16k for medium, and Liba-16 for larger entrances. In general, we aimed to minimize modifications to the entrance, thus, we integrated the light barrier within the existing opening if it was possible. If the opening was too small, we mounted the light barrier on the inner wall of the hibernaculum, directly behind the back of the opening (compare the positioning of the Liba-16k and Liba-16 in Eldena, Figure 8A). When the light barrier could not be installed directly at the entrance, mainly due to safety reasons, it was installed in a wooden frame before any branching or alternative paths, maximum a few meters away from the entrance (Figure 8B). At sites with multiple entrances, all openings were monitored, so no bat could enter or leave the site without flying through a light barrier.

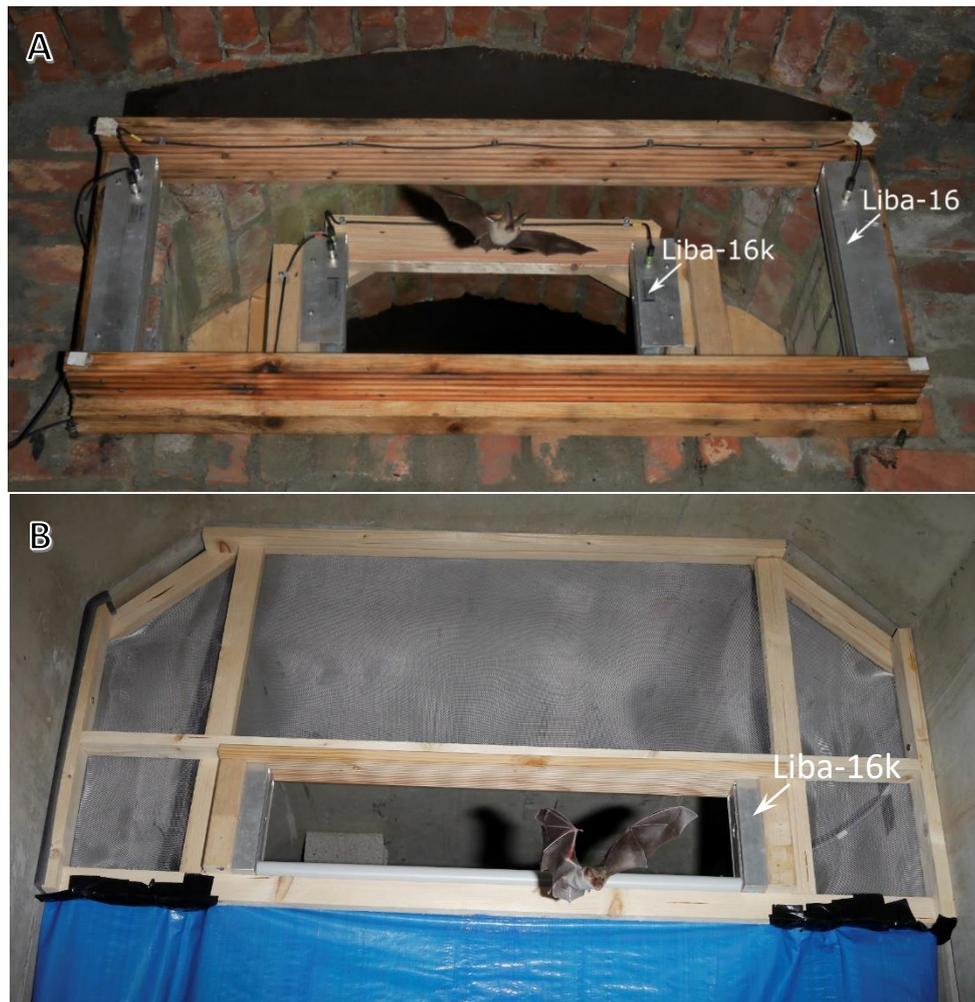


Figure 8. A) The main entrance of a hibernaculum (Eldena) showing two light barrier models in situ: a Liba-16k integrated within the existing opening and a Liba-16 mounted on the inner wall of the hibernaculum, directly behind the back of the opening. The camera trap was triggered by a brown long-eared bat (*Plecotus auritus*). **B)** A Liba-16k light barrier installed in a wooden frame a few meters away from the entrance of the hibernaculum. The camera trap was triggered by a greater mouse-eared bat (*Myotis myotis*).

The monitored hibernation sites largely differed in terms of their inner structural complexity. Purpose-built or concrete-made hibernacula, such as Demmin and Comthurey, had little to no crevice formation where the bats could potentially hide during the visual counts (Figure 9A). In cellars and bunkers, the amount of deep crevices was highly variable between sites. Some sites, like Eldena and Trollenhagen, had so-called cavity walls (i.e., wall with hollow center) that are accessible to bats via small openings, which made it impossible to visually count them (Figure 9B). Certain hibernacula, like the one in Strasburg, had inaccessible sections because parts of the site have collapsed (Figure 9C), while other sites were completely inaccessible for visual surveys, like the 60-m deep well shaft in Baumberge (Figure 9D).



Figure 9. The inner structural composition of different hibernacula: **A)** with little to no crevice formations, **B)** with deep cavity-walls (arrow indicates the opening where bats can enter into the hollow wall), **C)** with inaccessible sections, and **D)** a 60-m deep well that is inaccessible for visual surveys.

Besides the size and complexity of the hibernacula, the preference of different species to hide in small cracks and deep crevices also affects our ability to accurately count them during visual surveys. Based on their roosting preference, we can distinguish between free-hanging species that are relatively easy to count (Figure 10A), and crevice-dwelling species that can often hide quite effectively (Figure 10B, C). While these crevice-dwelling species are at best underestimated or not found at all during visual hibernation counts (Toffoli & Calvini, 2021), the light barrier and camera trap data is not affected by their visibility in the hibernacula. From the camera trap images, we can identify bats to species level, or as a species complex when the characteristics visible in the images are not sufficient to reliably distinguish between morphologically similar species (Box 3).



Figure 10. Bats in hibernation: **A)** a free-hanging mouse-eared bat (*Myotis myotis*), **B)** a brown long-eared bat (*Plecotus auritus*) hiding in a brick wall, and **C)** a Natterer's bat (*Myotis nattereri*) hibernating in a metal pipe.

Box 3. Bat species and species complexes identified from camera trap images in this study:

1) Barbastelle bat (*Barbastella barbastellus*), **2) Serotine bat** (*Eptesicus serotinus*), **3) Noctule bat** (*Nyctalus noctula*), **4) Bechstein's bat** (*Myotis bechsteinii*), **5) Pond bat** (*Myotis dasycneme*), **6) Daubenton's bat** (*Myotis daubentonii*), **7) Natterer's bats** (*Myotis nattereri*), **8) Geoffroy's bat** (*Myotis emarginatus*), **9) Common bent-wing bat** (*Miniopterus schreibersii*), **10) Whiskered bats:** *Myotis alcathoe*, *M. brandtii*, *M. mystacinus*, **11) Mouse-eared bats:** *Myotis blythii*, *M. myotis*, **12) Long-eared bats:** *Plecotus auritus*, *P. austriacus*, **13) Pipistrelle bats:** *Pipistrellus pipistrellus*, *P. pygmaeus*, and **14) Horseshoe bats:** *Rhinolophus ferrumequinum*, *R. hipposideros*.



Box 3. (cont.)**1.5. Objectives of the thesis**

The main goal of this thesis was to develop and evaluate automated methods for data collection and analysis that can improve bat monitoring and thus, conservation. Specifically, we investigated the use of infrared light barriers combined with camera traps to monitor bat population dynamics at hibernacula. Importantly, for this method to be applicable as a new large-scale bat monitoring technique, it had to meet the following three criteria: being minimally invasive because bats are sensitive to disturbance; being accurate because otherwise the misleading population monitoring data might lead to wrong or no conservation actions; and being scalable to increase the spatial and temporal resolution of the collected data.

The **first objective** was to evaluate the invasiveness of the monitoring method and investigate whether the white flash of the camera trap has any measurable short or long-term effect on bat activity and behavior. To do so, we ran an experimental field study over four months at four hibernacula in northern Germany. During the study, each hibernaculum entrance was monitored using a camera trap with white flash, an infrared light barrier, an infrared video camera and a full-spectrum acoustic logger. The flash of the camera trap was turned on and off every week at each site, which allowed us to compare the activity and behavior of bats between flash-on and flash-off nights. If the flash disturbs the bats, we expected 1) lower overall bat activity as measured by the light barrier, 2) change in the flight

direction of entering bats as measured using the infrared video data, and 3) change in the echolocation behavior of entering bats as a short-term response to the flash. In addition to this experimental approach, we also looked at the potential long-term effects of the flash at a site with two entrances, where one was monitored with a camera trap and a light barrier, and the other one only with a light barrier over six years. If the flash would have caused substantial disturbance to the bats, we expected that 1) bats should show a preference for the entrance monitored without a camera trap, or 2) in the worst-case scenario, some individuals may abandon the site, which would ultimately lead to the reduction of annual population size estimates in the long-term. Collectively these data allowed us to determine if this photo-monitoring system can be considered a minimally invasive bat population monitoring method, without any short- or long-term effects on natural bat behavior.

After evaluating and confirming that the monitoring method is minimally invasive, the **second objective** was to quantify how accurate the infrared light barriers are, and to develop a standardized data analysis pipeline that provides comparable and reproducible results for bat population monitoring. First, we quantified light barrier accuracy and described how it varies with model type, positioning, and season. To do so, we monitored six light barrier installations with infrared video cameras over 15 weeks during the autumn hibernation entry phase and 15 weeks during the spring emergence phase. For each pass registered by the light barrier, a corresponding short video clip was cut from the continuous video data. Subsequently, these video snips were manually evaluated to determine whether the light barrier registration was correct or not. Next, we standardized the estimation of population sizes using the light barrier data. This required evaluating the possibility of generating population size estimates based on the autumn entry or the spring emergence data, and developing a method to delineate the start and end dates of these different activity phases in a standardized way ([Box 2](#)). Using the developed pipeline, we then estimated bat population sizes at 12 hibernacula in northern Germany and compared these estimates to traditional visual counts at ten sites, and to population size estimates based on the infrared video data at five sites. Since light barriers are not affected by the visibility of bats in the hibernacula, unlike visual surveys, we expected that 1) winter counts underestimate bat population sizes to a varying degree, based on the complexity of the hibernacula (i.e., amount of crevice formations and cavity walls where bats remain invisible during visual surveys), and that 2) light barriers can accurately estimate the true bat population sizes, thus, will overlap with the video-based population size estimates. Finally, we used the video data to calculate a confidence interval around the population size estimates that accounts for the measurement error of the light barrier at each video-monitored site. Moreover, we developed a method that can approximate these confidence intervals around the light barrier-based population size estimates even at sites where video data were not available. Applying such a method to population size estimates over multiple years allowed us to distinguish true population trends from measurement uncertainty, which could not be quantified in the case of visual counts. Consequently, we used the light barrier-based population estimates with approximated confidence intervals to explore population dynamics at four sites for which 5-6 years of continuous light barrier data were available. Overall, these results enabled us to quantify the

accuracy of this novel, automated bat monitoring system and propose a standardized methodology to make use of the resulting data for site-level population monitoring purposes.

Finally, to integrate light barriers and camera traps into large-scale monitoring programs, we require efficient solutions to analyze the hundreds of thousands of camera trap images collected every year. Therefore, the **third objective** was to overcome the bottleneck of manual image analysis using automated solutions to identify bat species from camera trap images. To achieve this, we developed an open-source, deep learning-based tool (named *BatNet*) for automated identification of 13 European bat species or species-complexes, that encompass all species commonly observed at underground hibernacula in Germany. For the widespread adoption of this novel tool, it was crucial to demonstrate its ability to be retrained for new locations and to add new species from within a coding-free graphical user interface. The final model performance was evaluated in terms of 1) accuracy on test images of all 13 species from previously seen backgrounds, 2) accuracy on test images from six unseen backgrounds, before and after retraining the model on the site, 3) accuracy on test images (of the 13 original species and one new species) after retraining the model to recognize an additional species (*Miniopterus schreibersii*), and 4) in an ecological case study, in terms of species-level ecological metrics (i.e., diversity, relative abundance, and phenological estimates) compared between the human and *BatNet* results. Taken together, these results allowed us to evaluate the potential of *BatNet* to speed up the camera trap image analysis while achieving high accuracy, both in terms of species-level identifications and ecological measures that are relevant for bat monitoring and conservation.

2. Synthesis

2.1. General findings and discussion

This dissertation investigated the use of automated monitoring methods and data analysis tools to improve bat population monitoring and thus, conservation. Specifically, we focused on the use of infrared light barriers and camera traps to monitor bat population dynamics at hibernacula, as well as deep learning-based solutions to automate the analysis of the resulting large camera trap datasets. To support the widespread use of this automated bat monitoring system, we first evaluated the invasiveness of the white flash of camera traps that are connected to the light barriers. Second, we quantified the accuracy of infrared light barriers in terms of recording bat passes and estimating population sizes and trends. Finally, we developed standardized and automated data analysis pipelines, both for the light barrier and camera trap data, to support scaling up bat monitoring efforts both in space and time.

In **Publication I** we found that bats did not show any observable negative reaction to the use of camera traps with white flash at hibernation sites. Specifically, when comparing flash-on and flash-off nights, we found that nightly bat activity was not affected by the flash, but it decreased in relation to an abiotic environmental factor: the duration of rain per night. Similarly, flight direction of bats entering the hibernaculum was not affected by the flash, but we did observe changes based on the presence of other bats, likely due to chasing and avoidance behavior. Likewise, we observed no difference in the latency of the first echolocation call after the camera trap trigger between nights when the flash was turned on and off (i.e., no discernible startle reaction). In terms of the potential long-term effects of the flash, we did not find a decreasing trend in overall light barrier activity or winter hibernation counts, as would be expected if the flash caused substantial disturbance to the bats. Moreover, at a site with two entrances, bats showed no increasing preference over the years for using the entrance monitored only with a light barrier (without flash), over the entrance which was also monitored with a camera trap (with flash). Taken together, this multi-faceted experimental approach allowed us to establish that camera traps with a white flash, connected to infrared light barriers, are a minimally invasive method for monitoring bat populations, without any short- or long-term effects on natural bat behavior.

In **Publication II** we quantified light barrier accuracy and how it varies with light barrier model, positioning, and season. Using infrared video surveillance as a control, we found that light barriers with high sensor density integrated within the entrance opening could achieve nearly perfect accuracy in registering directional bat passes (i.e., entry and exit) during the spring emergence phase. Therefore, we developed an analysis pipeline to use the resulting spring data to estimate bat population sizes and trends in a standardized way. This involved developing a method to flexibly determine the start and end dates of the emergence phase based on the observed activity, which ensures that light barrier estimates are comparable even between years and between sites where the timing of the emergence varies due to the latitude and species composition. Next, we compared the population sizes estimated using light barrier data to video-based population estimates and traditional visual counts at several

hibernacula. We found that light barrier estimates overlapped with the video-based estimates, confirming that light barriers accurately estimated population sizes. In contrast, visual counts markedly underestimated population totals and recovered as little as 3% of all bats at the most complex hibernacula, such as natural caves. When comparing population trends over six years, the light barrier-based trends showed regional patterns of growth and decline that were not detectable using the visual count data. Moreover, we could use the light barrier accuracy to calculate confidence intervals around the light barrier-based population size estimates. This allowed us to distinguish true population trends from measurement error, which was not possible with the visual count data. Based on these results and the proposed analysis pipeline, we established that infrared light barriers can be used to estimate the population sizes and trends of hibernating bat assemblages with unprecedented accuracy and in a standardized way.

In **Publication III** we developed and presented a deep learning-based tool, *BatNet*, that can automatically identify bat species from camera trap images (for further details see [Box 4](#)). We showed that the baseline model performance was high across all 13 European bat species or species complexes on which the model was trained. The accuracy was high for trained sites, but also for untrained sites when the camera angle and distance from the entrance were comparable to the training images. At untrained sites with more atypical camera placements, we demonstrated the ability to retrain the baseline model in a coding-free environment within a graphical user interface. As a result of retraining, site-specific models achieved an accuracy comparable to the trained sites. Additionally, we showed that the baseline model can be also retrained to identify new species, while maintaining high classification accuracy for all original species. Finally, in an ecological case study, we established that *BatNet* can be used to accurately describe species-level ecological metrics from camera trap images, such as species diversity, relative abundance of species, and species-specific phenological estimates. Altogether, we demonstrated that *BatNet* can significantly speed up the camera trap image analysis process while achieving high accuracy, both in terms of species-level identifications and ecological measures that are relevant for bat monitoring and conservation. Moreover, the user-friendly interface, and the possibility to retrain the model for new sites and species within it, makes *BatNet* readily accessible for users without any programming knowledge.

Combining the results of these three publications, we established that the use of camera traps and infrared light barriers is a viable method to monitor bat populations at hibernacula. In order to understand the potential benefits and real-world application of this method, it must be contextualized and compared to other common monitoring methods, namely acoustic surveys, visual hibernation counts, and swarming captures. In the following section, we compare these methods in terms of their invasiveness, the type of data they provide, their accuracy, the associated costs and the effort required to collect and analyze the data, for individual sites and at large geographic scale (for summary see [Table 2](#)).

Box 4. Automated bat species identification from camera trap images using *BatNet*

Deep neural networks can be trained to automatize image-based species identification, and *BatNet* was specifically developed to automatically identify bat species from camera trap images. *BatNet* is composed of three distinct stages: detection, segmentation, and classification. First, an object detector network locates the bat in the image and places a bounding box around it (Figure 11A). Second, the image is cropped to the bounding box and a segmentation network removes the background (Figure 11B) to ensure that the actual bat characteristics are used for classification in the next step and not the background features. Third, an ensemble of three neural networks makes a prediction for the cropped bat image, which consists of a species label and a confidence value (0-100%), indicating the level of certainty in the species identification (Figure 11C).

In this study, the object detector and the segmentation network were trained on camera trap images collected at 32 hibernation sites (i.e., trained sites). Since both these steps require the networks to distinguish between the bats and the background, their performance is expected to be lower at new, untrained sites, where the background characteristics are different from those previously learnt from the training images. The classifier networks were also trained on these 32 backgrounds with images of 13 European bat species or species complexes, and we refer to this final model as the ‘baseline model’. This baseline model can be retrained in a graphical user interface to expand the species list that the classifier can identify. Moreover, this approach can be used to create site-specific models at new sites where the background does not resemble of those in the training dataset, which is expected to improve the performance of both the detector and the segmentation network.



Figure 11. The three main steps of automated species identification with *BatNet*: **A)** detecting the bat in the image with an object detector network, and placing a bounding box around it; **B)** cropping the image to the bounding box, and removing the background around the bat with a segmentation network; and **C)** identifying the bat species and the corresponding confidence level, using an ensemble of three classifier networks.

2.1.1. Invasiveness

Since bats are especially sensitive to human activities and disturbance (Kunz et al., 2009; Tuttle, 2003), when choosing a monitoring method, we have to first ensure that the process of data collection does not affect their natural behavior, and either causes them to abandon the monitored site or in the worst-case scenario, reduces their survival rate.

In terms of the disturbance caused to bats, passive acoustic surveys can be considered the least invasive monitoring method, because they do not require the presence of an observer on site or any direct contact with the monitored bats (Battersby, 2008).

In comparison, visual in-person hibernation counts can be considered potentially disturbing, because the non-tactile stimuli caused by the observers, such as light, noise, air currents and increase in temperature, may evoke arousal of hibernating bats (Davis, 1970; Pflitsch & Piasecki, 2003; Speakman et al., 1991; Thomas, 1995). These arousals can lead to less efficient hibernation and premature depletion of fat reserves in disturbed individuals (Johnson et al., 1998; Speakman et al., 1991; Thomas, 1995). Moreover, disturbed bats may also wake up other individuals within the hibernacula, spreading this energy stress across a larger part of the population as a cascade effect (Thomas, 1995), and potentially lowering the overwinter survival rate of the population (Johnson et al., 1998).

Compared to visual surveys, capturing bats with mist-nets or harp-traps is associated with potentially even higher degrees of disturbance because they require handling the bats (Battersby, 2008). Additionally, captures are sometimes combined with marking (e.g., rings, RFID-tags) and/or other sampling (e.g., wing punches, blood sampling). These actions expose the bats to even higher level of stress and may in some cases reduce long-term survival (e.g., Baker et al., 2001).

We argue that light barriers and camera traps are only marginally more invasive than passive acoustics, because they do not require the presence of an observer or direct contact with the bats. Moreover, in Publication I, we demonstrated that the use of camera traps with white flash has no measurable effect on the bats in terms of their activity or behavior. However, since the system does produce a potentially disturbing stimulus (i.e., flash) and requires some degree of entrance modification, further notes on its invasiveness are considered below.

At first glance, the lack of flash effect may appear surprising for two reasons. First, several other studies have found startle reactions in mammals to the white flash of camera traps (Gibeau & McTavish, 2009; Schipper, 2007; Séquin et al., 2003; Wegge et al., 2004). Second, the hibernacula investigated in this study were dominated by *Myotis* species that are generally considered light aversive (Voigt et al., 2018), and show a strong negative reaction to even low levels of constant illumination (Azam et al., 2018; Zeale et al., 2018). We posit that the lack of a flash effect observed in this study can likely be explained by the comparatively weak (power 1/16) and short duration (1/5500 s) of the flash. Therefore, it is important to note that changing the monitoring setup, in particular the brightness, length and frequency of the flash, could potentially result in undesirable consequences. For example, there might be a threshold in terms of the number of flashes per night, at which bats may start to react to the stimulus. While technically the camera trap and flash can be triggered every 20 ms, the sites included in this study were of moderate sizes (populations ranging from 100 to 400 bats), where the flash was 'only' triggered a maximum of 400-500 times per night during the highest activity

peak in autumn. However, at hibernacula with larger populations, particularly during the autumn swarming, the camera trap might be triggered up to 4000 times a night. This could potentially lead to such high frequency of flashes that it might be perceived as almost constant light by the bats. As we do not know whether this would have a negative effect on bats, we recommend being cautious at new sites that are expected to have large populations. This caution could be incorporated in practice by designing stepwise installation protocols for monitoring new sites where no previous information on activity is available. In such cases, one could first only install a light barrier to gather information about the general activity levels. At sites with substantially higher activity than those included in this study, as a next step, infrared video cameras could be temporarily installed together with the camera traps to observe the behavior of bats and investigate any potential reactions to the monitoring system.

Another important aspect to consider is that the effect of flash in this study was investigated only at a population level. The reason for this is that species-level identification could only be reliably achieved from camera trap images when the flash was turned on, and individual-level recognition was not possible from any of the data sources. Consequently, in rare cases, individual- or species-level aversive reactions may have remained undetected. In this context, it is worth noting that bats turning around and leaving the site right after being exposed to the flash (i.e., U-turns) were exceptionally rare, which was considered the most extreme potential behavioral reaction of an individual to the flash. Therefore, highly negative reactions to the flash appear unlikely for the species that were present at the hibernation sites in this study. This notably includes *Myotis myotis*, which is one of the most well-documented light aversive bat species (Voigt et al., 2018).

Beyond the flash of the camera trap, another potential impact that this automated monitoring system may have on bats is the reduction of the hibernacula opening to a size that allows installing a light barrier (currently max 35x300 cm). While such entrance modifications can be beneficial due to minimized anthropogenic disturbance, substantially reducing the size of the existing opening of a hibernaculum can affect both bat behavior and abundance (Pugh & Altringham, 2005; Spanjer & Fenton, 2005). Therefore, light barriers can be considered a minimally invasive monitoring method at hibernacula where their installation does not require large-scale entrance modifications. In many cases, hibernaculum entrances have already been reduced in size to limit human access, and thus, their entrances do not need to be substantially modified to enable light barrier installation.

Overall, despite being a permanent monitoring installation, the entrance modifications required to install a light barrier and the effect of camera traps with white flash appears to be minimal at most, certainly when compared to the alternative of winter hibernation counts and captures. Furthermore, the effect of the flash is limited to individuals that actively trigger the camera trap by flying in and out of the hibernacula, in contrast to the potential disturbance to bats in torpor caused by visual surveys.

2.1.2. Data type and accuracy

Next, we compare the data generated by different bat monitoring methods (i.e., swarming captures, acoustic surveys, winter counts, infrared light barrier combined with camera traps), in terms of their accuracy and the ecological metrics that they can provide. Specifically, the resulting data are most often used to describe species diversity, to estimate population sizes and trends, and to quantify changes in species-level activity patterns and phenology.

2.1.2.1. Species diversity

The first step of monitoring is to identify where the populations are that we want to protect and thus, monitor. Therefore, monitoring efforts should focus on the spatial distribution of species and site-level species composition.

Captures at underground sites during the autumn swarming phase have the advantage that the species of the captured individuals can be always reliably identified. This is also true for cryptic species, which can only be reliably distinguished in the hand (Dietz & von Helversen, 2004). Moreover, handling bats offers an opportunity to determine additional features of the captured bats, such as sex, age group, or body condition. However, it is important to consider that the capture probability of different species, demographic groups and even individuals may vary, with some being better at avoiding certain types of capture methods (Ferreira et al., 2021), which may bias the recorded species composition.

The ability to reliably identify species from acoustic data has massively improved over the past decade (e.g., Russ, 2021), but still faces several. Traditionally, acoustic data were collected actively, where the surveyor followed the bat with an acoustic detector and thus, could optimize the method by changing the orientation of the microphone relative to the bat (Britzke, 2004). As a result, this method yields high-quality echolocation calls that make species identification more reliable. More recently, passive acoustic sampling schemes have been developed to record bat activity throughout the entire year without the presence of a surveyor (Froidevaux et al., 2014). Although passive acoustic setups allow large data volumes to be collected with minimal human effort, the quality of the data is limited due to the fixed position and the lower quality of the microphone used in most passive recorders. While acoustic data can be used to reliably distinguish certain morphologically similar species, like pipistrelle bats (Russ, 2021), distinguishing calls of many other bat species is not always possible due to intraspecific call variation or interspecific overlap in the call features (Russo et al., 2018). For example, *Myotis* species that dominated the hibernacula monitored in this study can be challenging to reliably distinguish acoustically, especially when many individuals are concurrently calling (e.g., Bergmann et al., 2022). Moreover, reliable acoustic detection of some species is challenging because they emit short and quiet echolocation calls (e.g., *Plecotus* species, which are known as the “whispering bats”; Russ, 2021).

Visual count data from hibernacula can also be used to describe the species composition of hibernating bat assemblages. However, it is important to note that these data are affected by the visual detectability of bats, which varies with the available hiding places in the hibernacula, but also with the different preferences of bat species to hide in crevices. For example, free-hanging or clustering species, like *Myotis myotis*, are easier to count, while

crevice-dwelling species, like *Myotis bechsteinii*, are more likely to be undercounted or completely missed during visual surveys (Toffoli & Calvini, 2021). Moreover, some species (e.g., *Barbastella barbastellus*) are not necessarily present at underground sites for the entire hibernation period but shift between man-made objects and natural roosts, such as tree cavities, depending on winter temperatures (De Bruyn et al., 2021). As a result, winter hibernation counts might underestimate the importance of the site for some species that are not present or recorded on the given day when the visual survey is performed at the site.

In contrast to visual counts, camera traps installed at the entrance of hibernacula are not affected by the visibility of bats, thus can reliably detect rare and crevice-dwelling species as well. However, similar to acoustics and visual counts, certain morphologically similar species cannot be distinguished from camera trap images to species level, but only as a species complex (e.g., whiskered bats). Despite this limitation, camera traps outperform the other monitoring methods for describing species diversity at hibernation sites for several reasons. First, because the detection probabilities do not vary between species when using camera traps. Second, the fine temporal resolution of the data allows us to describe species-level use of underground sites across all seasons, even outside of the hibernation period. Finally, accurate identification of species and species complexes from camera trap images is also feasible using automated solutions.

Previously, the primary limitation to using camera traps for bat monitoring was the need for experts to manually identify thousands of images per site every year. We addressed this limitation by developing *BatNet*, a deep learning-based tool presented in Publication III. By using *BatNet*, we were able to automatically identify 13 European bat species and species complexes with high accuracy (F1-score range: 0.98-1) on test images. Using real-world data, we established an analysis pipeline to describe the species diversity at a hibernaculum in an automatized way using the output of *BatNet*. To do so, first we filtered out *BatNet* identifications with a confidence level below 95% to minimize the proportion of false positive errors while still retaining each species, including the rare ones. Next, we manually reviewed images for species that constituted less than 1% of the total dataset to eliminate the small number of remaining false positives. Applying this method, *BatNet* results provided the same species diversity as human experts at all study sites, and manual review was required for only 0.1% of all images.

2.1.2.2. Population size and trends

When the populations to be monitored are identified, the next goal is to gather information about the current size of the population, and track population trends over time.

For estimating the population size of hibernating bat species, the use of acoustic data is limited, because thus far the recorded activity data could only be used to generate a relative index of bat activity, but not to directly estimate absolute population sizes or absolute population trends. Similarly, swarming capture data collected at hibernation sites cannot be directly translated into population-level estimates. Potentially, comparing acoustic or capture data over several years could give an indication about the population trends compared to a reference year (i.e., indexed population trends), but the effect of confounding factors, such as

weather effects, remain unknown. Moreover, since species have different detection probabilities, these methods are not suitable to compare absolute abundance or activity levels between species (Thomas & Davison, 2022).

Currently, the most widespread method for assessing the population dynamics of European bat species is winter hibernation counts. These visual counts estimate the absolute number of hibernating bat assemblages and the abundance of each species within these assemblages. However, the main limitation of visual counts is that they are point estimates that reflect only the visible proportion of the population on a given day. In Publication II, we found that in comparison to light barrier estimates, visual counts account for as little as 3-10% of all the bats at the most complex hibernation sites with many hidden crevices and cavities. Predicting *a priori* the accuracy of a winter count at a site is not possible, for several reasons. First, we found that the visually counted percentage of the population substantially varied within a site between years and, even between hibernacula that have similar inner structural complexity. Second, besides the amount of crevice formations, it has been previously shown that the visibility of bats is also affected by observer effects (i.e., survey effort and experience; Dambly et al., 2021), weather conditions (Degn, 1987; Toffoli, 2021) and by the timing of the count within the season (Daan, 1973; Řehák et al., 1994; Zukal et al., 2017) as a result of internal bat movements within the hibernaculum. Finally, the roosting preferences of the species (i.e., free-hanging vs crevice-dwelling) may lead to biased relative abundances of species, and in some cases vast underestimates of the total hibernating population. Nevertheless, when data from many sites are combined, robust overall population trends are possible for some species (Van der Meij et al., 2015).

In this context, infrared light barriers constitute a vast improvement, as they provide absolute population size estimates and trends that are not affected by the visibility of bats. In Publication II, we found that the entries and exits registered with light barriers during the spring emergence phase can be summed to accurately estimate the number of bats that successfully emerged from hibernation. Using video recordings as a control, we found that light barriers with high sensor density and integrated within the entrance provide the most accurate population size estimates. As a result of the standardized analysis pipeline presented in Publication II, these light barrier-based estimates are also comparable between sites and years. Moreover, the light barrier activity data can be used to calculate a confidence interval around the light barrier-based population size estimates, which allows distinguishing true population trends from measurement error.

The primary limitation of these analyses is that they currently only measure site-level population estimates, and not species-level trends. To obtain species-specific estimates, a camera trap can be connected to the light barrier, which takes an image of each bat that enters or leaves the hibernaculum. Despite the possibility for automated species identification from the images, partitioning these site-level estimates into species-level population sizes and trends remains challenging for several reasons. First, collecting 10 camera trap images of a species could be either a result of one bat flying in and out of the hibernaculum 10 times, or 10 individuals entering the site once. Second, the number of times a bat flies in and out of the hibernaculum before finally emerging in spring may vary between individuals, species, sites, light barrier models and positioning, and it may also change with weather conditions. Therefore, we cannot just simply take the relative abundances of species from the camera

trap images and partition the total population estimate into species-level estimates. To overcome this challenge, a potential solution would be to install two camera traps (i.e., 'entry' and 'exit' camera) connected to the light barrier: one being triggered when bats enter the site, and the other when they exit. Taking the net number of images per species from the two cameras could provide species-specific population estimates. However, confidence intervals should be calculated around these estimates to account for light barrier error (i.e., missing images as a result of some passes not being registered by the light barrier, and extra images due to false triggers by bats circling on the inside of the light barrier). This could be achieved similarly as our proposed method to calculate confidence intervals around the site-level population size estimates based on light barrier accuracy.

2.1.2.3. Phenological estimates

Tracking changes in the phenology of species with strong seasonal dynamics is crucial to understand how different species optimize their behavior to avoid unfavorable environmental conditions (i.e., low temperature and reduced prey availability), but at the same time minimize the ecological and physiological costs of hibernation (Meyer et al., 2016; Willis, 2017). In this context, describing the hibernation phenology of temperate zone bats is essential to understand how global climate change might affect the population dynamics of different species (Festa et al., 2023; Reusch et al., 2022). From a conservation perspective, such phenological information can also help identify sensitive periods when disturbance should be avoided at hibernacula (e.g., Meier et al., 2022). Finally, investigating the hibernation length of bats is relevant for predicting the impact of emerging infectious diseases on temperate zone bat species, such as white-nose-syndrome (Puechmaile et al., 2011).

One way to investigate species-level phenology of temperate zone bats is passive acoustic surveys (Kotila et al., 2022; Thomas & Davison, 2022). However, this method remains challenging because call intensity and thus, acoustic detectability varies between species, and correctly distinguishing certain species only based on echolocation calls is not always feasible (Russ, 2021). Moreover, species-level identification of many individuals echolocating simultaneously (i.e., during autumn swarming) remains extremely difficult due to the high overlap between call sequences (Rydell et al., 2017), only allowing the detection of the most dominant species from swarming soundscapes (Bergmann et al., 2022).

Systematic repeated captures during the autumn or spring swarming phases allow for a general comparison of phenology within and between species (Furmankiewicz, 2008; Parsons et al., 2003; van Schaik et al., 2015). Besides the possibility for reliable species identification, captures at swarming sites provide additional information about the captured individuals (e.g., sex, age group), which may further affect their phenology. Similarly, repeated visual hibernation counts throughout the winter have been used to track the presence of species at underground sites (e.g., Daan, 1973). However, the biggest limitation of both methods is that the temporal resolution of the resulting data is very coarse. To increase the temporal resolution of phenological data, individualized RFID-tags can be implanted in captured bats which will be automatically registered by an antenna at the entrance of the hibernacula throughout the year. Despite the high data resolution provided by RFID-tags, such studies

have been limited to a few model species at single study sites (Meier et al., 2022; Norquay & Willis, 2014), because it is associated with extraordinarily high effort.

Infrared light barriers and camera traps allows us to track species-level activity patterns of bats at a spatial and temporal resolution that cannot be achieved by any of the other methods. Such an approach can be combined with automated deep learning-based species identification to speed up data processing. Indeed, in Publication III we found that phenological patterns of the investigated bat species (*Myotis daubentonii*, *M. myotis*, *M. nattereri* and *Plecotus auritus*) were nearly identical when images in a 5-month long dataset were identified by human experts versus automatically by *BatNet*. This means that the data from camera traps that are triggered by bats entering the hibernacula (i.e., 'entry' camera) can be automatically analyzed with *BatNet* to accurately describe species-level activity patterns of bats in autumn. However, to quantify and compare these autumn phenological estimates between species and years, the remaining challenge is that we lack standardized methods to separate the two overlapping autumn activity phases: the swarming phase and the hibernation entry phase. In contrast, the start and end dates of the spring emergence phase can be determined with the standardized methodology presented in Publication II. To investigate spring emergence phenology, ideally an additional camera trap should be connected to the light barrier that takes an image of each bat that leaves the hibernaculum (i.e., 'exit' camera), using the same setup as in autumn (i.e., 'entry' camera).

2.1.3. Scalability

Finally, monitoring population dynamics and phenology of bats at a small geographic scale allows only to identify local effects and threats for the populations, but to understand how different bat species are coping with the rapidly changing environment on a larger scale, we have to monitor many sites simultaneously. When choosing the most suitable data collection method for such large-scale monitoring programs, it is important to consider both the monetary and human costs associated with them.

While swarming captures are relatively low-cost (i.e., travel costs, capture equipment), handling bats is time intensive and requires experts to be present at the site for the entire survey night. Due to this high human effort associated with swarming captures, both their spatial and temporal scalability is relatively limited.

On the contrary, winter hibernation counts require a single visit per year by surveyors, who have experience with the morphological differences between hibernating bat species. The expenses and human effort associated with visual surveys are relatively low; therefore, it is a suitable method for large-scale, long-term bat monitoring. Indeed, the earliest bat population monitoring data available are based on winter counts and even today visual surveys are the most widespread monitoring method to track population dynamics of temperate zone bat species (Van der Meij et al., 2015). Moreover, these counts also serve an important public outreach function by engaging citizen scientists and volunteers in bat monitoring and conservation.

In contrast to these traditional monitoring methods, automated data collection methods can increase the spatial scale of monitoring efforts, because they do not require the presence of surveyors on-site. For example, passive acoustic surveys can provide continuous monitoring data, potentially at several sites and even over the entire year. Since passive acoustic detectors are medium to low-cost devices, large-scale acoustic surveys can be considered cost-effective. While passive detectors allow large volume of acoustic data to be collected with minimal maintenance effort, the resulting data quality is lower compared to active acoustic surveys and manually identifying the echolocation calls from the collected huge data volume is labor and time intensive. To reduce manual workload, several automated classifiers of echolocation calls have been developed (see examples in Rydell et al., 2017). Such tools can speed up data analysis, but it is important to consider that different species are expected to have different probabilities to be correctly identified based on their echolocation calls (Russo et al., 2018; Rydell et al., 2017; Thomas & Davison, 2022), especially when many bats of multiple species are calling simultaneously (Bergmann et al., 2022).

Similarly, infrared light barriers combined with camera traps also offer an automated monitoring approach that can be implemented at many hibernacula simultaneously, without the need for an observer to be present at the site. While the initial installation cost of such systems might be higher than for acoustic surveys, later the system can run with relatively low maintenance cost. Maintaining such monitoring systems consists of monthly visits to exchange power supplies at remote sites and backup the data. This effort is comparable to what would be needed for long-term acoustic monitoring; however, the data storage requirements are significantly lower for light barriers and camera traps. Moreover, in hibernacula with direct connection to electricity, the light barrier can be accessed remotely, providing constant access to the data, and allowing us to immediately identify technical issues

or potential threats for the bats. For example, near real-time activity data from hibernacula can be used to detect when the light barrier is blocked for an extended time (e.g., by a spiderweb) and cannot register bat passes. This feature is also useful for detecting sudden changes in bat activity that may be caused by imminent threats to the population, such as the persistent presence of a predator (e.g., an owl at the entrance) or unusual constant illumination of the entrance (e.g., during construction work directly adjacent to a hibernaculum entrance).

Increasing the spatial scale of monitoring efforts enables us to collect large volumes of monitoring data but interpreting such “big data” also requires efficient and standardized analysis tools. Therefore, in the context of light barrier data analysis, we proposed a standardized pipeline that ensures that the light barrier-based population estimates are comparable between sites and years, and that confidence intervals capture the measurement error. Moreover, this methodology includes recommendations on detecting potential outliers and dealing with missing data. In terms of efficiently analyzing camera trap images, we presented a highly accurate, deep learning-based solution for automated species identification. However, there are several important aspects to consider when using *BatNet* in practice to automatically analyze camera trap datasets.

First, performance of the baseline model (i.e., final *BatNet* model trained with 13 species on 32 backgrounds) may drop when we use it on images from a new site that was not included in the training data. This is particularly the case at sites where the camera placement (i.e., angle and distance from the entrance) are different from the training images. Overall, new installations are expected to be similar to the ones included in the training data, because the infrared light barriers and camera traps used in this study have default settings. Nevertheless, in cases when the baseline model performance is not satisfactory, retraining the model on new backgrounds is possible within the graphical user interface, without any programming knowledge. However, it is important to note that before inference, these new site-specific models should be first carefully evaluated on a batch of images that were identified by a human expert.

Second, we also have to consider that the baseline model can currently distinguish 13 species or complexes. In cases when the model is presented a completely new, untrained species, it can only assign a species from the list of trained species (i.e., no indication that it is a new species). However, when we have enough training images, it is possible to retrain the baseline model to recognize an additional species in the future. Indeed, we showed in Publication III that using only 58 local annotations, we can achieve high accuracy for a new species (F1-score: 0.99), while maintaining high classification accuracy for all other 13 species (F1-score range: 0.94-0.99).

Third, when using *BatNet* to describe ecological metrics, we have to decide on an approach on how to deal with the *BatNet* identifications that have a low confidence threshold, and the extra identifications of bats that are in the background and are normally not scored by humans. In this study, we decided to simply exclude all identification that were below a pre-defined confidence threshold (70%) and include all identifications above it, also including bats in the background. We found that this approach did not affect the results of *BatNet* compared to human experts in terms of species diversity, relative abundance, and species-specific phenology. However, depending on the purpose, manual review of identifications with low

confidence threshold might be required. Moreover, distinguishing the bat that triggered the light barrier from the bats flying in the background might be also necessary for certain applications. For example, to partition population-level estimates and trends into species-specific ones, we would need to identify all images from an entry and exit camera, including manual review of the ones that had low confidence thresholds. Although automatically isolating the bat that triggered the camera trap is currently not feasible, presumably the population size estimates based on the spring emergence are less affected by this issue, because images with multiple bats are most often taken during the autumn swarming period. Furthermore, even if images with low threshold and/or multiple bats are manually validated, the overall effort required to process camera trap datasets is substantially reduced when using *BatNet*.

Table 2. Overview of bat monitoring methods used at underground sites.

	Infrared light barrier and camera trap	Visual hibernation count	Bioacoustics	Swarming captures
Invasiveness	low (no effect of camera trap flash, but possible species- or individual level aversion in rare cases; minimal entrance modifications)	medium (arousals of hibernating bats evoked by light, noise, air currents, heat)	low (no direct contact with bats)	high (requires handling bats at minimum)
Species diversity	yes (even rare and elusive species, but some cryptic species can only be identified as a species complex)	yes (but crevice-dwelling species undercounted or missed completely)	yes (but misses species with faint calls, and certain species cannot be reliably distinguished)	yes (but capture probability varies between species and methods)
Data type and accuracy	yes (accurate because not affected by visibility of bats, confidence intervals account for measurement error; but only site-level estimates, potential for species-level estimates with double-camera trap setups)	yes (but inaccurate at large, structurally complex sites; potentially biased population trends; cannot quantify measurement error)	no (potential for indexed trends, but has not been investigated)	no (potential for indexed trends, but has not been investigated)
Phenology	yes (fine temporal scale, can be applied at many sites simultaneously)	no (usually only point estimate, in rare cases repeated with coarse resolution)	yes (large-scale, but misses species with faint calls, and certain species cannot be reliably distinguished)	yes (but coarse temporal resolution, limited to few sites)
Monetary cost	high (equipment and maintenance cost)	low (basic equipment and travel costs)	medium (equipment and maintenance cost)	medium (equipment and travel costs)
Scalability	medium (maintenance relatively high, but automated analysis of light barrier and camera trap data)	low (requires single yearly visit by group of experts or trained volunteers)	medium (maintenance relatively low, potential for semi-automated analysis to detect and identify calls)	high (requires the presence of experienced experts for full nights)

2.2. Conclusions and perspectives

Overall, the results of this thesis demonstrate that infrared light barriers combined with camera traps are a minimally invasive bat monitoring method that improves our ability to accurately monitor population dynamics and phenology of temperate zone bat species at underground sites. Moreover, the resulting camera trap images can be automatically analyzed with a deep learning-based image analysis tool (*BatNet*) to vastly scale up monitoring efforts over time and space. Based on the comparison with other monitoring methods outlined above (Table 2), it is evident that infrared light barriers and camera traps should be integrated into the toolbox of bat monitoring and have the potential to completely change how we think about bat monitoring. However, the real challenge still lies ahead of us: how do we implement this method in practice, and translate the resulting “big data” into evidence-based conservation?

2.2.1. Practical recommendations

Based on the results of this thesis and our experiences, we share some practical advice on how to best use infrared light barriers and camera traps, in combination with other monitoring methods, to improve our ability to collect accurate bat monitoring data on large temporal and spatial scales. For infrared light barriers and camera traps to be an integral part of large-scale bat monitoring schemes, first we need to identify a network of suitable sites where this method is the most useful, but also feasible. In Publication II, we found that the biggest gain of using light barriers as a monitoring tool is at structurally complex hibernacula with large bat populations, particularly at sites that are either completely inaccessible for visual surveys or have many areas that cannot be visually counted (e.g., cavity walls and deep crevices), and thus, hibernation counts are expected to be less accurate. To identify such sites, swarming captures, visual inspections or passive acoustic surveys can serve as useful “scouting methods”.

Beyond having high structural complexity, it is also important to consider the number of entrances at a site and their dimensions, when choosing light barriers as a monitoring method. First, sites with one or two entrances should be prioritized, because all openings have to be monitored with a light barrier to provide accurate population estimates. Second, infrared light barrier models used in this study (ChiroTEC, Germany) are only suitable to monitor sites with relatively small entrances (max 35x300 cm). Although this somewhat limits the range of suitable sites, most underground sites have already been closed off from the public, and thus, their entrances are suitably sized for light barrier installation. If the entrance dimensions are too large, light barriers can also be installed within a wooden frame a few meters away from the entrance, before any branching paths. For such installations, it is essential to cover the area around the light barrier with a fine mesh. This setup does not substantially change the microclimatic conditions of the site since it allows continuous airflow, but it ensures that all bats fly through the light barrier when entering or leaving the site. In such cases, the recorded bat activity is expected to be higher due to internal movements of the bats within the hibernaculum, which ultimately increases the number of collected camera trap images. Moreover, in addition to the standard ‘entry’ camera, such installations also allow us to connect an ‘exit’ camera to the light barrier on the inside of the hibernaculum, by minimizing the risk of theft or damage. While these double-camera setups further increase the number

of camera trap images to be analyzed, the automated species identification pipeline presented in Publication III can overcome this bottleneck of manual image analysis.

Overall, the benefits of using light barriers in comparison to winter counts are clearly demonstrated in this study. Nevertheless, it is not realistic to expect that in the future every bat hibernaculum will be monitored with such an automated monitoring system, due to the high cost and effort related to installation and maintenance. In contrast to light barrier monitoring, winter counts are a low-cost method, that can be relatively easily performed at large spatial scales. This is because visual counts require only a one-day effort every year from a few experienced people who are able to distinguish hibernating bat species. Moreover, as shown in Publication II, at smaller sites with comparatively few deep crevices and cavities, winter hibernation counts did not differ substantially from the light barrier-based population size estimates, suggesting that such sites can be reliably monitored by visual surveys.

Taken together, in the future, infrared light barriers should be considered a powerful complementary method to traditional visual counts that can cost-effectively monitor many smaller hibernation sites but are notoriously difficult and inaccurate at larger, more complex sites. To implement this approach in practice, we should first designate a network of hibernacula that meet the criteria of light barrier monitoring being both useful and feasible, and consider performing visual surveys for the remaining sites. The combination of these two methods allows us to accurately monitor the population dynamics of species that predominantly make use of underground hibernacula, notably including those that have previously been difficult to detect (e.g., *Myotis bechsteinii*; Toffoli & Calvini, 2021). Nevertheless, in certain cases we would still need alternative solutions. For example, monitoring hibernation sites where there are several huge entrances (e.g., some natural caves), or where we do not know all possible entrances (e.g., rock crevices in the ground; Lemen et al., 2016) cannot be monitored with either of these methods. For such sites, the most feasible methods we currently have for monitoring bat populations are passive acoustic surveys and captures.

2.2.2. Scientific and conservation implications

Besides ensuring that the monitoring method is non-invasive, accurate and scalable, it is also essential to consider how can the collected data be used to gain better understanding about the monitored ecological systems, and to contribute to data-driven conservation and management decisions (Yoccoz et al., 2001). Therefore, in the following section we outline the scientific and conservation implications of using light barrier and camera trap data collected at bat hibernacula.

Monitoring at hibernation sites of temperate zone bats species enables us to investigate how different bat species respond to climate change. This information is crucial both for better understanding bat biology and for designing effective conservation strategies (Festa et al., 2023). Thus far, studies about how hibernation timing differs between bat species were mainly based on individualized RFID-data, but such studies are limited to a few model species at single study sites due to their invasiveness and high monitoring effort (e.g., Meier et al., 2022; Norquay & Willis, 2014). Although light barrier and camera trap data do not provide such individual-level information, the method can be implemented over large geographic scales to

investigate climate change-related deviations in the hibernation phenology of different bat species. Such phenological changes may affect the population dynamics of hibernating species (Reusch et al., 2022; Wells et al., 2022), thus the light barrier and camera trap data can also be used to predict the future impact of global environmental changes on the persistence of different bat species. While such information is crucial to support biodiversity conservation, scientific knowledge on its own - even if it is based on accurate, large-scale monitoring data - might not be enough to make an impact. To make a positive change for biodiversity conservation, such scientific findings must be translated into conservation policy and on-the-ground action (Gluckman, 2016).

To achieve conservation success, biodiversity monitoring should not be considered as a standalone activity, but as a component of a larger process that creates a link between monitoring objectives and conservation actions (Lindenmayer et al., 2012; Robinson et al., 2018). Although most monitoring programs identify conservation as a rationale for collecting data, these programs very often do not lead to real-world changes in conservation practice. This suggests that there is a barrier that prevents the collected data and scientific evidence from being used by policy makers and managers (Walsh et al., 2019), which ultimately results in the lack of evidence-based conservation. Here, we highlight several ways through which we could overcome this barrier and successfully incorporate monitoring data into conservation practice.

First, we need to ensure that the scientific evidence is robust, and the quality of the data and their analysis is clearly communicated to practitioners in the field. Despite the limitations of some traditional monitoring methods, researchers and conservationists may be reluctant to adapt new methods when lacking relevant and transparent performance data (Hall & Fleishman, 2010). To reduce the uncertainty regarding the use of this new automated bat monitoring system, we demonstrated that infrared light barriers greatly improve the accuracy of population size estimates and trends of hibernating bat assemblages, in comparison to traditional visual surveys. Moreover, we showed that the combination of this automated monitoring system with deep learning-based species identification can improve our ability to accurately monitor species-level activity patterns and phenological estimates. However, such deep learning solutions are also often referred to as a “black box” (i.e., they are said to lack transparency or interpretability of how input data are transformed into model outputs), which is a key concern for their wider application. Consequently, the lack of sufficient evaluation of novel deep learning-based methods may prevent their acceptance by users in other disciplines than computer science (Hall & Fleishman, 2010). To overcome this challenge, we evaluated the performance of *BatNet* both in terms of traditional accuracy metrics on test images, but also using real-world camera trap datasets, in terms of ecological metrics (i.e., species-diversity and species-level phenology) that are relevant for conservation.

Second, jointly framing research questions and conservation goals can also contribute to closing the gap between science and conservation (Young et al., 2014). Such approach is essential, because it is extremely challenging to try to retrofit research outcomes to meet the needs of decision makers. In the context of bat conservation, the most basic objective is often to quantify population trends and begin to understand the drivers of population declines. Accurate monitoring at local and broader geographic scales (e.g., within the catchment area

of a species vs between distant catchment areas) can help identify the spatial scale of such declines. For example, if a decline is observed at an individual site within a local network, this suggests that the driver might be site-specific, such as predation or anthropogenic disturbance. On the other hand, if all the sites within a local network show similar declines in comparison to other regional networks, this suggests that the driver might be related to wide-scale impacts, such as extreme climatic events or changes in land-use and habitat quality. In this respect, light barrier monitoring can be scaled up to provide accurate population monitoring data from a network of hibernacula both on local and broad spatial scales. While this approach does not explicitly tell us what is causing the decline, it is an important first step to identify the potential drivers affecting bat population dynamics. In contrast, with inaccurate and biased monitoring methods, we would not even be able to detect such declines in the first place.

Third, even if we implement accurate and objective-oriented monitoring, the results must reach the right audience to be translated into practical action, and ultimately, to make any difference for biodiversity conservation. However, such evidence-based conservation cannot be achieved without improving the access of stakeholders to scientific evidence (Kadykalo et al., 2021). Unfortunately, research results are often exclusively reported in scientific publications, which can be hard to comprehend for stakeholders that are involved in developing policy and its implementation. For example, if the conclusions from the development of a new monitoring method are not communicated to those who might implement it, then the research effort was wasted. Therefore, it is crucial to maintain good communication between the data collectors and the stakeholders, and to tailor the language of communicating these results to audiences and policy contexts (van den Broek et al., 2020). For example, thus far, population trends of temperate zone bats were assessed based on winter counts, that are simple measures, making the results easy to comprehend. If we want to transform bat conservation to incorporate “big data”, such as infrared light barrier and camera trap data, then we have to make sure that the results are similarly easy to interpret. To achieve this goal, in Publication II, we presented a pipeline that provides standardized population size estimates using the light barrier data, which are comparable between sites and years, and account for measurement error. In Publication III, we presented an automated analysis pipeline for camera trap images, including opportunities for retraining the network for new sites and species without any programming knowledge, which makes the results readily accessible for conservationists and researchers. This is a huge step towards making these automated bat population monitoring data accessible to stakeholders and thus, translatable to conservation action.

To ultimately conclude, this study demonstrates the potential of a novel, automated monitoring approach to non-invasively and accurately track population dynamics and phenology of temperate zone bats at scale and illustrates how the collected data can be successfully applied to address real-world conservation issues. Such an approach is essential for bats in the Anthropocene, where the environment is changing so rapidly that the window of opportunity for action to prevent further biodiversity loss is becoming extremely narrow.

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4. Publication list

Accepted in peer-reviewed journals:

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Publication I

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**Camera traps with white flash are a minimally invasive method for
long-term bat monitoring**

ORIGINAL RESEARCH

Camera traps with white flash are a minimally invasive method for long-term bat monitoringGabriella Krivek¹ , Brian Schulze², Peter Zs. Poloskei³, Karina Frankowski¹, Xenia Mathgen¹, Aenne Douwes¹ & Jaap van Schaik¹ ¹Applied Zoology and Nature Conservation, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany²Animal Ecology, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany³Feldstrasse 79, Greifswald 17489, Germany**Keywords**Chiroptera, light barrier, long-term monitoring, mammals, *Myotis*, remote sensing, wildlife conservation**Correspondence**

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Remote Sensing in Ecology and Conservation 2022, **8** (3):284–296**Abstract**

Camera traps are an increasingly popular survey tool for ecological research and biodiversity conservation, but studies investigating their impact on focal individuals have been limited to only a few mammal species. In this context, echolocating bats are particularly interesting as they rely less on vision for navigation, yet show a strong negative reaction to constant illumination. At hibernacula, camera traps with white flash could offer an efficient alternative method for monitoring threatened bat species, but the potential negative impact of white flash on bat behavior is unknown. Here, we investigate the effect of camera traps emitting white flash at four hibernation sites fitted with infrared light barriers, infrared video cameras, and acoustic recorders over 16 weeks. At each site, the flash was turned off every second week. We quantified whether flash affected (1) nightly bat passes using generalized linear mixed models, (2) flight direction of entering bats using permutational multivariate analyses, and (3) latency of the first echolocation call after the camera trap trigger using randomization tests. Additionally, we quantified and corrected for the potential impact of confounding factors, such as weather and social interactions. Overall, white flash did not influence short- or long-term bat activity, flight direction or echolocation behavior. A decrease in nightly bat activity was observed with an increasing proportion of hours with rain. Moreover, flight direction was affected by the presence of other bats, likely due to chasing and avoidance behavior. Our findings highlight the potential of camera traps with white flash triggered by infrared light barriers as a minimally invasive method for long-term bat population monitoring and observation of species-specific phenology. Such automated monitoring technologies can improve our understanding of long-term population dynamics across a wide range of spatial-temporal scales and taxa and consequently, contribute to data-driven wildlife conservation and management.

Introduction

Reliable and cost-effective population monitoring of bats is critical given their key role in ecosystem functioning and as effective ecological indicators of environmental changes (Jones et al., 2009). Moreover, monitoring of bat population trends is legally required in most European countries (Battersby, 2010). Nevertheless, obtaining accurate estimates of population trends is challenging due to

the cryptic nature of bats (i.e. small size, nocturnal habits, ability to fly and hide effectively in crevices), and sensitivity to disturbance. Traditional bat monitoring relies heavily on time-consuming and invasive techniques such as captures with mist-nets and visual counts at winter and summer roosts (Kunz et al., 2009). Over the last decades, several automated bat counting techniques have been developed, offering more accurate and less invasive alternatives to traditional bat surveying methods. For example,

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thermal and infrared cameras combined with automated video processing methods have been used to accurately estimate population sizes during nocturnal emergence from roosting sites (Corcoran et al., 2021; Elliott et al., 2006; Frank et al., 2003; Sabol & Hudson, 1995), but due to high power and data storage requirements these methods remain unfeasible for widespread or long-term monitoring.

Remotely triggered cameras have become important survey tools for automated wildlife monitoring and their rapid improvement in recent years has transformed ecological research and biodiversity conservation (Wearn & Glover-Kapfer, 2019). Camera traps can be used to monitor population trends and community composition, which are fundamental requirements for efficient wildlife management and setting conservation priorities (Battersby, 2010). Although camera traps are often considered less invasive alternatives to traditional sampling methods that require capture or human presence (e.g. Sollmann et al., 2013), their novelty in an environment, as well as the light and sound they emit may also affect wildlife (Caravaggi et al., 2020; Meek et al., 2014). Several studies have found varying responses to camera traps among mammal species, including both avoidance behavior (e.g. coyotes, Séquin et al., 2003; kinkajous, Schipper, 2007; tigers, Wegge et al., 2004; wolves, Gibeau & McTavish, 2009) as well as curious visual exploration behavior (e.g. large felids, Kelly et al., 2012; apes, Kalan et al., 2019; feral cats, Meek et al., 2016; stoats, Glen et al., 2013). The use of infrared flash may constitute a less invasive alternative to white flash (Wearn & Kapfer, 2017), however, reliable and accurate identification of co-occurring species with similar characteristics is often more difficult (Burns et al., 2018). Improved understanding of how camera traps affect the behavior of focal individuals is necessary to minimize disturbance of automated monitoring methods.

Cameras with white flash automatically triggered by infrared motion detectors (Daan, 1970; Degn et al., 1995; Hope & Jones, 2013; Kugelschafter et al., 2014; Lubczyk & Nagel, 1995) and manually triggered cameras (Rydell & Russo, 2015) have been proposed as a viable method to monitor bat activity throughout the year. These studies either suggested that white flash has no negative impact on focal bats, or did not consider disturbance altogether. On the other hand, flash photography of bats emerging from roosts is banned at several touristic sites (e.g. Carlsbad Cavern, US) or permitted only with an appropriate license (e.g. United Kingdom), because the unexpected bright light might disturb the bats and may alter their emergence behavior. Similarly, Zukal et al. (2017) have asserted that the use of flash will negatively affect natural bat behavior. However, while the impact of light

pollution (i.e. continuous illumination) on bat roosting, commuting, and foraging activity has recently received increased attention (e.g. Barré et al., 2021; Russo et al., 2017; Spoelstra et al., 2018; Stone et al., 2015), to our best knowledge, the potential negative impact of white flash on bat behavior has never been systematically evaluated.

Here, we comprehensively examine the effect of camera traps with white flash on bats in an experimental field study over 4 months at four hibernacula in Northern Germany. At each site, an infrared light barrier was installed to measure nightly bat activity and trigger the camera trap. Additionally, flight behavior was recorded using an infrared camera, and echolocation calls were recorded using a full-spectrum acoustic logger. We first evaluated whether the flash elicited any avoidance or attraction behavior using the light barrier data. We posit that if bat activity deviates on flash-on nights compared to flash-off nights, while correcting for weather and temporal effects, then data from camera traps with white flash may not be informative proxies of bat activity, and in the worst case may even cause a substantial disturbance and cause individuals to abandon the site. We subsequently categorized flight direction of bats and latency of the first echolocation call after the camera trigger to investigate whether bats are startled by the flash, even if this does not immediately lead to a reduction in the overall number of bat passes. We hypothesized that if entering bats are disturbed by the flash, this may either lead to a change in the relative abundance of specific flight directions observed within a site, or that bats might call earlier (i.e. surprise reaction) or later (i.e. shock reaction) as compared to flash-off nights. Finally, we investigated the long-term effects of a year-round camera trap installation at a fifth site where only one of two entrances has been monitored with a camera trap and flash for the past 6 years. We hypothesized that if the flash disturbs the entering bats, they should preferentially make use of the other entrance, or that the overall number of bats counted at the site in winter should decline.

Materials and Methods

Study site and experimental design

Infrared light barriers with camera traps (ChiroTEC, Lohra, Germany), self-built infrared video cameras, and Audio-Moth acoustic loggers were installed at the entrance of four hibernacula sites in Northern Germany (Table 1; entrance schematics in Fig. S1). At each site, the white camera trap flash was turned on and off in a weekly alternating manner for 16 weeks between August and December 2020, with two sites exposed to 'flash-on' treatment and two to 'flash-off' during any given week (Table 1). The digital camera

was left turned on continuously, taking black images on flash-off weeks and thus the camera shutter emitted a detectable sound irrespective of flash treatment. The timing of the experiment coincided with the busiest activity period at the hibernacula, the autumn swarming phase and onset of hibernation. At two sites, Anklam and Peenemuende, the camera trap installation took place in autumn 2019, thus the adult bat population at these sites was exposed to flash for a whole year before the experiment. At the other two sites, Demmin and Friedland, camera trap installation occurred directly prior to this experiment, therefore bats were completely naive to the flash.

Light barriers consist of a sensor array with 4 (Liba-4) or 16 (Liba-16 and Liba-16k) pairs of infrared emitters and corresponding receivers. Adjacent sensors are 2.1 cm apart in the Liba-4 and Liba-16, and 1.1 cm apart in the Liba-16k model.

The camera trap consists of a Panasonic Lumix G5 digital mirrorless camera and a Metz 58 AF-2 digital white flash (aperture 5.6, power 1/16, zoom 70). The camera trap is connected to the light barrier, and it is triggered on each entry registered by the light barrier.

Self-built infrared video cameras were used to record the flight direction of bats entering the hibernacula. Each camera was built using a Raspberry Pi 3, an 8 MP camera, and an 850 nm infrared illuminator (see <https://gabik-bat.github.io/FlederCam/> for full construction details). Cameras were mounted on top of the camera traps and recorded continuously between sunset and sunrise (800 × 600 resolution, 25 frames per second). The video cameras were modified to additionally receive a signal from the light barrier for each registered event. Due to technical problems, infrared video recordings were unsuccessful on 8 of 64 deployments (Table 1).

Acoustic recordings were collected with AudioMoth acoustic loggers (v1.0.1, LabMaker; Hill et al., 2018). Each device recorded continuously every day between sunset and sunrise from week 3 onwards at a sample rate of 192 kHz on the medium gain setting. Devices were

encased in a protective case and mounted directly on top of the infrared video cameras, with the microphone facing the entrance of the hibernaculum.

Hourly air temperature (°C) and precipitation data (mm) for the study period were obtained from the German Meteorological Service (DWD) from the closest local weather stations to each hibernaculum: weather station in Anklam (Anklam), Tribsees (Demmin), Trollenhagen (Friedland), and Karlshagen (Peenemuende).

Video analysis: flight direction and video-based bat activity

From the c. 5400 h of raw infrared video recording, 6-second-long video snips were isolated for each event registered by the light barrier. In total, 53 134 video snips were manually analyzed to quantify video-based bat activity. The camera flash was visible on the video snips as one or two overexposed frames. To reduce potential observers' bias in the video processing, these frames were detected based on white pixel threshold values and replaced by a merged image of the pre- and post-flash frames with 50% transparency. This pre-processing step ensured that when the videos were scored for flight direction, the observer was unable to tell whether the focal bat was exposed to the flash or not.

For each video snip of an entering bat, we categorized bat behavior based on the direction of its flight after the camera trap trigger. If a bat turned <180° during the time it remained in the field of view, it was scored based on the edge of the frame where it left the camera's field of view: **up**, **down**, **left**, **right**. If a bat turned more than 180° within the field of view of the camera but remained within the hibernacula, it was scored a **loop**. If a bat turned around and left the hibernacula, it was scored a **U-turn**. Finally, if the bat approached and triggered the light barrier while circling on the inside without leaving the hibernacula (i.e. inspection flight), it was scored an **innerloop**. Innerloops were excluded from the flight

Table 1. Description of the study sites, including location, light barrier model and its installation date, flash treatments on even and odd weeks, number of weeks with infrared video data per flash treatment (maximum number per treatment is eight; missing weeks due to video camera failure: Anklam week 4, 9, 10, 11; Friedland week 5, 6, 7; Peenemuende week 13), and species recorded at each site during the winter census in January 2020.

Location	LB model	LB installation	Even/odd weeks	Video weeks	Species composition
Anklam	Liba-4	18.10.19	On/off	6/6	<i>Myotis dasycneme</i> , <i>M. daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Pipistrellus pipistrellus</i>
Demmin	Liba-16k	31.07.20	Off/on	8/8	<i>Myotis daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
Friedland	Liba-16	31.07.20	On/off	7/6	<i>Myotis daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
Peenemuende	Liba-4	18.10.19	Off/on	8/7	<i>Myotis brandtii</i> / <i>M. mystacinus</i> , <i>M. dasycneme</i> , <i>M. daubentonii</i> , <i>M. nattereri</i> , <i>M. myotis</i> , <i>Plecotus auritus</i>

direction comparisons to restrict the analysis to individuals entering the hibernacula from outside and from the video-based activity counts to avoid overestimating overall activity. In addition to the direction, we also categorized if an event was 'social' or not, based on whether any other bats were seen during the 6-second video snip.

Audio analysis: echolocation call latency

All audio files were processed in Python v3.7.3 to automatically identify each recorded camera trap trigger based on the audible shutter sound. We downsampled each audio file from the original 192 to 96 kHz and cut it into fragments, consisting of 2^{17} datapoints. We computed the *short-time Fourier transform (STFT)* of each fragment using a Gaussian window with 256 data points. The normalized, squared magnitude of STFT was integrated between 450 and 550 Hz, to obtain the bandpower of the corresponding peak intensity range of the shutter sound. To detect the typical trimodal intensity peaks (see Fig. S3) of the camera trigger, we used the *find_peaks* function (prominence 0.2, SciPy package, v1.6.3; Virtanen et al., 2020). The precise moment of the camera trigger was defined as the middle intensity peak, which also corresponds to the flash event on flash-on nights.

To measure the potential change in echolocation behavior of bats, we calculated the time from the camera trigger onset to the first echolocation pulse, which we refer to as the first echolocation call latency. We selected this measure as we hypothesized that bats may either react to the flash by calling faster ('surprise'), or slower ('shock'). Other measures such as call activity prior to entry or inter-call-interval after entry varied strongly in repetition rate and amplitude between sites due to large differences in hibernacula entrance dimensions and between flight directions and therefore could not be quantified reliably using automatic detection thresholds.

We created 2-second-long audio snips centered around the detected camera trigger signals. Based on the corresponding video snips, we excluded those scored as 'social', because the detected call may not have been produced by the entering bat, and additionally bats may modify their echolocation behavior in the presence of other bats. To detect echolocation calls, spectrograms were computed using STFT of the original 192 kHz audio data and bandpower was calculated between 35–65 kHz, representing the broad frequency range of species present at the study sites (Barataud et al., 2015). We identified all peaks corresponding to individual pulses after the camera trigger using the *find_peaks* function (prominence 0.005) and calculated the time between the camera trigger and the first subsequent echolocation call. Spectrograms of all events, with marks denoting the timing of the

automatically detected camera trigger and echolocation pulses (see Fig. S3), were inspected manually. All events with failed detections or calls of multiple bats were excluded from the analysis, resulting in a total of 3272 events (1568 flash-on, 1704 flash-off).

Long-term monitoring

At a fifth hibernaculum with two entrances (Eldena), the main entrance has been monitored using a camera trap with white flash and a light barrier (Liba-16), while the nearby side entrance (± 20 m) has been monitored with only a light barrier (Liba-4) since autumn 2015. Annual hibernation surveys were carried out once per year in January.

Statistical analyses

All statistical analyses were conducted in R (v3.6.1; R Core Team, 2019).

Activity data

Light barrier-based bat activity was measured as the total number of bat passes registered each night by the light barrier. In addition, we quantified the video-based bat activity by counting the passes observed in the infrared video data. We investigated the impact of flash on nightly bat activity registered by the infrared light barrier using a generalized linear mixed model with negative binomial distribution (*glmer.nb* function, package lme4, v1.1-26; Bates et al., 2015). To account for temporal variation (e.g. higher activity in August than in December), weather variation (e.g. higher activity on warmer and dry nights), and variation across sites, we included study week, precipitation, and temperature in the full model as fixed effects and study site as random effect. However, study week was excluded from the final model due to collinearity with temperature (Pearson's correlation coefficient, $r = -0.8$). Temperature was defined as the ambient temperature at sunset as Parsons et al. (2003) postulated that temperature at emergence may be the primary driver of swarming activity at underground hibernacula. Precipitation was defined as the proportion of hours with rain between sunset and sunrise, because we expected lower bat activity with longer duration of rain, irrespective of the amount of rainfall. We standardized both weather parameters prior to analysis. Model assumptions were examined using the DHARMA package (v0.4.1; Hartig, 2021). Odds ratios with 95% confidence intervals were calculated for each fixed effect with the *confint* function of the stats package (R Core Team, 2019). An additional model was run with identical model structure but using the video-based activity data to investigate whether

excluding innerloops could improve the strength of correlation between activity and weather parameters.

Behavioral data

Possible flight directions were highly dependent on the layout of the hibernacula entrance and positioning of the video camera (Fig. S1), thus we performed only within site comparisons of flight directions. Social interactions often included chasing and avoidance behavior and are therefore likely to influence the flight direction categories. Thus, we compared the compositional similarity of flight directions between flash-on and flash-off nights, as well as social and non-social events using a non-parametric permutational ANOVA (PERMANOVA; Anderson, 2014) with the *adonis* function of the *vegan* package (v2.5-7; Oksanen et al., 2020). Pairwise comparisons were based on Bray-Curtis dissimilarity matrices with 10^4 random permutations and adjusted for multiple comparisons (*p.adjust* function, 'bonferroni' method, stats package; R Core Team, 2019). All calculations used flight direction totals per group as an abundance-based measure of dissimilarity to account for overall reduction of flight activity as a potential effect of disturbance.

Permutational multivariate analysis of variance is sensitive to heterogeneous group dispersions. The activity data showed that weekly flight direction totals had unequal variances due to temporal and weather effects. Therefore, we grouped nightly flight direction totals by day of the week, thereby pooling days (e.g. all flash-off Mondays) across the entire sampling period, to create samples with homogenous variance between flash treatments and social groups. Homogeneity of dispersion between compared groups (flash-on vs. flash-off, social vs. non-social) was tested using the *betadisper* function of the *vegan* package (v2.5-7; Oksanen et al., 2020).

Acoustic data

Mean first call latency, the mean time in milliseconds from the camera trigger to the first echolocation pulse, was compared between flash-on and flash-off nights using a randomization test with 10^4 permutations. Since the echolocation behavior of bats can be affected by the dimension of the hibernacula entrance, the analysis was restricted to within site comparisons.

Results

Activity data

Nightly bat activity did not differ between flash-on and flash-off nights (Fig. 1A, Table 2). Bat activity decreased with a higher proportion of hours with rain between sunset and sunrise (Fig. 1B, Table 2), but ambient temperature at sunset did not uniformly impact bat activity (Fig. 1C, Table 2). Specifically, nightly bat activity increased in Demmin and Peenemuende, and decreased in Anklam and Friedland with higher temperature at sunset (Fig. 1C). Results were comparable when using the video-based activity data (Fig. S2, Table S1).

Behavioral data

The composition of flight directions differed between sites, with up and down being the most common flight directions, and U-turns being the rarest events overall (Table S2). During non-social events, bats entering hibernacula with narrow entrances (i.e. Anklam and Peenemuende) most often left the frame at the bottom, presumably due to losing height after gliding through the entrance (in 74.84% and 62.95% of the non-social events, respectively; Table S2). In Demmin, where the entrance is slightly taller, and Friedland, where the camera was placed further away due to the entrance's height above ground, bats primarily left the frame at the top (in 65.67% and 37.92% of the non-social events, respectively; Table S2).

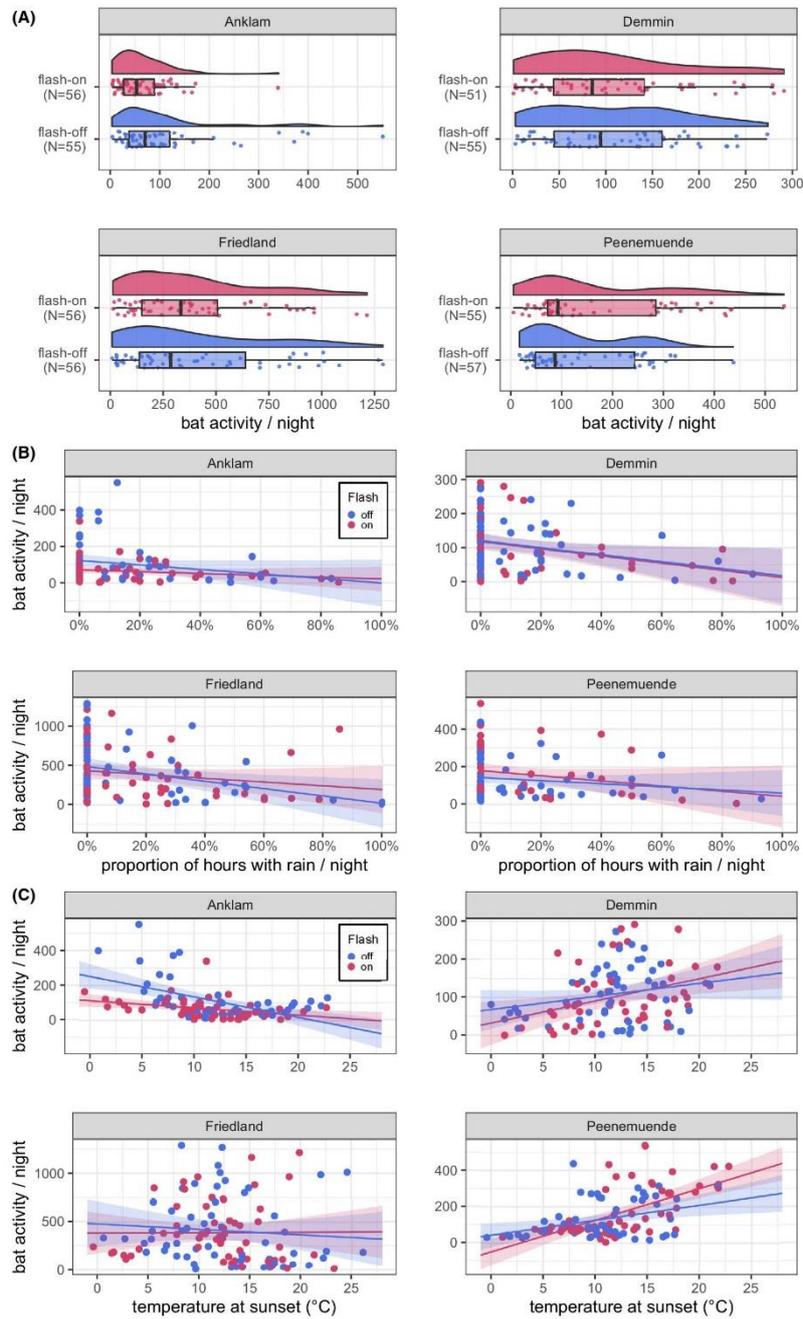
Tests of the homogeneity of group dispersions indicated that variances of nightly flight direction totals were homogenous for both flash and social variables at all hibernacula, except in Peenemuende, where variance was non-homogenous between flash treatments (Table 3).

The composition of flight directions of bats entering the hibernacula was different between social and non-social events but did not differ between flash-on and flash-off nights (Fig. 2, Table 3). PERMANOVA results between flash treatments in Peenemuende trended toward significance, but this is likely the result of heterogeneous group dispersion (Table 3). Social events reduced the number of bats entering in the 'default' direction (Fig. 2), and chasing (i.e. both bats leaving the frame in the same direction in close succession) or avoidance (i.e. flying away from the

Figure 1. Comparison of light barrier-based (A) nightly bat activity on flash-on and flash-off nights, and the relationship between bat activity and (B) proportion of hours with rain per night between sunset and sunrise, and (C) ambient temperature at sunset. Each point corresponds to the total number of bat passes recorded per night using infrared light barriers. Data points are colored based on flash treatments for illustrative purpose only, as no differences were found between flash-on and flash-off nights. Solid lines with shading represent the regression line and 95% confidence interval.

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Effect of White Flash in Camera Traps on Bat Behavior



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Table 2. Results of the generalized linear mixed model showing the effect of the proportion of hours with rain between sunset and sunrise, ambient temperature at sunset and flash on light barrier-based nightly bat activity.

Fixed effects							
Variable	Estimate	SE	z-value	Odds ratio	LCL	UCL	P-value
(Intercept)	5.12	0.33	15.74	166.63	75.16	370.59	<0.001
Proportion rain	-0.30	0.05	-6.28	0.74	0.67	0.81	<0.001
T_{sunset}	0.09	0.11	0.85	1.10	0.89	1.36	0.394
Flash	-0.08	0.08	-0.95	0.93	0.79	1.08	0.341
Random effect							
Variable	N	Variance	SD	Observations			
Site	4	0.37	0.61	441			

When the 95% confidence interval (between lower – LCL – and upper – UCL – confidence level) of the odds ratio (OR) contains 1, the variable is considered to have no measurable effect. An interval above 1 indicates a positive, and below 1 a negative effect of the predictor. Bold values indicate statistical significance at $P < 0.05$ level.

other bat observed in the frame without the other bat pursuing) behaviors were frequently observed.

Acoustic data

Mean first call latency did not differ on flash-on and flash-off nights at each site based on randomization tests (Anklam $P = 0.092$, Demmin $P = 0.258$, Friedland $P = 0.955$, Peenemuende $P = 0.562$), with a maximum difference of 4.61 ± 1.7 ms in Demmin (Fig. 3).

Long-term monitoring

The proportional use of the main entrance (with camera trap) and side entrance (without camera trap) did not change substantially over the past 6 years. On average 67% of the bats entered the hibernacula via the main entrance (Fig. 4), suggesting that this is the preferred entrance, despite bats being exposed to the white flash upon entrance. Moreover, the number of hibernating individuals counted during the winter census increased from c. 250 in the year prior the installation of the monitoring system, to over 300 individuals in recent years.

Discussion

In several mammal species, distinct startle and attraction responses elicited by the white flash of camera traps have been observed (Séquin et al., 2003; Wegge et al., 2004, e.g. Gibeau & McTavish, 2009; Schipper, 2007.), potentially biasing population monitoring. In this experimental field study investigating the reaction of bats to white flash at the entrance to four hibernacula, we did not observe

an effect of flash on bat activity, flight direction, or echolocation behavior.

Successful conservation of threatened species relies on accurate assessment of long-term population trends. In temperate-zone bats, monitoring of hibernacula has the distinct advantage that many species make use of these sites, thereby bundling monitoring efforts. However, traditional winter counts only yield a single population estimate per year. Automated monitoring methods, such as light barriers that measure activity and camera traps that allow for species identification, have the potential to vastly improve monitoring via accurate, year-round sampling of nearly all entering bats. The system is particularly applicable to situations where several hundred to several thousand bats hibernate in a site with a comparatively narrow entrance, but where traditional winter counts are impossible for safety reasons or ineffective due to uncountable sections or crevices (e.g. bunker complexes, tunnels, shale mines).

Activity models accounting for temporal and weather parameters showed no negative impact of flash on nightly bat passes. Unsurprisingly, bat activity was negatively affected by precipitation that corresponded to findings of previous studies (e.g. Parsons et al., 2003). Visual inspections suggested that higher temperature at sunset increased or decreased nightly bat activity depending on the site, which can be partially explained by differences in species-specific behavior. For example, a highly synchronized winter arrival of *Pipistrellus* was observed during the last and coldest study week in Anklam, thereby shifting the overall correlation. In addition, due to the collinearity between temperature and study week, the correlation between activity and temperature may also encompass temporal effects. It should be noted that given the lack of an independent temporal variable in the

Table 3. Homogeneity of group dispersions of nightly flight direction totals (top) and non-parametric permutational ANOVA using Bray-Curtis dissimilarity matrices of nightly flight direction totals (bottom) between flash-on versus flash-off nights and social versus non-social events.

BETADISPERSION Site	Social		Flash	
	F-value	P-value	F-value	P-value
Anklam	0.02	0.896	2.79	0.107
Demmin	1.10	0.304	0.09	0.765
Friedland	0.76	0.391	0.02	0.896
Peenemuende	0.02	0.903	18.69	<0.001

PERMANOVA Site	Social		Flash	
	R ²	P-value	R ²	P-value
Anklam	67.72	<0.001	3.10	0.363
Demmin	24.55	0.004	5.60	0.530
Friedland	24.24	0.003	4.85	0.659
Peenemuende	78.12	<0.001	4.24	0.068

All PERMANOVA were performed with 10⁴ random permutations; a ‘*bonferroni*’ correction was applied to adjust P-values for multiple comparisons. Bold values indicate statistical significance at P < 0.05 level.

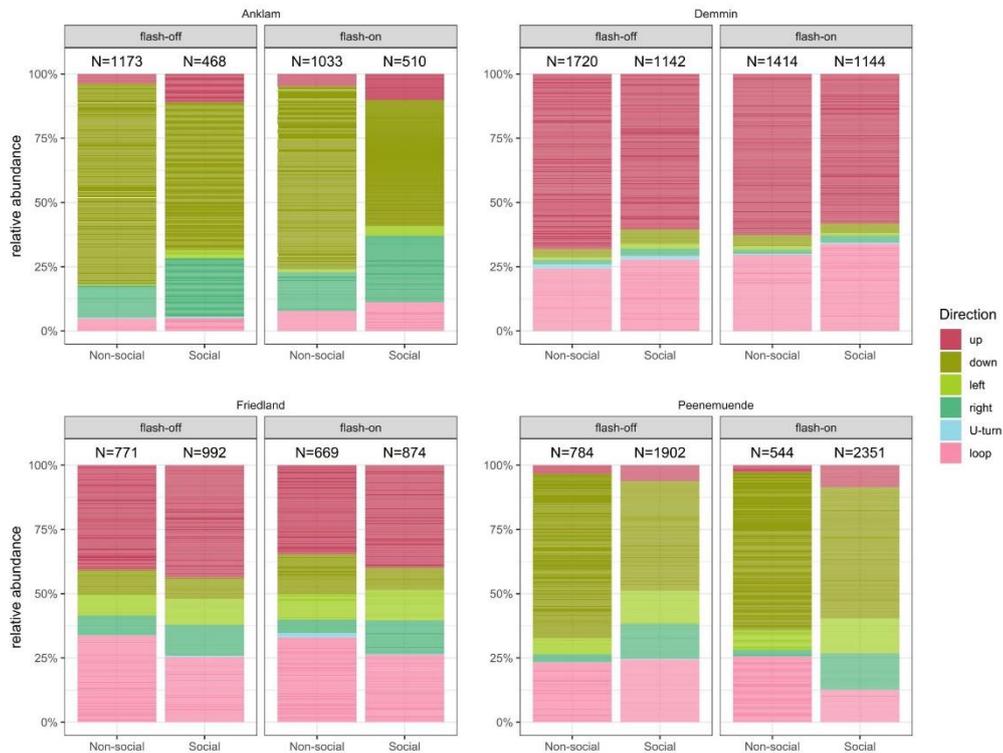


Figure 2. Proportion of social and non-social flight directions of bats entering four hibernation sites on flash-on and flash-off nights. Social events indicate the presence of other bats in the scored 6-second-long infrared video snips.

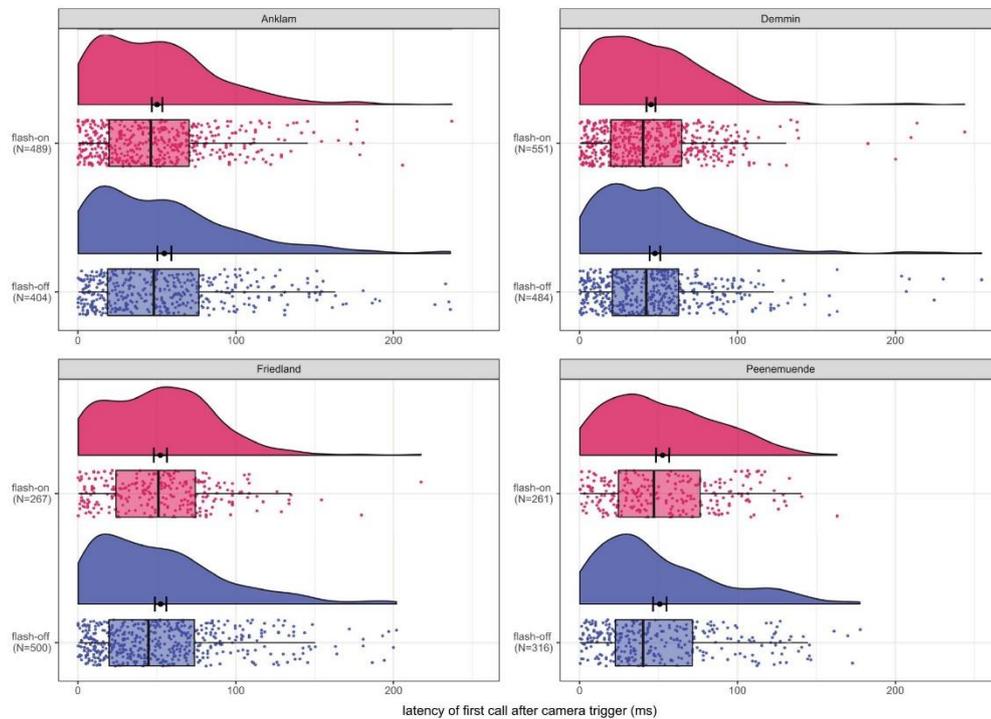


Figure 3. Comparison of latency of the first echolocation call after camera trigger in milliseconds between flash-on and flash-off nights. Mean values with 95% confidence intervals are indicated by black points and error bars.

activity model, we cannot exclude possible habituation effects, but critically, if individuals were to habituate, this would nevertheless yield reliable monitoring results.

As in the overall activity, flash had no effect on the flight direction of bats entering the hibernacula, but we did observe changes based on social context. The presence of other bats is expected to alter the flight behavior of bats, particularly during the swarming season when chasing behavior is common (Parsons et al., 2003). Although bats circling inside the hibernacula were excluded from the behavioral analyses, flash did not deter bats from doing innerloop ‘inspections’ prior leaving the hibernacula, indicating no substantial impact of flash on their natural behavior. All analyses were performed at the population level, and therefore rare individual reactions or aversions could go undetected. In this context, it is worth noting that the number of U-turns, expected to be the most extreme behavioral reaction of an individual, were exceptionally rare on flash-on nights at all sites (in total 30 U-turns out of 14 920 events across all sites).

Likewise, we observed no difference in first call latency after a camera trigger between flash-on and flash-off nights. Bats are capable of extremely fast reaction as echolocation allows for very short reaction times due to higher temporal processing of the auditory system. In fact, a behavioral startle response to a sudden acoustic cue can only take 20 ms (Geberl et al., 2015). Therefore, although we only measured latency of the first call, we expect that most bats entering the hibernacula had enough time to process the visual information of the flash before emitting their first echolocation call.

While vision may be more important for bats than previously supposed (Voigt et al., 2018), they certainly do not rely on it as heavily as other nocturnal mammals. Nevertheless, light stimuli can be highly disturbing to bats. For example, evidence from summer roosts suggest that constant illumination during emergence may cause bats to use alternative entrances if available, or in the worst case, bats may abandon the site (Stone et al., 2015). Although this experimental study was limited to a single

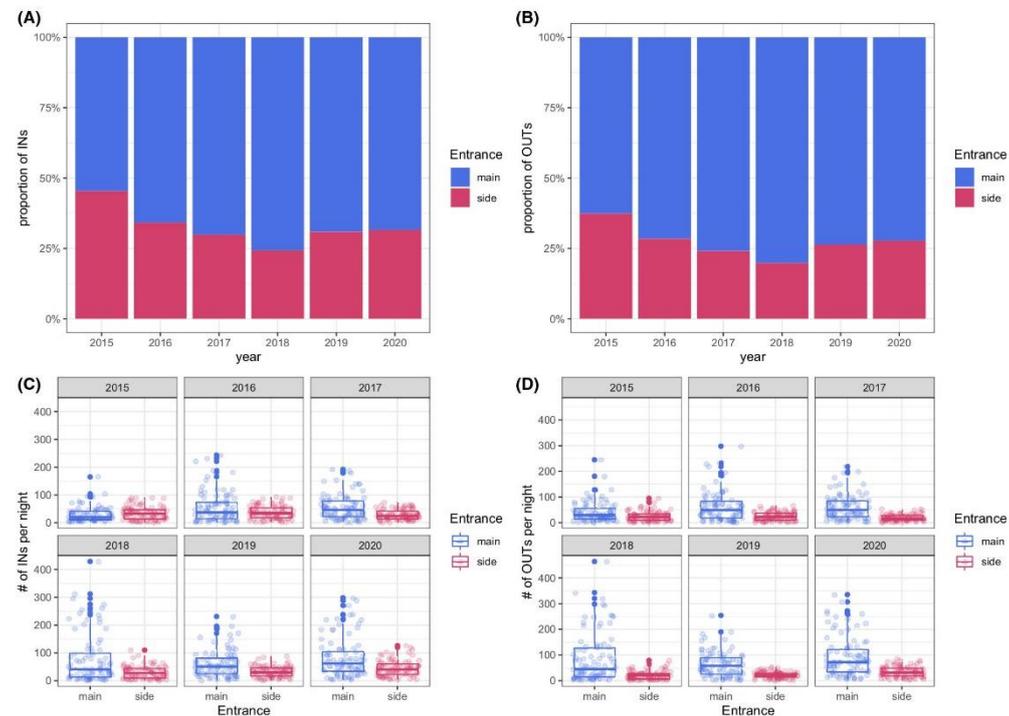


Figure 4. Proportion of bats (A) entering and (B) exiting the hibernaculum through the main entrance (with camera trap) and side entrance (without camera trap), and the total number of (C) 'in' events and (D) 'out' events per night registered by light barriers at the two entrances in Eldena between August and December from 2015 to 2020.

season, long-term winter census data from Eldena, where one entrance is monitored with a camera trap and the other only with a light barrier, showed no increasing preference for the entrance without flash, or decreasing trend in overall light barrier activity or hibernation census counts over the past 6 years. In addition, it is notable that the investigated hibernacula were dominated by *Myotis* species that are considered particularly light-averse (Voigt et al., 2018) and even low levels of constant light can disrupt their natural behavior (Azam et al., 2018; Zeale et al., 2018).

The discrepancy between these findings and the lack of an effect observed here can likely be explained by the comparatively weak (power 1/16) and short duration (1/5500 sec according to manufacturer specifications) of the flash. Similarly, white camera flash had no negative effect on other light-sensitive mammals (Heaslip & Hooker, 2008), in contrary with the negative impact of continuous illumination observed in several mammals. In this context,

it is important to note that our results may not be directly applicable to professional photography, where the use of full illumination or several synchronized flashes arranged around the camera should be carefully evaluated.

Finally, this study additionally highlights the potential of low-cost video cameras, built with off-the-shelf components, as a powerful monitoring tool (Droissart et al., 2021; Klemens et al., 2021; Wilkinson et al., 2021), particularly in conservation projects with limited budget. The self-built, Raspberry Pi-based infrared cameras used in this study can be adapted to a wide range of contexts with minimal technical and programming skills. Further technological improvements, combined with recent computer vision technologies for automated data processing, are expected to support the more widespread application of camera traps in monitoring and applied conservation of other threatened mammals (McCallum, 2013), particularly during times with restrictions on fieldwork activity (Blount et al., 2021).

Conclusion

Our multi-faceted experimental design showed no change in overall bat activity, flight, or acoustic behavior when exposed to white camera trap flash. Thus, camera traps are a promising minimally invasive tool to monitor bat populations. While in rare cases individuals may still react adversely, the overall effect of the monitoring technique appears to be minimal at most, certainly when compared to the alternative of winter hibernation counts. To optimally take advantage of these new techniques, the accuracy of the species-specific monitoring achieved by the camera traps should be evaluated and compared to the data obtained through traditional techniques. The development and application of camera traps and other automated monitoring technologies can help advance our understanding of changing population trends, species composition, and phenology across a wide range of spatial-temporal scales and taxa. Understanding long-term population dynamics can inform conservation decision making, contribute to data-driven wildlife conservation and management, and help to better understand and tackle the global biodiversity crisis.

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Data Availability Statement

Detailed instructions regarding the construction and configuration of the self-built infrared video cameras can be found at: <https://gabik-bat.github.io/FlederCam/>. All data and scripts used for statistical analyses are available at: <https://github.com/GabiK-bat/BatFlash>.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Figure S1. Layout of the entrance and monitoring setup at four hibernation sites in (A) Anklam, (B) Demmin, (C) Friedland and (D) Peenemuende, indicating the light barrier model, width (W), height (H) and height above ground of the light barrier, height of the camera trap above ground and the width and height of the infrared video camera's field of view (FOV).

Figure S2. Comparison of infrared video-based (A) nightly bat activity on flash-on and flash-off nights, and the relationship between bat activity and (B) proportion of hours with rain per night between sunset and sunrise and (C) ambient temperature at sunset.

Figure S3. Power spectrum of the broad frequency range of species present at the study sites (35–65 kHz) and zoomed in view of the normalized amplitude of the typical trimodal intensity peaks of the camera trigger (red dashed lines) between 450 and 550 Hz (top).

Table S1. Results of the generalized linear mixed model showing the effect of the proportion of hours with rain between sunset and sunrise, ambient temperature at sunset and flash on infrared video-based nightly bat activity recorded at four hibernation sites in Northern Germany between August and December 2020.

Table S2. Social and non-social flight direction totals of bats entering hibernation sites on flash-on and flash-off nights based on infrared video snips.

Supporting Information

G. Krivek, B. Schulze, P. Zs. Poloskei, K. Frankowski, X. Mathgen, A. Douwes, J. van Schaik (2021)

Camera traps with white flash are a minimally invasive method for long-term bat monitoring,

Remote Sensing in Ecology and Conservation.

Corresponding author: Gabriella Krivek, krivek.g@gmail.com

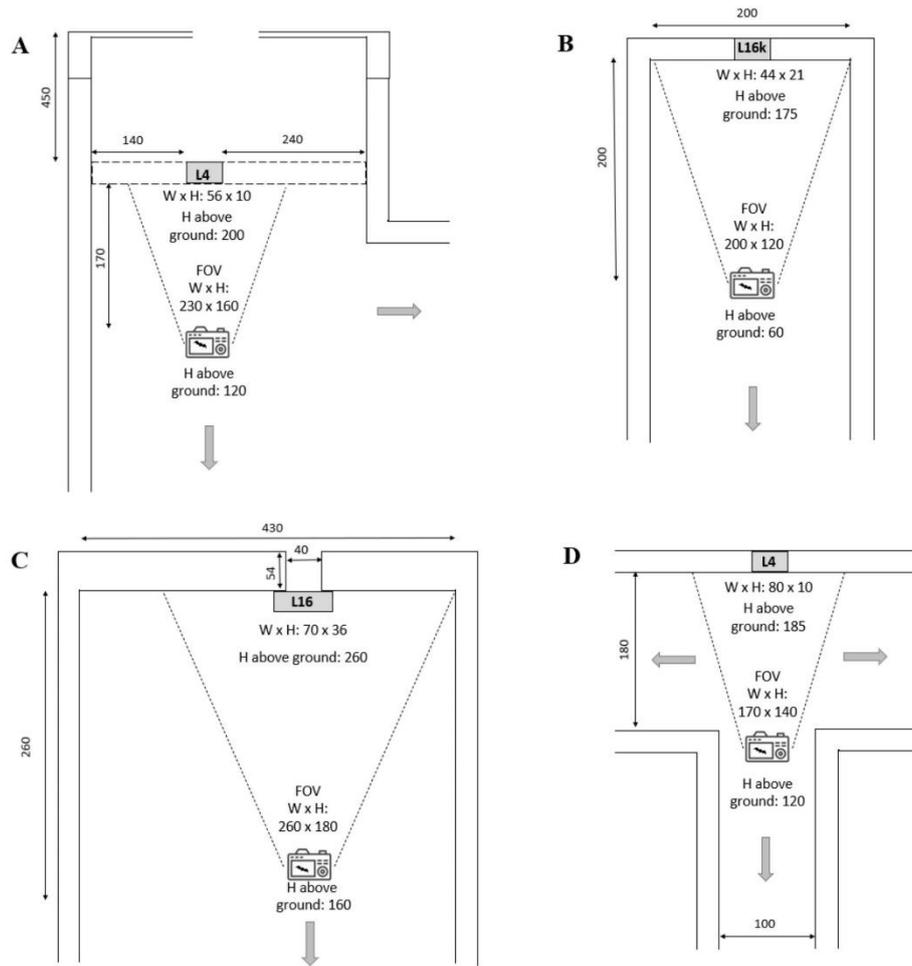


Figure S1 Layout of the entrance and monitoring setup at four hibernation sites in A) Anklam, B) Demmin, C) Friedland and D) Peenemuende, indicating the light barrier model, width (W), height (H) and height above ground of the light barrier, height of the camera trap above ground and the width and height of the infrared video camera’s field of view (FOV). The diagram is not up to scale and only the vicinity of the entrance is illustrated with gray arrows indicating the directions where the room continues.

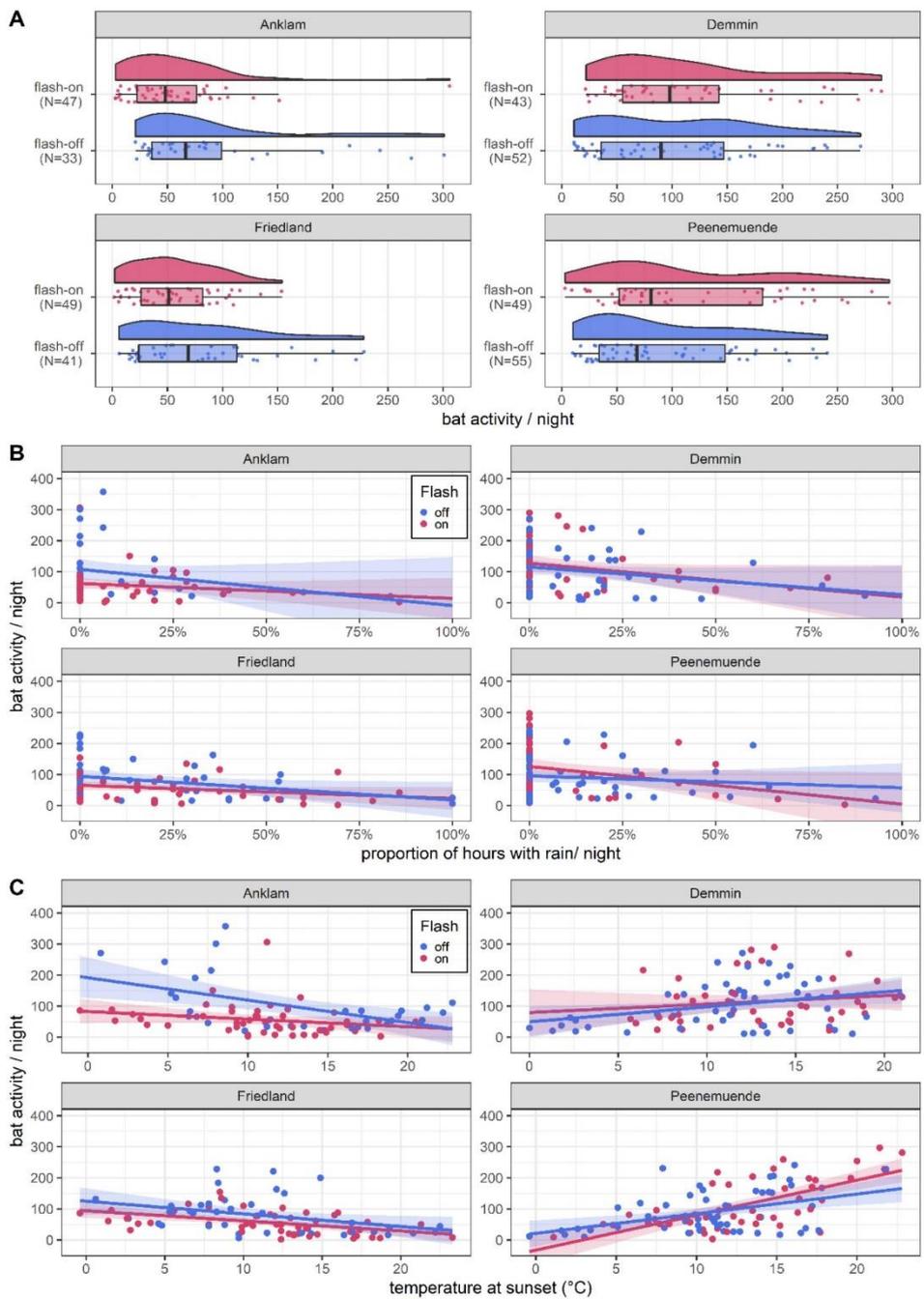


Figure S2 Comparison of infrared video-based A) nightly bat activity on flash-on and flash-off nights, and the relationship between bat activity and B) proportion of hours with rain per night between sunset and sunrise and C) ambient temperature at sunset. This is the same analysis as presented in Figure 2 in main text, but instead of using light barrier-based activity data, here each point corresponds to the total number of bat passes recorded per night using infrared video cameras. Due to the missing weeks of video data, this analysis does not include all days for all study sites. Data points are colored based on flash treatments for illustrative purpose only, as no significant differences were found between flash-on and flash-off nights. Sample size (N) indicates the number of sampling nights with available infrared video recording per flash treatment group. Solid lines with shading represent the regression line and 95% confidence interval.

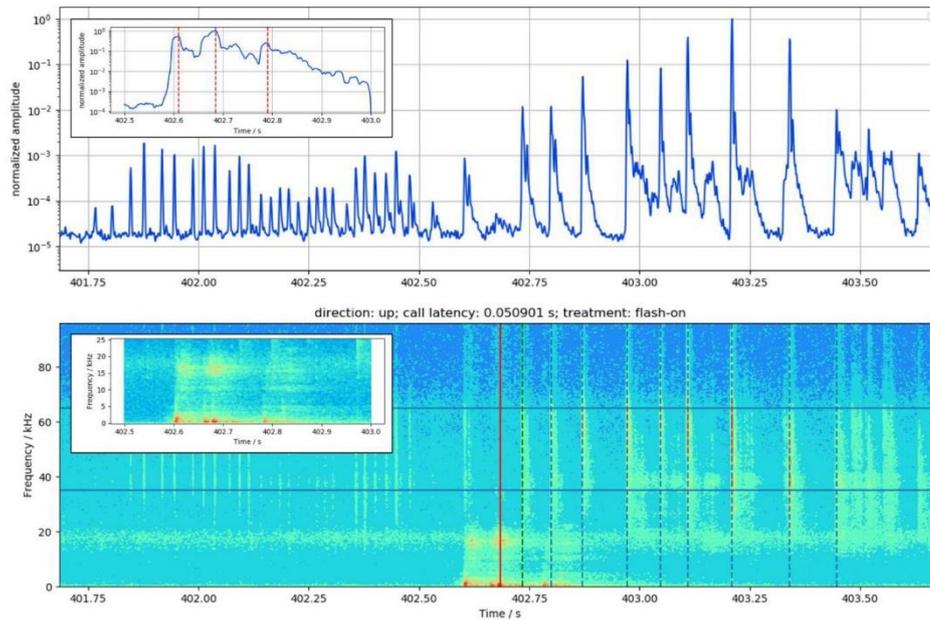


Figure S3 Power spectrum of the broad frequency range of species present at the study sites (35-65 kHz) and zoomed in view of the normalized amplitude of the typical trimodal intensity peaks of the camera trigger (red dashed lines) between 450 and 550 Hz (top). The first peak corresponds to the shutter closing to block the sensor, the middle peak is the opening of the shutter and exposing the sensor to the flash and the closing of the shutter, which stops the exposure to light and takes the picture. The last peak indicates that the shutter opens again, and the camera is in default state. Spectrogram of the echolocation calls of a bat entering the hibernacula and leaving the video camera frame towards up after being exposed to white camera flash and zoomed in spectrogram of the camera trigger in the low frequency range (bottom). The precise moment of the camera trigger was defined as the middle peak (red vertical line), that matched the exact time of the flash trigger on nights when the flash was turned on. Echolocation pulses following the camera trigger (vertical dashed lines) were detected between 35 and 65 kHz (horizontal solid lines).

Table S1 Results of the generalized linear mixed model showing the effect of the proportion of hours with rain between sunset and sunrise, ambient temperature at sunset and flash on infrared video-based nightly bat activity recorded at four hibernation sites in Northern Germany between August and December 2020. When the 95% confidence interval (between lower – LCL – and upper – UCL – confidence level) of the odds ratio (OR) contains 1, the variable is considered to have no measurable effect. An interval above 1 indicates a positive, and below 1 a negative effect of the predictor.

Fixed effects

Variable	Estimate	SE	z-value	Odds ratio	LCL	UCL	p-value
(Intercept)	4.56	0.14	33.14	95.59	72.64	129.03	<0.001
Proportion of rain	-0.31	0.05	-6.36	0.73	0.67	0.81	<0.001
T _{sunset}	0.10	0.11	0.94	1.11	0.89	1.38	0.349
Flash	-0.12	0.08	-1.49	0.89	0.76	1.04	0.136

Random effect

Variable	N	Variance	SD	Observations
Site	4	0.02	0.16	369

Table S2 Social and non-social flight direction totals of bats entering hibernation sites on flash-on and flash-off nights based on infrared video snips. Events were scored using infrared video recordings collected at four hibernation sites in Northern Germany between August and December 2020. Social events indicate the presence of other bats in in the scored 6-second-long infrared video snips.

Site	Flash	Social	Total	down	left	loop	right	U-turn	up	innerloop
Anklam	off	non-social	1193	915	5	57	147	4	45	20
Anklam	off	social	501	269	15	23	106	3	52	33
Anklam	on	non-social	1062	736	12	80	156	0	49	29
Anklam	on	social	533	250	19	57	132	0	52	23
Demmin	off	non-social	1726	56	17	417	28	30	1172	6
Demmin	off	social	1145	64	21	316	32	18	691	3
Demmin	on	non-social	1418	62	19	418	23	6	886	4
Demmin	on	social	1158	41	13	387	30	7	666	14
Friedland	off	non-social	3302	74	63	260	59	1	314	2531
Friedland	off	social	2400	81	101	251	119	6	434	1408
Friedland	on	non-social	4025	104	66	220	35	12	232	3356
Friedland	on	social	2640	75	104	229	114	3	349	1766
Peenemuende	off	non-social	930	501	49	182	24	1	27	146
Peenemuende	off	social	2536	813	244	463	259	6	117	634
Peenemuende	on	non-social	674	335	41	139	13	1	15	130
Peenemuende	on	social	3410	1202	319	296	333	1	200	1059

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Counting in the dark: estimating population size and trends of bat assemblages at hibernacula using infrared light barriers

Counting in the dark: estimating population size and trends of bat assemblages at hibernacula using infrared light barriers

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Keywords

bat conservation; Chiroptera; hibernation census; population dynamics; remote sensing; winter count.

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Abstract

Accurate population estimates are crucial to developing successful conservation policy, but the underlying data remain difficult to collect for many taxa. This is especially true for elusive species, such as temperate-zone bats, where visual counts in hibernacula underestimate their population to an unknown extent. Infrared light barriers that count all entering and exiting bats at the entrance of a hibernaculum could offer a more accurate alternative for bat population monitoring. We used infrared video recordings to quantify light barrier accuracy (i.e., concordance between light barrier and video registration of entries and exits) at five hibernacula over 30 weeks during autumn and spring. Subsequently, we developed a standardized methodology to estimate light barrier-based population sizes based on the number of emerging bats in spring, and compared these estimates to visual counts at 12 sites. Finally, we calculated confidence intervals around the estimated population sizes, and used these to evaluate population trends using 6-year-long light barrier datasets from four sites. Light barrier accuracy varied based on the model and precise location of the installation, with the best combination achieving near-perfect accuracy across the entire emergence phase. When compared to the resulting light barrier-based population estimates, winter hibernation counts markedly underestimated population totals, recovering less than 10% of the bats at the most complex sites. Moreover, light barrier-based population trends showed regional patterns of growth and decline, which were not evident in the visual counts. This study demonstrates that light barriers can estimate the population size and trends of bat assemblages with unprecedented accuracy, even at large, complex, or inaccessible hibernacula that cannot be precisely assessed with visual hibernation counts. Installing light barriers at a representative network of sites, where their installation does not require large-scale entrance modifications, has the potential to revolutionize bat monitoring and contribute to data-driven conservation.

Introduction

Accurate estimates of population sizes and trends are central to assessing the conservation status of species and the effects of global change on animal populations (Williams, Nichols, & Conroy, 2002). Despite a legal obligation to monitor species of conservation interest (e.g., EU Habitats Directive), as well as advances in statistical methods for determining population trends (e.g., TRIM analyses; Pannekoek & Van Strien, 2001), the underlying data required for such estimates often remain sparse and difficult to collect (Frick, Kingston, & Flanders, 2020). As such, there is an urgent need to establish new monitoring approaches to accurately and efficiently assess population sizes over large temporal and spatial scales.

Monitoring of bat populations is challenging because bats are small, elusive, nocturnal mammals that are sensitive to disturbance (Frick *et al.*, 2020). Many temperate-zone bats make use of underground sites for hibernation in winter and congregate at these sites in autumn, a behavior known as autumn swarming. Since the same underground sites are used by multiple species, and by individuals from different maternity colonies (e.g., Dekeukeleire *et al.*, 2016), these sites provide an opportunity to efficiently monitor regional population dynamics. At such roosting sites, passive acoustic recording devices can be used to non-invasively monitor bat activity throughout the year (e.g., Thomas & Davison, 2022). However, the recorded call activity can only be used as an index of bat abundance, but not to estimate absolute population size. Autumn swarming captures using mist nets or harp

traps can similarly be used to describe species-level activity and abundance patterns (e.g., Van Schaik *et al.*, 2015), but again cannot be used to reliably estimate population size. Therefore, estimations of bat population dynamics are most often based on winter hibernation counts (Van der Meij *et al.*, 2015), which have several notable shortcomings. For one, visual surveys necessarily exclude sites that are unsafe or inaccessible. For those sites that are counted, population size estimates from different years are susceptible to bias from observer effects (Dambly *et al.*, 2021), timing of the count (Daan, 1973; Řehák, Zukal, & Kovařík, 1994; Zukal *et al.*, 2017) and weather conditions (Degn, 1987; Meschede & Rudolph, 2004; Toffoli, 2021). Additionally, winter counts are limited to the visible proportion of hibernating bats (Battersby, 2008), which varies with hibernacula size, hibernacula complexity (Zöphel, Wilhelm, & Kugelschafter, 2001), and species composition. Moreover, visual counts may be further biased by the roosting preferences of different species. For example, crevice-dwelling species (e.g., *Myotis bechsteini*) are often undercounted or cannot be recorded at all (Toffoli & Calvini, 2021), potentially leading to vast underestimates of the total hibernating population. The discrepancy between the visually observed and the actual population size of bat assemblages in hibernacula remains to be accurately quantified, along with its effects on the resulting site-level population trends.

Infrared light barriers, installed at the entrance of hibernacula, have the potential to accurately count the number of bats that enter and exit a site throughout the year. Since light barriers are directional (i.e., distinguish between bats flying in and out), a net sum of bats entering in autumn, or exiting in spring, can be used to estimate the total hibernating population size. While such techniques have been experimented with for several decades (Kolb, 1959; Böhme & Natuschke, 1967; Daan, 1970; Degn, Andersen, & Baagøe, 1995; Lubczyk & Nagel, 1995), the method has not been widely adopted, most likely due to low accuracy of several early designs, both in terms of correctly counting the number of bat passes (Sedgeley, 2012) and registering their directionality (Klinger, Adler, & Fiedler, 2002; Redell *et al.*, 2006). Thus, to the best of our knowledge, the resulting light barrier data have been used only as a proxy for nightly bat activity (Berková & Zukal, 2010), but not to estimate population sizes. The second potential limitation to such an approach is that the entrance dimensions of the site cannot exceed the maximum dimensions that can be covered by the light barrier (current maximum dimensions of the model deployed in this study: 35 × 300 cm). Nevertheless, the entrances of many hibernation sites have already been altered to minimize human access and disturbance, thereby restricting their dimensions so that light barrier installation requires no additional modification.

In this study, we assess the accuracy of a light barrier system and introduce an analysis pipeline to estimate population sizes and trends using light barrier data. Specifically, we quantify light barrier accuracy by comparing the recorded passes from six light barrier installations to simultaneous infrared video recordings over the course of 30 weeks during

the autumn entry and the spring emergence phase. Based on our observations, we propose a standard methodology to estimate site-level population sizes using the sum of net exits during the spring emergence phase. Subsequently, we estimate bat population sizes at 12 sites and compare these estimates to the traditional visual counts at 10 of them (two are inaccessible). Finally, we develop a method to calculate a confidence interval around the population size estimate, including approximation for sites where no additional video recording has taken place, and explore population dynamics at four sites for which 5–6 years of continuous data were available.

Materials and methods

Infrared light barriers

Infrared light barriers consist of a sensor array of infrared LEDs and corresponding receivers that create two parallel sets of infrared light beams, called 'curtains'. We used several light barrier models produced by ChiroTEC (Lohra, Germany). These systems cycle through each transmitter–receiver pair at a frequency of 1 kHz within each curtain. The curtain is considered blocked when any single receiver does not register the photoelectric signal from any of the individual LEDs during a cycle. The direction of the passes through the light barrier can be distinguished based on the order that the curtains are blocked (i.e., outer, then both, then inner curtain blocked indicates an entry; for further details regarding light barrier event registration see [Supplementary File](#)). In addition to recording passes, the light barrier software can save a log of all individual curtain triggers (referred to here as 'curtain data'), which enables the identification of times when a curtain is blocked for an extended period of time (e.g., by a leaf or spiderweb in the entrance).

In this study, we investigated three light barrier models, which differ in the height of the monitored opening (Liba-4: 9.7 cm; Liba-16: 35.5 cm; Liba-16k: 20.1 cm), the number of sensors per curtain (Liba-4: 4 sensors; Liba-16 and Liba-16k: 16 sensors) and sensor density (Liba-4 and Liba-16: 2.2 cm between sensors, Liba-16k: 1.3 cm). Light barriers were either powered by car batteries (12 V, 100 Ah), or directly via power outlet.

Study sites

In total, we considered 12 hibernation sites, distributed across northern Germany, where light barriers were installed at all entrances (Table 1, map of sites Fig. S1). For the assessment of light barrier accuracy, five sites were concurrently monitored with continuous infrared video of the entrance for 30 weeks (Table 1a). At one site, Eldena, two light barriers were placed in sequence within the same entrance (Liba-16k and Liba-16; Fig. 1) to compare and cross-reference activity between light barrier models. A further seven sites were only monitored using light barriers (Table 1b).

All selected sites have one or two narrow entrances, and the dimensions of the existing opening were not substantially

Table 1 Description of the bat hibernation sites monitored with (a) infrared light barriers and infrared video cameras and (b) only with light barriers

Method	Site	Federal state ^a	Roost type	Light barrier model	Light barrier positioning	Available year(s)	Species composition
(a) light barrier + infrared video monitoring	Anklam	MV	Cellar, accessible	Liba-4	Within opening	2021	<i>Myotis dasycneme</i> , <i>M. daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Pipistrellus pipistrellus</i>
	Demmin	MV	Purpose-built hibernaculum, accessible	Liba-16k	Within opening	2021	<i>Myotis daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
	Eldena	MV	Cellar, accessible	Main: Liba-16/ Liba-16k Side: Liba-4 ^b	Back of opening/ within opening	2016–2021	<i>Myotis brandtii</i> / <i>M. mystacinus</i> , <i>M. daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
	Friedland	MV	Cellar, accessible	Liba-16	Within opening	2021	<i>Myotis daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
	Peenemünde	MV	Bunker, accessible	Liba-4	Back of opening	2021	<i>Myotis brandtii</i> / <i>M. mystacinus</i> , <i>M. dasycneme</i> , <i>M. daubentonii</i> , <i>M. nattereri</i> , <i>M. myotis</i> , <i>Plecotus auritus</i>
(b) light barrier monitoring	Comthurey	MV	Bunker, accessible	Liba-4	Back of opening	2021	<i>Myotis daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i>
	Putbus	MV	Cellar, accessible	Liba-4	Within opening	2019	<i>Myotis daubentonii</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
	Strasburg	MV	Cellar, accessible	Liba-4	Within opening	2021	<i>Myotis daubentonii</i> , <i>M. nattereri</i>
	Trollenhagen	MV	Bunker, accessible	Main: Liba-16 Side: Liba-16k	Within opening	2016–2021	<i>Myotis brandtii</i> / <i>M. mystacinus</i> , <i>M. daubentonii</i> , <i>M. myotis</i> , <i>M. nattereri</i> , <i>Plecotus auritus</i>
	Kalkberghöhle	SH	Natural cave, accessible	Main: Liba-16k Side: Liba-16k	Within opening	2020	<i>Myotis bechsteinii</i> , <i>M. brandtii</i> / <i>M. mystacinus</i> , <i>M. dasycneme</i> , <i>M. daubentonii</i> , <i>M. nattereri</i> , <i>M. myotis</i>
	Baumberge 1	NRW	Well, inaccessible	Liba-16	Within opening	2016–2020	NA
	Baumberge 2	NRW	Well, inaccessible	Liba-16	Within opening	2016–2021	NA

For each site, we indicate the type of underground structure and if it was accessible or not for visual surveys (roost type), the light barrier model installed at each entrance at the site (Liba-4/16/16k), and the positioning of the light barriers relative to the entrance (integrated within the opening, or mounted on the wall at the back of the opening). Species composition refers to the bat species recorded during the last winter count (NA: sites are inaccessible); bold species represent 25% of the visually counted population.

^aThe sites were distributed across three federal states in northern Germany. Federal state abbreviations: MV – Mecklenburg-Western Pomerania, NRW – North Rhine-Westphalia, SH – Schleswig-Holstein.

^bThe side entrance in Eldena was not monitored with an infrared video camera; for population estimates the Liba-16k dataset was used.

altered to fit the light barrier. When possible, light barriers were integrated within the opening. When the opening was smaller than the light barrier, the light barrier was mounted on the inner wall of the hibernaculum, directly against the back of the opening so that no bats could enter or exit the site without passing the light barrier (Table 1; and compare Liba-16k and Liba-16, Fig. 1). At sites with multiple entrances, light barrier data from the main and side entrances were summed.

Light barrier-based population size estimates were compared with winter counts from the 10 accessible sites (Table 1). Visual surveys were carried out by local conservationists between end of January and beginning of March, under the coordination of the respective Federal state government.

Site-level population trends were evaluated at four sites (Eldena, Trollenhagen, Baumberge 1 and 2) where light barrier data were available for a 5- or 6-year period (2016–2020/21). At two sites (Eldena and Trollenhagen), winter count data were also available over these years and were used to compare the population trends observed by the two methods.

Infrared video monitoring and scoring

At each hibernaculum, we collected 15 weeks of video data from August 1 to 4, December 2020 (here referred to as ‘autumn’), and another 15 weeks from 20 February to 4 June 2021 (here referred to as ‘spring’) using self-built infrared video cameras (as described in Krivek *et al.*, 2022; see

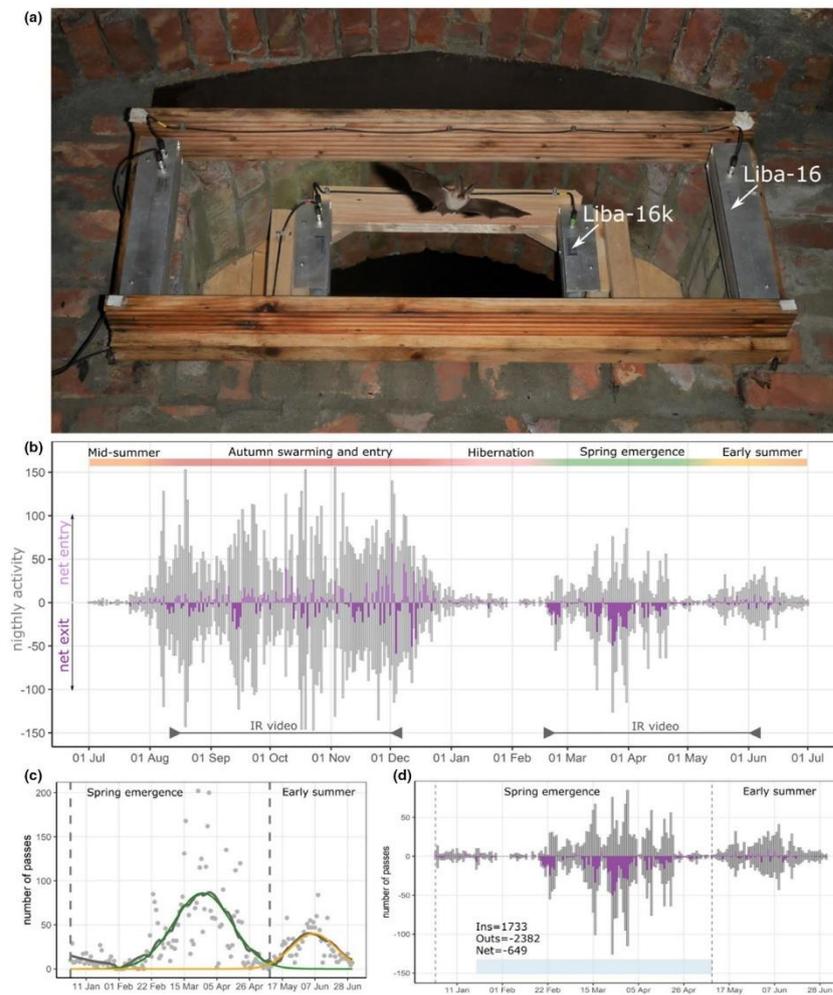


Figure 1 (a) Main entrance of a bat hibernaculum (Eldena, Germany) showing two light barrier models in situ: a Liba-16k integrated within the opening and a Liba-16 mounted on the inner wall of the hibernaculum, at the back of the opening. (b) Annual activity of temperate-zone bats can be divided into five broad, partially overlapping phases (colored bar on top). The number of passes registered each night by the light barrier is indicated with gray bars (entries on the positive axis and exits on the negative axis). The nightly net sum of all entries and exits is represented by the purple bars (net entry: light purple bars; net emergence: dark purple bars). The duration of infrared video monitoring in autumn and spring is denoted along the bottom. (c) To isolate spring emergence from early summer activity, two Gaussian curves were fitted to the emergence activity peak (green) and early summer activity peak (yellow). The gray solid line shows the smoothed number of passes per night and the points show the raw number of passes per night. The crossing point between these curves defines the end of the search window for the emergence-based population size estimate (dashed vertical lines; start of search window: January 1 at all sites). (d) The emergence phase (blue bar) is defined as the contiguous time period that yields the maximum number of emerging bats within the previously defined search window (dashed vertical lines). The population size is calculated by taking the absolute value of the sum of all entries (total ins = 1733) and exits (total outs = -2382) between the start and end of the emergence phase, yielding 649 emerging bats

<https://gabik-bat.github.io/FlederCam/> for construction details). From the resulting 11 868 hours of raw infrared video recording, 6-second-long video clips were isolated for each event registered by the light barrier. This method yielded 62 914 video clips from autumn and 20 124 video clips from spring. We manually determined the direction of each bat pass (i.e., entry or exit), or whether the bat triggered the light barrier while circling on the inside without leaving the hibernacula, which we refer to as an ‘innerloop’. Subsequently, we scored each event recorded by the light barrier as correct or incorrect (for details on how innerloops were scored, see Supplementary File).

If the light barrier failed to register a pass, no video clip was created. To quantify the occurrence of such missed registrations, we cross-referenced each bat pass recorded at the main entrance of Eldena, which was simultaneously monitored with two consecutive light barriers. Since the logic behind how a pass is triggered does not differ between the light barrier models (see Supplementary File), we assume that this rate of missed passes can be considered an accurate proxy for all other sites.

Light barrier accuracy in autumn and spring

The activity of bats at underground hibernacula can roughly be divided into five phases (e.g., Zúkal *et al.*, 2017): (1) *mid-summer*, with low activity at sites that are not also used as summer roosts; (2) *autumn swarming and entry*, characterized by a period of very high flight activity, gradually transitioning into a period of net entrance into the site; (3) *winter hibernation*, with very low activity; (4) *spring emergence*, with extensive departure activity, and (5) *early summer*, with relatively high activity without significant net entry or exit (Fig. 1b). Importantly, the precise timing of these phases differs between species, sexes, age classes, sites, and years (e.g., Meier *et al.*, 2022), and thus cannot be delineated by fixed dates, but must be inferred separately for each dataset.

Estimating population sizes of bat assemblages with light barriers can either be achieved by counting the net number of bats that enter the hibernaculum during the autumn swarming and entry phase or by counting the net emergence during the spring emergence phase. By scoring each entry as +1 and exit as -1, the resulting positive net sum over the autumn entry phase corresponds to the number of bats that entered the site for hibernation, and the negative net sum over the emergence phase corresponds to the number of bats that have left the hibernaculum in spring. To assess the feasibility and accuracy of both estimates, we first compared light barrier accuracy during autumn and spring using video count data. We used Lin’s concordance correlation coefficients (CCC) with 95% confidence intervals to quantify the agreement between nightly net passes obtained by light barriers and the manually counted video data, separately for both 15-week autumn and spring datasets. Based on these results (see *Results: Light barrier accuracy in autumn and spring*), we focused on the spring period for all subsequent analyses.

Population size

Prior to estimating the population size, we applied filters to remove passes during daytime (9 am–3 pm; removed 0.2–3% of passes), and to identify and correct for light barrier technical defects (one night; see Supplementary File and Fig. S2 for details). To quantify the population size based on spring emergence data, we first aimed to separate the emergence phase from the early summer activity phase (Fig. 1b, c). For this, we identified the peaks of the smoothed nightly number of passes (Savitzky–Golay filter, $x = 51$, 2nd order polynomial) between January 1 and July 1 using the *find_peaks* function (prominence = max activity/night \times 0.1, SciPy package, v1.6.3; Virtanen *et al.*, 2020). Subsequently, we fit two Gaussian curves to these peaks with the *lmfit* package (Newville *et al.*, 2016, Fig. 1c), and defined the search window for the emergence phase as starting on January 1 and ending at the point where these two curves intersected. In some cases, there was little to no early summer activity, and this method failed to find a second activity peak. In these cases, the 99th percentile of the first activity peak was used as a cutoff for the end of the emergence phase. Within this search window, we calculated the sum of nightly net passes for each possible combination of start and end dates, and identified the maximum number of emerging bats within a contiguous timeframe. If this maximum value was reached by multiple timeframes, we selected the shortest one. For example, in Eldena in 2021 (see Fig. 1d), this yielded a site- and year-specific emergence phase between January 20 and May 9 (blue bar), a population size estimate of 649 bats (absolute value of the sum 1733 ins and 2382 outs), and a total of 4115 passes during the emergence phase.

Site-specific confidence intervals

We quantified the error rate of the light barriers at each site, to generate a site-specific confidence interval around the population size estimate. Specifically, we used infrared video data to measure the mean number of incorrect registrations per 100 passes at each site, and used the consecutive light barriers in Eldena to estimate the number of passes missed by each of the light barriers. These components were plugged into the general formula: $\bar{x} \pm z * \sigma/\sqrt{n}$, where \bar{x} is the mean net error, σ is the standard deviation of the mean net error, n is the number of passes, and z represents the appropriate z -value for the desired confidence level (here: 95%). Finally, the confidence interval was adjusted using the curtain data to account for periods when the light barrier was blocked. A detailed description of the calculation is provided in the Supplementary File.

The video-based estimate was supplemented with a correction for the missed registrations and blocked periods, as these events were not scored in the video data. Additionally, when the emergence phase started earlier than the video monitoring (February 20), the video estimate was supplemented by the light barrier estimate and confidence interval for the days prior to the video recording.

Table 2 Overall light barrier accuracy summarized for six bat hibernation sites in Northern Germany, per light barrier positioning (integrated within opening, or mounted directly against the back of the opening), and light barrier model

Site	Light barrier positioning	Light barrier model	Total errors	Innerloop-related errors	CCC (mean \pm SD)	
Anklam	Within opening	Liba-4	79	38 (48%)	0.97	0.95 \pm 0.03
Peenemünde	Back of opening		907	765 (84%)	0.93	
Eldena	Back of opening	Liba-16	954	919 (96%)	0.85	0.82 \pm 0.05
Friedland	Back of opening		1010	989 (98%)	0.78	
Eldena	Within opening	Liba-16k	12	2 (17%)	1.00	1.00
Demmin	Within opening		14	8 (57%)	1.00	

For each site, we calculated the total number of light barrier errors (i.e., light barrier registration does not match the bat pass observed in the corresponding infrared video data), the proportion of errors caused by innerloops (i.e., when a bat triggers the light barrier while circling on the inside without leaving the hibernacula), and Lin's concordance correlation coefficients (CCC; indicating the agreement between light barrier and video-based nightly net activity) per site and summarized per light barrier model.

Approximation of the confidence interval

For nonvideo-monitored sites, it was not possible to calculate the mean net error and its standard deviation. The only available information was the population size estimate, the total number of passes during the emergence phase, and the curtain data where this option was turned on. However, when scoring the video clips, we observed that the primary source of error (up to 98% of the total error; Table 2) occurred when bats performed innerloops (i.e., triggering the light barrier while circling at the entrance but not entering or leaving the site). While we cannot calculate the proportion of innerloops at sites without video monitoring, this behavior inflates the mean number of passes observed per emerging bat (Table 3). Thus, we used linear regression to correlate the ratio of total passes:population size estimate to the site-specific standard deviation (Fig. S3a; $R^2 = 0.78$) and to the number of passes missed by the light barrier (Fig. S3b; $R^2 = 0.79$). This was done for all six light barriers that had been installed at the five video-monitored sites (two light barriers in Eldena). The resulting estimates were cross-validated using a jackknife procedure (Fig. S3c) and were subsequently used to approximate the standard deviation and the number of missed passes for sites where no video monitoring took place.

Regarding the mean net error, we found no systematic in or out bias at the video-monitored sites. The mean net error was particularly low (-0.17 – 0.34 , Table S1) for light barriers integrated within the entrance, which was the case for all but one of the sites without video monitoring. As a conservative estimate, we used a mean net error of ± 1 (i.e., -1 was used for the lower bound of the CI, and $+1$ for the upper) to ensure that the true error of the site is contained within the estimate in all but the most extreme cases. Finally, for sites where curtain data were available (Comthurey and Strasburg), we estimated and corrected for the passes missed during the blocked periods, as in the original site-specific formula. For further details on both confidence interval calculations, see Supplementary File. All calculations were performed in R (v.4.1.3; R Core Team, 2022), and an annotated scripts of the analysis

pipeline are provided on GitHub (<https://github.com/GabiK-bat/LightBarrier>).

Population trends

Confidence intervals indicate an uncertainty range around the population size estimate that account for light barrier errors, and consequently, these can be used to detect population trend changes while taking into account measurement uncertainty. To explore site-level population trends, we defined the confidence interval of the first year for which data were available as our baseline. We considered the population to be growing or declining if the confidence interval of the population size estimates in subsequent years exceeded the upper or lower bounds of this baseline.

Results

Light barrier accuracy in autumn and spring

Overall, bat activity was up to 11 times higher during the autumn swarming and entry phase than during spring emergence, with over half a million passes registered in autumn at the largest site (Table 3). At the video-monitored sites, the total number of passes during the autumn swarming and entry phase ranged from 12 497 up to 47 584, and during the spring emergence phase from 1223 to 4672. The concordance between light barrier and video-based net activity was remarkably high, with variation depending on the season, model, and positioning of the light barrier (Fig. 2, Table 2). Specifically, the agreement between nightly net passes recorded by light barriers and counted from infrared videos was in general very high, but lower in autumn (Fig. 2a, $CCC = 0.84 \pm 0.16$) than in spring (Fig. 2b, $CCC = 0.92 \pm 0.09$). The higher sensor density of the Liba-16k resulted in a nearly perfect registration of nightly net passes in spring (Fig. 2b; $CCC = 1.0$). Likewise, the positioning of the light barrier integrated within the opening, rather than mounted on the wall at the back of the opening, greatly reduced the number of innerloop-related errors and

Table 3 Overview of the light barrier-based total bat activity, population size estimates, and comparison to visual winter counts for all 12 investigated hibernation sites in Northern Germany

Site	Year	Total number of passes			Mean passes/bat		Light barrier population size estimate (confidence interval)	Visual winter count
		Autumn swarming and entry	Emergence	Early summer	Autumn swarming and entry	Emergence		
Anklam	2021	12 497	2611	1454	40	8	311 (262–360)	139 (45%)
Demmin	2021	13 040 ^a	1223	661	69	6	189 (169–231)	150 (79%)
Eldena	2021	17 484	4115	1381	27	6	649 (586–714)	271 (42%)
Friedland	2021	47 584 ^a	4672	1069	211	21	225 (103–354)	198 (88%)
Peenemünde	2021	21 653	2846	5126	74	10	292 (237–350)	131 (45%)
Comthurey	2021	13 650 ^a	1377	2948	119	12	115 (78–152)	99 (86%)
Putbus	2019	4734 ^a	1530	399	23	8	204 (172–236)	114 (56%)
Strasburg	2021	38 005	5369	NA	52	7	735 (651–819)	61 (8%)
Trollenhagen	2021	180 100	23 754	16 087	49	7	3642 (3335–3949)	278 (8%)
Kalkberghöhle	2020	527 046	76 850	36 435	16	2	33 164 (32 310–34 018)	1017 (3%)
Baumberge 1	2020	288 244	48 839	65 985	37	6	7723 (7127–8319)	NA
Baumberge 2	2020	36 744	5167	6759	43	6	849 (770–928)	NA

The total number of bat passes (i.e., sum of all ins and outs) registered by light barriers at each site were separated into three phases: autumn swarming and entry phase (broadly defined here as July 1–January 31), emergence phase (site- and year-specific dates, estimated independently for each dataset), and early summer activity (site and year-specific start – July 1). The light barrier population size estimates represent the net number of bats emerging from the hibernacula during the emergence phase. The confidence intervals represent the approximated confidence interval calculated when no video data were available. The mean number of passes recorded per bat represents the total number of passes per phase divided by the population estimate. The visual winter count denotes the number of bats counted during a single hibernation survey (parentheses indicate the visually counted proportion of the light barrier-based population size estimate; NA: sites are inaccessible).

^a Light barriers were installed on July 30 in Demmin, Friedland, and Comthurey, and on September 22 in Putbus.

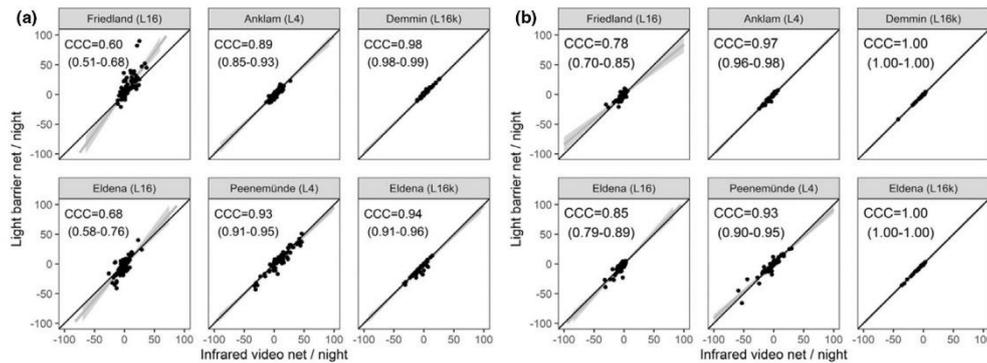


Figure 2 Comparison of the nightly net passes of bats recorded by the infrared light barriers and counted from video recordings in (a) autumn (August 1–December 4, 2020) and (b) spring (February 20–June 4, 2021). Data were collected at five hibernacula entrances in northern Germany that were monitored with one of three light barrier models (Liba-16, Liba-4, and Liba-16k). In Eldena, the entrance was monitored with two light barriers (Liba-16 and Liba-16k) that were assessed independently in terms of their accuracy. Accuracy was quantified using Lin’s concordance correlation coefficients (CCC) with 95% confidence intervals (gray solid line with ribbon), which measures the agreement between nightly net passes obtained by the two methods. These coefficients indicate how far the observed data deviate from the line of perfect concordance (black solid line)

increased concordance (compare Anklam and Peenemünde, Eldena Liba-16k and Liba-16; Table 2).

Population size

The light barrier-based population size estimates (i.e., number of bats emerging in spring) ranged from 115 to 33 164

at the 12 study sites (Table 3). Where available, winter counts recorded far fewer bats (range 61–1017), which represented 3–88% of the light barrier estimates. At sites with little to no crevice formation where bats could hide during the visual counts, such as the purpose-built hibernaculum in Demmin or the concrete-made hibernaculum in Comthurey, the proportion of counted bats in winter was relatively high

(79% and 86%). It was more variable (45–88%) at cellars and bunkers with differing amounts of deep crevices (Anklam, Friedland, Peenemünde, Putbus). The visual counts accounted for a markedly lower proportion of the light barrier-based population estimates at large sites with so-called cavity walls (Eldena 42% and Trollenhagen 8%), inaccessible sections (Strasburg 8%), and at a natural cave (Kalkberghöhle 3%).

Confidence interval

Light barrier-based population size estimates with site-specific confidence intervals overlapped with the range of the video estimates at all sites (Fig. 3). However, in Peenemünde the infrared video data revealed that the light barrier slightly underestimated the population size, only counting 79% of the video-based population estimate (compare: 35% for winter count). Site-specific confidence intervals varied in size (range: 15.9–66.2% of the population estimate), correctly reflecting the differences in light barrier accuracy between sites. Notably, approximated confidence intervals similarly reflected this difference and mirrored the relative differences seen across sites in the site-specific confidence intervals. The approximated confidence intervals

contained the full range of the video-based estimates at all sites but Peenemünde (Fig. 3), where it underestimated the population size.

Population trends

Light barrier-based population sizes and trends varied considerably over the monitored 5- or 6-year period (range of population estimates in Eldena: 498–649; Trollenhagen: 2730–3905; Baumberge 1: 7126–7723; and Baumberge 2: 549–958; Fig. 4). In Eldena and Trollenhagen, populations showed a pronounced decrease in 2018, with a subsequent recovery, resulting in a stable overall population trend relative to the baseline year (2016). This population decrease in 2018 was not observed at Baumberge 1 and 2, which are located 600 km further to the west (map of sites Fig. S1). At Baumberge 1, the population has grown steadily but has not exceeded the confidence interval of the baseline year. At Baumberge 2, the population has grown significantly relative to 2016.

In Eldena and Trollenhagen, these population trends were not reflected in the winter counts. Variation across winter counts was lower (range in Eldena: 224–303 and Trollenhagen: 248–362), and in Eldena the winter count even peaked in 2018, when the light barrier-based population size estimate was the lowest. The proportion of the light barrier-based estimates observed during the visual surveys varied substantially between years (Trollenhagen 6–10% and Eldena 36–61%).

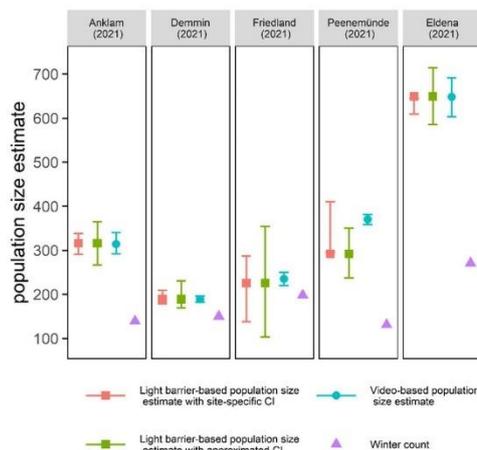


Figure 3 Population sizes estimates at five hibernacula in northern Germany, monitored with a light barrier, infrared video camera, and counted visually. For each site, we provide the light barrier-based population size estimates with site-specific confidence intervals (based on estimates of mean net error and standard deviation from video data; red square with error bar), the same population estimate with approximated confidence intervals (based on approximations of mean net error and standard deviation using only light barrier data; green square with error bar), the video-based population size estimates with confidence intervals (accounting for missed registrations, blocked periods, and periods without video monitoring; blue circle with error bar), and the visual winter counts (purple triangles).

Discussion

Automated monitoring approaches can drastically improve our ability to estimate the size and trends of wild animal populations (e.g., Hodgson *et al.*, 2018; McCarthy *et al.*, 2021). Here, we first quantified the accuracy of light barrier systems installed at the entrances of bat hibernacula based on 30 weeks of infrared video monitoring. We found that light barriers are able to monitor the number of bats entering and leaving the hibernaculum with unprecedented accuracy. Thus, for the first time, we can obtain realistic population size estimates of bat assemblages at complex and/or inaccessible hibernacula that can house up to tens of thousands of hibernating bats, enabling us to truly count in the dark. Moreover, as we can approximate the measurement error of a light barrier system, confidence intervals can be calculated around the population size estimates to distinguish true population trends from measurement uncertainty.

Light barrier accuracy

Overall, accuracy was higher than in any previously assessed light barrier system (e.g., Klinger *et al.*, 2002; Redell *et al.*, 2006; Sedgeley, 2012), but comparable to the accuracy of the same light barrier model installed at summer maternity colonies and evaluated using single nights of video observations (Matthäus, Kugelschäfer, & Fietz, 2022). We found that population estimates based on the number of bats

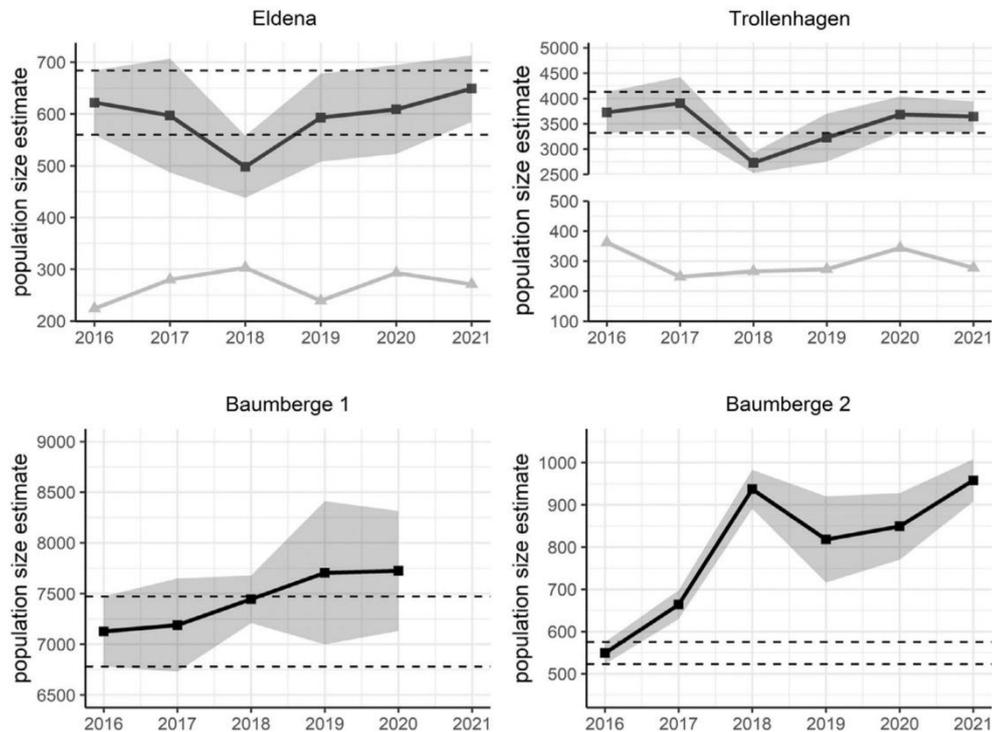


Figure 4 Light barrier-based bat population size estimates with approximated confidence intervals (black squares and gray ribbon) and winter counts (gray triangles) for two underground hibernacula in Mecklenburg-Western Pomerania, Germany (Eldena, Trollenhagen), and two in North Rhine-Westphalia, Germany (Baumberge 1 and 2; inaccessible for winter counts). The range of the confidence interval in the first year was taken as the baseline (dashed horizontal lines) and used to evaluate population trends (significant growth or decline: entire confidence interval exceeds baseline range positively or negatively, respectively).

emerging from a hibernaculum in spring are the most accurate, due to the lower mean number of passes per bat compared with the autumn swarming and entry phase. This is further aided by our ability to separate the emergence phase from early summer activity using a standardized methodology. Accuracy varied depending on the positioning and model of the light barrier. Strikingly, models with high sensor density (Liba-16k) nearly perfectly registered nightly net passes over the entire emergence phase. Likewise, integration of light barriers within the hibernacula entrance increased accuracy by reducing the proportion of recorded innerloops (i.e., bats circling on the inside without leaving the hibernacula), which was the primary source of error.

Accuracy differed between the video-monitored sites, and these differences were correctly reflected in the range of the site-specific confidence intervals. Interestingly, as the vast majority of errors were caused by innerloops, the ratio of total passes to the population size estimate can be used to approximate a confidence interval at sites without video monitoring. Although these approximated confidence

intervals are comparatively conservative, their relative size between sites was comparable to the site-specific confidence intervals that were based on video monitoring. In rare cases, the approximated confidence intervals may not include the true population size, if the mean net error is substantially larger than the approximated parameter (± 1). For example, the positioning of the light barrier at the back of a very narrow opening in Peenemünde led to more registered innerloops, which resulted in a larger mean net error than estimated. While this may lead to slight under- or overestimation of the population sizes, these estimates are likely still far closer to the true population sizes than visual counts in the hibernaculum. Moreover, assuming that the site-specific bias remains constant, as would be expected with consistent biases due to the positioning of the light barrier, population trends at a given site can still be informatively assessed.

By providing an accurate population size estimate with a confidence interval, light barriers overcome the primary downside of traditional winter counts – that we know they underestimate the actual population size, but that we do not

know by how much. This is exemplified here by the considerable variability between sites in the visually counted proportion of the light barrier-based population size estimates, even at sites that are broadly comparable in terms of their structural complexity. Contrary to previous claims (e.g., Battersby, 2008; Van der Meij *et al.*, 2015), the proportion of bats counted in winter varied within a site between years. Thus, the accuracy of winter counts cannot reliably be predicted a priori. As a result, population trends based on winter count data should be interpreted with extreme caution. Importantly, light barriers and visual counts capture fundamentally different population size estimates: Winter counts quantify the number of bats that can be visually counted in the hibernaculum, whereas light barriers measure the number of bats emerging from the hibernaculum. Thus, in years with substantial winter mortality, the winter count may be proportionally larger if they were performed before the bats died.

Implementation recommendations

Several recommendations can be drawn from our results concerning where light barriers may be best implemented to aid bat population monitoring. We find that installing light barriers within the entrance rather than mounted directly on the wall behind it yields the best accuracy. It must be noted that any modifications to a site entrance should be performed with extreme caution (Pugh & Altringham, 2005), and that the system is naturally unsuitable for larger entrances (currently maximum 35 × 300 cm), or sites with numerous entrances. Conveniently, in Germany and many other countries, some hibernaculum entrances have already been reduced in size to limit human access, and light barriers may offer an ideal minimally invasive method to monitor these sites. Beyond the entrance specifications, light barriers may be more useful at some sites than others. The gains are greatest at sites that are either inaccessible for visual surveys or with many areas that cannot be visually counted (e.g., cavity walls, crevices, and karstic formations). Indeed, we found that visual counts only recover a fraction of the true population (as low as 3%) at such complex sites. By contrast, at smaller sites that are purpose-built as bat hibernacula or with comparatively few deep crevices, light barrier-based population size estimates did not differ substantially from winter hibernation counts, suggesting that such sites can be reliably and cost-effectively monitored by visual surveys.

Conservation applications

Site-level population estimates and trends have several important conservation applications. For example, accurate population sizes for complex or inaccessible sites can help identify hibernacula of regional importance. This may be especially relevant in the context of monitoring population dynamics during emerging infectious disease outbreaks, where eliminating the need to physically enter the site may be highly valuable (e.g., white-nose syndrome; Boyles, 2017). Furthermore, site-level population trends could be used to track natural population dynamics and allows immediate

conservation action when local populations unexpectedly decline. Here, populations in Trollenhagen and Eldena (both in Mecklenburg-Western Pomerania) showed a pronounced decline in 2018, whereas such a decline was not observed in Baumberge 1 and 2 (in North Rhine-Westphalia). Notably, these declines were not detected in the winter counts at either Eldena or Trollenhagen, but similar large differences between winters have been observed in other populations using data from individually marked bats (Fleischer *et al.*, 2017; Reusch *et al.*, 2019), suggesting that these trends are indeed realistic. Had this population decline only been observed at a single site within a region, this could indicate harmful human disturbance or predation at the site, and conservation action could be warranted. Although here we quantified population size estimates only based on emergence in spring, the observed accuracy of the light barrier at some sites might be sufficient to also calculate population size estimates based on entry in autumn, thereby allowing for estimates of site-level mortality during the hibernation phase. In addition to improved population size estimates, given that the light barrier records activity continuously, the data can also be used to track site-level phenology of bat populations, and investigate the effects of weather and climate on bat hibernation. Taken together, correlating site-level population trends from a diverse network of sites monitored with light barriers could help to quantify the components affecting population dynamics and distinguish between site-level effects (e.g., mitigation and conservation measures, partial site collapse, predator effects, and changes in microclimate) and regional effects (e.g., land-use modification and weather).

Future challenges

To make these population estimates actionable for species-level conservation and monitoring objectives, they must be partitioned into species-specific estimates. At sites with up to several thousand bats, a camera trap triggered by a light barrier can be used to obtain relative species abundances. Along these lines, we have previously shown that such custom-built camera traps are minimally invasive and do not noticeably affect bat behavior (Krivek *et al.*, 2022). At large and remote sites, camera trap monitoring may not always be feasible due to power limitations and data volume. Alternatively, species composition could be roughly approximated using other methods, such as passive acoustic recordings (Froidevaux *et al.*, 2014) or repeated captures during the swarming season (Van Schaik *et al.*, 2015). Notably, even such approximations constitute a substantial improvement for undercounted crevice-dwelling species (e.g., *Myotis bechsteinii*; Toffoli & Calvini, 2021), as even Europe-wide TRIM analyses have thus far been unable to provide population trend estimates for such species (Van der Meij *et al.*, 2015).

Conclusion

In conclusion, the light barrier systems investigated here provide a minimally invasive, automated approach to accurately monitor bat population dynamics, even at sites that are

inaccessible for visual counts. Using the standardized methodology proposed here, the resulting data can be used to estimate the population size of bat assemblages in hibernacula and a confidence interval around it that reflects the site-specific measurement error. This automated monitoring tool offers a powerful complementary method to traditional winter counts, which can cost-effectively monitor many smaller sites but are notoriously difficult and inaccurate at larger, more complex sites. A monitoring network, composed of a subset of such sites where the entrance does not need to be substantially modified to enable light barrier installation, would allow us to estimate population sizes and trends accurately, and track year-round activity at varying spatial scales. This constitutes a massive leap forward for bat monitoring and hence bat conservation.

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Authors' contributions

GKr, JvS, and GKe contributed to the conception. GKr and JvS contributed to the study design. GKr, JvS, and FM contributed to the light barrier data collection. GKr contributed to the infrared video data collection. EPNM and GKr contributed to the video processing. GKr and JvS contributed to the writing with input from all authors.

Data availability statement

Data and scripts are available at: <https://github.com/GabiK-bar/LightBarrier>.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Map of Germany with the 12 hibernation sites that were monitored with infrared light barriers in three federal states (MV – Mecklenburg-Western Pomerania, NRW – North Rhine-Westphalia, SH – Schleswig-Holstein).

Figure S2. (a) Nightly passes and net activity in Friedland in spring 2021, including the outlier night (28 March 2021) and (b) after excluding the outlier night, and replacing the nightly passes and net activity with an average of the three preceding and following nights. (c) Number of curtain registrations per night in Friedland in spring 2021, where the red lines mark the erroneous, outlier night. (d) Four classical goodness-of-fit plots to visually evaluate the presence of potential outlier nights in the light barrier data, based on the deviation from a gamma distribution fitted to the nightly curtain registrations. Arrows denote the outlier night.

Figure S3. Linear regression with 95% confidence interval, showing the correlation between the ratio of total passes: population size estimate to the (a) the site-specific standard deviation, and (b) the total number of passes missed by the light barrier as a result of simultaneous entries and inner-loop-related false negative errors based on six video-monitored light barrier installations. (c) At new sites, the standard deviation and number of missed passes were approximated based on the regression estimates across all six datapoints. The robustness of these estimates was investigated using a jack-knife procedure, where each observation was systematically left out from the dataset, and a regression estimate was calculated over the remaining observations.

Table S1. Summary of estimates used to calculate site-specific confidence intervals for the population size estimates using the emergence data: mean net error per 100 passes (\bar{x}_1) with standard deviation (SD_1), accounting for incorrect events, false positive error, simultaneous entries, and inner-loop-related false negative errors, quantified using infrared video data; mean net error per 100 passes (\bar{x}_2) with standard deviation (SD_2) due to false negative errors, quantified using the cross-validated data from two consecutive light barriers (Eldena, Liba-16k and Liba-16); combined standard deviation (SD_{comb}) using Gaussian error propagation; total number of

registered passes divided by 100 (n), missed passes expressed per 100 passes as a result of simultaneous entries (MP_1), innerloop-related false negatives (MP_2), false negative errors (MP_3) and during blocked periods (MP_4); blocked hours (minutes rounded to the closest hour; B_{hours}) when the light barrier was not registering any events during the emergence phase, mean passes per hour (B_{mean}) and mean net passes per hour ($B_{net,mean}$) with 95% confidence interval during emergence phase. \bar{x}_2 , SD_2 and MP_3 could only be quantified in Eldena, therefore the worst-case scenario was used as an approximation for the other sites, indicated by stars.

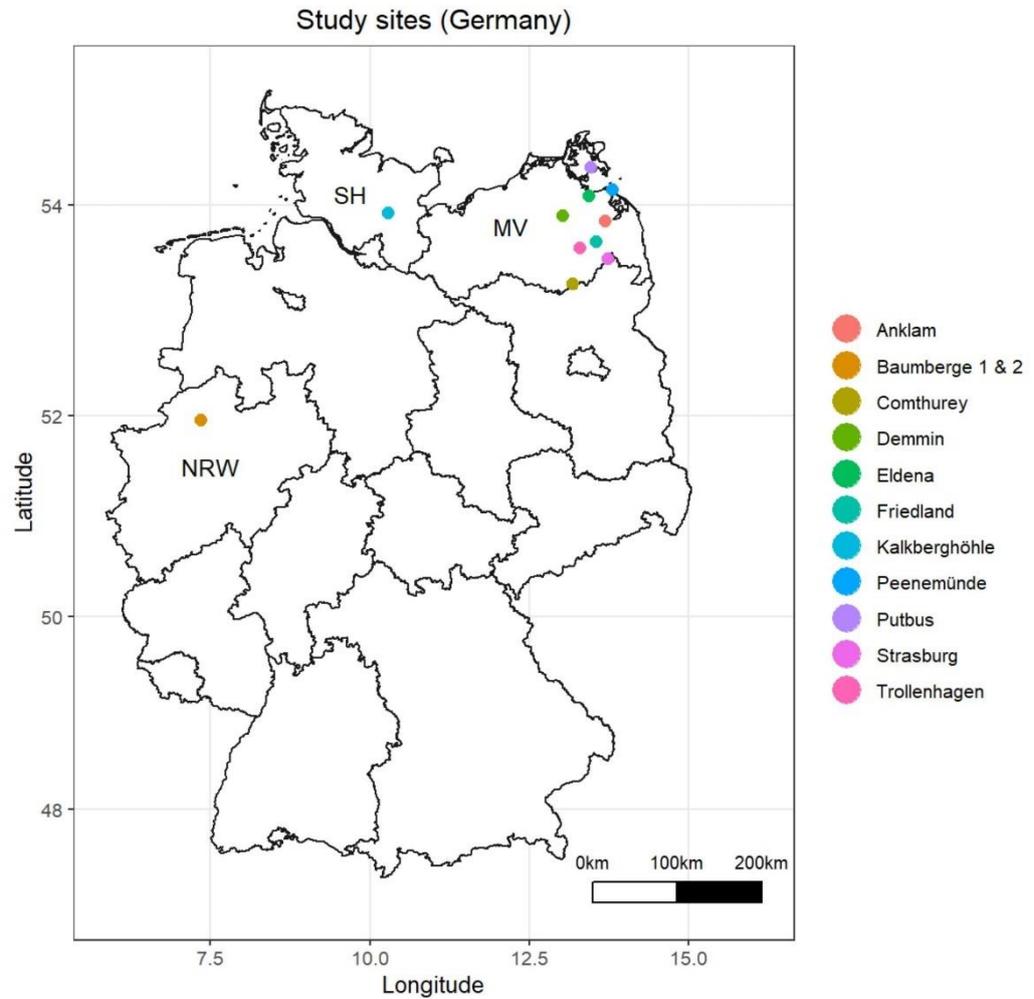
Supplementary materials to:

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Figure S1 Map of Germany with the 12 hibernation sites that were monitored with infrared light barriers in three federal states (MV - Mecklenburg-Western Pomerania, NRW - North Rhine-Westphalia, SH - Schleswig-Holstein).



Light barrier event registration

Light barriers record four possible status options: 0 – no curtain is blocked, 1 – outer curtain is blocked, 2 – inner curtain is blocked, 3 – both curtains are blocked. A series of curtain events is considered an entry or an exit if the first and the last registration flanking a null registration are from opposite curtains. In the simplest case, the sequence 0-1-3-2-0 indicates an entry and 0-2-3-1-0 an exit, but sequences may also cycle through multiple status changes (e.g., 0-1-3-1-3-2-3-2-0 is also considered an entry). If a series of curtain registrations starts and ends with a registration from the same curtain, the activity is not logged as a pass (i.e., 0-1-3-2-3-1-0). If there is no null registration recorded for an extended period of time, the light barrier is considered blocked, and no passes can be registered during this time. Bats circling on the inside without leaving the hibernacula trigger an exit and an entry in quick succession (0-2 seconds), particularly when the light barrier is installed behind the entrance; we refer to these events as ‘innerloops’. See below examples for each case.

When the option to log all curtain information is turned on, each individual curtain trigger is logged as an event with a timestamp, and a separate pass status is generated when the sequence matches the definition of an entry or an exit. This was the case for all sites used in the infrared video accuracy assessment. For sites with higher bat activity or in places where data is not collected regularly, only the summary passes (entries and exits) are recorded to reduce storage requirements.

Date	Time	Event
15.9.2021	22:44:58	LS1 Estatus 0
15.9.2021	22:45:01	LS1 Estatus 1
15.9.2021	22:45:01	LS1 Estatus 3
15.9.2021	22:45:01	LS1 Estatus 2
15.9.2021	22:45:01	LS1 Estatus 0
15.9.2021	22:45:01	LS1 Einflug entry

Date	Time	Event	
15.9.2021	22:45:02	LS1 Estatus 0	
15.9.2021	22:47:22	LS1 Estatus 2	
15.9.2021	22:47:22	LS1 Estatus 3	
15.9.2021	22:47:22	LS1 Estatus 1	
15.9.2021	22:47:22	LS1 Estatus 0	
15.9.2021	22:47:23	LS1 Ausflug	exit
15.9.2021	22:56:13	LS1 Estatus 1	
15.9.2021	22:56:13	LS1 Estatus 0	
15.9.2021	22:56:13	LS1 Estatus 1	
15.9.2021	22:56:13	LS1 Estatus 0	
15.9.2021	22:56:13	LS1 Estatus 1	
15.9.2021	22:56:13	LS1 Estatus 3	
15.9.2021	22:56:13	LS1 Estatus 2	
15.9.2021	22:56:13	LS1 Estatus 0	
15.9.2021	22:56:13	LS1 Einflug	entry with multiple status changes
15.9.2021	22:54:11	LS1 Estatus 0	
15.9.2021	22:54:11	LS1 Estatus 2	
15.9.2021	22:54:11	LS1 Estatus 3	
15.9.2021	22:54:11	LS1 Estatus 1	
15.9.2021	22:54:11	LS1 Estatus 3	
15.9.2021	22:54:11	LS1 Estatus 1	
15.9.2021	22:54:11	LS1 Estatus 3	
15.9.2021	22:54:11	LS1 Estatus 1	
15.9.2021	22:54:11	LS1 Estatus 0	
15.9.2021	22:54:11	LS1 Ausflug	exit with multiple status changes
15.9.2021	21:03:02	LS1 Estatus 0	
15.9.2021	21:04:16	LS1 Estatus 1	
15.9.2021	21:04:16	LS1 Estatus 3	
15.9.2021	21:08:03	LS1 Estatus 1	
15.9.2021	21:08:03	LS1 Estatus 3	
15.9.2021	21:13:04	LS1 Estatus 1	
15.9.2021	21:13:04	LS1 Estatus 3	
15.9.2021	21:17:42	LS1 Estatus 2	
15.9.2021	21:17:42	LS1 Estatus 3	
15.9.2021	21:17:42	LS1 Estatus 1	
15.9.2021	21:17:42	LS1 Estatus 3	
15.9.2021	21:17:42	LS1 Estatus 1	
15.9.2021	21:18:02	LS1 Estatus 0	blocked period for 15 minutes
15.9.2021	22:51:36	LS1 Estatus 0	
15.9.2021	22:51:55	LS1 Estatus 2	
15.9.2021	22:51:55	LS1 Estatus 3	
15.9.2021	22:51:55	LS1 Estatus 1	
15.9.2021	22:51:55	LS1 Estatus 0	
15.9.2021	22:51:55	LS1 Ausflug	
15.9.2021	22:51:55	LS1 Estatus 1	
15.9.2021	22:51:55	LS1 Estatus 3	
15.9.2021	22:51:55	LS1 Estatus 2	
15.9.2021	22:51:55	LS1 Estatus 0	
15.9.2021	22:51:55	LS1 Einflug	“innerloop” (exit-entry)

Scoring of light barrier accuracy – innerloops

When scoring the accuracy of light barriers, innerloops pose a particular challenge. In the simplest cases:

A bat either fully passes (exit) and returns (entry) in quick succession (see ‘innerloop’ in the example log excerpt above). This was scored as 2 ‘correct’ passes.

OR

A bat never fully passes the light barrier, and the curtain log starts and ends with a registration from the same curtain (i.e., 0-1-3-2-3-1-0), no passes are recorded, thus nothing was scored.

However, in some cases, the angled flight trajectory and rapid wing flapping resulted in:

The registration of only one half of the exit-entry pass combination.

OR

In additional spurious registrations (e.g., 2 exits and 1 entry).

In these cases, the accuracy was scored in reference to the ‘correct’ sequence of exit and then entry. For example, if only an exit was registered, but no entry, this was scored as 1 ‘correct’ and 1 ‘incorrect’ (missing) pass; or if 1 exit and 2 entries were registered, this was scored as 2 ‘correct’ and 1 ‘incorrect’ (the extra entry) pass.

Date	Time	Event
27.9.2021	20:56:02	LS1 EStatus 2
27.9.2021	20:56:02	LS1 EStatus 3
27.9.2021	20:56:02	LS1 EStatus 1
27.9.2021	20:56:02	LS1 EStatus 0
27.9.2021	20:56:02	LS1 Ausflug exit part of ‘innerloop’ (correct)
27.9.2021	20:56:02	LS1 EStatus 1
27.9.2021	20:56:02	LS1 EStatus 0
27.9.2021	20:56:02	LS1 EStatus 2
27.9.2021	20:56:02	LS1 EStatus 3
27.9.2021	20:56:02	LS1 EStatus 2
27.9.2021	20:56:02	LS1 EStatus 0 missing entry part of ‘innerloop’ (incorrect)

Date	Time	Event	
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 0	
27.9.2021	23:18:16	LS1 Ausflug	exit part of 'innerloop' (correct)
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 0	
27.9.2021	23:18:16	LS1 Einflug	entry part of 'innerloop' (correct)
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 0	
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 0	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 1	
27.9.2021	23:18:16	LS1 EStatus 3	
27.9.2021	23:18:16	LS1 EStatus 2	
27.9.2021	23:18:16	LS1 EStatus 0	
27.9.2021	23:18:16	LS1 Einflug	extra entry related to an 'innerloop' (incorrect)

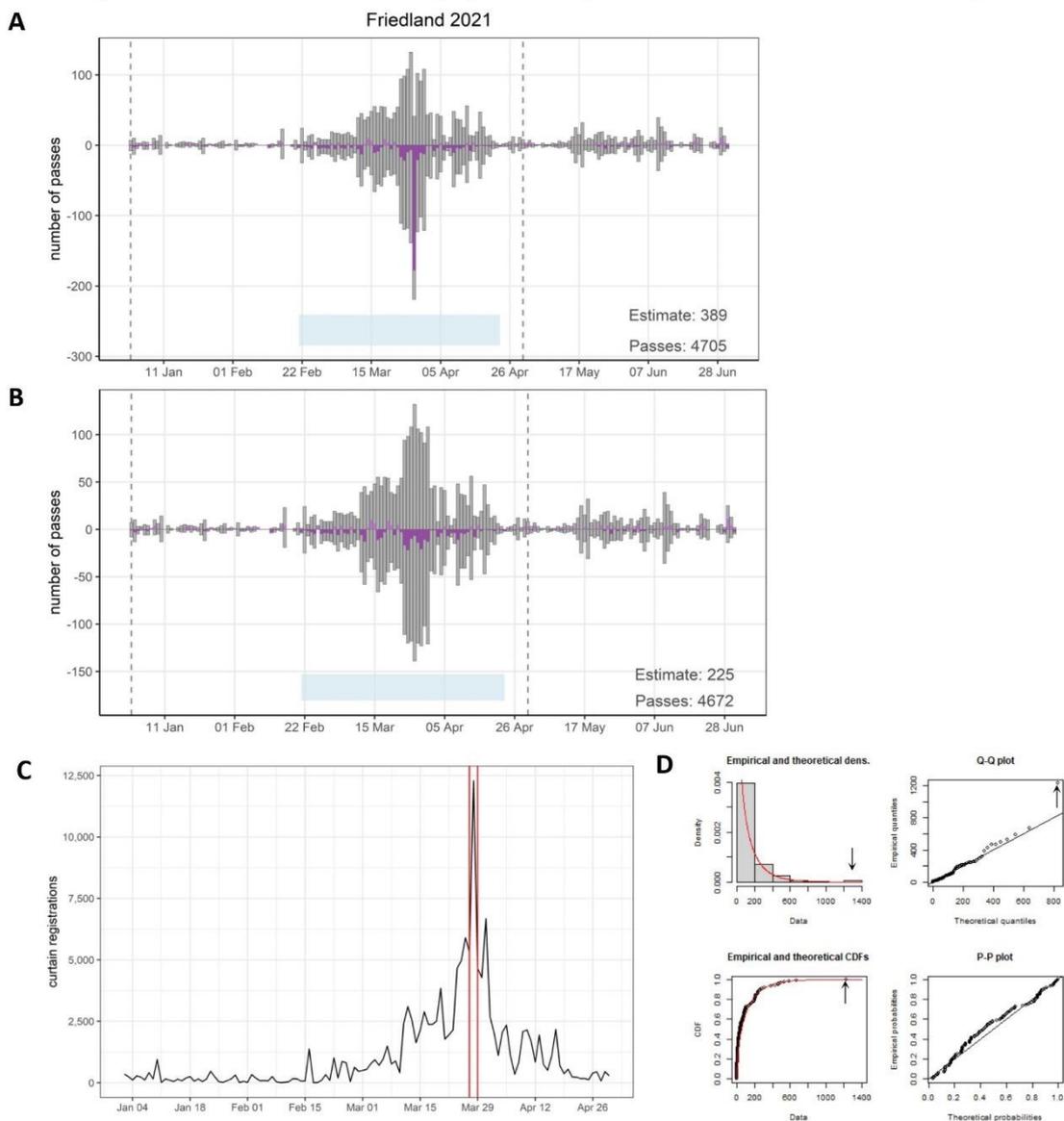
Data preparation

Prior to estimating population size, we applied two filters. First, we removed all events registered between 9 am and 3 pm (1 hour after earliest sunrise, and before latest sunset time, respectively), as these were generally caused by diurnal species and not by bats (0.2-3% of the total passes at the study sites). This filter was not applied where the light barrier was installed in a frame a few meters away from the entrance (Anklam, Strasburg), as here these passes represented daytime activity of bats inside the hibernaculum.

Outlier night detection and estimation

In very rare cases a technical defect or the rapid movement of an object in one of the light barrier curtains (e.g., a spider in a web vibrating in the wind) can lead to a severe bias in the nightly emergence estimate. Over 30 weeks of infrared monitoring of six light barriers, we only observed a single such case, on 28 March 2021 in Friedland (Fig. S1A). For all further calculations, the total passes and net activity of this night were replaced with an average of the three nights preceding and following the event (Fig. S1B). Based on visual inspection, the exceptionally high net activity (Fig. S1A), and large volume of curtain registrations (Fig. S1C) on this night, appeared highly suspect. This was confirmed by the infrared video data (light barrier = 260 passes, -178 net; video = 415 passes, -17 net). In an attempt to standardize the detection of such events, we fit a gamma distribution to the nightly curtain registrations (package: `fitdistrplus`, function: `fitdist`, `distr = "gamma"`, `method = "mle"`). Using this approach, this night could indeed be visually detected due to its significant deviation from the estimated gamma distribution (Fig. S1D). However, as we currently only have a single outlier observation, and detecting outliers in gamma distributions is not trivial, the method will require further evidence before it can be evaluated. Thus, for now such outlier nights were identified by visual assessment, and the curtain log files were referenced to confirm whether the recorded activity on such nights is plausible or likely erroneous.

Figure S2A) Nightly passes and net activity in Friedland in spring 2021, including the outlier night (28 March 2021) and **B)** after excluding the outlier night, and replacing the nightly passes and net activity with an average of the three preceding and following nights. **C)** Number of curtain registrations per night in Friedland in spring 2021, where the red lines mark the erroneous, outlier night. **D)** Four classical goodness-of-fit plots to visually evaluate the presence of potential outlier nights in the light barrier data, based on the deviation from a gamma distribution fitted to the nightly curtain registrations. Arrows denote the outlier night.



Site-specific confidence interval calculation

Starting from the basic formula: $\bar{x} \pm z * \sigma/\sqrt{n}$, the mean net error (\bar{x}), standard deviation of the mean net error (σ) and number of passes (n) were estimated as follows from the video data:

1. All the events, which were assessed for accuracy based on the video data, were randomly divided into 100-pass blocks 1000 times to calculate a site-specific mean net error per 100 passes (\bar{x}_1) and standard deviation (SD_1). This standard deviation represents the error produced by incorrect events (i.e., event registered in the wrong direction), false positives (i.e., event registered by the light barrier, but there was no event), simultaneous entries (i.e., two bats entered simultaneously and only a single pass was registered) and innerloop-related false negative errors (i.e., half of an ‘innerloop’ was missed, but the other half was correctly registered). For the latter two sources (simultaneous entries and innerloop-related false negatives), we also calculated the mean number of missed passes per 100 passes, and added them (MP_1 and MP_2) to the total number of recorded passes divided by 100 (n).
2. In addition to the events that were scored in the video data, we also accounted for false negative errors (i.e., bat passes that were not registered by the light barrier). For this, we estimated the number of missed passes per 100 registered passes by cross-referencing the activity data from two consecutive light barriers installed 30cm apart within the same entrance (Eldena main entrance, Liba-16k and Liba-16). This yielded a mean net error (\bar{x}_2), standard deviation of the mean net error (SD_2), and a number of missed passes (MP_3).
3. Finally, we calculated the total amount of time that one or both curtains were blocked for longer than 1 minute, as passes occurring during these times are not registered. Blocked periods were detected in the light barrier status log files, summed, and rounded to the nearest hour throughout the emergence phase (B_{hours} ; range 0-66 hours). To correct the estimate for these times, we calculated the mean passes per hour (B_{mean}), the mean net passes per hour ($B_{\text{net_mean}}$) with 95% confidence level for the entire emergence phase

(Table S1). Then we multiplied the lower (B_{CI-L}) and upper (B_{CI-U}) confidence levels by the total number of blocked hours and added their absolute value to the lower and upper limits of the confidence interval. The estimated number of passes missed due to the light barrier being blocked ($MP_4 = B_{hours} * B_{mean}$) was added to the total number of passes.

To combine these, means were summed ($\bar{x}_{sum} = \bar{x}_1 + \bar{x}_2$), as were the number of registered passes and all missed passes ($n_{sum} = n + MP_1 + MP_2 + MP_3 + MP_4$). The standard deviations were combined using Gaussian error propagation ($\sigma_{sum} = \sqrt{SD_1^2 + SD_2^2}$). We calculated the z-value for Student's t-distribution, to correct for small sample sizes at some sites, using the following formula: $qt(1-(\alpha/2), df=n_{sum}-1)$, where $\alpha=0.05$ for the 95% confidence interval. This yields the following formulas to calculate a site-specific confidence interval for the population size estimate:

$$\text{Lower bound: } population\ size - \left((\bar{x}_{sum} - z \times \frac{\sigma_{sum}}{\sqrt{n_{sum}}}) \times n_{sum} \right) + |(B_{hours} * B_{CI-L})|$$

$$\text{Upper bound: } population\ size + \left((\bar{x}_{sum} + z \times \frac{\sigma_{sum}}{\sqrt{n_{sum}}}) \times n_{sum} \right) + |(B_{hours} * B_{CI-U})|$$

where all values are expressed in 100-pass units (i.e., 600 passes is $n_{sum} = 6$). Table S1 provides a complete overview of each estimate for all video-monitored sites.

Table S1 Summary of estimates used to calculate site-specific confidence intervals for the population size estimates using the emergence data: mean net error per 100 passes (\bar{x}_1) with standard deviation (SD_1), accounting for incorrect events, false positive error, simultaneous entries, and innerloop-related false negative errors, quantified using infrared video data; mean net error per 100 passes (\bar{x}_2) with standard deviation (SD_2) due to false negative errors, quantified using the cross-validated data from two consecutive light barriers (Eldena, Liba-16k and Liba-16); combined standard deviation (SD_{comb}) using Gaussian error propagation; total number of registered passes divided by 100 (n), missed passes expressed per 100 passes as a result of simultaneous entries (MP_1), innerloop-related false negatives (MP_2), false negative errors (MP_3) and during blocked periods (MP_4); blocked hours (minutes rounded to the closest hour; B_{hours}) when the light barrier was not registering any events during the emergence phase, mean passes per hour (B_{mean}) and mean net passes per hour (B_{net_mean}) with 95% confidence interval during emergence phase. \bar{x}_2 , SD_2 and MP_3 could only be quantified in Eldena, therefore the worst-case scenario was used as an approximation for the other sites, indicated by stars.

Site	\bar{x}_1	\bar{x}_2	SD_1	SD_2	SD_{comb}	n	MP_1	MP_2	MP_3	MP_4	B_{hours}	B_{mean}	B_{net_mean} (95% CI)
Anklam	0.34	-0.4*	2.08	1*	2.31	26	0.06	1	1*	0	0	1.33	-0.15 (-0.19, -0.11)
Demmin	-0.08	-0.4*	1	1*	1.41	12	0	1	1*	70.6	66	1.07	-0.17 (-0.23, -0.11)
Eldena (L16)	-0.75	-0.4	5.23	0.9	5.31	37	0.05	12	1	28.6	10	2.86	-0.29 (-0.36, -0.22)
Eldena (L16k)	-0.17	-0.2	0.96	0.8	1.25	12	0	0	0.74	1.64	2	0.82	-0.24 (-0.29, -0.19)
Friedland	0.10	-0.4*	4.83	1*	4.93	46	0	15	1*	41.2	10	4.12	-0.34 (-0.58, -0.10)
Peenemünde	2.26	-0.4*	4.82	1*	4.92	28	0.21	13	1*	13.4	5	2.68	-0.27 (-0.37, -0.17)

Approximation of the confidence interval to non-video monitored sites

Starting from the same basic formula: $\bar{x} \pm z * \sigma/\sqrt{n}$, the mean net error (\bar{x}), standard deviation of the mean net error (σ) and total number of passes (n) for non-video monitored sites were approximated as follows:

1. We applied a mean net error of ± 1 (i.e., -1 was used for the lower bound of the CI, and +1 for the upper) based on the following reasoning. First, mean net error was particularly low (-0.17 – 0.34, Table S1) at the video-monitored sites for light barriers installed within the frame of the entrance, which was the case for all but one of the non video-monitored sites. Second, the mean net error was not systematically biased in either direction at all but one of the video-monitored sites. We did observe a larger, in-biased mean net error in Peenemünde (2.26), as a result of the particularly poor positioning of the Liba-4 light barrier behind a very narrow entrance that altered the normal flight behavior of bats. Therefore, we consider ± 1 to be a realistic estimate that will likely overestimate the error at the vast majority of sites but may nevertheless fail to capture the true error in some exceptional cases.
2. The first component of the standard deviation of the mean net error (SD_1) accounts for incorrect events (i.e., event registered in the wrong direction), false positives (i.e., event registered by the light barrier, but there was no event), simultaneous entries (i.e., two bats entered simultaneously and only a single pass was registered) and innerloop-related false negative errors (i.e., half of an ‘innerloop’ was missed, but the other half was correctly registered). At non video-monitored sites, it was estimated using a linear regression without intercept that correlates the ratio of total passes:population size estimate to the site-specific standard deviations from the video-monitored sites (estimated slope = 0.22; Fig. S2A). Although this regression is based on a low number of samples, the slope remains reasonably constant over a jackknifing procedure (Fig. S2C).

The second component of the standard deviation of the mean net error (SD_2) accounts for false negative errors (i.e., bat passes that were not registered by the light barrier). This could only be quantified in Eldena, where the worst value was 0.9. Therefore, we used a rounded-up value of 1 as an approximation at non-video monitored sites. The two standard deviation components were combined using Gaussian error propagation ($\sigma_{sum} = \sqrt{SD_1^2 + SD_2^2}$).

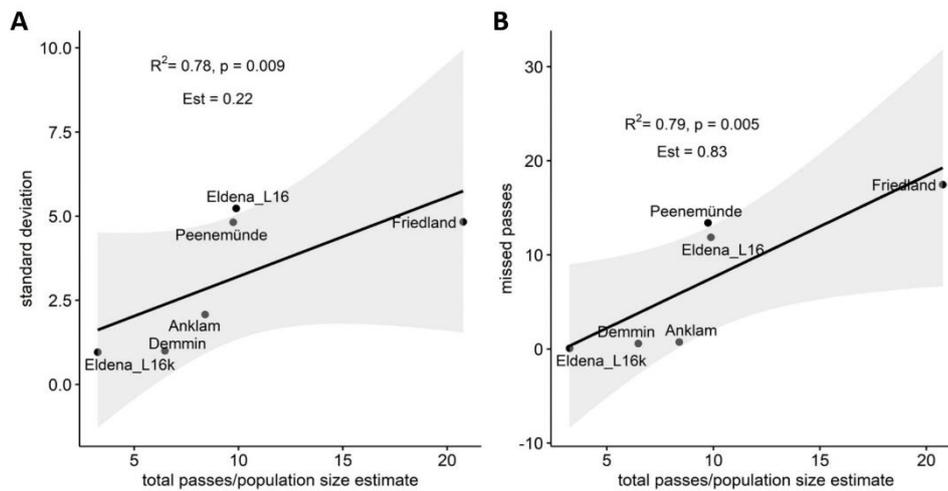
3. Missed passes due to simultaneous entries (MP_1) and false negative errors (MP_3) cannot be quantified without video monitoring, therefore we used the worst-case scenarios from the video-monitored sites for approximation ($MP_1=0.2$ missed pass/100 pass, $MP_3=1$ missed pass/100 pass; Table S1). Innerloop-related missed passes (MP_2) were estimated using a linear regression without intercept that correlates the ratio of total passes:population size estimate to the site-specific missed passes from the video-monitored sites (estimated slope = 0.83, Fig. S2B). As with the standard deviation, although based on a low sample size, the estimate remained comparable across jack-knifing (Fig. S2C). Missed passes due to the light barrier being blocked (MP_4) were calculated, if curtain logging data were available, by multiplying the blocked periods (minutes rounded to the nearest hour) with the mean pass per hour (B_{mean}). If curtain data was not available, we assumed the blocked hours to be 0. The total number of passes (n_{sum}) was a sum of registered passes (n) and all missed passes ($MP_1+MP_2+MP_3+MP_4$).
4. As in the original calculation, to correct for the passes occurring when the light barrier was blocked, we calculated the blocked periods (B_{hours}) and the mean net pass per hour (B_{net_mean}) with 95% confidence level for the entire emergence phase, when curtain data was available and allowed us to identify blocked periods. Then we multiplied the lower (B_{CL}) and upper (B_{CU}) confidence levels by the total number of blocked hours and added the absolute value of these to the lower and upper limits of the confidence interval.

The following formulas were used to calculate an approximated confidence interval for the population size estimate:

$$\text{Lower bound: } \text{population size} - \left((-1 - z \times \frac{\sigma_{sum}}{\sqrt{n_{sum}}}) \times n_{sum} \right) + |(B_{hours} * B_{CI-L})|$$

$$\text{Upper bound: } \text{population size} + \left((1 + z \times \frac{\sigma_{sum}}{\sqrt{n_{sum}}}) \times n_{sum} \right) + |(B_{hours} * B_{CI-U})|$$

Figure S3 Linear regression with 95% confidence interval, showing the correlation between the ratio of total passes:population size estimate to the **A)** the site-specific standard deviation, and **B)** the total number of passes missed by the light barrier as a result of simultaneous entries and innerloop-related false negative errors based on six video-monitored light barrier installations. **C)** At new sites, the standard deviation and number of missed passes were approximated based on the regression estimates across all six datapoints. The robustness of these estimates was investigated using a jack-knife procedure, where each observation was systematically left out from the dataset, and a regression estimate was calculated over the remaining observations.



Excluded site	Standard deviation			Missed passes		
	Adjusted R ²	P-value	Estimate	Adjusted R ²	P-value	Estimate
Demmin	0.82	0.012	0.24	0.86	0.007	0.88
Peenemünde	0.77	0.021	0.20	0.83	0.012	0.75
Anklam	0.79	0.018	0.23	0.89	0.005	0.91
Friedland	0.73	0.031	0.28	0.66	0.050	0.82
Eldena_L16	0.80	0.016	0.19	0.80	0.015	0.78
Eldena_L16k	0.79	0.018	0.23	0.84	0.011	0.84
ALL	0.78	0.009	0.22	0.79	0.005	0.83

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***BatNet*: a deep learning-based tool for automated bat species
identification from camera trap images**

BatNet: a deep learning-based tool for automated bat species identification from camera trap images

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Running headline: Automated bat species identification

Keywords: automated monitoring, bat conservation, camera trap, Chiroptera, deep learning, infrared light barrier

Abstract

Automated monitoring technologies can increase the efficiency of ecological data collection and support data-driven biodiversity conservation. Camera traps coupled with infrared light barriers can be used to monitor temperate-zone bat assemblages at underground hibernacula, where many species and thousands of individuals can aggregate in winter. However, the broad-scale adoption of such photo-monitoring techniques is limited by the bottleneck of manual image processing. Here, we present *BatNet*, an open-source, deep learning-based tool for automated identification of 13 European bat species from camera trap images. *BatNet* includes a user-friendly graphical interface, where the baseline model can be retrained to add new bat species or to create site-specific models to improve detection accuracy at new sites. Model accuracy was evaluated on test images from both trained and untrained sites, and in an ecological context, where 5-month camera trap datasets were used to compare species-level metrics (species diversity, relative abundance, and phenology) between human experts and *BatNet*. At trained sites, baseline model performance was high across all species (F1-score range: 0.98-1). At untrained sites, overall classification accuracy remained high (96.7-98.2%), when the camera placement was comparable to the training images. For atypical camera placements, retraining the baseline model with 500 local annotations achieved an accuracy of over 95% at all sites. In a case study encompassing the complete hibernation-entry phase at three sites, all three species-level metrics were nearly identical between human and *BatNet* identifications. Finally, we retrained *BatNet* to identify a new species, *Miniopterus schreibersii*, achieving an F1-score of 0.99 while maintaining high classification accuracy for all original species. *BatNet* can be implemented directly to scale up the deployment of camera traps in Europe and enhance bat population monitoring. Moreover, the pretrained model can be used as a baseline for transfer learning to automatize the identification of bat species worldwide.

Introduction

Effective conservation depends on the ability to quantify biodiversity and monitor species-level population dynamics in our threatened ecosystems (Primack, 1995). Bats are an integral part of nearly all terrestrial ecosystems, where they provide essential ecosystem services and act as ecological indicators of general ecosystem health (Kunz et al., 2011). Despite their essential ecological role, bat populations across the globe face a daunting diversity of threats, such as the loss and degradation of suitable roosting and foraging sites, the introduction of new infectious diseases, and global warming coupled with increasingly unpredictable climatic conditions (Frick et al., 2020). These effects are especially problematic for species with slow life histories, such as bats, where populations may require decades to recover from individual mortality events (Fleischer et al., 2017). Therefore, the need for accurate estimates of population trends and a fundamental understanding of how these effects are changing bat behavior and life history has never been more pressing.

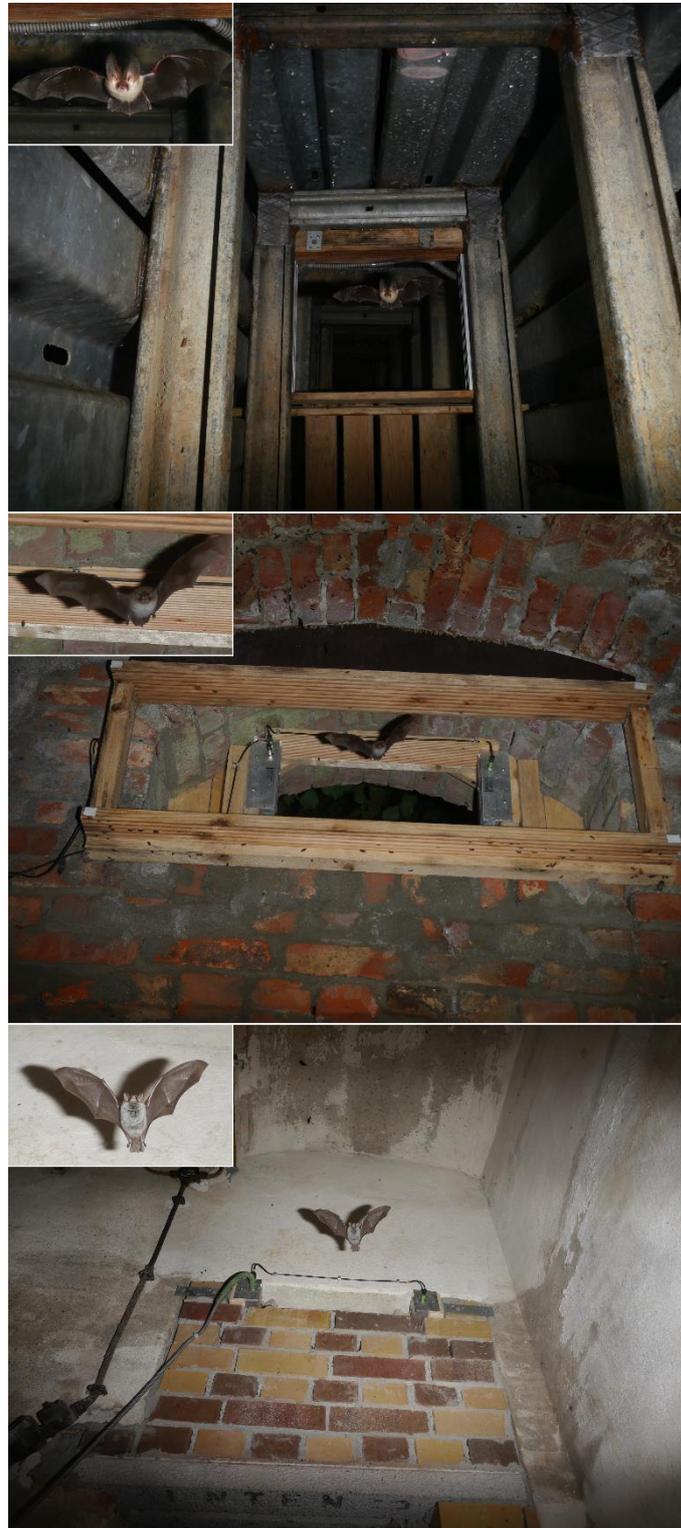
One of the primary monitoring techniques used for temperate-zone bat populations is counting bats at winter hibernacula. Hibernation sites are attractive for monitoring as they are used by individuals of multiple species, and by individuals from multiple summer maternity colonies (Dekeukeleire et al., 2016). However, as bats are small and many prefer to hibernate in crevices, there can be large discrepancies between winter hibernation counts and actual population sizes (Battersby, 2008), and some species may be entirely missed (e.g., Toffoli & Calvini, 2021). More accurately monitoring bat activity and population dynamics at hibernacula is possible with custom-made camera traps that are triggered by bats flying through an infrared light barrier installed at the entrance of the site. Unlike other nocturnal species, bats do not change their behavior in response to the fast, white flash of such camera traps (1/5500 s, 1/16 power), making these photo-monitoring systems suitable as a minimally invasive method for bat monitoring (Krivek et al., 2022). The resulting camera trap images (e.g., Fig. 1) allow

species-level identification and can thus be used to describe the diversity, relative abundance, and phenology of bat species at a hibernaculum.

Manual bat species identification from camera trap images is a time-consuming and monotonous task that requires extensive experience with the subtle morphological differences between the species. Given that a site with around 600 hibernating bats may yield up to 30,000 camera trap images per year (Krivek et al., unpublished data), manual identification of images represents a substantial hurdle for large-scale monitoring projects. While deep learning-based species identification from camera trap images is now commonplace for many terrestrial mammals and birds (e.g., Norouzzadeh et al., 2018; Tabak et al., 2019), such resources do not exist for bats.

Here, we present *BatNet*, an open-source, deep learning-based tool for automated bat species identification from camera trap images. *BatNet* consists of three main stages: a detector that localizes bats in the image, a segmentation network that removes the background, and a classifier that uses the image crop for species identification. We used 16,333 camera trap images from 32 hibernation sites to train the baseline model to identify 13 bat species or species-complexes, encompassing all species commonly observed at hibernacula in Northwestern Europe. Model performance was evaluated in three ways: 1) accuracy on test images of all species from trained sites; 2) accuracy on images from new, untrained sites; and 3) in an ecological case study, where species-level ecological metrics (i.e., diversity, relative abundance, and phenology) were compared between human and *BatNet* identifications in 5-month datasets encompassing the hibernation-entry phase. Finally, we highlight the ability to retrain the detector and the classifier for new locations and species within a coding-free graphical user interface. *BatNet* is freely available under a CC BY-NC-SA 4.0 license (<https://github.com/GabiK-bat/BatNet>).

Figure 1. Camera trap image of bats entering a hibernaculum in Batzbach (*Plecotus auritus*), Eldena (*Myotis daubentonii*) and Comthurey (*Myotis nattereri*) in Germany, where the entrance is monitored with a custom-made camera trap that consists of a digital camera and a white flash, and it is triggered by bats flying through an infrared light barrier.



Methods

Training data and model architecture

In total 18,496 images of bats were collected at the entrance of 32 hibernacula across Germany using custom-built camera traps that are triggered by infrared light barriers (ChiroTEC, Lohra). For each image, two human experts classified the bat to species level (*Barbastella barbastellus*, *Eptesicus serotinus*, *Myotis bechsteinii*, *M. dasycneme*, *M. daubentonii*, *M. emarginatus*, *M. nattereri*, and *Nyctalus noctula*) or to species-complex (the whiskered bats: *Myotis alcaho*, *M. brandtii*, *M. mystacinus*; the mouse-eared bats: *M. blythii*, *M. myotis*; the long-eared bats: *Plecotus auritus*, *P. austriacus*; the pipistrelles: *Pipistrellus pipistrellus*, *P. pygmaeus*; and the horseshoe bats: *Rhinolophus ferrumequinum*, *R. hipposideros*). The location of each bat in all images was annotated with a bounding box. A random subset of 3,685 images were subsequently manually traced to crop the bat out from the background. From the total dataset, 90% (N=16,333) was used to train the detector and the classifier, and 10% (N=2,163) was used for testing final model performance (for sample sizes per species see Table S1). All networks were trained for 30 epochs with a learning rate of 0.05 and a stochastic gradient descent (SGD) optimizer.

BatNet is composed of three distinct stages: detection, segmentation, and species classification (Fig. S1). First, a Faster-R-CNN object detector (Ren et al., 2015) with a ResNet50 (He et al., 2016) Feature Pyramid Network (Lin et al., 2017) places a bounding box around all bats detected in the image. Second, the image is cropped to the bounding box and a U-Net segmentation network (Ronneberger et al., 2015) with a MobileNet V3 backbone (Howard et al., 2019) removes the background. Because deep learning models have the tendency to learn static background features (Miao et al., 2019), this segmentation step ensures that the actual bat characteristics are used for classification in the next step and not the background features. Finally, the segmented crop of the image is classified by an ensemble of

three MobileNet V3 networks (Howard et al., 2019). This configuration was selected, because ensemble networks are less prone to make highly confident yet incorrect predictions than a single neural network (Li & Hoiem, 2020). Each network classifies the original and the flipped version of the image crop and the predictions of the individual networks are then averaged. This technique, called test-time augmentation, is known to improve the performance of image classification models (e.g., Kim et al., 2020). The final output of the classifier is composed of the predicted identification for each detected bat and a confidence value between 0 and 1 for each prediction. Confidence values indicate the level of certainty in the species identification and predictions with confidence values below a user-defined threshold are flagged.

Since transfer learning is an established technique to improve neural network performance and reduce training time (Yosinski et al., 2014), the object detector was pretrained on the COCO (Common Objects in Context) dataset (Lin et al., 2014), and all other networks were pretrained on ImageNet (Russakovsky et al., 2015). In addition, the training dataset was augmented with random horizontal flips of the original camera trap images, and we included additional image crops of bats and of the background. Since outlier exposure is commonly used to improve detection performance at untrained background locations (Hendrycks et al., 2018), random images were also included from the ImageNet dataset (Russakovsky et al., 2015) as negative examples (i.e., images of anything else than a bat).

Within the *BatNet* graphical user interface, both the object detector and the species classifier can be retrained on new images in a coding-free environment. In both cases, new images can either be manually labeled with bounding boxes and species identifications, or the baseline model output can be corrected and used directly. All training parameters (i.e., species of interest, number of epochs, learning rate) are adjustable, and the resulting retrained model can be selected from a drop-down menu within the user interface. Further details and documentation are provided on GitHub (<https://github.com/GabiK-bat/BatNet>).

Evaluation on test data

As an initial evaluation, we quantified *BatNet* performance on the 2,163 test images that were withheld from the training dataset but were taken at trained background locations. To evaluate the performance of the object detector, we compared the intersection between the predicted and true bounding boxes around each labelled bat. We considered predictions as true positive above 0.4 Intersection over Union (IoU; 0 – no overlap, 1 – perfect overlap) and false negative if the overlap was below the threshold. Predicted bounding boxes without any bats were considered false positive errors. To evaluate classifier performance, identifications were considered true positive when the human and predicted classifications were the same, false negative when the species of interest was incorrectly classified as a different species, and false positive when a different species was incorrectly classified as the species of interest.

Object detection and classifier performance were quantified by three accuracy metrics: precision (i.e., ratio of correctly predicted positive observations to the total predicted positive observations; minimizes false positive errors), recall (i.e., ratio of correctly predicted positive observations to all observations in the actual class; minimizes false negative errors) and F1-score (i.e., weighted average of precision and recall; used for evaluation when both false negative and false positive errors are equally undesirable).

Untrained sites and model retraining

Next, we evaluated the baseline model performance on 49,873 images from seven untrained sites that were spatially and temporally independent from the training data. Untrained sites were categorized based on their similarity to the training dataset and included three typical sites (camera angle and distance from the entrance similar to the training images), two sites with atypical camera angle and one with atypical camera distance (Fig. S2). Images from the untrained sites were classified by one human expert and annotated with bounding boxes and species labels.

In addition to the baseline model, the object detector was retrained for each site (10 epochs, learning rate 0.001) with varying numbers of local annotations (25, 50, 100 or 500). As for the baseline detector model, F1-scores were calculated for each of these site-specific detector models.

Ecological case study

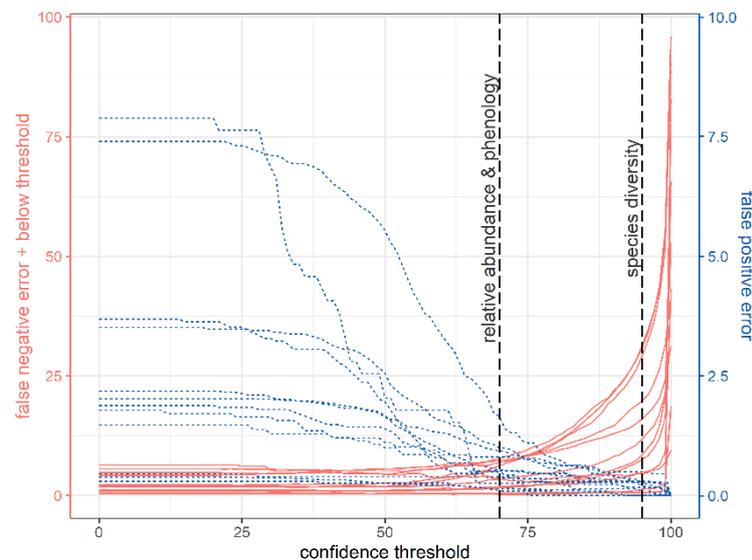
Finally, we explored the utility of *BatNet* for describing species-level ecological metrics using a contiguous 5-month camera trap dataset comprising the complete hibernation-entry phase (01 August – 01 January) from one trained (Eldena) and two untrained locations (Batzbach, Comthurey). In these datasets (N=54,748 images) only the species of the bat that triggered the camera trap was identified by one human expert, and bats flying in the background were not considered. This represents the typical manual identification procedure, where the primary goal is to quantify the number of entering bats per species. For images from Eldena (trained) and Batzbach (untrained, typical camera angle) model predictions were based on the baseline model. For Comthurey (untrained, atypical camera angle) images were identified using a site-specific model that was trained with 500 local annotations.

In addition to the overall accuracy as described above, we focused on three ecological metrics: species diversity (i.e., which species are present at a site), relative abundance (i.e., the percentage of identifications attributed to each species at a site) and species-specific phenology (i.e., the activity pattern of individual species throughout the hibernation-entry phase). For these applications, different confidence thresholds can be applied to the output of *BatNet* to optimize the balance between increased accuracy (i.e., F1-score) and decreased sample size.

To generate an optimal confidence threshold for each application, we first evaluated the proportion of false positive errors versus the false negative errors and identifications below the confidence threshold for species with more than 100 identifications at a site, across all confidence thresholds (Fig. 2). Based on these results, species diversity at a hibernaculum was

determined using a 95% confidence threshold, which minimizes the proportion of false positive errors while still retaining each species, including the rare ones. To eliminate the small number of remaining false positives, we manually reviewed all identifications of species that constitute less than 1% of the total dataset based on the *BatNet* output. To estimate the relative abundance of each species and describe species-specific phenology, we selected a 70% confidence threshold. At this threshold the proportion of false positive errors is strongly reduced, but the proportion of identifications that are discarded as below the threshold has not started to exponentially increase yet (Fig. 2).

Figure 2. Proportion of false positive errors (blue dashed lines) versus the proportion of false negative errors and identifications below threshold (red solid lines) across all confidence thresholds. Each line represents a species at a site with more than 100 identifications. Vertical dashed lines indicate the confidence thresholds used for describing relative abundance and phenology of species (70%) and species diversity (95%) using camera trap images.



To describe overall accuracy for the ecological case studies, we generated confusion matrices using a 70% confidence threshold for the *BatNet* output. Since *BatNet* provides predictions for all bats detected in an image, some images yielded multiple bat identifications. Since true species labels were missing for the bats that were not considered to have triggered the camera trap by the human evaluator, *BatNet* predictions for these images were manually corrected so that only the bat that triggered the camera trap was retained for the accuracy assessment (if it exceeded the 70% threshold). To correct for human error, if there was a mismatch between the human label and the prediction above 70% confidence threshold (N=243 out of 54,748 images), two additional human experts manually reviewed the identifications. Based on this consensus scoring, the original human identification was either considered correct (i.e., *BatNet* prediction was incorrect, 76.5%), or incorrect (23.5%) and thus, the original human label was corrected.

For all ecological metrics, all *BatNet* predictions above the confidence threshold were considered, including cases where multiple bats per image met these criteria. To investigate the ability of *BatNet* to accurately describe species diversity, we compared the list of species identified by *BatNet* with the species that were truly present at a site based on human identifications. For relative abundance, we compared the percentage of the dataset assigned to each species by human identification (i.e., the bat that triggered the image) and by *BatNet* (i.e., including multiple identifications per image when both were above threshold). Finally, we compared the phenology of the four most common bat species at the investigated sites (*Myotis nattereri*, *M. daubentonii*, *M. myotis* and *Plecotus auritus*) between a human expert and *BatNet*. Specifically, we quantified the differences in the phenological estimates by calculating the date at which certain percentiles (5, 25, 50, 75 and 95%) of the total number of identifications had been reached per species and per site. Additionally, we used Lin's concordance correlation

coefficients (CCC) to quantify the agreement between the human expert and *BatNet* regarding the number of identifications per species per night throughout the season.

Classifier retraining: adding new species

We explored the feasibility of adding a new species to the classifier, while maintaining the classification accuracy for all other species. The baseline classifier was retrained with 58 annotations of a new species (*Miniopterus schreibersii*) and 40-50 annotations per species (complex) originally included in the baseline training. The classifier was retrained for 10 epochs at a learning rate of 0.001. These parameters were selected to ensure high classification accuracy for the new species, and to minimize forgetting the species learnt from the original training data. The performance of the retrained model was evaluated on 1,143 test images of *Miniopterus schreibersii*, in addition to the original 2,163 test images of the other 13 bat species.

Results

Test dataset evaluation

Out of the 2,163 *BatNet* identifications on test images from trained background locations, 15 were incorrect (12 misidentifications and 3 missed detections), yielding an overall classification accuracy of 99.3% (CI 98.9-99.6%). Precision, recall and F1-score ranged from 0.97 to 1.00 for all 13 bat species (for confusion matrices see Fig. S3).

Untrained sites

Object detection performance of the baseline model, quantified using the F1-score, ranged from 0.95 to 1.00 at five of six untrained locations. It was noticeably lower at one site (0.38 in Calw; Fig. 3), where the camera trap was situated further from the entrance than usual (around 3.5 meters instead of 1.5 meters). After retraining the baseline detector using 500 local annotations for each of the six previously untrained sites, the F1-score of the site-specific object

detection model increased to 0.94 in Calw and to over 0.98 at the other five previously untrained locations.

Classification accuracy of the baseline model varied depending on the camera angle and the distance between the camera and entrance (Table 1; example camera trap images: Fig. S2, confusion matrices: Fig. S4). Classification accuracy was high (96.7-98.2%) at untrained locations with typical backgrounds (i.e., similar camera angle and distance to the training dataset). It was markedly lower and more variable at sites with atypical camera placement (17.8%, 86.3% and 90.8%; Table 1), presumably because many bats were not detected or incorrectly segmented. Notably, after retraining the detector with 500 local annotations for each of the six unseen sites, classification accuracy improved to over 95% at all sites (Table 1; 95.5-99.9 %).

Figure 3. The object detection performance, quantified by the F1-score, on images from six untrained locations using the baseline model (no retraining) and using site-specific models after retraining the general detection model with a varying number of local annotations (25, 50, 100 or 500).

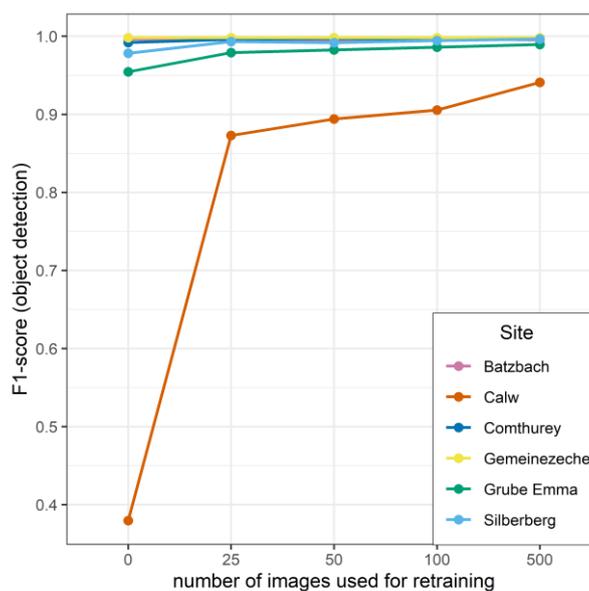


Table 1. Classification accuracy with 95% confidence interval at six untrained background locations using the baseline model and the site-specific models retrained with 500 local annotations (r500), and the number of images used for evaluation. Sites were categorized based on their similarity to the training dataset in terms of camera angle and distance from the entrance.

Site category	Site	N _{images}	Accuracy (95% CI) baseline	Accuracy (95% CI) r500
typical	Batzbach	39,430	98.2 (98.1 – 98.3)	98.1 (97.9 – 98.2)
	Gemeinezeche	997	97.6 (96.4 – 98.5)	99.9 (99.4 – 100)
	Silberberg	1,000	96.7 (95.4 – 97.7)	99.8 (99.3 – 100)
atypical angle	Comthurey	6,472	90.8 (90.1 – 91.5)	97.3 (96.9 – 97.7)
	Grube Emma	979	86.3 (84 – 88.4)	97.5 (96.3 – 98.3)
atypical distance	Calw	995	17.8 (15.5 – 20.3)	95.5 (94 – 96.7)

Ecological case study

Species diversity

To determine species diversity at a site using *BatNet*, we applied a 95% confidence threshold to the output and manually reviewed images for species that constituted less than 1% of the total dataset. *BatNet* detected all species that were identified by human experts at all three sites (Batzbach, Comthurey, Eldena; Table 2). Across the three evaluated datasets (N=54,748), manual review was required for 62 images (0.1% of total), resulting in the confirmation of three true positive species (N=60) and one false positive species (N=2).

Relative species abundance

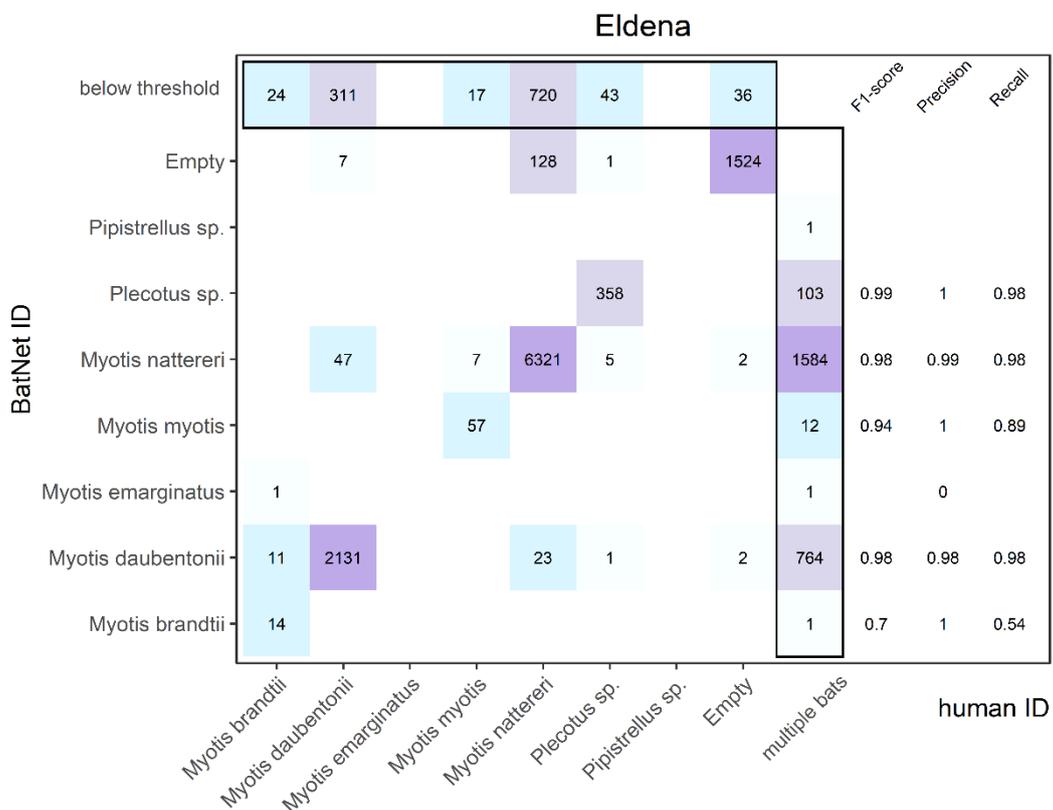
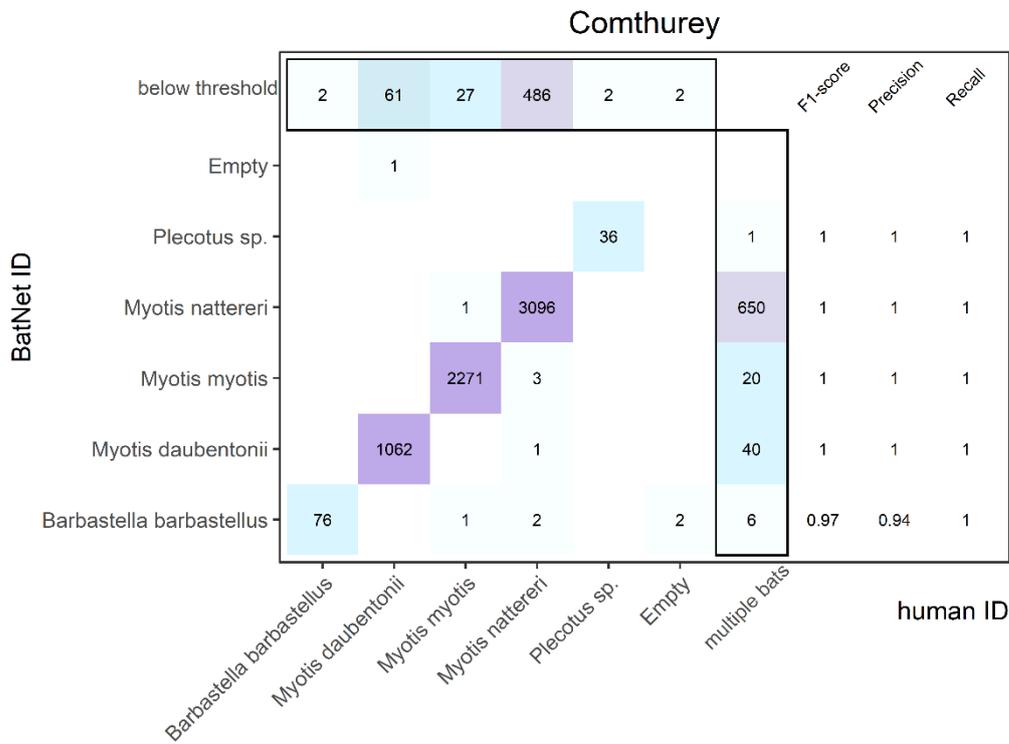
To describe species abundance, we used a 70% confidence threshold that maintained, high precision for all species (Fig. 4) and retained over 90% of the dataset at all sites (Eldena 90.1%, Batzbach 93.7%, Comthurey 92.1%). The difference in the relative abundance of all species was within 1.1% at all three sites when comparing *BatNet* predictions with 70% confidence threshold to human identifications (Table 3).

Table 2. Species diversity based on *BatNet* predictions with 95% confidence threshold and human expert identifications at three sites. Bold text indicates that the total proportion of predicted labels for that species was below the 1% threshold, thus these images required manual review by a human expert.

Site	Species	N _{algorithm}	N _{human}
Batzbach	<i>Myotis nattereri</i>	13,304 (43.2%)	19,416
	<i>Myotis bechsteinii</i>	11,077 (36%)	11,901
	<i>Plecotus sp.</i>	2,202 (7.16%)	2,191
	<i>Myotis daubentonii</i>	1,827 (5.94%)	2,666
	<i>Myotis myotis</i>	1,470 (4.78%)	1,653
	<i>Myotis brandtii</i>	879 (2.86%)	1,363
	<i>Myotis dasycneme</i>	2 (0.01%)	0
Comthurey	<i>Myotis nattereri</i>	2,836 (45.7%)	3,019
	<i>Myotis myotis</i>	2,239 (36.1%)	2,263
	<i>Myotis daubentonii</i>	1,024 (16.5%)	1,071
	<i>Barbastella barbastellus</i>	73 (1.18%)	76
	<i>Plecotus sp.</i>	36 (0.58%)	37
Eldena	<i>Myotis nattereri</i>	5,542 (72.4%)	6,403
	<i>Myotis daubentonii</i>	1,743 (22.8%)	2,192
	<i>Plecotus sp.</i>	345 (4.51%)	375
	<i>Myotis myotis</i>	19 (0.25%)	71
	<i>Myotis brandtii</i>	5 (0.07%)	51

Table 3. Relative species abundance based on *BatNet* predictions with 70% confidence threshold and human identifications at three sites.

Site	Species	<i>BatNet</i> %	Human %
Batzbach N _{images} =39,190	<i>Myotis nattereri</i>	48.40	49.50
	<i>Myotis bechsteinii</i>	31.40	30.40
	<i>Myotis daubentonii</i>	6.67	6.80
	<i>Plecotus</i> sp.	5.86	5.59
	<i>Myotis myotis</i>	4.32	4.22
	<i>Myotis brandtii</i>	3.28	3.48
	Other	0.04	0.00
Comthurey N _{images} =6,466	<i>Myotis nattereri</i>	47.30	46.70
	<i>Myotis myotis</i>	34.70	35.00
	<i>Myotis daubentonii</i>	16.20	16.60
	<i>Barbastella barbastellus</i>	1.24	1.18
	<i>Plecotus</i> sp.	0.55	0.57
Eldena N _{images} =9,092	<i>Myotis nattereri</i>	70.90	70.40
	<i>Myotis daubentonii</i>	24.30	24.10
	<i>Plecotus</i> sp.	3.99	4.12
	<i>Myotis myotis</i>	0.64	0.78
	<i>Myotis brandtii</i>	0.16	0.56
	Other	0.02	0.00



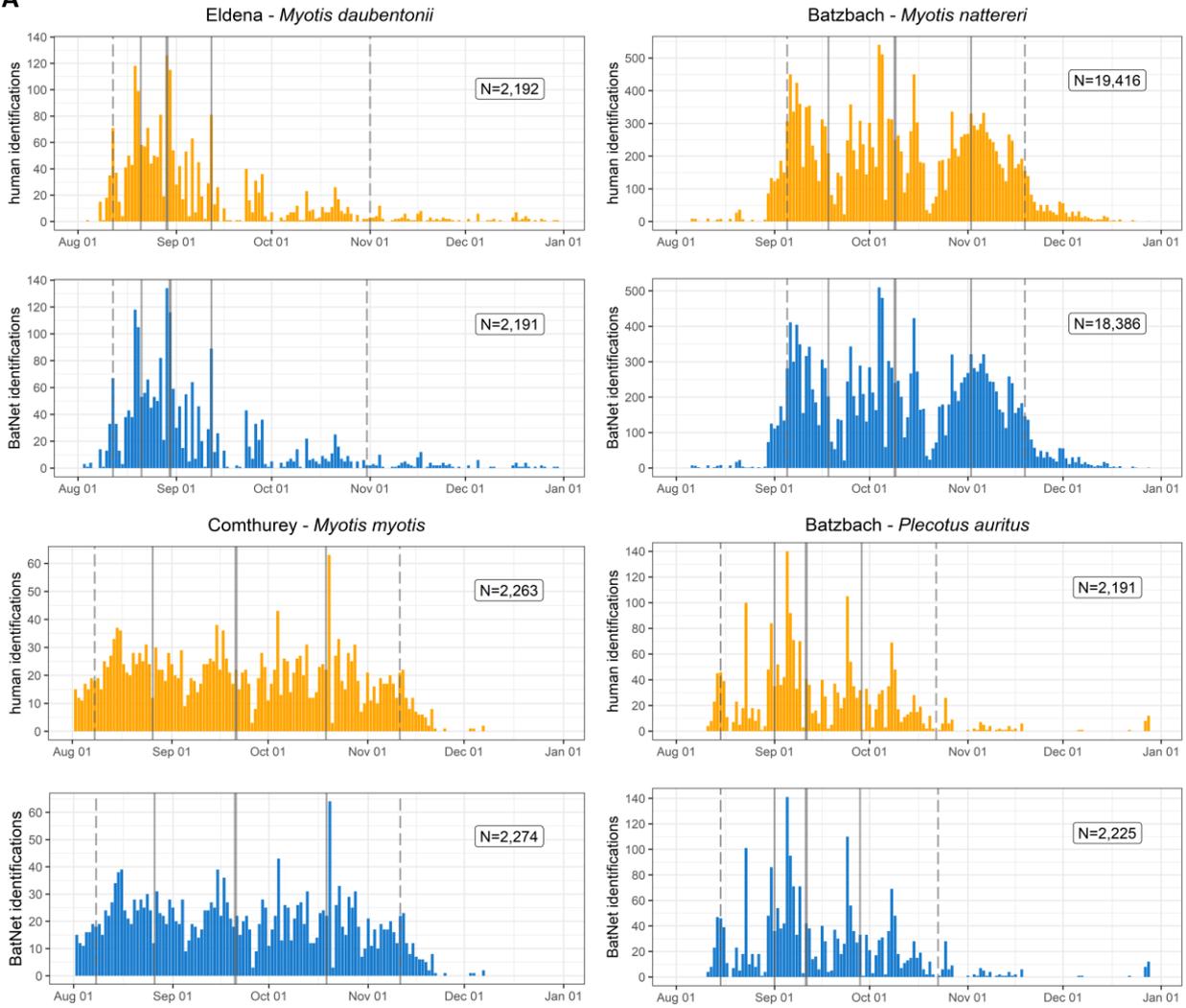
Species-specific phenology

Phenological patterns of the four investigated species (*Myotis daubentonii*, *M. myotis*, *M. nattereri* and *Plecotus auritus*) across a 5-month period were nearly identical between the human and *BatNet* identifications (see Fig. 5A for one example per species, all other combinations in Fig. S5A). When phenology was quantified using percentiles (5, 25, 50, 75 and 95%), the human and *BatNet* datasets differed by less than three days across all species for all percentiles (N=50) but one. In this case, a 6-day discrepancy was observed in the 95th percentile (*Myotis daubentonii* in Comthurey; Fig. S5A).

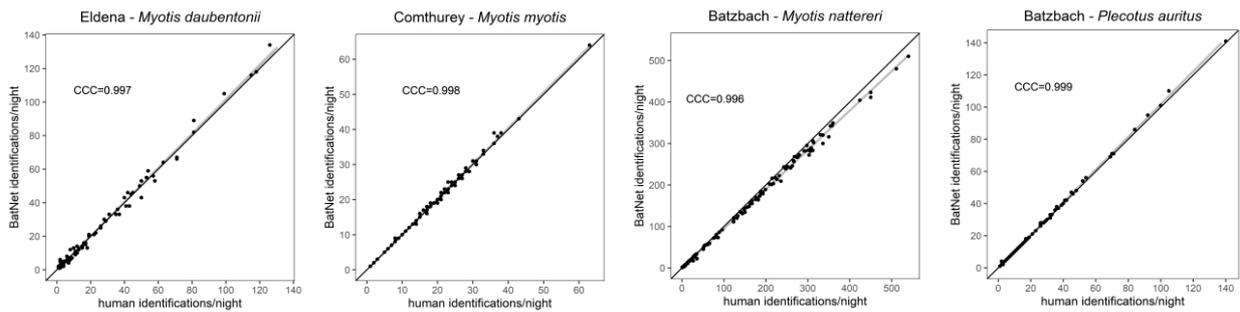
The overall sample sizes between the human and *BatNet* datasets differed due to classifications being discarded below threshold (reduces the *BatNet* sample size), and the classification of multiple bats per image where humans only scored a single bat per image (increases the *BatNet* sample size). Despite these differences, we observed high concordance between human and *BatNet* classifications per species, per day (range: 0.989-0.999; Fig. 5B and Fig. S5B).

Figure 5A) Phenology of *Myotis daubentonii*, *M. myotis*, *M. nattereri* and *Plecotus auritus* based on species identification by human experts (orange) compared to *BatNet* predictions with 70% confidence threshold (blue). To quantify the differences between the phenological patterns of the two methods, we used percentiles (5, 25, 50, 75 and 95%; indicated with vertical gray lines) across a 5-month dataset over the hibernation-entry phase (01 August – 01 January). The sample size (N) indicates the total number of identifications across the season. **B)** Concordance plots indicate the agreement between the number of human and *BatNet* identifications per species per night, quantified by the Lin's concordance correlation coefficient (CCC, range from 0 to 1).

A



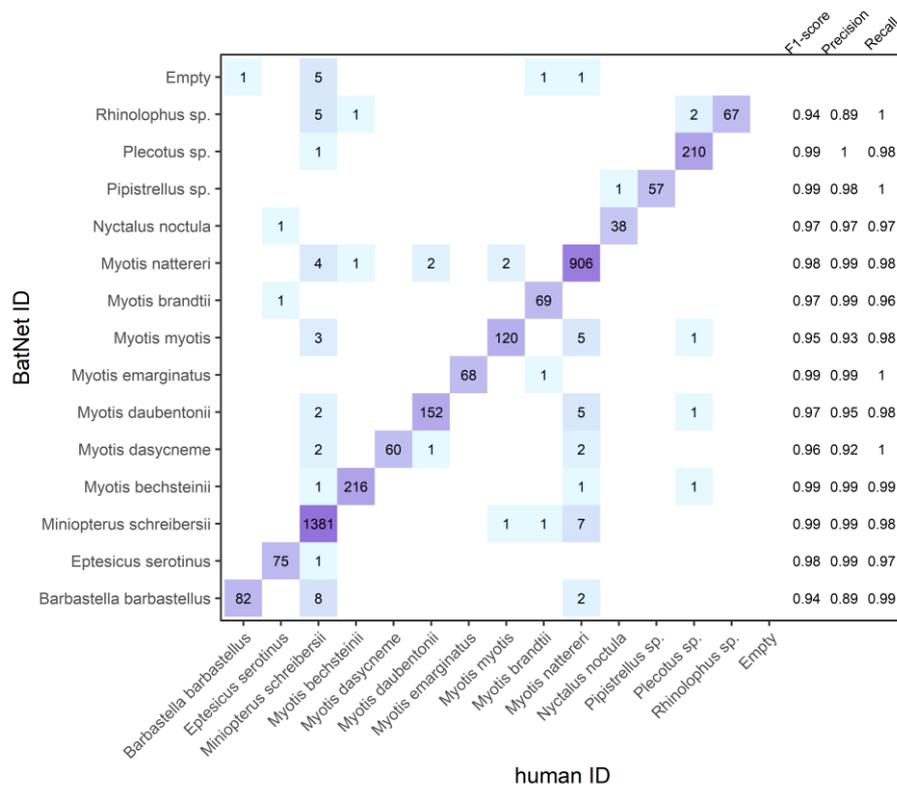
B



New species

After retraining the baseline model with 58 annotations of a new species (*Miniopterus schreibersii*), *BatNet* achieved an F1-score of 0.99 for the new species (Fig. 6). The performance for the original 13 species remained high (F1-score range: 0.94-0.99). Overall classification accuracy of the model was 98% (CI 97.3-98.3%). When applying a 70% confidence threshold, out of the 1,413 *Miniopterus schreibersii* identifications 196 were below the threshold and only 1 identification was incorrect (F1-score 1.00; Fig. S6).

Figure 6. Confusion matrix of *BatNet* predictions and human identifications after retraining the baseline model to be able to identify a new species, *Miniopterus schreibersii*, in addition to the 13 species included in the original training data.



Discussion

BatNet is a deep learning-based tool for automated identification of 13 Northwestern European bat species, that can be retrained to adjust to new sites and to include new species within a coding-free environment. On test images from trained locations, the baseline model achieved high species-level classification accuracy across all 13 bat species (F1-score range: 0.98-1.00). Overall classification accuracy of the baseline model remained remarkably high at untrained sites (96.7-98.2%), where the camera angle and distance from the entrance were comparable to the training images. At untrained sites with an atypical camera setup, site-specific models reached an overall classification accuracy above 95% after retraining with 500 annotations. This possibility to retrain the object detector and create site-specific models with minimal manual annotation effort allows *BatNet* to overcome detection difficulties related to new backgrounds and camera setups. Beyond overall accuracy, we showed that *BatNet* yields nearly identical results to manual identification when used to quantify ecologically relevant species-level metrics, such as species diversity, relative abundance, and phenological patterns. Finally, retraining the baseline model with an additional, morphologically similar, new species resulted in high classification accuracy, both for the new species (F1-score: 0.99), and for all other 13 species (F1-score: 0.94-0.99). Consequently, *BatNet* represents an accurate and highly adaptable platform for automation of camera trap-based bat monitoring.

Improving the speed and scalability of camera trap-based monitoring of bats has large implications considering the improvement this method constitutes over the alternative methods available for quantifying bat population dynamics. Importantly, camera traps attached to infrared light barriers are able to detect all species entering the site, including those that are often vastly undercounted or not detected at all during visual surveys (e.g., crevice-roosting species; Toffoli & Calvini, 2021). Moreover, relative species abundances from the automatically identified camera trap images can be combined with the light barrier-based

population size estimates (Krivek et al., unpublished data) to accurately measure species-level population trends, which remains difficult for many species (Van der Meij et al., 2015). In addition, the continuous nature of camera trap-based monitoring allows us to describe the phenological patterns of different species, which can be quantified using activity percentiles. The 5th and 95th percentiles can serve as a reliable measure of the start and end of the species-specific activity during a particular phase (i.e., hibernation-entry or emergence), while the combination of 25th, 50th and 95th percentiles can indicate the peak activity of different species. These measures can be used then to compare phenological patterns between species, sites, years in a standardized way. Exploring these fine-scale changes in bat activity can help describe how species differ in their hibernation phenology and in terms of their response to changing weather conditions (cf. Meier et al. 2022) and contribute to data-driven conservation actions. Finally, the installation of camera traps with light barriers could be a promising new survey method to minimize direct contact with bats and thus, prevent human disturbance and possible introduction of pathogens to new sites (e.g., WNS, Covid-19; Blehert et al., 2009; Kingston et al., 2021).

The primary limitation to the implementation of the method is that the light barriers that are used to trigger the camera trap can only monitor entrance sizes of up to 35x300 cm. However, the entrances of many large complex mines and caves, where gains in monitoring resolution are expected to be greatest (Krivek et al., unpublished data), have already been reduced to limit human disturbance and access. Thus, although modifications to the entrance should always be performed with caution (e.g., Pugh & Altringham, 2005), the method may be nevertheless widely applicable.

Comparison with other automated species identification approaches

The accuracy of *BatNet*, both at trained and untrained sites, is remarkably high in comparison to other deep learning solutions for automated, image-based mammal species identification (e.g., Norouzzadeh et al., 2018; Tabak et al., 2019). In large part this may be explained by several key differences between classic wildlife camera trap setups and the camera traps triggered by light barriers here used for bat monitoring. First, these custom-made camera traps are installed at the entrance of hibernation sites that are nearly exclusively used by bats. Therefore, only a relatively narrow species range had to be considered for training the networks. Second, since these camera traps are triggered by bats flying through an infrared light barrier, their distance from the camera when the image is taken remains highly consistent. Thus, the camera can be manually focused at a fixed depth to ensure that most bats appear sharp on the images. Third, the environment is often comparatively simple and artificial, and the bats are only rarely partially occluded, which contrasts sharply with the complex, vegetation-rich backdrop of most camera trap studies. This allows for relatively simple segmentation and isolation of the target from the background. Finally, the use of white flash with standardized settings provides a fixed amount of white light in an otherwise completely dark environment. This results in a better and more standardized image quality than afforded by infrared flashes and variable lighting conditions in most traditional wildlife camera setups. The resulting high image quality allows identification of different bat species with high certainty, even though the morphological differences between bat species are far more subtle than between most other mammals.

The performance of *BatNet* was further improved by implementing techniques that have been rarely used in other automated species identification pipelines. First, deep learning models can learn the background features of specific camera trap stations instead of the focal animals (Miao et al., 2019), which introduces bias. To ensure that the classifier focuses on the

characteristics of bats instead of the common background features, we trained a U-Net segmentation network to automatize background removal. While such approaches may be more difficult to implement for datasets with more complex backgrounds, they may nevertheless be worthwhile. Second, single neural networks are more prone to make highly confident yet incorrect predictions (Li & Hoiem, 2020). Here, we used an ensemble of three neural networks for classification, where each network classified the original and the flipped version of the image (i.e., test-time augmentation). This resulted in informative confidence levels that could be reliably used for data filtering. Exploring the adoption of these techniques in other deep learning-based species identification approaches may similarly improve their performance.

Application in bat monitoring and conservation

Automated monitoring of hibernacula combined with the implementation of *BatNet* has the potential to improve bat population monitoring worldwide. In Northwestern Europe, the ability to retrain *BatNet* for new locations allows it to be directly applied to vastly scale up camera trap monitoring while maintaining high accuracy. In other regions, the pretrained model of *BatNet* can be used as a baseline for transfer learning to automatize identification of a broad range of bat species, beyond our target species list. In adjacent regions this may only require minor modification of the species list, to add species such as illustrated here for *Miniopterus schreibersii*. In other areas, using the pretrained model as a baseline is expected to produce more accurate and stable results with less computational expense than pretraining on conventional image datasets, because of the general features it learnt from a diverse, yet bat-specific camera trap dataset. However, all modification should be carefully evaluated using a subsample of manually identified images to detect new or hidden biases, prior to ecological inference for new sites and species. Such revisions and modifications to the model can be performed within the user-friendly graphical interface of *BatNet*.

Given the numerous stressors affecting global bat populations (Frick et al., 2020) and the legal obligation to monitor bat populations worldwide, a greater flow of monitoring data is essential to support data-driven wildlife management and conservation decisions. *BatNet* drastically improves our ability to achieve these objectives.

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Author Contributions

Conception: GKe, JvS, GKr; Training data collection: GKr, MH, MF, JvS; Training data preparation: GKr, KF, LT, LM; Human image identification: KF, MH, GKr, JvS; Software development: AG, UFvL; Evaluation: GKr, JvS; Writing: GKr, JvS. All authors commented on the manuscript and gave final approval for publication.

Data Availability

BatNet is freely available under a CC BY-NC-SA 4.0 license at <https://github.com/GabiK-bat/BatNet>, along with data and scripts used for evaluation, under a CC BY-NC-ND 4.0 license.

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Supplementary materials to:

Krivek et al. (submitted) **BatNet: a deep learning-based tool for automated bat species identification from camera trap images**

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Table S1. Number of camera trap images per species used for training BatNet and testing the baseline model performance.

Species/species-complex	Training images	Test images
<i>Barbastella barbastellus</i>	760	83
<i>Eptesicus serotinus</i>	677	77
<i>Myotis bechsteinii</i>	2,132	218
<i>Myotis dasycneme</i>	596	60
<i>Myotis daubentonii</i>	2,157	155
<i>Myotis emarginatus</i>	626	68
<i>Myotis myotis/M. blythii</i>	1,065	123
<i>Myotis alcathoe/M. brandtii/ M. mystacinus</i>	712	72
<i>Myotis nattereri</i>	3,576	929
<i>Nyctalus noctula</i>	372	39
<i>Pipistrellus pipistrellus/ P. pygmaeus</i>	733	57
<i>Plecotus auritus/P. austriacus</i>	2,203	215
<i>Rhinolophus ferrumequinum/ R. hipposideros</i>	724	67
Total	16,333	2,163

Figure S1. Schematic overview of *BatNet*, a deep learning-based tool that automatically identifies bat species from camera trap images in three steps: bat detection (object detector), background removal (segmentation network) and species classification (ensemble of classifiers). The final output includes a species prediction with a confidence level. Optionally, low-confidence predictions can be manually reviewed in the graphical user interface by human experts.

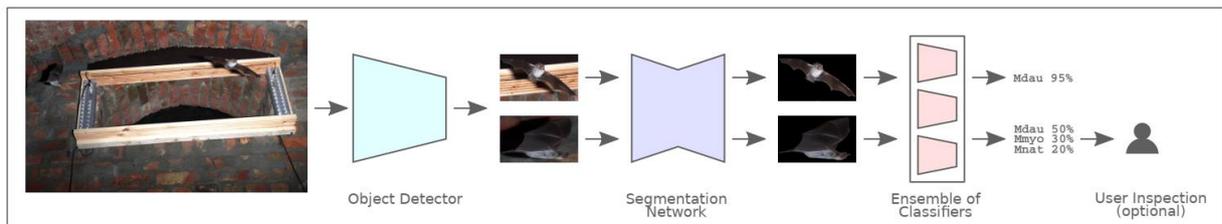


Figure S2. Example camera trap images from untrained locations that were categorized based on their similarity to the training dataset, including three typical sites (camera angle and distance from the entrance similar to the training images; A - Batzbach, B - Gemeinezeche, C - Silberberg), two sites with atypical camera angle (D - Comthurey, E - Grube Emma) and one with atypical camera distance (F - Calw).

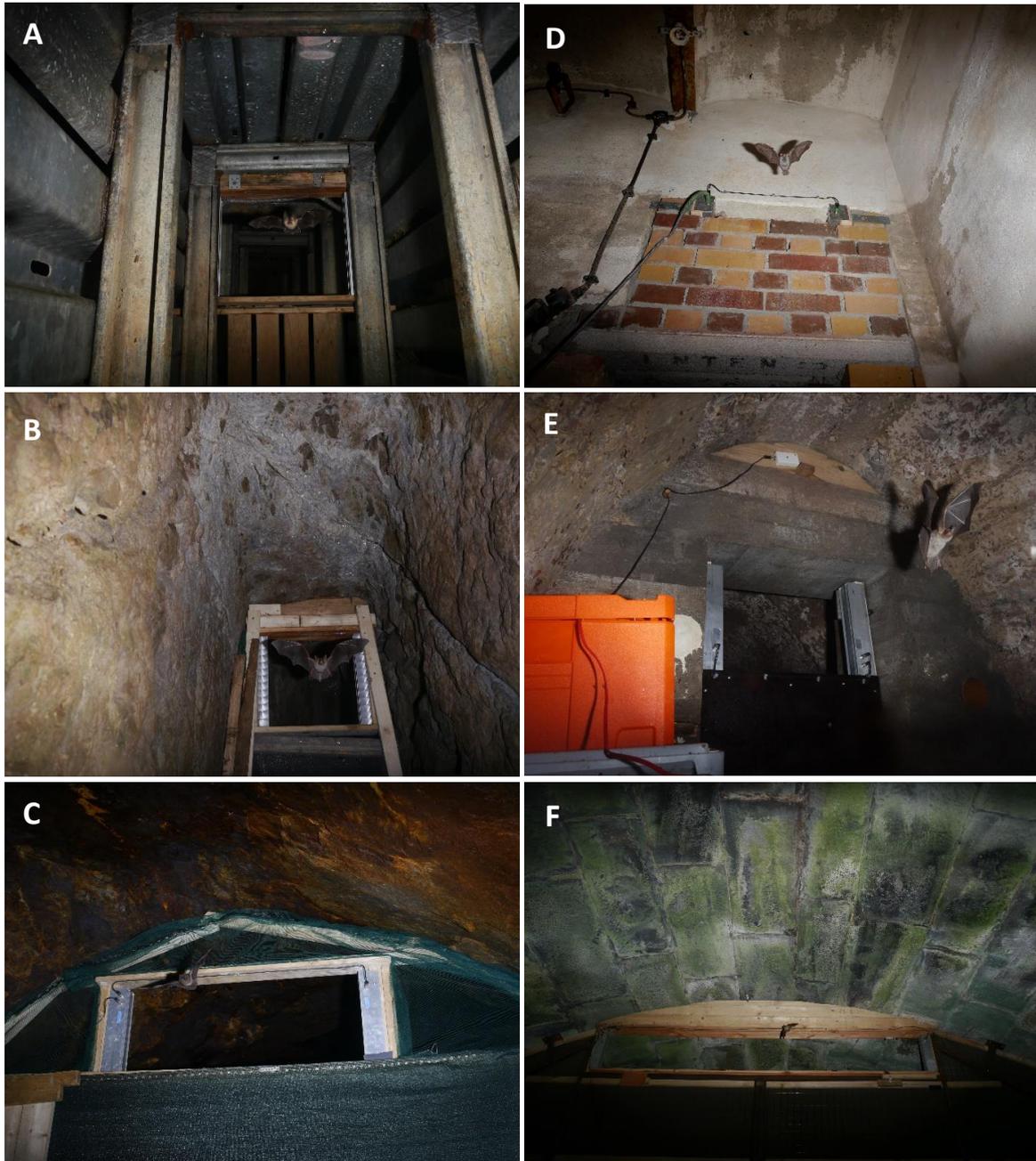


Figure S3. Confusion matrix of *BatNet* predictions and human identifications for test images from trained background locations. The confusion matrix shows the distribution of classification error within a species, where accurate classifications are across the diagonal and all other cells on the matrix describe error (missed detection or misclassification).

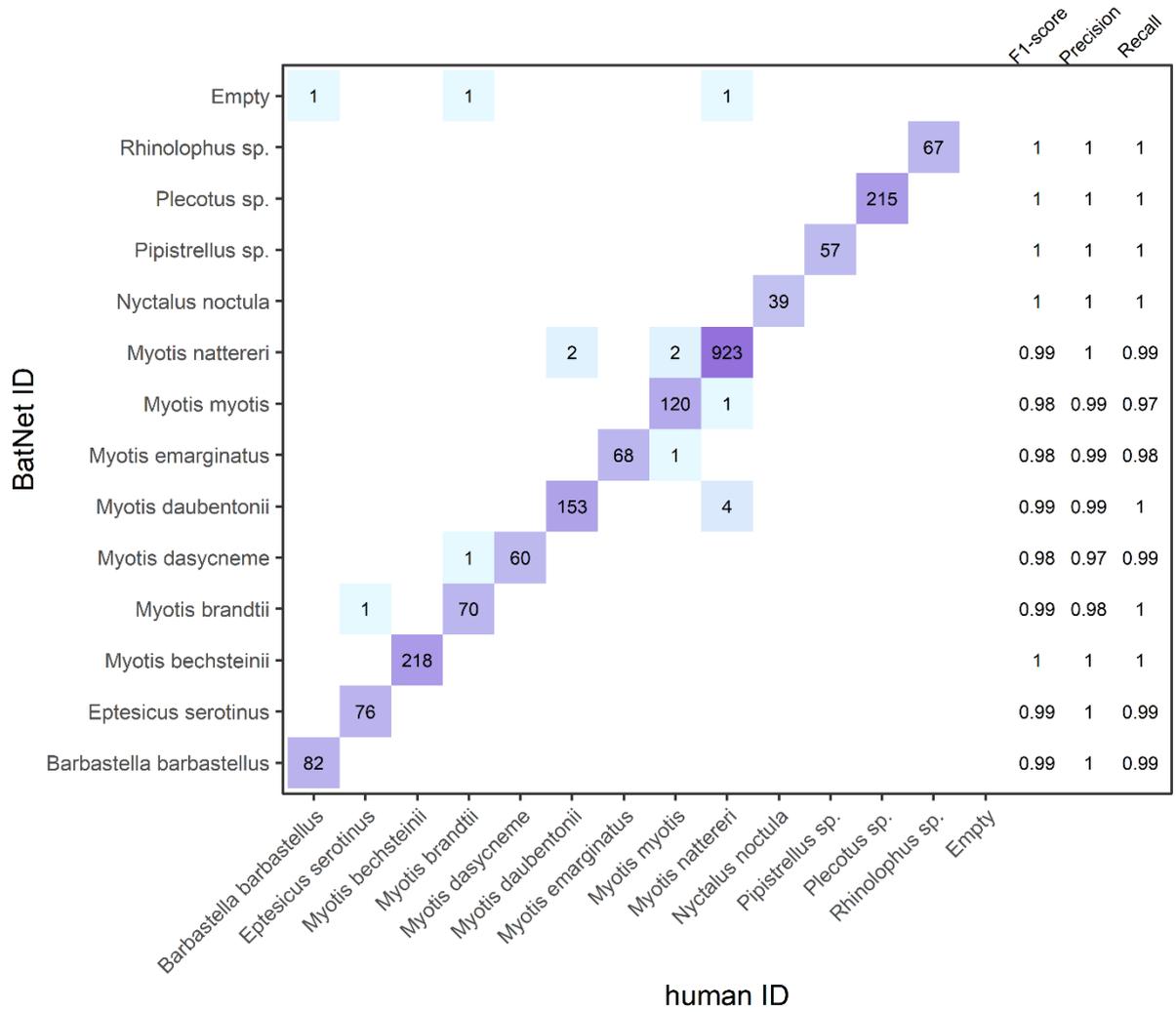
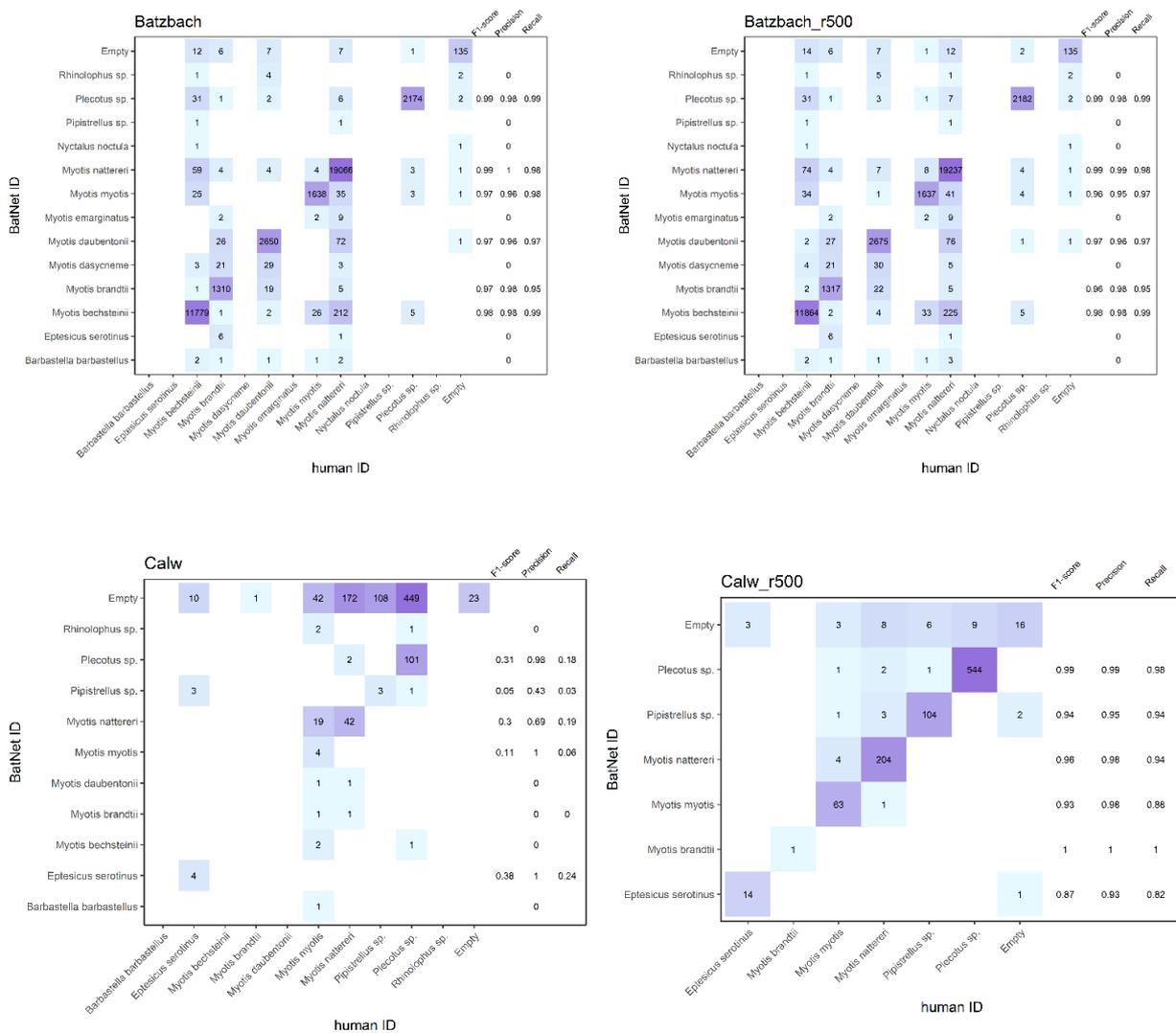
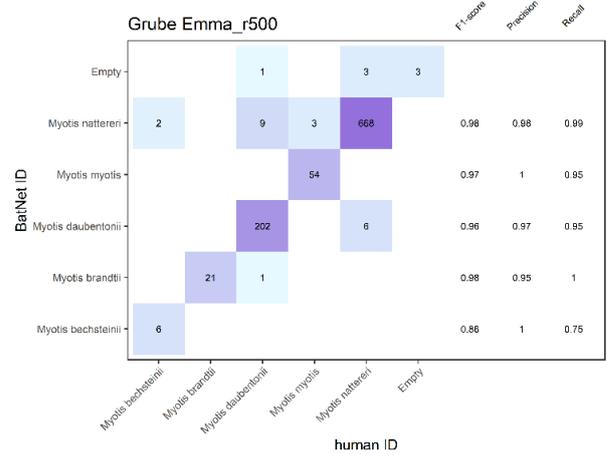
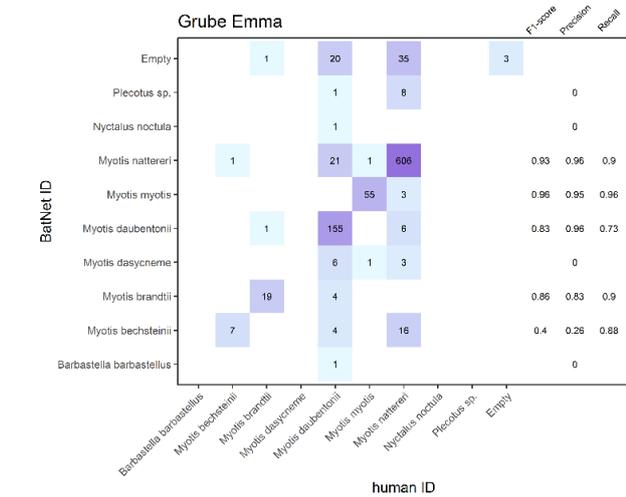
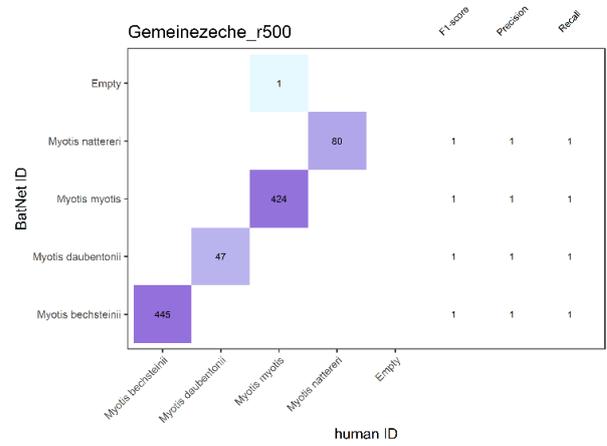
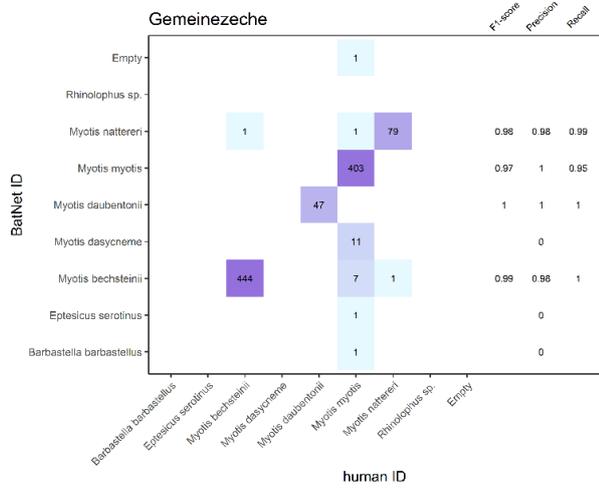
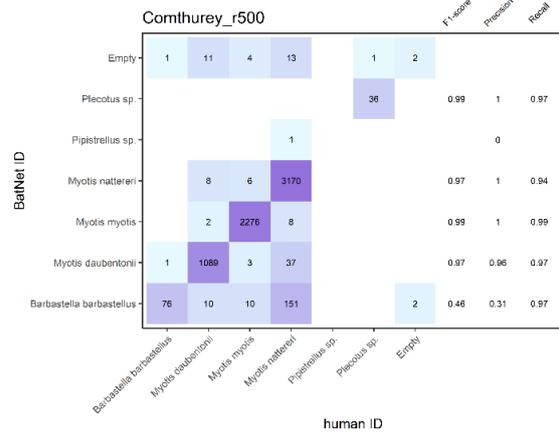
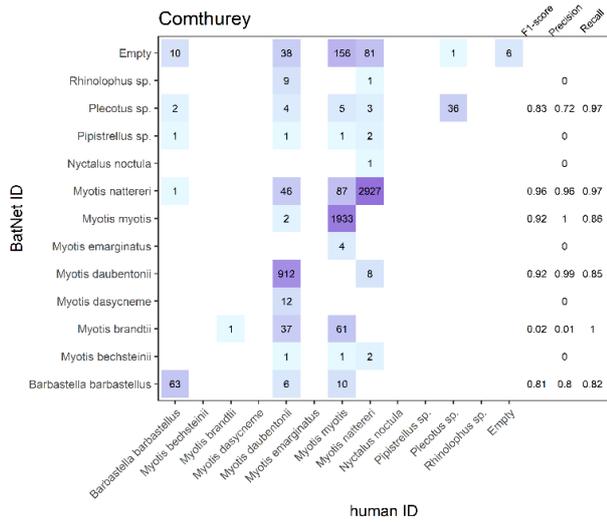


Figure S4. Confusion matrix of *BatNet* predictions and human identifications for camera trap images from six untrained background locations using the baseline model and the site-specific models retrained with 500 local annotations (r500). The confusion matrix shows the distribution of classification error within a species, where accurate classifications are across the diagonal and all other cells on the matrix describe error.





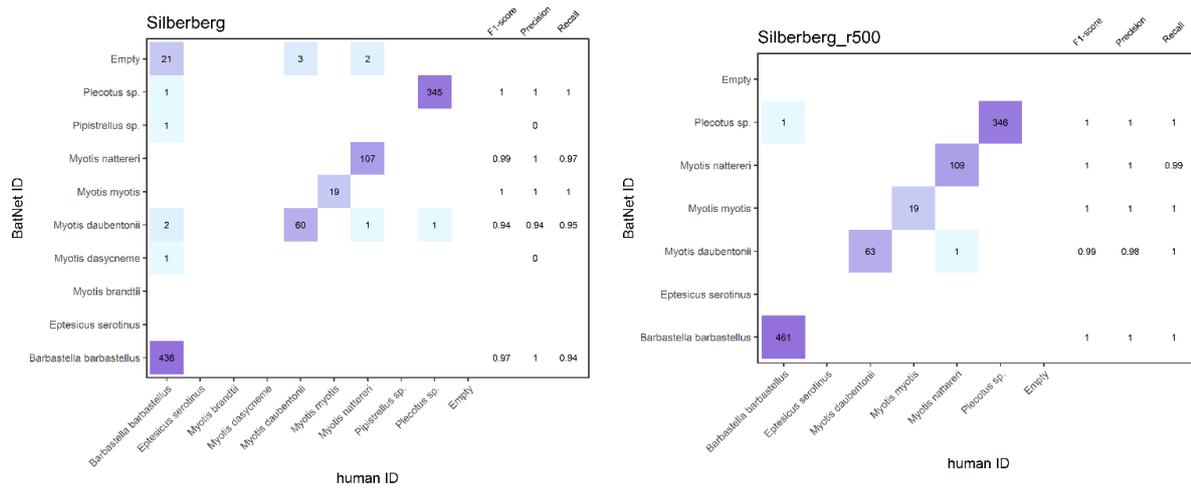
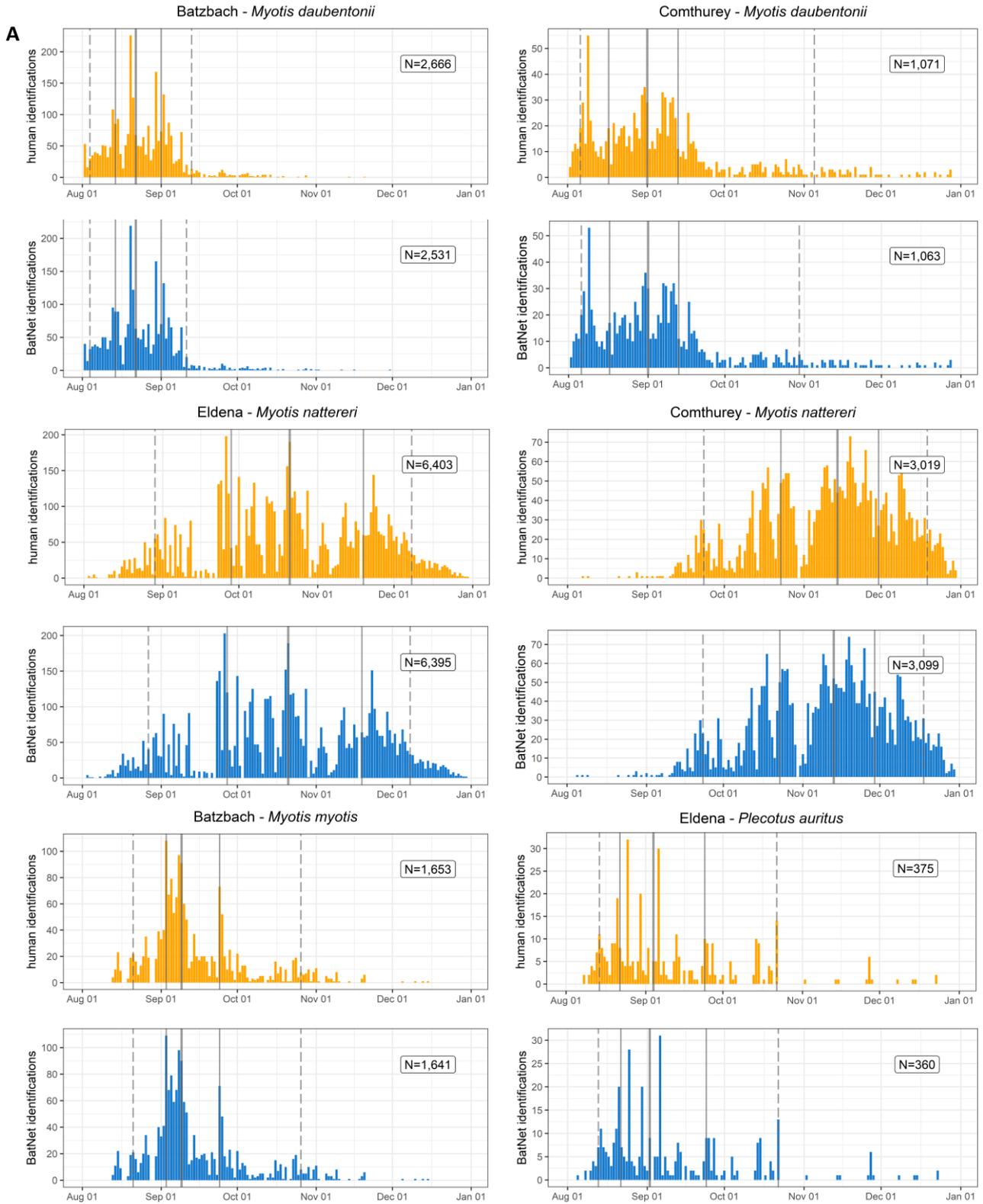


Figure S5. A) Phenology of *Myotis daubentonii*, *M. myotis*, *M. nattereri* and *Plecotus auritus* based on species identification by human experts (orange) compared to *BatNet* predictions with 70% confidence threshold (blue). For quantifying the differences between the phenological patterns of the two methods, we used percentiles of the nightly identifications (5, 25, 50, 75 and 95%; indicated with vertical gray lines) across a 5-month dataset over the hibernation-entry phase (01 August – 01 January). The sample size (N) indicates the total number of identifications across the season. **B)** Concordance plots indicating the agreement between the number of human and *BatNet* identifications per species per night, quantified by the Lin’s concordance correlation coefficient (CCC, range: 0-1).



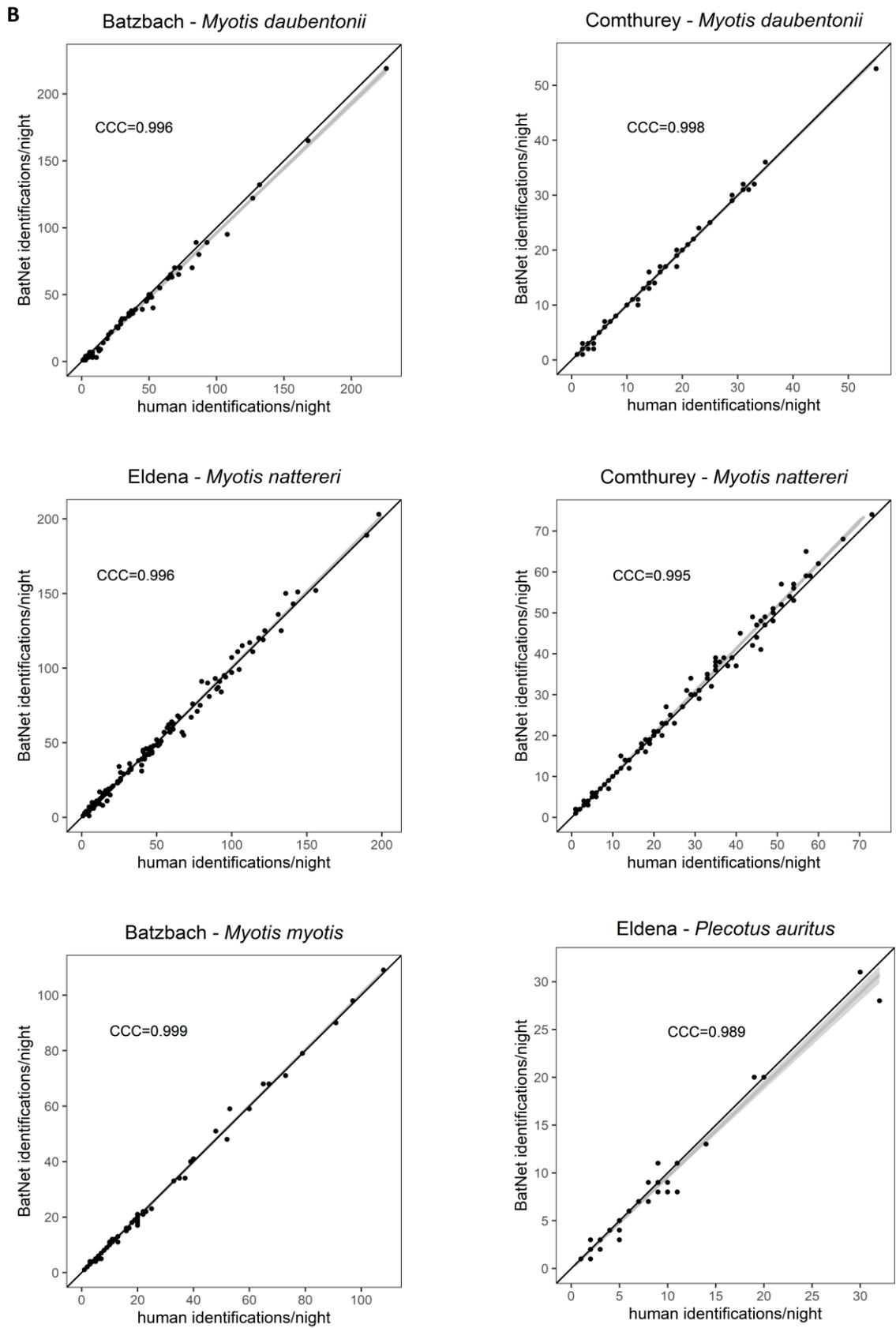
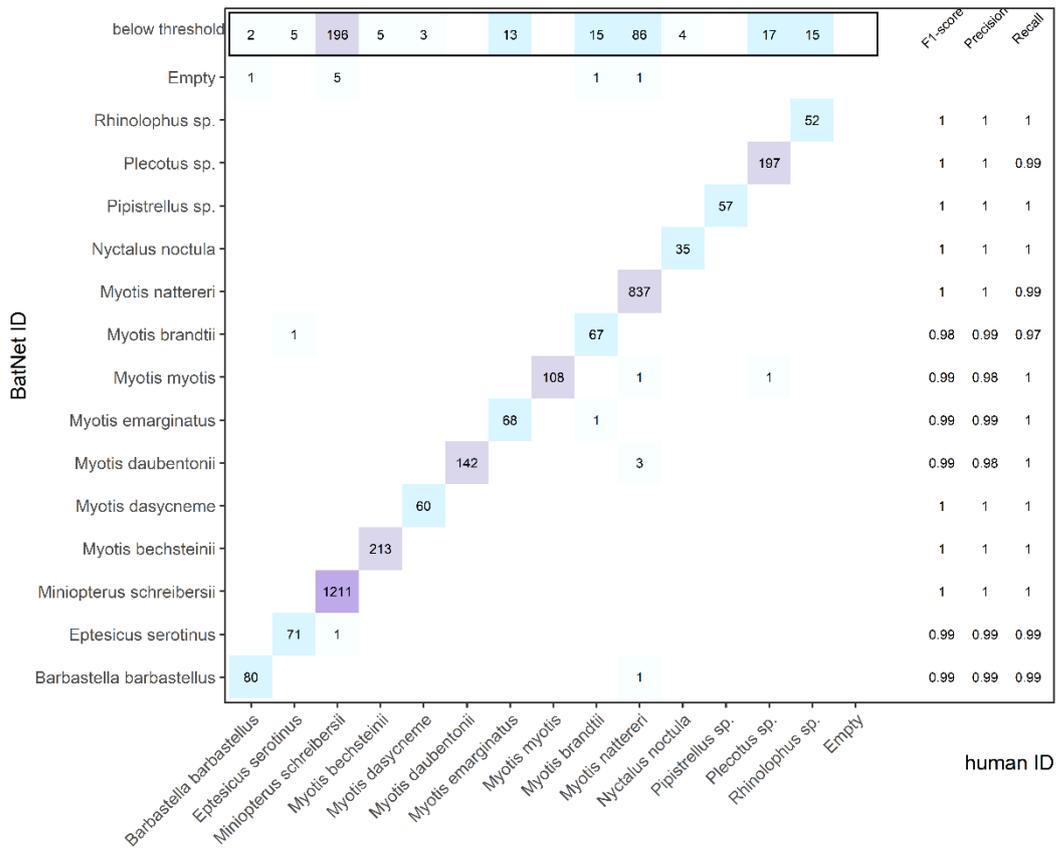


Figure S6. Confusion matrix of *BatNet* predictions with 70% confidence threshold and human identifications after retraining the baseline model to be able to identify a new species (*Miniopterus schreibersii*) in addition to the 13 species included in the original training data. The confusion matrix shows the distribution of classification error within a species, where accurate classifications are across the diagonal and all other cells on the matrix describe error (missed detection or misclassification). Additionally, identifications below the confidence threshold are summarized according to their true species label.



5. Contribution to Publications

- I) Krivek G., Schulze B., Poloskei P.Zs., Frankowski K., Mathgen X., Douwes A., van Schaik J. (2022). Camera traps with white flash are a minimally invasive method for long-term bat monitoring. *Remote Sensing in Ecology and Conservation*, 8(3): 284-296.

Study design & methodology: **GKr**, JvS; Data collection: **GKr**; Designing and building of infrared video cameras, video processing: **GKr**, BS; Audio processing: **GKr**, PPZS; Behavioral scoring: **GKr**, KF, XM, AD; statistical analysis: **GKr**, JvS; Writing: **GKr**, JvS with input from all authors.

- II) Krivek G., Mahecha E. P. N., Meier F., Kerth G., van Schaik J. (2023) Counting in the dark: estimating population size and trends of bat assemblages at hibernacula using infrared light barriers. *Animal Conservation*.

Conception: **GKr**, JvS, GKe; Study design: **GKr**, JvS; Light barrier data collection: **GKr**, JvS, FM; Infrared video data collection: **GKr**; Video processing: EPNM, **GKr**; Writing: **GKr**, JvS with input from all authors.

- III) Krivek G., Gillert A., Harder M., Fritze M., Frankowski K., Timm L., Meyer-Olbersleben L., Freiherr von Lukas U., Kerth G., van Schaik J. (2023) *BatNet*: a deep learning-based tool for automated bat species identification from camera trap images.

Conception: GKe, JvS, **GKr**; Training data collection: **GKr**, MH, MF, JvS; Training data preparation: **GKr**, KF, LT, LM; Human image identification: KF, MH, **GKr**, JvS; Software development: AG, UFvL; Evaluation: **GKr**, JvS; Writing: **GKr**, JvS.

Dr. Jaap van Schaik

Gabriella Krivek

6. Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde. Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Unterschrift des*der Promovend*in

7. Acknowledgements

Throughout my thesis, I used the term “we” very often, because research is a collaborative effort, and none of this work would have been possible without the support from so many wonderful people, who I am really thankful to.

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This project was funded by a joint research project *DIG-IT!* supported by the European Social Fund (ESF/14-BM-A55-0014/19), and the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern, Germany. Many thanks to my fellow colleagues from *DIG-IT!*, and first and foremost to Alexander Gillert for doing an amazing job with listening to all my crazy ideas and make them happen nevertheless! I am also grateful for the opportunity to be an associate member of the RESPONSE.

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How a neural network imagines my fieldwork now:
monitoring bats with camera traps.

- image generated using MidJourney AI 2023 -