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

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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 bechsteinii*) 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	*Myotis dasycneme*, ***M. daubentonii***, *M. myotis, M. nattereri*, ***Pipistrellus pipistrellus***
	Demmin	MV	Purpose-built hibernaculum, accessible	Liba-16k	Within opening	2021	***Myotis daubentonii***, *M. myotis*, ***M. nattereri***, *Plecotus auritus*
	Eldena	MV	Cellar, accessible	Main: Liba-16/ Liba-16k Side: Liba-4[b]	Back of opening/ within opening Within opening	2016–2021	*Myotis brandtii/M. mystacinus*, ***M. daubentonii***, *M. myotis*, ***M. nattereri***, *Plecotus auritus*
	Friedland	MV	Cellar, accessible	Liba-16	Back of opening	2021	*Myotis daubentonii, M. myotis*, ***M. nattereri***, *Plecotus auritus*
	Peenemünde	MV	Bunker, accessible	Liba-4	Back of opening	2021	*Myotis brandtii/M. mystacinus, M. dasycneme*, ***M. daubentonii***, *M. nattereri, M. myotis, Plecotus auritus*
(b) light barrier monitoring	Comthurey	MV	Bunker, accessible	Liba-4	Back of opening	2021	*Myotis daubentonii*, ***M. myotis***, ***M. nattereri***
	Putbus	MV	Cellar, accessible	Liba-4	Within opening	2019	***Myotis daubentonii***, ***M. nattereri***, *Plecotus auritus*
	Strasburg	MV	Cellar, accessible	Liba-4	Within opening	2021	*Myotis daubentonii*, ***M. nattereri***
	Trollenhagen	MV	Bunker, accessible	Main: Liba-16 Side: Liba-16k	Within opening	2016–2021	*Myotis brandtii/M. mystacinus*, ***M. daubentonii***, *M. myotis*, ***M. nattereri***, *Plecotus auritus*
	Kalkberghöhle	SH	Natural cave, accessible	Main: Liba-16k Side: Liba-16k	Within opening	2020	*Myotis bechsteinii, M. brandtii/ M. mystacinus, M. dasycneme*, ***M. daubentonii***, ***M. nattereri***, *M. myotis*
	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.

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

[b]The 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

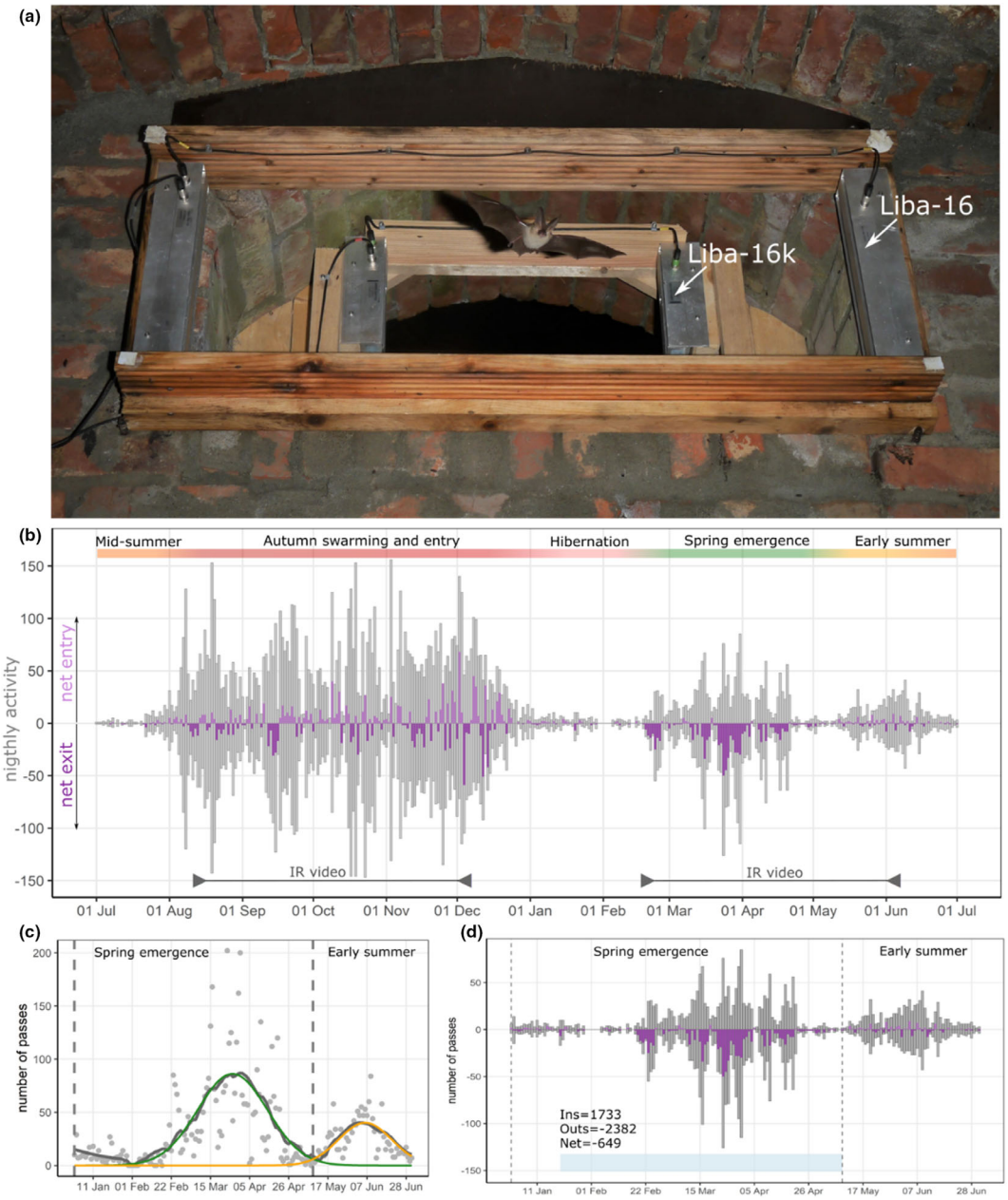

Figure 1 (a) Main entrance of a bat hibernaculum (Eldena, Germany) showing two light barrier models in situ: a Liba-16k integrated within the opening and a Liba-16 mounted on the inner wall of the hibernaculum, at the back of the opening. (b) Annual activity of temperate-zone bats can be divided into 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 smoothened 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., Zukal *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 smoothened 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 × 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 ± SD)	
Anklam	Within opening	Liba-4	79	38 (48%)	0.97	0.95 ± 0.03
Peenemünde	Back of opening		907	765 (84%)	0.93	
Eldena	Back of opening	Liba-16	954	919 (96%)	0.85	0.82 ± 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 ± 0.16) than in spring (Fig. 2b, CCC = 0.92 ± 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 increased concordance (compare Anklam and Peenemünde, Eldena Liba-16k and Liba-16; Table 2).

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

		Total number of passes			Mean passes/bat			
Site	Year	Autumn swarming and entry	Emergence	Early summer	Autumn swarming and entry	Emergence	Light barrier population size estimate (confidence interval)	Visual winter count
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.

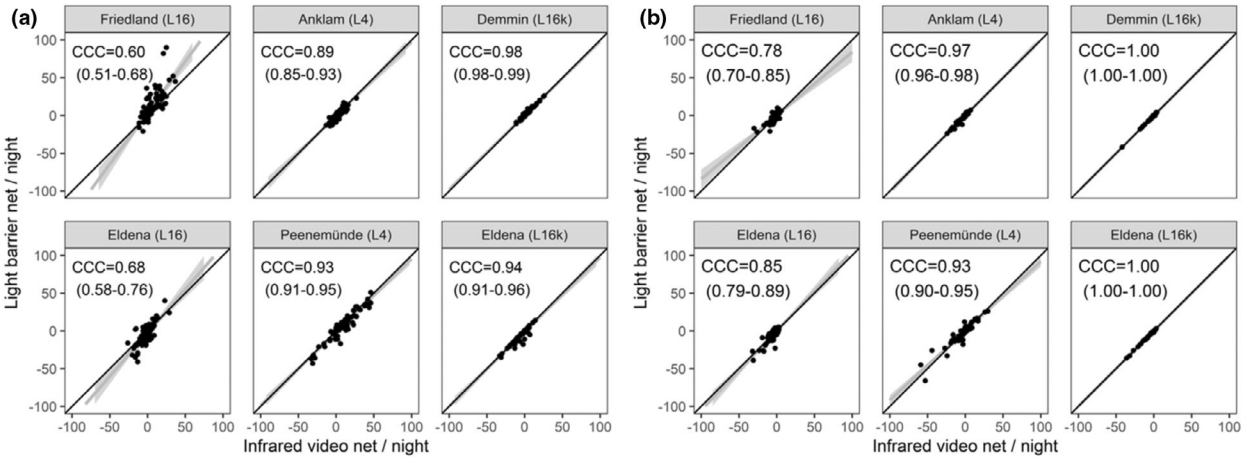

Figure 2 Comparison of the nightly net passes of bats recorded by the infrared light barriers and counted from video recordings in (a) autumn (August 1–December 4, 2020) and (b) spring (February 20–June 4, 2021). Data were collected at five hibernacula entrances in northern Germany that were monitored 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).

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.

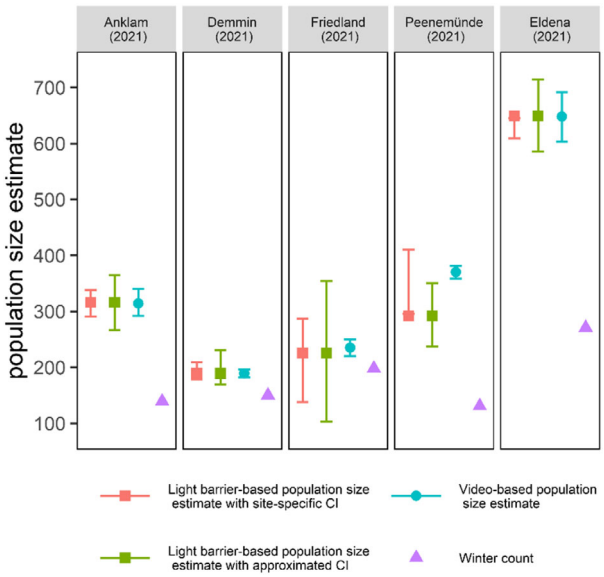

Figure 3 Population sizes estimates at five hibernacula in northern Germany, monitored with a light barrier, infrared video camera, and counted visually. For each site, we provide the light barrier-based population size estimates with site-specific confidence intervals (based on estimates of mean net 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).

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 decreases 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%).

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, Kugelschafter, & Fietz, 2022). We found that population estimates based on the number of bats

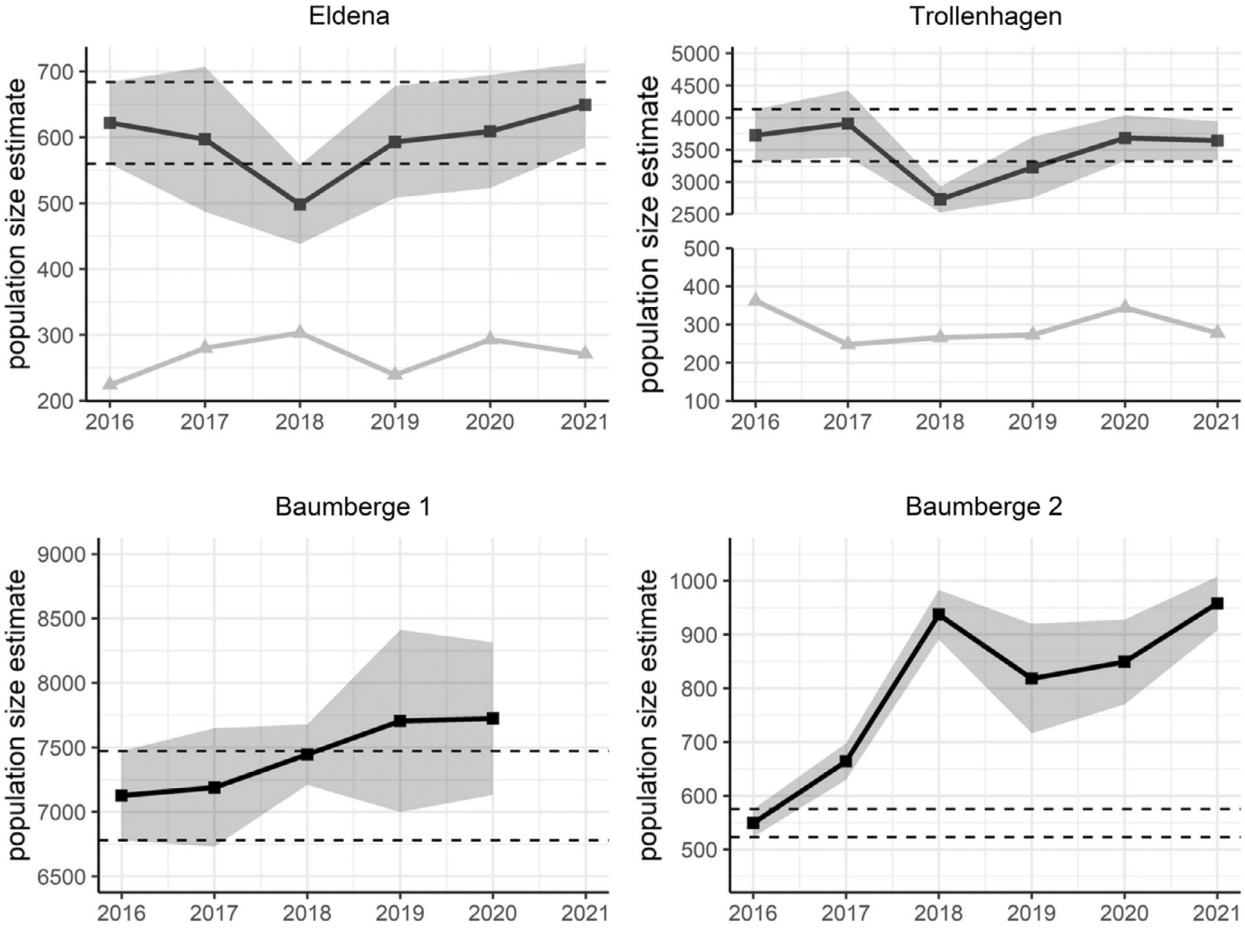

Figure 4 Light barrier-based bat population size estimates with approximated confidence intervals (black squares and gray ribbon) and winter counts (gray triangles) for two underground hibernacula in Mecklenburg-Western Pomerania, Germany (Eldena, Trollenhagen), and two in North Rhine-Westphalia, Germany (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 sizes 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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Author 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-bat/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.