Long-term trends of bats hibernating in small artificial hibernacula in northern Limburg and population dynamics explained by climatic conditions

Joris J.F. Verhees[1], Jan C. Buys, Paul H. van Hoof, Henk W.G. Heijligers & Willem F. de Boer[1]

[1] Wildlife Ecology and Conservation Group, Wageningen University, Droevendaalsesteeg 3a, NL-6708 PB Wageningen, the Netherlands, e-mail: jjf.verhees@hotmail.com

Abstract: Hibernation site selection by bats depends on various environmental and physiological factors, microclimatic conditions, and species-specific requirements. For successful overwinter survival, efficient use of stored fat reserves is crucial. Current predicted climate change may alter microclimatic conditions of hibernacula, stimulate arousal, and cause shifts in hibernation site use. A better understanding of these effects is needed to improve bat conservation. Between 1986–2020, we annually monitored 18 small (mean: 70 m^3) and artificial hibernacula, assessed long-term trends, and analysed population dynamics of hibernating bats in northern Limburg, the Netherlands. In *Myotis daubentonii* and *M. nattereri*, we found a significant population increase in these hibernacula, with a yearly mean growth of 5.8% and 11.7%, respectively. The trend of *Plecotus auritus* remained stable throughout the study period. Yearly population growth rate dynamics of these species could be best explained by climatic factors in terms of winter severity. In *M. nattereri* and *P. auritus*, yearly population growth rates positively correlated with the number of ice days per winter, but not in *M. daubentonii*. In all three species, temperature fluctuations during winters did not significantly affect population dynamics. Our results show that population growth rates of both *M. nattereri* and *P. auritus* increased during more severe winters. However, during relatively mild winters, *M. nattereri* but especially *P. auritus* was less abundantly present in the monitored hibernacula. As winters are expected to become warmer as a results of climate change, this species most likely select more exposed hibernacula such as tree cavities. Hence, availability of different types of hibernacula that provide a wide variety of microclimatic conditions throughout the winter could improve future conservation of this cold dwelling bat species.

Keywords: climate change, Chiroptera, heterothermy, hibernacula, hibernation, population dynamics, population growth rate, thermoregulation, torpor, TRIM.

Introduction

To bridge winter, bat species differ in their strategy to overcome this period of low temperatures and low food availability. For bats in the temperate zone, a common strategy is to enter a state of torpor, which is characterized by low body temperatures and reduced metabolic rates (Geiser et al. 2004). In the temperate zone in Europe, bats are predominantly insectivorous and all species

hibernate during winter, hence they can only rely on stored fat reserves when their food source is mostly unavailable. To use these reserves efficiently, temperate bats hibernate in sites that have reduced exposure to harsh environmental conditions, such as in bunkers, caves, cellars, fortifications, and tree cavities (Dietz & Kiefer 2016). The selection of suitable hibernation sites differs between bat species (Altringham 2011) and depends on factors such as season (Ransome 1990), microclimatic conditions (de Boer et al. 2013), and ambient temperature (De Bruyn et al. 2021). Hibernation is not a continuous process throughout winter; arousal in for example *Rhinolophus ferrumequinum* is synchronized with dusk (Park et al. 2000) and depends on external temperature (Ransome 1971). Moreover, arousal depends on a bat's body condition (Boyles et al. 2007) and can be induced for physiological processes such as drinking, excretion and reproduction, but also occurs spontaneously (Altringham 2011). Bats that wake up from torpor bouts frequently switch to different parts within the same -or less frequently- between hibernation sites (Daan 1973). However, arousal from a deep state of torpor and subsequent flights come at a cost: they are highly energy consuming (Currie et al. 2015), in particular when induced by a perturbation such as human activity (Speakman et al. 1991).

Under current predicted climate change, winters are expected to become milder with higher temperatures and fewer frost days (IPCC 2021). Bat species that hibernate at low temperatures, such as *Barbastella barbastellus* and *Plecotus auritus*, are expected to face major reductions in their distribution area (Rebelo et al. 2010). In *B. barbastellus*, warmer winters have recently led to a shift in hibernation site as this species selects colder (Gottfried et al. 2020) and more stable hibernacula (De Bruyn et al. 2021). Despite this plasticity, changes in winter conditions due to climate change are expected to have an impact on hibernation ranges of bats (Humphries et al. 2002) and may alter microclimatic conditions within roosts (Rebelo et al. 2010). Since hibernation patterns are closely related to the reproductive cycle in temperate bats (Racey & Entwistle 2000), climate change is likely to have a significant impact on reproduction (Jones et al. 2009) and overwinter survival (Pryde et al. 2005). Because temperature is one of the main drivers of winter energy requirements in heterothermic bats (Humphries et al. 2002), also larger temperature fluctuations may facilitate passive warming and stimulate costly arousal during torpor bouts (Geiser et al. 2004). However, the impact of climate change on patterns of hibernation and torpor in bats is complex (Sherwin et al. 2012) and not fully understood. A better understanding of the effects of climate change on species population sizes and both site and species-specific hibernation site use is therefore urgently needed (Staswki et al. 2014).

We expect that yearly population growth rates of bats hibernating in small artificial hibernacula depend on winter climatic conditions. Here, we define *population growth rate* as the yearly change in total numbers of hibernating bats in these hibernacula and assume that these rates change in response to winter climatic conditions. Demographic stochasticity, i.e., changes in birth or death rates, could not be quantified, as we had no information on the number of hibernating bats outside these hibernacula. We hypothesize that population growth rates of hibernating bats increase during severe winters, as for example observed in *R. hipposideros* hibernating in a large traditional hibernaculum (Martínková et al. 2020). Cold-dwelling bat species that hibernate in more exposed roosts, such as *B. barbastellus* (Gottfried et al. 2020) and *P. auritus* (Altringham 2011), are expected to shift to hibernacula that offer more stable conditions during severe winters (Swift 1998, De Bruyn et al. 2021). Contrary, due to milder winters, hibernacula are more subject to passive warming, and since hibernating at warmer temperatures increases energy expenditure

(Boyles et al. 2007, Geiser et al. 2014), this is expected to negatively affect growth rates. Furthermore, population growth rates are hypothesized to decrease during winters that are subject to larger temperature fluctuations because this increases flight activity during hibernation (Berková & Zukal 2010). After arousal induced by temperature fluctuations, and dependent of ambient temperature and season, we expect bats to select different hibernation sites to compensate for increased arousal frequency (Ransome 1971), which may lead to reduced growth rates. Since *P. auritus* is, compared to two of our other studied *Myotis* species, more cold-dwelling (Swift 1998), we discuss that the surveyed hibernacula will become less suitable in the near future and that other available hibernation site types will become more important for *P. auritus*. To test these hypotheses we carried out the above analyses on a 35 year database of bats hibernating in small artificial hibernacula in northern Limburg, the Netherlands, between 1986–2020.

Material and methods

Study area

Surveying bat hibernacula by counting individual hibernating bats is a reliable method that allows assessing species trends and conservation statuses (van der Meij et al. 2015). In the Netherlands, annual surveys of bat hibernacula have resulted in long-term databases (e.g. Weinreich & Oude Voshaar 1992, Dijkstra et al. 2006). This particularly applies to the monitoring of bats hibernating in limestone quarries in the South Limburg region, that traditionally has been carried out for more than 80 years (Daan 1980, Akkermans et al. 2016, Weinreich & Verheggen 2022, in this issue). Apart from these limestone quarries, there are approximately 60 bat hibernacula of another type scattered throughout the province of Limburg (Verboom 2006). The monitoring of 18 of these hibernation sites, which are all situated in the north of the province of Limburg (figure 1), is carried out annually since 1986 (Buys 1991). This resulted in a long-term database with annual numbers of bats hibernating in small hibernacula. The 18 surveyed hibernation sites primarily consist of relatively small and single space cellars (n=15) with a mean volume of 70 (sd ± 90) m^3. Other surveyed hibernation site types are one former brick kiln, a bunker, and a water reservoir (table 1). Human use of the sites differs; some are specifically assigned as a bat roost while others are parts of the year in use for recreational purposes (e.g., Verhees et al. 2019). The surveyed hibernation sites are surrounded by a landscape that primarily consists of agricultural land, fragmented forests and dispersed villages (Schmitz 2010).

Fieldwork

All hibernation sites were monitored almost annually from 1986 onwards or later when a site became accessible to bats (table 1). Yearly surveys took place between 25 December and 13 February. From 1992 and onwards, the monitoring was more standardized by annually surveying all sites during one day in January and by the same group of people. Hibernation sites were systematically searched for the presence of hibernating bats with the use of torches. Bats were identified while hibernating and without handling. In case that insufficient characteristics were observed or no consensus was reached by the observers, bats were identified as "species unknown". No distinction was made between *Myotis brandtii* and *M. mystacinus*, since identification to species level is not possible without handling (Dietz & Kiefer 2016). The surveys were carried out as part of a national bat monitoring programme (NEM) (La Haye et al. 2020) and under provision of an annual permit issued by the Dutch Mammal Society.

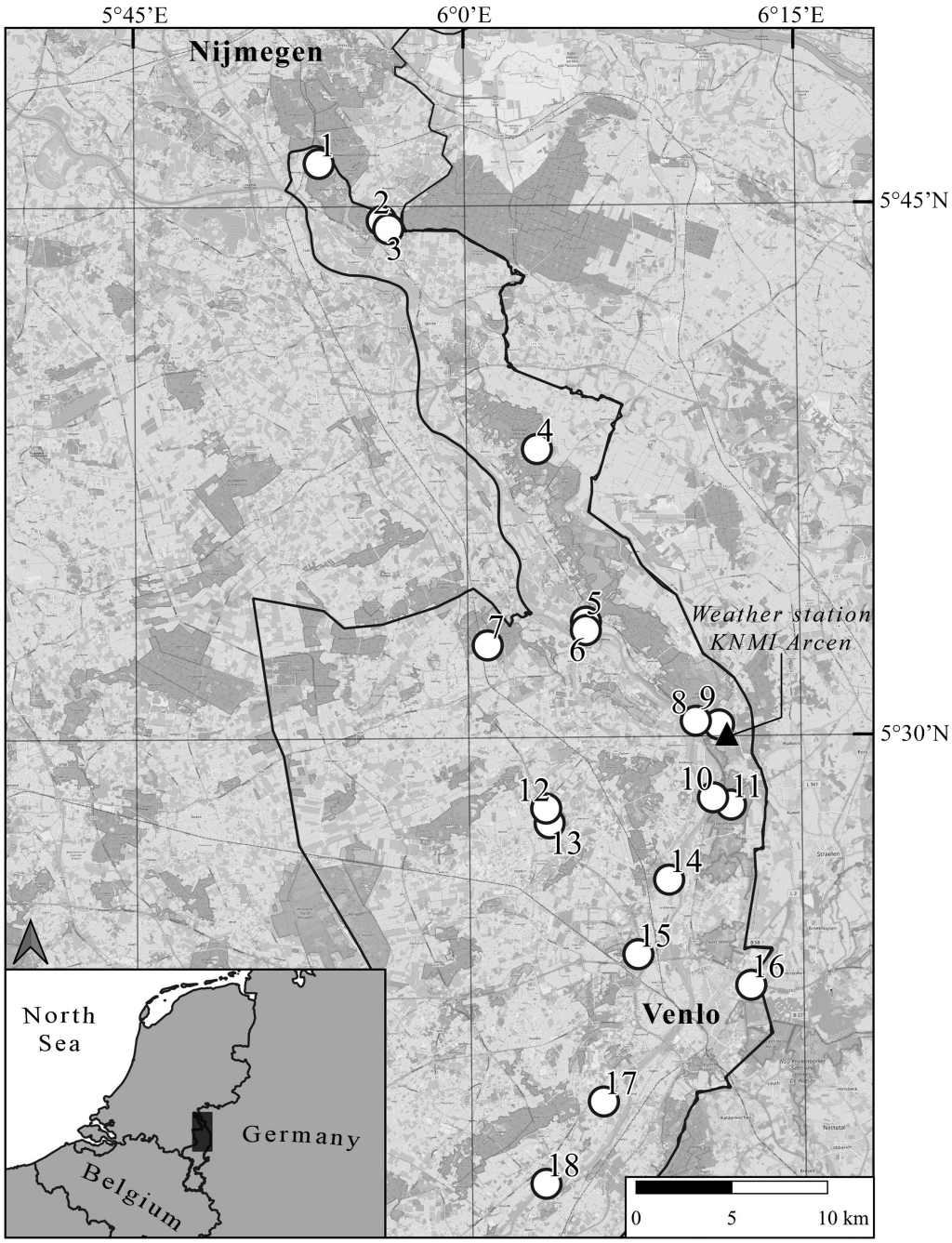

Figure 1. Locations of the 18 hibernation sites in northern Limburg under study and the Royal Netherlands Meteorological Institute (KNMI) weather station in Arcen (triangle). The black line indicates the province of Limburg. Site numbers: see table 1.

Table 1. Hibernation site characteristics (site type, volume and number of separate spaces), year since accessible to bats, start year of the monitoring and total number of years that the monitoring took place. Site numbers correspond to those in figure 1.

Site number	Hibernation site name	Site type	Volume (m^3)	Number of separate spaces	Accessible to bats since	Monitored since	Years monitored
1	Wildkelder Bethanië	Cellar	30	1	<1986	1992	29
2	IJskelder Sint-Jansberg	Cellar	20	1	<1986	1986	34
3	Waterkelder Sint-Jansberg	Cellar	5	1	<1986	1991	30
4	Ruïne Bleijenbeek	Cellar	125	4	<1986	1986	34
5	Kruitkelder kasteel Well	Cellar	100	1	<1986	1988	33
6	Tunnel kasteel Well	Cellar	20	1	<1986	1989	32
7	Waterreservoir Boshuizerbergen	Water reservoir	5000	1	2017	2017	4
8	Keldertje De Hamert	Cellar	10	1	<1986	1987	15
9	Vleermuiskelder Walbeckerheide	Cellar	20	1	2017	2017	4
10	Keldertje Oranjerie Arcen	Cellar	300	1	<1986	1988	30
11	IJskelder Arcen	Cellar	20	1	<1986	1987	34
12	Kasteel Huys ter Horst	Cellar	250	5	<1986	1986	34
13	Vleermuiskelder A73 Horst	Cellar	40	1	1996	1996	24
14	Bakoven Kaldenbroek	Cellar	15	1	<1986	1986	32
15	Vleermuiskelder A73 Zaarderheiken	Cellar	40	1	1996	1996	25
16	Bunkertje Herungerberg III	Bunker	20	1	<1986	1987	11
17	Kelder kasteel d'Erp Baarlo[a]	Cellar	20	1	<1986	1986	7
18	Kelder kasteel Keverborg Kessel[b]	Cellar	50	5	<1986	1986	8

[a]Accessible to bats until 2005.

[b]Accessible to bats until 1994.

Trend analysis of hibernating bats

Our database contains counts of hibernating bats at 18 sites over 35 years (1986–2020). The long-term changes in numbers of hibernating bats were analysed using *rtrim* v2.1.1 (Bogaart et al. 2020). This package is a reimplementation of the software TRIM (Trends and Indices for Monitoring Data), originally developed to analyse temporal variation in monitoring data (Pannekoek & Van Strien 2005). Using loglinear Poisson regression, *rtrim* estimates populations trends based on incomplete count data (only 11.2% of the data i.e. site and year combinations were missing). The analysis was conducted using 'model 2', assuming populations vary across sites but show similar growth everywhere (Bogaart et al. 2020). Models were deployed with a stepwise selection of changepoints and with a correction for both overdispersion and serial correlation. The trend of *P. auritus* was improved by including a binary covariate for hibernacula that consists out of a single space or concern relatively large sites (>50 m^3) consisting out of multiple separate spaces. Due to the lack of positive counts in a few site/year combinations, this covariate could not be included to model trends of *M. daubentonii* and *M. nattereri*. No trends could be calculated for other bat species due to low numbers. Calculated trends were classified into one out of six categories depending

on the yearly rate of change. A yearly significant change of >5% represents a strong in- or decrease; <5% a moderate in- or decrease, and non-significant changes of <5% as stable (Bogaart et al. 2020). Any other case was classified as an uncertain trend.

Climate data

Temperature data from 1990–2020 were obtained from the Royal Netherlands Meteorological Institute (KNMI) in Arcen (WGS coordinates 51.501, 6.191), situated at an average distance of 14.6 (sd ± 10.8) km to the hibernation sites. The daily mean, minimum, and maximum temperatures were obtained based on 24 hourly measurements (KNMI 2021). Since this station is operative since 1990, no temperature data were available during the first four years of the study period (1986–1989). As a measure of winter severity, four parameters were calculated based on temperature data between 1 November and the annual census date, on average after 76 (sd ± 7.5) days. These are: 1. Mean winter temperature. 2. Average minimum winter temperature. 3. Number of frost days (days with a minimum temperature below 0 °C). 4. The number of ice days (days with a maximum temperature below 0 °C). As a fifth parameter, the sd of daily mean winter temperature was calculated as a measure of temperature fluctuation.

Effects on species-specific yearly growth rates

In order to analyse population abundance dynamics, the yearly population growth rate was calculated per species using the equation: $\lambda = (N_{t+1} - N_t) / N_t * 100$, where 'N' represents the population at year 't'. Subsequently, λ was included as a response variable in General Linear Models (GLMs). Since all winter severity parameters were highly correlated to each other (Pearson's r (29) >0.823, P<0.001), only one of each was included as an explanatory variable, together with the sd of the mean winter temperature. An interaction term between the winter severity parameter and the sd of the mean winter temperature was also included as a potential term in all full models. Before analysis, all explanatory variables were log+1 transformed. Model selection was based on Akaike's Information Criterion (AIC), considering models significantly better with an AIC value of two or more units lower. All

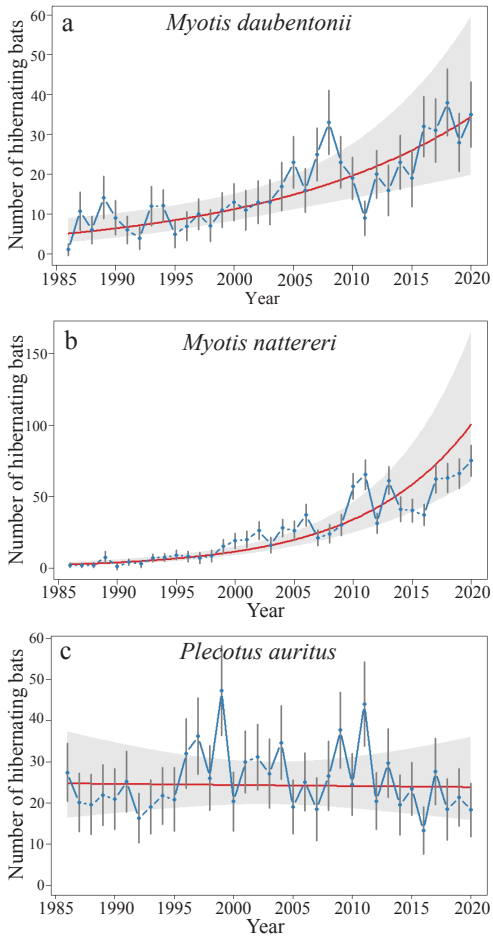

Figure 2. Trends of hibernating *Myotis daubentonii* (a), *Myotis nattereri* (b), and *Plecotus auritus* (c) between 1986–2020. Vertical lines represent the ± standard errors and the grey areas display the 95% confidence intervals. Note the differences in y-axes.

Table 2. Trend estimate of the multiplicative slope and standard errors (± S.E.) of the number of hibernating *Myotis daubentonii*, *Myotis nattereri*, and *Plecotus auritus* in northern Limburg between 1986–2020. The slope values represent the mean yearly rate of change, for example 0.997 implies an average decrease of 0.3% while 1.118 implies an average increase of 11.8%. Trend descriptions are based on the classification of Bogaart et al. (2020).

Species	Multiplicative slope	± S.E.	Trend description	AIC
Myotis daubentonii	1.058	0.016	Moderate increase (P<0.001)	-12.20
Myotis nattereri	1.118	0.018	Strong increase (P<0.001)	-133.84
Plecotus auritus	0.997	0.009	Stable (P=0.782)	2.63

analyses were conducted in R v4.0.3 (R Core Team 2020).

Site-specific species composition

Two principal component analyses (PCA) were performed to display changes in the species composition of hibernating bats between sites and during the study period. Based on splitting the dataset in two approximately equal periods, the mean number of hibernating bats per site and per species from 1986–2004 was used as a response variable for the first PCA, whereas the second PCA used this data from 2005–2020. To calculate the mean number of hibernating bats per site, only those years that a hibernation site was accessible for bats were included. In case of missing counts, the imputed values calculated with *rtrim* were used for that year. Before analysis, response variables were log+1 transformed, species were centred and standardized, and plots were standardized to norm. Both PCAs were conducted in Canoco5 v5.12 (ter Braak & Šmilauer 2018).

Results

General results and long-term trends

During the study period of 35 years (1986–2020), hibernating bats were found in 17 out of 18 monitored sites (94,4%; supplementary table S1[1]). In total 2372 bat observations were done, divided over five species and one species group (*M. brandtii/mystacinus*). In decreasing order of observations these were: *M. nattereri* (*n*=920), *P. auritus* (*n*=818), *M. daubentonii* (*n*=561), *M. brandtii/mystacinus* (*n*=3), *P. austriacus* (*n*=2), and *Eptesicus serotinus* (*n*=1). Sixty-five times, a bat observation was noted as 'species unknown', and twice as *Plecotus* sp. (supplementary table S1[1]). The total number of hibernating bats per year varied among years between 13–138 individuals, mainly due to a relatively recent increase in the numbers of *M. daubentonii* (figure 2a) and *M. nattereri* (figure 2b). Between 1986–2020, the overall trend of *M. daubentonii* showed a significant moderate increase with a mean growth of 5.8% per year (table 2). *Myotis daubentonii* was found hibernating in half of all surveyed sites, of which 44.4% got occupied from 2000 and onwards (supplementary table S1[1]). The trend of *M. nattereri* significantly strongly increased by 11.8% per year on average (table 2). Also this species was found present in half of all sites (supplementary table S1[1]). *Plecotus auritus* occupied most roosts and was found hibernating in 17 out of all surveyed sites (supplementary table S1[1]). The trend of *P. auritus* was classified as stable, but overall this species slightly decreased by 0.25% per year on average (figure 2c; table 2).

[1] https://www.Zoogdiervereniging.nl/publicaties/2022/lutra-65-1-2022

Table 3. Final models including parameters, coefficients plus standard errors (± S.E.), and corresponding *t*, *P*, and AIC-values of the yearly population growth models per species. *: $P<0.05$; **: $P<0.01$.

Species	Final model parameters	Coefficients	± S.E.	*t*-value	*P*-value	AIC
Myotis daubentonii	Intercept	107.19	62.47	1.716	0.079	350.18
	Number of frost days (log+1 transformed)	-28.39	19.95	-1.423	0.166	
Myotis nattereri	Intercept	-27.47	24.26	-1.133	0.267	350.13
	Number of ice days (log+1 transformed)	26.0	10.68	2.435	0.021*	
Plecotus auritus	Intercept	-29.907	13.864	-2.516	0.039*	315.45
	Number of ice days (log+1 transformed)	18.894	6.102	3.096	0.004**	

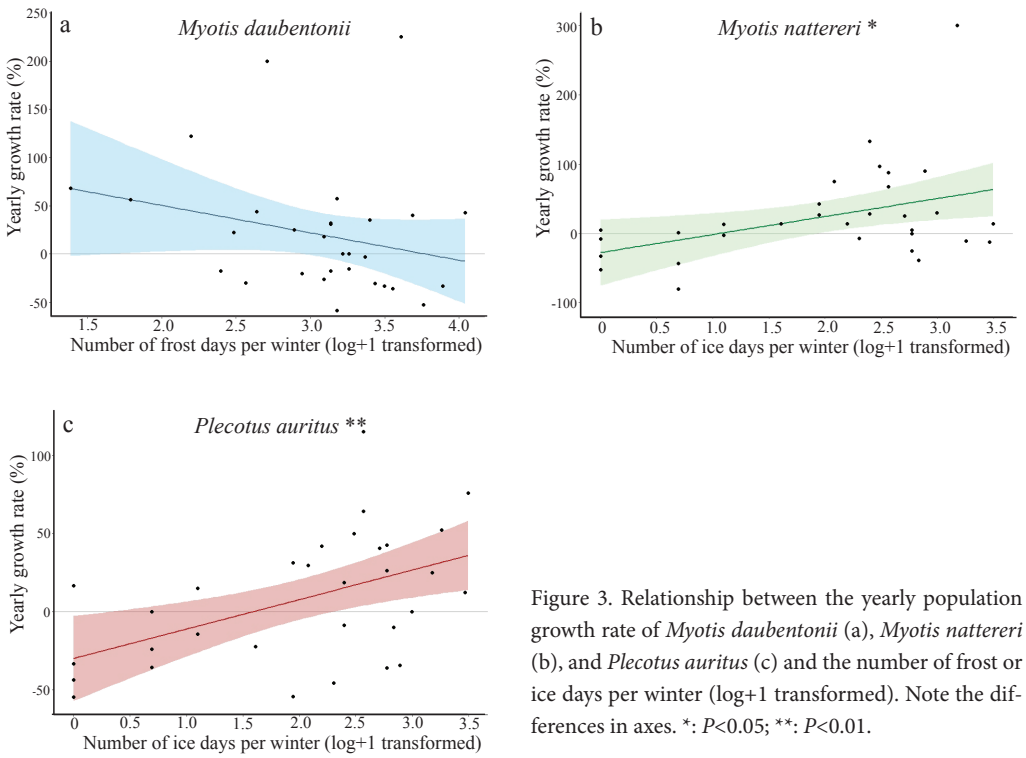

Figure 3. Relationship between the yearly population growth rate of *Myotis daubentonii* (a), *Myotis nattereri* (b), and *Plecotus auritus* (c) and the number of frost or ice days per winter (log+1 transformed). Note the differences in axes. *: $P<0.05$; **: $P<0.01$.

Climatic factors and species specific population growth rates

In *M. daubentonii*, no relation was observed between the yearly population growth rate and any of the four winter severity parameters, the sd of the mean winter temperature, nor their interaction (table 3). The model with the lowest AIC only included the number of frost days, but this was not significant (GLM, t=-1.423, P=0.166; figure 3a). In *M. nattereri*, a significant positive correlation was found between the yearly population growth rate and the number of ice days (GLM, t=2.435, $P< 0.05$; figure 3b), but not when the sd of the mean winter temperature nor their interaction term were included. The yearly population growth rate of *P. auritus* significantly correlated with all four winter severity parameters. The model with the lowest AIC included the number of ice days and this correlated significant and positively with the yearly population growth rate (GLM, t=3.096, $P<0.01$; figure 3c). No parameters correlated significantly with

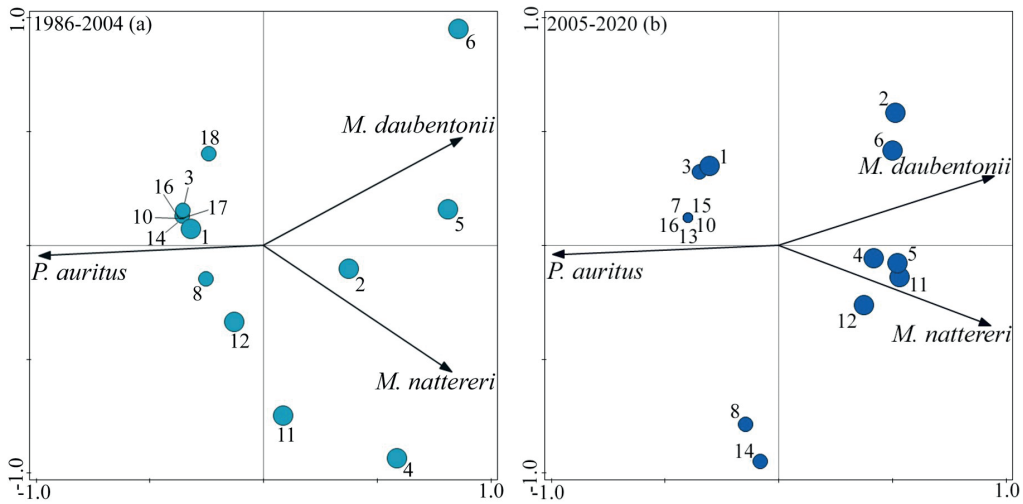

Figure 4. Graphical visualisation of two PCAs for the periods 1986–2004 (a) and 2005–2020 (b). The size of the symbol indicates the number of displayed species (1–3) that make use of the hibernacula. Site numbers: see table 1.

yearly growth rates when the sd of the mean winter temperature nor their interaction were included in these models. Hence, yearly population growth rates of bats hibernating in the surveyed sites are not significantly affected by temperature fluctuations.

Shifts in species composition

The PCA on the species composition during the first period of the study (1986–2004) was rather similar to the one from 2005–2020 (figure 4). All but one accessible hibernation sites were occupied by *P. auritus*, and in six sites this was the only species present. Hence, *P. auritus* strongest correlated to the first axis. In the first period, the total variation explained by the PCA was 42.0%. The first PCA axis explained 81.0% of that variation, the second axis 17.8%. During 2005–2020, more sites became occupied by both *M. daubentonii* and *M. nattereri* (figure 4b). In hibernacula where these species already were present, their numbers increased and sites became relatively more important for these species, while the abundance of *P. auritus* remained approximately equal. The total variation in the PCA during 2005–2020 was 29.0%, with the first axis explaining 81.9% of that variation, the second axis 17.4%.

Conclusion and discussion

General

To analyse long-term bat trends and population dynamics, we surveyed 18 artificial hibernation sites in northern Limburg by annually counting individual hibernating bats between 1986–2020. Even though this method is widely applied to monitor hibernating bats in underground hibernacula (Haysom et al. 2013), it is also subject to bias since underestimations frequently occur (Kugelschafter 2009). However, we expect deviations from the actual number of hibernating bats during our survey to be small or even negligible. Even though hibernating bats trends can deviate from those in summer (La Haye et al. 2020), we assume that our estimations represent local populations trends *M. daubentonii*, *M. nattereri*, and *P. auritus* in northern Limburg.

In the surveyed sites, we found a total of five different bat species and one species group.

The latter concerns only three observations of *M. brandtii/mystacinus*. Even though this species group is often present in comparable hibernation sites throughout the Netherlands that accommodate a similar species composition (La Haye & van der Meij 2022, in this issue) its nearly absence is somewhat remarkable. Even though the surrounding habitat does seem appropriate and the hibernacula accommodate a wide variety of microclimatic conditions, it is unclear why this species group hardly makes any use of hibernation sites in northern Limburg.

Population trends

Our findings are very similar to previously reported *M. daubentonii*, *M. nattereri*, and *P. auritus* trends in central and western Europe (Uhrin et al. 2010, Haysom et al. 2013, van der Meij et al. 2015, Bernard et al. 2019). Between 1986–2020, the same overall population trends of *M. daubentonii* and *M. nattereri* are also observed in the Netherlands (van der Meij et al. 2015). However, since approximately 2010 and onwards, the national trend of *M. daubentonii* has flattened (La Haye et al. 2020), hence deviating from the continuously growing *M. daubentonii* population trend in our study area. Also the stable trend of *P. auritus* deviates from the overall increasing trend in the Netherlands (La Haye et al. 2020), but recently this species is moderately declining. The exact causes for the observed population increases in *M. daubentonii* and *M. nattereri* are difficult to determine and are probably affected by various factors. Several bat species, including *M. nattereri*, are possibly still recovering from a dramatic decline between 1940–1980 (Daan 1980), a result of negative effects caused by an intensive bat banding programme and severe disturbances during hibernation (Akkermans et al. 2016). Furthermore, the banning of certain pesticides and wood preservatives most likely contributed to population recoveries from this period and onwards for various bats species in the Netherlands, including *M. daubentonii* and *M. nattereri* (Weinreich & Oude Voshaar 1992, Akkermans et al. 2016). Additionally, in the Netherlands and elsewhere in Europe, a plausible theory of Kokurewicz (1995) is that the increase of *M. daubentonii* is a result of higher prey availability, caused by eutrophication of water bodies. However, Boonman et al. (1998) found that eutrophication also can reduce foraging possibilities of this species, therefore possibly resulting in negative effects on *M. daubentonii* populations. Since current water management strategies aim for less eutrophicated water bodies through improved water quality, it is unlikely this is an important factor nowadays. *Myotis daubentonii* and *M. nattereri* likely also benefitted from improved forest management, as a shift from forests mainly maintained for production to more natural forests took place (Limpens et al. 1997). A management strategy that focusses on the aging of trees, forest composition and structure, preservation of dead trees, and an abundance of tree cavities as well as loose bark results in improved foraging conditions as well as increased roosting possibilities for all forest-dwelling bat species.

Lastly, in our study area, both *M. daubentonii* and *M. nattereri* assumingly also benefitted from hibernation sites refinements, such as minimizing human disturbance, improvements of microclimatic conditions, and increased hiding possibilities (i.e., Buys 1991, Verhees et al. 2019). A combination of these factors likely enhanced the selection and occupation of newly suitable hibernation sites during the study period, therefore contributing to the population increases of *M. daubentonii* and *M. nattereri*.

Yearly population growth rates and climatic factors

We found no significant relationship between abundance dynamics in terms of yearly population growth rates and climatic factors in *M.*

daubentonii, since no winter severity parameter and/or the sd of the mean winter temperature correlated with these rates. These results are comparable to those of Fuszara et al. (2010), who also did not find a relationship between ambient temperature and numbers of hibernating *M. daubentonii* during a comparable long-term study. However, our results do show a pattern that population growth rates are lower during severe winters. During such winters, *M. daubentonii* possibly crawls further away into deep crevices allowing them to hibernate less exposed to environmental conditions, hence lowering detectability during surveys.

In *M. nattereri*, yearly population growth rates were positively correlated with the number of ice days per winter. Even though all four winter severity parameters highly correlated to each other, only the number of ice days significantly correlated with yearly growth rates of this species. This indicates that the presence of higher numbers of *M. nattereri* are only in response to climatic conditions during relatively severe winters (with many ice days), and that other factors are likely to affect yearly growth rates in this species during milder winters. Lower growth rates during relatively mild winters are possibly the result of foraging conditions that are still appropriate (Hope et al. 2014) and allow roosting during daytime in other site types. The winter diet of *M. nattereri* in England predominantly consists of lepidopteran larvae, indicating that this winter prey is caught by a 'gleaning' foraging strategy, complementary to their way of foraging (aerial hawking/gleaning) in summer (Hope et al. 2014). Since *M. daubentonii*, a trawling bat species (Boonman et al. 1998), is perhaps not able to complement or shift this strategy during winter, this possibly explains the observed contrasting patterns in population growth rates in both *Myotis* species in relation to winter severity.

In *P. auritus*, growth rates significantly correlated with all four winter severity parameters, but strongest, and positively, with the number of ice days. A significant relationship between hibernating numbers of *P. auritus* and ambient temperature was previously reported by e.g., Klys & Woloszyn (2005), and is also found by Bekker (2022, in this issue) and Van Zuijlen & Groenendijk (2022, in this issue) in similar sized hibernacula. After arousal and in response to changes in ambient temperatures (Bezem et al. 1964), individual *P. auritus* likely leave their hibernacula to forage (Hays et al. 1992, Blomberg et al. 2021) and to (temporarily) roost elsewhere under more suitable conditions for thermoregulation (Swift 1998). This plasticity shows that *P. auritus* actively selects and thus relies more on stable hibernacula with reduced exposure to environmental conditions during severe winters (Bezem et al. 1964). Especially in such winters, apparently our surveyed hibernation sites offer these requirements, however during mild winters, mainly other hibernacula (types) are preferred.

Climate change and conservation implications

The vulnerability of species to climate change depends on various factors such as exposure, sensitivity, and adaptive capacity (Aguiar et al. 2016). Mammalian species that have already evolved the capacity to remain torpid during relatively warmer temperatures may thus be favoured by natural selection (Frank 2012). However, in slowly reproducing species such as bats, the evolutionary response to climate change may take considerable time to become apparent (Sherwin et al. 2012). Negative effects of climate change are expected to affect many bat species, but especially those that are cold-dwelling (Rebelo et al. 2010). In order to mitigate the first stages of climate change, a partial habitat shift in response to warmer winters was observed in *B. barbastellus* (De Bruyn et al. 2021), a typical cold-dwelling Eurasian bat species. In response to warmer winters, such plasticity is also likely to occur in one of our studied cold-dwelling

species, *P. auritus* (Swift 1998). During severe winters, we found significant higher population growth rates, and in terms of absolute abundances, the numbers of hibernating individuals were approximately four times higher than those during relatively mild winters. *Plecotus auritus* prefers hibernating at lower temperatures compared to *Myotis* species (Daan 1980, Siivonen & Wermundsen 2008) and generally at more exposed sites (Altringham 2011), such as close to hibernation site entrances and in tree cavities (Dietz & Kiefer 2016). Since hibernation depends on a bat's body condition (Boyles et al. 2007), but also on microclimatic conditions (de Boer et al. 2013) and ambient temperature (Blomberg et al. 2021), it is expected that due to warmer winters our monitored sites become relatively less important for this cold-dwelling bat species. Currently, relatively little is known about alternative roosts that may serve as appropriate hibernacula, such as tree cavities (Cryan & Veilleux 2007), likely due to their inaccessibility for humans. However, it is likely that during warmer winters these more exposed tree cavities become increasingly important for such bat species in the near future. In forest management, therefore, trees and their importance as bat hibernacula should be integrated in conservation strategies of forest-dwelling bat species. Prior to the harvesting of trees, which is typically done in winter, it is of crucial importance to determine the presence of hibernating bats. Even tough forest management recently has shifted from production forest as primary objective to more natural forests (Limpens et al. 1997), retention of high numbers of tree cavities should be included and improved in management practices (Lučan et al. 2009). The availability of different types of hibernacula, including tree cavities, allow bats to respond to altered winter conditions and enable them to select hibernacula that (temporarily) meet the right microclimatic requirements for thermoregulation, vital for overwinter survival. Further optimalization of these hibernacula and minimization of overall human disturbances that cause perturbations of bats (Speakman et al. 1991) could enhance future safeguarding of these regional bat populations in northern Limburg.

Acknowledgements: We thank the Dutch Mammal Society for annually provisioning us with the permit to survey all sites. Additionally, they supplied us with missing data on bats hibernating in the cellars of the castle "d'Erp" and the bunker "Herungerberg III", of which the latter was surveyed by Jan van de Put† between 1999–2005. We also thank two editors of Lutra that provided us with constructive comments on an earlier version of the manuscript. Lastly, all site owners are thanked for their allowance to carry out the monitoring.

References

Aguiar, L.M.S., E. Bernard, V. Ribeiro, R.B. Machado & G. Jones 2016. Should I stay or should I go? Climate change effects on the future of Neotropical savannah bats. Global Ecology and Conservation 5: 22–33.

Akkermans, R.W., J.P. Bekker & (H.)G.J. Bekker 2016. Zoogdieren in een veranderend landschap. In: S. Broekhuizen, K. Spoelstra, J.B.M. Thissen, K.J. Canters & J.C. Buys (eds). De Nederlandse zoogdieren: 29–68. Natuur van Nederland 12. Naturalis Biodiversity Center & EIS Kenniscentrum Insecten en andere ongewervelden, Leiden, the Netherlands.

Altringham, J.D. 2011. Bats: from Evolution to Conservation. Oxford University Press, Oxford, UK.

Bekker, J.P. 2022. The relation between numbers of hibernating brown long-eared bats (*Plecotus auritus*) and weather conditions (ambient temperatures and precipitation). Lutra 65 (1): 189-199.

Berková, H. & J. Zukal 2010. Cave visitation by temperate zone bats: effects of climatic factors. Journal of Zoology 280: 387–395.

Bernard, R., R. Jaros, J. Samoląg & J.Z. Kosicki 2019. Long-term monitoring of a winter bat assemblage revealed large fluctuations and trends in species abundance. European Journal of Ecology 5 (2): 72–78.

Bezem, J.J., J.W. Sluiter & P.F. van Heerdt 1964. Some

characteristics of the hibernating locations of various species of bats in South Limburg. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen 67 (5): 325–350.

Blomberg, A.S., V. Vasko, M.B. Meierhofer, J.S. Johnson, T. Eeva & T.M. Lilley 2021. Winter activity of boreal bats. Mammalian Biology 101: 609–618.

Bogaart, A.P., M. van der Loo & J. Pannekoek 2020. Rtrim: Trends and Indices for Monitoring Data. R Package Version 2020, 2.1.1. Available online: https://cran.r-project.org/web/packages/rtrim/index.html.

Boonman A.M., M. Boonman, F. Bretschneider & W.A. van de Grind 1998. Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. Behavioral Ecology and Sociobiology 44 (2): 99–107.

Boyles, J.G., M.B. Dunbar, J.J. Storm & V. Brack 2007. Energy availability influences microclimate selection of hibernating bats. Journal of Experimental Biology 210: 4345–4350.

Buys, J.C. 1991. Overwinterende vleermuizen in Noord-Limburg. Natuurhistorisch Maandblad 80 (6): 109–113.

Cryan, P. & J.P. Veilleux 2007. Migration and use of autumn, winter, and spring roosts by tree bats. In: M.J. Lacki, J.P. Hayes & A. Kurta (eds). Bats in forests: Conservation and management: 153-175. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Currie, S.E., K. Noy & F. Geiser 2015. Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands. American Physiological Society 308: 34–41.

Daan, S. 1973. Activity during natural hibernation in three species of Vespertilionid bats. Netherlands Journal of Zoology 23 (1): 1–71.

Daan, S. 1980. Long term changes in bat populations in the Netherlands. Lutra 22: 95–105.

de Boer, W.F., S. van de Koppel, H.J. de Knegt & J.J.A. Dekker 2013. Hibernation site requirements of bats in man-made hibernacula in a spatial context. Ecological Applications 23 (2): 502–514. https://doi.org/10.1890/1051-0761-23.2.502

De Bruyn, L., R. Gyselings, L. Kirkpatrick, A. Rachwald, G. Apoznański & T. Kokurewicz 2021. Temperature driven hibernation site use in the Western barbastelle *Barbastella barbastellus* (Schreber, 1774). Scientific Reports 11: 1464. https://doi.org/10.1038/s41598-020-80720-4

Dietz, C. & A. Kiefer 2016. Bats of Britain and Europe. Bloomsbury, London, UK.

Dijkstra, V.A.A., L.S.G.M. Verheggen, J.A. Weinreich & B. Daemen 2006. Wintertellingen van vleermuizen in Limburg. Natuurhistorisch Maandblad 95 (1): 36–40.

Frank, C.L. 2012. The Relationship between climate warming and hibernation in mammals. In: K.B. Storey & K. Tanino (eds). Temperature adaption in a changing climate: Nature at risk: 120–230. CPI Group, UK.

Fuszara, E., M. Fuszara, M. Kowalski, G. Lesiński, J.P. Cygan, T. Nitkiewicz, A. Szarlik & B. Wojtowicz 2010. Population changes in Natterer's bat *Myotis nattereri* and Daubenton's bat *M. daubentonii* in winter roosts of central Poland. Polish Journal of Ecology 58: 769–781.

Geiser, F., R.L. Drury, G. Kortner, C. Turbill, C.R. Pavey & R.M. Brigham 2004. Passive rewarming from torpor in mammals and birds: energetic, ecological and evolutionary implications. In: B.M. Barnes & H.V. Carey (eds). Life in the cold: evolution, mechanisms, adaptation, and application: 45–62. Twelfth International Hibernation Symposium, 25 July to 1 August 2004. Institute of Arctic Biology, University of Alaska Fairbanks, USA.

Gottfried, I., T. Gottfried, G. Lesiński, G. Hebda, M. Ignaczak, G. Wojtaszyn, M. Jurczyszyn, M. Fuszara, E. Fuszara, W. Grzywiński, G. Błachowski, J. Hejduk, R. Jaros & M. Kowalski 2020. Long-term changes in winter abundance of the barbastelle *Barbastella barbastellus* in Poland and the climate change – Are current monitoring schemes still reliable for cryophilic bat species? PLoS One 15 (2): e0227912. https://doi.org/10.1371/journal.pone.0227912

Hays, G.C., J.R. Speakman & P.I. Webb 1992. Why do brown long-eared bats (*Plecotus auritus*) fly in winter? Physiological Zoology 65 (3): 554–567.

Haysom, K.A., J. Dekker, J. Russ, T. van der Meij & A. van Strien 2013. European bat population trends. A prototype biodiversity indicator. EEA Technical Report No. 19/2013. European Environment

Agency, Copenhagen, Denmark.

Hope, P.R., K. Bohmann, M.T.P. Gilbert, M.L. Zepeda-Mendoza, O. Razgour & G. Jones 2014. Second generation sequencing and morphological faecal analysis reveal unexpected foraging behaviour by *Myotis nattereri* (Chiroptera, Vespertilionidae) in winter. Frontiers in Zoology 11 (39): 1–15.

Humphries, M.M., D.W. Thomas & J.R. Speakman 2002. Climate-mediated energetic constraints on the distribution of hibernating mammals. Nature 418: 313–316.

IPCC 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu & B. Zhou (eds)]. Cambridge University Press, Cambridge, UK.

Jones, G., D.S. Jacobs, T.H. Kunz, M.R. Willig & P.A. Racey 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8: 93–115.

Klys, G. & B.W. Wołoszyn 2005. The influence of weather and interior microclimate on the hibernation of Common long-eared bats (*Plecotus auritus*). Opole Scientific Society Nature Journal 38: 57–68.

Kokurewicz T. 1995. Increased population of Daubenton's bat (*Myotis daubentonii* Kuhl, 1819) (Chiroptera: Vespertilionidae) in Poland. Myotis 32 (33): 155–161.

KNMI 2021. Royal Netherlands Meteorological Institute. Daggegevens van het weer in Nederland. https://www.knmi.nl/Nederland-nu/klimatologie/daggegevens

Kugelschafter, K. 2009. Fledermauserfassung in vier bayerischen Winterquartieren. Gutachten i. A. des Bayerisches Landes amt für Umwelt, Augsburg, Germany.

La Haye, M., E. Korsten, M. van Oene, J. van Zweden & T. van der Meij 2020. NEM wintertellingen vleermuizen. Telganger (2): 20–24.

La Haye & T. van der Meij 2022. Hibernating bats in the Netherlands in 1986-2020, based on the National Monitoring Scheme of Bat Hibernacula. Lutra 65 (1): 7-21.

Limpens, H.J.G.A., K. Mostert & W. Bongers 1997. Atlas van de Nederlandse vleermuizen. Onderzoek naar verspreiding en ecologie. KNNV Uitgeverij, Utrecht, the Netherlands.

Lučan, R.K., V. Hanák & I. Horáček 2009. Long-term re-use of tree roosts by European forest bats. Forest Ecology and Management 258 (7): 1301–1306.

Martínková, N., S.J.E. Baird, V. Káňa & J. Zima 2020. Bat population recoveries give insight into clustering strategies during hibernation. Frontiers in Ecology 17 (26): 1–11.

Pannekoek, J. & A. van Strien 2005. TRIM 3 Manual (TRends and Indices for Monitoring data). CBS Statistics Netherlands, Voorburg, the Netherlands.

Park, K.J., G. Jones & R.D. Ransome 2000. Torpor, arousal and activity of hibernating greater horseshoe bats (*Rhinolophus ferrumequinum*). Functional Ecology 14: 580–588.

Pryde, M.A., C.F.J. O'Donnell & R.J. Barker 2005. Factors influencing survival and long-term population viability of New Zealand long-tailed bats (*Chalinolobus tuberculatus*): implications for conservation. Biological Conservation 126: 175–185.

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Racey, P.A. & A.C. Entwistle 2000. Life-history and reproductive strategies of bats. In: E.G. Crichton & P.H. Krutzsch (eds). Reproductive biology of bats: 363– 414. Academic Press, London, UK.

Ransome, R.D. 1971. The effect of ambient temperature on the arousal frequency of the greater horseshoe bat, *Rhinolophus ferrumequinum*, during hibernation, in relation to environmental factors. Journal of Zoology 164 (3): 353–371.

Ransome, R.D. 1990. The natural history of hibernating bats. Christopher Helm, London, UK.

Rebelo, H., P. Tarroso & G. Jones 2010. Predicted impact of climate change on European bats in relation to their biogeographic patterns. Global Change Biology 16: 561–576.

Schmitz, H.A.J.M. 2010. De landschappen van Limburg. In: C.E. Huizenga, R.W. Akkermans, J.C. Buys, J. van der Coelen, H. Morelissen & L.S.G.M.

Verheggen. Zoogdieren van Limburg, verspreiding en ecologie in de periode 1980–2007: 16–31. Stichting Natuurpublicaties Limburg, Maastricht, the Netherlands

Sherwin, H.A., W.I. Montgomery & M.G. Lundy 2012. The impact and implications of climate change for bats. Mammal Review. https://doi.org/10.1111/j.1365-2907.2012.00214.x

Siivonen, Y. & T. Wermundsen 2008. Characteristics of winter roosts of bat species in southern Finland. Mammalia 72: 50–56.

Speakman, J.R., P.I. Webb & P.A. Racey 1991. Effects of disturbance on the energy expenditure of hibernating bats. Journal of Applied Ecology 28 (3): 1087–1104.

ter Braak, C.J.F. & P. Šmilauer 2018. Canoco reference manual and user's guide: software for ordination, version 5.10. Microcomputer Power, Ithaca, USA.

Uhrin, M., P. Benda, J. Obuch & P. Urban 2010. Changes in abundance of hibernating bats in central Slovakia (1992–2009). Biologia 65 (2): 349–361.

van der Meij, T., A.J. van Strien, K.A. Haysom, J. Dekker, J. Russ, K. Biala, Z. Bihari, E. Jansen, S. Langton, A. Kurali, H. Limpens, A. Meschede, G. Peterson, P. Presetnik, J. Pruger, G. Reiter, L. Rodrigues, W. Schorcht, M. Uhrin & V. Vintulis 2015. Return of the bats? A prototype indicator of trends in European bat populations in underground hibernacula. Mammalian Biology 80: 170–177.

Verboom, B. 2006. Winterverblijven voor vleermuizen in Limburg. Rapport 2006.033. Zoogdiervereniging VZZ, Arnhem, the Netherlands.

van Zuijlen & D. Groenendijk 2022. Effects on hibernating bats of ambient temperatures and the characteristics of winter roosts in a dune area. Lutra 65 (1): 153-161.

Verhees, J.J.F., P.H. van Hoof, J.C. Buys & H.W.G. Heijligers 2019. Kasteel Huys ter Horst als winterverblijfplaats voor vleermuizen. Aantalsontwikkeling van soorten en veranderingen van de kasteelruïne gedurende de periode 1986-2019. Natuurhistorisch Maandblad 108 (12): 357–366.

Weinreich, J.A. & J.H. Oude Voshaar 1992. Population trends of bats hibernating in marl caves in the Netherlands (1943–1987). Myotis 30: 75–84.

Weinreich, J.A. & L.S.G.M. Verheggen 2022. The monitoring of hibernating bats in marl quarries in the period 1979–2020. Lutra 65 (1): 23-45.

Received: 8 January 2022
Accepted: 29 March 2022