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# Bats and Caves: Activity and Ecology of Bats Wintering in Caves

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Additional information is available at the end of the chapter

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## Abstract

Temperate regions of the world undergo a marked range of seasonal variation, most becoming extremely cold during the winter. Bats are the only group of vertebrates that have successfully exploited caves as permanent shelter. Although bats may use caves throughout all year, their most important role in ecology of temperate bats is as hibernacula. Here, we summarize various aspects of bat hibernation ecology, including variation in flight activity at the cave entrance; patterns of bat hibernation behaviour; site selection in hibernacula, including the importance of temperature during hibernation; and level of bat movement activity inside the cave. In addition, we review present knowledge on white-nose syndrome, one of the most important threats to cave-dwelling bats.

**Keywords:** Chiroptera, hibernation, flight activity, seasonal use, white-nose syndrome

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## 1. Introduction

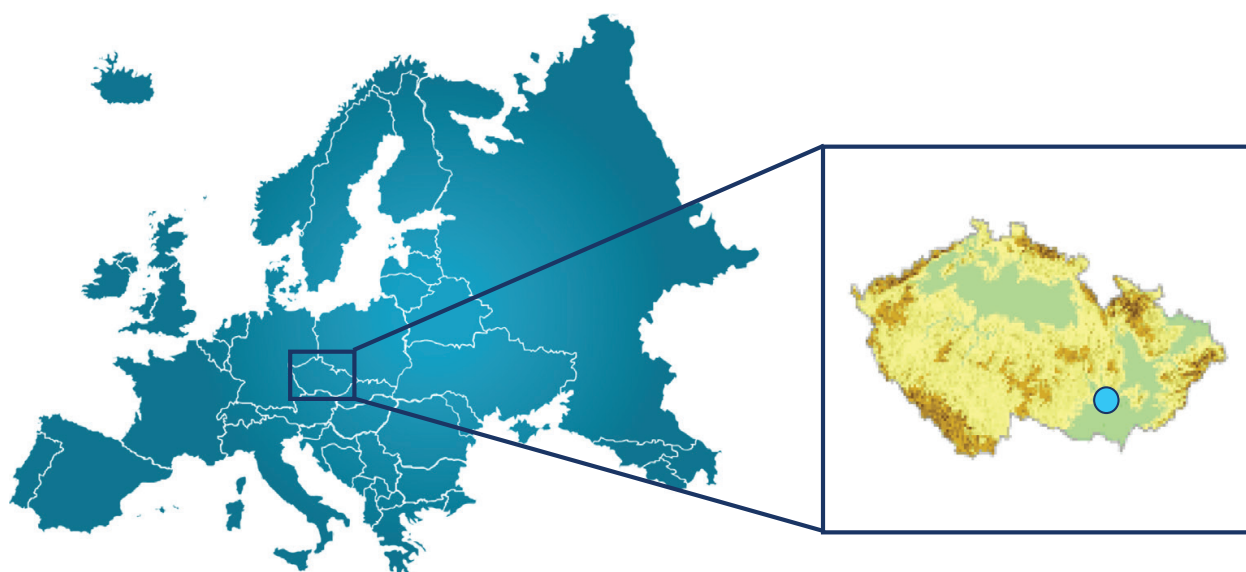
When one thinks about caves, the first image that comes to mind is that of a dark place full of stalactites and stalagmites, with lots of bats hanging on the walls. Bats are mysterious and scary creatures for most people but extremely interesting and enigmatic animals for zoologists. Not only their night activity, longevity, underground roosting, and active flight make them a fascinating species to study but the actual methods used to study them are also of interest [1]. Up to the 1990s, almost all bat research was closely associated with their roosts [2]; animals being captured at the roost entrances, measured, and marked. As bats have high roost fidelity, they can be here caught and recorded repeatedly [3]. Recent developments in

ultrasound detectors and miniaturized telemetry, however, have significantly expanded the range of possible bat research topics to include subjects as time of foraging activity [1].

While microchiropteran bats are able to use a wide range of natural and mad-made structures as roosts, roost availability and presence of an abundant food supply are often the main limiting factors for bats, particularly in temperate zone. Roost availability can influence species distribution, foraging behaviour, social and mating behaviours, population size, diversity, and even bat morphology or physiology [4]. While providing many benefits (e.g. protection against bad weather and predators, effective thermoregulation, higher probability of mating and rearing young, lower foraging costs or information transfer), roosts also represent a major evolutionary pressure regarding the survival and reproductive success of each individual bats.

Bats spend a significant proportion of their life hidden in roosts, though their requirements may differ through the year or even at different times of the day. As such, the diversity of bat roosts is very high, ranging from short-term ephemeral to long-term permanent sites. Almost half of the approximately 1200 species of living bats, including all European bats, use permanent roost sites such as buildings, caves, mines, tunnels, tree hollows, or rock crevices [5]. Caves and similar underground spaces offer temperate bats long-term roost sites with specific microclimatic conditions that fulfil two crucial factors: a relatively stable above-freezing temperature (close to the mean annual surface temperature for the area) and high humidity [6].

In this review, we focus on the ecology of temperate zone bats roosting in caves of the Moravian Karst, Czech Republic (**Figure 1**), habitats that supply many of the bats' needs and that can be used year-round. In doing so we summarize the results of our research on various aspects of bat ecology over winter, including variation in flight activity at the cave entrance, factors affecting site selection within hibernacula, and level of bat movement activity inside the cave. In addition, we summarize present knowledge on white-nose syndrome (WNS). The



**Figure 1.** Map of Europe with the location of the Czech Republic indicated. The circle within the inset map indicates the location of the Moravian Karst.

study of these factors, along with a general understanding of bat hibernation, are essential prerequisites to understanding the impact of disturbance on hibernating bat populations and for providing focus to future conservation efforts [7].

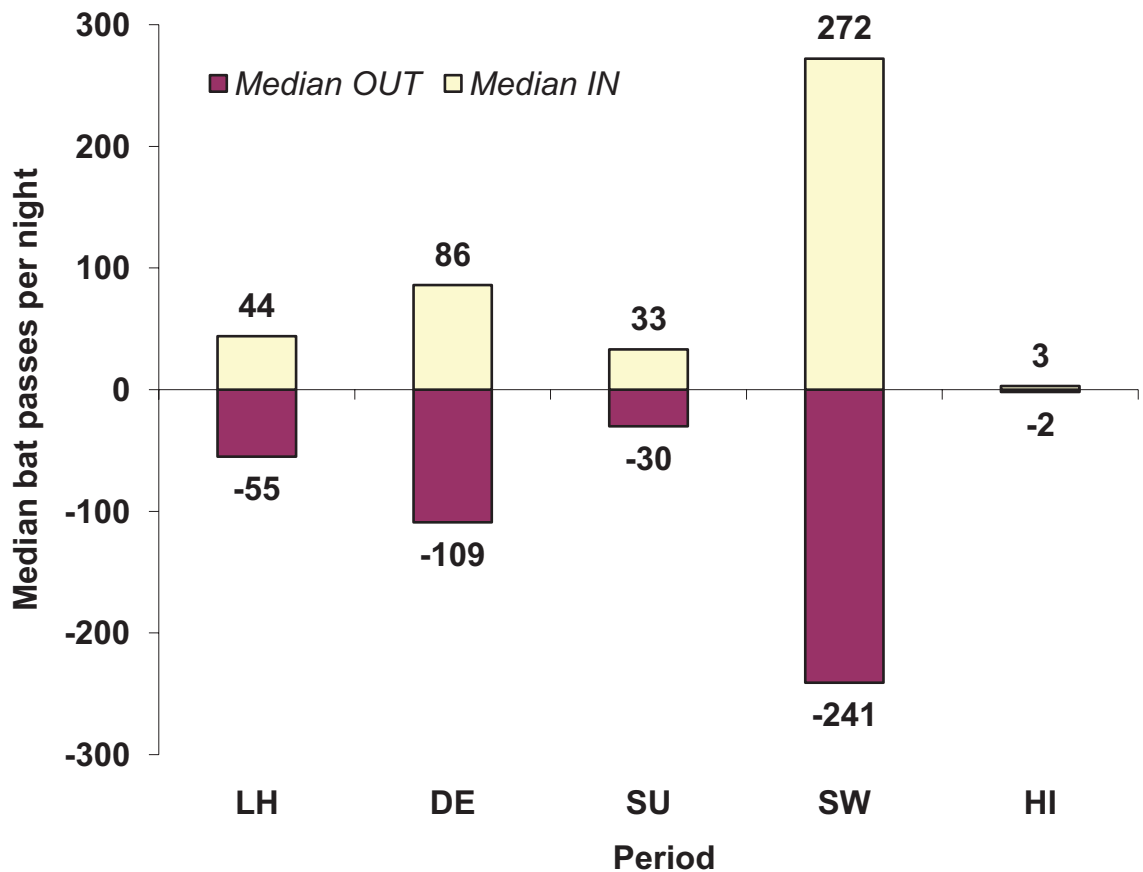
## 2. Variability in cave use by bats (flight activity at the cave entrance)

The ecology and behaviour of temperate zone bats are fundamentally affected by seasonal changes in day length and other associated climatic variables [8], the effect of which become more pronounced at increasing latitudes. In order to remain nocturnal, therefore, bats must display behavioural flexibility in circadian and circannual activity patterns. We have been investigating nightly and seasonal changes in bat flight activity at the entrance of a natural karstic cave (Kateřinská cave, Czech Republic), an important hibernaculum monitored for hibernating bats since 1970 [9, 10]. Activity was recorded using a double infrared-light (IR) automatic logging system that allows discrimination between bats leaving the cave and those entering. Recently, automatic loggers capable of collecting large quantities of data over long periods are increasingly being used to monitor activity at European hibernacula, e.g. in the Netherlands, Denmark and Germany (e.g. [11–13]). The use of such IR automatic loggers has been shown to provide a reliable index of activity levels [14, 15] and, unlike netting, they have the advantage of not disturbing or interfering with the bats' normal activity. Their main drawback, however, is that they are unable to distinguish between individual bats or bat species [16, 14]. If the study is focused on the activity of the bat assemblage as whole, however, this is a minor problem. Connection of an IR logging system to a camera can help in later species identification, though the use of flashlight will affect natural bat behaviour. Note, however, that some authors (e.g. [15]) state that species identification using this method can be unreliable. Ultrasound bat detectors can also be connected to IR logging systems and these have been used to monitor activity of a single species (e.g. the lesser horseshoe bat *Rhinolophus hipposideros* [17], the greater horseshoe bat *Rhinolophus ferrumequinum* [18]) or overall activity of all species in a locality (e.g. [14, 15, 19]). Unfortunately, this method is not very reliable at distinguishing echolocation calls of individual *Myotis* species [20].

The level of bat activity (at the cave entrance) varies seasonally and five periods have been defined (**Figure 2**), all showing a non-random temporal distribution with flight activity concentrated around a specific time [21]. In each case, activity level is influenced by a range of climatic factors, the effect and contribution of which change nightly and over the year [22].

(1) *Hibernation period (mid-November–early March)*. Bats show very low or almost no activity and departures from the cave are very rare. Interruption of lethargy in these bats is most often caused by (i) changes in ambient conditions outside cave, (ii) changes in the physiological state of the hibernating bat (e.g. dehydration), or (iii) direct disturbance [23, 24]. During hibernation, average temperature and daily temperature range (i.e. the difference between daily maximum and minimum temperatures) are key factors predicting the general level of flight activity [16, 18, 22]. As temperature increases, so the percentage of nights with bat activity also increases. Similarly, an increase in temperature fluctuation during the day will also

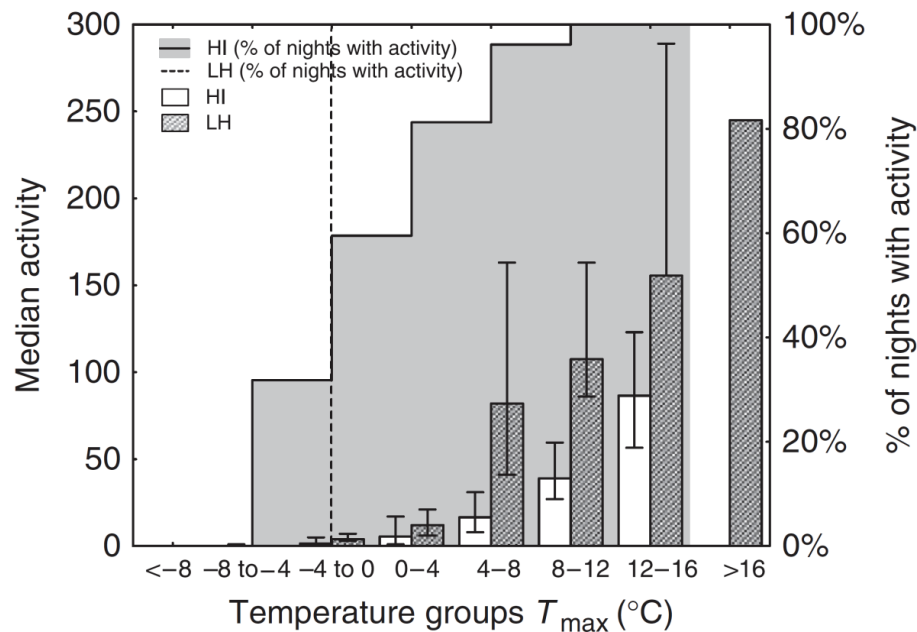




**Figure 2.** Out-flight (negative values) and in-flight (positive values) medians for each defined period as monitored by double IR-light barrier between March 2000 and November 2002. Explanation: HI, hibernation period (mid-November–beginning of March); LH, late hibernation (March–mid-April); DE, departure (and transition) period (mid-April until beginning of June); SU, summer period (mid-June–end of July); and SW, swarming period (late July–mid-November).

result in bat arousals and increased flight activity. Note, however, that bat activity at the cave entrance has been recorded at temperatures as low as  $-13.2^{\circ}\text{C}$  (cf. [25]). Daily recordings were positive at maximum daily temperature exceeding  $6.2^{\circ}\text{C}$ , when some bat species are able to forage [16]. The activity within defined temperature groups [22] was significantly lower during deep hibernation period than during late hibernation (**Figure 3**). Opinions on the level of activity desynchronization at sunset and loss of nocturnality during hibernation differ and the results of research are inconsistent, some supporting desynchronization and others not (e.g. [18, 26–28]). Our own data [22] clearly indicate that activity at the cave entrance is synchronized with sunset, even in winter, and that a concentration of activity occurs between 3 and 3.5 h after sunset. No change in activity patterns has been recorded following the emergence of white-nose syndrome (WNS) in Europe, suggesting that the hibernation behaviour model described, including changes in activity, could represent a behavioural adaptation that has prevented fatal impact of the disease observed in North America [29].

(2) *Late hibernation period (March–mid-April)*, with intensive departure activity during the first quarter of the night. Movement activity inside the cave is relatively high and the bats are



**Figure 3.** Activity levels (median values  $\pm$  interquartile range) in individual temperature group during hibernation (15 November–4 March) and late hibernation (5 March–14 April). The percentage of nights on which activity occurred in individual temperature groups is indicated by the grey area for the hibernation period and the dashed line denotes late hibernation. Source: [22].

probably already preparing themselves for departure from the hibernaculum [30]. Flight activity is positively affected by average daily temperature, and negatively so by minimum temperature during the preceding day. Bats react very quickly to temperature changes from day to day, with activity decreasing or increasing if temperatures drop or rise by more than 2°C. Such rapid changes in activity level become feasible as the bats move towards the hibernaculum entrance, enabling them to register fluctuations in ambient temperature [19, 30] and, as a consequence, potential changes in insect abundance. Bats are capable of foraging at very low temperatures, e.g. Daubenton's bat *Myotis daubentonii* at temperatures as low as -3.3°C [31]. In some species, the activity increases during late hibernation period, presumably, as food availability is already higher and foraging effectively compensates for any energy loss [32].

(3) *Spring migration (mid-April–early June)*, a period of relatively high activity. At this time, the cave may serve as a transitional roost during the spring migration and, from around May, as a temporary roost for males as females already start to form summer colonies. Emergence activity in the first quarter of the night is high, and a small number of bats may re-enter the cave in the last part of the night. Average daily temperature and average daily atmospheric pressure at this time has a significant positive influence on overall flight activity. The degree of variability in activity explained by such climatic factors is the lowest during this period, however, suggesting that either temperature is no longer a limiting factor, or that endogenous rhythms have a strong influence on departure from the hibernaculum [11, 22]. However, the use of underground roosts including caves in the spring may be species specific; it may differ by region, and can also depend on roost structure [6, 18, 33].

(4) *Summer period (mid-June–end of July)*. During this period, the cave is used only sporadically (**Figure 2**), though the bats visiting the roost stay the whole night, i.e. they enter before mid-night and leave after midnight. This type of activity suggests that, during this period, the cave may be being used as a night roost between peaks in foraging activity or as a transitional day roost [11, 18]. At this time, the cave entrance is visited almost exclusively by males [34, 35] as adult females occupy maternity roosts during lactation and return to these between foraging bouts, night roosts being used sporadically and for brief periods [36, 37].

Flight activity at the night roost entrance is influenced by fluctuation in ambient temperature, rather than any absolute temperature threshold, the higher the difference between maximum and minimum daily temperature, the higher the activity level. This corresponds with a model proposing that activity changes in temperate insectivorous bats reflect changes in insect activity [8], i.e. if day-insect abundance is high due to warmer nights, bat foraging activity may continue overnight with no visits registered at the cave entrance (low activity). On the other hand, when nights are cooler and the daily temperatures range is higher, bats will tend to spend more time in the night roost. Foraging activity is highest at dusk and just before dawn, after which the bats return to the day roost [36]. This model is also supported by the influence of rainfall, with flight activity at the cave entrance increasing as rainfall increases whether the nights are warm or cold.

(5) *Autumn migration or swarming period (late July–mid-November)*. This period is typified by very high general activity and an increasing number of bats entering the cave. With the break-up of the summer breeding colonies, activity at the cave entrance gradually increases as adult females and juveniles arrive [9, 38], often in small groups of 2–12. The majority of bats does not roost in the cave and probably arrive after the first foraging period; hence, peak activity tends to occur around midnight. Activity around the cave entrances in autumn probably enables juveniles to recognize potential hibernacula and to meet individuals of the opposite sex, which live separately during summer (e.g. [15]). Activity level is positively related to average daily temperature, atmospheric pressure and rainfall. Thus, when nights are warm and insect activity is high (high atmospheric pressure), the bats will quickly catch enough prey and will search for the cave entrances (swarming sites) in order to mate or obtain shelter if it be raining [14, 22].

### 3. Caves as hibernacula

Hibernation, an optimal adaptation to a prolonged fall in temperature and reduction in prey availability, is a characteristic of the annual cycle of insectivorous temperate zone bats [16]. Selection of a suitable hibernation site is crucial for overwinter survival and, in temperate zone, caves and mines tend to be the most common hibernacula. Caves can be divided into three basic types based on microclimate and use by bats: (1) warm caves used during the summer, including maternity colonies, (2) relatively cold hibernacula with a stable microclimate and (3) caves used during the autumn swarming [6]. Of course, both warm caves and hibernacula can also be used during the spring and autumn migrations too. At higher



latitudes, cave temperatures are too low and they tend to be used mainly during torpor and hibernation. Note, however, that while thousands of bats can hibernate at such sites, those sites with lower numbers may be very important locally and their overall contribution to bat population great [7].

More than 1200 caves are located in the Moravian and Javoříčský Karst regions of the Czech Republic, many of which host significant and regularly monitored bat hibernacula (**Figure 4**). Three of these cave systems (Javoříčské, Sloupsko-šošůvské and Býčí skála) represent the largest bat hibernacula in the Czech Republic [39], with 17 bat species registered during hibernation, including rare species such as *Rhinolophus ferrumequinum*, the northern bat *Eptesicus nilssonii*, and the pond bat *Myotis dasycneme*. A similarly rich bat fauna has only been found in caves in the Slovak Karst and the Muránská Planina [40], both of which are also located along the northern distribution border of some bat species (e.g. Geoffroy's bat *Myotis emarginatus* or the lesser mouse-eared bat *Myotis blythii*).

Both of these karst systems have a long history of bat research, beginning with speleological research of caves made by Dr. Friedrich Anton Kolenati in the second half of nineteenth century [41]. Modern bat research in the region was initiated by Prof. RNDr. Jiří Gaisler in the 1950s and it continues, including our long-term research of bat hibernation, to the present day. As a result, some of these hibernacula have been monitored for almost 50 years [42].



**Figure 4.** Main entrance of Sloupsko-šošůvské cave representing one of the largest bat hibernacula in the Czech Republic. Photo by Leos Stefka.

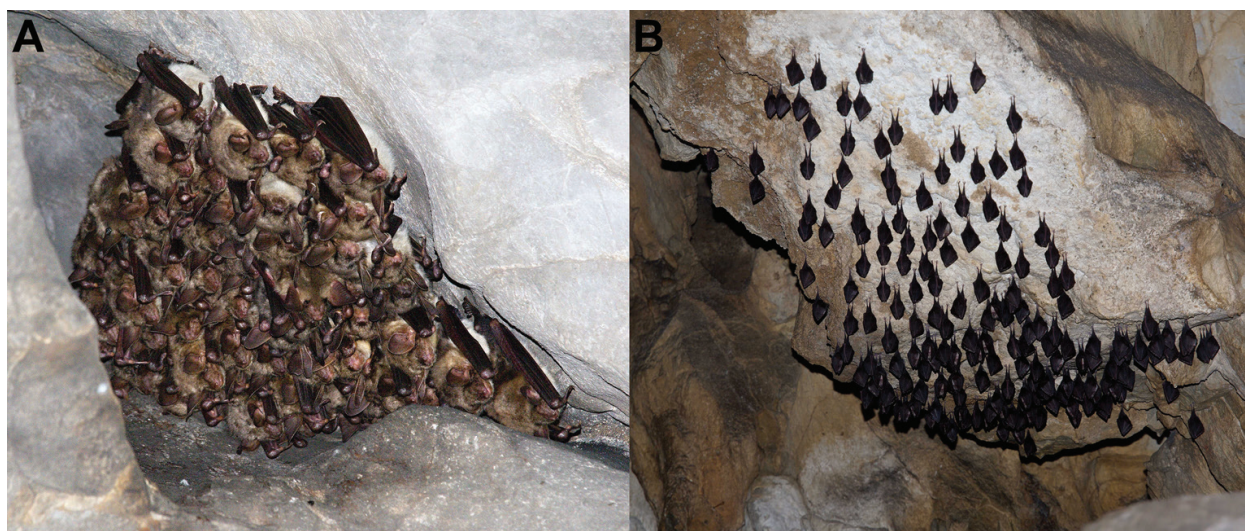


As one of the main requirements of our own research was to avoid any disturbance to hibernating bats, we used visual censuses only (including night censuses using Pathfinder 2000s night-vision scope) with no handling or marking [10, 29]. Thermal profiles were also undertaken to evaluate physiological condition. Fur surface body temperature, which is correlated with core body temperature, was measured using a Raynger MX2 non-contact IR thermometer (Raytek Corporation, USA). Two major model species were regularly monitored in the caves, the greater mouse-eared bat (*Myotis myotis*) and the lesser horseshoe bat (*R. hipposideros*) (Figure 5), these being typical members of the bat community hibernating in the Moravian Karst [9, 10, 43].

### 3.1. Model of bat hibernation in natural caves

In late summer and early autumn, bats undergo a preparation phase for hibernation during which they rapidly accumulate body fat deposits [44] needed for surviving the torpor period. The fat is accumulated by energy savings achieved through increasingly longer daily torpor bouts during the diurnal resting period. Hibernation is usually interrupted by periodic arousals [45, 46], usually related to drinking, feeding (in mild periods) or even mating [23, 35]. As part of the fat deposits must be metabolized for torpid individuals to become physiologically active during winter, such arousals are energetically costly [47, 48].

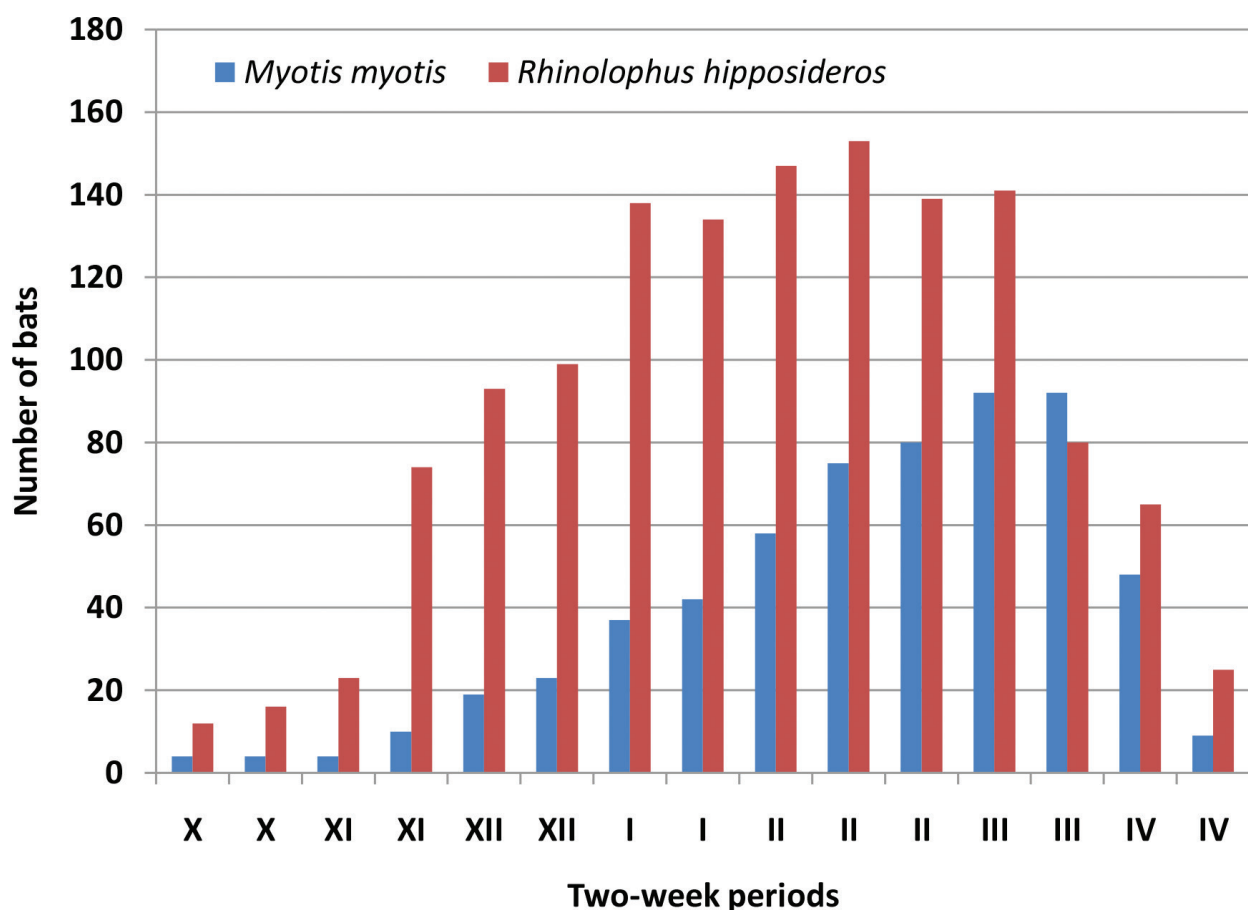
These arousals, and any subsequent activity, will be mirrored in ecological parameters such as community structure, bat population abundance, shelter selection or total movement activity. Monitoring of hibernating bats in the Moravian Karst has confirmed that the ratio of 'visible' bats changes through the winter, i.e. bats may move from inaccessible shelters to places where they can be monitored by investigators [9, 49]. The total number of hibernating bats grows continuously from October, with highest abundances occurring in February or



**Figure 5.** Hibernating clusters of two bat species regularly monitored in the Moravian Karst. (A) The greater mouse-eared bat (*Myotis myotis*) (body length of 6.5–8 cm) and (B) the lesser horseshoe bat (*Rhinolophus hipposideros*) (body length of 3.5–4.5 cm).

March, depending on community structure. Any increase in abundance will be influenced by immigration of newcomers during the pre-hibernation period only (mid-November–mid-December). Switching of hibernation sites during the deep hibernation period (i.e. leaving the hibernaculum) has only been registered exceptionally [21]. In April, there is a gradual but relatively rapid emergence from the hibernation sites (approximately 3 weeks), with bat abundance in cave decreasing to a minimum.

Movement activity of bats inside the hibernaculum, expressed as the percentage of new findings during a visit, is registered throughout the winter, with levels fluctuating in our species-specific models. Hibernation activity of *R. hipposideros*, for example, could be divided into three distinct periods reflecting early, deep, and late hibernation; while *M. myotis* movement activity remained relatively high throughout the season [30]. A continuous arrival of bats at the hibernaculum means that *R. hipposideros* abundance increased gradually over the 6–8 weeks leading to mid-December, and decreased again from mid-March as they gradually left (Figure 6). The deep hibernation period was characterized by low movement activity in the cave and minimal changes in abundance, as also confirmed by detection of ultrasound signals [17, 27]. Even in the middle of winter, when the conditions outside were suitable, some awakened *R. hipposideros* became aroused and left the cave, shortly to return again [17, 50].



**Figure 6.** Changes in abundance of two model species during hibernation period 2011/2012. Data presented in 2-week periods.



Abundance of *M. myotis*, on the other hand, increased continuously throughout the winter, eventually dominating the bat community by the end of hibernation period. In comparison, this species leave the hibernacula rapidly, all bats having disappeared over a period of just 3 weeks [49, 51].

Our two model species accounted for more than 80% of all bat observations in the caves. Bat netting at the cave entrances during spring and autumn migrations, however, confirmed a much higher diversity than during hibernation, with other bat species showing a higher dominance. Small species of genus *Myotis*, such as *M. emarginatus*, *M. daubentonii*, Natterer’s bat *Myotis nattereri*, and Bechstein’s bat *Myotis bechsteinii*, are often underestimated during winter monitoring [9] as they tend to use more or less inaccessible roost sites (e.g. deep crevices) [34], depending on the local microclimate, species-specific requirements, season or weather. We found that around 20% of all bats hibernating in natural caves need to be monitored during winter as the cumulative number of bats entering the cave (calculated using a double IR-light logging system) was much higher (Figure 7).

3.2. Shelter selection during hibernation

As roost site characteristics can play an important role in bat thermoregulation, choice of site will undoubtedly influence bat fitness and survival. Ransome [52] classified caves used

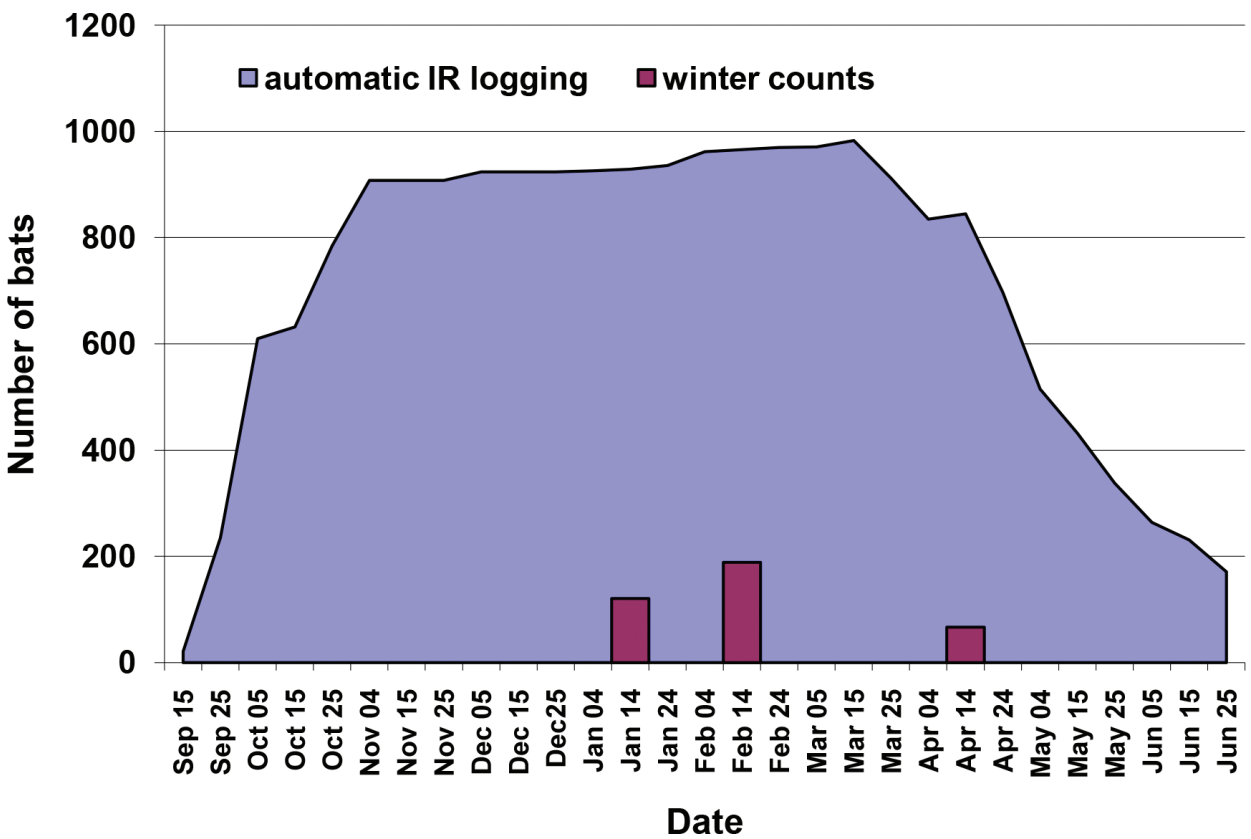
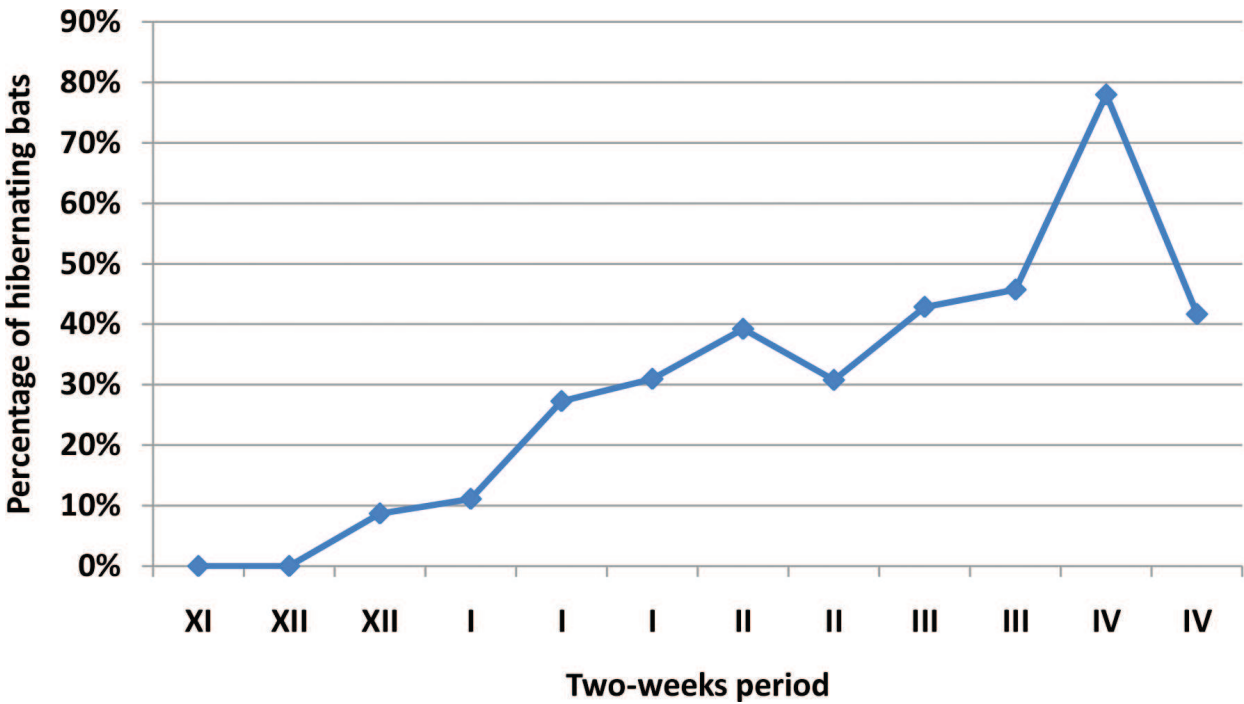


Figure 7. Cumulative number of bats entering the cave (winter season 2000/2001) recorded by the automatic IR-light logging system (area) and the numbers of bats hibernating inside the cave monitored during winter counts (bars).

as hibernation sites into three basic types depending on temperature fluctuation: (1) caves displaying a constant temperature regime, (2) caves with dynamic temperatures and (3) caves with fluctuating low temperatures. Note, however, that numerous factors affect the climate of individual caves; and that each cave will be unique in its geomorphologic and microclimatic parameters [6]. Caves with more or less constant temperatures over the year (averaging between 6 and 10°C) usually have just one entrance and temperature fluctuation tends to occur in the outer entrance parts only due to high air flow. Thermally dynamic caves are characterized by large passages with different temperatures. Such caves tend to have two or more entrances, their mutual positions influencing internal temperature conditions. As any two caves will differ significantly, therefore, it will be difficult to specify an average annual temperature. In general, average annual temperature will be in the range of 3–14°C. Hibernating bat communities sheltering in such caves tend to show the most stable abundances. The third cave type always tends to display fluctuating temperatures, despite usually having just one entrance. During winter, air temperature will decrease significantly due to cold air flowing in from the cave entrance [53, 54].

Survival of hibernating bats will be influenced not only by the selection of a suitable hibernaculum but also by the specific microhabitat conditions within. The correct choice will be crucial for the efficient use of stored energy and for the appropriate timing of flight activity. Indeed, studies have shown that bats are able to regulate length and depth of torpor by selecting favourable sites [46]. During our own monitoring of hibernating bats, we monitored a range of parameters including site type (exposed, semi-exposed and hidden), relative height above the floor and position in the cave [30]. During hibernation, *R. hipposideros*, a thermophilic species, were registered in practically all parts of the caves under study (Kateřinská and Sloupsko-šošůvské), with the exception of the entrances, which have more dynamic microclimates [30, 49]. The bats tended to prefer low shelters (under 3 m from the floor) and always hibernates hanging free in open unprotected sites, regardless of season or hibernaculum type. The sites selected by *R. hipposideros* had stable temperature and humidity conditions with minimal air flow and, as a result, the species showed very low movement activity levels during deep hibernation. The pre- and post-hibernation periods, on the other hand, are typified by high movement activity. Changes in shelter types during winter corresponded to phases in the annual cycle and the physiological status and behaviour of bats only and not to any changes in the environment, there being no temperature fluctuation in the deep cave sections used by *R. hipposideros* [16, 55]. Despite hibernating next to a footpath frequently used by tourists or speleotherapy patients, we failed to register any vulnerability of *R. hipposideros* to human activity.

Euryvalent *M. myotis*, on the other hand, were registered throughout the cave systems during hibernation, using all shelter types indiscriminately (exposed and hidden, ceiling and walls) and showing high seasonal dynamics. During deep hibernation (mid-December–early April), these bats are continuously moving into the outer parts of the cave where they select specific sites for the formation of clusters (**Figure 8**). Over 80% of all *M. myotis* hibernating in the Sloupsko-šošůvské caves, for example, were found in one specific area during late hibernation [49]. A shift towards the cave entrance has also been reported in other European hibernacula [56, 57]. Movement activity of *M. myotis* was relatively high in hibernacula throughout the hibernation period and could not be divided into specific periods [30]. In the absence of



**Figure 8.** Percentage of the greater mouse-eared bats *Myotis myotis* hibernating in the Kateřinská cave entrance during winter 1992/1993. Data presented in 2-week periods.

food, *M. myotis* select sites with a constant temperature for deep hibernation in order to maximize energy savings. On the other hand, the bats most likely shift to sites with more dynamic temperature regimes during the late hibernation period as the changes in ambient temperature help bats synchronize arousals with actual weather conditions. In doing so, aroused bats are able to couple emergence activity with favourable climatic conditions for foraging.

It is apparent that neither all individuals nor all populations have the same model of hibernation [58]. Our studies suggest that *M. myotis*, at least at population level, may not follow the same hibernation model and that a range of hibernation strategies (i.e. level of movement activity, preference for different shelter types) may be used depending on the prevalent different microclimate profile (i.e. dynamic or stable). Populations in different hibernacula will exhibit responses tuned to that environment, while individuals of the same species may vary in the strategy used to survive hibernation. In this way, the bats optimize their depletion of energy reserves and improve their chances of surviving in the winter [59]. High fidelity to particular underground shelters also suggests that the adopted hibernation strategy may limit bats to repeated use of particular hibernacula [60].

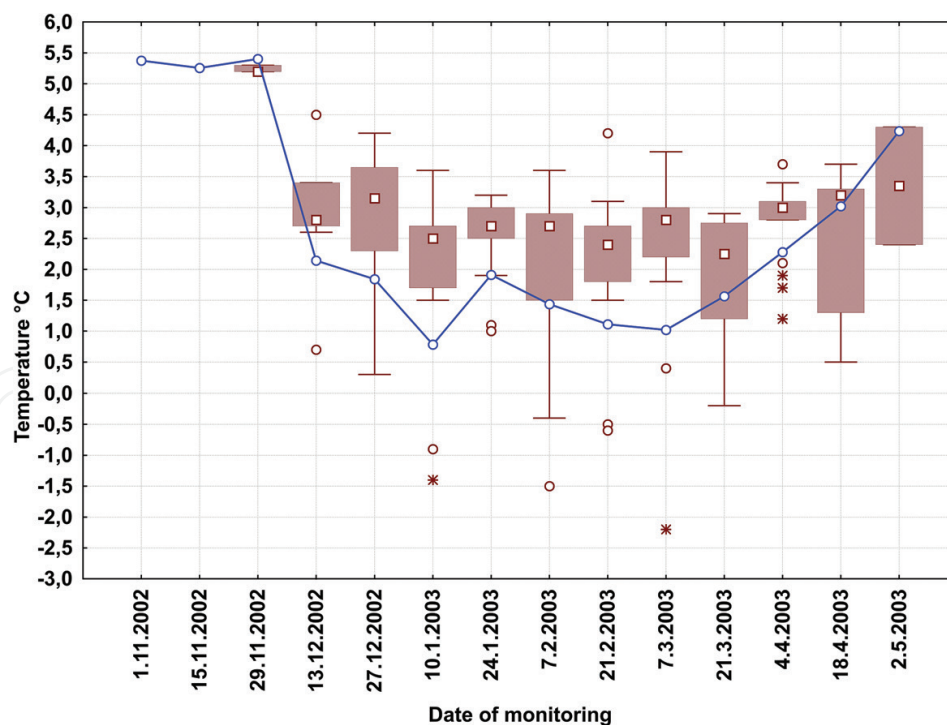
### 3.3. Cave temperature and bat hibernation

The length of time that temperate bats can survive without feeding will be dictated by the temperature, at which they hibernate. In general, by hibernating in caves where temperatures are low but above freezing (i.e. between 2 and 5°C), the bat's metabolism rate is maintained at an efficient level. While the actual temperatures at which different bats hibernate is species

specific [61, 62], the interspecific differences are very small due to the low metabolism and small body mass of temperate bats. Such species-specific differences vary seasonally, being somewhat smaller during deep hibernation and greater during the pre- and late hibernation periods. Bats also display intraspecific variations in preferred temperature, as individuals will select locations based on their energy reserves [63].

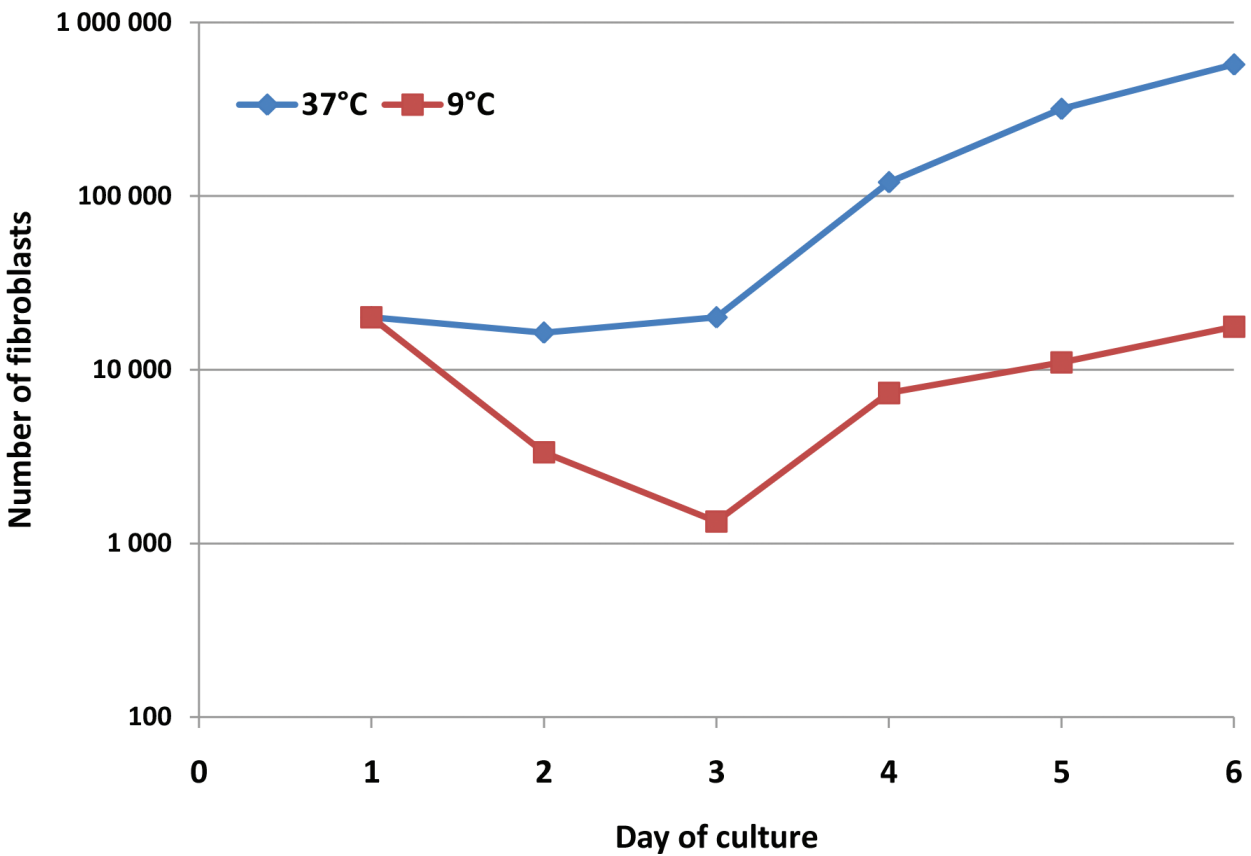
Bat arousal may occur as a result of temperature changes in hibernacula, following which the bats may move to a more suitable location [64]. In general, bats prefer to start hibernation at sites with higher temperatures as those with low temperatures may reach freezing point over the coldest months. An optimizing strategy of such type has been observed in *M. myotis* in natural karstic caves (**Figure 9**). As bats often return to the same sites year-after-year, this could suggest the use of prior experience, learning from others, and/or olfactory clues in microhabitat selection. Arousals are also temperature dependent, with the length and frequency of bat arousals increasing with temperature increases over 10°C [46].

During hibernation, bat body temperature falls to within 1–2°C of ambient temperature and metabolic processes slowdown, thereby reducing energy requirements. As a result, hibernation incurs physiological costs, including the build-up of metabolic wastes, dehydration, reduced motor function, altered immune response, and sleep deprivation [65]. Hibernation may also impose ecological costs such as decreased detection and response to predators [66] and an increased likelihood of freezing [67]. At the cellular level, cold stress changes cellular membrane lipid composition and suppresses the rate at which protein synthesis and cell proliferation takes place [68]. We examined



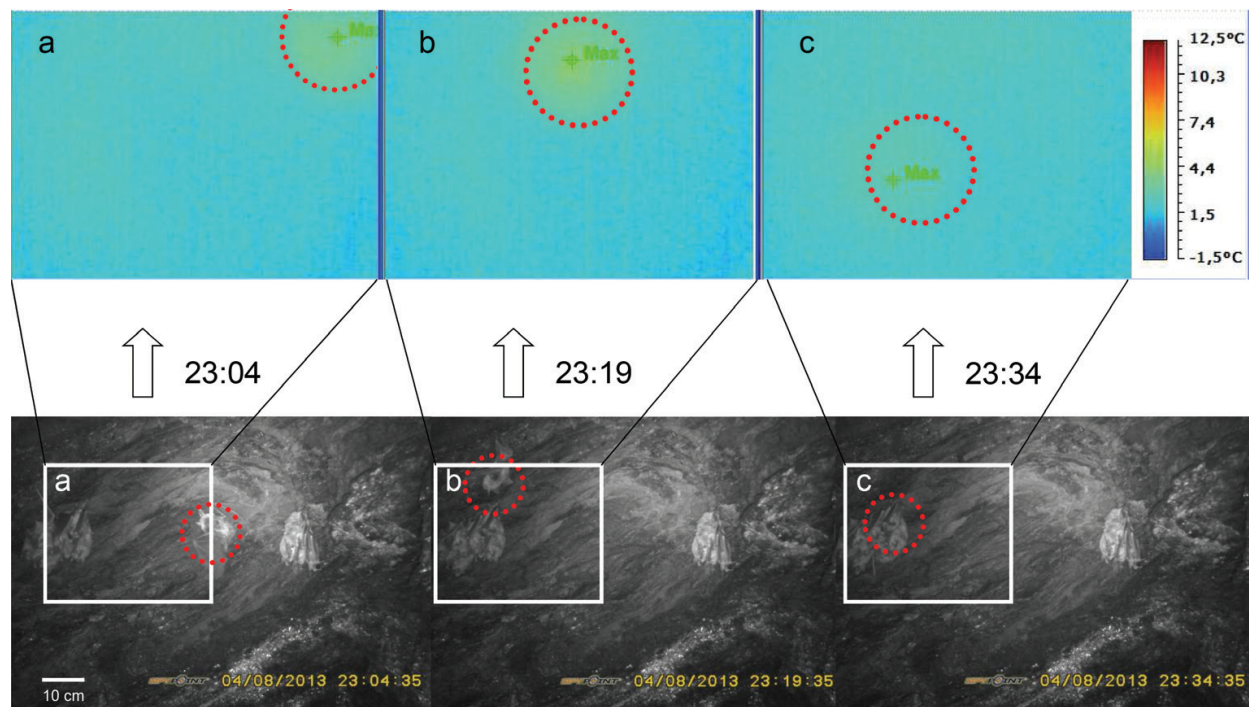
**Figure 9.** Changes in greater mouse-eared bat *Myotis myotis* body temperature and shelter temperature during the winter of 2002/2003. Explanations: box = interquartile range; middle point = median; whiskers = non-outlier range; circles = outliers; stars = extremes; continuous line = average shelter temperature.

the ability of primary skin fibroblast cells from the flying membrane of a hibernating *M. myotis* to proliferate under torpor and euthermia. After loosening the tissue mechanically (without proteases), the cells were identified as fibroblasts based on their spindle shape, positive staining for the vimentin mesenchymal marker, and the presence of typical stress-fibre organization in the actin cytoskeleton. Cell numbers for the assay started with 20,000 cells per well and these were incubated at 9 or 37° C for 6 days in a 5% CO<sub>2</sub> humidified environment for the experiment. Cells were detached from the cultivation wells and recalculated daily with 30 times repetition. While bat fibroblasts cultured at 37°C were elongated reached high numbers in 6 days, and attached successfully to the well substrate; those cultured at 9°C were spherical, reduced in number and took time to attach (**Figure 10**). Extrapolation from this cellular *in vitro* model suggests that bat fibroblasts have some proliferative capacity at the temperature conditions prevalent during torpor, though wound healing capacity would be much slower than in euthermic animals. Such a physiological response of bat cells may help explain the movements registered in *M. myotis* at low fur temperatures ( $T_{\text{flow}} < 5^{\circ}\text{C}$ ) [69], which would allow bats to save energy long-term and prolong torpor bouts. All  $T_{\text{flow}}$  events were recorded during late hibernation, when bats are faced with an acute shortage of energetic reserves and enormous metabolic requirements. In most cases,  $T_{\text{flow}}$  events were represented by slow displacements between clusters of bats, though departure or arrival to and from clusters was also recorded with no elevation in body temperature (**Figure 11**). Repeat appearances suggest that  $T_{\text{flow}}$  movements may represent a regular part of bat hibernation tactics.



**Figure 10.** Numbers of primary cell (fibroblast) cultured at two different temperatures showing some proliferative capacity at the temperature conditions prevalent during torpor.





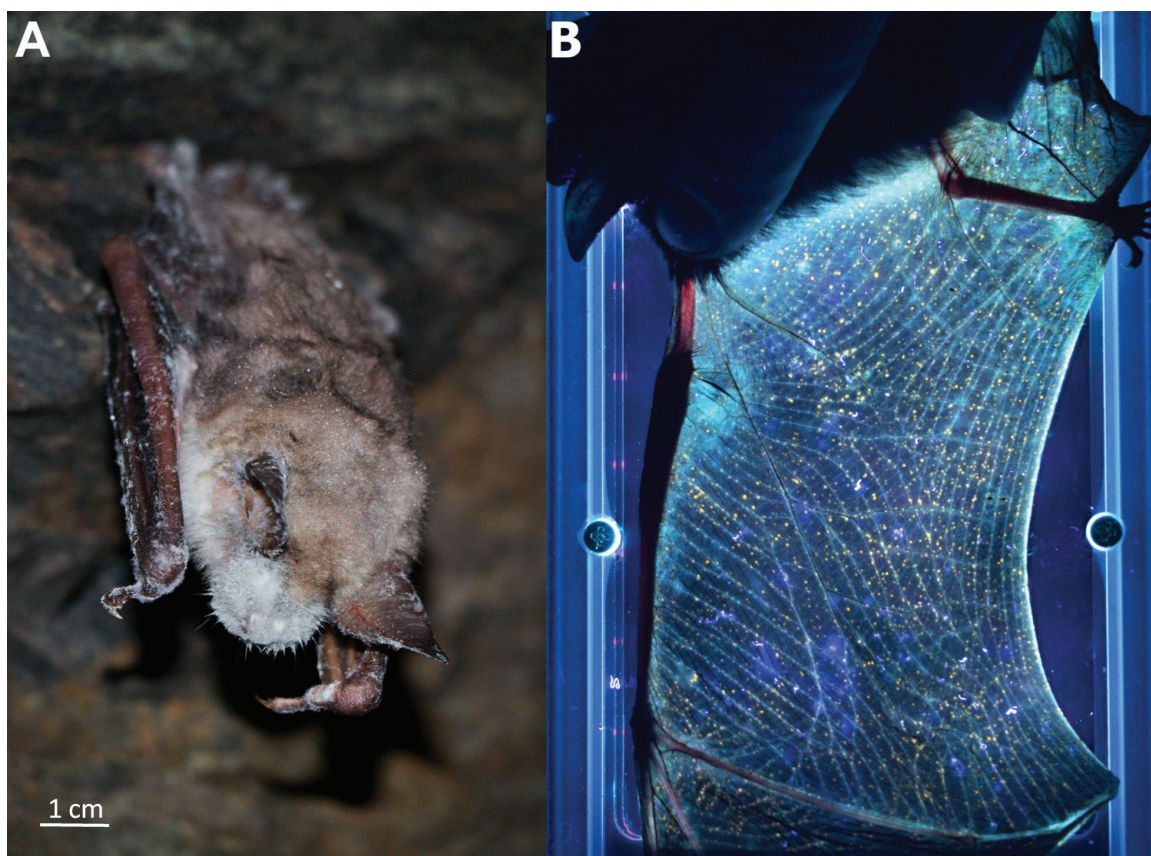
**Figure 11.** Examples of low body temperature movements, showing the bat moving between clusters from left to right. The upper thermal images correspond with lower images from photo-traps recorded simultaneously (a–c). The rectangles in the lower photo-trap images indicate the position of the thermal image, while the dotted circles indicate the moving bat. Source: [69].

#### 4. White-nose syndrome: a threat to bat populations hibernating in caves

Bats are threatened by a range of both natural and anthropogenic stressors, including predation, lack of food, pathogenic agents, climate change, habitat loss, ecological disasters, illegal trade, chemical and light pollution, roosts and hibernacula disturbance, and wind turbine construction. Considering their economic importance to agriculture, the general decline in bat populations documented around the world is of some importance [70]. Recently, a novel threat to insectivorous bat species hibernating in caves and mines has been recognized in North America [71, 72]. White-nose syndrome (WNS), a fungal infection characterized by fungal growth on the bat's muzzle (**Figure 12A**), has caused a dramatic decline in American bat populations. *Pseudogymnoascus destructans*, the causative agent, is a psychrophilic fungus [73] that thrives at the body temperatures displayed by bats in torpor [74].

Despite intensive research, the origin of the pathogenic agent associated with this disease remains unknown and it is still not known why the disease appeared so suddenly [75]. The disease was first registered as a point-source epidemic at Howe's Cave, Albany, New York, in 2006 [71], since when it has spread westward at approx. 200–900 km annually [76]. Based on the 'novel pathogen hypothesis', Europe was initially thought to be the source of the agent which the following findings tending to suggest that WNS did indeed originate in Europe: (1) a single *P. destructans* genotype was identified in North American hibernating bats [77]; (2) the WNS fungal agent was also found in bats in European bats [78]; (3) no mass mortality events

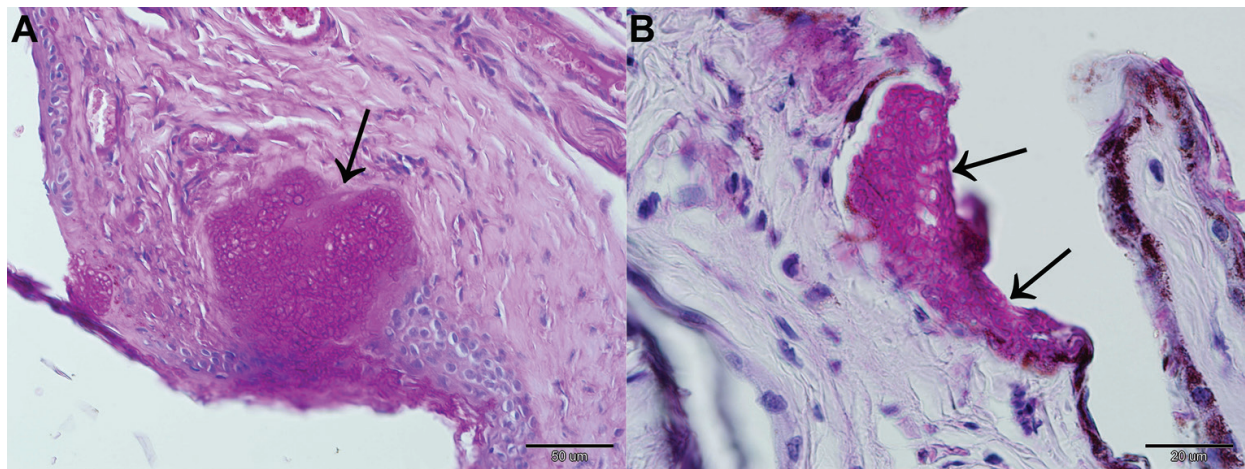




**Figure 12.** Skin infection with white-nose syndrome fungus. Greater mouse-eared bat (*Myotis myotis*) showing fungal growth on the muzzle, ears and forearm photographed in April 2016 (A). Transillumination of the wing membrane by ultraviolet light: a technique to visualize and detect wing membrane lesions based on yellow fluorescence of riboflavin produced by the white-nose syndrome agent *Pseudogymnoascus destructans* in the skin of affected bats (B). The scale is the same for both pictures. Photos by Jiri Pikula.

were reported in European hibernating bats harbouring the causative agent [79–81]; (4) inoculation with European fungus isolates induced WNS in the North American the little brown bat *Myotis lucifugus* [82]; and (5) European *P. destructans* isolates exhibited higher genetic diversity [83, 84]. However, recent findings of both the causative agent and WNS infection in Asia have refuted most of the hypothesis arguments and pushed the search for the pathogen source to non-European hibernacula [85, 86]. Likewise, the detection of bat WNS in western North America in March of 2016 does not fit the previously documented pattern of *P. destructans* geographic spread [76].

Diagnosis of WNS is based on identification of the fungal agent growing on bats using cultivation, morphological characteristics (e.g. crescent shaped conidia), and molecular assays [73, 87]. One of the most useful diagnostic methods is wing membrane transillumination with ultraviolet (UV) light, which reveals fluorescent lesions produced by the infection [88]. The method is non-lethal, can be used under field conditions and, in combination with photography, can be used to estimate infection intensity (**Figure 12B**) as it is highly sensitive and specific for WNS. Histopathology findings of typical cupping erosions (**Figure 13**) are the gold standard of WNS diagnosis [89, 90].



**Figure 13.** Microscopic appearance of white-nose syndrome infection. Thick-type skin of the muzzle extensively infected with *Pseudogymnoascus destructans* fungus (black arrow). Greater mouse-eared bat (*Myotis myotis*) sampled in a hibernaculum (Czech Republic) in May 2016 (A). Thin-type skin of the wing membrane with a white-nose syndrome pathognomonic cupping erosion that contains densely packed fungal hyphae (black arrows, B). The same bat specimen as in (A). Both skin sections stained with periodic acid-Schiff stain.

Surprisingly, the WNS fungal infection is restricted to the skin only, with no evidence of systemic fungal invasion in infected bats [71, 89]. Hence, bat mortality is thought to follow complex pathophysiological mechanisms, and a multi-stage WNS model has recently been proposed to explain the disease's progression [91]. Hibernating bats positive for WNS have been reported as displaying abnormal behaviour, higher arousal frequency from torpor, emaciation and fat depletion, dehydration, acidosis and electrolyte disbalance [82, 92–94]. The extent of wing pathology in infected bats appears to be directly related to mortality [95]. In general, Palearctic bats tend to have a lower disease intensity (measured as the percentage of wing membrane area affected by WNS lesions) than Nearctic bat species [96], possibly explaining the intercontinental differences in bat mortality.

Riboflavin or vitamin B2 is the main compound responsible for the distinctive orange-yellow fluorescence observed under UV light (**Figure 12B**) after invasive *P. destructans* growth has replaced living tissues. Pathogenic *P. destructans* strains produce considerably more riboflavin than non-pathogenic *Pseudogymnoascus* spp. strains. Importantly, high riboflavin concentrations accumulated in WNS skin lesions are toxic to cells under conditions typical for bat hibernation and euthermia. As such, riboflavin may act as a key virulence factor for WNS [96].

As *P. destructans* is a generalist pathogen, all bat species hibernating within contaminated caves may be at risk of infection [97]. However, adverse population-level effects depend on the species and appear to differ considerably between North America and Eurasia. Hibernating Palearctic bats appear to have evolved infection tolerance mechanisms to cope with the endemicity and extensive spatial distribution of virulent *P. destructans* in the Palearctic region [86]. These mechanisms include behavioural adaptations, such as specific patterns of hibernation and shelter selection [29] that ensure low pathogen impact. While our knowledge of this threat is growing, there are still numerous unanswered questions that require study, at the local and global levels.



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