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Numerous cold arousals and rare arousal cascades as a hibernation strategy in European *Myotis* bats

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ABSTRACT

Hibernating bats optimise the duration of torpor bouts and arousals in relation to hibernaculum microclimatic conditions and fat reserves. Clustering has significant physiological and ecological benefits, promoting successful hibernation of individuals. Such aggregations may help maintain optimal temperatures, allowing better energy utilisation than in solitarily bats. However, aroused bats in a cluster could conceivably disturb those still hibernating, starting an energy-demanding arousal process. Our study was conducted over two winters in two different hibernacula (cave and mine) in the Czech Republic, where Greater mouse-eared bats (*Myotis myotis*) have previously been diagnosed with white-nose syndrome. In 118 arousal episodes we recorded 193 individual arousals in which a warming phase was observed, 135 (69.9%) being cold arousals, where bats ceased increasing their body temperatures at $\leq 10\,^{\circ}$C. The remaining arousals were standard normothermic arousals, where body (fur) surface temperatures reached $> 20\,^{\circ}$C. Cold arousals occurred during the mid- and late hibernation periods, suggesting they were a response to disturbance by a neighbour in the same cluster. Arousal cascades, where bats aroused in series, were rare (12.7%) and reached a maximum in mid-January. Our data suggest that *Myotis* bats prolong their torpor bouts using numerous cold arousals but few arousal cascades. Upon arrival of a bat, the clustered bats show tolerance to disturbing by conspecifics.

1. Introduction

Although hibernating mammals can rewarm from torpor passively as ambient temperature increases (Geiser et al., 2004) many, such as temperate bats, can also change their body temperature from low values during torpor to high normothermic values using endogenous heat production. However, increasing body temperature during arousal above the ambient temperature is energetically costly (Boyles et al., 2006). To manage energy expenditure over the period of hibernation, hibernating mammals optimise roosting site selection and torpor bouts and arousals in relation to both temperature in the hibernaculum and their fat reserves (Geiser and Kenagy, 1988). The selection of roosting site, and hence the temperature at the site, determines the both metabolic rate and energy consumption (Boyles et al., 2007). When fat reserves dwindle, or ambient conditions change, bats may change roost sites during arousal, within or even between hibernacula (Twente 1955; Berkova and Zukal, 2006; Zukal et al., 2016a).

Clustering is a behavioural phenomenon with significant physiological and ecological consequences for successful hibernation. Bats hibernating underground benefit from clustering behaviour during normothermic periods (Boyles et al., 2008), where clusters of just five individuals show reduced energy consumption (Brown, 1999; Boyles and Brack, 2009) and loss of water through evaporation (Thomas and Geiser, 1997, Boratynski et al. 2015). Bats can further decrease their energy expenditure during arousals if individuals in a cluster synchronise arousals in an arousal cascade, thereby sharing the costs via social thermoregulation (Boyles et al., 2008, Boyles and Brack, 2009; Boratynski et al. 2012; Czenze et al., 2013). However, cluster formation can also prove risky to bats already hibernating at the roosting site where the cluster develops. Following the assumption that bats hibernating in clusters conserve energy, individuals should be sensitive to activity in a cluster in order to facilitate arousal cascades; however, bats

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Nomenclature

Ts	Body (fur) surface temperature
Tmax	Maximum bat Ts during an arousal (°C)
ΔTs	Difference between Tmax and Ts for the same individual 24 h before arousal in torpor (°C)
Duration	Time between first and last image in an arousal (in minutes)
Days	Rank of days since the beginning of hibernation (November 1st)
max.cluster	Maximum size of cluster recorded during an arousal episode
active.n	Total number of bats showing ΔTs during an arousal episode
frequency	Percentage of active bats in a cluster during an arousal episode
max active.f	Maximum ratio of the number of bats with alleviated temperatures and the number of bats present in the specific image during an arousal episode
arrive	Total number of bats joining a cluster during an arousal episode
depart	Total number of bats leaving a cluster during an arousal episode
move	Total number of cases where bats changed position during an arousal episode (see text for details)
total.act	arrive + depart +move

should also be able to avoid disturbance from a neighbour when arousals are not physiologically or energetically beneficial (Czenze and Willis, 2015). Consequently, arousal cascades should be rare during periods of cluster formation, and their frequency should increase during late hibernation when the frequency of arousals increased linearly with ambient temperature (e.g. Johnson et al., 2012).

Bats also alter their behaviour during hibernation in reaction to infection. *Pseudogymnoascus destructans* fungus, known cause of white-nose syndrome, forms lesions on the wing tissue, the lesions being more extensive in the Nearctic than Palearctic (Bandouchova et al., 2015; Zukal et al., 2016b), though the duration of normothermic arousals does not differ between infected and healthy bats (Warnecke et al., 2012; Brownlee-Bouboulis and Reeder, 2013). Strategies that facilitate energy conservation are likely to face selection pressure. One such strategy is social thermoregulation, where individuals that live within groups limit their activity during arousal and reduce the number of individuals in clusters (Wilcox et al., 2014; Bohn et al., 2016). Arousal cascades, synchronised arousals, if they are advantageous, should be very common and represent most of the arousals during hibernation (Czenze and Willis, 2015). During arousal cascade bats may be able to rewarm at the same time and reduce individual costs by sharing heat energy with other group members (Boyles et al., 2008). However, temperature preference is not a fixed phenomenon but depends on many intrinsic and extrinsic factors, including the reduction of individual costs, thermal conditions available in the environment, and cluster size (Boyles et al., 2007; Wojciechowski et al., 2007). Following mass mortalities of WNS-infected bats in the Nearctic, fewer bats were found in hibernacula, which may be adaptation to WNS (Frick et al., 2015), despite of the fact that not all bats react the same. The effective size of the cluster could also be affected by maintaining a body temperature lower than normothermic temperature, which may represent a strategy noted by Jonasson and Willis (2012) as cold arousals, where individuals hibernating in a cluster reduce the negative impact with other conspecifics (Czenze et at. 2013).

Given that bats with WNS in the Nearctic exhibit aberrant behaviour during hibernation, we decided to investigate the behaviour of bat species tolerant to WNS (Zukal et al., 2016b). The greater mouse-eared bat (*Myotis myotis*, body mass 20–35 g) is a common species in European underground hibernacula (Řehák et al., 1994) and prevalence of WNS in the species is one of the highest in the Palearctic (Zukal et al., 2014). Despite their infection tolerance, however, an increased *P. destructans* infection intensity of over 300 lesions disrupt blood homeiostasis (Bandouchova et al., 2018). *Myotis myotis* often hibernate in clusters, though these tend to be contain fewer individuals than in some North American species (Frick et al., 2015). Considering the relative lack of knowledge on hibernation behaviour under pathogen pressure, we investigated 1) the pattern of arousals in hibernating WNS-tolerant bats, 2) the frequency of arousal synchronisation concerning the "arousal hypothesis" of Boyles et al. (2008), which suggests that clustering minimises heat loss, and 3) whether synchronised rewarming and number of arousals increase late during the season of hibernation, when fat reserves are more likely to be depleted.

2. Material and methods

2.1. Study sites

This study was conducted over the winters of 2012/13 and 2013/14 in the Šimon and Juda Mine near Malá Morávka in the Jeseníky Mountains (50°03′N, 17°18′E; 900 m a.s.l.) and the Kateřinská Cave in the Moravian Karst (49°21′N, 16°48′E, 345 m a.s.l.), Czech Republic. The two hibernacula are among the most important hibernacula for *M. myotis* in the Czech Republic, with approximately 500–700 individuals hibernating in the mine and 50–120 in the cave from mid-November to mid-April.

The Šimon and Juda Mine system (SJM) has two gated entrances and consists of a complicated labyrinth of galleries. Entrance to the Kateřinská Cave (KC) is over 5 m high, with a gated area of 35 × 15 cm. Deeper portions of both hibernacula have quite stable temperatures throughout the year, i.e. about 5 °C in the mine and 8 °C in the cave. Average steady-state Ts during hibernation was 4.1 ± 1.3 °C in the mine and 4.5 ± 1.4 °C in the cave. In both hibernacula, prevalence of *P. destructans* infection is high (ca. 80%; Zukal et al., 2014). About 42% of *M. myotis* hibernate in clusters in SJM and 81% in the KC, in comparison to the median of 19% in the Czech Republic as a whole (Czech Bat Conservation Society, unpublished data). The number of bats forming clusters differs between seasons and localities, with a winter-long median of 5 (2–16) individuals in SJM and 4 (2–36) in KC.

2.2. Video surveillance of hibernating bats

The thermal behaviour of hibernating *M. myotis* clusters was recorded using Guide M8 thermographic cameras (Wuhan Guide Infra-red Co. Ltd., Wuhan, China). Images of bat clusters were recorded from a distance of ca. 1.2 m every five minutes from January to April (for details see Bartonička et al., 2017). We then selected the largest clearly observable cluster for further analysis, technical limitations of camera stability and visibility range preventing data acquisition from some clusters. To control for a) torpid individuals invisible to a thermal camera and b) thermal cameras not capturing the complete behaviour of hibernating bats, we installed HD-10 photo traps (Spypoint, Victoriaville, Canada) in SJM and an IPCorder KNR-1004 Standalone infrared camera (Prague, Czech Republic) in KC parallel to the thermal camera. Cameras and photo traps were synchronised to photograph bats every 10 min in the SJM and every 5 min in the KC. In SJM, batteries and data storage devices were replaced bi-weekly, while the cameras in KC used power from the show cave infrastructure with data being downloaded weekly.

All observations in the hibernacula were performed in compliance with Czech Law No. 114/1992 on Nature and Landscape Protection

(00356/KK/2008/AOPK, 866/JS/2012) and the study was validated under South Moravian Regional Authority Permit JMK 24451/2013. The authors are authorised to handle free-living bats under Certificate of Competency No. CZ01297 (§17, law No 246/1992).

2.3. Extraction of arousals

The time series of images from thermographic cameras were analysed using Guide IRAnalyser software (freeware for Guide cameras, Wuhan Guide Infra-red Co. Ltd.). Arousal episodes covered activity of whole cluster, therefore one arousal episode could include arousals of more than just one bat, for example arousal cascade. Arousal episodes are represented by thermal changes in consecutive images, starting from the first image with a change in body surface temperature (Ts) in one bat until the last image where at least one bat in a cluster exhibited a body surface temperature distinguishable from the ambient temperature. In principle, these arousals represent an inverse of Jonasson and Willis's definition of torpor bouts (Jonasson and Willis, 2012). During both winter periods, all arousals and arousal episodes were recorded with the date, start time, duration, Ts, Tmax and bat activity information (for details see Bartonička et al., 2017). Individuals were identified with help from infrared images and their Tmax at each photographed time point was recorded. Bat activity information included 'arrive', meaning that a bat was absent on a previous image, 'depart', meaning that the bat was absent on a subsequent image, and 'move' meaning that the individual changed position compared to the previous image (cf. Abbreviations).

During each arousal episode, we recorded the maximum number of bats present (max.cluster) and, at each time increment, we recorded the frequency of bats with increased Ts relative to the number of bats present at the time (frequency). For temperature change data, we summarised the time series of images, with the maximum frequency of active bats was used as a change summary statistic (active.f) and the total number of bats exhibiting arousals (active.n). We recorded Tmax of any bat involved in the process and then summed the behavioural activities. We categorised arriving, departing and moving bats, summing the respective cases for the change summary characteristics (arrive, depart, move; see above for details) and recorded total activity as a sum of all cases (total.act). We recorded 118 arousal episodes, where in a warming phase was defined by a rapid increase in Ts of ≥ 5 °C over 20 min. In 37 cases, we were unable to recognise the stabilised Ts of > 25 °C necessary to specify the strict boundary between torpor and normothermia (Jonasson and Willis, 2012) due to bat departure from the cluster.

2.4. Data analysis

We tested both the distribution frequency of differences between arousal times and sunset time (in radians) and whether these times differed from a random distribution using Rayleigh's test (Czenze et al., 2013). Factors of potential influence were assessed using a generalised linear model (GZLM) with a Gaussian distribution and a log link function. GZLM were constructed by fitting explanatory variables, i.e. covariates (days, duration and max.cluster) and random factor (year), that could potentially influence ΔTs.

We assumed that an increase in Ts in the second bat was dependent on the first bat being active during an arousal episode. We developed a predictive classification model to quantify the intuitive selection criteria for an arousal cascade identified manually by the authors, using a support vector machine (SVM) classification model with a radial kernel. The SVM model was tuned with 10-fold cross-validation at a cost parameter range of between 10^{-1} and 10^3 and a γ parameter range of between 0 and 2, with four equidistant values per parameter (exponents in cost). Summary statistics used in the SVM model were selected from exhaustive grid variable selection using logistic regressions compared with the Akaike information criterion. We investigated behavioural patterns in arousals using segmented linear regression.

All analyses were performed using R (R Development Core Team, 2011) with packages *e1071* (Meyer et al., 2015), *segmented* (Muggeo, 2003) and *zoo* (Zeileis and Grotherndieck, 2005).

3. Results

3.1. Arousal episode characteristics

In total, 71 794 thermal images were recorded of four different bat clusters (one per year in both SVM and KC), each with 3–16 individuals. Arousals lasted up to 525 min, with a median of 70 min (Tab. S1). We found no evidence of any relationship between time of arousals and sunset (Rayleigh's test: $Z = 0.61$, $p = 0.364$, mean vector $= 0.27$, $n = 193$).

We recorded individual arousals of 193 bats. In 135 (69.9%) arousals, bats cease lowering their Ts at a steady-state with $\Delta Ts \leq 10\,^{\circ}C$ (cold arousals according to Mayberry et al., 2018). Cold arousal Ts ($\Delta Ts = 5.5 \pm 1.4\,^{\circ}C$) differed significantly from that of normothermic arousals ($\Delta Ts = 18.7 \pm 4.6\,^{\circ}C$; $n = 58$; *t*-test, $Z = 30.21$, $p < 0.001$). Cold arousals occurred throughout the hibernation period (early January to mid-April; Fig. 1). Of the 118 arousal episodes, 75 consisted only of cold arousals, 36 of normothermic arousals, and seven episodes of both cold and normothermic arousals.

We observed no trend in the frequency of arousals with oncoming spring (Fig. 1). We recorded 33 (23.9%) arrivals, 40 (29%) departures and 65 (47.1%) bat movements during normothermic arousal episodes (Table 1a), and 24 (24.2%) arrivals, 35 (35.4%) departures and 40 (40.4%) movements during cold arousal episodes (Table 1b). We recorded 57 (76%) cold arousals when a neighbouring bat moved within one hour (30 min time window before and after the beginning of cold arousal). In 48 cold arousals (35.6%), bats warm, groomed, wiggled, and cooled down, while remaining in the same position throughout the cold arousal episode. We found positive correlation between all summed activity (arrival, departure and relocation) of bats in hour period during which a cold arousal episode occurred and number of occurred cold arousal episodes in the same hour (Spearmann correlation coefficient, $r_s = 0.251$, $p < 0.05$).

GZLM significantly explained variation in ΔTs during an arousal episode (Intercept $F = 4.11$, $p < 0.042$; Table 2), with ΔTs significantly influenced by duration of the episode ($F = 5.54$, $p = 0.019$) and interaction between year and duration ($F = 4.35$, $p = 0.037$; Table 2).

Using segmented linear regression (Fig. 4), we estimated the

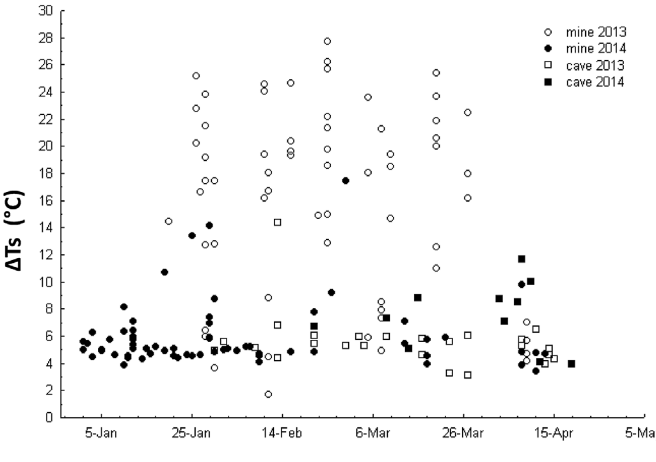

Fig. 1. Difference (ΔTs) between maximum surface temperature (Tmax) and body surface temperature (Ts) in the same individual 24 h before an arousal in the Šimon and Juda Mine (dots) and the Katerinska Cave (squares), Czech Republic, over the winters of 2013 (white) and 2014 (black).

Table 1
Summary statistics describing arousal episodes during *Myotis* hibernation, where only a) cold (N = 75) and b) normothermic arousal episodes (N = 36) were detected.

a) Cold arousal episodes

Variable	Mean	SD	Median	Minimum	Maximum
max active.f	0.2	0.1	0.2	0.1	1.0
Ts	2.8	1.4	2.3	1.3	9.9
Tmax	8.2	0.7	8.0	7.4	9.9
frequency	0.2	0.1	0.2	0.1	0.8
active.n	1.3	0.7	1.0	1.0	4.0
max.cluster	6.9	1.9	7.0	3.0	11.0
duration	72.6	57.2	60.0	20.0	310.0
move	0.5	0.8	0.0	0.0	3.0
depart	0.5	0.7	0.0	0.0	3.0
arrive	0.3	0.5	0.0	0.0	2.0
total.act	1.3	1.3	1.0	0.0	6.0

b) Normothermic arousal episodes

Variable	Mean	SD	Median	Minimum	Maximum
max active.f	0.2	0.1	0.1	0.1	0.3
Ts	1.6	1.0	1.4	0.5	5.4
Tmax	16.4	5.4	15.0	10.0	28.2
frequency	0.2	0.2	0.2	0.1	0.7
active.n	2.1	1.9	1.0	1.0	10.0
max.cluster	9.3	4.1	7.5	3.0	17.0
duration	140.4	112.8	115.0	20.0	410.0
move	1.8	4.0	0.0	0.0	18.0
depart	1.1	1.8	1.0	0.0	10.0
arrive	0.9	0.9	1.0	0.0	3.0
total.act	3.8	6.1	1.5	0.0	31.0

Table 2
Results of generalised linear modelling analysis (full factorial design) for the ΔTs of arousal as a function of year (2013, 2014), days (days since the beginning of the hibernation), duration (time between first and last image of arousal episode) and max.cluster (maximum size of cluster recorded during an arousal episode).

Effect	Wald	p
Intercept	**4.11**	**0.043**
year	3.55	0.059
duration	**5.54**	**0.019**
days	1.55	0.212
max.cluster	2.81	0.094
year*duration	**4.35**	**0.037**
year*days	0.72	0.395
days*duration	2.92	0.087
year*max.cluster	2.09	0.147
duration*max.cluster	3.34	0.067
days*max.cluster	0.49	0.482
year*days*duration	1.66	0.197
year*duration*max.cluster	2.78	0.095
year*days*max.cluster	0.11	0.735
days*duration*max.cluster	1.68	0.195
year*days*duration*max.cluster	0.71	0.397

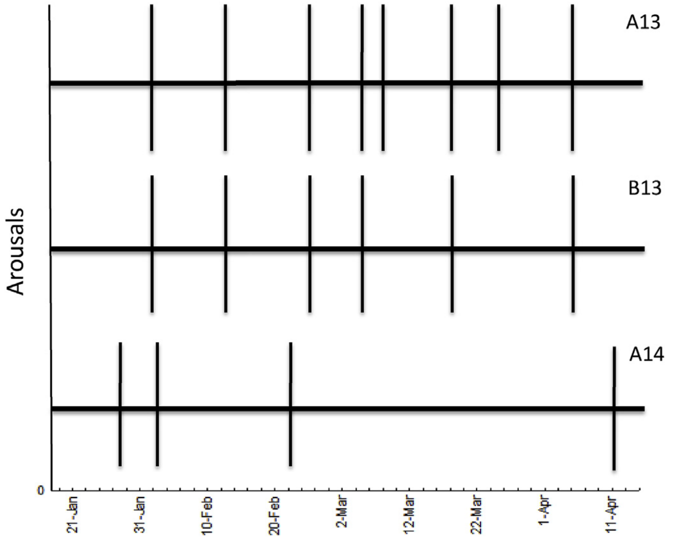

Fig. 2. Frequency of arousals and torpor periods in three individuals forming part of a cluster for more than two months.

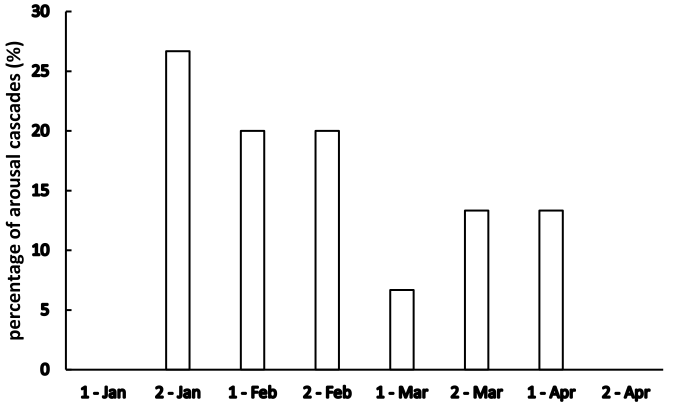

Fig. 3. Percentage of arousal cascades in all arousal episodes. Data have been grouped in 14 days intervals (1st half and 2nd half of the month).

breakpoint for Tmax as a function of number of individuals and the breakpoint at 8.9 individuals in a cluster was found (st. error = 1.03). The slope b of the first segment did not differ statistically from 0 ($b = 0.16$, $t = 0.51$, $p > 0.05$), whereas the relationship in the second segment showed a significant increase in Tmax with an increasing number of individuals in the cluster ($b = 1.47$, $t = 6.83$, $p < 0.05$).

We did not observe shortening of torpor bouts between arousals in the late winter weeks while investigating the frequency of arousals in three individuals present within the camera field for more than two months. Torpor bout duration remained broadly stable in two individuals (11.1 ± 3.8, 14.8 ± 3.5 days) with each synchronising arousals (12 of 14 arousals). The third individual (A14) remained in long torpor bout between arousals over February 21 and April 11 (53 days; Fig. 2).

3.2. Arousal cascades

The number of bats in each arousal episode ranged from 1 to 10, while 72.1% (N = 85) had only one bat. Arousal cascades (Turner et al., 2015) were only observed in the KC. A typical arousal cascade affected 17% of seven individuals in a cluster, with the animal usually not relocating. Arousal cascades were more frequent late in the season of hibernation (December–March), with maximum occurrence in mid-January (Fig. 3). Only two (13.3%) cascades were caused by the arrival of a new bat to the cluster.

Exhaustive grid model comparison with logistic regression indicated that the optimal model included max.cluster, active.f, active.n and arrive (AIC = 10.0) as parameters. The optimal hyperparameter settings for the SVM model with radial kernel included cost equal to 1 and γ equal to 0.2. Classification with 37 support vectors relied on feature weights of −9.33, −5.76, −15.95 and 1.99 for active.f, max.cluster, active.n and arrive, respectively, using a ρ parameter of 0.136. Specificity of the SVM model, where expert changes were considered positive, was 97.9% and sensitivity 100%, meaning that the predictive properties of the model were robust.

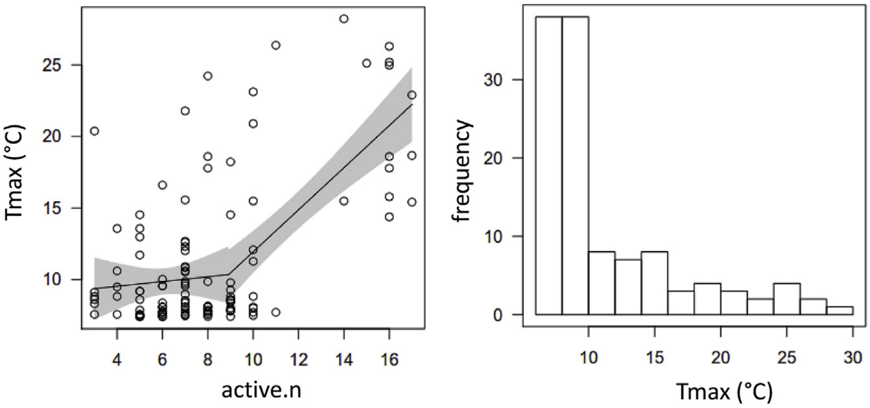

Fig. 4. a) Using segmented linear regression, we estimated a breakpoint at 8.9 individuals in a cluster for which Tmax increased rapidly, and b) frequency of Tmax in arousal episode.

4. Discussion

We observed 70% of arousal episodes where bats ceased Ts temperature elevation at < 10 °C. We called these "cold arousals" as they were characterised by obvious warming and cooling phases, clearly differing from low Ts changes lacking a temperature elevation (Bartonička et al., 2017). During cold arousals, Ts increased by ≤ 6 °C compared to ≥18 °C for normothermic arousal episodes. While bats frequently relocated after normothermic arousal episodes, they did not move during cold arousal episodes. Although many arrivals and departures were based on 'the sudden' appearance of a bat between successive thermal images (cf. Bartonička et al., 2017), synchronisation of the thermal imaging camera with IR recordings in KC showed bats crawling, flapping wings, and even gliding when arriving or departing from the clusters during cold arousal episode. Cold arousal episodes were recorded more frequently in January (Berkova and Zukal, 2010), although some also occurred late in the season in mid-April, like cold arousals observed by Mayberry et al. (2018), who suggested that cold arousals were a reaction to disturbance by bats within a cluster. They found most of the cold arousals within two weeks of the end of hibernation, when they could be highly beneficial to avoid spending the energy required to rewarm to normothermia; however, there were few cold arousals occurred ≥20 days before the end of hibernation. In our recordings, we recorded cold arousals spread over whole monitored period of hibernation; hence, we suggest that such arousals are better explained as responses to disturbance in the neighbourhood of the cluster. Our findings of disturbance in 60.3% of cold arousal episodes and 13.3% of arousal cascades caused by the arrival of a new bat to the cluster lead us to conclude that the cold arousal episodes observed reflect a controlled energy-saving behaviour allowing bats to avoid unnecessary normothermic arousals. Twente and Twente (1987) considered that cold arousals [described already by Twente and Twente (1978) as inhibited arousals], occur at the time of expected normothermic arousals initiated by brief bursts of cardiac rate as a proximate factor. We could speculate that the ultimate mechanism of this behaviour could be the ability to warm-up slightly, and then quickly re-enter deep torpor, allows the bats to differentiate risky disturbances (e.g. predator or smoke presence; Doty et al., 2018) from those that are not dangerous (e.g. conspecific movement).

Mayberry et al. (2018) suggested that cold arousals were not associated with WNS as they observed them in both diseased and healthy bats. Further, the interval between cold and normothermic arousal episodes was shorter than that between two successive normothermic arousal episodes. This suggests that bats were still able to re-warm completely over the following days. Hence, we suggest that the cold arousals and activity events without Ts elevation described in Bartonička et al. (2017) may prolong torpor bouts between two successive normothermic arousals. Although bats moved very slowly and were unable to fly during Ts elevation changes, bats during cold arousal episodes were clearly able to glide. Hence, we believe that cold arousals reflect a physiological and behavioural adaptation which may, under some circumstance, help avoid energetic constraints of clustering that can lead to fat reserve exhaustion. A few individuals have been found to be able to initiate normothermic arousal after less than 3 days from cold arousal. This would indicate that both types of arousals do not substitute each other, but perform a different function.

We found no evidence of arousal synchronisation with dusk, as found in locations where bats arouse periodically from hibernation to forage (e.g. Twente and Twente, 1978; Thomas, 1993). However, in hibernacula with stable microclimates, such as large caves, and in mid-winter synchronisation is unlikely (Czenze et al., 2013; Czenze and Willis, 2015; Zukal et al., 2016a). Indeed, the absence of the 'time-giver' ('zeitgeber') that entrains circadian rhytms is likely to increase individual variation in duration of torpor bouts (Thomas, 1993; Körtner and Geiser, 2000; Ruby, 2003). While we failed to document any synchronisation between arousals and dusk, it would appear that bats synchronise arousals with each other. Czenze et al. (2013), for example, observed a high proportion of synchronised arousals, with many bats aroused on the same day, though not all at the same time.

If regular normothermic arousals are necessary during mid-winter, then it is difficult to explain differences in torpor bout duration between individuals (Grieneisen et al., 2015; Lilley et al., 2016) although they clearly exist (Twente et al., 1985). Our study was not focused on individual bat Ts, and hence the number of bats monitored long-term is small (n = 3; > 3 months). Nevertheless, it is apparent that individuals A13 and B13 displayed a similar interval between two successive arousals, between 11 and 14 days (cf. Harmata, 1994), while bat A14 stayed in torpor four times longer. In comparison, Jonasson and Willis (2012) recorded some *M. lucifugus* torpor bouts lasting as long as 48 days, Grieneisen (2011) even 76 days.

High variability in torpor length and Ts could be caused by a wide range of factors; however, our results suggest that differences in ΔTs during arousals was most likely influenced by both location and season. This mirrors inter-individual differences, i.e. pathogen pressure depending on hibernaculum-dependent physiological effects, hibernation conditions, gender, age and previous experience with hibernation (Bandouchova et al., 2018). Our preliminary data show that juveniles arouse frequently for short periods, are likely to change position within the hibernaculum more than adults and tend to experience more normothermic arousals (T. Bartonička, unpublished). Moreover, Geiser and Broome (1991) showed adults of pygmy possum (*Burramys parvus*) commenced hibernation earlier and hibernated longer than juveniles. Inexperienced juveniles may be more prone to disturbance during torpor and first have to learn to differentiate 'dangerous' from 'safe' disturbances in order to avoid exhausting their fat reserves too quickly (Czenze et al., 2013; Doty et al., 2018).

4.1. Synchronised rewarming

We tested the "arousal hypothesis" of Boyles et al. (2008), which suggests that clustering minimises heat loss during a series of associated normothermic arousals and subsequent periods of normothermy. Boyles and Brack (2009) assumed that clustering lowered energy loss by up to 40% of that expected when bat hibernates solitarily. In our study, bats showed no synchronisation with sunset, though we did observe synchronisation with conspecifics, as did Czenze et al. (2013). In their study, many bats aroused over a two-day period, with just 38% of arousals occurring in isolation. Although this behaviour shows evidence of synchronised arousal, it does not explain the energy-savings identified in the study of Boyles and Brack (2009), in which all bats arouse at the same time, with each bat rewarming other individuals. Synchronised re-warming, therefore, should prove useful during all normothermic arousals, but should not occur when forming clusters and should be rare during periods of hibernation late in the winter (December–March), when bats hibernating in clusters should not be disturbed by individuals that arrive from elsewhere, i.e. bats should have evolved not to react to new arrivals. We observed arousal cascades relatively rarely (just 13% of all arousal episodes); however, we started our study in January when large clusters had already formed. In autumn, bats forage intensively and have large energy reserves, so energy conservation may not by apriority (Kunz et al., 1998; Kokurewicz and Speakman, 2006, Boratynski et al. 2012). Czenze and Willis (2015), for example, recorded just 12.5% of overlapping (synchronised with conspecifics) arousals in early winter, when bats start to form clusters. The bat's ability to induced warming by conspecifics must be flexible, therefore, as shown by the difference in number of cold arousal episodes during late winter. The higher number of cold arousal episodes at the beginning of January may be related to the period when bats form clusters and a need to withstand disturbance caused by the activity of other bats in the cluster. In contrast, cold arousal episodes were detected in higher numbers at the end of hibernation (mid-April) when extension of the torpor bout could help individuals with less reserves to survive until the end of winter (Czenze and Willis, 2015).

WNS-tolerant bats in Europe may conserve more energy by resisting conspecific disturbance more effectively (e.g. using cold arousals) and, therefore, arousal cascades maybe less common compared to bats in North America. While we observed no signal of daily periodicity in *M. myotis* arousals, Hayman et al. (2017) recorded a strong circadian pattern, with *M. sodalis* arousal activity recorded via remote surveillance video. When the first obvious WNS mortality events were observed, this species declined more slowly (< 30%) than others (e.g. *Eptesicus fuscus* ~50%, *Perimyotis subflavus* and *M. lucifugus* > 90%; IDNR 2016). The discovery of daily arousal periodicity in survivors of *P. destructans* infection is surprising, however, as had was thought that WNS increased arousal activity, with associated energy depletion leading to high mortality (Reeder et al., 2012; Warnecke et al., 2012). Moreover, Hayman et al. (2017) often observed arousal cascades in diseased *M. sodalis*, suggesting that such periodicity was triggered by WNS and helped bats to spend less time at a Ts supporting fungal growth. On the other hand, in WNS-tolerant bat species such as *M. myotis*, periodic circadian arousals cease during late hibernation period (March) in harsh winters (Pohl, 1961; Daan, 1973; Zukal et al., 2016a), so bats avoiding being aroused up by conspecifics through the use of cold arousals.

Few arousal cascades and numerous cold arousals upon arrival of a new bat allow *M. myotis* to prolong their torpor bouts, lowering their pathological immune response to tissue invasion (Moore et al., 2013).

Thus, we suggest that thermoregulatory behaviour among clustering hibernating bats may be more variable than previously postulated. *P. destructans* infection survivors and even WNS-tolerant bat species all show different hibernation strategies (Lilley et al., 2016; Hayman et al., 2017), however, mechanisms of arousal timing remain poorly understood and require further attention with regard to individual patterns when energetically challenged.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.04.002.

Author contributions

TB, JZ and ZŘ conceived the idea and designed the study; JB, HBa, HBe, JZ and JP.organised the field trips and provided material; NM, PŠ, TB and JB analysed the data; JB, TB and NM wrote the manuscript, to which all authors contributed.

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