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Conspecific disturbance contributes to altered hibernation patterns in bats with white-nose syndrome

James M. Turner [a,1], Lisa Warnecke [a,1], Alana Wilcox [a], Dylan Baloun [a], Trent K. Bollinger [b], Vikram Misra [c], Craig K.R. Willis [a,*]

[a] Department of Biology and Centre for Forest Interdisciplinary Research, University of Winnipeg, Winnipeg, MB R3B2E9, Canada
[b] Department of Veterinary Pathology and Canadian Wildlife Health Cooperative, University of Saskatchewan, Saskatoon, SK S7N5B4, Canada
[c] Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N5B4, Canada

HIGHLIGHTS

- Bats infected with *Pseudogymnoascus destructans* synchronised arousals from torpor.
- Bats often aroused sequentially, but normothermic phases did not overlap entirely.
- Rewarming rates did not differ between infected and control bats.
- Rewarming rate was not affected by clustering behaviour.
- We suggest disturbance by aroused, infected bats affects hibernation patterns.

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ABSTRACT

The emerging wildlife disease white-nose syndrome (WNS) affects both physiology and behaviour of hibernating bats. Infection with the fungal pathogen *Pseudogymnoascus destructans* (*Pd*), the first pathogen known to target torpid animals, causes an increase in arousal frequency during hibernation, and therefore premature depletion of energy stores. Infected bats also show a dramatic decrease in clustering behaviour over the winter. To investigate the interaction between disease progression and torpor expression we quantified physiological (i.e., timing of arousal, rewarming rate) and behavioural (i.e., arousal synchronisation, clustering) aspects of rewarming events over four months in little brown bats (*Myotis lucifugus*) experimentally inoculated with *Pd*. We tested two competing hypotheses: 1) Bats adjust arousal physiology adaptively to help compensate for an increase in energetically expensive arousals. This hypothesis predicts that infected bats should increase synchronisation of arousals with colony mates to benefit from social thermoregulation and/or that solitary bats will exhibit faster rewarming rates than clustered individuals because rewarming costs fall as rewarming rate increases. 2) As for the increase in arousal frequency, changes in arousal physiology and clustering behaviour are maladaptive consequences of infection. This hypothesis predicts no effect of infection or clustering behaviour on rewarming rate and that disturbance by normothermic bats contributes to the overall increase in arousal frequency. We found that arousals of infected bats became more synchronised than those of controls as hibernation progressed but the pattern was not consistent with social thermoregulation. When a bat rewarmed from torpor, it was often followed in sequence by up to seven other bats in an arousal "cascade". Moreover, rewarming rate did not differ between infected and uninfected bats, was not affected by clustering and did not change over time. Our results support our second hypothesis and suggest that disturbance, not social thermoregulation, explains the increased synchronisation of arousals. Negative pathophysiological effects of WNS on energy conservation may therefore be compounded by maladaptive changes in behaviour of the bats, accelerating fat depletion and starvation.

1. Introduction

Pathogenic infections trigger behavioural responses of hosts. These can reduce severity of disease, benefitting the host [1–3], or increase parasite survival or transmission, disadvantaging the host [1,3–5]. For example, a common response of hosts to infection is to increase body

* Corresponding author.
E-mail address: c.willis@uwinnipeg.ca (C.K.R. Willis).
[1] Present address: Department of Animal Ecology and Conservation, Biocentre Grindel, Hamburg University, Hamburg 20146, Germany.

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temperature (T_b) outside the pathogen's optimal thermal zone [6]. In ectothermic animals, this response often involves selection of ambient temperatures (T_a) that are warmer than hosts normally experience [7–10]. On the other hand, selecting a cooler T_a can slow the growth of a parasite and reduce the chance of successful development within the host [11]. However, the relationship between T_a and host/parasite survival is seldom linear [12] and environmental constraints might further limit hosts' responses if a suitable T_a is not available.

Many mammalian and avian species save energy during adverse weather or resource scarcity by using torpor, a controlled physiological state of reduced T_b and metabolism [13]. Torpid animals are usually only capable of slow, poorly co-ordinated movements and must, therefore, select roost or nest sites with suitable microclimates for torpor expression while they are still normothermic. Hibernating mammals use long-term bouts of torpor that can last days to weeks. During these long torpor bouts, T_b is typically thermoconforming and microclimates selected by many hibernators are often highly stable. Immune responses of the few hibernating mammals that have been examined are down-regulated [14,15]. An immune response might occur during normothermia but, for many hibernators, maximising time in torpor is critical for winter survival. The combination of diminished temperature-dependent physiological processes, restricted behavioural movements, extreme energy limitation and a narrow range of T_a could make torpid mammals particularly susceptible hosts for pathogens that can tolerate low T_a.

The cold-adapted fungus *Pseudogymnoascus destructans* (*Pd*, formerly *Geomyces destructans*; [16]) appears to be such a pathogen, and is the first known pathogen that appears to specialise on torpid mammalian hosts. *Pd* causes white-nose syndrome (WNS) [17,18], an infectious disease that has devastated bat populations in eastern North America [19,20]. *Pd* invades exposed skin of torpid bats during hibernation and this infection appears to disrupt regular torpor patterns resulting in the premature exhaustion of fat reserves and starvation [18,21–23]. Bats affected by WNS show a progressive increase in the frequency of periodic arousals from torpor (i.e., decrease in torpor bout duration) compared to control animals [18,23] which could reflect increased fluid and electrolyte loss across damaged wing tissue [24,25]. In addition to these physiological changes, infected bats also display behavioural changes including altered activity levels and reduced clustering [20,26,27]. Overall, *Pd* affects physiological and behavioural aspects of bat hibernation in ways that disrupt the tight winter energy budgets of bats.

Compared to the torpid state, periodic arousals to normothermia consume a disproportionally large fraction of a hibernator's winter fuel supply and account for ~85% of the over-winter energy budget [28]. Many heterotherms decrease arousal costs by passively rewarming from torpor with increasing T_a or solar radiation [29–31] but environmental conditions in hibernacula of WNS-affected bat species are highly stable, eliminating this possibility. Some species such as little brown bats (*Myotis lucifugus*) often roost in large clusters which could help them conserve water and prolong torpor bouts, reduce thermoregulatory energy expenditure during torpor if hibernaculum T_a falls below the lower critical torpid T_b, and/or reduce energy expenditure during arousals if individuals tightly synchronise rewarming and share costs via social thermoregulation [32–36]. There is some evidence for synchronised arousals in *M. lucifugus* and, although their energetic implications are not fully understood, this could reflect social thermoregulation [36]. By passively absorbing heat from adjacent, normothermic individuals during rewarming, individual bats could reduce energy expenditure during arousals and conserve fat reserves [33]. This behaviour could increase the chance of survival for WNS-affected bats if it helps them endure the increased arousal frequency associated with *Pd* infection.

There are other explanations for synchronised arousals in hibernating bats. First, in many hibernators the timing of arousals follows a circadian pattern entrainable to the light–dark cycle (e.g., [37–39]) and synchronised arousals could reflect an active circadian rhythm. Some hibernating bats time periodic arousals with their usual foraging time around sunset [40,41] while, for other species like *M. lucifugus*, that store large fat reserves and hibernate in caves with few external environmental cues, the rhythm can be weakened [42] or absent [36,43–45]. During mid-hibernation, periodic arousals of free-ranging, healthy *M. lucifugus* do not coincide with sunset but occur any time during the day or night and are often at least partially synchronised with cluster-mates [36,42]. This synchronisation could be beneficial if it allows social thermoregulation but could also reflect a detrimental consequence of roosting in large groups. Torpid bats are sensitive to even non-tactile disturbance [46–48] and it is possible that synchronised arousals reflect disturbance by normothermic, active conspecifics [47].

Potential interactions between physiological (i.e., increased arousal frequency) and behavioural changes (i.e., reduced clustering behaviour) that occur with WNS have not been investigated and no data have addressed how WNS may affect temporal patterns of arousal in hibernating bats. Therefore, we investigated arousal timing and rewarming rates in relation to clustering behaviour in *M. lucifugus* inoculated with *Pd*. We tested two competing hypotheses about effects of WNS on the behaviour and physiology of arousal. First, we tested whether infected bats adjust arousal physiology as part of an adaptive response to help compensate for the increased frequency of energetically expensive arousals. This hypothesis leads to three predictions: 1) Infected bats will more tightly synchronise arousals with colony-mates compared to controls and share arousal costs by rewarming simultaneously; 2) rewarming rates during synchronised arousals, or during arousals of clustered bats, will be slower than those during unsynchronised arousals, or arousals of solitary bats, because passive rewarming exploiting an exogenous heat source (e.g., an adjacent bat) occurs at a slower rate than active rewarming [29]; and 3) unsynchronised arousals by infected bats will be characterised by faster rewarming rates than unsynchronised arousals of controls because, in the absence of a passive heat source, faster rewarming is less costly than slow rewarming [49]. Second, we tested the alternative hypothesis that changes in arousal timing for bats with WNS are maladaptive consequences of infection which partially reflect disturbance of torpid bats by infected conspecifics. This hypothesis leads to two predictions: 1) As for the social thermoregulation hypothesis above, a greater proportion of arousals by infected individuals will occur at about the same times compared to those of controls. However, if disturbance rather than social thermoregulation influences arousal behaviour, arousals of infected colony-mates should occur in a sequence or “cascade” rather than simultaneously, with relatively few individuals normothermic at precisely the same time; and 2) neither arousal synchrony nor clustering will influence rewarming rates of infected or control bats.

2. Materials and methods

2.1. Housing

Details on methods and other data from this experiment have been published previously [18,25,27] and so are presented briefly here. The study was carried out at the Western College of Veterinary Medicine, University of Saskatchewan, Canada between November 2010 and March 2011. 54 male *M. lucifugus* were brought into captivity from a WNS-free cave in central Manitoba, Canada and housed in nylon mesh enclosures (Reptarium; Apogee, Dallas, TX, USA) within environment chambers (VWR BOD 2020; VWR International, Mississauga, ON, Canada). Consistent with many natural hibernacula, chambers were maintained at 7 °C and >97% relative humidity and kept in complete darkness without provision of food. Water was available ad libitum.

2.2. Inoculation

Bats were divided into three groups (n = 18) and inoculated by applying 20 μl of a conidial suspension containing 500,000 *Pd* spores in PBS-Tween20 to the dorsal side of both wings (for details see [18]). Group NA*Pd* was inoculated with a *Pd* isolate from North America (designated type isolate 20631-21; see [50]); Group EU*Pd* was inoculated with a European *Pd* isolate (MmyotGER2; see [51]); and Group CO was sham-inoculated with a PBS-Tween20 solution lacking conidia. Each group was maintained within a separate environmental chamber and left undisturbed for the duration of the experiment.

2.3. Skin temperature measurement

To monitor torpor patterns we recorded skin temperature (T_{sk}) of bats, which is closely correlated with T_b [52]. Each individual was outfitted with either a temperature-sensitive data logger (DS1922L-F5 Thermochron iButton; Maxim Integrated Products, San Jose, CA, USA; n = 9 each group; or iBBat; Alpha Mach, Sherbrook, QC, Canada; n = 3 each group) or radio transmitter (LB-2NT; Holohil Systems, Carp, ON, Canada; n = 6 each group). Data loggers recorded T_{sk} every 15 min and radio transmitter pulses were recorded every 15 min to a datalogging receiver (SRX400; Lotek Wireless, Newmarket, ON, Canada). We used two different methods of T_{sk} collection for several reasons. First, transmitters allowed us to monitor real-time T_{sk} of a subset of bats without disturbance so we could be sure that they re-entered torpor. However, the time required to scan and record transmitter signals by our datalogging receiver for all 18 bats in each cage would have dramatically reduced our sampling resolution (i.e., increased the time between T_{sk} measurements), causing us to miss recording some arousals. Therefore, we also used modified iButtons. Second, we were unsure how each type of device would cope with an extended period at such high humidity so we used two types to increase the chance of obtaining useable T_{sk} data. iButtons were modified after Lovegrove [53] and Reeder et al. [23], wrapped in plastic film and waterproofed using a rubber sealant (Plasti Dip; Plasti Dip International, Blaine, MN, USA). All devices were attached to bats between the shoulder blades using a latex-based adhesive (Osto-Bond; Montreal Ostomy Centre, Vaudreuil-Dorion, QC, Canada).

2.4. Behavioural observation

We monitored behaviour using infrared video cameras (VL650IRVFS; Speco Technologies, Amityville, New York, USA). Recording was triggered by movement of bats in the hibernaculum and video data were written to a digital video recorder (SHR-3040; Samsung Techwin, Ridgefield Park, New Jersey, USA). Video files were later viewed on a personal computer using software supplied by the recorder manufacturer.

2.5. Torpor and arousal definitions

We defined arousals (i.e., normothermic periods) following Jonasson and Willis [54] and Warnecke et al. [18]. The rewarming period was obvious in T_{sk} traces because T_{sk} during torpor was highly stable. We defined the onset of rewarming as the time at which T_{sk} began to increase from the stable, torpid minimum and the end of the rewarming phase as the time at which a stable, elevated T_{sk} was obvious in the trace.

We were also able to define torpor bouts and arousals using video data. We defined the onset of arousal as the time when a bat was observed making continuous co-ordinated movements (e.g., grooming or moving from its roosting position during torpor). We defined the end of an arousal as the time at which the bat had re-roosted and remained still for a least 5 min. To be conservative in our inference about social thermoregulation, for all arousals identified based on video alone, we added 15 min (i.e., the time between two consecutive T_{sk} data points) to the beginning and end of the arousal to ensure any overlap in arousal times of multiple individuals was included in our analyses.

2.6. Data handling and analyses

To analyse temporal organisation of torpor expression we divided the 105-day study period into four intervals (26.3 days each) beginning one week after inoculation (see [18]). We sometimes lost radio transmitter signals due to radio-interference in the laboratory, and the memory on our data loggers was filled by late February. Therefore, our analyses of rewarming rates focus on the first three months of the experiment using only iButton-based data. Bats exhibited advanced disease by three months [18] so these data reliably reflect arousal patterns of bats with WNS. We tested whether the timing of arousals from torpor differed from a random distribution using a Rayleigh's test [55]. We used one-way ANOVA with Student–Newman–Keuls (SNK) post-hoc tests to test for differences in the timing of arousals relative to sunset time among groups, and repeated measures ANOVA to test for changes in arousal timing over the course of the study within groups.

We calculated mean rewarming rate (°C min^{-1}) as the rate of increase in T_{sk} between torpor and normothermia (i.e., rewarming phase) during a periodic arousal. Maximum rewarming rate was defined as the fastest increase over 15 min. We were not able to measure metabolism in this study so we could not determine whether individuals depended entirely on endogenous heat production to rewarm to normothermia (i.e., active rewarming), or whether they shared arousal costs (i.e., passive rewarming). However, passive rewarming typically occurs much more slowly than active rewarming [29] and the stable T_a in each incubator ensured that the only available exogenous heat source would be adjacent individuals.

We compared mean and maximum rewarming rates among groups using one-way ANOVA and assessed changes in rewarming rates within groups over time using repeated measures ANOVA. We used a univariate general linear model (GLM), controlling for interval (i.e., time), to determine if mean rewarming rate differed between bats that were roosting singly or in a cluster during an arousal (i.e., "cluster" = two or more bats roosting in physical contact with each other). We also used GLMs to determine if the order in which bats rewarmed within a series or "cascade" of arousals (see below) influenced rewarming rate (covariate = Interval) and whether overlapping arousals influenced the rewarming rate of individual bats (covariate = overlap yes/no).

We examined arousal events during which multiple bats aroused at about the same times and defined these "arousal cascades" as events during which three or more individuals exhibited arousals that were at least partially overlapping in time. We counted the number of bats in an arousal cascade using video data, therefore including all bats in all four intervals (i.e., independent of T_{sk} data, which were only used for Intervals 1–3). However, we could not reliably identify individual bats consistently using the video because bats were often obscured from view by others roosting in the same cluster. Therefore, we could not control for individual in the cascade analysis and defined arousal as our experimental unit. We used ordinary least-squares regression to examine the relationship between numbers of bats in a cascade and cascade duration and a chi-squared goodness-of-fit test to see whether the number of cascades within an interval differed from expected proportions among intervals for each group. We also used GLM to control for effects of interval (i.e., time) and compare the proportion of an individual bat's arousals that overlapped with the arousals of at least one other bat for treatment vs. control bats.

We used SPSS v21 to test for data normality and *statistiXL* v1.9 for all other analyses. Data presented are means for each individual ± SD; n indicates the number of individuals and N the number of measurements. All methods were approved by the University Committee on Animal Care and Supply of the University of Saskatchewan (Protocol #20100120) under Manitoba Wildlife Scientific Permit WB11145.

3. Results

3.1. Timing of arousal

During Interval 1 the distribution of arousals times for all three groups did not differ from a random circular distribution (Table 1, Fig. 1). However, for both inoculated groups the synchronisation of arousals increased as infection progressed, demonstrated by a non-random distribution of arousal times during Intervals 2 and 3 (Table 1, Fig. 1). Arousal timing of control bats remained random throughout the study (Table 1, Fig. 1). The time that bats aroused relative to sunset (i.e., their normal foraging time outside winter) did not differ among groups within any interval (Interval 1: $F_{2,19} = 0.18$, $p = 0.84$, $n = 22$; Interval 2: $F_{2,30} = 0.96$, $p = 0.40$, $n = 33$; Interval 3: $F_{2,29} = 1.44$, $p = 0.25$, $n = 32$), and did not change over the course of the study for any group (CO: $F_{2,6} = 1.30$, $p = 0.34$, $n = 8$; NA*Pd*: $F_{2,6} = 1.28$, $p = 0.35$, $n = 8$; EU*Pd*: $F_{2,4} = 0.54$, $p = 0.62$, $n = 6$).

3.2. Arousal cascades

After a bat had rewarmed from torpor it was often followed by the rewarming of one or more other bats, so that several arousals were at least partially overlapping and occurred in an apparent cascade (Fig. 2). During an arousal cascade a bat was normothermic at the same time as up to four (CO), six (NA*Pd*) or five (EU*Pd*) other individuals (Fig. 2). The number of cascades within an interval ranged from 0–3 (CO), 0–7 (NA*Pd*) and 0–12 (EU*Pd*) and accounted for 26.3% (CO), 27.3% (NA*Pd*) and 39.5% (EU*Pd*) of the total number of arousals. The number of cascades did not differ from expected proportions across intervals for CO bats ($\chi^2 = 3.33$, $p = 0.34$), whereas the proportion of cascades did increase across intervals for NA*Pd* ($\chi^2 = 8.23$, $p = 0.04$) and EU*Pd* groups ($\chi^2 = 11.47$, $p = 0.003$) (Fig. 3). Within each group, the number of bats arousing during a cascade was positively related to the cascade's duration (CO: $R^2 = 0.85$, $F_{1,4} = 23.29$, $p = 0.008$, $n = 6$; NA*Pd*: $R^2 = 0.67$, $F_{1,11} = 22.16$, $p = 0.001$, $n = 13$; EU*Pd*: $R^2 = 0.42$, $F_{1,17} = 12.38$, $p = 0.003$, $n = 19$; Fig. 4). The proportion of each individual's arousal within a cascade that overlapped with the arousal of at least one other individual did not differ among groups or intervals ($F_{3,158} = 0.36$, $p = 0.79$; CO: $N = 26$, NA*Pd*: $N = 53$, EU*Pd*: $N = 83$). In other words, arousals were not more likely to overlap in time as hibernation or infection progressed.

3.3. Rewarming rates

Mean rewarming rate (Table 2) did not differ among groups within any of the three intervals (Interval 1: $F_{2,19} = 0.42$, $p = 0.67$, $n = 22$; Interval 2: $F_{2,30} = 1.30$, $p = 0.29$, $n = 33$; Interval 3: $F_{2,29} = 2.75$, $p = 0.08$, $n = 32$) or over the course of the trial for any of the three groups (CO: $F_{2,6} = 0.09$, $p = 0.92$, $n = 8$; NA*Pd*: $F_{2,6} = 0.75$, $p = 0.51$, $n = 8$; EU*Pd*: $F_{2,4} = 3.58$, $p = 0.13$, $n = 6$). There was also no difference in rewarming rate between the bat that aroused first in each cascade and those that followed it ($F_{3,30} = 1.53$, $p = 0.23$; CO: $n = 11$, NA: $n = 5$, EU: $n = 8$). Mean rewarming rates of individuals whose rewarming phases overlapped with the normothermic period of at least one other bat did not differ from rewarming rates of bats that rewarmed by themselves for any group ($F_{3,55} = 1.69$, $p = 0.18$; CO: $n = 11/10$ overlapped/alone, NA: $n = 9/9$, EU: $n = 11/9$). Moreover, whether a bat rewarmed while it was in a cluster or roosting solitarily had no effect on rewarming rate ($F_{3,40} = 1.08$, $p = 0.37$; CO: $n = 12/2$ clustered/alone, NA: $n = 10/1$, EU: $n = 11/8$). Maximum rewarming rate (Table 2) did not change with time for the CO ($F_{2,6} = 1.58$, $p = 0.28$, $n = 8$) or NA*Pd* ($F_{2,6} = 1.39$, $p = 0.32$, $n = 8$) groups, but decreased in Interval 3 for EU*Pd* bats ($F_{2,4} = 8.98$, $p = 0.03$, $n = 6$). Consistent with this result, maximum rewarming rate did not differ among groups within Intervals 1 ($F_{2,19} = 0.50$, $p = 0.61$, $n = 22$) or 2 ($F_{2,30} = 0.97$, $p = 0.39$, $n = 33$), but in Interval 3 ($F_{2,29} = 3.47$, $p = 0.045$, $n = 32$) the maximum rewarming rate of EU*Pd* bats was slower than that for CO bats ($p = 0.03$, SNK).

Table 1
Average times of day that bats were aroused from torpor during each Interval for each Group and results of Rayleigh's tests indicating whether these times differed from a random circular distribution. Times that were non-randomly distributed (i.e., synchronised) are in bold.

Interval	Group	n	Time of day	SD (h)	r	z	p
1	CO	8	00:33	6.0	0.28	0.66	>0.5
	NA*Pd*	8	09:47	7.8	0.13	0.13	>0.5
	EU*Pd*	6	10:24	7.0	0.19	0.21	>0.5
2	CO	12	03:07	5.4	0.36	1.57	>0.2
	NA*Pd*	**10**	**06:14**	**2.5**	**0.81**	**6.62**	**<0.001**
	EU*Pd*	**11**	**03:08**	**3.8**	**0.61**	**4.14**	**<0.02**
3	CO	12	02:03	9.6	0.04	0.02	>0.5
	NA*Pd*	**10**	**07:32**	**3.8**	**0.60**	**3.62**	**<0.05**
	EU*Pd*	**10**	**01:50**	**2.1**	**0.86**	**7.34**	**<0.001**

4. Discussion

Our results demonstrate that infection with *Pd* leads to changes not just in the frequency of periodic arousals throughout hibernation, but also in the timing of arousals by individuals and the synchronisation of arousals within groups of bats. We found no evidence to support our first hypothesis that changes in arousal physiology reflect an adaptive response to help compensate for energetic costs of increasing arousals as disease progresses. Instead, our results support our second hypothesis that changes in arousal physiology do not help bats compensate for increasing thermoregulatory energy expenditure and, instead, an increased synchronisation of arousals appears to reflect disturbance of torpid bats by infected conspecifics. This disturbance may contribute to the overall increase in arousal frequency and could negatively impact survival.

The arousal timing of both groups of inoculated bats, but not controls, became partially synchronised as infection progressed (Fig. 1), although this synchronisation was not consistent with social thermoregulation/energy savings. In warmer regions than central Canada where our study animals were captured, some hibernating bat species time periodic arousals with sunset, even in the absence of photoperiod cues in the hibernaculum, so they can leave hibernacula to forage on warm nights [40,41]. In Intervals 2 and 3, also in the absence of any photoperiod cue, our NA*Pd* bats began to arouse in the early morning while EU*Pd* bats aroused in the second half of the night (Table 1). This timing could indicate that bats resumed a circadian pattern to arousal, synchronising their arousals with normal foraging time as their energy reserves declined. Although free-ranging *M. lucifugus* from the same population as our bats abandon a circadian pattern to arousal in the middle of winter [36,42], Czenze and Willis [56] found that they began to time arousals from torpor with sunset towards the end of hibernation, when fat reserves would have been running out but prey may have started to become available. However, if waning energy reserves served as a cue for reinstating a circadian pattern to arousal towards the end of hibernation in *M. lucifugus*, we would have expected to see the same response in our control bats, and this was not the case. Therefore, in the absence of photoperiodic cues, it is unlikely that bats aroused at night in an attempt to forage and instead we suggest that the observed patterns reflect disturbance by normothermic individuals (see below).

Our first hypothesis predicted that synchronised arousals would occur at relatively slow rewarming rates because passive rewarming rates are typically dramatically slower than active rates [29,33,57] and faster rewarming rates would be less energetically expensive for solitary individuals [49]. On the contrary, we found no difference in rewarming rates of bats within clusters vs. those roosting alone, nor did we find a difference in warming rates for arousals that occurred in a cascade vs. those that occurred in isolation. Indeed, there were no

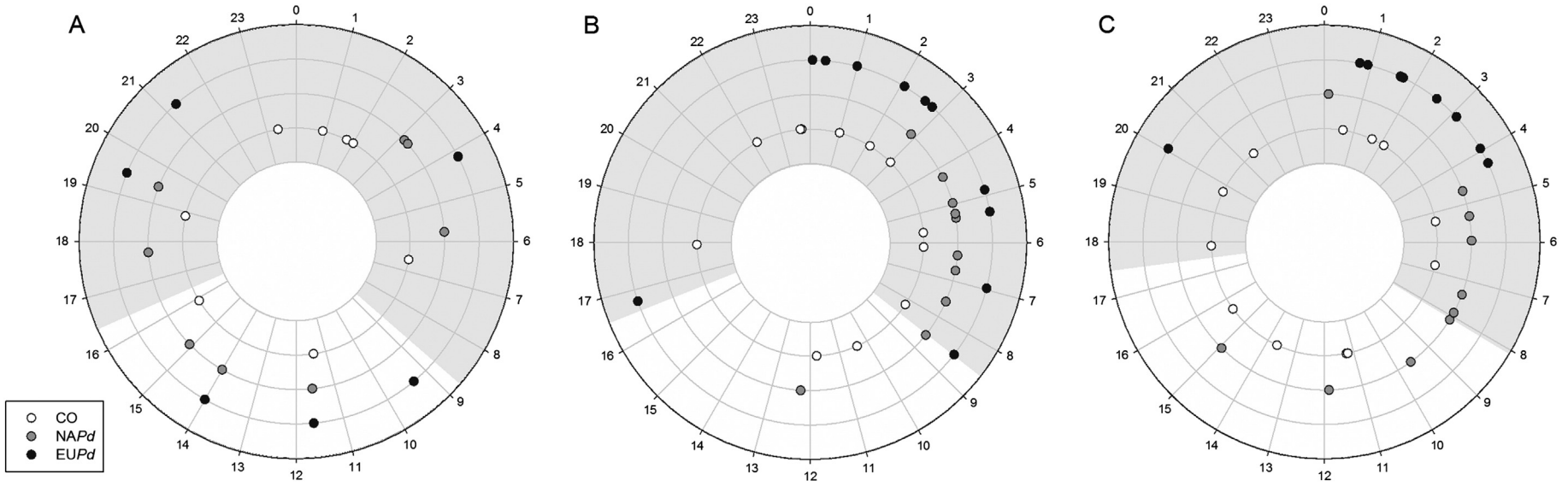

Fig. 1. Time of day of arousals from torpor (individual means) during Intervals 1, 2 and 3 (A, B and C, respectively) for bats inoculated with North American *Pd* (NA*Pd*; grey circles), European *Pd* (EU*Pd*; black circles) and the sham-inoculated Control group (CO; white circles). Times of arousals for each treatment group occupy a single ring on the radial axis and their relative positions are only for display purposes. The shaded area indicates the average scotophase during each interval that corresponds with the site from which the bats were collected. Arousal time was non-randomly distributed for both the NA*Pd* and EU*Pd* groups in Intervals 2 and 3; see text for details.

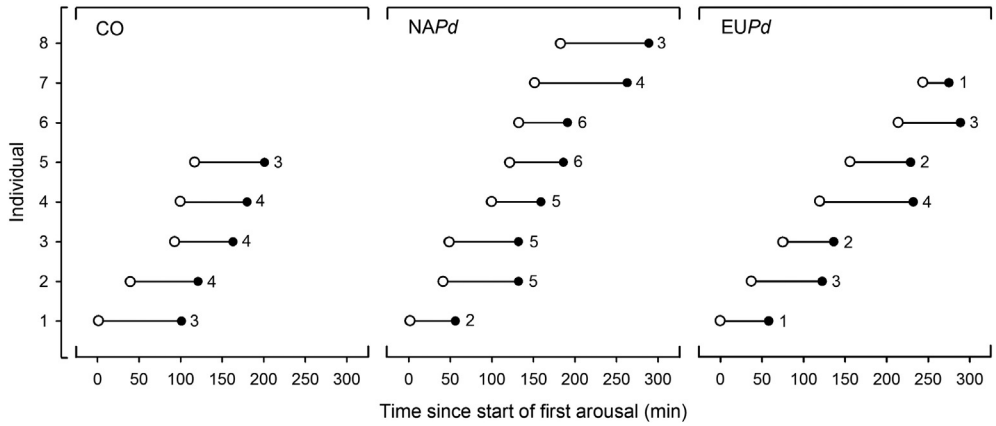

Fig. 2. An example of an "arousal cascade" for each treatment group, determined from video data. An open circle indicates the start of an individual arousal; the closed circle indicates the end. The number to the right of each arousal shows the number of other arousals with which it overlaps. The time that the first bat began to arouse within each cascade is Time = 0 min.

differences in rewarming rates among any of the three treatment groups or across time. The only change in maximum rewarming rate we observed was a decrease for EU*Pd* bats in Interval 3 (Table 2), likely indicating a reduced capacity for endogenous heat production owing to diminished brown fat reserves and difficulty fuelling thermogenesis. During this interval several EU*Pd* animals became moribund and necropsy revealed that their brown and white adipose reserves were completely exhausted [18]. Additionally, if synchronisation reflected an energy-saving strategy, the overlap of arousals within cascades should have increased for infected bats over the course of hibernation as more individuals warmed up simultaneously to share thermoregulatory costs. Instead, the sequential pattern of arousals in cascades was consistent regardless of infection status or time and, overall, a relatively small proportion of arousals overlapped in time. Taken together, these results run counter to the hypothesis that changes in arousal physiology and behaviour of infected bats reflect an adaptive, energy-saving response to infection.

Our results were consistent with our second hypothesis that changes in arousal timing are maladaptive consequences of infection and that increased arousal synchrony reflects disturbance of torpid bats by normothermic individuals within the hibernaculum, as suggested for healthy, free-ranging bats by Czenze et al. [36]. The increase in arousal frequency would bring normothermic, active bats into contact with torpid individuals more often, potentially inducing more premature arousals from torpor [47,58]. Hibernating bats are highly susceptible to extrinsic disturbance and may arouse in response to both tactile and non-tactile disturbance by humans (e.g., [46–48,59,60]). Moreover, Thomas [47] found that the majority of induced arousals of hibernating bats following a hibernaculum visit by researchers did not appear to occur as a direct consequence of the visit, but were likely triggered hours later by other bats within the cave, the result of a "cascade effect of disturbance" over time. Golden-mantled ground squirrels (*Callospermophilus lateralis*) are more susceptible to noise disturbance towards the end of a torpor bout [43]. As hibernation progressed and torpor bouts of our infected bats shortened, arousal frequency may have increased as a result of bats being more susceptible to disturbance more often, snowballing the detrimental effects of *Pd* on arousal frequency. Altered vocalisation of the bats could be another trigger for disturbance. Although we did not record vocalisations, normothermic individuals infected with *Pd* may have produced social or echolocation calls which could have disturbed torpid individuals and induced arousals [61–63].

Reduced clustering behaviour has now been observed in both free-ranging [20] and captive [27] bats affected by WNS. Both studies hypothesised that this change in behaviour could be part of a suite of behaviours known as "sickness behaviour", which has been identified in multiple animal taxa (e.g., [64–66]). The absence of an effect of clustering status on rewarming rates in our study suggests that, although

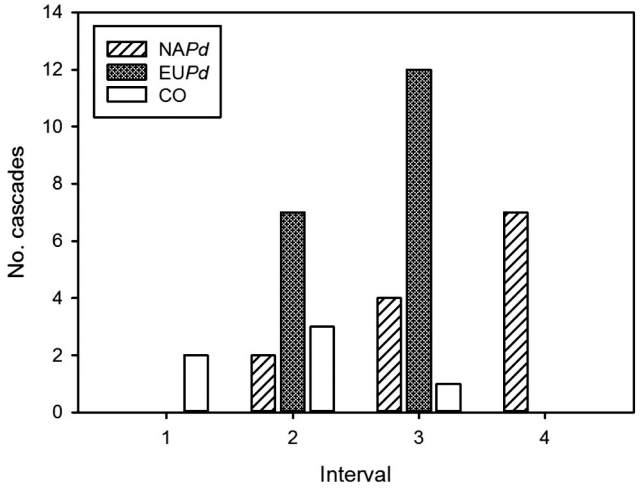

Fig. 3. The number of arousal cascades, where bats rewarmed from torpor consecutively and their normothermic periods overlapped at least partially in time, for each group within each Interval. The EU*Pd* group was terminated at the beginning of Interval 4 owing to severe mortality/morbidity [14].

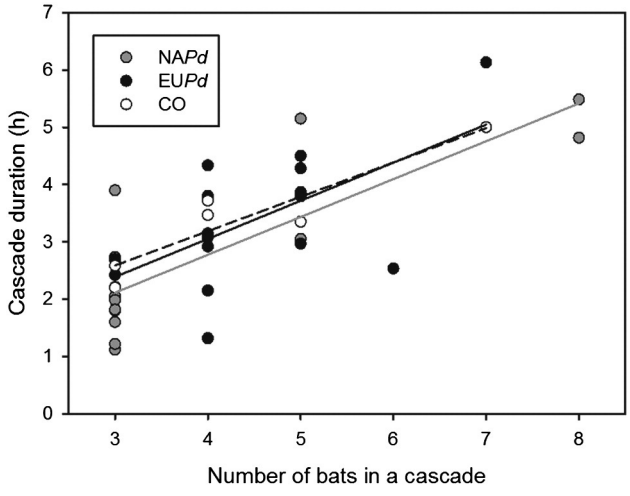

Fig. 4. The duration of arousal cascades for each group as a function of the number of bats arousing during the cascade (NA*Pd*; grey circles and line, EU*Pd*; black circles and line, CO; white circles and dashed line). Regression details are as follows: NA*Pd*: $R^2 = 0.67$, $F_{1,11} = 22.16$, $p = 0.001$, $n = 13$; EU*Pd*: $R^2 = 0.42$, $F_{1,17} = 12.38$, $p = 0.003$, $n = 19$; CO: $R^2 = 0.85$, $F_{1,4} = 23.29$, $p = 0.008$, $n = 6$.

Table 2
Average rate of rewarming from torpor (°C min^{-1}) and average maximum rewarming rate (°C min^{-1} over a 15-min period) of bats during each Interval for each Group. An asterisk indicates a rewarming rate significantly different from others in the group.

Interval	Group	n	Rewarming rate (°C min^{-1})	Max. rewarming rate (°C min^{-1})
1	CO	8	0.41 ± 0.12	0.84 ± 0.21
	NA*Pd*	8	0.45 ± 0.13	0.75 ± 0.18
	EU*Pd*	6	0.47 ± 0.14	0.74 ± 0.20
2	CO	12	0.46 ± 0.14	0.78 ± 0.23
	NA*Pd*	10	0.52 ± 0.13	0.79 ± 0.13
	EU*Pd*	11	0.43 ± 0.12	0.76 ± 0.16
3	CO	12	0.43 ± 0.12	0.74 ± 0.17
	NA*Pd*	10	0.49 ± 0.11	0.77 ± 0.13
	EU*Pd*	10	0.37 ± 0.10	0.56 ± 0.12*

roosting alone was not beneficial, it may have been less detrimental than remaining in a cluster. Clustering during hibernation is usually considered energetically advantageous [32,34,35] but, for bats with WNS, avoiding other individuals in the hibernaculum by roosting alone could be even more important by reducing an individual's risk of being disturbed by normothermic conspecifics. Therefore, in addition to potentially reducing *Pd* transmission, self-isolation and reduced clustering behaviour could represent part of an adaptive response by bats to *Pd* infection. We recommend further studies quantifying the susceptibility of infected and uninfected bats to disturbance at various times throughout torpor bouts, and throughout the hibernation season, to better understand implications of sociality and disturbance for arousal frequency and survival. For example, the disturbance hypothesis predicts that the density of bats in a given hibernaculum should be positively correlated with both the increase in arousal frequency and mortality that occurs as a result of WNS. In some hibernacula, multiple species may hibernate together, including in mixed-species clusters (e.g., [20,42]). Thus, we also recommend studies of the potential for negative effects of heterospecific disturbance.

Although our environment chambers were designed to imitate the bats' natural hibernacula, they were still an artificial habitat in which captured bats were confined and maintaining bats in an enclosed space may have increased the likelihood of disturbance. Nevertheless, we are confident in our data for several reasons. First, the hibernaculum from which bats were collected has a small volume and the entrance can be completely snow-covered for months at a time during hibernation, confining bats inside. Second, our data are consistent with those collected from free-ranging *M. lucifugus*. T_{sk} patterns of our captive control bats [18] closely matched those of individuals hibernating in the wild [54] and the approximately 3-fold increase in arousal frequency that we observed for these bats with infection [18] was virtually identical to that observed for naturally-infected bats [23]. Additionally, clustering density of the bats in our study [27] was comparable to that exhibited by free-ranging animals [67,68], and the decrease in clustering that occurred for the infected bats we studied [27] was consistent with that observed in the wild by Langwig et al. [20]. Thus, we were able to control both bat density and critical environmental variables (i.e., relative humidity, T_a), which would be exceedingly difficult to achieve in the field. Therefore, we are confident that the patterns we observed reflect infection with *Pd* and provide a realistic approximation of potential effects of disturbance by conspecifics in natural hibernacula.

Pathogen transmission and disease pathology can be affected by the behavioural and physiological changes that occur within hosts. Our study suggests that the complexity of these changes may be amplified by the normally aggregative nature of colony-roosting bats and between-individual interactions. Our findings suggest that behavioural disturbance accounts for at least part of the increase in time spent normothermic for bats with WNS. One prediction that arises from our results is that severity of disturbance will be positively correlated with colony size, accelerating fat depletion for individual bats and contributing to density-dependence in the severity of mortality from WNS.

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