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Warming up and shipping out: arousal and emergence timing in hibernating little brown bats (*Myotis lucifugus*)

Zenon J. Czenze[1] · Craig K. R. Willis[1]

Abstract Phenology refers to the timing of events in the annual cycle of organisms. For temperate-zone mammals, hibernation is one such event, but little is known about its phenology. Hibernation consists of energy-saving torpor bouts interspersed with energetically expensive arousals to normothermic T_b, and hibernators should benefit from mechanisms which reduce arousal costs and help them time arousals to coincide with foraging opportunities. In a previous study, we showed that, in contrast to hibernating bats from warmer climates, little brown bats (*Myotis lucifugus*) from central Canada abandon a circadian pattern to arousal in the middle of winter when there is no chance of feeding. Here, we used temperature telemetry to test whether they would re-synchronize arousals with normal foraging time (i.e. sunset) during late winter as the chance of foraging or emergence opportunities improves, and whether they would synchronize arousals with conspecifics, possibly to exploit social thermoregulation. We also used passive transponders to test whether energy reserves and/or sex differences in reproductive timing influence phenology and the sensitivity of emergence timing to environmental cues. In contrast to patterns in mid-winter, after 7 April 2013, bats synchronized arousals with sunset and with conspecifics. Females emerged earlier than males, and females in the best condition emerged first while body condition had no influence on male emergence timing. Both male and female bats appeared to time emergence with falling barometric pressure, a cue that predicts favourable foraging conditions for bats but which, unlike outside temperature, would have been readily detectable by bats inside the hibernaculum. Our results highlight hibernation traits associated with extreme winter energy limitation for insect-eating bats in cold climates and illustrate the influence of reproductive timing and environmental conditions on hibernation energetics and phenology.

Keywords Periodic arousal · Circadian rhythms · Hibernation phenology · Chiroptera

Introduction

Phenology refers to the timing of events in the annual cycles of organisms and describes how these events are influenced by seasonal and inter-annual variation in climate (Both et al. 2009). Shifts in phenology associated with climate change have received recent attention as some avian species have failed to advance migration in step with changing peaks in resources (reviewed by Visser and Both 2005). Mammals should also face selection pressure to adjust timing of life cycle events but less is known about their phenology. Few mammals exhibit long-distance latitudinal migrations to avoid winter and, instead, many use hibernation to survive seasonally unfavourable conditions (Geiser 2004, 2013). Some species hibernate for up to 8 months and winter duration likely impacts over-winter survival and fitness in the following reproductive season (Lyman 1982; Twente et al. 1985; Wilkinson and South 2002; Lane et al. 2012; Norquay and Willis 2014). Over-winter survival and reproductive success may also be impacted by emergence phenology. During warmer springs, yellow-bellied marmots (*Marmota flaviventris*) emerge from hibernation

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earlier and experience greater reproductive success than in colder years (Ozgul et al. 2010). Spring weather varies inter-annually, and cold years may delay emergence adding additional costs that must be balanced by over-winter energy reserves (Lane et al. 2012).

Before entering hibernation, hibernators accumulate a food cache or fat store to fuel over-winter energy demands and then reduce energetic demands during hibernation via multiday bouts of torpor [i.e. dramatically reduced metabolic rate (MR) and body temperature (T_b)] (Geiser 2004). Torpor bouts can last for days to weeks depending on species, and are typically interspersed with brief (typically <24 h) periodic arousals to normothermic T_b, which account for up to 90 % of the winter energy budget (Wang 1989; Thomas et al. 1990; Geiser 2004; but see Lovegrove et al. 2014). Hibernators likely face selection pressure to budget the frequency, duration and timing of arousals to ensure they emerge from hibernation in adequate condition to support survival and reproduction (Jonasson and Willis 2011; Norquay and Willis 2014).

Mechanisms underlying the timing of arousal are still not fully understood (reviewed by Körtner and Geiser 2000). Circadian mechanisms are thought to be inhibited during multiday torpor bouts in hibernation (Tøien et al. 2011; Williams et al. 2012a, b). However, in the United Kingdom (U.K.), cave-dwelling, greater horseshoe bats (*Rhinolophus ferrumequinum*) and Natterer's bats (*Myotis nattereri*) maintain a circadian rhythm to arousals in the absence of photoperiod cues and rewarm from torpor at sunset. This is likely because mild winter nights (>10 °C) with flying insects available occur relatively often and bats may emerge to forage (Park et al. 2000; Hope and Jones 2012). In contrast, little brown bats (*Myotis lucifugus*) hibernating in central Canada where ambient temperature (T_a) on winter nights rarely exceeds 0 °C, appear to abandon any circadian rhythmicity to arousals (Czenze et al. 2013).

Despite use of torpor, hibernators may face additional pressure to reduce thermoregulatory costs in winter. Huddling with conspecifics reduces exposed surface area, and therefore, loss of both heat and water (Boyles et al. 2008; Boratyński et al. 2014). Groups of individuals could maximize thermoregulatory benefits of huddling if they synchronize arousals with each other and exploit social rewarming (Arnold 1993). Little brown bats tend to arouse at about the same times as conspecifics but overlap in arousal times has not been quantified in the wild (Czenze et al. 2013). Given the potential sensitivity of hibernating bats to disturbance by other individuals in their hibernaculum (Thomas 1995; Turner et al. 2015), it is unclear whether similarly timed arousals by multiple bats result in energetic benefits via social rewarming or reflect a response to disturbance by colony mates (Czenze et al. 2013; Turner et al. 2015).

Emergence timing is a key phenological trait for fat-storing hibernators and may be influenced by fat reserves and environmental cues (e.g. spring weather patterns) which signal spring conditions and food availability (Murie and Harris 1982; Arlettaz et al. 2001; Schorr et al. 2009; Sheriff et al. 2011; Norquay and Willis 2014). The influence of energy reserves on emergence phenology may also be influenced by sex differences in the timing of reproductive investment. Most fat-storing bats mate before hibernation and delay fertilization until spring. Female little brown bats are thriftier with fat and emerge from hibernation earlier than males presumably to exploit microclimates in maternity roosts (Jonasson and Willis 2011; Norquay and Willis 2014). Warm temperatures expedite gestation (Racey and Swift 1981) and, despite more variable in T_a than hibernacula, maternity roosts will be warmer on many spring days than hibernacula. Males, whose reproductive investment takes place before and during early hibernation (Thomas et al. 1979), may remain in hibernation longer and delay emergence until fat reserves are nearly exhausted, reducing exposure to inclement weather and other sources of mortality like predation (Stawski and Geiser 2010; Norquay and Willis 2014).

We used temperature radio-telemetry, and passive transponders (PIT tags) to test hypotheses about links between energetics, torpor-arousal cycles and emergence phenology in hibernating little brown bats. We first revisited Czenze et al.'s (2013) finding that little brown bats exhibit no circadian pattern to arousals during hibernation and tested the hypothesis that, as opposed to during deep winter, opportunities for feeding in late winter/early spring influence arousal timing. We predicted that, as warmer nights with flying insects became more common, bats would reinstate a non-random circadian pattern to arousal. Second, we tested whether hibernation period would affect the propensity of bats to synchronize arousals with conspecifics. We predicted that, as energy reserves decline towards spring and relative benefits of social rewarming increase, individuals would be more likely to rewarm simultaneously to share arousal costs. Finally, we tested the hypothesis that sex, energy reserves, and environmental conditions inside and outside hibernacula influence hibernation phenology. We predicted that females should emerge earlier than males and that energy reserves (assessed based on body condition) should affect hibernation period differently for males and females. Males in the best condition should hibernate longest to minimize exposure to extrinsic mortality while females in the best condition should emerge earliest because they will be best equipped to balance costs of cold, unpredictable spring weather against benefits of early gestation. We also predicted that if bats can detect cues inside the hibernaculum that reflect favourable foraging conditions outside (e.g. warm T_a, insect availability), they should adjust emergence timing to coincide with these conditions.

Methods

This study was conducted about 50 km north of the town of Grand Rapids (53°30′N; 99°24′W), in central Manitoba, Canada. Eight caves are known little brown bat hibernacula including our study site, Dale's Cave. Dale's Cave houses 80–200 bats and is accessed by a 40–50-cm diameter, 3-m deep sinkhole/tunnel leading to a bell-shaped chamber (approximately 7 m in length, 5 m in breadth with a 4 m ceiling). Most of the bats in Dale's Cave hibernate in 3–4 large clusters on the ceiling 6–7 m from the entrance with a few smaller clusters of 2–3 bats or solitary bats scattered throughout the chamber (Czenze and Willis unpub. data). Bats in Dale's cave may experience dim photoperiod cues during winter, especially those roosting close to the entrance tunnel. However, the long, narrow entrance to the cave significantly limits light penetration, and snow and ice cover around the entrance throughout the winter, further reducing the potential for photoperiod cues.

All procedures were approved by the University of Winnipeg Animal Care Committee and conducted under Manitoba Conservation Wildlife Scientific Permit WB0612. Temperature data loggers (HOBO Micro Station—H21-002, Onset Computer Corporation, Cape Cod, Massachusetts, USA) were used to record cave T_a and humidity every 2 h. We entered the cave and handled bats during hibernation, but to minimize disturbance, we only entered once and limited the duration of our visit to less than 2 h. Thirty torpid bats were captured by hand on 24 January 2013, sexed and weighed to the nearest 0.01 g (Durascale-100, MyWeigh, Ontario, Canada). Forearm length was measured to the nearest 0.05 mm.

For small bats, skin temperature (T_{sk}) provides a good approximation of T_b during torpor (Audet and Thomas 1996; Barclay et al. 1996; Willis and Brigham 2003). To record T_{sk}, we clipped a small (<1 cm^2) patch of fur between the shoulders and attached a temperature-sensitive radio-transmitter (0.80–0.90 g, BD-2NT; Holohil Systems Ltd, Carp, Ontario) using a non-toxic, latex-based adhesive (Osto-Bond, Montreal Ostomy, Vaudreuil, Quebec, Canada). Transmitters represented between 6.7 and 10.4 % of body mass, greater than the 5 % guideline suggested by Aldridge and Brigham (1988) but similar to the size which Jonasson and Willis (2012) demonstrated had no negative impact on body condition in hibernating little brown bats. Before use, we calibrated transmitters to the nearest 0.1 °C in a water bath at 5 °C increments from 0.3 to 45 °C against a thermometer traceable to the National Institute of Standards and Technology.

We deployed a data logging receiver (Lotek SRX600; Lotek Engineering Inc Newmarket, Ontario, Canada) powered by two 64 W solar panels (US-64, Uni-solar, Auburn Hills, MI) and connected to a solar charge controller (Sunsaver 10, Morningstar, Newtown, PA) in a weather-proof box outside the cave. The receiver was connected to three yagi antennas positioned inside the cave and recorded T_{sk} of each individual at 10-min intervals. To assess individual emergence date, passive transponders (PIT tags, Trovan Ltd. ID 100-01, <1 g, Douglas, UK) were attached to each transmitter with epoxy. The mouth of the cave was outfitted with two 475 × 400 × 40 mm panel antennas (ANT-612 Large Panel Antenna, Trovan Ltd., Douglas, UK). Panels were placed so that their detection range exceeded the diameter of the cave entrance.

Large differences between cave T_a and normothermic T_{sk} made arousals obvious in T_{sk} traces, and we followed Jonasson and Willis (2012) and Czenze et al. (2013) to define phases of torpor and arousal. We defined torpor bouts as periods of reduced stable T_{sk} between the obvious warming and cooling phases associated with each arousal. Occasionally, Holohil BD-2NT transmitters exhibit a gradual increase in temperature after initial entry into a torpor bout, which appears to be an artefact (e.g. Jonasson and Willis 2012; Czenze et al. 2013, Fig. 1) as it does not occur for other types of temperature data loggers attached to hibernating bats (e.g. Warnecke et al. 2012; Reeder et al. 2012). We could readily identify this artefact in our T_{sk} traces, and it did not affect our analyses. Occasionally, T_{sk} also appeared to decline and was occasionally undetectable during deep torpor bouts, especially late in the season presumably as battery power declined (Fig. 1). However, signals were always detectable during arousals so this artefact also had no effect on our analysis. We followed Jonasson and Willis (2012) and classified the warming phase as an abrupt increase in torpid T_{sk} that eventually stabilized at a normothermic level that was readily distinguishable from warming or cooling phases of a torpor bout. The cave's cold T_a appeared to increase ambient cooling of external transmitters, leading to relatively low T_{sk} values during arousal (Willis and Brigham 2003), but this effect was similar across individuals and did not interfere with our ability to detect arousals. The normothermic period was defined as the time between the end of the warming phase and the initiation of cooling which was readily obvious as an abrupt decline in T_{sk} preceding steady-state torpor. We excluded data from the first 24 h after capture to avoid the influence of this disturbance on our results.

All analyses were conducted in R version 2.10.1 (R Development Core Team 2009). Values are reported as the mean ± SD with N equal to the number of animals and n equal to the number of events/arousals. We recorded the date and time of each arousal from the T_{sk} trace and converted time of arousal from minutes before or after sunset into radians. We then used Rayleigh's test for circular distributions to determine if the daily distribution of arousal times was significantly different from random. We used an

Fig. 1 Representative T_{sk} traces for six little brown bats (*Myotis lucifugus*) in Dale's Cave in Central Manitoba. *Dotted lines* represent cave T_a. Break in the *x*-axis represents a period between 10 February and 12 March when we were not able to record data due to equipment failure. The gradual decline in torpid T_{sk} represents transmitter battery drain and did not affect our analysis because arousals could still be detected. *Dashed lines* represent periods when we lost the transmitter signal due to transmitter battery drain
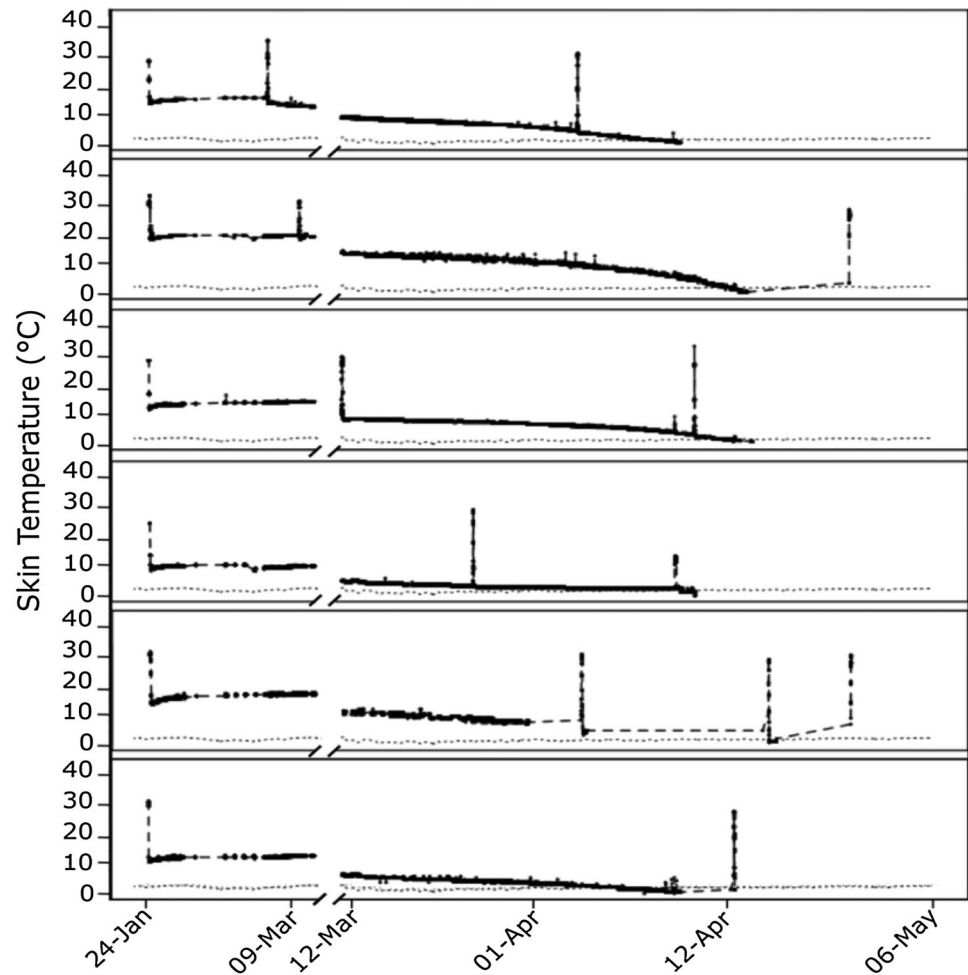
iterative process to determine if arousal timing shifted from a random pattern during mid-winter to a pattern of clustered arousals around the time of sunset during late winter/spring. We began by running Rayleigh's test on the first ten arousals we recorded after tagging the bats on 24 January 2013 (i.e. Arousal 1 to Arousal 10). We then shifted our analysis window forward by one arousal (i.e. Arousal 2 to Arousal 11) and re-ran the test. We continued this process until our final analysis window included the last 10 arousals we recorded at the end of the winter. This allowed us to investigate potential changes in temporal patterns of arousal from torpor.

To determine which bat-specific variables best predicted emergence phenology, we used a general linear model (GLM) with hibernation period as the response variable and sex and body condition index [BCI; body mass (g)/forearm length (mm)] at capture as predictors. We defined hibernation period as the number of days after 15 September 2012 [i.e. the average recorded immergence date for little brown bats in our study area (Norquay and Willis 2014)]. We used our Hobo data loggers and archived climate data from the Grand Rapids weather station 37 km from Dale's Cave

(53°11′N; 99°16′W) (National Climate Data and Information Archive 2013) to obtain values of weather and microclimate variables that we predicted might influence emergence timing: cave T_a (T_{cave}), which will be buffered and much less variable compared to outside T_a but could provide a reflection of outside conditions that bats can assess (Perry 2012); and changes in barometric pressure (BP), which reflect passing weather fronts and have been shown to correlate with both insect and bat activity (Paige 1995; Turbill 2008). In our small cave, BP should rapidly equilibrate inside and outside the hibernaculum (Pflitsch et al. 2010) and could be used by bats to predict whether insects might be available (Paige 1995; Turbill 2008). We used a GLM including several temperature and BP variables that we predicted a priori should be most relevant to emerging bats: (1) mean daily T_{cave} for the 24-h period before each night when at least one bat emerged (mean T_{cave}); (2) 24-h change in daily mean T_{cave} prior to nights when at least one bat emerged (ΔT_{cave} = mean T_{cave} on Day 1 − mean T_{cave} on Day 0); and (3) 24-h change in daily mean BP prior to nights when at least one bat emerged (ΔBP). Prior to analysis, we tested for normality with Shapiro–Wilks tests and

homogeneity of variances with Levene's test. We assessed significance at the $P < 0.05$ level.

Results

Body mass of all bats captured on 24 January 2013 was 9.4 ± 1.1 g, and BCI was 0.25 ± 0.02 g/mm ($N = 22$ males, 8 females). Males (9.1 ± 0.7 g, $N = 22$) were lighter ($P = 0.02$, $t = 2.9$, $df = 9$) than females (10.5 ± 1.2 g, $N = 8$), and average BCI of males (0.24 ± 0.02 g/mm, $N = 22$) was lower ($P = 0.02$, $t = 2.8$, $df = 10.2$) than females (0.27 ± 0.03 g/mm, $N = 8$).

We recorded 1276.8 bat days of T_{sk} data from 27 individuals over 81 days (24 January–10 February, 22 February–24 February, 12 March–13 May) following transmitter attachment (mean 48.2 ± 18.5 days per bat). Gaps in the data occurred during deep snowfalls when we could not access the system, snow accumulated on the solar panels and the SRX600 battery failed. During periods when we did record un-interrupted T_{sk} data, bats exhibited long bouts of torpor averaging 20.07 ± 14.55 days with the longest continuous bout lasting 60.25 days (Fig. 1). Torpor bouts were interspersed with periodic arousals averaging 190 ± 90 min (Fig. 2). We recorded 46 arousals before the final transmitter battery failed on 13 May 2013.

We detected a clear shift in the timing of arousals relative to sunset (Fig. 3). Prior to 7 April (i.e. 25 January–6 April), 92 % (11/12) of the 10-arousal analyses, we performed indicated that arousal timing was not significantly different from a random distribution. After 7 April (i.e. between 7 April and 13 May), 100 % (13/13) of our 10-arousal analyses indicated that arousal timing was significantly different from a random distribution (Fig. 3). We, therefore, combined all arousals prior to 7 April and all arousals after 7 April for two subsequent analyses. Before 7 April, bats aroused within 5.3 ± 3.6 h of sunset, and the circular frequency distribution for the timing of all arousals relative to sunset was not significantly different from a random distribution (Fig. 4a, b, $Z = 2.02$, $P = 0.13$, mean vector = 0.31, $N = 17$ bats, $n = 21$ arousals). We recorded multiple arousals from some individuals so this analysis was technically pseudoreplicated, but we still found no relationship when we calculated average arousal time for each individual ($Z = 0.83$, $P = 0.44$, mean vector = 0.24) or limited the analysis to each individual's first arousal ($Z = 1.14$, $P = 0.32$, mean vector = 0.29). In contrast, there was a relationship between arousal times and sunset when we combined all arousals recorded after 7 April during late winter/spring. During this period, bats aroused within 0.6 ± 3.3 h of sunset, and the circular distribution of arousal times relative to sunset was significantly non-random (Fig. 4c, d, $Z = 14.8$, $P < 0.01$, mean vector = 0.77,

$N = 15$, $n = 25$). As above, this analysis included multiple arousals for some individuals, but we still found the relationship when we only used each individual's first arousal during this period ($Z = 10.6$, $P < 0.01$, mean vector = 0.77) or calculated the average arousal time for each individual ($Z = 12.8$, $P < 0.01$, mean vector = 0.84).

Prior to 7 April, 35 % (6/17) of individuals aroused at least once with a conspecific, and about 38 % (8/21) of arousals were at least partially overlapping in time with those of other individuals. Arousals tended to occur closer together in time during late winter/spring compared to winter. After 7 April, the arousals of 80 % (12/15) of individuals were at least partly overlapping with those of at least one conspecific, and 80 % (20/25) of all arousals were at least partially overlapping in time with arousals of other individuals, a significantly greater proportion than that before 7 April ($Z = -2.4$, $P = 0.02$, $N = 46$). Five bats were detected rewarming both singularly and synchronously. Four of these five bats aroused by themselves prior to 7 April but alongside conspecifics after 7 April. We also observed three instances of what we term "arousal cascades" during which one individual aroused followed by more than one additional individual. All arousal cascades occurred during late winter and involved up to eight individuals. Despite apparent arousal synchrony, bats did not necessarily completely synchronize their arousals. During early winter, only 12.5 % (1/8) of the overlapping arousals referred to above were completely synchronized in time meaning that bats would not have maximized thermoregulatory benefits of social thermoregulation. During late winter, the number of arousals which was completely synchronized in time increased but was still less than half of all overlapping arousals (45 %, 9/20).

Males emerged from hibernation 22.7 ± 6.9 days later than females (Fig. 5a, $P < 0.01$, $t = 8.4$, $N = 17$). The effect of an interaction between sex and BCI on hibernation period approached significance ($P = 0.09$), so we examined the effect of BCI for each sex separately. We found no effect for males ($P = 0.41$, $t = 0.8$, $N = 12$ bats), but females with better BCI emerged significantly earlier (Fig. 5b, $P = 0.03$, $t = -3.7$, $N = 5$ bats).

During the emergence period (27 April–16 June), average daily BP ranged from 96.75 to 101.23 kPa, T_{cave} ranged from 2.5 to 4.5 °C, and outside T_a ranged from -15.7 to 26.6 °C. ΔT_a measured outside the cave resulted in a temperature signal inside the cave, reflected by a positive correlation between ΔT_a and ΔT_{cave} ($r = 0.45$, $t = 3.5$, $df = 49$, Fig. 6a) although the range of ΔT_{cave} values was small (Fig. 6a). ΔBP measured outside the cave was negatively correlated with ΔT_a such that rapidly falling BP signalled large increases in T_a ($r = -0.63$, $t = -0.57$, $df = 49$, Fig. 6b), Mean T_{cave} gradually increased throughout the emergence period reflected by a positive correlation

Fig. 2 Representative skin temperature traces from six little brown bats recorded on 15 April 2013. *Dotted vertical lines* represent sunrise and sunset, and *dashed horizontal line* represents cave T_a. *Black horizontal bars* represent dark phase, and *white horizontal bars* represent light phase
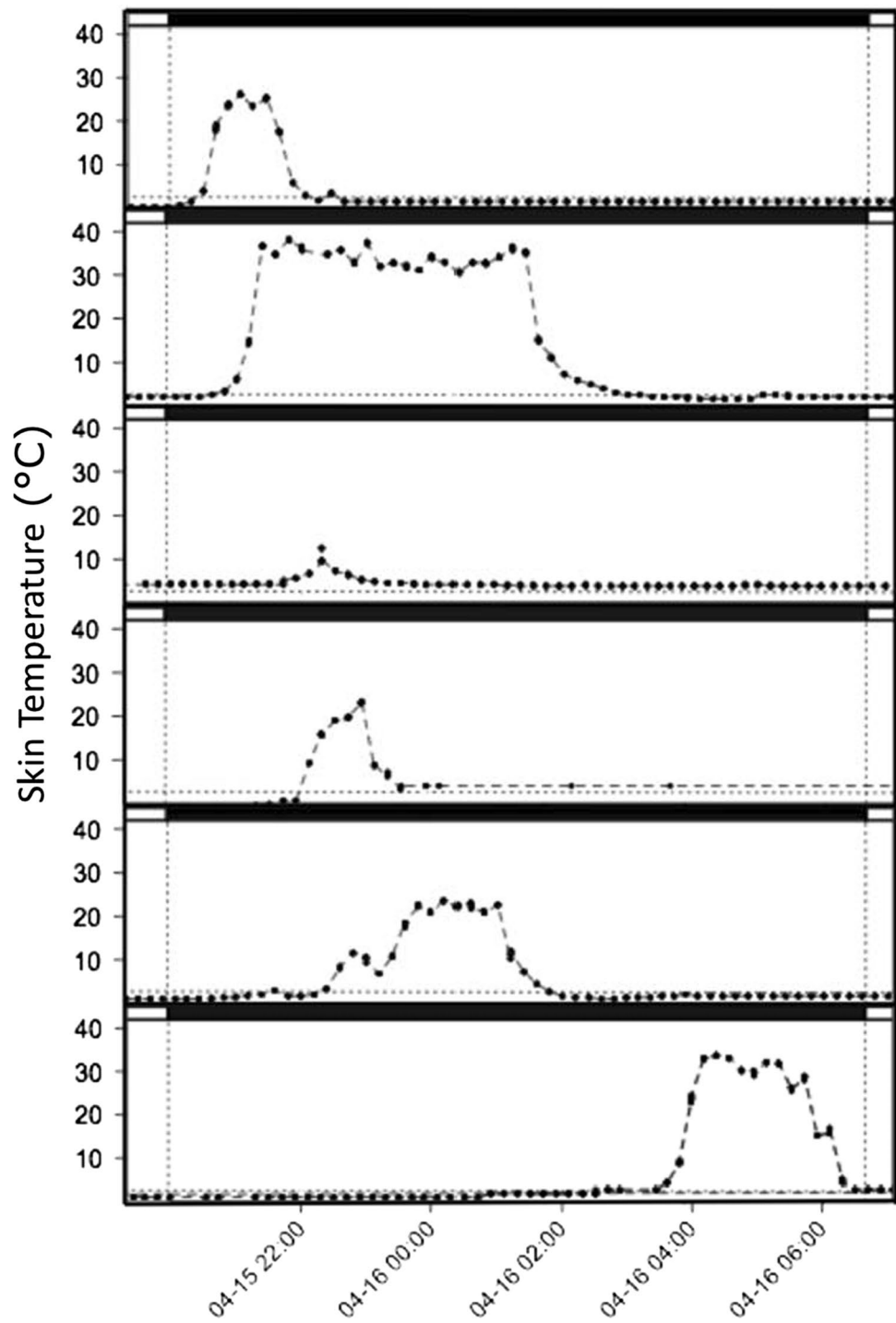
between T_{cave} and hibernation period for all bats ($P < 0.01$, $t = 14.2$, $N = 17$). Interactions between sex and ΔT_{cave} ($P = 0.03$, $t = -2$, $N = 17$) and sex and ΔBP ($P < 0.01$, $t = 5.1$, $N = 17$) on hibernation period were significant so we examined effects of these predictors separately for each sex.

We found no correlation between ΔT_{cave} and hibernation period for females ($P = 0.14$, $t = -1.9$, $N = 5$ bats). Male

hibernation period was positively correlated with ΔT_{cave} ($P = 0.05$, $t = 2.25$, $N = 12$), but the effect was strongly influenced by one outlier and disappeared when we removed this data point ($P = 0.39$, $t = 0.89$, $N = 11$). The effect of ΔBP appeared stronger. All females emerged on days when ΔBP was negative (i.e. falling) and females that emerged latest in the spring (i.e. those with the lowest BCI, see above), tended to emerge on days with the greatest fall
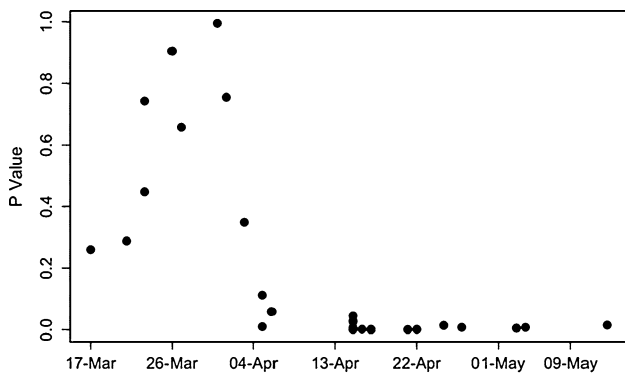
Fig. 3 *P* values generated from repeated analyses testing whether the timing of ten consecutive arousals by hibernating little brown bats, relative to sunset, differed from a random circular distribution. Arousal timing relative to sunset was calculated for each arousal and converted to radians prior to analysis with Rayleigh's test. *Horizontal axis points* represent the time of the 10th arousal in each ten-arousal sample. See "Methods" for details

in BP (Fig. 7a, $P = 0.04$, $t = -3.4$, $N = 5$). Males emerging early in their emergence period, when conditions would have been relatively harsh, also tended to emerge on days with falling BP (Fig. 7b, $P < 0.01$, $t = 4.3$, $N = 12$).

Discussion

We found strong support for the hypotheses that declining energy stores in late hibernation influence arousal timing and arousal synchrony, and that the timing of investment in reproduction influences hibernation phenology. As we reported previously for little brown bats from a different cave a year before this study was undertaken (Czenze et al. 2013), hibernating little brown bats exhibited no circadian pattern to arousal during the coldest months of the year (January–March). Here, we also found that between 24 January and 6 April, bats aroused randomly with respect to sunset suggesting that they were either no longer sensitive to, or had abandoned maintenance of, their circadian rhythm. During this period, mean daily T_a outside the cave ranged from −31.4 to −1.7 °C (National Climate Data and Information Archive 2013), eliminating any opportunity for bats to forage. This could help explain the exceptionally long torpor bout (60.25 days) we observed. Costs of maintaining a circadian rhythm in the absence of an energetic benefit could explain the random timing of arousals with respect to sunset (Czenze et al. 2013). After 7 April, however, bats resumed a circadian pattern with arousals clustered after sunset. Some mammalian hibernators appear to maintain a circadian rhythm of arousals (Zervanos et al. 2009; Grahn et al. 1994; Heller and Ruby 2004; Park et al. 2000; Hope and Jones 2012), but, to our knowledge, this is the first report of a hibernator abandoning a circadian arousal pattern and then later resuming that pattern prior to emergence.

The resumption of the circadian rhythm was closely correlated with increasing air temperature and, during this period, mean T_a outside the cave ranged from −12.2 to 13.2 °C (National Climate Data and Information Archive

Fig. 4 Histograms showing the timing of arousals relative to sunset for little brown bats during hibernation before (**a**) and after (**b**) 7 April 2013. *Clock faces* represent circular distributions of arousals (*dotted line* represents mean sunset time) before (**c**) (mean sunset = 19:02, $Z = 2.02$, $P = 0.13$, mean vector = 0.31, $N = 17$, $n = 21$) and after (**d**) (mean sunset = 20:47, $Z = 14.8$, $P < 0.01$, mean vector = 0.77, $N = 15$, $n = 25$) 7 April 2013

(a)

(b)

P= 0.03, t= -3.7, df= 3
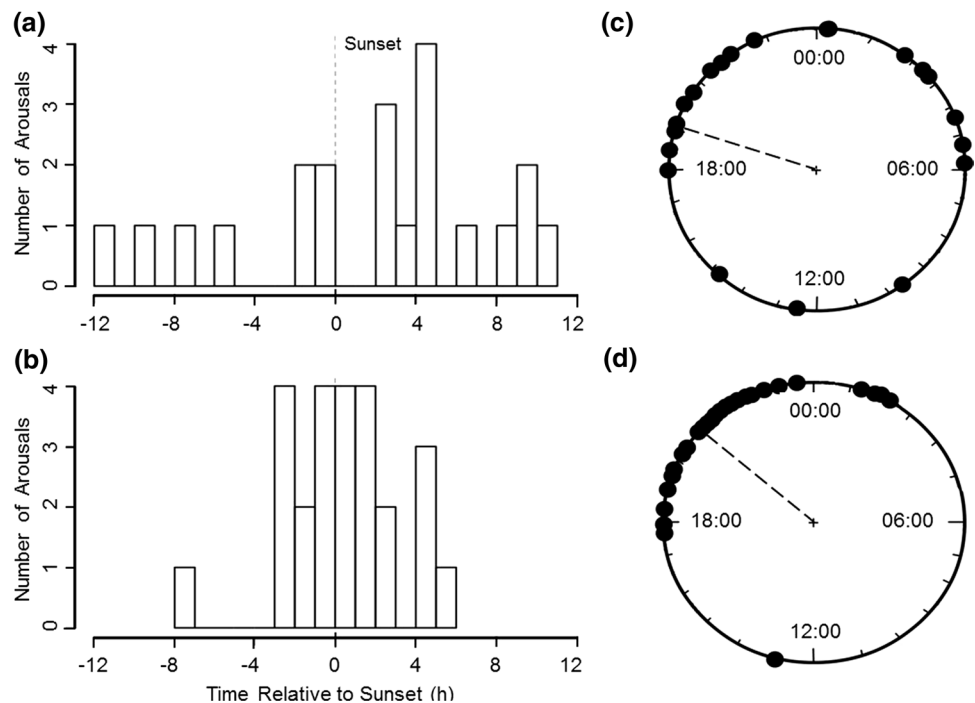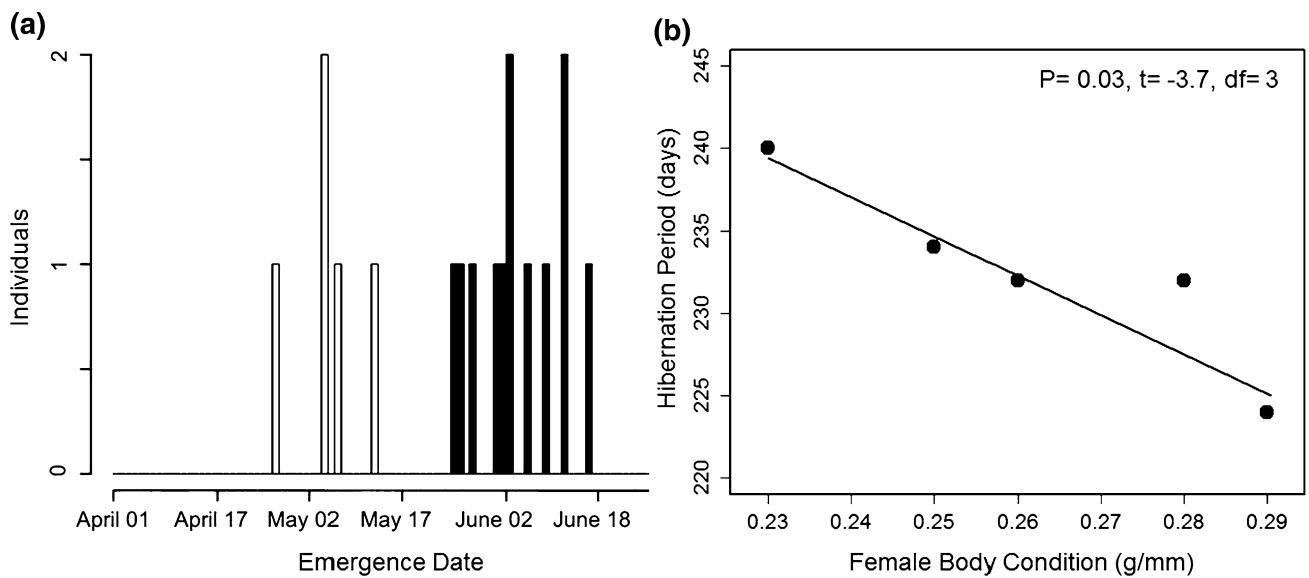
Fig. 5 Histogram (**a**) of recorded emergence dates for individual little brown bats. *White bars* represent females, and *black bars* represent males ($N = 5$ females, 12 males) and **b** Ordinary least squares regression *line* of the body condition [body mass (g)/forearm length (mm)] and emergence timing of hibernating female little brown bats. *Hibernation period* represents the number of days since 15 September 2012

(a)

(b)

P< 0.05, t= 3.5, df= 49

P< 0.05, t= -5.4, df= 49
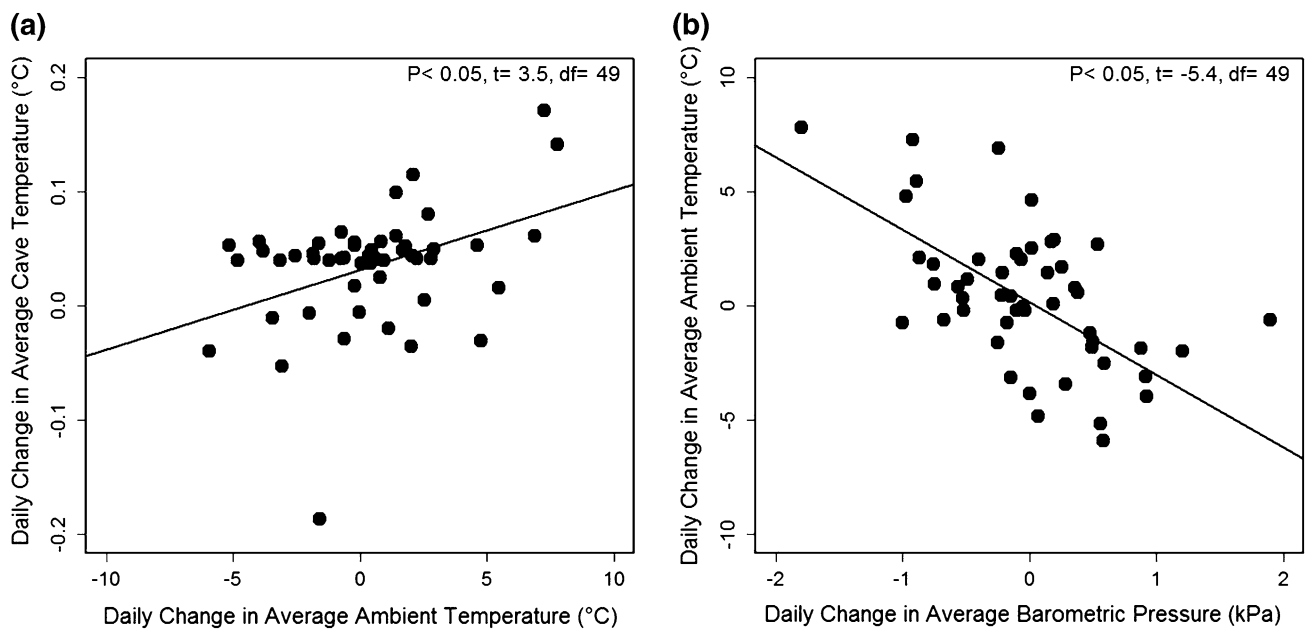
Fig. 6 Ordinary least squares regression of **a** daily change in average ambient temperature and daily change in average cave temperature and **b** daily change in average ambient temperature and daily change in average barometric pressure recorded during the emergence period 27 April–16 June 2013

2013). Although there were many nights during which T_a was below 10 °C, a threshold thought to be predictive of flying insect availability (Paige 1995), on 21 % (7/33) of nights between 7 April and 13 May, outside T_a at sunset exceeded 10 °C. This suggests that, during late winter/early spring, there is at least some opportunity for bats to exploit flying insects and individuals may gain an energetic benefit from the chance they might arouse on a warm evening.

Mechanisms underlying suppression and subsequent resumption of a circadian rhythm could be found in the supra-chiasmatic nucleus (SCN) of the hypothalamus (Ruby et al. 2002). The expression of circadian rhythms in

(a) Female

P= 0.04, t= -3.3, df= 3

(b) Male

P< 0.01, t= 4.3, df= 10
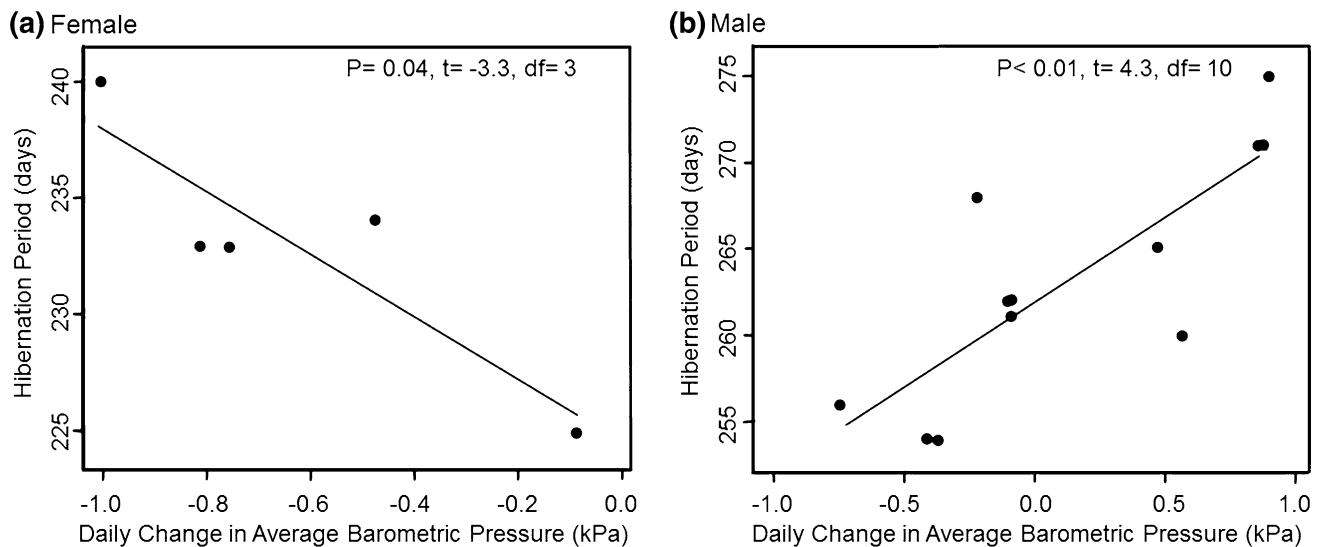
Fig. 7 Ordinary least squares regression *lines* are shown here to illustrate the relationship, which makes up part of a larger model (see "Results"), between the change in barometric pressure (kPa) over 24 h and *hibernation period* for **a** *female* and **b** *male* little brown bats. *Hibernation period* represents the number of days since 15 September 2012

bats may be similar to that of hibernating European hamsters (*Cricetus cricetus*) for which the expression of SCN clock genes is halted during low T_b associated with deep torpor (Revel et al. 2007) possibly because protein synthesis is depressed during long torpor bouts (Storey and Storey 2004). Alternatively, like in arctic ground squirrels (*Urocitellus parryii*), SCN oscillators may still function rhythmically during hibernation, but output pathways may be inhibited even if the animal experiences photoperiod cues prior to emergence (Williams et al. 2012a, b). We observed arousals during the day throughout the winter and, although bats are unlikely to obtain detectable photoperiod cues where they roost in the cave, it is possible photoperiod was detectable especially near the entrance. Thus, bats may have aroused before flying insects are readily available to obtain photoperiod cues and begin the process of resuming their circadian rhythm in time for the active season.

In addition to scheduling arousals around sunset, we also found that bats synchronized arousals with each other, especially after 7 April when the proportion of arousals that were partially overlapping in time nearly tripled. Part of this effect could reflect the fact that bats were synchronizing their arousals with sunset and, therefore, their arousals overlapped in time. However, rewarming at sunset does not necessarily require them to arouse on the same days, and it is unlikely that the arousal synchrony we observed is completely explained by resumption of the circadian rhythm. We hypothesized that synchronized arousals would result from energy limitation during late winter when savings provided by arousal synchrony and huddling could become especially important. For bats to realize potential energy

savings of social thermoregulation, multiple bats should rewarm and remain normothermic at precisely the same times to share the costs of arousal and normothermia. We could not tell which individual bats were huddling together in the same clusters, but we did find that some evidence bats were sharing arousal costs, especially after 7 April when 45 % of group arousals overlapped completely with those of another individual. This suggests that social thermoregulation provides at least a partial, although perhaps not complete explanation for greater arousal synchrony in late winter.

An alternative explanation for arousals by multiple bats at about the same time is disturbance by conspecifics. Some hibernating bats are sensitive to non-tactile disturbance, arousing from torpor when human visitors enter caves (Thomas 1995). Interestingly, Thomas (1995) found that individual bats continued arousing for hours, even after a human visitor left the hibernaculum, which suggests some bats rewarmed because of disturbance by colony mates. Even if only a few bats are sensitive to non-tactile disturbance, their subsequent re-entry into huddles could result in tactile disturbances to huddle mates, un-scheduled arousals, and increased energy expenditure (Speakman et al. 1991; Turner et al. 2015). This suggests that part of the arousal synchrony effect we observed could reflect a maladaptive consequence of colonial hibernation in bats—the potential for increased energy expenditure as a result of disturbance from conspecifics (Turner et al. 2015). Additional studies are needed, potentially using thermal infrared imaging, to help tease out potential costs of disturbance from benefits of social thermoregulation.

In addition to a resumption of a circadian pattern to arousals and increased arousal synchrony among individuals in late winter, we found support for the hypothesis that sex, energy reserves, and environmental conditions influence emergence phenology. Female bats emerged earlier than males, likely to exploit more variable but potentially warmer microclimates in maternity roosts and occasional warm spring nights for foraging, both of which could expedite gestation and, in turn, benefit sub-adult survival (Racey and Swift 1981; Frick et al. 2010). This strategy may be risky, however, as weather during early spring is unpredictable with a high likelihood of cold T_a and spring snowstorms. Despite a small sample size, we found a strong relationship between female BCI and emergence date, with females in the best condition emerging first. Norquay and Willis (2014) reported the same pattern during a different winter, at a different hibernaculum 200 km south of our study site. Females in better condition may be better equipped to tolerate unpredictable harsh spring weather and balance risks of early emergence against the benefit of an earlier start to gestation (Jonasson and Willis 2011; Norquay and Willis 2014).

Emergence phenology differed for male bats. We predicted that males in the best condition should emerge later because larger fat reserves would permit longer hibernation, reducing their risk of predation or harsh spring weather (Stawski and Geiser 2010; Geiser and Brigham 2012; Bieber et al. 2014) and because of relaxed selective pressure for early emergence (Norquay and Willis 2014). We found no significant relationship between BCI and hibernation period even though our sample size for males, while small was larger than that for females. High interindividual variation, combined with a small sample size, may have masked a potentially weak relationship between BCI and emergence phenology of males. We recommend future studies tagging larger samples of male bats to determine potential effects of BCI, and other variables, on emergence patterns of males.

Emergence timing of hibernators can be affected by exogenous cues like winter severity, snow cover, snow depth, food availability, and T_a (Murie and Harris 1982; Inouye et al. 2000; Arlettaz et al. 2001; Schorr et al. 2009; Sheriff et al. 2011). For bats, outside T_a will influence thermoregulatory costs and flying insect availability (Anthony et al. 1981; Negraeff and Brigham 1995). Although hibernaculum microclimates are typically much more stable than conditions above ground, large, short-term (i.e. 24 h) changes in T_a outside a cave could still result in small changes within a cave (Perry 2012) that could be detectable by bats. Outside ΔT_a and ΔT_{cave} were correlated in our study (Fig. 6a). However, the magnitude of ΔT_{cave} values we measured was extremely small (i.e. tenths to hundredths of °C, Fig. 6a), and we found no evidence that bats

used this subtle signal of outside conditions to time their emergence.

Changes in BP, unlike temperature, can quickly equilibrate inside and outside a hibernaculum particularly if the cave volume is small and openings are un-obstructed (Perry 2012; Pflitsch et al. 2010). The magnitude of ΔBP can predict approaching weather fronts (Linacre and Geerts 1997) with falling BP predictive of passing warm fronts, warmer T_a and lower chance of precipitation (Lutgens and Tarbuck 2013). During winter, sub-tropical Australian bats timed periods of activity with falling BP, which predicted warm nights (Turbill 2008), while periods of low BP corresponded with the highest activity of both flying insects and North American tri-colored bats (*Perimyotis subflavus*) (Paige 1995). In our study, falling BP was a strong predictor of increasing T_a (i.e. warm fronts) (Fig. 6b) and could have signalled favourable conditions for emergence to bats. All female bats emerged during warm fronts when BP was falling, and the latest-emerging females, which had the lowest energy reserves and, therefore, may have faced the greatest threat from harsh spring conditions, emerged on days when the drop in BP was greatest (Fig. 7a). Males also appeared to respond to ΔBP. Males that emerged earliest, and thus during periods when the weather was likely to be less favourable overall, ended hibernation during warm fronts when BP was falling. Taken together, our results suggest that BP, but not T_{cave}, provides a signal which bats can detect within a hibernaculum and use to improve the chance of emergence during favourable conditions.

We found that little brown bats abandoned a circadian pattern to arousal during the coldest parts of the winter but resumed this pattern in late winter presumably because of increased opportunity for energy acquisition. Bats synchronized arousals with conspecifics more often during late hibernation which could enhance energy savings via social rewarming or reflect energetically costly disturbance. Despite a small sample, we found that females in the best body condition during winter emerged earliest in spring but that male condition did not affect emergence timing. Both males and females appeared to fine-tune the timing of emergence based on BP which signalled warm fronts and favourable conditions. Our findings highlight adaptations of little brown bats for extreme energy limitation in winter (Jonasson and Willis 2012) while also highlighting the strong influence of sex differences in reproductive timing on hibernation energetics and emergence phenology (Jonasson and Willis 2011; Norquay and Willis 2014).

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