Lack of evidence for a biological alarm clock in bats (*Myotis* spp.) hibernating under natural conditions

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Noting that captive hibernating big brown bats (*Eptesicus fuscus*) aroused from bouts of torpor at precise times of the day (17:00–19:00), Twente and Twente (J. W. Twente and J. Twente. 1987. Can. J. Zool. 65: 1668–1674) hypothesized the existence of a temperature-compensated, non-free-running biological alarm clock that regulated the timing of arousals in bats. I tested this hypothesis in a natural hibernaculum used by little brown bats (*Myotis lucifugus*), northern long-eared bats (*Myotis septentrionalis*), and big brown bats (*E. fuscus*) by recording the timing of periods of activity with an ultrasonic detector and an electronic datalogger. Although there was significantly (2.5–4.0 times) more activity during the night than during the day, the pattern of activity did not conform with that observed in the laboratory. Bats were found to be active at all times of the day and there was little evidence of a concentration of activity around the period 17:00–19:00 as observed in the laboratory. Over three measurement periods in early winter and midwinter, the mean activity times were 18:54 ± 4.5 h, 23:48 ± 5.0 h, and 23:18 ± 5.2 h. The high variation around the mean times indicates that bats aroused and were active at most times of the night. If a biological alarm clock exists in bats, it is only weakly expressed under natural conditions.

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Lorsqu'ils ont constaté qu'en captivité la Sérotine brune (*Eptesicus fuscus*) se réveillait de l'hibernation à des heures précises (17h00 à 19h00), Twente et Twente (J. W. Twente and J. Twente. 1987. Can. J. Zool. 65: 1668–1674) ont émis l'hypothèse que les réveils étaient contrôlés par un rythme endogène. Ce rythme semblait être à la fois indépendant de la température et d'une période rigide de 24 h. Pour vérifier cette hypothèse dans un site naturel d'hibernation utilisé par la Vespertilion brun (*Myotis lucifugus*), la Vespertilion nordique (*Myotis septentrionalis*) et la Sérotine brune (*E. fuscus*), j'ai employé un détecteur ultrasonique muni d'une enregistreuse électronique. Même s'il y avait significativement (2.5–4.0 fois) plus d'activité la nuit que le jour, le schema d'activité ne se conformait pas à celui observé dans le laboratoire. Les chauves-souris ont démontré une certaine activité à toutes les heures de la journée et il y avait peu d'évidence d'une concentration d'activité autour de la période de 17h00 à 19h00. Lors de trois périodes d'observation au début et au milieu de l'hiver, les temps moyen d'activité étaient de 18h00 ± 4.5 h, 23h00 ± 5.0 h et 23h18 ± 5.2 h. La grande variation autour des durées moyennes démontre que les chauves-souris pourraient se réveiller et devenir actives à n'importe quel moment de la nuit. S'il existe un rythme endogène comme celui proposé par Twente et Twente, il n'est exprimé que faiblement dans des conditions naturelles.

[Traduit par la rédaction]

Introduction

Hibernating bats and other mammals arouse periodically through the winter. Thomas et al. (1990) calculated that for the little brown bat (*Myotis lucifugus*) the process of arousal, homeothermy, and reentry into torpor requires about 107.9 mg fat, or the energetic equivalent of about 67 days of torpor. Thus, arousals are energetically very costly, typically consuming 83–90% of the fat reserves used during hibernation (Wang 1978). Clearly, identifying the processes that cause or regulate arousals is central to any understanding of the energy requirements and constraints faced by hibernators.

The occurrence of periodic arousals from torpor by hibernating bats and other mammals remains an enigma. Although in some cases reproductive status may influence the timing and duration of arousals (Barnes et al. 1986, Michener 1983), in most cases the underlying causes are poorly understood. Metabolic rate, evaporative water loss, and endogenous biological rhythms have variously been proposed as factors implicated in the occurrence and frequency of winter arousals in hibernators, but none has received conclusive experimental support (Willis 1982; Thomas and Cloutier 1992).

One factor that appears to have a nearly universal effect on arousals is temperature. Twente and Twente (1965) and Twente

et al. (1985) showed that the frequency of arousals decreased as both ambient and body temperature declined. While this does not help to identify which physiological processes underlie arousals, it shows that the arousal process is not normally independent of internal and external conditions.

In contrast, however, Twente and Twente (1987) provided evidence for the existence of a persistent endogenous temperature-independent biological "alarm" clock in hibernating big brown bats (*Eptesicus fuscus*). Under constant laboratory conditions, arousals of *E. fuscus* were tightly clustered around dusk (17:00–19:00) and they remained so over varying temperatures (2–16°C), long periods of torpor (up to 38 days), and numerous successive arousals. The timing of arousals did not appear to be correlated with either exogenous factors such as barometric pressure or sound, or with endogenous factors such as cardiac rhythm or autonomic nervous activity. The existence of a temperature-independent biological rhythm that is not free-running is extremely unusual and raises the question of whether these results are attributable to laboratory conditions.

The purpose of this study was to determine whether there was evidence of a similar tight regulation of the timing of arousals in a natural hibernating population of bats. If arousals

Feb. 07 - Feb. 28

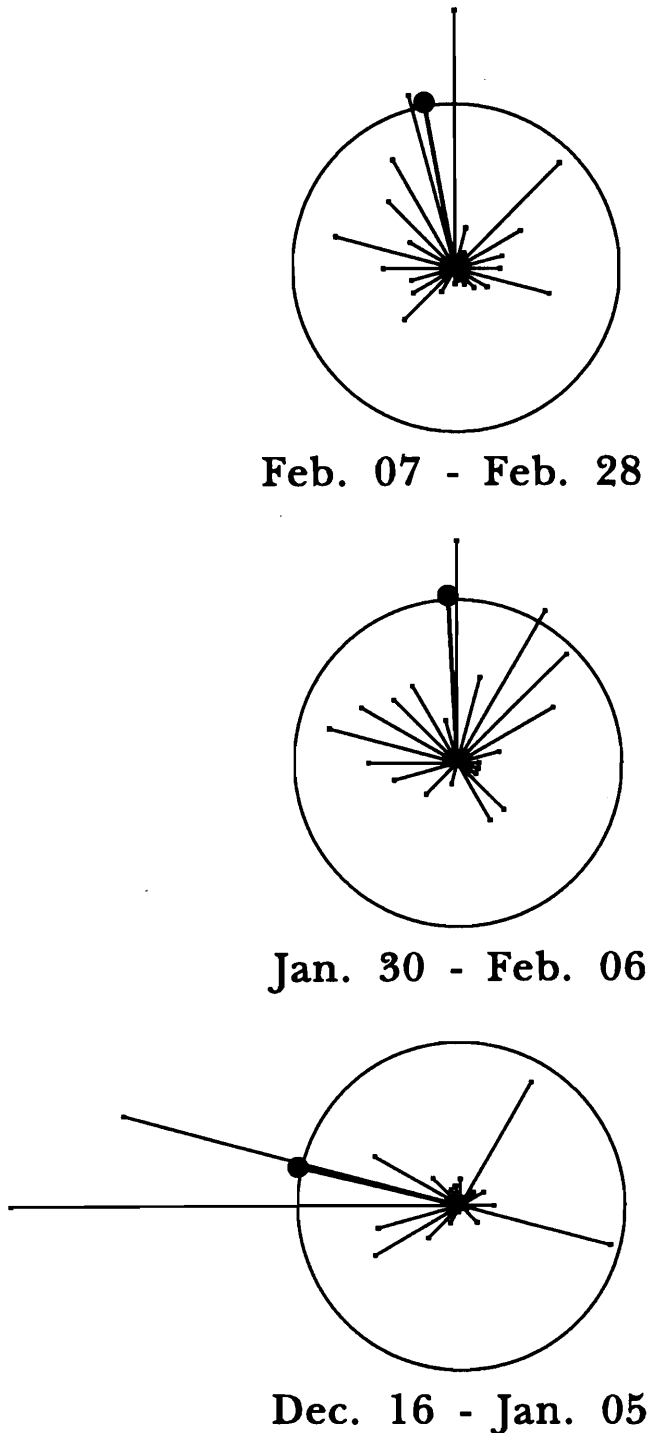## Jan. 30 - Feb. 06

Dec. 16 - Jan. 05

FIG. 1. Temporal distribution of bat activity over a 24-h day during three sampling periods at a bat hibernation site. The day is portrayed on the 24-h clock with 24:00 and 12:00 at the top and bottom, respectively, of each circle. The length of each line represents the percentage of the total activity occurring at each hour. The distance to the perimeter of the circle indicates 10% of the total activity. The mean activity time is indicated by the line joined to the solid circle.

were controlled by an endogenous biological alarm clock, I predicted that a hibernating population would exhibit a marked peak in activity associated with arousals between 17:00 and 19:00 as was observed by Twente and Twente (1987) in the laboratory.

Materials and methods

This study was conducted between 14 December 1991 and 15 February 1992 at a disused slate mine in Windsor County, Quebec. Bats hibernated in a straight, 280 m long drainage adit having no side passages and where ambient temperatures were $2-5°C$. A census of bats in this mine in 1990 indicated that it was used by approximately 1000 little brown bats (*M. lucifugus*), 300 northern long-eared bats (*Myotis septentrionalis*), and five big brown bats (*Eptesicus fuscus*).

I monitored the timing of activity for the hibernating population, using an automated system consisting of an ultrasonic bat detector coupled with an electronic datalogger. Bats that aroused and emitted echolocation signals in approximately the distal 50% of the mine tunnel were detected by a broadband microphone and "divide-by-10" bat detector (Miller and Andersen 1984; Thomas and West 1989). The output from the bat detector was sent to a simple timing circuit that signaled an event to a Trailmaster 1500 datalogger (Trailmaster, Lenexa, Kans.) and then remained unresponsive for 15 s. In this way, the datalogger recorded the date and time of any echolocation signal that would signify an arousal, but it did not trigger on each individual call in an echolocation sequence. This detector system did not discriminate between bat species, although the majority of detections were likely to have been of *M. lucifugus* because of the dominance of this species in the hibernating population.

I placed the detector system in the mine on 16 December, 30 January, and 7 February (hereafter referred to as periods 1, 2, and 3), and left it undisturbed for the following $7-21$ d to register bat activity. At the end of each period there were no new tracks in the snow at the mine entrance, indicating that there was no human disturbance of the hibernating population apart from my own visits. At the end of each period, data were down-loaded to computer for analysis. To analyse the activity pattern over a 24-h period, I used angular statistics described in Zar (1984). The mean time of activity is calculated from the mean angle where the 24 h of the day are converted to circular angles. The resultant (r) of the mean time is a measure of the concentration of data around the mean. Variation around the mean time is presented as the circular standard deviation, an equivalent of the parametric standard deviation.

Results

Initial inspection of the data for the three periods indicated that my brief entry into the mine provoked a large number of arousals and that activity remained abnormally high for up to 24 h. Therefore, I considered only data collected >48 h after a visit. Sample sizes used in these analyses were 564, 76, and 445 detections which were collected over 18, 5, and 19 d for periods 1, 2, and 3, respectively.

Figure 1 shows the distribution and mean time of activity over a 24-h day. In all three periods, bat activity was non-randomly distributed through the day (Watson's U^2-test: $U^2 > 5.1$; $P < 0.01$ for all periods). Dividing the 24-h day into night ($18:00-06:00$) and day ($06:00-18:00$) confirmed a nonrandom distribution ($\chi^2 > 27.0$, $P < 0.001$ for all three periods). On average, activity was 3.1 times higher at night than during the day (range $2.5-4.0$).

The dispersion of activity over the 24-h day, however, was extremely high. The resultants (r) for the mean time of activity were only 0.50, 0.43, and 0.40, respectively, for the three periods, which indicates little concentration of activity around any one time. Indeed, some activity was measured in all hours of the day. The mean times of activity for the three periods were 18:54 ± 4.5 h, 23:48 ± 5.0 h, and 23:18 ± 5.2 h. This means that in all three periods activity occurred throughout most of the night and was not concentrated in any one time period.

Discussion

In this study, I used the incidence of echolocation calls to indicate the presence of active (= aroused) bats. Although I have no direct proof that all bats echolocated when they aroused, I believe that most did owing to the low light levels. Most, if not all, bats that flew and echolocated in the mine tunnel would be detected so there are no obvious biases in the data collection. Although this method differed from that used by Twente and Twente (1987), it should be sensitive to any synchrony in the timing of arousals. If bats aroused at a fixed time of day, this would result in a peak in detections at this time. Bats remain homeothermic and potentially active for only 1.5 h following arousal (Twente and Twente 1987), so this should result in a only minor spreading of the peak in activity.

These results do not support those of Twente and Twente (1987), based on a laboratory study of *E. fuscus*. Twente and Twente (1987) found that arousals were extremely concentrated around 17:00 – 19:00 as was indicated by r-values >0.90 for 16 individuals held at 5°C. In the laboratory, none of these bats aroused later than 21:48 or earlier than 13:54. In contrast, bats hibernating in a natural hibernaculum aroused and were active at all times of the day, although activity tended to occur more frequently at night. The low r-values (0.43 – 0.50) and the high circular standard deviations (4.5 – 5.2 h) together indicate that arousals and subsequent activity were generalized throughout the night.

These results suggest that if the temperature-compensated, non-free-running biological rhythm described by Twente and Twente (1987) exists, it is not expressed to a high degree under natural conditions. This places in question how accurately laboratory studies of hibernation and arousal reflect the natural situation. While a general tendency to arouse does occur in the field, the temporal patterning is often different from that described from laboratory studies. For example, the mean duration of torpor bouts for hibernating *Spermophilus columbianus* was 56% longer under natural conditions (27.7 d) than was observed in the laboratory (17.7 d; Twente and Twente 1965; Young 1990; see also Wang 1978). Similarly, the mean duration of torpor bouts for *M. lucifugus* were 55% longer in natural hibernacula (19.7 d) than in the laboratory (12.7 d; Brack and Twente 1985; Twente et al. 1985). Also, in the field, *S. columbianus* did not exhibit the progressive shortening of torpor bouts at the end of the winter that is characteristically seen in the laboratory (Pengelley and Fisher 1961; French 1986; Geiser et al. 1990; Young 1990). Clearly, more field data are required before the temporal patterns of hibernation and arousal and, hence, of winter energy expenditures can be characterized.

It remains, however, that bats did tend to be more active during the night. It may be that bats arouse at all times of the day, yet are more likely to remain active for longer periods during the night. This pattern would correlate well with observations that some bats move between hibernacula and may even feed during the night when conditions permit (Avery 1985; Whitaker and Rissler 1992).

Acknowledgements

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