HIBERNATION OF THE EASTERN PIPISTRELLE, *PERIMYOTIS SUBFLAVUS*, IN AN ABANDONED MINE IN VERMILLION COUNTY, INDIANA, WITH SOME INFORMATION ON *MYOTIS LUCIFUGUS*

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ABSTRACT. We studied the number of hibernating eastern pipistrelles, *Perimyotis subflavus,* in Copperhead "Cave," an abandoned mine in Vermillion County, Indiana, just across the river from Montezuma, Parke County. The number of bats increased through early December, remained high through April, then declined through May. A few bats remained at the same spots in the mine for up to 18 weeks or throughout the entire winter, but we also observed numerous short bouts of torpor. Pipistrelles usually hibernated singly (observed 3628 times), but were in pairs 108 times, triplets 10 times, and 4 bats were together on one occasion. Winter mass losses averaged 2.65 grams for males and 2.50 g for females. Pipistrelles generally hibernated in different chambers than did *Myotis lucifugus.* Few (5) pipistrelles emerged from the mine in winter (December through February) and few exited in spring (0.4 per night) until May. In fall, they were active through October, with the end of the major swarming in mid-October.

Keywords: Hibernation, Eastern Pipistrelle, *Perimyotis,* bats, mines

INTRODUCTION

Bats, like other mammalian hibernators, arouse periodically between torpor bouts during hibernation (Folk 1940; Krzanowski 1959; Daan 1973). During arousal, bats fly within and occasionally leave hibernacula (Guthrie 1933; Hooper & Hooper 1956; Mumford 1958; Tinkle & Patterson 1965; Daan 1973; Whitaker & Rissler 1992*a,b*).

Individual pipistrelles, *Perimyotis subflavus,*[2] usually hibernate in relatively small numbers in caves and mines, and they use more caves and mines for hibernation than do other species of bats in the eastern United States (Brack et al. 2003, 2004). Hibernacula are apparently usually within about 100 km of the summer roosting area, as the longest migration recorded for this species was 85.0 km (Griffin 1940). Brack and Mumford (1984) showed that pipistrelle distribution in Indiana was coincident with the southern edge of the Wisconsinan glaciation and the northern edge of the cave region, which suggests that the presence of suitable hibernacula may influence geographic range in this species.

Males and females hibernate in the same caves and mines, although most individuals are solitary during hibernation (Guthrie 1933; Hitchcock 1949; Hall 1962; McNab 1974). Mumford and Whitaker (1982) found eastern pipistrelles hibernating in contact with another bat in only one instance. Laval and Laval (1980) suggested that pipistrelles are the earliest bats to enter hibernacula in autumn, and the last to emerge in spring.

Brack and Twente (1985) marked bats with fluorescent paint and then recorded their individual locations three times per week between December and March for 2 years. They found that pipistrelles were more variable in the length of time spent at one location than big brown bats, *Eptesicus fuscus,* or little brown myotis, *Myotis lucifugus.* Brack and Twente (1985) recorded 20 instances of bats spending greater than 50 days in the same spot, three over 98 days, and one of 111 days in their sample of 155 individuals, although undetected movements could have occurred. Harden and Hassell (1970) and Daan (1973)

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[2] *Pipistrellus subflavus* has now been placed into its own genus, *Perimyotis* Menu, 1984 (Hoofer and Van Den Bussche 2003; Hoofer et al. 2006).

likewise suggested that bats may arouse, move, and return to the same location. Twente et al. (1985) reported that individual pipistrelles hibernating at 5 °C stayed in one place for time periods of up to 27 days. They concluded that bats tended to employ longer torpor bouts at lower temperatures. Menaker (1964) noted that of six periods of hibernation at 3–5 °C, 26 individuals hibernated for less than 5 days, whereas two hibernated more than 80 days.

Mean ambient temperatures (T_a) to which pipistrelles are exposed during hibernation are influenced by latitude: 6.8 °C in Minnesota (Swanson & Evans 1936) to 14–18 °C in some caves in Florida (Rice 1957). Rabinowitz (1981) argued that both high and low temperatures were avoided. McNab (1974) argued that these bats choose the warmest temperatures, which are different depending on the geographic location of the hibernaculum. Pipistrelles have been consistently recorded hibernating at temperatures higher than those used by the little brown myotis. Hall (1962) and Henshaw & Folk (1966) observed that little brown myotis hibernate between 1–13 °C. They usually occupy deeper parts of caves or mines where temperatures are relatively stable (Hitchcock 1949; Hall 1962; Fitch 1966). Given their small size, it is not surprising that the hibernation strategy used by the eastern pipistrelle is to seek out areas with stable temperatures, where they can remain in prolonged torpor (Davis 1964; McNab 1974). Fitch (1966) found that male eastern pipistrelles had a steady decrease in mass of 39% between September and April, and that females had a decrease of 29% from September to March.

Most caves and mines in Indiana contain a few eastern pipistrelles in winter, but Copperhead "Cave," actually a long abandoned mine, has one of the largest hibernating populations in the state (Brack et al. 2003, 2004; Whitaker & Rissler 1992*a*). Whitaker and Rissler (1992*a*) found that Copperhead mine serves as a hibernaculum for approximately 200 eastern pipistrelles, 130 little brown myotis, and 900 northern myotis, *Myotis septentrionalis*. They assessed activity at the mouth of the mine for 15 months (including 2 winters) using weekly harp trapping and found little movement of pipistrelles in and out during winter. They captured only five individuals from 30 October to 17 April, 1989–1990, and two individuals from 19 October to 5 April, 1990–1991. Northern myotis and little brown myotis were much more active during these time periods, with 91 little brown myotis captured in 1989–1990 and 419 in 1990–1991, and 474 northern myotis in 1989–1990 and 210 in 1990–1991. The absence of pipistrelles at the mine entrance in winter suggested much less inter-cave, and possibly intra-cave, activity than by the little brown myotis during the hibernation period.

Because of Copperhead mine's large hibernating population of pipistrelles, it provides a useful opportunity to learn more about the behavior and activity of this species during hibernation and to address questions from the literature about hibernation in pipistrelles and bats in general.

Our objectives were to: 1. Quantify the timing of hibernation onset, the timing of increasing, population peak and decreasing population size, and the timing of the end of hibernation. 2. Determine the length of time pipistrelles remained in specific roost sites within Copperhead during hibernation. 3. Assess clustering during hibernation. 4. Quantify winter mass loss by comparing the mass of bats entering the mine in fall with those exiting in spring. 5. Compare T_a at pipistrelle hibernation sites within Copperhead with that of sites used by the little brown myotis.

METHODS

Copperhead "Cave" is actually an abandoned clay mine in a bluff, 1 km west of the Wabash River and 1.5 km northwest of Montezuma, Vermillion County, Indiana (St. Bernice Quadrangle N 39°48′08″ W 87°23′17″; elevation 235 m). Bats were studied from 7 October 1994 to 17 June 1995, and from 6 September 1995 to 3 May 1996. The "growing season" in this locality extends from early May to early October. There are no natural caves in the area. The mine is 120 km northwest of the unglaciated cave country of southern Indiana (Whitaker & Rissler 1992*a,b*). The entrance is 1.4 m high and 2.5 m wide, and opens into a series of chambers occupying approximately 400 m². The first six rooms, and passages connecting them, were used for the study (Fig. 1). The three back rooms were not studied because of safety concerns. Data collection stopped in spring when females left for maternity colonies. Data were taken approximately once per week the first year, and

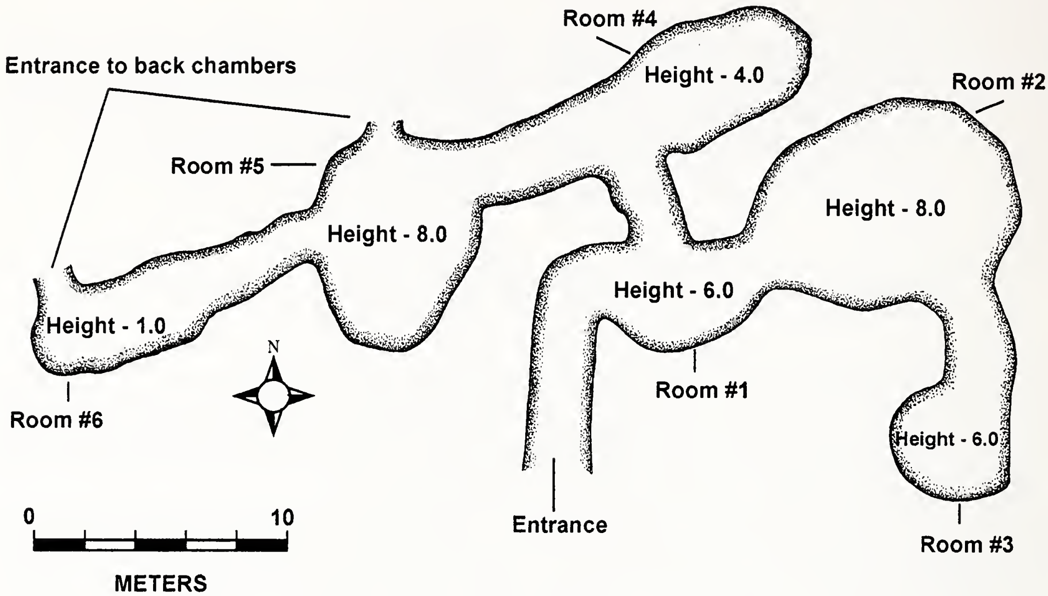

Figure 1.—Copperhead Cave, an abandoned mine in Vermillion County, Indiana. Heights at maximum points in meters.

every third week the second year. Less frequent visitations in the second year was an attempt to determine if frequent observation affected the arousals, as reported by Thomas (1995). On each visit we captured bats at the mine entrance in a double frame harp trap. The area around the edge of the trap was covered with bird netting to force bats through rather than around the trap. The trap was continuously tended dusk to midnight. We recorded species, gender, reproductive status, and mass of each bat captured. All bats were released unharmed.

To gather data on movements of pipistrelles inside the mine, we painted white numbers on the mine wall immediately below and as close to the hibernating bats as possible, and recorded these locations on maps. We observed each spot on every visit to determine if bats continued to use these locations. The T_a was recorded at the mine wall near the bats using a Taylor pocket thermometer. From 5 January 1995 through the end of the study, numbers were placed under some little brown myotis at the same time and in the same manner in order to compare the roost T_a selected by the two species. Our definition of group behavior or clustering was that bats had at least part of their body in contact with one or more bats.

We tested whether mean T_a, T_a stability, and T_a range were correlated with numbers of bats in each chamber to determine which factors influenced bat numbers. Temperature stability for a given room was defined as the number of times the temperature fluctuated between visits within a room during the time period of the study. Total stability for each room was calculated by counting the number of times a temperature occurred in the room and multiplying it by designated rank (the lowest rank number, 1, was assigned to the temperature that occurred most often in that room). For example, in Room 1, 12 °C occurred seven times, therefore was multiplied by 1 (rank). These values were then added, to establish an overall stability value for each room. Room 3 had the lowest stability scores of 16 (calculated based on Kendall's ranking procedure) in the first year and 8 in the second year, and was therefore the most stable of all rooms during both years. Room 4 had ranks of 21 and 10, the second lowest. We used Kendall's coefficient of concordance (Zar 1984) to determine how average temperature, range, and stability were related to numbers of bats in each room. Rooms with the most stability were determined by removing variables systematically and testing for significance of remaining variables.

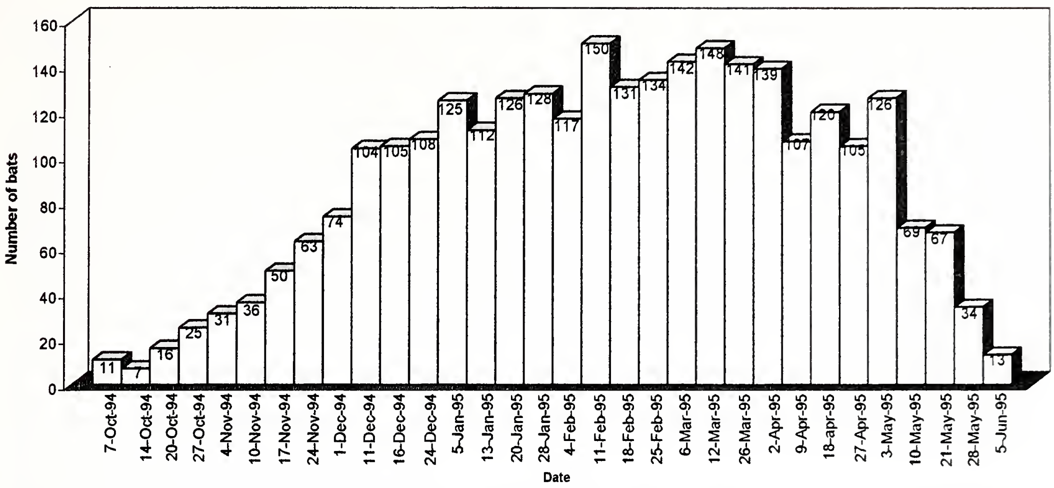

Figure 2.—Total number of *Pipistrellus subflavus* within Copperhead, an abandoned mine for 1995–1996, located in Vermillion County, Indiana.

RESULTS

We caught 119 eastern pipistrelles in the bat trap during the two years, and our capture rates were similar to those in a previous study at the same mine (Whitaker and Rissler 1992*a*). Eastern pipistrelles were common at the entrance in August, and significantly decreased in 1994–95 from September through December (χ^2 = 51.8, 3 df). Unlike little brown and northern myotis which flew in and out of the entrance throughout the winter, only five pipistrelles were caught from 1 December to 31 March during the 2 years of study. Few pipistrelles exited until May in either year, averaging 0.4 per night in April and 7.3 per night in May.

Hibernation onset.—Bats caught in the harp trap but especially numbers observed in the mine (Fig. 2) were used to determine the timing of hibernation onset and the gradual increase in numbers of hibernating bats. No trapping was done in August, but in September, numbers averaged 8.0 per night, falling to 6.6 in October. The major swarming period ended in mid-October.

Eleven eastern pipistrelles were found in the mine on 7 October in year 1, when data collection started (Fig. 2). Numbers then fluctuated throughout the winter but increased to 125 by 5 January and peaked at 150 on 11 February. They steadily declined until all had left the mine for the summer (Fig. 2).

The data for the second year were not presented, since observations were made every third week. However, they showed a similar pattern. In the second year, 3 bats were present on 6 September, and this number increased to 139 bats by 7 February. There were fewer fluctuations recorded than in year 1, but that was clearly due to less frequent visits.

Duration of time bats spent in one place.—Bats did not show strong long-term fidelity to specific roost spots within the mine. Often bats were in a roost site for only one observation. This occurred 1153 times. Many individuals were marked, 1103 were seen in the same position one week later, 277 were seen two weeks later, 254 were in place for 3–5 weeks, 54 for 6–9 weeks, 23 for 10–12 weeks, 13 for 13–17 weeks, and 7 for 18–25 weeks. The longest periods were 23 and 25 weeks (1 bat each). It is likely, of course, that sometimes a bat awoke then came back to the same position, or that one bat replaced another.

Decline in numbers of bats with approaching spring.—In year 1, there was an initial spring decline of 32 bats between 2 April and 9 April, followed by fluctuations from 18 April to 3 May, prior to the large decline (54.8%) between 3 May and 10 May (Fig. 2). In the second year, the largest decline was between 10 April and 1 May (N = 63 bats; 63%), indicating that most bats were leaving for summer roosts. No pipistrelles were caught in the trap on 10 April, suggesting that most bats left after this date, as compared with the next trap date, 9 May, when 4.25 pipistrelles per hour were caught. For both years combined, April averaged 0.4 bats per night and May 7.3 bats per night. Thus, pipistrelles exited the mine at the end of April through May.

Clustering during hibernation.—We found eastern pipistrelles in hibernation 3747 times. Most hung singly (N = 3628 ; 96.8%), but pairs were observed 108 times, 3 bats 10 times, and 4 bats once. In contrast, little brown myotis were found hibernating singly in Copperhead Mine on 957 occasions (43.9%), and in groups of up to 15 individuals.

Overwinter mass loss.—Male pipistrelles exhibited a significant average mass loss between fall (October) and spring (May), at about 2.65 grams (t = 3.14, df = 57, $P >$ 0.01). Females showed a similar loss of about 2.5 grams (t = 2.49, df = 34, $P >$ 0.02).

Roost temperature preference.—Pipistrelles were found more often in rooms 3, 4, and 5 during both years (Table 1). The number of eastern pipistrelles per room/per visit, in year 1, from highest to lowest was Room 4 ($\bar{x}$ = 32.4), Room 5 ($\bar{x}$ = 29.4), Room 3 ($\bar{x}$ = 19.0), Room 2 ($\bar{x}$ = 10.4), and Room 1 ($\bar{x}$ = 4.6). In temperature stability, again highest to lowest, they were Room 3 (stability value = 16), Room 4 (21), Room 1 (24), Room 5 (24), and Room 2 (33). There was a significant influence by temperature variables in the rooms (χ^2 = 14.82, df = 5, $P <$ 0.025) for year 1. Rooms with the most stable temperatures tended to harbor the greatest number of pipistrelles (Table 1). The variables of stability and range, combined, as compared to number of bats within rooms, were significant (χ^2 = 11.55, df = 5, $P <$ 0.05). Range of temperature had the second most influence in bat numbers within rooms.

Temperature variables did not influence sites used by little brown myotis, but percentages for both years show that little brown myotis more frequently hibernated in Room 2 (Table 1). Between January and May 1995, 73.5% of little brown myotis hibernated in Room 2. This value was 87.0% in 1996. Mating of eastern pipistrelles occurs mostly in fall and spring (Fugita & Kunz 1984). However, eastern pipistrelles were observed copulating on three occasions in the hibernacula: 18 and 28 February, and 16 March.

DISCUSSION

Numbers of pipistrelles increased in the mine in the fall, reached highest numbers from November through March, and then decreased (Fig. 2). This is logical as bats gather in the fall to swarm and breed; some stay for hibernation, and in spring, leave for summer roosts.

Present data are similar to those of Whitaker and Rissler (1992*a*), although the latter authors did not catch any eastern pipistrelles at the mine entrance past October in 1990, whereas during the present study 1.3 bats per night were caught in both November and December. No pipistrelles were caught by trapping in January, February, or March.

Whitaker and Rissler (1992*a*) found that eastern pipistrelles exited the mine between 27 April and 26 May 1990. They caught bats at a rate of 0.4 per night in March, 3.2 per night in April, and 6.1 per night in May, again similar to data from the present study.

The increases of pipistrelles in the mine in fall and early winter (November and December) did not correlate well with the number of bats caught in the trap. This disparity may arise from several sources. Bats may have been entering and exiting from the back rooms. (Earlier visits indicated pipistrelles did occupy these rooms.) A few pipistrelles may occasionally have been out of sight in cracks and crevices, but most hibernating pipistrelles were easily seen. However, the main reason was probably that many of the bats entering in late autumn/early winter remained in the mine, whereas earlier, many of the bats caught at the entrance probably included many more individuals moving through rather than hibernating in the mine.

Bats and other hibernating mammals arouse periodically during hibernation (Lyman et al. 1982). Thomas (1995) used infrared cameras to determine effects of disturbance on hibernating little brown myotis in a cave. Some individuals aroused and stayed active for 2.5–7.5 hours after he entered a cave containing about 1300 hibernating bats. He indicated that light, sound, and possibly a rise in temperature associated with presence of humans in the hibernacula provoked arousals in a portion of the population of hibernating bats. The addi-

Table 1.—Mean number (±SE) of bats and mean temperature (°C) per day by month (±SE) in Copperhead "Cave," Vermillion County, Indiana.

Month	Number of visits	Rooms	1	2	3	4	5	6	Totals
Oct.	5	mean temp	18	17	14	16	17	16	
		P. subflavus x̄/day	3	1	2	5.2	5.6	0.6	15.6
		M. lucifugus x̄/day	0	2.3 ± 0	0.2	0	0	0	3.4
Nov.	6	mean temp	15 ± 4	17 ± 1	14 ± 0	15 ± 1	15 ± 1	16 ± 0	
		P. subflavus x̄/day	2.2 ± 1	4.3 ± 0	6.2 ± 0.5	17.5 ± 10	15.3 ± 5	2.7 ± 0.5	48.2
		M. lucifugus x̄/day	0	6.8 ± 5.5	0.3	0.2	0	0.5 ± 1.5	7.8
Dec.	5	mean temp	11 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0	
		P. subflavus x̄/day	4.6 ± 0	12 ± 0	18.6	37.4 ± 0	25.8 ± 0	4.8 ± 0	103.4
		M. lucifugus x̄/day	0	6.8 ± 0	0	0.2	0	0.6	7.6
Jan.	5	mean temp	13.8 ± 1.2	13.8 ± 0.6	12	13.5 ± 0.2	13.6 ± 0.3	13.3 ± 0.3	
		P. subflavus x̄/day	6.2 ± 1.7	11.8 ± 1	24.4	41.4 ± 6.9	38.2 ± 4.2	9.2 ± 1.8	131.2
		M. lucifugus x̄/day	4.4 ± 2.7	54.6 ± 5.7	2.6	12.2 ± 3.9	13.2 ± 3.2	0.2	87.2
Feb.	6	mean temp	11.5 ± 0.3	11.8 ± 0.3	11.6	12 ± 0.2	12.6 ± 0.2	13.3 ± 0.2	
		P. subflavus x̄/day	7.8 ± 1.6	16.5 ± 0.8	24.3	44.5 ± 3.3	44.8 ± 3.3	9 ± 1	147
		M. lucifugus x̄/day	12.3 ± 3	94.8 ± 2.6	1.2	1.5 ± 0.8	4.3 ± 1.3	0.2 ± 0.2	114.3
Mar.	5	mean temp	11 ± 0.3	12 ± 0.3	12 ± 0	11.8 ± 0.2	11.7 ± 0.4	11.5 ± 0.4	
		P. subflavus x̄/day	4.8 ± 1.4	15.6 ± 1	28 ± 2.6	44 ± 2.2	44.4 ± 2.2	6.4 ± 1.4	143.2
		M. lucifugus x̄/day	14.8 ± 3	111.2 ± 6	5.4 ± 1.5	1.4 ± 0.4	1.4 ± 1	0	134.4
Apr.	4	mean temp	12.3 ± 0.4	12 ± 0.6	12 ± 0	11.8 ± 0.3	12 ± 0.3	11.8 ± 0.3	
		P. subflavus x̄/day	5 ± 1.1	10.5 ± 1.9	31 ± 0.4	42.3 ± 6	37.5 ± 2	5.5 ± 1.2	131.8
		M. lucifugus x̄/day	11.5 ± 6.4	52.5 ± 35	4.3 ± 0.8	3 ± 1.2	4 ± 2.5	0	75.3
May	2	mean temp	12 ± 0	13 ± 0	12 ± 0	12 ± 0	11.5 ± 0	11.5 ± 0	
		P. subflavus x̄/day	6 ± 2.5	12.5 ± 0.5	24.5 ± 14	23 ± 19	18.5 ± 13	2 ± 2	86.5
		M. lucifugus x̄/day	0.5 ± 0.5	24 ± 4	0	0.5	0	0	25.0
Total numbers of bats per room during the 38 visits									
		P. subflavus							
		Total no.	176	394	721	1228	1118	201	
		No. per day	4.6	10.4	19.0	32.4	29.4	5.3	
		M. lucifugus							
		Total no.	217	1747	68	92	115	8	
		No. per day	5.7	46.0	1.8	2.4	3.0	0.2	

tive effect of arousals provoked by human disturbances may deplete fat stores and reduce chances for survival of individual bats. Thomas (1995) reported that arousals in his study were limited to a relatively small number of individuals. Because Thomas (1995) indicated that some bats were awakened by his presence in the hibernaculum, we reduced disturbance the second year to see if weight losses were different between the two years of study. Interestingly, mass loss was higher for both males and females in the first year. Less weight loss in the second year (although not significant) suggests that disturbance from research activity may have negatively affected the mass of the bats. Unpublished mass loss data were available from the 1990–1991 field season at Copperhead. The average mass loss for males was 2.73 grams, and the average for females was 2.53 grams. These are comparable to the average mass loss found in the present study. Johnson et al. (1998) found a relationship between human visitation and weight loss for the Indiana myotis (*Myotis sodalis*). In contrast to the eastern pipistrelle, the Indiana myotis hibernates in large clusters, and disturbance from aroused neighboring bats may contribute greatly to the disturbance from human visitation.

Whitaker and Rissler (1992*a,b*) reported that the influx of eastern pipistrelles began in August and swarming tapered off through September and October. Present data were similar to those of Whitaker and Rissler, with few pipistrelles caught in winter. However, more were captured in April.

Whitaker and Rissler (1992*a*) counted all bats in the mine, including the back rooms, on four occasions. They found 201 eastern pipistrelles on 23 February 1988, 113 on 14 January 1989, 92 on 27/29 December 1989 and 170 on 3 March 1991, or an average of about 144 eastern pipistrelles hibernating within the mine. These were similar to the maximum numbers counted during the present study.

We expected that most pipistrelles would stay relatively inactive, and that individuals would remain loyal to specific locations in the cave throughout hibernation. However, we observed a great deal of movement between locations throughout the winter, interspersed between relatively long periods in one spot. Harden and Hassell (1970) found that eastern pipistrelles might return to the same location after moving. During the present study, there was no way to determine if a particular bat had awakened, and returned to the same location, or if another bat had replaced it at that location.

Little brown myotis and Indiana myotis cluster during hibernation. Twente (1955) remarked that this clustering behavior probably aids thermoregulation. McNab (1974) suggested that clustering species will cope better with colder cave temperatures than nonclustering species because solitary animals have a greater surface to volume ratio and dissipate heat more quickly than clustered animals. Eastern pipistrelles are a small bat that does not cluster, apparently mitigating the impact of temperature on its small size by hibernating in thermally stable environments.

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