2004. Proceedings of the Indiana Academy of Science 113(1):60–65

COLONIES AND REPRODUCTIVE PATTERNS OF TREE-ROOSTING FEMALE EASTERN PIPISTRELLE BATS IN INDIANA

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ABSTRACT. We investigated colonies and reproductive patterns of adult female eastern pipistrelles, *Pipistrellus subflavus*, in Indiana during the non-hibernation season in 1999–2000. Females began to form small colonies (range, 1–8 individuals) during early May, and parturition occurred during late June and early July (sex ratio of young = 1:1). Young pipistrelles began to fly during middle to late July. Colonies of adult females began to disband soon after the young became volant. After leaving colonies, the adult females roosted singly for the remainder of the summer, and then moved to hibernation sites from late August through September. This study is the first detailed description of tree colonies and reproductive patterns of eastern pipistrelles roosting in natural structures.

Keywords: Bats, eastern pipistrelle, *Pipistrellus subflavus*, colonies, reproduction, tree roosts

Eastern pipistrelles are common across the mid-western United States (Barbour & Davis 1969), and in summer prefer wooded habitats near permanent water sources such as streams and ponds (Mumford & Whitaker 1982). The eastern pipistrelle is usually a tree-roosting species, spending the day in clusters of dead or live foliage (Veilleux et al. 2003). Although eastern pipistrelles roost mainly in tree foliage, they sometimes roost in buildings; and to date studies investigating populations and reproduction of eastern pipistrelles in summer are based solely on colonies located in man-made structures (Allen 1921; Cope et al. 1961; Jones & Pagels 1968; Jones & Suttkus 1973; Whitaker 1998; Winchell & Kunz 1996). In this study we examined colonial behavior and reproductive patterns of eastern pipistrelles roosting in trees.

Female eastern pipistrelles leave hibernacula during April through mid-May (Whitaker & Rissler 1992), presumably migrate a relatively short distance (perhaps 50–100 km), and then begin to form summer colonies (Hoying & Kunz 1998; Whitaker 1998). After a gestation period of approximately 44 days (Wimsatt 1945) females give birth to two young (Lane 1946; Whitaker 1998; Wimsatt 1945) in late June to early July (Hoying 1983; Whitaker 1998). However, parturition may occur as early as late May at more southern latitudes (Jennings 1958). In many litters only one pup survives due to predation (Hoying & Kunz 1998) or other unknown factors. Young fly when approximately three weeks old and are able to effectively forage about one week later (Fujita & Kunz 1984). Adults leave the maternity roost once the young are fully weaned (Hoying 1983; Whitaker 1998), although the young may remain in the roost for longer periods. Whitaker (1998) reported mean summer colony size in man-made roosts to be 15 individuals (range 7–29), and Hoying & Kunz (1998) report the largest summer colony on record at approximately 55 bats.

The purpose of our study was to investigate colonial behavior (arrival at summering grounds, colony size and fluctuations, summer colony breakdown, migration to hibernacula following the summer roosting season) and describe reproduction events (dates of pregnancy and parturition for adult females, and first flight and weaning of young) for eastern pipistrelles roosting in trees. We also compare these data with similar data from pipistrelle colonies located in buildings.

METHODS

Data were collected mainly during April through September 1999 and 2000 at Prairie

Creek in southwest Vigo County, in southwest Indiana (UTM 43.48.000N, 4.54.000E), although information from several mist-netting samples collected during 1997 and 1998 also is included (see Veilleux et al. 2003 for details of habitat and netting areas). Mist-netting and radio-telemetry were used to gather data on dates of arrival and departure from summering grounds, dates and duration of female reproductive stages (i.e., pregnancy, lactation, post-lactation), and dates of first flights of juveniles.

Mist-netting consisted of using two mist-nets, one above the other, for a combined surface area of 45 m^2 (9 m long, 5 m high). Mist-nets were typically manned from dusk until midnight. Once captured, each bat was weighed and assessed for sex, age, and reproductive status. Age class (adult/juvenile) was determined by the degree of ossification of epiphyseal plates on the phalanges (Anthony 1988). Adult females were classed as pregnant by the degree of distension of the abdomen, and as lactating if milk could be expressed after gentle pressure was applied to the teat (Racey 1988). Bats were considered non-reproductive if the abdomen was not distended (typical of bats caught very early in the year, i.e., late April to early May), or if no milk could be expressed from teats that had been obviously suckled (hereafter termed post-lactating). Bats were fitted with a numbered plastic or aluminum wing band (Barclay & Bell 1988) to allow future identification of recaptured individuals. Bands were fitted to the left wing of females and right wing of males.

Radio-telemetry was used to locate tree roosts of female pipistrelles. Adult female pipistrelles were fitted with small (0.45 g) radio-transmitters (Model LB-2, Holohil Inc., Ontario, Canada; Model LTM, Titley Electronics, New Ballina, Australia). A small amount of fur was trimmed from between the scapula, and the transmitter was glued into place using a non-toxic surgical adhesive (Skin-Bond, Smith + Nephew, Largo, Florida). Mean percent body mass of transmitters was 7.0%. Following processing, bats were immediately released at their point of capture. Radio-tagged bats were tracked to their day roosts on the following day, and each day thereafter until the transmitter battery failed or the transmitter detached from the bat.

Bats were tracked to roost trees using a radio-receiver (Model TRX2000S, Wildlife Materials, Carbondale, Illinois) and a 3-element Yagi antenna. After a roost tree was located, the location of the bat was estimated using the receiver; and up to four hours were spent searching for the specific roost location using binoculars and a spotting scope. Daily emergence counts were made at each roost to gather data on colony size; and on several occasions, to determine whether young were present. Emergence counts were conducted until 10 min after the last bat emerged. To avoid potential bias (i.e., the effect of disturbance during radio-tagging), if bats were tracked to a roost tree on the first day of monitoring, and on subsequent days never re-used that roost, data from that roost were not included in later analyses. Data were reported as mean ±SD. Student's *t*-test and binomial analyses were performed using SPSS v. 10 statistical software.

RESULTS

Arrival at summer habitat and colonies.—Data describing capture rates of eastern pipistrelles are presented in Table 1. The earliest date that female pipistrelles were captured at Prairie Creek was 29 April in 1999 and 1 May in 2000. Some bats radio-tagged in late April and early May were probably spring migrants in transit. Single individuals radio-tagged on 29 April, 6 May, and 9 May in 1999 and on 2 May 2000, were never located after the day following capture, and were assumed to have continued migration and left the study area.

Females began to establish summer residence at Prairie Creek during the first two weeks of May in both 1999 and 2000. The first female pipistrelles that remained at the Prairie Creek study site were radio-tagged on 9 May 1999 and on 3 May 2000. Colonies began to form in early May; and their composition was quite fluid, as indicated by the behavior of several radio-tagged bats. The earliest female to remain at the study site, radio-tagged on 3 May 2000, roosted singly during the entire observation period (4–9 May). The first radio-tagged females found roosting in a colony occurred on 9 May 1999 and 7 May 2000. The individual from 1999, a female in early pregnancy, roosted singly on three days, and with other bats (approximately 2–5 bats;

Table 1.—Biweekly captures of *Pipistrellus subflavus* at mist-net sites. Bats per net night is the number of bats captured divided by number of net nights during each 2-week period.

	April 15–30	May 1–15	May 16–31	June 1–15	June 16–30	July 1–15	July 16–31	August 1–15	August 16–31	Sept. 1–15	Sept. 16–31
# Net nights	11	13	10	13	9	12	25	14	12	7	2
Adult female											
No. of bats	2	20	8	10	5	8	4	3	2	0	0
No. per net night	0.2	1.5	0.8	0.8	0.6	0.7	0.2	0.2	0.2	0.0	0.0
Adult male											
No. of bats	0	1	5	3	1	2	5	0	4	2	1
No. per net night	0.0	0.1	0.5	0.2	0.1	0.2	0.2	0.0	0.3	0.3	0.5
Juvenile female											
No. of bats	0	0	0	0	0	0	6	3	4	0	0
No. per net night	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.0	0.0
Juvenile male											
No. of bats	0	0	0	0	0	0	8	3	1	0	0
No. per net night	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.0	0.0

exact number of individuals could not be counted) on five occasions. In 2000, a female in early pregnancy was monitored between 7–13 May 2000, and roosted with 4–7 other bats.

Pregnancy.—A total of 35 pregnant pipistrelles was captured: 10 between 9 May-B19 June 1999, and 25 between 6 May-B23 June 2000. The first pipistrelles with slightly distended abdomens (indicating early pregnancy) were observed on 9 May 1999 and on 6 May 2000. By 16 June 1999 and 13 June 2000 pregnant females had large, distended abdomens, with body masses between 8–11 g.

Parturition.—Earliest dates of parturition were estimated by direct observation of non-volant young remaining at the roost when mothers left to forage in the evening. On 29 June 1999, at least one young bat was present in a roost located within the live foliage of a hackberry (*Celtis occidentalis*) tree, indicating that at least one adult female had given birth. On 26 June 2000, at least one young bat was present in a roost located in live foliage of a sugar maple (*Acer saccharum*), again indicating at least one female had given birth. This roost remained occupied for the next six days, and adults were apparently giving birth during this period because 4–6 young were visible by the fourth day.

Seven lactating pipistrelles were captured: 1 in 1999 and 6 in 2000. The earliest capture dates for lactating females were 29 June 1999 and 30 June 2000. The latest capture of a lactating female occurred on 6 July 2000. Additional lactating females were likely present past this date because pregnant pipistrelles were captured as late as 23 June, and lactation lasts at least 3–4 weeks (Hoying 1983; Whitaker 1998).

Size of reproductive colonies.—Data are reported for 16 reproductive colonies; 9 pre-maternity colonies (only pregnant females present) and 7 maternity colonies (pre-weaned young and/or lactating females present). Pre-maternity colonies that were observed toward the end of the pregnancy period may have included some lactating females. Early maternity colonies could have contained bats in late pregnancy, and late maternity colonies may have contained newly volant young. Mean size of pre-maternity colonies was 3.7 ±1.9 bats; range 1–7. Mean size of maternity colonies was 4.4 ±2.4 bats; range 1–8. Size difference between pre-maternity and maternity colonies was not significantly different ($t = -0.717$, $df = 14$, $P = 0.48$).

First flight and weaning.—We estimated the date of first flight of juvenile pipistrelles by capturing newly volant young in mist-nets. The earliest volant juveniles were captured on 26 July 1999 and 16 July 2000.

Disbanding of summer colonies.—By 20 July 1999 and 6 July 2000, colony size began to fluctuate; and eventually females roosted singly for the remainder of the summer. For example, the number of bats roosting with a radio-tagged post-lactating female between 20–30 July 1999 varied daily: day 1 = two bats; day 2 = one bat; day 3 = 1 bat; day 4 = four bats; day 5 = two bats; day 6 = unknown; day 7 = one bat; day 8 = three bats; day 9 = four bats; day 10 = unknown; day 11 = three bats. A lactating pipistrelle monitored between 6–14 July 2000 exhibited similar fluctuations in the number of bats she roosted with across several days. She was probably near the end of the lactation period, and the number of bats at the roost varied: day 1 = seven bats; day 2 = three bats; days 3 = two bats, day 4 = two bats; day 5 = two bats; day 6 = unknown; day 7 = one bat. Six individuals were captured and radio-tagged after 4 August 1999 (n = 1) and 25 July 2000 (n = 5); each was post-lactating and roosted singly.

Migration to hibernacula.—The number of adult bats captured in mist-nets declined by late July (Table 1). Most adult female pipistrelles appear to have left their summering grounds (i.e., Prairie Creek) for hibernacula by late August.

Radio-tagged adult female pipistrelles were last observed at the study area on 18 August 1999 and 22 August 2000. The last adult females captured by mist-net occurred on 25 August 1999 and 15 August 2000 (the radio-tagged individual from 22 August 2000). The adult female captured on 25 August 1999 was radio-tagged, but was not relocated, possibly indicating the bat was a migrant in transit.

The last juveniles were captured on 3 August 1999 and 18 August 2000. Occasional captures of adult males continued for weeks past the last capture of adult females. The latest capture of adult males occurred on 25 September 1999.

Sex ratios.—There were 86 adult and 26 juvenile pipistrelles captured between 1997 and 2000. Among adults, females (n = 62) were more commonly encountered than males (n = 24) by a nearly 3:1 ratio ($P < 0.01$ for binomial test of 1:1). Juveniles were captured at nearly equal numbers (n = 12 males; n = 13 females).

DISCUSSION

Female eastern pipistrelles begin to settle at their summer range (Prairie Creek) during early May, and begin to form small colonies as additional individuals arrived. This behavior is similar to that observed for eastern pipistrelles colonizing man-made roosts early in the summer roosting season. Whitaker (1998) examined dates of first arrival and colony accretion for six man-made roosts in Indiana over a four-year period. As the colonies grew, most individuals (14 of 16 observations) arrived at these roosts singly, or in pairs, over several weeks.

In Indiana, pipistrelles that roost in man-made structures appear to arrive earlier at these roosts, as compared with pipistrelles roosting in trees at the Prairie Creek study site. Whitaker (1998) reported the earliest date of arrival for pipistrelles utilizing man-made roosts at 13 April, and 14 of 19 observations of arriving pipistrelles were between 13–29 April. On several occasions first arrivals were late: 17 May, 21 May, and 3 July, respectively. Whitaker (1998) speculated that these individuals might have initially joined colonies at other locations before moving on to their respective man-made roosts.

Tree-roosting females may arrive at summer ranges relatively late because foliage roosts are not available early in the spring (whereas roosts in man-made structures are presumably always available). Initial leaf-out of trees at the Prairie Creek study site begins in late April, but leaves are not fully formed on most tree species until mid-May (pers. obs.; last frost date for the study area averages 10 May). Hence, colony formation may be delayed compared to man-made roosts due to a lack of suitable tree-roost sites (i.e., leaf clusters) early in the year.

At roosts located in man-made structures, pipistrelle colony size gradually increases until reaching a maximum size after approximately 22 ±10 days (Whitaker 1998). In our study site, during both 1999 and 2000, maximum colony size was approached 11 days past first residency at tree roosts. It appears that colony size of tree-roosting bats reaches maximum size earlier compared to colonies from man-made roosts. The longer time needed for colonies in man-made roosts to reach maximum size may occur because more time is needed for all bats to reach the colony (building colonies contain, on average, nearly four times the number of bats found in tree roosts).

Size of reproductive colonies differs between tree roosts and man-made roosts (colony data for man-made roosts from Whitaker, 1998) in *P. subflavus*. Whitaker (1998) found colonies averaged 15 individuals (range 7–29), nearly four times the average colony size for tree-roosting pre-maternity groups, and 3.5 times the average size of tree-roosting maternity colonies. Other studies of roosting behavior of pipistrelles in buildings also have reported larger colony sizes compared to those observed in tree-roosting colonies. For example, Allen (1921), Cope et al. (1961), and Hoying & Kunz (1998) reported pipistrelle colony sizes located in man-made roosts at 18, 30, and 55 bats, respectively.

Two factors could contribute to the differences observed between size of colonies in tree roosts and in man-made roosts. First, the relative permanency of buildings compared to tree roosts may allow greater numbers of pipistrelles to congregate at a particular roost over several years. Tree roosts, particularly in dead leaf clusters, are not available for more than a single season and therefore may hinder many bats from forming reliable social bonds at a roost site. Second, the relatively small size of foliage roosts may simply limit the number of bats that are able to roost together.

Whitaker (1998) observed pipistrelles giving birth as early as 30 May, and as late as 11 July, with 92.2% of births occurring between 12 June and 1 July. During our study, we did not detect evidence of parturition until late June. Man-made roosts may be warmer than foliage roosts because buildings, but not foliage, are often protected from climate conditions such as wind and rainfall. Because gestation rate increases with increasing temperature (Racy 1973), this may account for the later parturition at tree roosts.

Whitaker (1998) found that most adult females left maternity roosts in buildings approximately one week after young became volant (although the adult females may have remained in the same area but at different roosts). Similar colony abandonment by tree-roosting adult females may explain the decrease in their capture rates beginning in mid-July and continuing through August.

In Indiana, male Indiana bats (*Myotis sodalis*) do not travel as far from hibernacula as females. (Whitaker unpubl. data). If adult male pipistrelles also remain nearer to hibernacula, it could explain their relatively low numbers at Prairie Creek. Whitaker & Rissler (1992) monitored year round bat activity at a hibernaculum located at Copperhead Cave (Vermillion County, Indiana). Adult female pipistrelles were not captured at the mine during early summer, whereas adult males were observed at the mine during both June and July. Additionally, males may forage alone, whereas females may forage in small groups (Wilkinson 1992; Fleming 1982), perhaps increasing the probability of capturing relatively more females due to local concentration of females.

ACKNOWLEDGMENTS

We thank the landowners at the Prairie Creek study site, in particular, H. Clark, J. Strain, and B. and J. Evans, who allowed access to their properties, and to A. Krochmal, J. Duchamp, and C. Ritzi for help with fieldwork. We also thank the Life Sciences Department of Indiana State University for financial assistance and use of field vehicles and equipment. Additional funding was provided by grants from Bat Conservation International, the American Museum of Natural History (Theodore Roosevelt Fund), the Indiana Academy of Science, and the Indiana State University Graduate School Research Fund to Jacques Pierre Veilleux.

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Manuscript received 22 January 2003, revised 10 February 2004.